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"EVO-DEVO AS A COMPLEX APPROACH TO BIOLOGICAL EVOLUTION"

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CONFERENCE

A01

EVOLUTION AND DEVELOPMENT OF THE OLFACTORY SENSORY SYSTEM: AN UNUSUAL NEUROENDOCRINE INTERFACE

Whitlock, KE
Centro Interdisciplinario de Neurociencia de Valparaíso, Institute of Neuroscience, Facultad de Ciencias, Universidad de Valparaíso, Chile. Kathleen.whitlock@uv.cl

The olfactory sensory system is a fascinating sensory system with unique among the sensory systems for its lifelong neurogenesis and special interface with neuroendocrine systems that respond to both internal and external signaling. We work with zebrafish, which as a teleost fish represents approximate 50% of the vertebrates on the planet. Using imaging techniques in both living and fixed tissues coupled with gene and protein expression, we have investigated development of the olfactory sensory system and associated gonadotropin releasing hormone Gnrh) neuroendocrine cells. Traditional models, based on morphological studies of fixed tissues, subdivided the peripheral nervous system into "ectodermal placodes" located at the border of the neural plate. We have shown that the olfactory epithelia arise from large fields of cells within the neural plate that are continuous with telencephalic precursors, and that cell movements, not cell division, underlie olfactory placode morphogenesis. (Whitlock, 2008; Torres-Paz and Whitlock, 2014; Torres-Paz et al 2020). Thus, similar to the visual system, the olfactory epithelia and bulbs arise from a common neurectodermal region and develop in concert both in developmental and evolutionary time. Concurrently we have shown that Gnrh neuroendocrine cells of the hypothalamus, originally thought to arise from the olfactory organ, arise from neural crest, a characteristic that was later shown to be partially conserved in mammals. Previously we have shown that olfactory imprinting, a specific type of long-term memory, is correlated with a transcriptional response in the olfactory organs that include upregulation of genes associated with the immune system unique. Subsequently we identified a population of neutrophils in the olfactory organs that are associated with both the olfactory epithelia and the lymphatic vasculature suggesting a dual olfactory-immune function for this unique sensory system (Palominos & Whitlock 2021; Palominos et al 2022). In our most recent work we examined the Gnrh signaling pathway to better understand reports in the literature demonstrating that loss of function of Gnrh genes in zebrafish does not affect fertility. In contrast to inactivating the Gnrh ligand we targeted the Gnrh receptors and showed that loss of function Gnrh receptor3 mutants are infertile, a result we explain by the ability of exogenous Gnrh to stimulate the hypothalamic axis (Tine et al 2025). These findings support a mechanism of environmental compensation for gene loss in a teleost fish.

YOUNG SCIENTISTS' SYMPOSIUM

A02

EVO-DEVO AND TRANSGENERATIONAL PLASTICITY: HOW PARENTS AND ENVIRONMENTAL PREDICTABILITY SHAPE PHYSIOLOGICAL RESPONSES.

Salinas, S
Department of Biology, Kalamazoo College, Kalamazoo, MI 49006, USA

Transgenerational phenotypic plasticity (TGP) occurs when environmental conditions experienced by parents influence the phenotype of their offspring without underlying genetic changes. This phenomenon, central to evolutionary developmental biology (evo-devo), can shape adaptive responses to rapid environmental change, such as global warming. I present experimental evidence of thermal TGP in the sheepshead minnow (*Cyprinodon variegatus*), testing how parental temperature and environmental predictability affect offspring growth and maturation across multiple generations. Our results show that TGP occurs in several populations and that its magnitude is linked to the temporal and spatial predictability of thermal variation. These findings highlight the need to integrate evo-devo, ecological, and population-level approaches to better understand adaptive mechanisms in a changing world.

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A03

THE DUAL NATURE OF FLORAL ONTOGENY: OPPORTUNITY AND CONSTRAINT IN THE EVOLUTION OF ANGIOSPERMS

Strelin, M M
Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), Córdoba, Argentina.
Email: mstrelin@imbiv.unc.edu.ar

My research questions the traditional view of floral evolution, which often underestimates the importance of floral ontogeny as a source of variation, opportunity, and constraint for evolutionary change. In the first part of my talk, I will address the concept of floral ontogeny as a source of opportunities for evolutionary change. I will present a summary of my studies on the evolution of development in Loasoideae (Loasaceae), a widely distributed family of angiosperms in the Andes. This family underwent transitions from bee pollination to vertebrate-mediated pollination (hummingbirds and rodents) during the uplift of the Andes. This change in pollinator type is related to the creation of high-altitude niches where insects are less abundant. Regarding the evolution of floral development in this subfamily, I will explain how a simple change in the ontogeny of the flowers—a delay in the maturation of the epidermal cells of the petals—could be responsible for the origin of larger corollas with a more "juvenile" (closed, like a flower bud) shape in vertebrate-pollinated species. In the second part of the talk, I will address the concept of floral ontogeny as a constraint on evolutionary change. I will use the floral biology of soybeans as an example, a crop that has been subjected to intense artificial selection and genetic improvement. This crop exhibits cleistogamy, which means that some of its flowers do not open upon reaching reproductive maturity and self-pollinate. I will discuss how, despite breeding efforts, the morphology of soybean flowers and their dependence on pollinators are strongly influenced by developmental constraints common to various lineages of cleistogamous angiosperms. These constraints could have a significant large-scale impact on crop yield. Through this presentation, I aim to demonstrate that by considering development as a source of evolutionary opportunity (for example, simple changes in conserved developmental trajectories can generate large phenotypic changes and adaptation to new pollinators) and evolutionary constraint (in a crop as "modified" as soybeans, certain important floral attributes could not be altered), we gain a more complete picture of the basis of biological diversity, in both natural and artificial systems.

A04

THE CONSTRUCTION OF THE AVIAN FOOT: FROM EMBRYONIC DEVELOPMENT TO EVOLUTIONARY DIVERSITY

Carril J
CONICET; Instituto de Morfología y Patología Animal (IMPA, FCV, UNLP); julyetacarril@gmail.com

The evolutionary trajectory of birds led to a constructional pattern that characterizes their body plan (*bauplan*) and the adaptive morphological variation of their feet at lower taxonomic levels (orders and families). The diverse foot types (*e.g.,* anisodactyl, didactyl, zygodactyl, webbed) are accompanied by an also diverse musculoskeletal system, enabling birds to adopt different primary lifestyles (*i.e.,* terrestrial, arboreal, aquatic, and hyperaerial) and to exhibit a wide range of locomotor and manipulative skills (*e.g.,* walking, running, jumping, wading, perching, climbing, swimming, diving, hanging upside down, grasping). Moreover, avian hatchlings exhibit remarkable differences in their locomotor activity, spanning a broad altricial–precocial spectrum. Historically, research focused on the anatomy of foot types and their potential link with avian primary lifestyles, locomotor abilities, post-hatching developmental modes, and evolution. However, within the evo-devo paradigm, research gaining relevance emphasizes the role of developmental reprogramming as a key evolutionary process between (random) mutations and (non-random) natural selection. The current state of the art regarding these questions includes research about: (1) the molecular mechanisms of foot development, (2) the foot skeletogenesis and ossification sequences searching for heterochronies, (3) digit retroversion and the epigenetic influence of musculature on skeletal development, and (4) the anatomy and connectivity patterns of anatomical elements of the foot during development and their link to the phylogenetic history. Taken together, these approaches allow us to understand birds' feet not only as adaptive structures but also as windows into the interplay between development and evolution. As the Catalan biologist Pere Alberch once reflected: natural selection may decide the winner of the game, but development non-randomly defines the players.

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BIOLOGY SOCIETIES SYMPOSIUM

A05

STUDYING CARDIAC DEVELOPMENT IN ZEBRAFISH MODEL

Lombardo VA
[1]*Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR)* [2]*Centro de Estudios Interdisciplinarios (CEI-UNR)*
lombardo@ibr-conicet.gov.ar

Cardiac morphogenesis is a highly conserved process among vertebrates. The zebrafish (*Danio rerio*) is utilized as a model organism to study cardiac development and its associated pathologies. In our laboratory, we study an early phase of cardiogenesis known as cardiac looping, a process that depends on both intrinsic and extrinsic factors, and whose disruption can lead to congenital heart defects in humans. To study this, we used transgenic zebrafish lines that express fluorescent proteins within cardiac tissues, and we combined fluorescence and confocal microscopy techniques with photoconversion assays, hemodynamic force perturbation, and pharmacological inhibition. We established morphological parameters to quantify the impact of different treatments on the cardiac looping degree. Our results show that endocardial growth occurs through cell proliferation, without the addition of extracardiac cells. This proliferation is independently regulated by both blood flow and BMP signaling. We also observed that this signaling pathway is differentially activated in cardiac tissues, regulating cell proliferation in the endocardium and modifying cell morphology at the superior atrio-ventricular canal within myocardium, facilitating cardiac tube folding. We are currently applying these methodologies to analyze how alterations in reactive oxygen species homeostasis affect normal cardiac development. This work contributes to a comprehensive understanding of the morphological, cellular, and molecular events involved in cardiac looping, essential knowledge for elucidating the etiology of congenital heart defects in humans.

A06

RHO GTPASE SIGNALING IN NEURONAL POLARIZATION AND MORPHOGENESIS: DUAL FUNCTIONS OF RHOA AND EMERGING ROLES OF RHOD

Bisbal M
Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Córdoba (UNC), Córdoba, Argentina.
mbisbal@immf.uncor.edu

Small Rho GTPases are essential regulators of neuronal cytoskeletal dynamics, cellular polarization, and morphogenesis of developing neurons. We will present research lines from our laboratory exploring the roles of RhoA and RhoD in these processes. In a recent study, we demonstrated that RhoA exerts both inhibitory and stimulatory functions in axon formation and elongation. Using FRET-based biosensors, we observed that RhoA activity is high in growth cones of minor neurites and elongating axons, but low in newly specified axons. ROCK-dependent signaling restricts axon formation, whereas the RhoA–mDia pathway promotes microtubule stability and axonal extension, revealing a dual spatio-temporal regulation. In parallel, we initiated the functional characterization of RhoD, an atypical, fast-cycling Rho GTPase expressed exclusively in mammals. Through knockdown, dominant-negative mutants, and morphometric analyses, we found that RhoD is essential for the transition to polarized neurons, the differential growth of axons, and dendritic complexity during development. Our findings expand the functional landscape of the Rho family, highlighting that distinct members act complementarily and at specific developmental stages to coordinate cytoskeletal remodeling and neuronal morphology.

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A07

ONTOGENY, EVOLUTION, AND DOMESTICATION: PERSPECTIVES ON CRANIAL DEVELOPMENT IN MAMMALS

Flores, DA
Instituto de Vertebrados, Fundación Miguel Lillo. Unidad Ejecutora Lillo (CONICET-Fundación Miguel Lillo). Email: daflores@lillo.org.ar

Knowledge of cranial developmental patterns is essential to understanding the evolution of cranial form and function in this clade. Previous studies have shown the effect of historical legacy on mammalian skull growth patterns. As demonstrated in this taxonomically broad study of mammals, domestication, through directed selection, modifies the allometric pattern of postnatal cranial growth in the group. Skull shape was characterized in 1,128 individuals using 14 functionally relevant linear measurements, comparing 13 pairs of wild versus domesticated forms representing different clades within Mammalia. Among wild forms, the wild boar, rabbit, and wolf showed the highest proportion of allometric growth, partly explaining the great morphological diversity of the domesticated forms of these species. Wild forms exhibited more isometric growth than their domesticated counterparts. Multivariate comparisons showed that dogs and llamas presented the greatest differences in ontogenetic trajectories relative to their wild counterparts, while the smallest differences were recorded in wild boar–pig, camel, and horse pairs. Bivariate analyses of ontogeny revealed that most domesticated forms had different growth trajectories from their respective wild counterparts with respect to slopes, or growth rates. In pigs and camels, slopes were shared while intercepts differed. An extension of the trajectory was observed in most domesticated herbivores, while the opposite pattern was found in carnivores. However, there is no single universal or global pattern of paedomorphosis or any other type of heterochrony underlying the morphological diversification associated with domestication in Mammalia.

EXPERT SCIENTISTS SYMPOSIUM

A08

EVOLUTION AND CONSEQUENCES OF POSTMATING MOLECULAR INTERACTIONS

Luciano M. Matzkin
University of Arizona, Department of Entomology, BIO5 Institute, Department of Ecology and Evolutionary Biology, lmatzkin@arizona.edu

Sexual selection and conflict can drive the evolution of traits involved in postmating prezygotic interactions. Incompatibilities of the postmating prezygotic interactions can lead to the formation of reproductive barriers and possibly the origins of species. Working with cactophilic *Drosophila*, we uncovered a previously unrecognized form of postcopulatory inter-organismal communication driven by the transfer of mRNA from males to females. Although RNA is found in the seminal fluid of diverse organisms, it was unknown whether this RNA is functional within females. We developed an experimental proteomic method called VESPA (Variant Enabled SILAC Proteomic Analysis) to test the hypothesis that *Drosophila* male seminal fluid RNA is translated by females. We find strong evidence for 67 male-derived, female-translated proteins (mdFTPs) in female lower reproductive tracts at six hours postmating, many with predicted functions relevant to reproduction. CRISPR gene knockout (KO) experiments indicate that genes coding for mdFTPs play diverse roles in postmating interactions, with effects on fertilization success, and the formation and persistence of the insemination reaction mass, a trait hypothesized to be involved in sexual conflict. We have been developing a suite of genomic and transgenic resources in cactophilic *Drosophila* to facilitate the use of this system to answer a myriad of biological questions. We are now using our newly generate UAS/Gal4 transgenic tool kit in these species to determine the role of accessory gland derived exosomes in the transfer of these mdFTP into females. In addition to RNA, a variety of seminal fluid proteins (SFPs) are transferred during mating. Our work indicates extensive divergence between species pairs in seminal fluid and female reproductive proteomes at protein sequence, compositional, and quantitative levels. Using CRISPR KO we assessed the functional effects of divergently evolving SFPs. Using protein-protein interaction modeling we predicted that conserved male-female protein interactions are maintained in heterospecific crosses despite extensive protein sequence divergence. Furthermore, our work indicates that disrupted protein interactions in heterospecific crosses are predicted to arise from compositional changes to reproductive proteomes. Given the diverse species known to carry RNA and SFP in seminal fluid, these discoveries have broad significance for understanding molecular mechanisms of cooperation and conflict during reproduction. These findings advance our understanding of reproduction by revealing novel mechanisms of postmating molecular interactions between the sexes that strengthens and extends male influences on reproductive outcomes in previously unrecognized ways.

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A09

SOME MACROEVOLUTIONARY PERSPECTIVES ON THE EVOLUTION OF DEVELOPMENT

Soto, IM
Laboratorio de Biología Integral de Sistemas Evolutivos. Departamento de Ecología, Genética y Evolución, FCEyN, UBA. IEGEBA-CONICET. soto@ege.fcen.uba.ar

Understanding how the evolution of development generates morphological diversity requires integrating ontogenetic perspectives with macroevolutionary analyses. How does the synthesis of paleobiology, comparative anatomy, and phylogeny decipher the mechanisms governing morphological diversification in deep time? Through case studies, we will explore how developmental processes channel and constrain evolution across lineages. We will analyze the interaction between developmental constraints, selective pressures, and ecological opportunities. In birds, we will demonstrate how craniofacial modularity and the evolution of cranial kinesis acted as prerequisites for the adaptive radiation of Furnariidae, allowing independent evolution of functional components. Furthermore, in the genus *Tyrannus*, we will show how allometric relationships and evolutionary lines of least resistance directed the evolution of exaggerated sexual traits. From the fossil record, we will illustrate how ontogenetic trajectories in Cretaceous bivalves underpin macroevolutionary patterns, and how constraints imposed by critical body mass influenced the emergence and extinction of giant sloths. These cases demonstrate that ontogenetic and modular variation constitutes a fundamental dimension structuring morphological evolution, and its study at a macroevolutionary level represents a complementary and necessary perspective to the experimental approaches of Evo-Devo

A10

SAND STORIES: SPIDERS THAT BREAK PARADIGMS IN THE SOUTHERN CONE

Aisenberg A
Departamento de Ecología y Biología Evolutiva, Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Avenida Italia 3318, Montevideo, Uruguay. anita.aisenberg@gmail.com aaisenberg@iibce.edu.uy

Due to their great diversity of known and yet-to-be-discovered species, and the wide variety of behavioral strategies that allow for combining experiments in natural settings with elegant laboratory manipulations, spiders are invaluable research models for testing hypotheses on Evolutionary biology and Ecology. At Latin American Arachnology, we have created long-standing collaborative networks between researchers from diverse disciplines, promoting a multidisciplinary approach distinctive for evolutionary biology studies. *Allocosa marindia* and *Allocosa senex* are wolf spiders of the subfamily Allocosinae that build burrows in fluvial, estuarine, and oceanic sandy beaches in Argentina, Brazil, and Uruguay. Unlike the patterns reported for spiders, in both species, females are the mobile sex that initiates courtship, and males are larger than females. These species provide a great opportunity to study the origin and evolution of sexual traits in spiders and to test the impact of environmental conditions on them. We studied mobility and sexual size dimorphism in spiders of the subfamily Allocosinae inhabiting fluvial, estuarine, and oceanic coastal ecosystems, as well as coastal volcanic and grassland ecosystems in South America. We reviewed scientific collections, and conducted three consecutive nights of field samplings to collect adults and determine the nocturnal surface activity of each species. We recorded genetic, taxonomic, morphometric, environmental, and behavioral data for each species and/or morphotypes. A total of 1,071 adult Allocosinae spiders belonging to 18 species or morphotypes were analyzed. We reported new species for Uruguay, and described new genera and species for science. We found new records of coastal Allocosinae with novel sexual traits. All spiders with atypical patterns belonged to coastal environments, but not all coastal spiders displayed novel sexual dimorphism patterns or courtship behaviors. We confirmed behavioral plasticity of *A. senex* regarding its sexual strategies and burrow construction. Future studies will focus on two species of this subfamily: the coastal *A. senex* and the grassland spider *Paratrochosina amica*, determining their genetic, morphological, and behavioral diversity throughout their wide distribution. Due to their status as biological indicators and their charismatic traits, these species have potential as flagship species for the conservation of the implied South American ecosystems.

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A11

EVOLUTION OF FORM AND FUNCTION IN MAMMALIAN SPERMATOZOA

Roldan, RS
Departament of Biodiversity and Evolutionary Biology,
Museo Nacional de Ciencias Naturales (CSIC), 28006-Madrid, Spain;
roldane@mncn.csic.es

Mammalian spermatozoa display an extraordinary diversity in morphology, ranging from subtle variations in head shape and flagellar length to extreme specializations in size, architecture, and motility patterns. This diversity has long been interpreted as the outcome of postcopulatory sexual selection, particularly sperm competition and cryptic female choice, which favour traits enhancing fertilization success under different mating systems. Comparative analyses across mammals have revealed significant associations between sperm form, velocity, and competitive regimes, suggesting strong adaptive pressures on sperm performance. However, an exclusive focus on selection overlooks the potential role of developmental and phylogenetic constraints in shaping sperm design. Recent advances in evolutionary developmental biology (evo-devo) and molecular genetics have begun to uncover how the differentiation of sperm components (nucleus, acrosome, flagellum) depends on complex gene expression networks and cytological processes that may limit or bias morphological evolution. Integrating these perspectives reveals that sperm form and function result from an interplay between adaptive modification and inherited developmental architecture. This integrative framework not only refines our understanding of sperm evolution but also provides insights into the balance between selection, constraint, and innovation in reproductive diversification. The presentation will summarize comparative evidence and discuss current approaches linking functional morphology, molecular regulation, and evolutionary dynamics of mammalian sperm.

SHORT COMMUNICATIONS

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 1

A12

EFFECT OF ENERGY RESTRICTION ON THE FERTILIZING ABILITY OF CATSPER KNOCKOUT SPERM

González SN[1], Rebagliati Cid A[1], Sulzyk V[1], Weigel Muñoz M[1], Visconti P[2], Cuasnicú PS[1]
[1] Instituto de Biología y Medicina Experimental (IByME-CONICET), Vuelta de Obligado 2490, C1428ADN, Ciudad Autónoma de Buenos Aires, Argentina. Tel. +54 11 4783-2869 / Fax. +54 11 4786-2564
[2] Department of Veterinary and Animal Sciences, University of Massachusetts, 01003 Amherst, MA, USA

The principal sperm Ca^{2+} channel, CatSper (Cation Channel of Sperm), is essential for male fertility in both mice and humans as well as for the development of hyperactivation (HA), a vigorous motility pattern required for sperm to reach the fertilization site and penetrate the resilient zona pellucida (ZP) surrounding the egg. Recent results from our group revealed novel roles for CatSper not only in the previous stage of cumulus oophorus penetration but also in the subsequent step of gamete fusion. Based on previous findings showing that sperm energy restriction followed by nutrient reintroduction (SER) improves motility and fertilization rates in severely subfertile mice, we investigated whether SER treatment could rescue the inability of CatSper mutant sperm to penetrate the cumulus and/or fuse with the egg. To this end, control and CatSper KO sperm were incubated in standard TYH medium containing BSA, HCO_3^-, and nutrients (glucose and pyruvate) (CAP) or, alternatively, in nutrient-free TYH medium (SER) for 40 min (restriction phase), followed by the addition of nutrients and an additional 5 min incubation (rescue phase). CAP- and SER-treated sperm were then stained with Hoechst and co-incubated with cumulus-oocyte complexes to assess the number of fluorescent sperm within the cumulus (cumulus penetration), or with ZP-free eggs to evaluate the fertilization rate (gamete fusion). Results showed that while SER treatment did not affect the number of sperm detected within the cumulus, it significantly increased the percentage of fertilized ZP-free eggs. Further studies aimed at understanding the mechanisms underlying these observations revealed that SER did not modify either the spontaneous or progesterone-induced acrosome reaction, nor the ability of sperm to bind to the oolemma of ZP-free eggs. However, CASA (Computer-Assisted Sperm Analysis) revealed that whereas SER did not restore HA, it induced significant changes in several motility parameters associated with more linear trajectories, supporting the role of CatSper in promoting a specific motility pattern critical for sperm–egg fusion.

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A13

STUDY OF THE EFFECT OF cBiMPs, A CYCLIC ADENOSINE MONOPHOSPHATE (cAMP) ANALOG, ON MURINE SPERM PHYSIOLOGY.

Dalessandro Sellanes C, Oscoz-Susino N, Luque GM, Buffone MG, Marín Briggiler CI.
IBYME-CONICET, Buenos Aires, Argentina. E-mail: camiladsellanes@gmail.com

Capacitation is a complex process, essential for sperm to fertilize an oocyte. It is associated with the development of a vigorous motility pattern, known as hyperactivation, and with the occurrence of acrosomal exocytosis (AE). During capacitation, there is an increase in cyclic adenosine monophosphate (cAMP) concentration, which triggers the phosphorylation of protein kinase A substrates (pPKAs) and of tyrosine residues (pY). Phosphodiesterase (PDE) inhibitors increase cAMP concentration by blocking its degradation, thereby amplifying PKA activation and promoting the biochemical changes associated with capacitation. This can be reproduced *in vitro* by incubating sperm under appropriate conditions. In previous studies by our group, it was reported that the cAMP analog Sp-5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole 3'-5'-cyclic monophosphorothioate (cBiMPs) stimulates hyperactivated motility in human sperm. However, its potential clinical application requires safety testing using animal models. The aim of our work was to analyze the effect of cBiMPs on mouse sperm capacitation-related events. Epididymal spermatozoa were incubated for one hour with different concentrations of cBiMPs (0.3, 1, 3, 10, 30, and 100 µM), dimethyl sulfoxide (DMSO, 0.2%) as a negative control, and 8-bromoadenosine 3',5'-cyclic monophosphate (8-Br-cAMP, 1 mM), a known cAMP analog, as well as 8-Br-cAMP together with isobutyl-1-methylxanthine (IBMX, 0.2 mM), a known PDE inhibitor, as positive controls. Protein phosphorylation events were evaluated by Western blot, motility parameters using a CASA system (SCA, Microptic, Spain), and AE by Coomassie blue staining and optical microscopy. The results showed an enhancement in protein phosphorylation levels of pPKAs and pY when increasing cBiMPs concentrations, which were higher than those observed in spermatozoa incubated with 8-Br-cAMP and IBMX (n = 4). Likewise, a significant increase in kinematic parameters and in the percentage of hyperactivation was observed in spermatozoa incubated with cBiMPs compared to the negative control, although no differences were found in total or progressive motility percentages among the different treatments (n = 7). Regarding AE, although a trend toward an increase was observed with higher concentrations of cBiMPs, the values were lower than those obtained with 8-Br-cAMP and IBMX (n = 6). Taken together, these findings indicate that cBiMPs stimulate various physiological parameters associated with capacitation in mouse sperm, making it a compound with great potential to be used as a cAMP analog in studies related to mammalian fertilization. This work was supported by funding from CONICET and ANPCyT, and with financial assistance from the René Barón, Williams, and Honorio Bigand Foundations.

A14

COMBINED USE OF DIFFERENT ENERGY SUBSTRATES IN THE *IN VITRO* MATURATION OF BOVINE OOCYTES

Gutnisky C[1,2], Cetica P[1,2]
[1]Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Instituto de Investigación y Tecnología en Reproducción Animal (INITRA), Buenos Aires, Argentina. [2]Universidad de Buenos Aires - CONICET, Instituto de Investigaciones en Producción Animal (INPA). E-mail cgutnisky@fvet.uba.ar

Most metabolic studies related to the *in vitro* maturation (IVM) of bovine oocytes have been carried out in culture media containing different energy substrates, so the relative role of each substrate in the female gamete metabolism remains unclear. The aim of the present study was to evaluate the relative contribution of different oxidative substrates to the IVM of bovine oocytes. Cumulus-oocyte complexes (COCs) were obtained by aspiration of antral follicles from ovaries of slaughtered cows. IVM of COCs was performed for 22 hours in a defined medium (mSOF) in the presence of different oxidative substrates: a) absence of substrates, b) glucose (G), c) amino acids (AA), d) L-carnitine (L-car, an activator of fatty acid β-oxidation), e) G+AA, f) G+L-car, g) AA+L-car, and h) G+AA+L-car. Glucose consumption and ammonia production from AA deamination by the COCs during IVM were determined in microdroplets of culture media and measured spectrophotometrically. Endogenous lipid consumption was assessed by incubating denuded oocytes in a Nile Red solution and quantifying fluorescence from digital micrographs obtained with an epifluorescence microscope. Nuclear IVM of the oocytes was determined by the presence of metaphase II after staining with the fluorochrome Hoechst 33342 and observing under an epifluorescence microscope. Glucose consumption by COCs was higher in media supplemented with G, G+AA, and G+AA+L-car ($p<0.05$) compared with the other groups. Ammonia production was higher in media supplemented with AA and AA+L-car compared to media containing G+AA and G+AA+L-car ($p<0.05$). Endogenous lipid content decreased significantly in medium supplemented with G+AA+L-car compared with medium containing only G ($p<0.05$). Nuclear IVM rates were higher in media supplemented with G+AA and G+AA+L-carnitine ($p<0.05$). In conclusion, the combination of G and AA appears to be the most relevant for nuclear maturation of bovine oocytes, and this combination also promotes increased utilization of endogenous lipids by the oocyte.

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A15

DISRUPTIVE EFFECT OF TROPHOBLAST-DERIVED EXTRACELLULAR VESICLES FROM HYPOXIA-REOXYGENATION EXPOSED HUMAN PLACENTA ON PLACENTAL MICROVASCULAR CELL MIGRATION: PROTECTIVE ROLE OF MELATONIN

Etcheverry T[2], Canela Delas Y[2], Cabral I[1], Di Giusto G[1], Beltramone N[1], Sar J[3], White V[2], Farina M[2], Martínez N[1]
[1]Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO-UBA-CONICET), [2]Centro d Estudios Farmacológicos y Botánicos (CEFyBO-UBA-CONICET), [3]Servicio de Obstetricia, Hospital Naval Cirujano Mayor Dr. Pedro Mallo.
E-mail: noraalicia.martinez@hotmail.com

Proper development of the placental microvasculature, formed by fetal capillaries within the chorionic villi, is essential for efficient maternal-fetal exchange of gases and nutrients and, consequently, for normal fetal growth. Preeclampsia (PE) is a pregnancy disorder characterized by hypertension, proteinuria, and in severe conditions, multi-organ damage. Impaired remodeling of the uterine spiral arteries results in ischemia-reperfusion injury, generating hypoxia-reoxygenation (HR) episodes that promote inflammation, oxidative stress, and endothelial dysfunction. In PE, both the architecture and function of the placental microvasculature are altered, accompanied by an increased release of EVs that may contribute to endothelial impairment. Trophoblast cells synthesize large amounts of melatonin and express its receptors. Melatonin acts in an autocrine and paracrine manner within this organ, and it has been associated with the regulation of angiogenesis under different pathological and physiological conditions. This study was approved by the Ethics Committee of the Naval Hospital (Res. 110/22). Placentas were obtained from healthy term pregnancies (n=4). Placental explants were cultured under normoxia (N) or hypoxia-reoxygenation (HR) conditions, in the presence or absence of melatonin (1-20 μM). Extracellular vesicles (EVs) were isolated from explant-conditioned media by sequential centrifugation, filtration, and ultracentrifugation. EVs were characterized by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and western blot for CD63 and HSP70 proteins. Primary cultures of human placental microvascular endothelial cells (hPMEC), obtained from healthy placental villi, were exposed to EVs or EV-depleted supernatants (SN) from the different experimental conditions. Endothelial migration was evaluated using the wound-healing assay. Tissue or cell viability was assessed by MTT assay. Placental explant viability was not affected by HR. HR exposure increased EV release from trophoblast explants. The secretome derived from trophoblasts cultured under normoxia (N) significantly promoted hPMEC migration ($p<0.01$). Both EVs and EV-depleted supernatants (SN) obtained from HR-treated trophoblasts reduced hPMEC migration compared with those from N explants ($p<0.01$). EVs derived from explants cultured under HR in the presence of melatonin restored hPMEC migration compared to HR-derived EVs ($p<0.05$). These findings indicate that both EVs and soluble factors released by trophoblasts contribute to the crosstalk between trophoblasts and the placental microvasculature, modulating hPMEC migration. This regulatory mechanism is disrupted under HR conditions, whereas melatonin confers a protective effect by preserving endothelial cell function. Thus, HR-induced alterations in trophoblast-derived EVs may contribute to the vascular dysfunction seen in preeclampsia, whereas melatonin may prevent these effects.

A16

IDENTIFICATION OF MITOCHONDRIAL PROTEINS AS POTENTIAL CANDIDATES TO DEVELOP NON-HORMONAL MALE CONTRACEPTIVES

Candela Senosiain, Lucas N González, Vanina G Da Ros
Instituto de Biología y Medicina Experimental (IBYME - CONICET), E-Mail: csenosiain2002@gmail.com

The lack of alternatives for male contraception compared with the variety of female contraceptives highlights the need for new options for them, like those blocking sperm function. In this sense, given that the increase in mitochondrial activity is essential for sperm function, the goal of this project was to identify sperm specific proteins related to mitochondrial function that could work as potential contraceptive targets for non-hormonal male contraceptives. To achieve this goal, a bioinformatics-based strategy was used, comparing human (CitDBase) and mice (Skerrett-Byrne *et al*., 2022) proteomic databases. After this comparison, we selected proteins step by step based on defined criteria. In the first criteria we chose orthologous proteins between humans and mice using an Excel comparison. This analysis yielded 129 proteins. Thereafter, we used this list to determine which of them were specific from germ cells or male reproductive tract. Each protein was reviewed using PubMed and THPA. Results indicated that 106 proteins met this criterion. Out of this last list of proteins we selected those which were located only in sperm mitochondria by using PubMed and Uniprot. This search yielded 6 proteins (DNAJC5B, PDHA2, PPP3R2, SMCP, SPATA19 and UBL4B). After this, we analyzed if there was bibliographic evidence of infertility phenotypes in mice knockout models and genetic variants causing infertility in humans, this was completed using Uniprot and ClinVar. In this case we observed that only DNAJC5B, PDHA2, PPP3R2, SMCP and SPATA19 had a role in fertility and that PDHA2 also presented infertile patients with mutations in its coding gene. Subsequently, we evaluated if these proteins exhibited a low number of

ubiquitous homologs using BLATP (p<0,05). Through this analysis we observed that DNAJC5B, PDHA2 and PPP3R2 presented somatic homologs that could be blocked, while SMCP and SPATA19 had no homologs. Afterwards, we investigated whether the selected proteins possessed enzymatic activity. To this end, we simultaneously used PubMed searches and the UniProt database, detecting only DNAJC5B, PDHA2 and PPP3R2 with enzymatic activity. Finally, the three selected proteins were evaluated for the presence of potentially druggable pockets using the PockDrug algorithm, which identifies pockets based on each protein 3D structure. Results showed that the final proteins exhibited possible drug-susceptible pockets with different intrinsic characteristics. These results suggest that DNAJC5B, PDHA2 and PPP3R2 are potential candidates as contraceptive targets. Furthermore, the fact that mitochondrial proteins are essential for fertility highlights the role of this organelle for sperm function.

A17

RELATIVE ROLE OF OXIDATIVE SUBSTRATES IN PORCINE OOCYTE *IN VITRO* MATURATION AND THEIR EFFECT ON EMBRYO DEVELOPMENT

Morado S[1,2], Camporino A[1,2], Valle Ponce M[2], Cetica P[1,2]
[1]Instituto de Investigaciones en Producción Animal (INPA, UBA-CONICET), [2]Instituto de Investigación y Tecnología en Reproducción Animal, Facultad de Ciencias Veterinarias, UBA. E-mail: acamporino@fvet.uba.ar

It has been postulated that porcine oocyte-cumulus complexes (OCCs) can use endogenous lipids, glucose or amino acids present in the culture media as energy substrates during *in vitro* maturation (IVM). However, studies have been performed in complex media, so the relative use of each oxidative substrate may be overlapped. The objective of this study was to study the implication of the metabolism of endogenous lipids, glucose, amino acids or their respective combinations as oxidative substrates during IVM of porcine oocytes on subsequent embryo development. Cumulus-oocyte complexes (OCCs) were obtained by aspiration of antral follicles from ovaries of slaughtered gilts, selecting those with an intact and dense cumulus. COCs were matured in a defined medium (NCSU-37) in the presence of different oxidative substrates: a) without substrates; b) glucose; c) L-carnitine (activator of fatty acid β-oxidation); d) amino acids; e) glucose+L-carnitine; f) glucose+amino acids; g) L-carnitine+amino acids; h) glucose+L-carnitine+amino acids. Endogenous lipid consumption was assessed by staining oocytes with Nile Red and then quantifying their fluorescence form digital microphotographs obtained with an epifluorescence microscope. Glucose and ammonia levels in the residual medium were determined by specific spectrophotometric assays for each metabolite. Nuclear maturation percentages were assessed by epifluorescence microscopy after staining with Hoechst 33342, and embryo production by observing cleavage at 48 h and blastocysts at day 7 after *in vitro* fertilization. P values < 0.05 were considered statistically significant. Oocytes matured in the presence of L-carnitine showed an increase in endogenous lipid consumption compared to those cultured in its absence ($p<0.05$); while those cultured in the presence of amino acids as unique substrates showed an increase in ammonia production compared to the other groups ($p<0.05$). Glucose consumption, on the other hand, did not differ between treatments. It was observed that the groups matured with the combined use of 2 or 3 substrates presented significant differences in nuclear maturation and embryo cleavage compared to those matured in the absence or presence of a single substrate ($p<0.05$). However, all groups reached similar percentages of blastocysts, except for the one lacking oxidative substrates ($p<0.05$). These results suggest that endogenous lipids, amino acids, and glucose present in the culture medium can be used by porcine COCs as unique energy substrates during IVM to sustain subsequent embryo development to the blastocyst stage.

A18

CRISP PROTEINS AS TARGETS FOR A CANINE CONTRACEPTIVE VACCINE

Sulzyk, V, Robert F, González SN Weigel Muñoz M and Cuasnicú PS
IBYME-CONICET. (robertfacundo2@gmail.com)

The uncontrolled growth of canine populations represents a problem for both public health and animal welfare, indicating the need to develop new and better contraceptive methods. An attractive option is the development of a contraceptive vaccine against proteins that are critical for fertility. In this regard, CRISP proteins (Cysteine-Rich Secretory Proteins) stand out for their evolutionary conservation and their relevance in fertilization and fertility. Previous studies carried out in our laboratory using an antibody against the human epididymal protein CRISP3 (anti-hCRISP3) revealed the expression of at least one canine CRISP protein in the epididymis, its presence on the surface of ejaculated spermatozoa from animals of different breeds, and its permanence even after capacitation, supporting its possible participation in the fertilization process. Based on this, the objective of the present work was to carry out the functional characterization of the identified canine CRISP protein in order to evaluate its potential use as a contraceptive target. As a first step, we decided to express the recombinant canine protein for the subsequent production of specific antibodies against it and the future immunization of dogs. By using a plasmid carrying the corresponding gene transformed into the expression strain *Escherichia coli* Origami 2 and induction with IPTG, the recombinant canine CRISP protein was obtained within inclusion bodies. In parallel to the expression and purification of the recombinant protein, we

continued our functional characterization studies of the identified protein through the use of the anti-hCRISP3 antibody. For this purpose, ejaculated spermatozoa from animals housed in the Faculty of Veterinary Sciences (UBA) were washed and incubated under capacitating and non-capacitating conditions and in the presence or absence of the antibody, and several functional parameters analyzed thereafter. Results showed that the presence of the antibody during capacitation affect neither the occurrence of tyrosine phosphorylation, evaluated by Western blot, nor the acrosome reaction, evaluated by Coomassie blue staining, nor sperm motility analyzed by the computerized CASA system. As another approach, we analyzed the effect of the antibody on the ability of spermatozoa to penetrate the cumulus oophorus surrounding the ovulated eggs. For this purpose, capacitated canine spermatozoa previously stained with Hoechst were co-incubated with mouse cumulus-oocyte complexes (COC), and the number of fluorescent spermatozoa within the cumulus was evaluated 15 minutes later. Under these conditions, the presence of the antibody produced a significant decrease in the ability of spermatozoa to penetrate the murine cumulus compared to the controls. Taken together, these results support the participation of the identified canine CRISP protein in the fertilization process as well as its potential use as a target for the development of a canine contraceptive vaccine for both sexes.

A19

EFFECT OF MATERNAL ENVIRONMENTAL ENRICHMENT ON THE OFFSPRING EXPOSED TO CHRONIC MILD PRENATAL STRESS.

De la Cruz Borthiry FL[1], Krawczyk MC[2], Medina C[2], Beltrame JS[1], Cañumil VA[1], Negri Malbrán M[3], Boccia MM[2], Leguizamon GF[3], Franchi AM[1], Ribeiro ML[1].

[1]Laboratorio de Fisiología y Farmacología de la Reproducción. Centro de Estudios Farmacológicos y Botánicos (UBA-CONICET), Buenos Aires, Argentina. fdelacruz@fmed.uba.ar.[2]Laboratorio de Neurofarmacología de los Procesos de Memoria. Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina.[3]CEMIC-CONICET, Dirección de Investigación, Sección Medicina Materno-Fetal, Departamento de Obstetricia y Ginecología. Buenos Aires, Argentina.

Previously, we observed that maternal exposure to an enriched environment had beneficial effects on gestation, while chronic mild maternal stress resulted in metabolic changes in the mother and alterations in the vascular adaptations and immunological balance of the maternal-fetal interface. Besides, chronic mild prenatal stress affected the behavior of the offspring. Therefore, we investigated whether maternal exposure to environmental enrichment improves the effect of chronic mild maternal stress on the progeny. Female BALB/c mice were exposed to control conditions or a chronic mild stress protocol before and during gestation until labor. The environmental enrichment protocol was applied to a group of stressed mothers from day 0 of gestation. Fetuses and offspring were evaluated. Environmental enrichment reversed the fetal and offspring's weight reduction caused by the stress protocol ($p<0,05$). Also, it reduced the total cholesterol increase in females ($p<0,05$), while no differences were observed for males. Moreover, it did not improve the performance of the progeny in the passive avoidance test. This was observed in females and males. In the case of the open field test, environmental enrichment showed a tendency to reverse several determinations in the females, although they did not reach significance. In conclusion, environmental enrichment showed beneficial effects on the offspring's growth and metabolism. However, the detrimental effects of prenatal stress on behavioral tests were not reversed by the enriched protocol.

BIOCHEMISTRY, PHYSIOLOGY AND NEUROSCIENCE

A20

EFFECTS OF DECOCTIONS OF *Oxalis erythrorhiza* AND *Tessaria absinthioides* ON ANXIETY-LIKE BEHAVIOR IN JUVENILE RATS

Guibelalde M[1], Kruse MS[1], Coirini H[1], Tapia A[2], Rey M[1]

[1]Laboratorio de Neurobiología, IBYME-CONICET, [2]Instituto de Biotecnología-Instituto de Ciencias Básicas-Dpto. Ingeniería Agronómica, Fac. de Ing.-UNSJ. e-mail: mariana.rey@ibyme.conicet.gov.ar

In our country, the endemic flora is highly diverse. *Oxalis erythrorhiza* and *Tessaria absinthioides* are two of the Andean species most commonly consumed by the population for health purposes. In previous studies, we reported the beneficial effects of these plants in a rat model of insulin resistance. The objective of this study was to evaluate whether decoctions of these plants produce effects on anxiety-like behavior in control rats. Juvenile male Sprague-Dawley rats received water (CON), *O. erythrorhiza* decoctions at 5 or 10% w/v (OE5 and OE10), or *T. absinthioides* decoctions at 5 or 10% w/v (TA5 and TA10) as drinking fluid for 40 days. Anxiety-like behavior was evaluated using the open field (OF) and elevated plus maze (EPM) tests. The total distance traveled (DT), distance (DIST), number of entries (ENT), and time spent (TIME) in virtual zones (central, intermediate, and peripheral) were determined in the OF, and in the closed (CA), open (OA), and center (CEN) arms of the EPM. In addition,

the frequency (F) and TIME of grooming (G), rearing (R), and risk-taking (RT, only in EPM) were evaluated. No differences were found between OE5 and OE10 or between TA5 and TA10, so they were pooled as OE and TA, respectively. In the OF, no differences were found between groups in any of the analyzed parameters. In the EPM, no differences were observed in DT ($p=0.056$). TA showed higher DISTOA than CON and OE (127.55% and 59.30%, respectively; $p=0.0053$). Compared to CON, TA showed higher TIMEOA (92.77%; $p=0.0186$) and lower TIMECA (27.09%; $p=0.0247$). In addition, TA showed lower TIMECA than OE (22.75%; $p=0.0247$). TA and OE presented lower FR (54.93% and 35.05%, respectively; $p=0.0002$) and lower TIMER (64.77% and 48.29%, respectively; $p<0.0001$) than CON. Based on the results obtained, it can be concluded that TA may exert an anxiolytic-like effect, evidenced by greater permanence and distance traveled in the anxiogenic zones of the EPM (OA). Future studies will be necessary to validate these effects and to propose TA as a source of bioactive compounds applicable to the treatment of anxiety-related disorders (PICT2019-623; PIP-0390/2023).

A21

LONG-TERM CHANGES IN ANXIETY-LIKE BEHAVIOR FOLLOWING STEVIA EXPOSURE IN JUVENILE AND ADULT RATS.

Obeid MA, Rey M, Coirini H, Kruse MS.
[1]*Instituto de Biología y Medicina Experimental (IBYME-CONICET). E-mail: m.kruse@ibyme.org.ar*

We previously demonstrated that rats with unlimited access to sugary beverages during youth exhibit lasting behavioral impairments, including increased anxiety-like behavior. Here, we investigated the long-term effects of unlimited access to a stevia-sweetened beverage during the juvenile stage (childhood–adolescence) and adulthood. Sprague Dawley male rats (PD 25 or PD 75) were given ad libitum access to water (control), and a 10% sucrose solution (SUC), or a 4% aqueous Stevia rebaudiana Bertoni decoction (stevia). After the 25-day treatment period, all rats received only water and were then tested for anxiety using the open field test. Animals were allowed to freely explore a 75 × 75 × 30 cm arena for 5 minutes, and sessions were recorded. The arena was digitally divided into three zones: central (zone 1), intermediate (zone 2), and peripheral (zone 3), and the number of entries, distance traveled, and rearing behavior was analyzed using ANY-maze© software. No differences were found in the total distance traveled among the six groups (juvenile control–sucrose–stevia and adult control–sucrose–stevia). However, significant treatment × age interactions emerged for the zones visited and the distance traveled in central zones 1 and 1+2 ($p < 0.05$). Post-hoc analysis revealed that animals exposed to stevia showed increased entries into central zones 1+2 and greater distance traveled there ($p < 0.05$). Rearing behavior was also altered in stevia-exposed animals with a significant treatment × age interactions for both frequency and duration of rearing ($p<0.01$). Taken together, these findings suggest that early exposure to stevia is associated with a reduced long-term anxiety-like profile, regardless of the age at which this consumption was produced, in contrast to the effects observed with sucrose consumption. PICT-1695/2020.

A22

NEUROENDOCRINE REGULATION BY CCHAMIDE-1 IN *Rhodnius prolixus*: INSIGHTS INTO FEEDING AND EXCRETION PHYSIOLOGY

Padin I [1], Ons S[1], Capriotti N[1]
[1] *Lab. Neurobiol. Insectos (G.V. AL CENEXA. CREG-FCE-UNLP).*
E-mail: nataliacapriotti@conicet.gov.ar

Rhodnius prolixus, an hematophagous insect in the Reduviidae family, is medically significant as a vector for *Trypanosoma cruzi*, the etiological agent of Chagas disease affecting millions across Latin America. This study aims to functionally identify and characterize CCHamide-1 (CCHa1) in *R. prolixus*. CCHa1 is a highly conserved neuropeptide in various arthropods, known to regulate critical physiological functions like feeding, metabolism, growth, and reproduction. Understanding its neuroendocrine system is essential for effective vector control interventions. Gene expression analysis by qRT-PCR under different nutritional conditions and at several time points after blood feeding (3h, 12h, 24h) revealed that CCHa1 expression is modulated by the insect's nutritional status, showing a significant change 24 h post-feeding. To evaluate its function, RNA interference (RNAi) assays were performed, which significantly reduced the expression of CCHa1. Additionally, the modified Ramsay technique was used to measure the secretion rate in Malpighian tubules of fifth-instar nymphs. Postprandial expression studies did not show significant changes following blood ingestion. Likewise, RNA interference (RNAi) and Malpighian tubule secretion assays showed consistent trends, although the observed differences were not statistically significant. These results suggest that CCHa1 may be involved in the modulation of feeding and excretory processes, highlighting the need for additional studies to confirm its physiological role and to elucidate its potential participation in homeostatic regulation in *R. prolixus*.Together, these findings indicate that CCHa1 plays a crucial role in the biology of *R. prolixus* and position it as a promising molecular target for the design of new vector control strategies.

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A23

ANXIOLYTIC ACTION OF *Pistacia vera L.* OIL IN A MURINE MODEL OF ACUTE STRESS

Obeid MA[1], Mercogliano MF[2], Kruse MS[1], Schillaci R[2], Tapia A[3], Coirini H[1], Rey M[1]
[1]Laboratorio de Neurobiología, IBYME-CONICET, [2]Laboratorio de Inmunología Tumoral, [3] Instituto de Biotecnología-Instituto deCiencias Básicas-Dpto. Ingeniería Agronómica, Fac. de Ing.-UNSJ.e-mail: mariana.rey@ibyme.conicet.gov.ar

The consumption of *Pistacia vera* L. in Argentina is increasing, and its derivatives present a high content of unsaturated fatty acids, tocopherols, and phenolic compounds. Several studies report beneficial effects on lipid profile and endothelial function, highlighting its antioxidant capacity. On the other hand, during acute stress, an increase in oxidative status occurs, accompanied by alterations in anxiety-like behavior. The objective of this work was to evaluate whether *P. vera* L. oil (PVO) modifies the behavior of mice subjected to acute restraint stress (R). Juvenile male BALB/c mice received oral water (control group, CON) or PVO (0.54 µL/g body weight, PVO group) for 40 days. Then, half of the animals from each group were subjected to R for 2 h during three consecutive days. Anxiety-like behavior was evaluated in the groups CON, CONR, PVO, and PVOR, using the open field (OF) and elevated plus maze (EPM) tests. The total distance traveled (DT), distance (DIST), number of entries (ENT), and time spent (TIME) in the virtual zones (central (1), intermediate (2), and peripheral (3)) were determined in OF, and in the closed (CA), open (OA), and center (CEN) arms of the EPM. In addition, in EPM, the frequency (F) and TIME of risk assessment (RA) were evaluated. In OF, two-way ANOVA revealed differences only in TIME by condition and treatment. The R groups showed lower TIME2 and higher TIME3 than non-R groups. The PV groups showed higher TPO2 and lower TPO3 than water-treated groups. In EPM, two-way ANOVA revealed interaction effects in some parameters. Post hoc analyses showed that CONR had lower TIMEOA (−38.58%; p=0.0184), higher TIMECA (+69.69%; p=0.0013), and lower FRA (−28.67%; p=0.034) than CON. PVOR showed higher TIMEOA (+94.51%; p=0.0047), lower TIMECA (−44.88%; p=0.0040), and higher FRA (+94.11%; p<0.0001) than CONR. Comparisons among the remaining groups did not reveal significant differences. The results obtained indicate that PVO may exert preventive effects against acute stress, attenuating anxiety-like behaviors. Further studies will be necessary to validate these effects, determine the mechanisms involved, and propose *P. vera* L. as a potential treatment for anxiety-related disorders (PIP-0390/2023).

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 2

A24

RELEVANCE OF CRISP1 AND CRISP3 PROTEINS FOR FEMALE FERTILITY

Rebagliati Cid A, Simone J, Weigel Muñoz M, Cuasnicú PS
Instituto de Biología y Medicina Experimental (IBYME-CONICET),
E-mail: a.rebagliati@ibyme.org.ar

CRISP (Cysteine-Rich Secretory Proteins) family proteins are mainly expressed in the mammalian reproductive tract, play critical roles in the fertilization process and are relevant for fertility in both males and females. Previous studies from our laboratory showed that while single knockout (KO) females for each CRISP protein are fertile, double KO females for CRISP1 and CRISP3 (DKO 1/3) exhibit a significant decrease in fertility. Since CRISP1 and CRISP3 are expressed throughout the female reproductive tract, including the uterus, oviduct, ovary, and the cumulus cells surrounding the oocyte, the aim of this work was to investigate the mechanisms leading to the subfertility of the DKO 1/3 colony. To evaluate whether the absence of these proteins affects the estrous cycle, vaginal secretions collected during 20 consecutive days were analyzed microscopically. Results showed that, although the total cycle length was similar for both genotypes, DKO 1/3 females displayed a significant increase in the number of days in proestrus/estrus at the expense of a reduction in diestrus. We next analyzed the number and size of ovulated oocytes in both naturally cycling and superovulated females. While no differences were observed between genotypes in the first group, the number of ovulated oocytes was significantly higher in superovulated DKO 1/3 females compared to controls, with no changes detected in oocyte diameter. To assess the quality of the ovulated oocytes, superovulated females were mated with control males, and the percentage of fertilized eggs recovered from the ampulla the following day was evaluated. Although no significant differences were detected in fertilization rates, a marked decrease was observed in the ability of fertilized eggs from DKO 1/3 females to reach the blastocyst stage. Finally, *in vitro* fertilization experiments carried out to investigate the stage at which defects appear during embryo development revealed no differences at intermediate stages such as 2-cell, 4/8-cell, or morula but again a significant reduction in the percentage of blastocysts. Taken together, these results indicate that CRISP1 and CRISP3 proteins expressed along the female reproductive tract are relevant for fertility through their participation in different reproductive processes, including the estrous cycle, ovulation, and early embryonic development.

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A25

RELATIONSHIP BETWEEN PROGESTERONE CONCENTRATION AND EMBRYO QUALITY DURING COCULTURE OF PORCINE EMBRYOS WITH LUTEAL CELLS

MS Lorenzo[1-2], CG Luchetti [1-2], PR Cruzans[1], DM Lombardo[1-2].
1-Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Instituto de Investigación y Tecnología en Reproducción Animal. 2-CONICET

The coculture of gametes and embryos with somatic cells is a tool to optimize culture conditions with the aim of improving developmental rates and/or the quality of the embryos obtained. In previous studies, we demonstrated that the coculture with porcine luteal cells (PLC) improves embryo quality, increasing the cell number and decreasing the apoptosis in the blastocysts produced. PLC secrete progesterone, which supports embryonic–fetal development and maintains pregnancy. Pig embryos express progesterone receptors, and the in vitro supplementation with this hormone improves the quality of the embryos obtained. The aim of this study was to evaluate progesterone production by PLC during coculture with in vitro-derived pig embryos. For this purpose, embryos obtained by in vitro fertilization were cocultured during the first two days of development with a monolayer of PLC. In the control group, embryos were cultured without PLC. PLC purity was determined by the expression of 3β-hydroxysteroid dehydrogenase by immunocytochemistry. Culture supernatants were collected before embryo addition (day 0, control-0 n=5, PLC-0 n=9) and after coculture (day 2, control-2 n=5, PLC-2 n=5) and stored at –20 °C until use. Progesterone concentration was measured by chemiluminescent microparticle immunoassay. An ANOVA was performed, and differences were considered statistically significant at $p<0.05$. The lowest concentration was observed in the control-0 and control-2 groups (0.1 ng/mL). In PLC-0 group (5.98 ng/mL) the progesterone concentration was significantly higher than in the control-0 (0.10 ng/mL) and it significantly decreased between PLC-0 (5.98 ng/mL) and PLC-2 (0.93 ng/mL). On day 2, the progesterone concentration did not differ between PLC-2 (0.93 ng/mL) and control-2 (0.10 ng/mL). Therefore, the PLC in this culture system produce progesterone, and the levels of this hormone decrease during coculture, which could be explained either to its binding to receptors present in the embryos or to a reduction in its production under suboptimal conditions (e.g., absence of fetal bovine serum in coculture). Considering the data obtained in this study and our previous results, progesterone could be the factor that improves embryo quality.

A26

RESVERATROL AS A PROTECTIVE AGENT FOR THE OVARIAN RESERVE AGAINST SURGICAL DAMAGE

Ricci AG[1,2], del Valle S[1,2], Schwarz J[1], Villalba Diaz MV[1], Meresman GF[1,2], Bilotas MA[1]
[1]Instituto de Biología y Medicina Experimental (IBYME-CONICET), [2]Facultad de Medicina, Universidad del Salvador.
E-mail: agricci@ibyme.conicet.gov.ar

Endometriosis is a benign gynecological disease that affects approximately 10–15% of menstruating individuals, with pain and infertility as its main symptoms. It is characterized by the presence of endometrial tissue outside the uterine cavity, and the ovarian form is the most frequent presentation. The presence of ovarian endometriomas has been identified as a cause of infertility, either through alterations in folliculogenesis and/or granulosa cell function. However, several studies have reported that the surgical removal of endometriomas reduces ovarian reserve, creating a trade-off between the need to remove the cyst due to symptom severity and the potential detrimental impact on the ovary. Since mTOR signaling in pre-granulosa cells plays a crucial role in the activation of quiescent primordial follicles, its inhibition could protect the ovary from premature follicular activation and thereby preserve ovarian reserve. Given the need to develop fertility preserving strategies for these patients, and the lack of suitable models to study them, the aim of this study was to establish an experimental model of ovarian surgical injury that mimics cystectomy-induced damage, and to evaluate resveratrol (a polyphenol with demonstrated mTOR inhibitory activity) as a protective agent. Two-month-old female BALB/c mice underwent surgical excision of a small fragment of the left ovary (damaged), while the right ovary was left intact as a control. Resveratrol (7 mg/kg) was administered in drinking water for one week before and one week after surgery. Everolimus, a rapamycin analogue and mTOR inhibitor, was used as a positive control for the proposed protective mechanism. Animals were sacrificed in estrus 30 days after treatment initiation, and ovaries were collected and fixed in formalin. In hematoxylin–eosin–stained histological sections, early follicles, atretic antral follicles, and corpora lutea were counted. In the healthy ovary, resveratrol treatment did not produce significant differences compared with untreated controls. However, in the damaged ovary, treated animals showed a higher percentage of primordial ($p<0.01$) and primary ($p<0.05$) follicles compared to controls. Moreover, resveratrol treatment reduced the percentage of atretic antral follicles in both ovaries ($p<0.05$), without affecting the number of corpora lutea. These preliminary results suggest that resveratrol may represent an effective and novel strategy to protect the ovarian reserve from cystectomy-induced damage in patients with ovarian endometriomas.

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A27

EVALUATION OF THE INTERACTION BETWEEN ENDOMETRIAL FIBROBLASTS AND TROPHOBLASTS IN A MATERNAL-FETAL INTERFACE MODEL.

Cañumil VA[1], De la Cruz Borthiry FL[1], Scheffer F[1], Beltrame JS[1], Meresman G[2], Ribeiro ML[1].
[1]Laboratorio de Fisiología y Farmacología de la Reproducción. CEFYBO, Facultad de Medicina, UBA-CONICET, Argentina.
[2]IBYME (CONICET), e-mail: canumilvanesa@gmail.com

During the early stages of pregnancy, extensive tissue remodeling occurs at the maternal-fetal interface. This process involves cellular mechanisms such as proliferation, apoptosis, migration, and various forms of vascular adaptation. The interaction and communication between the different cell types that constitute the interface are crucial for pregnancy maintenance. We have previously demonstrated that endometrial stromal fibroblasts play a vascular role by modulating the behavior of the microvascular endothelium at the implantation site. The aim of this study was to evaluate the interaction between endometrial stromal fibroblasts and first-trimester trophoblasts. In particular, we investigated whether stromal fibroblasts regulate trophoblast function. As an experimental model, we used the T-hESC (human endometrial stromal fibroblasts) and HTR-8/SVneo (first-trimester human trophoblast) cell lines. T-hESC cells were cultured in 24-well plates until reaching confluence. Once a monolayer was formed, either a scratch wound was created or the monolayer was left intact and incubated for 15 h in culture medium. Supernatants were then collected. Metalloproteinase activity in the supernatants was assessed by zymography or used as conditioned media in assays involving HTR-8/SVneo cells. First, the migratory capacity of HTR-8/SVneo cells was evaluated using a wound healing assay. Cells were cultured until confluent, wounded, and incubated for 18 h with conditioned media derived from T-hESC cells. The percentage of wound closure was measured. Additionally, the ability of HTR-8/SVneo cells to form tubular structures was assessed using a tubulogenesis assay. Cells were seeded in 96-well plates on extracellular matrix (Matrigel) and incubated with T-hESC conditioned media. After 6 h, the number of branch points and total tube length were quantified. Differences were considered significant when $p<0.05$. We observed that supernatants from T-hESC cells exhibited metalloproteinase activity ($p<0.05$). Furthermore, conditioned media from migratory T-hESC cells stimulated the formation of capillary-like structures in the tubulogenesis assay using HTR-8/SVneo cells ($p<0.05$). No significant differences were observed in the migration assays. Taken together, our results suggest an interaction between endometrial stromal fibroblasts and first-trimester trophoblast cells in key processes involved in the establishment of the maternal-fetal interface.

A28

IMPACT OF *IN VIVO* INHIBITION OF THE MITOCHONDRIAL-DERIVED PEPTIDE HUMANIN ON OVARIAN PHYSIOLOGY

Conte JG[1], Sánchez Rojas I[1], Pidre ML[2], Sobarzo C[1,4], Celaya DB[1], Peluffo MC[3], Jaita G[1,4]
[1]CONICET – Universidad de Buenos Aires. Instituto de Investigaciones Biomédicas (INBIOMED). Buenos Aires, Argentina.
[2]Laboratorio de Virología Molecular, Instituto de Biotecnología y Biología Molecular (IBBM), Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), CONICET. [3]Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) -CONICET–FEI–División de Endocrinología, Hospital de Niños "Ricardo Gutiérrez". [4]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Biología Celular, Histología, Embriología y Genética. Buenos Aires, Argentina.
e-mail: julia_conte@hotmail.com

Proper regulation of ovarian physiology is essential both for the generation of a competent oocyte and for the maintenance of endocrine function. This regulation involves not only systemic factors such as gonadotropins, but also numerous intraovarian factors. Humanin (HN), a small mitochondrial-derived peptide, exerts cytoprotective effects in various tissues, including the ovary. Our laboratory has demonstrated the expression of HN in the rat ovary and shown that this peptide plays a key role in granulosa cell viability and apoptosis regulation. In the present study, we aimed to investigate the participation of HN in the regulation of ovarian physiology by evaluating the impact of its endogenous inhibition on follicular development and the ovarian microenvironment using a recombinant baculovirus encoding a specific shRNA against HN (BV-shHN) in an *in vivo* model. Cycling adult female Sprague Dawley rats (3–4 months old) were used. Ovaries were exposed and intrabursally injected (at estrus) with BV-shHN (n=5) or BV-control (a baculovirus that does not inhibit HN; n=5). Initial and final body weights were recorded, and the estrous cycle was monitored daily. After approximately two weeks, animals were sacrificed at metestrus, and ovaries were collected, photographed, and fixed in 4% PFA for 4 h for subsequent histological analysis. Ovaries were entirely sectioned at 5 µm, and follicle quantification was performed every 50 µm. Additionally, ovarian fibrosis (Masson's trichrome) and vascularization (PAS) were evaluated. Results showed no significant differences ($p>0.05$) in estrous cycle patterns, ovarian size, or body weight between control and treated animals. However, BV-shHN–treated rats exhibited an increased percentage of antral follicles ($p<0.01$). Regarding the ovarian microenvironment, increased ovarian fibrosis and a reduced number of arterioles ($p<0.05$) were observed in the BV-shHN group. In conclusion, our findings suggest that HN may participate in the regulation of ovarian physiology by modulating folliculogenesis and the ovarian microenvironment. Therefore, this peptide could play a role in intraovarian regulation with potential impact on fertility.

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A29

USE OF 1,10-PHENANTHROLINE AS A STRATEGY TO IMPROVE THE EFFICIENCY OF PORCINE IN VITRO FERTILIZATION

Holzmann L[1], Fernández-Martin R[1], Morado S[1,2], Ratner L[1].
[1]Laboratorio de Biotecnología Animal, FAUBA. Instituto de Investigaciones en Producción Animal (INPA- CONICET-UBA).
[2]Instituto de Investigación y Tecnología en Reproducción Animal (INITRA, FCV, UBA). E-mail: lucianaholzmann21@gmail.com

The in vitro production of porcine embryos represents a fundamental tool for the generation of genetically modified biomedical models; however, its efficiency remains limited, mainly due to the high incidence of polyspermy and the low embryonic quality obtained. The aim of the present study was to evaluate the effect of 1,10-phenanthroline (PHEN), a zinc chelator capable of inducing porcine oocyte activation, as a strategy to improve in vitro fertilization (IVF) outcomes.
Cumulus–oocyte complexes (COCs) were collected from antral follicles and matured in vitro for 44 h. Subsequently, IVF was performed with or without PHEN treatment (IVF+PHEN or IVF control, respectively), along with a haploid parthenogenetic activation control (HC). Semen from a genetically modified boar was used for fertilization. This boar was mosaic for the *galactosyl-alpha 1,3-galactose* (*GGTA1*) and *growth hormone receptor* (*GHR*) genes. The final sperm concentration was adjusted to 250.000 sperm/mL, and gametes were co-incubated for 3.5 h. After fertilization, presumptive zygotes were denuded and cultured to the blastocyst stage in porcine zygote medium (PZM). Zygotes from the IVF+PHEN group were denuded and incubated for an additional 30 min with 1,000 µM PHEN, washed in TALP-Hepes, and then cultured in PZM. For the haploid control, oocytes were denuded with hyaluronidase, incubated for 30 min with 1,000 µM PHEN, washed in TALP-Hepes, and cultured in PZM. Cleavage and blastocyst rates were analyzed by the Chi-square test on days 3 and 7, respectively. To determine the rate of parthenogenetic activation induced by PHEN, a nested PCR was performed using primers specific for the alleles present in the edited boar, and pronuclear staining was carried out using the vital dye Hoechst 33342. A significant increase in blastocyst formation rate was observed in the presence of PHEN compared with the control IVF group (IVF+PHEN: 14.5%, HC: 17.6% vs. IVF control: 4.5%; $p < 0.05$). Genotypic analysis confirmed the presence of at least one edited allele in blastocysts from both IVF groups, indicating that the embryos originated from fertilization rather than parthenogenetic activation. Additionally, the rate of polyspermy, determined by the presence of more than one edited allele for the same gene, was similar among experimental groups. No significant differences were observed in the number of pronuclei (one or two) between groups. In conclusion, the use of PHEN as an adjunct during porcine IVF represents a promising alternative to enhance system efficiency and may contribute to the development of more standardized and effective in vitro embryo production protocols.

A30

EVALUATION OF THE THERAPEUTIC POTENTIAL OF ROSEMARY EXTRACT IN CELLULAR MODELS OF ENDOMETRIOSIS

del Valle S[1,2], Ruiz I[3], Espinoza-Sánchez NA[4,5], Bilotas M[1], Villalba Díaz MV[1], Schwarz J[1], Leirós G[2,3], Greve B[4], Götte M[5], Ricci A[1,2], Meresman G[1,2]
[1]Instituto de Biología y Medicina Experimental (IBYME-CONICET), [2]Facultad de Medicina, Universidad del Salvador, [3]Instituto de Ciencia y Tecnología César Milstein-CONICET, [4]Department of Radiation Oncology, Münster University Hospital, [5]Department of Gynecology and Obstetrics, Münster University Hospital. E-mail: sdelvalle782@gmail.com

Endometriosis (EDT) is a chronic gynecological disease that affects 10–15% of menstruating people, for which therapeutic options remain limited. In the search for new treatments, rosemary (*Rosmarinus officinalis*) represents a promising natural alternative due to its antioxidant and anti-inflammatory properties. This study evaluated the effect of a dry rosemary extract (RE) rich in carnosic acid and carnosol on in vitro cellular models of EDT. Cell viability (WST-1 assay) was assessed in endometrial stromal (t-HESC) and endometriotic epithelial (12-Z) cells treated with various concentrations of RE (15–36 µg/ml). The migratory capacity of both cell lines was evaluated using a wound-healing assay after 20 h of exposure to 2, 8, and 15 µg/ml RE. In t-HESC cells, reactive oxygen species (ROS) production was quantified with 2',7'-dichlorofluorescein diacetate following treatment with 8–30 µg/ml RE. Cell cycle distribution (DAPI staining) and apoptosis (Annexin V/PI) were analyzed by flow cytometry in ST-T1b and 12-Z cells exposed to 2, 8, and 15 µg/ml RE. Finally, Western blot analysis was performed to determine the expression of cell cycle checkpoint proteins, including p21 and cyclin A, using GAPDH as a housekeeping control. RE significantly reduced cell viability in 12-Z cells from 25 µg/ml ($p<0.01$) and in t-HESC cells from 30 µg/ml ($p<0.01$). It also inhibited cell migration in 12-Z cells from 2 µg/ml and in t-HESC cells from 8 µg/ml ($p<0.05$). In t-HESC cells, RE decreased both basal ROS levels (from 15 µg/ml, $p<0.01$) and those induced by H_2O_2 (from 12 µg/ml, $p<0.01$). Cell cycle analysis revealed a reduction in the G1 phase and an accumulation of cells in the S phase at concentrations above 18 µg/ml ($p<0.05$), indicating S-phase arrest, which was associated with increased expression of p21 ($p<0.01$) and cyclin A ($p<0.01$). Finally, the highest RE concentration induced cell death in approximately 16% of the population in both 12-Z ($p<0.05$) and ST-T1b ($p<0.0001$) cells. In summary, RE shows significant antiproliferative, antimigratory, and antioxidant effects on endometrial and endometriotic cells, promoting S-phase arrest and cell death. This findings support RE as a promising natural therapeutic option for EDT

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Relevance of the interaction between CRISP proteins and the genetic background for the regulation of epididymal immune tolerance

Simone J, Rebagliati Cid A, Weigel Muñoz M, Cuasnicú PS
[1]Institute of Biology and Experimental Medicine (IBYME-CONICET)E-mail:julietasimone1@gmail.com

CRISP1-4 (Cysteine-Rich Secretory Proteins) are mainly expressed in the male reproductive tract of mammals and are critical for both the fertilization process and fertility. Consistent with their high homology with proteins that play immunoregulatory roles in other species, our results show that a proportion of multiple knockout (KO) animals for these proteins (i.e, double, triple, and quadruple) exhibits spontaneous epididymo-orchitis with clear defects in sperm viability, supporting the immunoregulatory role of CRISPs. It is interesting to note that all colonies presenting epididymo-orchitis lack the CRISP1 protein and have a mixed DBA/C57 genetic background, suggesting the importance of one or both of these conditions for the development of the inflammatory phenotype. In this regard, it should be noted that the DBA background is characterized by the lack of the CD94 receptor in NK cells, which are key in immune tolerance. Based on this, the objective of the present work was to investigate the interaction between CRISP and genetic background in order to clarify the mechanisms leading to the observed phenotype. To this end, we began by analyzing the presence of epididymo-orchitis in a new triple KO colony with deletions in *Crisp1/Crisp3/Crisp4* (TKO) in a DBA/C57 background recently generated in the laboratory. Interestingly, this subfertile colony showed that 41% of TKO males had visibly larger epididymides than controls, either unilaterally or bilaterally, as well as a significant increase (1.5 times) in their weight, with no changes in testicle size/weight compared to controls, indicating that this combination of deletions generates a different immune tolerance disruption profile than the previously described. Having once again observed the presence of the inflammatory phenotype in these animals lacking CRISP1, we decided to generate a colony of simple *Crisp1* KO animals in the DBA/C57 background by backcrossing. Although the mutant males in this colony did not show macroscopic evidence of epididymal or testicular inflammation, they did show a significant decrease in sperm count as well as sperm morphological alterations (loose heads and tails) in 30% of the cells, not observed in CRISP1-deficient males on a C57 background, indicating that the absence of CRISP1 on a mixed genetic background would be a necessary but not sufficient condition for the expression of the inflammatory phenotype (i.e. epididymitis/epididymo-orchitis), requiring the deletion of additional CRISPs. Taken together, these results indicate that CRISP proteins differentially regulate the immunotolerance of the male reproductive tract and that the resulting inflammatory phenotype depends on a delicate functional balance between the members of this family and their interaction with the genetic context.

A32

SEX-SPECIFIC EFFECTS ON PLACENTAL METABOLISM AND HYPOXIA IN A SUBCLINICAL INFECTION MODEL

Di Giacomo T1, Scheffer F1, de la Cruz Borthiry FL1, Cañumil VA1, Bogetti E1, Ribeiro ML1, Beltrame JS1.
1Laboratorio de Fisiología y Farmacología de la Reproducción. Centro de Estudios Farmacológicos y Botánicos, Facultad de Medicina, UBA-CONICET, Argentina. digiacomotomas@gmail.com - jbeltrame@fmed.uba.ar

The maternal environment shapes fetal development and metabolism, with growth deficiencies increasing the risk of chronic diseases later in life. Previously, we showed that low-dose lipopolysaccharide (LPS)-induced subclinical infection alters fetal growth, placental vascular processes, and has lasting effects on offspring. This study aims to further investigate the impact of fetal sex on molecular factors associated with placental metabolism and hypoxia in a LPS subclinical infection model. Wistar female rats received 4 intraperitoneal low doses of LPS on gestational days 6 to 9 (d6: 20ug/kg; d7-9: 50ug/kg). Placentas were collected on day 15 of gestation, sexed by PCR, and several molecular markers such as hypoxia-inducible factor (HIF1-alpha), O-GlcNAc Transferase (OGT), glucocorticoids receptor (GR), placental growth factor (PlGF), and apolipoprotein B (APO) mRNA levels were studied by qPCR. Differences were significant when $p<0.05$. Our results revealed that HIF1-alpha, OGT, GR and PlGF mRNA levels are increased in male placentas from LPS-treated mothers ($p<0.01$, $p<0.01$, $p<0.04$, $p<0.04$). Female placentas did not show significant differences in these mRNAs compared to controls, while PlGF showed a tendency to increase. APO did not vary, regardless of sex or treatment. Our findings show that male embryos from LPS-treated mothers exhibit distinct placental metabolic and hypoxic molecular profiles, indicating sex-specific susceptibility to subclinical maternal infection and its impact on fetal programming of disease risk in adult life.

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VETERINARY, BIOTECHNOLOGY AND ANIMAL PHYSIOLOGY

A33

HISTOMORPHIC CHARACTERIZATION OF THE FLIGHT MEMBRANE AND BODY INTEGUMENT OF THE VAMPIRE BAT (*Desmodus rotundus*)

Castro NN[1], Zanuzzi CN[1], Marchi BM[1], Barbeito CG[1], Krmpotic CM[1]
1- Instituto de Morfología y Patología (IMPA); Facultad de Ciencias Veterinarias (FCV), Universidad Nacional de La Plata. E-mail: castro.nicolas.nahuel@gmail.com
2-

Bats (Order: Chiroptera) play an essential role in various ecosystems. The diversity of their diet allows them to occupy a great variety of niches. They are the only mammals capable of active, flapping flight, using a complex skin structure called flight membrane or patagium, which extends between its body, its forelimbs (radius, metacarpus and fingers) and hindlimbs. Our study describes the histology of the integument in the common vampire bat, *Desmodus rotundus.* We compared the structure of the skin of the flight membrane with that of the rest of the body, and analyzed the disposition of its elastic and muscular fibers, and the morphology and distribution of hair follicles and glandules. Skin samples of specimens of *D. rotundus* belonging to a collection that were fixed in 10% formaldehyde and subsequently preserved in 70° alcohol were used for this study. Samples from different areas of the body and flight membrane (plagiopatagium, dactylopatagium, propatagium and uropatagium) were selected. The samples were processed for paraffin embedding, and serial sections 3-5 µm thick were taken. Finally, they were stained with routine histological techniques. Our first results revealed differences between the skin of the body and that of the flight membrane. The skin of the body consist of an epidermis, with no more than four cellular strata, a dermis that is not clearly differentiated in papillary and reticular layers, and a well-developed hypodermis. Regarding the integumentary appendages, simple hair follicles and sebaceous glands predominate. In contrast, the flight membrane consists of two layers of epithelial tissue with a thickness of two or three strata, and a dermis that contains, depending on the area, a variable amount of muscular and fibroelastic connective tissue. Particularly, the dactylopatagium (the area of the wing between the fifth and second fingers) is characterized by an absence of muscular tissue and the predominance of fibroelastic tissue. Regarding the annexes, the hair follicles are less abundant than in the body integument, and only sebaceous glands were found. The skin of the *D. rotundus* reveals a notable evolutionary specialization that goes far beyond its main function in flight. The absence of hypodermis and the variable distribution of collagenous, elastic, and muscular fibers in the different areas of the wings could be fundamental adaptations to provide further lightness and malleability during active flight. Future research will analyze in greater depth the correlation between these

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CHARACTERIZATION OF THE GLUTATHIONE ANTIOXIDANT SYSTEM IN STALLION EPIDIDYMAL AND TESTICULAR TISSUE

Moran KD1,2, Bilbao MG1,2, Luchetti CG1,3, Reumann AL4, Piccini JP2, Lombardo MD,3, Bartolome JA2, Ferrer MS5*
1 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); 2 Facultad de Ciencias Veterinarias, Universidad Nacional de La Pampa, Gral. Pico, La Pampa, Argentina; 3 Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Instituto de Investigación y Tecnología en Reproducción Animal, Buenos Aires, Argentina; 4 Conicet- Escuela de Producción Agroindustrial y Medicina Veterinaria de la Universidad Nacional de Rio Negro, Choele Choel, Río Negro, Argentina; 5Department of Large Animal Medicine, University of Georgia, Athens, GA, USA kmoran@vet.unlpam.edu.ar

Spermatozoa mature and are stored in the epididymis. They are highly susceptible to oxidative damage. Reduced glutathione (GSH), along with the enzymes glutathione peroxidase (GPx) and glutathione S-transferase (GST), work together as a central antioxidant system. GSH is the most important non-enzymatic antioxidant in cells. GPx uses GSH to catalyze the reduction of hydrogen peroxide and lipid hydroperoxides, while GST transfers it to other compounds for inactivation, thus protecting spermatozoa during their epididymal transit. GPxs are a family of selenium-dependent or -independent antioxidant enzymes that can be found both intracellularly and extracellularly. In addition to their role in antioxidant defense and cellular detoxification, GSTs are involved in sperm maturation, cellular signaling, and the biosynthesis of sex steroids and prostaglandins. These pathways have been extensively studied in murine and human models; however, available information in stallions remains limited. Therefore, the aim of this study was to describe the differential expression pattern of GPx and GST isoforms between the testis and the different regions of the epididymis, and quantify total GSH concentration in these tissues from reproductively normal stallions. For this purpose, testis, head, body, and tail of epididymis samples were collected from stallions (n = 3), over two years of age, with no history of infertility or reproductive disease, and processed for RNA-Seq. Genes related to GSH function were selected, and their differential expression between the testis and the three epididymal regions was analyzed. Additionally, samples from other stallions (n = 7), with the same criteria, were used to determine total GSH concentration (µmol/µl) in homogenates of the four tissues, using a modified Ellman's reaction in a microplate reader at 405 nm. Protein

content in each homogenate was quantified by nanovolume spectrophotometry and used to adjust GSH levels (µmol/mg of protein). Differential expression analysis was performed using DESeq.R. The Benjamini-Hochberg correction was applied to identify statistically significant differences (adjusted P-value). Total GSH content was analyzed using a general linear mixed model, considering tissue as a fixed effect and stallion as a random effect. Data analysis was conducted using R and RStudio. RNA-Seq analysis revealed constitutive expression of 15 GSH-related genes. Of these, 13 were differentially expressed in the epididymis compared to the testis. Genes upregulated in the epididymis (8/13) included: Gpx2, Gpx3, Gpx7, Gpx8, Gstk1, Gsta4, Gstp1, and Gstz1 (adjusted $P < 0.05$). Genes downregulated in the epididymis (5/13) included: Gsta3, Gstt4, Gstcd, Gsto1, and Gpx4 (adjusted $P < 0.05$). Total GSH content differed among tissues ($P = 0.030$). A significantly higher GSH levels were observed in the tail compared to the body of the epididymis ($P = 0.032$), and a trend toward increased levels in the epididymal tail compared to the testis was also detected ($P = 0.068$). This study demonstrated the importance of GSH-related enzymes in the reproductive tract of normal stallions. Surprisingly, Gpx1, a ubiquitous isoform of this enzyme family, was not detected. Similarly, Gpx5, previously reported in stallions and other models, was not expressed. An interesting finding was the upregulation of Gpx3 in the tail of epididymis, the only GPx isoform known to perform its detoxifying function extracellularly. This may suggest that secreted GPx3 helps neutralize oxidative stress within the epididymal lumen. In contrast, the higher relative expression of Gpx4, Gsto1, and Gsta3 in the testis aligns with their known functions in protecting developing sperm from oxidative damage, facilitating chromatin condensation, and participating in testosterone biosynthesis. Furthermore, the increased total GSH content in the tail of epididymis suggests enhanced antioxidant capacity in this region, strengthening the defense of sperm integrity and functionality by preventing oxidative damage to lipids, proteins, and DNA prior to ejaculation.

A35

TGFβ EXPRESSION IN BOVINE OVIDUCTAL EPITHELIUM IS ALTERED BY LPS AND MODULATED BY ESTROUS CYCLE HORMONES

De Boeck M[1,2], Choque FS[2], Vella MA[1], Roldán-Olarte EM[1,2], Valdecantos PA[1,2]
[1]Instituto Superior de Investigaciones Biológicas (INSIBIO-CONICET-UNT), [2]Instituto de Biología "Dr. Francisco D. Barbieri", Universidad Nacional de Tucumán (UNT), Facultad de Bioquímica, Química y Farmacia (FBQF).
E-mail: maximiliano.deboeck@fbqf.unt.edu.ar

The bovine oviduct provides a dynamic microenvironment, essential for fertilization and early embryo development, which is finely regulated by interactions between epithelial and stromal cells. This critical environment can be disrupted by external stressors, such as the pro-inflammatory endotoxin lipopolysaccharide (LPS), a component of *Escherichia coli* and other Gram-negative bacteria frequently associated with postpartum uterine infections and infertility. Bovine oviduct epithelial cells (BOEC) participate in the local immune response by producing various cytokines and growth factors. The aim of this study was to evaluate how LPS exposure modifies the expression of genes related to inflammatory processes (IL6, IL10, TNFα, NFκB) and their relationship with genes involved in tissue repair and regeneration mechanisms (TGFβ1, BMP7, ID3). Bovine ampullary tissue explants were cultured for 24 hours in DMEM medium supplemented with 0.1% fetal bovine serum at 38.5 °C and 5% CO_2, with or without LPS (100 ng/mL). To model the hormonal conditions of the estrous cycle, explants were incubated with 17 β-estradiol and progesterone simulating the Follicular Phase (SFF) (E_2 290 pg/mL; P_4 6 ng/mL) and the Luteal Phase (SFL) (E_2 86 pg/mL; P_4 120 ng/mL). BOECs were then recovered from these explants for gene expression analysis by RT-qPCR, including conditions with LPS treatment in the absence of hormones. The results showed a hormone-dependent response to LPS. Under SFF conditions, pro-inflammatory mRNA of IL6, TNFα, and NFκB were significantly upregulated compared to the hormone-free control ($p<0.05$), while TGFβ1, BMP7, and IL10 decreased ($p<0.05$). In contrast, in SFL conditions, IL6, IL10, NFκB, TNFα, TGFβ1, and BMP7 expression decreased relative to the control without hormones and without LPS. Notably, in the SFL+LPS cultures, no significant differences were found compared to the hormone-free control, except for IL6 and NFκB, which showed a significant decrease in their transcript levels, while the TGF/BMP-responsive gene ID3 was significatively upregulated ($p<0.05$). Since ID3 has a role in epithelial maintenance and regeneration, we performed functional cell adhesion assays using primary BOEC monolayers cultures on type I collagen matrix. LPS strongly decreased cell adhesion; however, this negative effect was significantly attenuated under both SFF and SFL conditions. The findings highlight that the oviductal response to LPS is dependent on the hormonal stage of the estrous cycle, modulating the expression of key genes coordinating inflammation and tissue repair, a crucial mechanism for maintaining oviductal homeostasis and reproductive success.

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THE PRE- AND POST-HATCHING STOMACH OF THE MONK PARAKEET (*MYIOPSITTA MONACHUS*): FOR THE SEARCH FOR HETEROCRONIES IN THE DEVELOPMENT OF ALTRICIAL VERSUS PRECOCIAL BIRDS

Arias N[1,2], Carril J [2,3], Badura E[1], Barbeito CG[2,3]

1. *Cátedra de Enfermedades de Aves y Pilíferos, FCV, UNLP. 2.Instituto de Morfología y Patología Animal (IMPA), FCV, UNLP. 3.CONICET, La Plata. nancyariasfcv@gmail.com*
2.

The stomach of modern birds (Neornithes) consists of two distinct parts: the proventriculus, whose primary function is chemical processing; and the gizzard, which is responsible for mechanical processing of food. Energy production and nutrient absorption depend on and are limited by the size of the digestive system. In altricial birds (those that are born with closed eyes, naked bodies, and remain in the nest and are fed by their parents), the chicks' energy is used almost exclusively for growth; while in precocial birds (those that are born with open eyes, a body covered with down, and are able to leave the nest and feed themselves), energy is also used for thermoregulation, activity, and locomotion. Thus, altricial birds grow at a faster rate than precocial birds. The objective of this study was to characterize and analyze the degree of stomach maturation pre- and post-hatching in the altricial *Myiopsitta monachus* (order Psittaciformes) and compare it with the information available for precocial Galliformes. To this end, two stage 40+ embryos (Hamburger & Hamilton 1951) and a 1-day-post-hatching *Myiopsitta* chick were processed. Histological techniques were performed using Hematoxylin-Eosin (HE), and histochemical staining using Periodic Acid-Schiff (PAS) and Alcian Blue (AB) at pH 0.5, 1, and 2.5 to characterize glandular secretion. Our results indicate that the structure of the proventriculus and gizzard at these stages resemble those of adult *Myiopsitta.* In the proventriculus, the apical region of the superficial glandular cells and the main ducts of the deep glands, both pre- and post-hatching, were PAS and AB positive, with the staining being of notable intensity at pH 2.5. The gizzard cuticle was PAS and AB positive, with greater staining intensity at higher pH. The superficial glands of the gizzard are canalized, and cuticle production is evident, observed as a homogeneous layer that is easily shed pre-hatching and as a thicker, laminar layer post-hatching. At the end of embryonic development, the stomach of *Myiopsitta* shows a degree of maturation similar to that observed in Galliformes. However, in pre-hatching quail embryos, the gizzard cuticle was described with a thickness and arrangement similar to that observed post-hatching in *Myiopsitta*, but not at the end of incubation. Future studies of the complete development of the gastric apparatus will allow us to establish which reprogramming processes (e.g., heterochrony) act in the development of precocial and altricial birds.

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Oryctolagus cuniculus AS AN ANIMAL MODEL FOR BIOREACTOR OR XENOTRANSPLANT; GENE EDITING TROUGH SMGT PLUS FTAI

Díaz Pumará PZ[1], Vazquez-Levin MH[1], Blanco DA[2]

[1] Institute of Biology and Experimental Medicine (IBYME-CONICET) Buenos Aires, [2] Faculty of Veterinary Sciences, University of Buenos Aires, Argentina. E-mail: diazpumara@biozootec.com

Sperm-mediated gene transfer (SMGT) with fixed-time artificial insemination (FTAI) has advantages over other livestock genetic modification methods, in terms of the number of viable genetically modified events (VGME) or in operational simplicity and cost-efficiency. Gene edition aimed to produce pharmacological molecules or organs for human xenotransplant look *Oryctolagus cuniculus* as a valuable model, owing to its post-translational glycosylation profile, which may render the target organ or biological fluid less prone to eliciting adverse immune reaction in primates. Objectives were to adapt the SMGT+FTAI technique, developed to obtain VGME in other species (*Ovis aries, Bos taurus*), to rabbits. Four healthy adult male rabbits and twelve multiparous adult females were used. Fluorescently labeled plasmids (Cy™3) carrying the green fluorescent protein (GFP) gene were applied. The experimental design included: (1) in males, semen collection, evaluation, and transformation protocol previously published; and (2) in females synchronization protocol for physiological (CF) and superovulated estrus (TSOV) adapted for FTAI. Given the induced-ovulation profile of the species, in the CF group (4 females), estrus was detected by vaginal mucosa exam, ovulation was induced with a variable dose of GnRH, from 4.2 to 12.6 µg/donor 12 h prior to insemination (AI). In the TSOV group (8 females), eCG was injected 48 h before AI at variable doses, 25 to 125 IU. 12 hours before AI, as in CF group GnRH was given. Semen was collected with artificial vagina, evaluated and co-incubated with plasmids 2 h before AI. Aliquots containing 10–20×10⁶ sperm, were delivered in two groups of females, treated and negative controls. For embryo collection and outcome evaluation, surgical ablation of the genital tract was performed. A total of 50 embryonic structures (ES) were collected, of which 35 were viable embryos (VES) and 15 non-viable (NVES). From the CF group, 14 VES were recovered, all of them viable. From the TSOV group, a total of 36 ES were collected, including 21 VES and 15 NVES. The difference in ES production between the CF group (14) and the TSOV group (36) was significant ($p < 0.01$). All

ES from both groups were evaluated by fluorescence microscopy to detect GFP expression. Among the six females inseminated with treated semen, 16 VES exhibited GFP fluorescence with no evidence of mosaicism, whereas six females from the negative control group yielded 19 VES and 15 NVES, all negative for GFP. A sperm-mediated transfection rate of 100% was acquired.

PHARMACOLOGY, ECOLOGY AND TOXICOLOGY

A38

ENDOTHELIAL RESPONSE *IN VITRO* TO AIRBORNE PARTICULATE MATTER OF DIFFERENT ORIGINS: URBAN, INDUSTRIAL, AND WILDFIRE SOURCES

Sotelos S[1], Masci I[2], Martínez N[3], Davidovsky L[4], Gelman Constantin J [1,4,5], Tasat D[2], Kurtz M[1].
[1]Escuela de Ciencia y Tecnología, UNSAM, [2]Laboratorio de Bio-Toxicología Ambiental, ITECA, UNSAM–CONICET.[3] Instituto De Fisiología y Biofísica Bernardo Houssay, UBA-CONICET [4]Comisión Nacional de Energía Atómica, [5]Consejo Nacional de Investigaciones Científicas y Técnicas. mkurtz@unsam.edu.ar

Air pollution (gases and particulate matter, PM) represents a global health issue. Its impact on health depends on the origin of the emissions (anthropogenic or natural), concentration, and physicochemical characteristics (size, shape, and composition). Currently, air pollution is recognized as a novel cardiovascular risk factor. Epidemiological and experimental evidence links PM with adverse effects mediated, in part, by endothelial damage. The aim of this study was to evaluate the impact of anthropogenic (urban and industrial) and natural (wildfire-WF) PM on human endothelial cells *in vitro*. Urban PM (UAP-BA) from Buenos Aires (Argentina) in a high vehicular traffic area, and industrial PM represented by Residual Oil Fly Ash (ROFA). WF-PM was collected from fire outbreaks in wetland areas (MP-IFp, Ciervo de los Pantanos National Park) and forest areas (MP-IFb, Nahuel Huapi National Park). PM samples were morpho-chemically characterized using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX). Human endothelial cells (EA.hy926 cell line) were exposed for 24 hours to different concentrations of UAP-BA, ROFA, PM-IFp, and PM-IFb (10–250 µg/ml). Cell viability was assessed by MTT assay, and secretion of the pro-inflammatory cytokine IL-8 by ELISA. Morpho-chemical analysis revealed that UAP-BA is mainly composed of carbonaceous nanoparticles, while ROFA is primarily composed of aluminosilicates with trace amounts of iron and vanadium. WF-PM showed heterogeneous particle sizes forming agglomerates, with carbonaceous cores and the presence of silicon (MP-IFp) and calcium (MP-IFb). All PM tested reduced endothelial cell viability and triggered a dose-dependent inflammatory response, with the highest IL-8 secretion induced by PM-IFp. Cytotoxic effects were more pronounced with ROFA, possibly due to the presence of transition metals. Together, the results show that both anthropogenic and natural PM possesses cytotoxic and pro-inflammatory potential, highlighting their role in endothelial damage and their emerging significance as a cardiovascular risk factor.

A39

INHIBITORY EFFECT OF COWPEA SEED EXTRACT ON THE PROTEOLYTIC AND HEMORRHAGIC ACTIVITIES OF *BOTHROPS ALTERNATUS* VENOM

Ruiz SA[1], Gomez AG[1], Maruñak SL[2], Peichoto ME[3], Gay CC[1]
[1]Instituto de Química Básica y Aplicada del Nordeste Argentino (IQUIBA-NEA, CONICET-UNNE),[2]Universidad Nacional del Nordeste (UNNE), Facultad de Ciencias Veterinarias, [3]Instituto de Biología Subtropical (IBS, CONICET-UNAM). E-mail: silvanaanalia12@gmail.com

In Argentina, *Bothrops alternatus* is the major species of medical importance in the northeastern region, whose venom is mainly composed of metalloproteinases (SVMPs), serine proteinases (SVSPs), and phospholipases A_2 (PLA_2s), which are responsible for causing hemorrhage, edema, inflammation, necrosis, and coagulation disorders. Currently, therapy is based on the administration of antivenom, which primarily counteract the systemic effects of envenomation but have some limitations on local damage. The use of plants represents an alternative for exploring compounds capable of inhibiting these enzymes, such as Fabaceae family, which is considered a rich source of phenolic compounds (PCs). The aim of the present study was to evaluate the inhibitory potential of cowpea seed extract (Vigna unguiculata) on activities induced by *B. alternatus*. The seeds were ground, sieved, and defatted with 10% (w/v) n-hexane. The solvent was evaporated to dryness, and 400 mg of flour was extracted with 40 ml of a 70% methanol-30% water (v/v) solution. The methanol was removed using a rotary evaporator, and the seeds were freeze-dried. The CF content was determined by Folin-Ciocalteu, and the protein concentration by Bradford. *In vitro* assays were performed to evaluate the neutralization of the venom activities (Ba) by pre-incubating different Ba:extract (ESVu) ratios (m:m) and controls (blank, Ba, and ESVu) for 30 min at 37 °C: (i) azocasein method and electrophoretic profile of casein degradation (12% SDS-PAGE); (ii) hemolysis in blood-agar plates; (iii) amidolytic activity using BApNA (N-benzoyl-DL-

arginine p-nitroanilide) and clotting time in citrated plasma (to assess SVMPs, PLA2s, and SVSPs, respectively). *In vivo* hemorrhagic activity was also determined using the pre-incubation method (hemorrhagic SVMPs). Densitometric analysis of electrophoresis bands/hemorrhagic halos (ImageJ) and statistical analysis using ANOVA followed by Tukey's test (Infostat; $p < 0.05$) were performed. A yield of 8.17 mg gallic acid equivalents per gram of dry extract was obtained. At the highest Ba:ESVu ratio assayed (1:1.5), a significant decrease in azocaseinolytic activity (46.63 ±2.48%) was observed compared to Ba. Similarly, in the electrophoretic analysis of casein (CN), Ba degraded its characteristic bands, with an αs2-CN band showing an intensity of 19.6%, whereas in the presence of the extract (1:18) the intensity was 58.0%, both relative to the CN control. The extract reduced venom-induced hemorrhage by 26.6% (1:3.8). However, it did not produce significant inhibition of hemolytic or amidolytic activities, nor did it prolong clotting time. These results demonstrate a specific inhibition of SVMPs by ESVu, the main cause of local damage in bothropic envenomation. Further experiments will be conducted to evaluate the effect of these compounds against purified SVMPs from the venom.

A40

IN VITRO CYTOTOXICITY OF ANTI-CHAGAS DRUGS IN 3T6 SWISS CELLS.

Carou MC[1], Gulin E[12], Davyt M[1], Lombardo DM[12]
[1]*Universidad de Buenos Aires. Facultad de Ciencias Veterinarias. Instituto de Investigación y Tecnología en Reproducción Animal (INITRA). Cátedra de Histología y Embriología.*
[2] *Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). mccarou@fvet.uba.ar*

Chagas disease, caused by *Trypanosoma cruzi*, is treated with nitroheterocyclics (nifurtimox, NFX; benznidazole, BZ), but evidence of cytotoxicity in non-tumoral models and prolonged exposures is scarce. Objective: to evaluate the cytotoxicity of NFX and BZ in 3T6 Swiss fibroblasts over 6–9 days using a multimodal approach. Methods: 96-well plates (500 cells/well); NFX or BZ 3.61–50 µM (six replicates), medium renewal with treatments on days 4 and 6. Mitochondrial activity (MTT, 570 nm) and adherent biomass (crystal violet, 595 nm) were measured on days 6 and 9; glucose consumption in supernatants (492 nm) on day 6; and colorimetric TUNEL at day 6 (3,3',5,5'-tetrametilbencidina, TMB, 450 nm), analyzed as (TMB − blank) with a zero floor. Analysis: one-way ANOVA + Tukey per plate (n=6) and comparisons versus pooled control (n=18) by Student's t; for TUNEL a directional non-parametric test was used(n=5). Significance was set at $p \leq 0.05$ and ≥10% difference vs control. Results: only NFX showed significant effects. MTT decreased 26% at 50 µM vs control. Crystal violet decreased 68% (50 µM) and 38% (36 µM). Glucose consumption decreased by 22% (13.44 µM), 26% (18.66 µM), 28% (25.92 µM), 47% (36 µM), and 72% (50 µM). TUNEL showed relative increases in DNA damage of ≈466% (13.44 µM), ≈894% (36 µM), and ≈689% (50 µM) versus control; other doses showed no significant changes. BZ and the DMSO vehicle produced no relevant effects. Altogether, NFX induces dose-dependent cytotoxicity in long-term 3T6 Swiss cultures at doses ≥13.44 µM. This model is suitable to explore protective strategies (e.g., antioxidants) under controlled conditions, and **glucose consumption** emerges as a **sensitive readout** for their screening.

A41

COMBINED EFFECT OF UNDERNUTRITION AND AIRBORNE PARTICULATE MATTER ON ALVEOLAR MACROPHAGE FUNCTION IN RESPONSE TO A VIRAL CHALLENGE

Masci I,[1] Lezón C,[2] Dugour A,[3] Figueroa JM,[3] Tasat DR,[1] Kurtz ML.[1]
[1]*Instituto de Tecnologías Emergentes y Ciencias Aplicadas,* [2]*Cátedra de fisiología, Facultad de Odontología, Universidad de Buenos Aires (UBA),* [3]*Centro de Biología Respiratoria, Instituto de CyT Dr. César Milstein Fundación Pablo Cassará.E-mail: imasci@unsam.edu.ar*

Malnutrition and air pollution are critical risk factors for children's health. These environmental stressors alter immune function and pulmonary homeostasis, primarily affecting alveolar macrophages (AM), key defensive cells of the respiratory system. AM are also targets of viral infections, which can further compromise immune responses and contribute to increased infant morbidity and mortality. The objective of this study was to evaluate the functional response of AM from an animal model of chronic undernutrition to Residual Oil Fly Ash (ROFA), a particulate matter surrogate, and to Poly I:C, a viral infection analogue. Wistar weanling rats were fed either *ad libitum* (Control, C) or with a diet restricted by 20% relative to C (Nutritional Growth Retardation, NGR) for four weeks. During this period, animals from both groups were intranasally instilled with ROFA (0.17 mg/kg body weight) or its vehicle (PBS) three times per week, resulting in four experimental groups: C, ROFA, NGR, and NGR+ROFA. AM were obtained through broncheoalveolar lavage and cultured for 24 h. Cell viability (MTT), superoxide anion production (NBT), antioxidant response activation (Nrf2 expression by immunofluorescence), phagocytosis (latex beads/cell; percentage and index), and TNF-α secretion (ELISA) were evaluated. In the cultures exposed to Poly I:C (10 and 50 µg/ml) for 4 h, cell viability and TNF-α production were assessed. Cell viability decreased in ROFA and NGR. Poly I:C induced a dose-dependent reduction of viability only in the NGR+ROFA group. The production of superoxide anion was increased in ROFA and

NGR+ROFA groups, while no changes were observed in the antioxidant response (Nrf2 expression). Neither of the experimental conditions showed differences in phagocytic activity. TNF-α levels increased in ROFA and NGR, and the exposure to Poly I:C induced dose-dependent secretion of this cytokine in all 4 groups. Nevertheless, this response was attenuated in the NGR+ROFA group. These results show that undernutrition compromises the viability and antiviral response capacity of AM, and that exposure to air pollution differentially modulates the inflammatory process.

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 3

A42

THE MITOCHONDRIAL-DERIVED PEPTIDE HUMANIN PROTECTS OOCYTES FROM *IN VITRO* AGING

Conte JG[1], Romani JE[2,3], Jaita G[1,4], Michaut MA[2,3]
[1]CONICET – Universidad de Buenos Aires. Instituto de Investigaciones Biomédicas (INBIOMED). Buenos Aires, Argentina. [2]Laboratorio de Biología Reproductiva y Molecular – Instituto de Histología y Embriología de Mendoza (IHEM, UNCuyo-CONICET). [3]Facultad de Ciencias Exactas y Naturales, UNCuyo. Mendoza, Argentina. [4]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Biología Celular, Histología, Embriología y Genética. Buenos Aires, Argentina.
e-mail: julia_conte@hotmail.com

The time interval between oocyte retrieval by follicular aspiration and the application of assisted reproductive technologies (ART) in fertility clinics has a direct impact on oocyte quality. Although *in vitro* culture conditions aim to mimic the physiological environment, prolonged exposure of oocytes to such conditions triggers a process known as *in vitro* aging. Humanin (HN) is a small mitochondrial-derived peptide with well-documented cytoprotective properties in various tissues, including the ovary. In this study, we aimed to evaluate the ability of HN to protect oocytes from *in vitro* aging by analyzing its effects on oocyte quality parameters that are altered during this process, such as spontaneous cortical granule exocytosis, cortical F-actin organization, and mitochondrial activity. Metaphase II (MII) oocytes were collected from superovulated female mice, denuded, and cultured for 4 h in the presence or absence of HN (1 μM). Freshly collected MII oocytes were used as non-aged controls. Oocyte quality parameters were assessed using confocal fluorescence microscopy in both live and fixed cells. In fixed oocytes, cortical granule density was determined using LCA-FITC lectin staining, while cortical F-actin was labeled with Phalloidin-Alexa Fluor 488. In live oocytes, mitochondrial membrane potential (MMP) was assessed using TMRE and MitoTracker Red probes, intracellular ATP levels with Quinacrine, and reactive oxygen species (ROS) levels with DCF-DA. Results showed that HN prevented spontaneous cortical granule exocytosis after 4 h of culture, maintaining densities comparable to control oocytes. Moreover, HN preserved cortical F-actin organization. Regarding mitochondrial activity, HN increased MMP relative to untreated aged oocytes, without altering ATP levels. However, HN significantly reduced ROS accumulation induced during *in vitro* aging. In conclusion, HN improves cytoplasmic parameters associated with oocyte quality and protects oocytes from deterioration caused by *in vitro* aging. These findings support the potential use of HN as a culture medium supplement to preserve oocyte quality during the pre-ART interval in fertility clinics

A43

INHIBITORY EFFECTS OF METFORMIN ON KEY CELLULAR MECHANISMS OF ENDOMETRIOSIS PATHOGENESIS

Schwarz J[1], Villalba Díaz MV[1], Ricci A[1,2], Bilotas M[1], del Valle S[1,2], Meresman G[1,2]
[1]Instituto de Biología y Medicina Experimental (IBYME-CONICET), [2]Facultad de Medicina, Universidad del Salvador (USAL).
E-mail: julietamariaschwarz@gmail.com

Endometriosis is a common gynecological disease that causes infertility and pelvic pain. Since currently available treatments are limited and often associated with side effects, safe and sustainable therapeutic alternatives are needed. Metformin has emerged as a promising candidate owing to its modulatory properties on cell growth and its reported anti-tumoral activity. In this study, the effect of metformin on cell viability and migration were evaluated using experimental in vitro models widely used in preclinical research on endometriosis. Cell viability was measured by the WST-1 colorimetric assay, which measures metabolic activity, in endometrial epithelial (ECC-1), endometrial stromal (T-HESC), and endometriotic epithelial (12Z) cells. Depending on the cell line, between 1 and 2 × 10^4 cells per well were seeded in 96-well plates and incubated for 24 h at 37 ºC and 5% CO_2. Afterwards, cells were stimulated with different concentrations of metformin (0.1; 0.5; 1; 5; 10 and 20 mM) for 24 h. Results are expressed as a percentage of basal control. The migratory capacity of endometrial stromal cells was evaluated using the wound-healing assay. A total of 5 × 10^4 cells were seeded in 24-well plates with DMEM-F12 medium, supplemented with 10% FBS and antibiotics.

Once 90% confluence was reached, a wound was made on the monolayer with a pipette tip, and cells were exposed to metformin (0,5; 1 y 10 mM) under serum-restricted conditions (1% FBS). Wound closure was recorded by photographing the cultures at 0 and 20 h, considering duplication time for that cell line (36 h). Migration was calculated as the reduction of the wound area between both time points using ImageJ software. Results shown that metformin significantly inhibited ECC-1 cell viability at concentrations of 10 mM ($p<0.01$) and 20 mM ($p<0.001$). Endometrial stromal cells exhibited reduced viability from 10 mM ($p<0.05$), while cells derived from endometriotic lesions showed inhibition at 10 mM ($p<0.01$) and 20 mM ($p<0.05$). In the wound-healing assay, metformin at 10 mM significantly reduced the migratory capacity of T-HESC cells ($p<0.01$). The results demonstrate an antiproliferative and antimigratory effect of metformin in experimental models of endometriosis. These findings highlight the potential repositioning of metformin, a drug widely used for the treatment of type II diabetes and polycystic ovarian syndrome, as a therapeutic alternative for endometriosis, offering new perspectives for patients affected by this disease.

A44

FUNCTIONAL CHARACTERIZATION OF A POLYCLONAL ANTIBODY AGAINST THE S2 REGION OF CRISP1 AS A STRATEGY FOR THE SCREENING OF GAMETE FUSION INHIBITORS

González SN[i], Sofía Bigliani[i], Robert F[i], Cuasnicú PS[i]
[1] Instituto de Biología y Medicina Experimental (IByME-CONICET), Vuelta de Obligado 2490, C1428ADN, Ciudad Autónoma de Buenos Aires, Argentina. Tel. +54 11 4783-2869 / Fax. +54 11 4786-2564

Members of the CRISP1–4 (Cysteine-Rich Secretory Proteins) family are mainly expressed in the mammalian reproductive tract, including humans, and play key roles in fertilization and fertility, representing attractive targets for the development of novel contraceptive methods. Studies from our laboratory showed that rat and human epididymal CRISP1 participate in gamete fusion by binding to egg-complementary sites through a conserved 12-amino acid consensus region of the CRISP family named Signature 2 (S2). Based on this, the aim of the present work is to develop an assay to analyze this interaction in order to identify small molecules capable of regulating the sperm fusogenic ability. To this end, we generated a polyclonal antibody directed against a synthetic peptide corresponding to the amino acid sequence of the S2 region of mouse CRISP1 (P2), named α-P2mC1. As a first approach, we evaluated the effect of this antibody on gamete fusion by co-incubating capacitated sperm and zona pellucida (ZP)-free eggs in the presence of the antibody. Our results revealed that α-P2mC1 produced a significant decrease in the fertilization rate, confirming the relevance of the S2 region for gamete fusion, and suggesting its potential accessibility within the native sperm protein to both specific antibodies (immunological inhibition) and small molecules (pharmacological inhibition) capable of binding and blocking this region. Next, we assessed the ability of α-P2mC1 to recognize CRISP1 under different experimental conditions. The results showed that α-P2mC1 was able to recognize the free P2 peptide, mouse and rat native CRISP1 and recombinant mouse CRISP1 by both Western blot (Native PAGE) and Dot blot. To optimize the affinity of α-P2mC1 for its antigen, we analyzed the effect of several experimental parameters on CRISP1 detection by Dot blot. We observed that incubation for 2 h at room temperature and the inclusion of 2% BSA and Zn^{2+} during the blocking and incubation steps increased the affinity of α-P2mC1 for its antigen by approximately 50-fold. Altogether, these observations support the use of a Dot blot assay using α-P2mC1 and recombinant CRISP1 as a screening tool to identify molecules capable of inhibiting this interaction and, potentially, gamete fusion, representing a promising strategy for the development of non-hormonal contraceptives.

A45

USE OF LOW DOSES OF eCG IN OVARIAN SUPERSTIMULATION PROTOCOLS IN SOUTH AMERICAN CAMELIDS: PRELIMINARY RESULTS

Amusquibar MV[1,2], Di Fonzo A[2], Carretero MI[1,2], Trasorras VL[1,2], Arraztoa CC[2]*
[1] National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina
[2] University of Buenos Aires, Faculty of Veterinary Sciences, Institute of Research and Technology in Animal Reproduction (INITRA), Buenos Aires, Argentina
**mamusquibar@fvet.uba.ar*

South American Camelids (SAC), comprising llama, alpaca, guanaco, and vicuña, are remarkable for their extraordinary adaptability, making them an invaluable source of economic resources for numerous families in the northern regions of our country. Llama production provides multiple benefits, including meat, fiber, and hides, among other products. Detailed study of these species not only deepens knowledge of their biology but also fosters their preservation and sustainable development.
Female SACs have a long gestation period of 335–360 days and produce only one offspring per year. Therefore, the implementation of reproductive biotechnologies such as follicular development synchronization, ovarian superstimulation, artificial insemination (AI), and subsequent embryo recovery offers the possibility of increasing the number of offspring and,

consequently, enhancing the proportion of genetically superior animals. This strategy aims to reduce the generational interval and accelerate genetic progress in SAC populations. Ovarian superstimulation (OS) has been successfully applied in various domestic species. This technique involves the controlled administration of hormones to induce the growth of multiple follicles. This increases the number of oocytes available for recovery through follicular aspiration for in vitro procedures, or by inducing ovulation as part of an embryo transfer program. In SACs, different doses and hormonal protocols have been tested to optimize ovarian response and improve reproductive outcomes. In particular, the use of equine chorionic gonadotropin (eCG) has been studied at doses ranging from 500 to 2000 IU, with better ovarian responses observed at higher doses, although with an increased probability of follicular cyst formation (>12 mm). In this context, the aim of the present study was to apply an OS protocol in llamas, using a low dose of eCG sufficient to induce multiple follicular development, and to evaluate the ovarian response in terms of follicle number and size. A group of adult llamas (n=8), averaging 7 years of age and weighing 125–170 kg, was used. Ovarian dynamics were monitored every 48 h by palpation and transrectal ultrasonography (Mindray® Z50 Vet, 5 MHz linear transducer). When a dominant follicle in the growth phase (≥7 mm) was identified, 8 µg of buserelin (synthetic GnRH analog, aGnRH) was administered intravenously (day 0) to induce ovulation. On day 2, 600 IU of eCG was administered intramuscularly, and on day 6 ovarian response was assessed, followed by 250 µg of PGF2α intramuscularly along with a second intravenous dose of aGnRH. Ovarian monitoring continued every 48 h to record follicle number and size. To date, 75% (6/8) of the females responded to OS, with a range of 2 to 4 follicles and an average of 3 ± 0.7 follicles/female, with more follicles observed in the left ovary compared to the right. In 25% of females (2/8), follicular cysts were detected. Based on these preliminary results, we can conclude that it is possible to implement an OS protocol in llamas using 600 IU of eCG, achieving multiple follicular development that provides the opportunity to recover oocytes for subsequent assisted reproduction techniques, such as in vitro fertilization.

A46

CHANGES IN THE MITOCHONDRIAL PROTEOMIC PROFILE IN MOUSE SPERMATOZOA DUE TO CAPACITATION

Cuniolo L[1], Gualano L[2], Cohen DJ[1], Battistone MA[3], Da Ros VG[1].
[1]Institute of Biology and Experimental Medicine (IBYME-CONICET) [2]Chascomús Technological Institute (INTECH)- National University of San Martín (CONICET-UNSAM) [3]Massachusetts General Hospital and Harvard Medical School, Boston, MA,USA.
E-mail: l.cuniolo@ibyme.org.ar

In mammals, ejaculated sperm acquire the ability to fertilize after completing the capacitation process in the female genital tract. This process involves the coordinated activation of signaling and metabolic pathways, along with structural and physiological changes that require efficient energy management. Several studies suggest an increase in mitochondrial activity during capacitation, although the mechanisms regulating this activation remain poorly understood. Given that sperm are transcriptionally and translationally inactive, the regulation of mitochondrial activity could depend on the selective relocation or degradation of pre-existing proteins. In this context, our objective was to identify changes in the mitochondrial proteomic profile in mouse sperm before and after capacitation. To this end, mitochondrial proteins from capacitated (CAP) and non-capacitated (NC) sperm were isolated and processed using a commercial kit, confirming the quality of the sample by Western Blot, where the mitochondrial protein Tom20 was detected and no cytosolic (GAPDHS) or plasma membrane (CAII) markers were observed. The purified proteins were identified by mass spectrometry (LC–MS/MS) with three biological replicates per condition. A total of 1,640 proteins were identified, and analysis using the Gene Ontology database confirmed their enrichment in oxidative phosphorylation and ATP synthesis processes and validated the mitochondrial origin of the samples. The R programming language (v.4.5.0) was used for data processing and statistical analysis using the ggplot2, ggrepel, and dplyr packages. After data curation (≥2 proteins per condition with abundance >0), 1049 proteins were retained, and for each one, the Fold Change (FC) per replicate (CAP/NC) was estimated and transformed to $\log_2$(FC). Differences between conditions were evaluated using Student's t-test ($p<0.05$; $|\log_2 FC|\geq 1$). The analysis revealed 11 proteins with increased relative abundance in the mitochondrial fraction during capacitation, associated with protein repair processes, methionine metabolism, redox balance, and flagellar reorganization, and 42 proteins with relative decrease, mainly linked to ubiquitin-dependent protein degradation and lipid metabolism. Together, these changes demonstrate a coordinated reprogramming of mitochondrial functions during sperm capacitation, which favors the adjustment of energy and redox metabolism necessary for the acquisition of fertilizing capacity. Our findings expand the understanding of sperm mitochondrial plasticity and lay the foundation for future research on its post-translational control and impact on fertility.

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A47

ROLE OF MITOCHONDRIAL LIPID BETA-OXIDATION IN THE REGULATION OF SPERM FUNCTIONALITY IN MICE

Álvarez Rossini V[1], Herzfeld JD[1], Cuniolo L[1], González, LN[1], Cuasnicú PS[1], Da Ros VG[1], Cohen DJ[1]
[1]Instituto de Biología y Medicina Experimental (IBYME-CONICET). E-mail: v.alvarezrossini@gmail.com

The influence of lipids and their metabolism on male fertility has been recognized for decades, although the contribution of lipid oxidation during capacitation to sperm functionality is not yet fully understood. With the aim of addressing this issue, in this study we used different complementary experimental strategies in the mouse model. On the one hand, we analyzed the mitochondrial membrane potential, progressive motility and fertilizing ability of sperm incubated in media with carbon sources from carbohydrates, but without the addition of fatty acids, using fatty acid-free albumin (BSA-FAF) or methyl-β-cyclodextrin and polyvinyl alcohol (MβCD-PVA). Medium supplemented with BSA containing fatty acids was used as a control. Results show that there were no differences in any of the parameters evaluated when spermatozoa were incubated in medium with BSA-FAF, while significant decreases ($p<0.05$) were observed in the presence of MβCD-PVA compared to the control. As a second approach, we evaluated the effect of L-carnitine, which mediates the transport of fatty acids to the mitochondria, in media without added carbon sources on progressive motility and acrosome reaction. While the addition of 0.5 mM L-carnitine had no effect on progressive motility when sperm were incubated in complete medium, its presence produced a substantial improvement in those sperm capacitated in substrate-free medium ($p<0.001$ vs control without substrates and without L-carnitine). In none of the cases did we observe differences in the percentage of reacted spermatozoa, suggesting that endogenous substrates of lipid beta-oxidation are not necessary for this functional event. These results suggest that fatty acids could play a role during sperm capacitation that could be masked by the presence of carbohydrates. Finally, the detection in a sperm mitochondrial proteome, carried out by our group, of various key proteins for beta-oxidation, such as CPT1b, CPT2, ACAA2, ACADL, ACADVL, SLC25A20, and CRAT, supported the experimental findings, as they indicate that the machinery for oxidizing long-chain fatty acids is largely intact and correctly localized in murine sperm. Taken together, these findings provide evidence that fatty acid beta-oxidation could generate the energy necessary for murine sperm motility in the absence of carbohydrates, supporting an active metabolic role for lipid oxidation during capacitation under certain metabolic conditions and reinforcing the plasticity of sperm to respond to different metabolic environments.

A48

ALTERATIONS IN TROPHOBLASTIC DIFFERENTIATION AND INVASIVENESS AND MATERNAL-PLACENTAL REMODELING INDUCED BY PERIGESTATIONAL ALCOHOL CONSUMPTION IN A MURINE MODEL.

Barril C [1,2,3], Pacheco Rodríguez L [1,2,3], Sobarzo CMA[,4,5], Gualdoni GS [1,2,3], Cebral E [1,2,3].
[1] Laboratorio de Reproducción y Fisiología Materno-Embrionaria. Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA)-CONICET, [2]Departamento de Biodiversidad y Biología Experimental (DBBE) [3], Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, [4] INBIOMED, [5] Facultad de Medicina-UBA, Buenos Aires, Argentina.
camilabarril1995@gmail.com

Full-term fetal development and postnatal health depend on the proper establishment and angiogenesis-vascularization of the placenta. Complex networks of trophoblastic signals, including the VEGF/receptor system and metalloproteinases (MMPs), regulate the differentiation, invasion and remodeling of the maternal-fetal interface. Failures in these processes are associated with intrauterine growth restriction, preeclampsia, or premature birth. Recently, it has been suggested that the craniofacial malformations, neuropathies, and neurobehavioral alterations characteristic of fetal alcohol syndrome (FASD) could originate, in part, from placental dysfunction. The objective was to analyze the effects of perigestational alcohol consumption (PAC) until organogenesis on the junctional zone of the placenta, evaluating the differentiation and invasiveness of trophoblastic giant cells (TGCs) and glycogenic cells (GCs), and their participation in decidual interstitial and vascular remodeling. It was administered 10% percent ethanol in drinking water to female mice for 15 days before and up to day 10 of pregnancy and gestation continued until day 13 without exposure (TF). Control females received water (CF). TF-placentas showed JZs with large edematous spaces, TGC delocalization and extensive PAS+-GCs islands in the labyrinth. The percentage of placentas with abnormal JZ increased ($p<0.05$), as did the fetal cephalic-facial malformation/abnormal JZ index (×20, $p<0.05$ vs CF). In TF-JZ, the proliferation of GC precursors (Ki67+/PAS+-non vacuolated) decreased ($p<0.001$), while mature GCs (Ki67-/PAS+, vacuolated) and their glycogenic content (DO GC-PAS/mm² JZ, ImageJ) increased. However, the migration of invasive GCs in the proximal decidua was reduced (Nr. GC-PAS+/JZ field, $p<0.05$). The TF-TGCs presented nuclear area, grade of polyploidy (DAPI) and VEGF/TGCs expression decreased ($p<0.05$), while the % of TGC-Ki67+- increased ($p<0.05$), indicating mitotic continuity and reduced differentiation. However, increased MMP-9/TGC expression ($p<0.05$) was associated with abnormal decidual interstitial

and endovascular remodeling, with loose extracellular matrix, edema and arteriolar endothelial damage. In overall, CPA induces alterations in trophoblastic differentiation and invasiveness and dysregulation of the VEGF/MMP system, compromising the angiogenesis of the placental maternal face, leading potentially to induction of fetal malformations typical of FASD.

A49

SUSTAINED ANANDAMIDE TONE AS POTENTIAL MEDIATOR IN THE ONSET OF HUMAN LABOR

Canela Delás Y [1] , Etcheverry T [1], Pacenza S [1], Saraco N [2], Sar J [3], Martínez N [4], De Laurentiis A [1], Farina M [1]
1. Centro de Estudios Farmacológicos y Botánicos (CEFYBO), UBA-CONICET 2.Laboratorio de Endocrinología, Hospital de Pediatría Juan Pedro Garrahan, CABA, Argentina.3.Servicio de Obstetricia, Hospital Naval Dr. Pedro Mallo, CABA, Argentina.4. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO),UBA-CONICET. Email: caneladelasyilena@gmail.com

Oxytocin (OT) is a key neuropeptide involved in the induction and progression of labor through the activation of its receptor (OTR), which stimulates uterine contractility. In the human placenta, placental aminopeptidase (P-LAP) catalize OT degradation, regulating the bioavailability in both maternal and fetal circulation, thereby preventing premature uterine activation. The endocannabinoid system (ECS), particularly anandamide (AEA), also plays a role in the regulation of pregnancy; its plasma levels increase at term, suggesting its involvement in the initiation of labor. However, the precise mechanisms linking AEA elevation to the modulation of the oxytocinergic system and placental P-LAP activity remain unclear. Therefore, we aimed to evaluate the effect of sustained anandamide tone on the expression and functionality of the oxytocinergic system and placental aminopeptidase (P-LAP) in the human placenta. Term placentas (37–40 weeks of gestation) from singleton pregnancies were collected and classified into two groups: vaginal delivery (VD, n=12) and cesarean section (CS, n=18) without prior uterine activity. Subsequently, placental explants from the CS group were incubated with AEA, URB-597 (a selective FAAH inhibitor), R-(+)-Methanandamide (Met-AEA, a non-hydrolyzable analog of AEA), and SR141716A (a CB1 receptor antagonist) to assess the impact of endocannabinoids on the OT/OTR system and P-LAP. As an in vitro model, the BeWo cell line was used to study the two main placental lineages: cytotrophoblast (CTB) and syncytiotrophoblast (STB). Syncytialization was induced with forskolin, followed by incubation with Met-AEA. OTR expression was evaluated by Western blot, qPCR, and immunofluorescence, while P-LAP expression was determined by qPCR and OT localization by immunohistochemistry. Our results revealed that VD placentas exhibited a significantly higher basal expression of OTR compared with CS placentas ($p<0.05$). Incubation of CS placental explants with Met-AEA or with sequential AEA pulses increased both protein and mRNA levels of OTR. These effects were prevented by co-incubation with SR141716. OTR mRNA was detected in both CTB and STB, but its expression significantly increased in the STB after Met-AEA stimulation, consistent with its localization observed by immunofluorescence. In contrast to OTR, CS placentas showed higher basal OT expression as determined by immunohistochemistry. Moreover, P-LAP mRNA levels increased following incubation with AEA. Our findings demonstrate that variations in AEA levels modulate OTR and P-LAP expression in the human placenta, suggesting that sustained AEA levels may act as signaling mediators contributing to the onset of labor

A50

EFFECT OF RESVERATROL ON THE NUCLEAR AND CYTOPLASMIC MATURATION OF THE BOVINE OOCYTE

[a]Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Instituto de Investigación y Tecnología en Reproducción Animal (INITRA), Buenos Aires, Argentina. [b]Universidad de Buenos Aires - CONICET, Instituto de Investigaciones en Producción Animal (INPA), Buenos Aires, Argentina. [*]tgadze@fvet.uba.ar

The production of reactive oxygen species (ROS) is a normal process that occurs in cells; however, certain stress conditions inherent to *in vitro* environments can disrupt the balance between ROS generation and their removal by intracellular antioxidant systems, resulting in oxidative damage and altered cellular function. The role of ROS and/or antioxidant compounds in the manipulation and culture of gametes and embryos *in vitro* is contradictory, with both detrimental and beneficial effects reported. New antioxidants, such as resveratrol, have emerged, and their properties are under investigation. There is not sufficient evidence regarding the concentrations at which this antioxidant may exert an effect on the *in vitro* maturation (IVM) of bovine oocytes. The aim of this study was to evaluate the effect of resveratrol on the IVM of bovine oocytes, focusing on their nuclear and cytoplasmic maturation. Ovaries were obtained from slaughterhouses and transported to the laboratory, where antral follicles were punctured and aspirated to recover cumulus-oocyte complexes (COCs). COCs were matured in 199 medium supplemented with gonadotropins and fetal bovine serum for 22 hours at 39°C in a humidified atmosphere of 5% CO_2 in air (control) or in the

same medium supplemented with 10 μM (R1), 25 μM (R2), or 50 μM (R3) resveratrol. Nuclear maturation was assessed under an epifluorescence microscope by the presence of metaphase II using the Hoechst 33432 fluorochrome. Cytoplasmic maturation was evaluated based on the ability of oocytes to cleave (≥2 cells) at 48 hours post in vitro fertilization (IVF) and their subsequent embryo development up to the blastocyst stage. For this purpose, COCs were co-incubated with thawed semen in IVF medium (mSOF + 10 IU heparin + BSA), and presumptive zygotes were then cultured in embryo culture medium until day 7. Data were expressed as percentages and analyzed using the chi-square test, considering $p<0.05$ as statistically significant. Nuclear maturation was not affected by the addition of resveratrol at the concentrations tested. In contrast, supplementation of the IVM medium with resveratrol significantly reduced cleavage rates and blastocyst formation at all concentrations tested compared to the control ($p <0.05$). In conclusion, although resveratrol during IVM does not affect nuclear maturation of bovine oocytes, it has a deleterious effect on their cytoplasmic maturation.

CELLULAR AND MOLECULAR BIOLOGY

A51

BIOACTIVE IONS WITH ANTITUMOR POTENTIAL: STUDY OF ION Mn^{2+} AS A CANDIDATE FOR INJECTABLE HYDROGELS APPLIED TO BREAST CANCER

Franzi L[1], Castro Romeo J[1], Barri Pérez P[1], Vellón L[1], Haro Durand LA[1]
[1]Laboratorio de Células Madre Tumorales y Reprogramación Metabólica- IByME-CONICET, Buenos Aires-Argentina.
E-mail: l.haro@ibyme.org.ar

Bioactive ions (BIs) are anions or cations that, when released *in situ* from a biomaterial, can modulate key cellular and molecular responses, including gene expression regulation, activation of signaling pathways, and cell cycle control. These ions play critical roles in diverse physiological processes and exhibit antibacterial, angiogenic, osteogenic, anti-inflammatory, and immunomodulatory properties. Surgical resection remains the standard treatment for solid tumors in breast cancer (BC); however, this procedure carries the risk of local recurrence and metastasis, which are the leading causes of postoperative mortality. This phenomenon is partly attributed to the persistence of residual cancer stem cells (CSCs), capable of proliferating locally or spreading through the bloodstream. Moreover, tumor removal inevitably affects surrounding healthy tissue, inducing inflammation, trauma, and infection risk, thereby compromising wound healing and tissue regeneration. In this context, the development of injectable hydrogels capable of controlled and localized release of bioactive ions with anti-CSC potential represents an innovative approach in regenerative oncology, offering a dual therapeutic action: preventing tumor recurrence and promoting tissue repair after surgery. The aim of this study was to evaluate the anti-CSC potential of ion Mn^{2+} in a BC-derived CSC line. ER^- (MDA-MB-231) cells were exposed to increasing concentrations of Mn^{2+} (0–300 μmol/L) for 48 h. Cell viability was assessed using an MTT colorimetric assay, proliferation was monitored in real time with the Sartorius Incucyte® system, and cell cycle progression was analyzed by flow cytometry. The results show that Mn^{2+} exerts a dose-dependent cytostatic effect on the studied CSC line, possibly by blocking the G2→M transition of the cell cycle. In addition, Mn^{2+} decreased mammospheres forming capacity in MDA-MB-231 cells, as observed and quantified by limiting dilution assay. Taking together, these findings suggest that ion Mn^{2+} possesses anti-proliferative and anti-CSC potential in BC cells and could be incorporated into an injectable hydrogel designed to prevent tumor recurrence and support tissue regeneration following oncologic surgery.

A52

A STUDY ON TELOMERIC CHROMOSOMAL DAMAGE IN INDIVIDUALS WITH AND WITHOUT MIGRAINE

Catanesi CI[1,2], Miranda SM[3,4], Giglio JA[5,6], Barandica MB[1,2], Baitella PA[1,2], Pratti Addiechi MA[1,2], Silbestro M[1], Bolzán, AD[1,2].
[1]Instituto Multidisciplinario de Biología Celular (IMBICE), La Plata, Argentina, [2]Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina, [3]CUCAIBA, La Plata, Argentina, [4]Consultorios Médicos París, La Plata, Argentina, [5]Fundación Dr. César Burry, La Plata, Argentina, [6]Instituto de Diagnóstico, La Plata, Argentina. E-mail: ccatanesi@imbice.gov.ar

Migraine is a chronic neurovascular disease characterized mainly by recurrent, intense, and highly disabling headaches. Previous studies indicate that individuals with migraine exhibit telomeric shortening compared to individuals without migraine. Taking it into account, the objective of this study was to investigate whether individuals with migraine present greater chromosomal damage at the telomeric level than individuals without migraine. We cultured peripheral blood lymphocytes from each individual

for 72 hours at 37 °C, obtained chromosomal spreads, and studied the presence of losses and duplications of telomeric signals per cell in 20 metaphases per individual, applying the Fluorescence In Situ Hybridization technique with pan-telomeric and pan-centromeric probes. The results obtained to date, analyzing 10 individuals (4 without migraine and 6 with migraine), indicate that there are no significant differences between individuals with and without migraine in the average number of telomeric signal duplications per cell (aberrations that indicate telomeric fragility), nor in the average number of telomeric signal losses per cell (aberrations that suggest telomeric shortening) ($p>0.05$). Nevertheless, we found that a control individual receiving medical treatment with an antimetabolite presented a very high frequency of telomeric signal losses per cell, and by excluding this individual from the analysis, we observed that the average frequency of chromosome-type telomeric signal losses (i.e., affecting both chromatids of the same chromosomal end) was significantly higher in individuals with migraine than in individuals without migraine ($p=0.02$). The difference between both groups of individuals in the average frequency of chromatid-type telomeric signal losses (i.e., affecting only one of the chromatids of the same chromosomal end) showed a similar trend, although not significant ($p=0.16$). Likewise, the percentage of lost telomeric signals and the total number of telomeric signal losses per cell were higher in individuals with migraine ($p=0.04$). These results suggest that individuals with migraine may have shorter telomeres than those who do not suffer from this condition, and that a greater presence of chromosomal arms without telomeric signals could be considered a biomarker to distinguish individuals with migraine from those who do not have it. Due to the number of individuals analyzed, we cannot yet establish definitive conclusions in this regard, but it is important to highlight that the results obtained in this study are the first worldwide concerning knowledge of telomeric chromosomal damage in individuals with migraine.

A53

TRANSCRIPTION FACTOR SP1 REGULATES MITOFUSIN 2 EXPRESSION, CONTRIBUTING TO MITOCHONDRIAL BIOGENESIS

Arona F[1,2], Rodrigo M[1,2,3], Dattilo M[1,2], Decono M[1,2], Mori Sequeiros Garcia M M[1,2], Benzo Y[1,2], Poderoso C[1,2]
[1]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Buenos Aires, Argentina.
[2]CONICET-Universidad de Buenos Aires, Instituto de Investigaciones Biomédicas (INBIOMED), Buenos Aires, Argentina. [3] Universidad de Buenos Aires, Facultad de Medicina, Hospital de Clínicas "José de San Martín". División Endocrinología.
E-mail: garona2018@gmail.com

Mitochondrial function is essential for angiotensin II (Ang II) and corticotropin (ACTH) signaling pathways, which stimulate the biosynthesis of aldosterone and glucocorticoids, respectively, in adrenocortical cells. We previously demonstrated that Ang II and the ACTH/cAMP pathway stimulate mitochondrial fusion in human adrenocortical H295R cells by increasing the expression of Mitofusin 2 (MFN2). MFN2 is a key protein involved in mitochondrial fusion as part of mitochondrial dynamics and plays a positive role in mitochondrial respiration and oxidative phosphorylation. These processes are regulated by mitochondrial biogenesis, which is necessary for maintaining mitochondrial function and activity. The human MFN2 promoter contains potential binding sites for various transcription factors, such as estrogen-related receptor α (ERRα) and specificity protein 1 (Sp1). The aim of this study is to explore the relevance of Sp1 in MFN2 expression and its role in mitochondrial biogenesis in human adrenocortical H295R cells. We observed that a decrease in Sp1 expression (through RNA interference) correlates with a reduction in MFN2 mRNA and protein expression under both basal ($p < 0.05$) and Ang II- or 8Br-cAMP–stimulated conditions (a membrane-permeable cAMP analog) ($p < 0.001$). Moreover, Sp1 participates in the hormone regulation of NRF-1 mRNA levels (a transcription factor that promotes mitochondrial biogenesis) ($p < 0.001$). We determined that the full-length MFN2 promoter is significantly activated by Ang II stimulation ($p < 0.001$), as shown by transfection and chemiluminescence assays. Deletion of the ERRα and Sp1 recognition sites resulted in decreased promoter activity under both basal and Ang II-stimulated conditions ($p < 0.001$), an effect that is reproduced when Sp1 is silenced. Finally, we observed that MFN2 silencing led to a decrease in NRF-1 and TFAM (another key mitochondrial biogenesis factor) protein levels ($p < 0.001$), in Ang II-stimulated H295R cells. Our results indicate that hormone stimulation promotes MFN2 transcription mediated by Sp1 and probably by ERRα in human adrenocortical H295R cells, and that MFN2 expression is required to maintain NRF-1 and TFAM levels, suggesting a role for MFN2 in mitochondrial biogenesis.

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A54

STUDY OF FERROPTOSIS IN LEYDIG CELLS AND ITS RELATIONSHIP WITH STEROIDOGENESIS

Benzo Y [1,2], Raggio MA[1,2], Mori Sequeiros Garcia MM [1,2], Gorostizaga A[1], Paz C [1,2], Theas MS [2,3], Ferrer Viñals D [2], Poderoso C[1,2], Maloberti PM [1,2]

[1]Universidad de Buenos Aires, Facultad de Medicina. Departamento de Bioquímica Humana., [2]CONICET – Universidad de Buenos Aires. Instituto de Investigaciones Biomédicas (INBIOMED),[3]Univetsidad de Buenos Aires, Facultad de Medicina. Unidad Académica de Embriología. E-mail: ybenzo@fmed.uba.ar

Male infertility is a global health issue frequently caused by alterations in the production of testosterone (T) and sperm. Both processes take place in the testes, where T synthesis occurs mainly in Leydig cells (LC). Iron is essential for testicular function, playing a crucial role in sperm production and in the synthesis of hormones such as T by LC. However, excess iron in testicular tissue can lead to reproductive dysfunction by inducing oxidative stress. Ferroptosis is a form of iron-dependent cell death triggered by the accumulation of oxidized phospholipids and is strongly associated with infertility, particularly through cysteine deficiency and inhibition of the enzyme glutathione peroxidase 4 (GPX4). To study this mechanism, two inducers are commonly used: Erastin, a compound that binds to the voltage-dependent anion channel 2/3 (VDAC2/3) and inhibits the cystine/glutamate antiporter, thereby reducing glutathione synthesis; and RSL3, which directly and selectively inhibits GPX4 activity. The aim of this study is to investigate ferroptosis in LC and its relationship with steroidogenesis, the main function of this cellular model. Using the mouse LC line MA-10, cell viability was evaluated through the MTT assay under different concentrations of Erastin and RSL3. A dose-dependent decrease in cell viability was observed, showing a reduction of 30–40% with 5 µM Erastin and 10 µM RSL3 ($p < 0.05$). Moreover, cell viability was drastically reduced in primary cultures of rat testicular interstitial cells treated with the same concentrations of the mentioned compounds. Ferroptosis was confirmed by determining lipid peroxidation using BODIPY C-11, showing a significant increase ($p < 0.05$). Ferroptotic effects were markedly reduced when cells were simultaneously incubated with ferroptosis inducers and human chorionic gonadotropin (hCG), a hormone that induces steroidogenesis. Additionally, the expression of genes involved in ferroptosis was evaluated by RT-qPCR in MA-10 cells. Both Erastin and RSL3 significantly modulated the expression of genes associated with ferroptosis and steroidogenesis, such as the StAR (Steroidogenic Acute Regulatory) protein, which is involved in the rate-limiting step of steroid hormone synthesis ($p < 0.05$; $p < 0.001$). Altogether, these results highlight the importance of studying ferroptosis in Leydig cells and suggest a direct relationship between ferroptosis and steroidogenesis, as well as a potential protective role of hormonal stimulation against the damage induced by this form of cell death.

A55

IMPLICATIONS OF THE INTERACTION BETWEEN ACIL-COA SYNTHETASE 4 AND THE ANDROGEN RECEPTOR IN BREAST CANCER

Dattilo MA[1,2], Lopez PZ[1,2], Mori Sequeiros MM[1,2], Bulian V[1,2], Arona F[1,2], Suberviola A[1], Mansini A[3], Hoeppner L[4], Poderoso C[1,2], Orlando UD[1,2], Maloberti PM[1,2]

[1]Universidad de Buenos Aires, Facultad de Medicina. Departamento de Bioquímica Humana, [2]CONICET – Universidad de Buenos Aires. Instituto de Investigaciones Biomédicas (INBIOMED). [3]Rush University - USA, [4]The Hormel Institute - USA. E-mail: mdattilo@fmed.uba.ar

The enzyme acyl-CoA synthetase 4 (ACSL4) has been linked to aggressive cancer behavior, as its overproduction encourages proliferation, migration, invasion, tumor growth and metastasis. In the context of breast cancer, our study investigated the regulation of ACSL4, revealing that the transcription factor ERRα controls its differential expression in triple-negative breast cancer (TNBC) cells, whereas the estrogen receptor (ERα) induces its repression. This regulation is reciprocal, as ACSL4 overexpression decreases ERα levels in TNBC cells. The androgen receptor (AR) has been investigated as potential therapeutic target in AR-positive TNBC tumors, with promising results emerging from clinical trials. However, therapeutic alternatives are limited in AR-negative TNBC tumors (QNBC). This study aimed to analyze the reciprocal regulation between ACSL4 and AR, and the efficacy of their combined inhibition as a therapeutic strategy in breast cancer. For this purpose, we used highly aggressive QNBC cell lines (MDA-MB-231 and BT-20), which are characterized by high levels of ACSL4 expression, as well as the AR-positive MCF-7 cell line, which has low levels of the enzyme. We observed that AR represses ACSL4: in MCF-7 cells, dihydrotestosterone AR activation decreased promoter activity, as measured by the *NLuc* reporter gene assay, while the antagonist bicalutamide (BICA) or site-directed mutation of the element increased it. Ectopic expression of AR in MDA-MB-231 and BT-20 cells reduced both promoter activity and ACSL4 levels, as determined by western blot analysis. Conversely, ACSL4 was found to negatively regulate AR: in MCF-7 cells, ACSL4 overexpression reduced AR expression; however, in MDA-MB-231 cells, silencing or inhibiting ACSL4 with PRGL493 restored AR expression. We investigated the combined effect of

PRGL493 and BICA in the MDA-MB-231 cell line. The treatment more effectively reduced cell proliferation (MTT assay), migration (wound closure) and invasion (spheroid invasion) than either drug alone. Furthermore, the zebrafish *Tg(fli1: EGFP)* model was used to evaluate extravasation, which is a key step in metastasis. We observed in their fluorescent vasculature in real time the behavior of xenografted, labelled MDA-MB-231 cells that had been preincubated with PRGL493 (1.5 µM), BICA (4 µM), or both. Maintenance of extravasation was observed in cells treated with either drug alone, whereas the combination blocked this process. Taken together, we demonstrated the therapeutic potential of simultaneous inhibition of ACSL4 and AR in QNBC breast cancer, a subtype currently lacking effective treatments.

A56

POTENTIAL ANTITUMOR EFFECT OF CHLOROGENIC ACIDS PRESENT IN *Ilex paraguariensis* (YERBA MATE)

Serrano AN[1], Cesario M[1], Cholich L[1], Olea G[2], Maiocchi M[1], Bustillo S[1]
[1]Grupo de Investigaciones Biológicas y Moleculares (GIByM), [2]Laboratorio de Investigaciones Bioquímicas (LIBIM). IQUIBA-NEA UNNE-CONICET. E-mail: natalia.serrano@comunidad.unne.edu.ar

Ilex paraguariensis (A.St.-Hil., 1822), commonly known as yerba mate (YM), is a plant species native to South America, whose natural distribution is limited to Brazil, Paraguay, and Argentina. Chlorogenic acids (CA) are the most abundant water-soluble components of YM and have been identified as the main contributors to the antioxidant activity of its traditional infusions (cebadura and mate cocido). The aim of the present study was to analyze the response of LM3 tumor cells to chlorogenic acid treatment, determining cell viability in comparison with the normal NMuMG cell line, and to evaluate proliferation through Ki-67 immunoexpression and the presence of apoptosis using the Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique. Briefly, LM3 and NMuMG cells were exposed to different concentrations of CA (0.25–4 mg/mL) (Sigma-Aldrich, purity >95%) for 24 h at 37 °C and 5% CO_2. Cytotoxicity was evaluated using the crystal violet assay, expressing the results as percentage of cell viability and determining the 50% cytotoxic concentration (CC_{50}). Experiments were performed in triplicate in at least two independent assays. Subsequently, LM3 cells seeded on coverslips were treated with 0.20 mg/mL of CA for 24 h for the analysis of proliferation and apoptosis. Proliferation was evaluated by Ki-67 immunostaining using the indirect biotin–streptavidin–HRP system (DAKO), with positive and negative controls, DAB chromogen revelation, and hematoxylin counterstaining. Apoptosis was analyzed using the TUNEL technique (POD kit, Roche), including negative controls (PBS) and positive controls (DNase). In both assays, preparations were observed under light microscopy, morphological features associated with apoptosis were analyzed, and the proliferation index (PI) and apoptotic index (AI) were calculated. The results showed that CA induces dose-dependent cytotoxicity in both cell lines; however, tumor cells exhibited greater sensitivity (CC_{50} = 0.20 mg/mL) than normal cells (CC_{50} = 0.50 mg/mL). In addition, a decrease in cell proliferation was observed after CA treatment, with a PI of 8% compared to 16% in control cells. The TUNEL assay revealed an AI close to 20% in treated cells, in marked contrast to the control group, which showed an AI lower than 1%, indicating a clear induction of apoptosis by CA. In conclusion, chlorogenic acid may exert a selective cytotoxic effect on tumor cells, leading to a decrease in their proliferation and the induction of apoptosis as a death mechanism involved. Future studies focused on the expression of key molecules related to programmed cell death will allow further progress in this line of research.

A57

ACSL4 MODULATES MICRORNA EXPRESSION AND PROMOTES AN AGGRESSIVE TUMORAL PHENOTYPE IN BREAST CANCER

Quevedo LM[1,2], Mori Sequeiros Garcia MM[1,2], Arona F[1,2], Poderoso C[1,2], Castillo AF[1,2]
[1]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Bioquímica Humana. Buenos Aires, Argentina.
[2]CONICET - Universidad de Buenos Aires. Instituto de Investigaciones Biomédicas (INBIOMED). Buenos Aires, Argentina.
E-mail:luquevedo.1997@gmail.com

The enzyme acyl-CoA synthetase 4 (ACSL4) participates in the activation of long-chain fatty acids, showing high affinity for arachidonic acid, and plays a central role in lipid metabolism. Its expression is increased in several carcinomas, including breast, prostate, colon, and hepatocellular cancers, and is associated with aggressive tumor phenotypes. Previous studies have demonstrated that ACSL4 overexpression induces a highly aggressive phenotype in breast and prostate cancer cells and promotes breast tumor growth in vivo. In this work, we investigated the molecular mechanisms driven by ACSL4 that may contribute to this phenotype, focusing on the regulation of microRNAs (miRNAs) as potential mediators. miRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally by binding to target mRNAs, promoting their degradation or inhibiting their translation. We performed mature miRNA sequencing (miRNA-seq) in MCF-7 breast cancer cells stably overexpressing ACSL4, identifying a differential miRNA expression profile compared with control cells (log2 fold change > |0.5|). Through bioinformatic pathway enrichment analyses (KEGG, WikiPathways), we explored the potential roles of these miRNAs in cellular processes and pathological conditions. After validating the results by RT-qPCR, we focused on miR-99a-3p, whose expression was inversely

correlated with ACSL4. In different breast cancer cell models, ACSL4 overexpression significantly decreased miR-99a-3p levels, whereas its silencing increased them. This inverse pattern was also observed for its primary transcript and for the complementary microRNA derived from the opposite strand, miR-99a-5p. To explore the regulatory mechanism, we analyzed the *MIR99AHG* locus and its promoter region, observing a significant reduction in promoter activity upon ACSL4 overexpression in reporter gene assays. Using PROMOTER 2.0 and FIMO (JASPAR database), we predicted potential transcription factor binding sites in the promoter region. Integration of these data with previous RNA-seq results and qPCR validation allowed the identification of transcription factors whose expression is modulated by ACSL4. Functional enrichment analyses (Gene Ontology, KEGG) indicated that several of these factors are associated with lipid metabolism. Altogether, our findings demonstrate that ACSL4 reshapes the miRNA expression profile in breast cancer and that its pro-tumoral activity may rely, at least in part, on the transcriptional repression of miR-99a-3p mediated by lipid metabolism-related transcription factors.

A58

ISOLATION OF ACIDIC PHOSPHOLIPASES A_2 (PLA_2s) FROM *Bothrops alternatus* VENOM

Moro R[1], Fusco L[2], Leiva L[1], Bustillo S[1]
1Grupo de Investigaciones Biológicas y Moleculares (GIByM), 2Grupo de Investigación en Proteínas (GrInPro). IQUIBA-NEA UNNE-CONICET. E-mail: ramiromoro55@gmail.com

Phospholipases A_2 (PLA_2s) are abundant enzymes in snake venoms of the Viperidae family, representing one of the main toxin families responsible for a wide range of biological and pathological effects. These enzymes catalyze the hydrolysis of the ester bond at the sn-2 position of glycerophospholipids, releasing fatty acids and lysophospholipids that contribute to membrane destabilization and the generation of lipid mediators. Proteomic analysis of *Bothrops alternatus* (yarará grande) venom have shown that PLA_2s represent approximately 6.85% of the total protein content. These enzymes occur as acidic and basic isoforms with markedly different functional properties. Acidic isoforms typically exhibit lower myotoxic activity but actively participate in processes such as inflammation, hemostatic disturbances, modulation of immune responses, and in some cases, anticoagulant or antiplatelet effects. Their study is essential not only for understanding envenomation mechanisms but also due to their potential pharmacological and biotechnological applications. The aim of this work was to isolate and partially characterize acidic PLA_2 isoforms from *B. alternatus* venom. Briefly, 70 mg of lyophilized *B. alternatus* venom were resuspended in 10 mM phosphate buffer (pH 7.2) and subjected to an initial chromatographic separation using an FPLC system (ÄKTA Avant 25, Cytiva) equipped with a HiPrep Q FF 16/10 strong anion exchange column. Fractions of 2 mL were collected and analyzed by SDS-PAGE (14%) and by radial indirect hemolysis assay to detect PLA_2-specific activity. Active fractions were further purified by size exclusion chromatography on a Superdex 75 Increase 10/300 GL column using the same FPLC system, collecting 0.8 mL fractions. Tubes showing only indirect hemolytic activity and lacking proteolytic activity were pooled. Molecular mass was estimated by SDS-PAGE, and an additional native agarose gel electrophoresis (1%, Tris-Glycine buffer, pH 8.6) was performed to assess electrophoretic mobility according to net charge. Finally, cytotoxicity was evaluated on murine myoblasts (C2C12 cell line) after 24 h of incubation. Our findings demonstrated that a pool of PLA_2 isoforms of approximately 28 kDa was obtained, as determined by SDS-PAGE under non-reducing conditions. The acidic nature of these isoforms was confirmed by native agarose gel electrophoresis. No cytotoxic effects were observed on myoblasts at the tested doses and incubation times, consistent with previous reports for acidic PLA_2s. Future studies will focus on isolating pure isoforms and evaluating how these enzymes influence muscle regeneration using differentiated myoblasts.

ENDOCRINOLOGY AND METABOLISM

A59

GENOTYPE-BY-ENVIRONMENT INTERACTIVE EFFECTS AND CONFLICT SOLVING DURING GONADAL SEX DIFFERENTIATION OF PEJERREY *Odontesthes bonariensis*, A FISH WITH DUAL GENOTYPIC/ENVIRONMENTAL SEX DETERMINATION

Chenyan Wu[1], Wakaba Baba[1], Ryuichi Nakagawa[1], Yoji Yamamoto[1*], Carlos Augusto Strüssmann[1]
[1] Tokyo University of Marine Science and Technology, Tokyo, Japan. [*] email: carlos@kaiyodai.ac.jp

Genotypic (GSD) and environmental (ESD) sex determination coexist in many species of reptiles, fish, and amphibians. Inherited genotypic signals and environmental factors conceivably interact as pro-testis or pro-ovary signals during sex determination, but how such interactions affect gonadal sex differentiation in these species remains largely unexplained. We used a model gonochoristic fish with coexisting GSD and ESD, the pejerrey *Odontesthes bonariensis*, to examine how synergism and antagonism between sex genotype (XX/XY) and thermal (feminizing/masculinizing) regimes interactively affect environmental

sensitiveness and the critical time of environmental sex determination and how genotype-by-environment conflicts are resolved. We show that convergence/divergence between genotypic and environmental signals advances/delays the critical time of sex determination and lowers/raises the degree of environmental sensitiveness, respectively, even when genotypic control is ultimately overridden. The results also evidence that ovarian formation is the default state regardless of genotypic sex and yet that commitment to femaleness is a lengthy, passive process requiring absolute seclusion from environmental pro-male stimuli. Testis formation, in turn, is the alternative state that can be imposed on this default by an extremely short environmental stimulus of sufficient strength at any time before ovarian commitment. We argue that this combination of developmental features is crucial to avoid ambiguous differentiation under conflicting genotypic/environmental signals. Overall, the results reveal genotypic sex-dimorphic critical periods of sex determination, show that it is "easier" to make males in pejerrey, and provide clues to understand how GSD+ESD species prevent discrepant sex determination/differentiation when genotype and environment diverge.

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ROLES OF *amhy* AND *amha* IN DUAL GENETIC AND TEMPERATURE-DEPENDENT SEX DETERMINATION SYSTEMS OF PEJERREY (*Odontesthes bonariensis*)

Katsuki Naoi[1], Larissa Monteiro de Vasconcelos[1], Yoji Yamamoto[1*], Carlos Augusto Strüssmann[1]
[1] Tokyo University of Marine Science and Technology, Tokyo, Japan* email: yyamam0@kaiyodai.ac.jp

Pejerrey exhibits a dual system of sex determination, integrating an XX/XY genetic sex determination (GSD) at the intermediate range of temperatures with temperature-dependent sex determination (TSD) at both extremes of the viable thermal range. Two paralogs of the *amh* gene, the Y-linked *amhy* and the autosomal *amha*, are implicated in male sex determination and differentiation, yet their precise roles in this dual system remain unresolved. To elucidate their functions, we performed loss-of-function analyses using the CRISPR/Cas9 system. F_0 mutants were generated by disrupting *amha* in XX embryos and *amhy* only or both genes in XY embryos and reared them either at sexually neutral temperatures (SNT; 22–25°C) or at a male-producing temperature (MPT; 29°C). High-efficiency mutants were identified by NGS, and gonadal phenotypes were examined histologically. All XY control (unmanipulated) fish developed as males at both rearing temperatures. 26.9% of the XY *amhy* mutants and all XY double (*amhy/amha*) mutants sex reversed to females at the SNT, whereas at the MPT, even double mutants developed as males. XX (unmanipulated) controls and *amha* mutants at the SNT were 67% and 100% female, respectively, but both groups were fully masculinized at the MPT. These results demonstrate that *amhy* and *amha* contribute to the establishment of the male pathway in XY and XX fish (*amha* only) under GSD (SNT conditions) but appear to be irrelevant for TSD masculinization at the MPT. This study highlights the environment-specific contributions of these two *amh* paralogs to the dual sex-determining system of pejerrey.

A61

OBSERVATIONS ON THE OTOLITH CHECK FORMATION IN YOUNG PEJERREY *Odontesthes bonariensis*

Yuki Gomi[1], Yoji Yamamoto[1], Carlos A. Strüssmann[1], Leandro A. Miranda[2], Dario C. Colautti[3], Gustavo E. Berasain[4]
[1] Laboratory of Population Biology, TUMSAT, Japan; [2] Laboratorio de Ictiofisiología y Acuicultura, INTECH; [3] Instituto de Limnología Dr. Raúl A. Ringuelet, UNLP; [4] Estación Hidrobiologica de Chascomús, MAAGBA* email: carlos@kaiyodai.ac.jp

The pejerrey *Odontesthes bonariensis* is an Atherinopsid fish species of considerable socioeconomic importance in the Pampas region of South America. This species combines genotypic (XX-XY system) and temperature-dependent sex determination at environmentally relevant temperatures. As a result, genotypic-phenotypic sex mismatches (sex reversal) and biased sex ratios are commonly observed in natural populations. These characteristics underscore the importance of monitoring the age structure, growth, and reproductive potential of wild populations for the conservation of natural pejerrey resources. Otolith analysis is an effective tool for obtaining life history information on fish populations. In pejerrey, it has been shown that otolith increments are formed on a daily basis but the timing and mechanism of the formation of "checks", which are often assumed to represent the age in years, remain unclear. This study examined the formation of the first otolith checks in young individuals collected between 2015 and 2025 during an ongoing survey of the pejerrey population in the Chascomús Lake, Buenos Aires, Argentina, in relation to their birth date (season), sex (phenotypic male or female), and the age and size at the time of check formation. The results reveal that first check formation in young pejerrey involves a complex interplay between factors such as birth date (season), daily age, and body size whereas phenotypic sex does not appear to have a significant influence. The study also reveals that checks may form twice a year in young fish under certain conditions. The possibility of a relation with reproductive activity is discussed.

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A62

SEX-DEPENDENT REGULATION OF PITUITARY FILAMIN A BY DOPAMINE AND ESTRADIOL: IMPLICATIONS FOR PROLACTINOMA DEVELOPMENT

Bornancini D, Segobia V, Peña-Zanoni M, Diaz-Torga G.
Instituto de Biología y Medicina Experimental (IBYME – CONICET). E-mail: dana.bornan@gmail.com

Filamin A (FLNA) is an actin-binding scaffolding protein that stabilizes and regulates the trafficking of several G-protein-coupled receptors, including the dopamine D2 receptor (D2R). In lactotrophs, FLNA is essential for proper D2R membrane localization and dopaminergic signaling. Reduced FLNA expression has been reported in dopamine-agonist-resistant prolactinomas. We aimed to investigate sex differences and local regulation of pituitary FLNA expression and its relationship with prolactinoma development. We analyzed two transgenic mouse models: a D2R knockout (KO model) and a βhCG-overexpressing (βhCG^{+} model), in which only females develop prolactinomas. In both models, pituitary FLNA expression was higher in males than in females, and decreased in transgenic mice of both sexes compared to wild-type animals. To assess hormonal regulation, wild-type mice were acutely treated in vivo with dopamine or estradiol. Estradiol decreased, whereas dopamine increased pituitary FLNA expression, indicating that FLNA is hormonally regulated. The estradiol-induced reduction and dopamine-induced stimulation of FLNA may underlie the sexual dimorphism in prolactinoma development. Impaired FLNA expression may thus contribute to dopamine-agonist resistance. These findings identify FLNA as a critical determinant of pituitary dopaminergic signaling and a potential therapeutic target to restore cabergoline responsiveness.

A63

EFFECTS OF THE NEONATAL EXPOSURE TO BISPHENOL A, BENZOPHENONES 2 OR 3 ON METABOLIC PARAMETERS IN C57BL/6 MICE

Riaño Gómez JM, Sorianello EM, Bianchi MS, Fernandez MO. Instituto de Biología y Medicina Experimental (IBYME), Vuelta de Obligado 2490, C1428ADN, CABA, Argentina. juanrianog@gmail.com

Bisphenol A, (BPA), monomer of polycarbonate plastics, and Benzophenones (BPs), UV filters, are endocrine disrupting chemicals (EDC). Previously we described effects of the neonatal exposure to BPA on the hypothalamic-pituitary-gonadal axis in female rats. In this study, we evaluated the effects of the neonatal exposure to BPA, BP2 or BP3 on insulin sensitivity and basal glucose levels in male and female C57BL/6 mice. Pups were dosed orally, daily, from PND (Postnatal day)1-PND10, with BPA (50 µg/kg), BP2 or BP3 (250 µg/kg), or vehicle (control). Animals were weighted weekly, and insulin tolerance tests (ITT) performed at PND45. For the ITT, mice were fasted for 3 h, ip injected with 1 U/kg insulin and glycaemia evaluated at 0,15, 30, 60, 90 and 120 min with a One-Touch Ultra glucose meter using tail blood. In females, exposure to neither BPA nor BP3 altered insulin sensitivity. Exposure to BP2 decreased insulin sensitivity (Two-way ANOVA: Main effect Treatment: $p<0.05$, Interaction: $p<0.05$). In males, exposure to BPA slightly decreased insulin sensitivity. Exposure to BP2 decreased insulin sensitivity (Two-way ANOVA: Main effect treatment: $p<0.05$). Exposure to BP3 altered the ability of peripheral tissues to respond to insulin (Two-way ANOVA: Interaction: $p<0.05$). Our results show that neonatal exposure to EDC found in plastics and personal care products alter insulin sensitivity in a sex-dependent manner. Supported by: CONICET, ANPCyT, Int. Soc. for Neurochemistry, Fund. R. Barón, Fund. Williams, Fund. H. Bigand.

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PATERNAL ALCOHOL EXPOSURE ALTERS HYPOTALAMIC AND PITUITARY D2 RECEPTOR ISOFORM EXPRESSION AND PROLACTIN REGULATION IN OFFSPRING: PROTECTIVE EFFECTS OF RESVERATROL

Victoria Ayelén Segobia, Lucila Gotfryd, Dana Micaela Bornancini, Milagros Peña Zanoni, Juan Carlos Calvo, Graciela Díaz-Torga, Vanina Andrea Fontana.
Instituto de Biología y Medicina Experimental (IBYME) – CONICET, Vuelta de Obligado 2490, Ciudad Autónoma de Buenos Aires, Argentina. E-mail: vickyegobia@gmail.com

High prolactin (PRL) levels have been described in alcohol-consuming subjects, but the mechanisms remain unclear. The dopamine D2 receptor (D2R) exists as short (D2S) and long (D2L) isoforms; a high D2S/D2L ratio enhances dopaminergic inhibition of PRL. Ethanol increases D2L and decrease D2S, favoring PRL release. Male mice (CF1) were exposed to 15% ethanol *ad libitum*, during 12 days before mating. A group received resveratrol treatment, administered every 24 hours via esophageal gavage for 12 days concomitant to ethanol exposure. In their offspring (at 60 days), hypothalamic and pituitary D2R isoforms, pituitary Pit1 expression, and serum PRL levels were evaluated. Offspring of alcohol-exposed males showed decreased

D2S and increased D2L expression in the hypothalamus and pituitary, resulting in a lower D2S/D2L ratio, more marked in females. Resveratrol reversed these effects. Paternal ethanol consumption also increased Pit1 expression and serum PRL levels in offspring, particularly in females, effects that were partially prevented by resveratrol. Paternal alcohol intake alters D2R splicing and dopaminergic regulation of PRL in offspring, while resveratrol exerts protective effects against these alterations.

A65

TESTOSTERONE EFFECT ON OXIDATIVE STATE IN SYRIAN HAMSTER SERTOLI CELLS

de Celis Sobral A [1], *Calandra RS*[1]; *Frungieri MB* [1], *Matzkin ME* [1], *Rossi SP* [1,2]
[1]*Instituto de Biología y Medicina Experimental (IBYME-CONICET), CABA, Argentina,* [2] *Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, CABA, Argentina. E-mail: s.rossi@ibyme.org.ar*

Oxidative stress is characterized by a delicate interplay between oxidative processes and antioxidant defenses known as redox homeostasis. Leydig cell-produced testosterone participates in the control of spermatogenesis by binding to intracellular and/or plasma membrane androgen receptors (AR) in Sertoli cells thus initiating functional responses to support spermatogenesis.
Taking into account that little is known about the role of androgens in the regulation of oxidative state in the testes, in this project we proposed to investigate the participation of testosterone in the regulation of the oxidative state in Syrian hamster Sertoli cells. For this purpose, Sertoli cells were isolated from 21 day-old Syrian hamsters (ihaSCs). After a 3 h incubation, testosterone (1 µM) significantly increased lipid peroxidation (control: 0.21 ± 0.02, testosterone: 0.32 ± 0.02*; determined by Thiobarbituric Acid Reactive Substances assay [TBARS]; pmol of malondialdehyde/µg protein; n=4; *p<0.05). Moreover, testosterone (1 µM) increased the activity of the antioxidant enzyme catalase (control: 2.95 ± 0.05, testosterone: 5.39 ± 0.36*; determined by a colorimetric assay; IU/mg protein; n=5, *p<0.05). In addition, following a 5 h incubation in the presence of testosterone (1 µM), levels of protein expression of catalase and peroxiredoxin 1 (Pxr1) was also increased (catalase; control: 1.00 ± 0.10, testosterone: 2.20 ± 0.20*; Pxr1; control: 1.00 ± 0.08, testosterone: 2.13 ± 0.30*determined by Western blot; n=3, *p<0.05).
When ihaSCs were incubated in the presence of a plasma membrane impermeable-testosterone conjugate (testosterone-BSA; 1 µM- 34 nM), testosterone-BSA related changes in lipid peroxidation, catalase activity and protein expression of catalase and Pxr1 were not observed (TBARs; control: 0.21 ± 0.02, testosterone-BSA: 0.17 ± 0.03, BSA: 0.18 ± 0.01; n=4, *p<0.05. Catalase activity; control: 2.95 ± 0.05, testosterone-BSA: 3.82 ± 0.2, BSA: 3.18 ± 0.41; n=5, *p<0.05. Protein expression: catalase; control: 1.00 ± 0.10, testosterone-BSA: 0.95 ± 0.15, BSA: 0.80 ± 0.20; Pxr1; control: 1.00 ± 0.08, testosterone-BSA: 1.23 ± 0.08, BSA: 1.20 ± 0.10; n=3, *p<0.05). Our results, while preliminary, indicate that although testosterone induces oxidative damage to some extent in ihaSCs, it also contributes to redox homeostasis by promoting antioxidant actions (increased catalase activity and increased antioxidant enzymes expression). Testosterone actions in ihaSCs might not be exerted through a non-classical mechanism, that involves the presence of plasma membrane-associated AR but rather by a canonical pathway involving intracellular AR. Further experiments are required to be clarified this.