

# **Sociedad de Biología de Cuyo**



## **Abstracts of the XXXIX Annual Meeting**

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**LECTURES**

**A1  
COVID-19 INFECTION FROM DIAGNOSTIC TO TRANSLATIONAL  
RESEARCH APPLICATIONS**

*Rosato AE*

*Riverside University Health System, Riverside, CA; University of California Riverside.*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and has caused a pandemic of acute respiratory disease named ‘coronavirus disease 2019 (COVID-19)’. It has threatened human health and public safety as it is a highly transmissible and pathogenic coronavirus. Being highly transmissible, COVID-19 has spread fast all over the world. It has overwhelmingly surpassed SARS and MERS in terms of both the number of infected people and the spatial range of epidemic areas. The ongoing outbreak of COVID-19 has posed an extraordinary threat to global public health. One of the major challenges in controlling the COVID-19 outbreak has been its asymptomatic transmission that remains mysterious and misidentified. In this seminar, we will cover aspects from accurate detection of SARS-CoV-2 to rapid methods of SARS-CoV-2 identification of VOI alpha and delta. We will also discuss key differences between symptomatic and asymptomatic SAR-CoV2 gene expression and the impact of the host microbiome in severity disease outcomes.

**A2  
ROLE OF INSULIN-LIKE GROWTH FACTORS AND SALT-INDUCIBLE KINASES  
ON THE REGULATION OF GRANULOSA CELL DIFFERENTIATION  
AND FEMALE FERTILITY**

*Stocco CO*

*Department of Physiology and Biophysics - University of Illinois at Chicago. USA. E-mail: costocco@uic.edu*

Infertility is a significant public health problem affecting 15% of couples, of which approximately 40% are unable to produce or release mature eggs at ovulation. Ovulation is the pinnacle of folliculogenesis, a process that requires granulosa cell (GC) proliferation and differentiation, both needed for preovulatory follicle formation. Steady follicle growth towards the preovulatory stage and prevention of follicular atresia depends on follicle-stimulating hormone (FSH), a prevailing drug for infertility treatments. However, a significant number of patients respond poorly to FSH; therefore, the doses and total FSH needed for optimal follicle growth differ considerably between patients undergoing controlled ovarian hyperstimulation. This uncertainty is of great concern as the effects of high doses of FSH are questionable if not harmful. Seeking to solve this significant clinical problem, we looked for regulators of FSH action in the ovary. We found that FSH and insulin-like growth factors (IGFs) cooperate to promote the differentiation of GCs. Strikingly, we also demonstrated that the IGF system does not merely enhance FSH action but is required for FSH-induced differentiation of human and rodent GCs. Next, we looked for factors downstream of IGF that may interact with FSH signaling. We observed that salt-inducible kinases (SIKs) interact with FSH to regulate GC function. Thus, pharmacological inhibition of SIK activity potentiates the stimulatory effect of FSH on markers of GC differentiation and estradiol production. In humans, SIK inhibition strongly enhances FSH actions in GCs of patients with normal or abnormal ovarian function. The knockdown of SIK2, but not SIK1 or SIK3, synergizes with FSH on the induction of markers of GC differentiation. *In vivo*, SIK inhibition boost gonadotropin-induced GC differentiation, while the genomic knockout of SIK2 leads to a significant increase in the number of ovulated oocytes. Conversely, SIK3 knockout female mice are infertile, FSH insensitive, and have abnormal folliculogenesis. These findings establish SIKs as critical regulators of ovarian function and show that SIK2 and SIK3 have opposite effects on female fertility. Our findings reveal novel roles for IGFs and SIKs in regulating folliculogenesis and ovulation in rodents and controlling GC differentiation in humans. Since SIK activity can be modulated pharmacologically, a better understanding of SIK-controlled mechanisms and pathways may facilitate the development of novel therapies to advance fertility, allowing safer and more effective ovulation induction in assisted reproductive technologies. SIK regulated mechanisms could also reveal new targets for the development of innovative contraception methods.

SYMPOSIUM

A3

**WHAT ARE THE SEASONALLY DRY TROPICAL FORESTS (SDTF)? FLORISTICS AND BIOGEOGRAPHY OF A BIOME WITH ITS OWN IDENTITY**

*Mogni VY<sup>1</sup>, Oakley LJ<sup>1</sup>, Mauro HM<sup>1</sup>, Galetti, LA<sup>1</sup>, Prado DE<sup>1,2</sup>*

*<sup>1</sup>Cátedra de Botánica, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Campo Experimental J. F. Villarino, Zavalla, Santa Fe, Argentina. <sup>2</sup>IICAR (Instituto de Investigaciones en Ciencias Agrarias de Rosario), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). E-mail: virginia.mogni@unr.edu.ar*

In Latin America and the Caribbean, the seasonally dry forests are a biome with a fragmented distribution across the territory. In South America these forests constitute a biogeographic domain named “Seasonally Dry Tropical Forests” (SDTF) that cross the continent in an “arc-shape” that is defined by a highly coincident distribution pattern between different woody taxa, with *Anadenanthera colubrina* “cebil, curupay” (Caesalpinioideae, Fabaceae) as the most paradigmatic. The SDTF domain comprises four nuclei with numerous species in common, each nucleus well defined based on their particular flora: Caatingas (NE Brazil), Misiones (sector of the old Jesuit missions in Brazil, Paraguay and Argentina), Chiquitano (SE Bolivia and NW Paraguay), and Piedmont (CS Bolivia and NW de Argentina); there are also remnant areas in the inter-Andean dry valleys of Bolivia and south-central Peru and several intrusions in the “Gran Chaco” and the Cerrado. The SDTFs comprise mostly deciduous vegetation associated with a strong climatic seasonality, with a well-defined dry season but of highly variable length, occurring on fertile soils with slightly acidic to alkaline pH. In these woody environments the Fabaceae dominate, accompanied by Bignoniaceae and Anacardiaceae, and the understory with abundant Cactaceae and Bromeliaceae. These forests have a notorious and unique floristic richness in the continent, and high levels of species endemism, which has been demonstrated in international studies both by analysis of the distribution patterns of their most important woody species and by comparing their vegetation. This new interpretation of the SDTF has had biogeographic impact (Pleistocene Arc Theory), evolutionary impact (at the molecular level, together with some paleoclimatic modelling studies), and influenced other sciences (e.g., Zoology). Nowadays, the biodiversity of the SDTF is seriously threatened by human activities and the advancement of the agricultural frontier. In sum, all this emphasizes the need for taxonomic, phylogenetic, and demographic studies that will contribute to the characterization and reconstruction of the evolutionary history of this ecosystem for the conservation of its genetic resources.

A4

**SPATIO-TEMPORAL DYNAMIC OF CURUPAY’S POPULATIONS (*Anadenanthera colubrina* var. *cebil*, LEGUMINOSAE) AS WITNESS OF THE PAST OF THE SDTFs**

*Barrandeguy ME<sup>1,2</sup>, Zerda H<sup>3</sup>, Savino C<sup>3</sup>, Dusset F<sup>1</sup>, Mogni V<sup>4</sup>, Prado DE<sup>4</sup>, García MV<sup>1,2</sup>*

*<sup>1</sup>Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Cátedra de Genética de Poblaciones y Cuantitativa. Departamento de Genética. Posadas Misiones, Argentina. <sup>2</sup>Instituto de Biología Subtropical (UNaM – CONICET). Consejo Nacional de Investigaciones Científicas y Técnicas. Misiones. Argentina. <sup>3</sup>Facultad de Ciencias Forestales. Universidad Nacional de Santiago del Estero. Santiago del Estero. Argentina. <sup>4</sup>Cátedra de Botánica, Facultad de Ciencias Agrarias, UNR e IICAR-CONICET. Campo Villarino. Zavalla. Santa Fe. Argentina. E-mail: ebarran@fceqyn.unam.edu.ar*

The current distribution of Seasonally Dry Tropical Forests (SDTFs) in South America is disjunct and there are different hypotheses about the evolution of these forests’ distribution. The paleo-scenario hypotheses raise questions about the long-term stability and dynamics of the SDTFs vegetation communities and the role of the Quaternary climate changes in their current patterns of genetic diversity and geographic distribution. In the current work, the spatiotemporal dynamics of the SDTFs were analyzed using different information regarding *Anadenanthera colubrina* var. *cebil*, the most paradigmatic species of these forests. Data of the current presence of the species, chloroplast DNA sequences, and the past presence of the species were considered. From the combination of different methods of data analysis, such as modeling analyses of species distribution to know the possible distribution of the species in the past considering the current and historical climatic conditions, the quantity and geographical distribution of chloroplast genetic variability considering the phylogenetic relationships between haplotypes and the information from the fossil record was showed on paleo-distribution maps. Also, a dated phylogenetic tree was performed using model-based methods considering accessions of four Caesalpinioideae species as an external group and, the tree was calibrated adding external information from the fossil record. The results show a fragmented distribution of the species from times before the Last Glacial Maximum, while the information from the fossil records shows the historical presence of the species in Argentina since the early Miocene. Phylogenetic relationships between the seven identified haplotypes depend on the geographical location of the individuals that carry them, and the most frequent haplotype of Misiones nucleus being in the central position. The dated tree grouped haplotypes of *A. colubrina* according to their SDTF nucleus of origin, while their divergence times within and between nuclei dated to Neogene. The divergence between the haplotypes of the genus

*Anadenanthera* Speg. as well as the divergence within the subfamily Caesalpinioideae date from the Paleogene. The estimated times support the hypothesis of rapid diversification and abundance of the Leguminosae in the Eocene, after the Cretaceous–Paleogene mass extinction event from which the origin of the family dates.

## A5

### WHAT CAN A REMNANT POPULATION OF *Anadenanthera colubrina* var. *cebil* (LEGUMINOSAE) TELL US ABOUT DISPERSAL DISTANCES?

Goncalves AL<sup>1,2</sup>, García MV<sup>1,2</sup>

<sup>1</sup>Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Departamento de Genética. Posadas Misiones, Argentina. <sup>2</sup>Instituto de Biología Subtropical (UNaM – CONICET). Consejo Nacional de Investigaciones Científicas y Técnicas. Misiones. Argentina. E-mail: alejandragoncalves@fceqyn.unam.edu.ar

Natural populations of forest tree species from fragmented biomes such as Seasonally Dry Tropical Forests (SDTF) show spatial patterns of genetic variation, which result of demographic and microevolutionary dynamics in the space–time context. Under isolation by distance, kinship levels between individuals are inversely correlated with the spatial distance separating them. Fine-scale spatial genetic structure (FSGS) is defined by the non-random spatial distribution of genotypes, which is generally associated with local family structures as a consequence of restricted gene dispersal. Fragmented landscapes from southern Misiones provide a framework that allows to develop FSGS studies of the native forest tree species *Anadenanthera colubrina* var. *cebil* (Leguminosae). The main aim of this work was to evaluate the spatial genetic structure in a remnant population of *A. colubrina* var. *cebil* from southern Misiones. As a starting point for spatial inference, a total of 119 individuals of two life stages (adults and saplings) were genotyped using eight specific nuclear microsatellite loci for characterizing the genetic diversity of that remnant population. Differences of genetic clusters and FSGS between stages were evaluated. High levels of genetic diversity and high inbreeding were detected. Genetic structure was stronger in saplings than in adult trees, probably due to assortative mating and density dependent mortality effects. Inbreeding levels and FSGS patterns suggest that the species shows a mixed mating system. Family structures defined by full-sib or half-sib saplings spatially clumped could be a consequence of restricted gene dispersal, limited mobility of propagules and spatial recruitment of seedlings. Gene dispersal of plants combine the effects of spatial scales and heterogeneity, temporal scales, and system complexity. Restricted seed dispersal distances, high tree densities and mixed mating system patterns directly influence FSGS levels. Therefore, the knowledge of gene dispersal distances of keystone forest tree species from SDTF is relevant to design sampling strategies in conservation and restoration projects.

## A6

### LOCAL CONTRIBUTIONS IN THE FORMULATION OF VACCINES AGAINST COVID-19

Sanchez MV

Laboratorio de Inmunología y Desarrollo de Vacunas. Instituto de Medicina y Biología Experimental de Cuyo, Centro Científico Tecnológico, CONICET, UNCuyo, Mendoza. E-mail: vicky\_sanchez\_@hotmail.com

The COVID-19 disease caused by the new SARS-CoV-2 virus, have proposed a great challenge for the entire global scientific community in order to respond to different needs. The production of knowledge and information exchange have occurred at a level of magnitude never seen before. At the local level, the same challenges were assumed, and several networks were generated between researchers to work collaboratively. In this context, new ties were generated, where each of the parties contributed, from their expertise, to generate new solutions or begin investigations that would respond to the “unknowns” given by this new disease. The Laboratory of Immunology and Vaccine Development of IMBECU participated in different networks with researchers in the country and thus began with vaccine formulation studies to develop a vaccine against COVID-19. The objective of this still current research is to provide knowledge on the immune responses induced by different formulations with the combination of adjuvants that help to induce robust immune responses and to elucidate the immune mechanisms necessary to generate more efficient immune responses to protect against SARS-CoV-2.

**A7**

**EVALUATION OF DIFFERENT TOTAL *Leishmania amazonensis* ANTIGENS FOR THE DEVELOPMENT OF A FIRST-GENERATION VACCINE FORMULATED WITH A TOLL-LIKE RECEPTOR-3 AGONIST TO PREVENT CUTANEOUS LEISHMANIASIS**

*Cargnelutti DE*

*Universidad Nacional de Cuyo, Facultad de Ciencias Médicas. Instituto de Medicina y Biología Experimental de Cuyo (IMBECU) CCT-Mendoza, CONICET, Mendoza, Argentina. E-mail: diegocargnelutti@hotmail.com*

Unfortunately, no vaccine against leishmaniasis has been developed for human use. Therefore, a vaccine based on total *Leishmania* antigens could be a good and economic approach, and there are different methodologies to obtain these antigens. However, it is unknown whether the method to obtain the antigens affects the integrity and immune response caused by them. Our objective was to compare the protein profile and immune response generated by total *L. amazonensis* antigens (TLA) produced by different methods, as well as to analyze the immune response and protection by a first-generation vaccine formulated with sonicated TLA (sTLA) and polyinosinic:polycytidylic acid [Poly (I:C)]. TLA were obtained by four different methodologies and their integrity and immune response were evaluated. Finally, sTLA was formulated with Poly (I:C) and their protective immune response was measured. sTLA presented a conserved protein profile and induced a strong immune response. In addition, Poly (I:C) improved the immune response generated by sTLA. Finally, sTLA + Poly (I:C) formulation provided partial protection against *L. amazonensis* infection. The protein profile and immune response depend on the methodology used to obtain the antigens. Also, the formulation sTLA + Poly (I:C) provides partial protection against cutaneous leishmaniasis in mice.

**A8**

**MANIPULATION OF VIRAL GENOMES BY BACTERIAL ARTIFICIAL CHROMOSOME (BAC) TECHNOLOGY: AN APPROACH FOR THE CONSTRUCTION OF RECOMBINANT VIRUSES AS POTENTIAL VACCINE CANDIDATES**

*Damiani AM*

*Biochemistry and Immunity Laboratory, School of Medicine, National University of Cuyo, IMBECU-CONICET, Mendoza, Argentina. E-mail: amdamiani9@gmail.com*

Bacterial artificial chromosomes (BACs) are recombinant DNA molecules developed for propagation of large and unstable foreign DNA fragment in *Escherichia coli*. The BAC technology has been adapted to the manipulation of viral genomes and became an effective alternative to traditional genetic engineering. The generated BACs can be then stably maintained in bacteria, in which genetic mutations of viral genomes (such as point mutations, deletions and insertions) can be easily introduced by different mutagenesis methods including a phage lambda-derived Red recombination system. The presentation describes the key elements in the development of the BAC system applied to herpesviruses, adenoviruses, and coronaviruses.

**A9**

**HETEROLOGOUS PRIME-BOOST VACCINATION BASED ON POLYMORPHIC PROTEIN D PROTECTS AGAINST INTRAVAGINAL *Chlamydia trachomatis* INFECTION IN MICE**

*Russi R<sup>1,2</sup>, Del Balzo D<sup>1</sup>, Veaute C<sup>2</sup>, Damiani MT<sup>1</sup>*

*<sup>1</sup>Biochemistry and Immunity Laboratory, School of Medicine, University of Cuyo, IMBECU-CONICET. <sup>2</sup>Experimental Immunology Laboratory, School of Biochemistry and Biological Sciences, National University of Litoral. E-mail: tdamiani@fcm.uncu.edu.ar*

The worldwide spread of sexually transmitted *Chlamydia trachomatis* (Ct) infection urgently demands the development of a preventive vaccine, owing to that there is no available one. Therefore, in this work, we designed a vaccine based on a fragment of polymorphic protein D (FPmpD), an autotransporter-like immunogenic surface-exposed protein with adhesin properties. FPmpD comprises amino acids from 693 to 1240 and displays three regions with B- and T-cell epitopes identified by *in silico* prediction. FPmpD immunogenicity was assessed in two strains of mice, BALB/c and C57BL/6, using a prime-boost strategy with DNA (FPmpD-pVAX1) followed by two doses of the recombinant protein (rFPmpD) with simultaneous systemic and mucosal administration routes. FPmpD proved to be immunogenic enough to generate a robust systemic and mucosal IgG humoral immune response that persisted over time in BALB/c and C57BL/6 mice. The sera from vaccinated mice strongly reacted against Ct infective bacteria by WB and rFPmpD by ELISA. FPmpD vaccine triggers anti-PmpD IgA synthesis at the genital tissues of some mice; however, the overall IgA in cervicovaginal washes did not significantly differ from non-immunized control mice. Besides, vaccination did not affect

mouse fertility indicated by unaltered fertility potential, pre-implantation, and post-implantation indexes. Next, we tested the vaccine in a mouse model of Ct intravaginal infection that resembles chlamydial genital infection in women. Anti-PmpD antibodies elicited by mice vaccination displayed potent neutralizing activity *in vitro*. Vaccination exerted protective effects in uterine tissues *in vivo*, resulting in decreased pathology (inflammation, edema, hyperemia, tubal stenosis, hydrosalpinx) of mice genital tracts after the Ct challenge. Notably, FPmpD-based vaccine effectively reduced Ct shedding into cervicovaginal fluids, bacterial burden at the genitourinary tract, and overall infectivity. A fundamental condition that a vaccine should accomplish is to generate memory lymphocytes capable of driving robust and fast immune responses when exposed to the pathogen. We showed that the FPmpD-based vaccine triggered the rapid production of high levels of specific anti-PmpD antibodies after the Ct challenge. Hence, the FPmpD-based vaccine might constitute an efficient tool to protect against Ct intravaginal infection and decrease the spreading of sexually transmitted Ct.

## LECTURE

### A10

#### SOME CHALLENGES OF TEACHING BIOLOGY IN THESE TIMES

*Adúriz-Bravo A*

*CONICET/Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, CeFIEC-Instituto de Investigaciones en Enseñanza de las Ciencias, Buenos Aires, Argentina. E-mail: aadurizbravo@cefiec.fcen.uba.ar*

Since the beginning of the 21st century, science teaching is increasingly being required to respond to new socially established, humanistic aims for general education, which are related to fostering the exercise of citizenship in democracy. These new, ambitious educational aims collectively set worldwide and, in our region, mean that science –and life, health and environment sciences in particular– need to be taught within recognizable, socially relevant real-life contexts where students will be able to understand phenomena, solve problems, make decisions, take courses of action, produce and understand discourse, engage in debate, and critically evaluate scientific processes and products. Researchers and innovators in the field of didactics of science (i.e., science education as an academic discipline) have been directing their efforts towards characterizing this kind of science teaching based on competences, which envisages young people who are empowered and autonomous in using science in their lives. In the meantime, the pandemic of COVID-19 has shown that such efforts are not only essential, but also urgent: even a minimal degree of scientific literacy has been reported to be alarmingly scarce in developed countries, and this has proven to be a grave obstacle for citizens to face the socio-sanitary crisis with some degree of efficiency and avoiding pseudo-science and denialism. This complex scenario thus results in a series of challenges for renewed biology teaching, which traverse compulsory and post-compulsory education and take new meaning at the University level. What should the main objectives be for teaching biology to citizens, to professionals, to scientists, to decision-makers? What content around life, health and environment best suits such objectives? And what would then be the most adequate strategies for a meaningful understanding of nature that enables responsible action? The theoretical notion of “school scientific activity”, which is here introduced and discussed, provides foundations for a learning of biology in context that supports the use of scientific models to make sense of the natural world when acting on it according to socially shared aims and values. Models, context, and inquiry conform a carefully designed teaching system where students answer questions, solve problems, and engage in projects where biology is seen as indispensable knowledge.

## SYMPOSIUM 3

### A11

#### PANDEMIC: FROM SCIENCE AND COMMON SENSE TO SCIENTISM

*Inserra F*

*Maimonides University, Buenos Aires, Argentina; advisor to the Academic Vice-Chancellor; Austral University, Buenos Aires, Argentina; director of Master Degree on Vascular Mechanics and Arterial Hypertension. E-mail: felipe.inserra@gmail.com*

Science could be defined as the art of breaking dogmas reconstructing transitory truths using the scientific method. However, scientists perfectly know that science is not dogmatic, and additionally its methodology is not infallible and that the truths of today will change for new ones tomorrow. Science coexists with common sense while new truths arrive. Common sense is a series of concepts and behaviors that have proven to be very useful for humanity. Nevertheless, some scientists think of the scientific method dogmatically, considering that it is the only method applicable in all aspects and

moments of human life and despises all other knowledge. The use of science and scientific methods in this way was an obstacle in times of pandemic because it delayed essential decisions, waiting for all the evidence to appear. The results could be catastrophic for humanity. There were many examples of the lack of common sense during the last COVID-19 pandemic. Many scientists and decision-makers in health seem to have not reacted according to the circumstances. They were elusive with the decisions in the middle of hundreds of thousands of people dying worldwide. Conclusion: during a pandemic, in the name of science, many decisions were made without the use of the common sense.

## A12 SCIENCE AND SCIENTISM

Ferder L

*Department of Pediatrics, Nephrology Division, Miller School of Medicine, University of Miami, Florida, USA; University of Maimónides, Buenos Aires, Argentina. E-mail: leoncho@hotmail.com*

The primary objective of science is to discover the laws that govern the phenomena of reality, understand, and explain them. The key to science is scientific thinking. “This is science” has been accepted as the seal of an unquestionable divine word. To do science we must work as a team, without selfishness, with adaptation to the times of a pandemic, without falling into dogmas, with serious information, to fundamentally reduce morbidity and mortality. Science lacks ideologies and requires common sense. “*The reality is not what is seen, but what should have been thought*” (Gaston Bachelard).

## A13 THE ECONOMIC SCIENCE DURING THE COVID 19 PANDEMIC – MAIN CHALLENGES AND OPPORTUNITIES

Elosegui P<sup>1</sup>

*<sup>1</sup>Banco Central de la República Argentina; University of Illinois at Urbana – Champaign, USA; Universidad Nacional de La Plata, Universidad Maimónides, Argentina. E-mail: elo@illinois.edu*

The COVID-19 pandemic has had a strong impact on the economies of both developed and developing countries. In this context, economics as a science and in its practical application faced strong challenges. Active policies, sometimes overlooked in academic research, became crucial to face an unprecedented economic and social shock. The economic science was challenged, and opportunities were created to bridge the gap between applied and theoretical research. The results of the policies implemented and their relative success in the various countries should be analyzed with caution. There exists an urgent need to reduce the gaps between poor, developing, and developed countries. Preliminary results show divergent paths that should be tackled by the economic science and the economists in the coming years. Actual problems should be matched with updated research and applied agenda.

## SYMPOSIUM 4

## A14 MICROBIOLOGICAL ASPECTS OF COVID-19

Torres C

*Dpto. de Microbiología. Facultad de Ciencias Exactas Físico Químicas y Naturales. Universidad Nacional de Río Cuarto. E-mail: ctorres@exa.unrc.edu.ar*

The emerging coronavirus SARS CoV-2 generates a respiratory condition COVID-19, a severe acute respiratory syndrome that in 2020 became one of the most serious global public health problems. This viral pathogen was first identified in an outbreak in Wuhan, China. At the end of December 2019, the Wuhan Municipal Health Committee, Hubei Province (China) reported cases of pneumonia in market workers to the World Health Organization (ISID, 2020; SAV, 2020). In the face of previous knowledge of events that occurred decades ago, studies were carried out in order to know if there was a relationship between the aforementioned viral agent with Influenza, SARS CoV-1, and MERS, and it was shown that it was a new coronavirus now called SARS CoV-2 (Hui, 2020; Zhou, 2020). Microbiological knowledge of SARS CoV-2 developed rapidly based on the antecedents produced by SARS CoV-1 decades ago in the same region. This pandemic situation caused the entire international scientific community to study the microbiological aspects of this viral infection in order to characterize the viral particle, sequence the viral genome, establish phylogenetic relationships, classify it taxonomically, design and produce diagnostic techniques, accurate for its determination in clinical samples. In addition, progress was made in the development of different vaccine formulations for their prevention and in determining the antiviral potential of chemotherapeutic agents applied in other viral infections. After these two pandemic years, it is evident that the virus in its new variants originated in different countries, has acquired the ability to transmit more quickly

and displace the previous variants. The rapid recognition of the viral particle structure, together with the history of SARS CoV-1, made it possible to achieve protective vaccines with different formulations that, together with other sanitary measures, allow the impact of the pandemic to be controlled. However, the knowledge of the microbiological aspects continues to question us about the intervention exerted on wildlife and the exposure to various viruses by contact with their reservoirs.

## A15

### REGENERATIVE MEDICINE USING TISSUE ENGINEERING STRATEGIES

Feldman S

LABOATEM (Laboratory of Osteoarticular Biology, Tissue Engineering and Emergent Therapies), School of Medicine, Rosario National University- CONICET/CIUNR. Santa Fe 3100. Rosario. E-mail: saryfeldmangmail.com

Tissue engineering is an interdisciplinary area that encompasses knowledge of materials engineering and biomedical sciences, as well as physics, chemistry, biology, and seeks to reconstitute, replace and/or regenerate specific tissues or organs through the implementation of effective and practical materials, which aim to maintain the existing residual structure, as well as to make tissue growth viable, acting as scaffolds that promote the proliferation of living tissues. At the same time, it is known that changes in the lifestyles of the population in general, as well as the shift of the population pyramids towards older ages, are related to increases in fracture rates, producing high impact socio-health problems. Orthopedic and odontostomatologic surgery, neurosurgery, maxillofacial surgery, and other medical practices require on numerous occasions the application of implant techniques or bone tissue repair or both. All this was the basis for our decision to dedicate ourselves to research in bone regenerative medicine by expanding tissue engineering strategies. Our work first focused on working with bone chips from cadaveric donors, but then we fully dedicated ourselves to working with scaffolds obtained in laboratories, either by chemical synthesis or by molecular biology strategies. We have developed collaborative work with several national and international laboratories, which has allowed us to participate in certain developments and synthesis of the same, verify their capabilities in *in vitro* studies, and subsequently observe the processes *in vivo*, developing for them experimental models of bone injury. We have had encouraging results working with hybrid scaffolds of bioactive glass with polyvinyl alcohol, composite matrices polymerized with chitosan. Subsequently, we started to work with a hydrogel scaffold obtained as elastin recombinamers, proving excellent biocompatibility, osteoinductive capacity, and bone neof ormation. We are currently deepening in the implementation of other elastin derivatives obtained by molecular biology, as well as obtained by chemical synthesis, polycapronlactone derivatives, as well as poly-lactide derivatives. In particular, our *in vivo* studies allow us to verify whether the scaffolds under study possess not only biocompatibility, but also whether they increase cell proliferation, do not generate undesirable inflammatory processes, and promote *de novo* bone tissue regeneration. Bone regenerative medicine applying tissue engineering strategies offers answers that in the near future will undoubtedly improve the response to medical practice.

## A16

### THE OTHER SIDE OF COVID-19 PANDEMIC: EFFECTS ON FEMALE FERTILITY

Herrero Y

Laboratorio de estudios de la Fisiopatología del Ovario. Instituto de Biología y Medicina Experimental (IByME) – CONICET, Buenos Aires, Argentina. E-mail: yamila.herrero@gmail.com

SARS-CoV-2 invades the target cell by binding to angiotensin converting enzyme 2 (ACE-2). In the human ovary, ACE-2 is expressed in stromal and granulosa cells. Our objective was to evaluate the effect of SARS-CoV-2 infection on female gonad. FF (follicular fluid) from patients undergoing ART (N = 80; 21–41 years old; November 2020–April 2021) were divided in two groups: FF from control patients and FF from recovered COVID-19 patients (asymptomatic and with mild symptoms). The levels of IgG antibodies against SARS-CoV-2, IL-1 $\beta$ , IL-10, and VEGF were measured in FF by ELISA. Using a granulosa cell line (COV434) and an endothelial cell line (EA.hy926), we studied the effect of FF from control and recovered COVID-19 patients. The expression of StAR, ER $\alpha$  and ER $\beta$ , 3 $\beta$ -HSD, VEGF, ANGPTs (angiogenesis-related proteins), and  $\gamma$ H2AX (DNA damage marker) was evaluated by WB. Proliferation was evaluated by a WST-1 assay. Endothelial cell migration was evaluated by a wound healing assay. We performed Student's *t*-test or one-way ANOVA. The results showed that 91.3% of post-COVID-19 FF was positive for IgG against SARS-CoV-2. Patients with higher levels of SARS-CoV-2 IgG showed a decrease in the number of retrieved oocytes ( $P < 0.05$ ). The levels of VEGF and IL-1 $\beta$  were lower ( $P < 0.05$ ) in post-COVID-19 FF, while IL-10 did not differ. In COV434 cells with post-COVID-19 FF, the expression of StAR, Er $\beta$ , and VEGF was decreased ( $P < 0.05$ ), while ER $\alpha$  and 3 $\beta$ -HSD did not change. In EA.hy926 cells with post-COVID-19 FF, a decrease in cell migration was observed ( $P < 0.0001$ ) without changes in the expression of ANGPTs. Both cell types showed higher expression of  $\gamma$ H2AX with post-COVID-19 FF ( $P < 0.05$ ). No differences were found in COV434 and EA.hy926 cell proliferation rates between the groups. In conclusion, these results describe that SARS-CoV-2 infection alters the follicular microenvironment, damaging ovarian function, and affecting reproductive performance in recovered COVID-19 patients.

**SYMPOSIUM 5**

**A17**

**EXPOSITION TO PESTICIDES IN PREGNANT WOMEN LIVING AT THE HIGH VALLEY OF RÍO NEGRO AND NEUQUÉN PROVINCES, THE PLACENTA AS A BIOMONITORING TOOL**

Vera B<sup>1</sup>, Ondarza P<sup>2</sup>, Guiñazú N<sup>3</sup>

<sup>1</sup>Facultad de Medicina, Universidad Nacional del Comahue, LIBIQUIMA, CITAAC-CONICET. E-mail: vera.bertha02@gmail.com. <sup>2</sup>Laboratorio de Ecotoxicología y Contaminación Ambiental, IIMyC, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata-CONICET. E-mail: pmondar@mdp.edu.ar. <sup>3</sup>Departamento de Ciencias del Ambiente, Facultad de Ciencias del Ambiente y la Salud, Universidad Nacional del Comahue. LIBIQUIMA, CITAAC-CONICET. E-mail: natanien@hotmail.com

The worldwide use of pesticides has greatly increased in the last 20 years. Pesticides are substances designed to kill, repel, or control plants, insects which impact negatively on agricultural productivity. The spread use of pesticides has favored their detection in environmental matrices as air, water, and soil, as well as their accumulation in non-target organisms, as human beings. In rural settings, the proximity to areas of intensive pesticide application is a risk factor favoring xenobiotic exposure. It is now recognized that first xenobiotic exposure begins *in utero*. The High Valley of Río Negro and Neuquén is an important agricultural area which intensively produce apples and pears, among other fruits. Pest control is performed with integrated pest management, the insecticides mostly used are organophosphates –OP- (chlorpyrifos), carbamates (carbofuran and pirimicarb), and neonicotinoids –NEO- (thiacloprid and acetamiprid). Pregnant women residing in rural locations (Plottier, Centenario, General Roca, Cipolletti, Cinco Saltos) and in Neuquén city, were included in different study groups from 2008 to the present. Participants were classified as they lived in a rural or urban setting. Matrices analyzed were maternal blood, placenta, and umbilical cord blood. Studies were first focused on OP exposure and changes in the classical pesticide exposure biomarkers as acetylcholinesterase, butyrylcholinesterase and carboxylesterases activities were determined. Also, the non-classical biomarkers of oxidative stress (glutathione content, antioxidant enzyme activity, genotoxic damage), and hormone levels were also observed in both groups. We demonstrated that the three matrices studied are impacted in the population residing in these rural locations. We confirmed women exposure to current (chlorpyrifos) and historical use insecticides (DDT, lindane, endosulfan). The placenta systems such as the cholinergic, mitochondria bioenergetics, steroidogenic and antioxidant functions were recognized to be important targets of pesticide toxicity in the current environmental exposure scenario. Toxic effects of the different insecticides families OP and NEO were also confirmed in the human trophoblast cell lines of third (JEG-3) and first trimester (HTR-8/SVneo), at representative levels of human environmental exposure.

**A18**

**USE OF FUNGI FOR THE RECOVERY OF CONTAMINATED SOILS**

Medaura MC

Facultad de Ingeniería, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Centro universitario, 5500 Ciudad de Mendoza, Argentina.

Mycoremediation is the use of fungi to degrade or remove toxins from the environment. In contaminated soils, its practice involves the sowing of mycelia, its introduction on a support material of vegetal origin, the selective stimulation of autochthonous species or a combination of these strategies. This oral presentation exhibits results of classic biostimulation, biostimulation and fungal bioaugmentation tests to evaluate the potential of fungal degradation on a soil contaminated with a complex matrix of petroleum hydrocarbons, rich in heavy saturated fractions and aromatics of high molecular weight (HMW-PAHs), previously treated by a classic bioremediation technique. From the same soil, 19 fungal strains were isolated and identified, most of them belonging to the Ascomycota division, which were used as fungal inoculum for bioaugmentation. Chemical (GC-MS and GC-FID), microbiological (profiling of ribosomal genes using DGGE), ecotoxicological (Microtox), and enzymatic tests were carried out to identify laccase and Mn-peroxidase activity. Two of the isolated strains showed polyphenoloxidase activity, capable of oxidizing aromatic compounds. Using fungal bioaugmentation, a degradation of total petroleum hydrocarbons of 68.90% and of PAHs 86.03% to 28.27% was achieved in the compounds of 3 to 6 rings respectively, causing a decrease in the toxicity of the soil leachates (Microtox) and a change in culture-determined eubacterial populations. The differences with respect to the classical biostimulation tests demonstrate the potential advantages that mycoremediation offers for the bioremediation of soils with heavy hydrocarbons.

LECTURE

A19

**STORIES OF BINOMIALS AND MICROEVOLUTIONARY PROCESSES: FROM GENOMES TO POPULATIONS AND ITS ENVIRONMENTS**

*García MV*

*Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Departamento de Genética. Posadas Misiones, Argentina. Instituto de Biología Subtropical (UNaM – CONICET). Consejo Nacional de Investigaciones Científicas y Técnicas. Misiones. Argentina. E-mail: vgarcia@fceqyn.unam.edu.ar*

The study of causes and consequences of microevolutionary processes action gives support to population genetics (PG). The mere mention of this biological discipline refers to thinking about the Hardy–Weinberg equilibrium and the simplified expression of  $p + q = 1$  reducing this vast discipline to an algebraic expression that only extrapolates Mendel’s 1st law to a population level. Since 1908 when this equilibrium was formulated, this challenging discipline has continuously been revitalized both in its theories and methodologies of data analysis, allowing it to be defined as the discipline that studies the origin, quantity, and distribution of genetic variability in populations and the fate of this variation through time and space. Thus, this definition includes both microevolutionary processes and historical-demographic events as well as both phenotypic and genomic variability. In this way, the crucial role of PG in the field of evolutionary biology is evident owing to it studies the mechanisms by which evolution happens. After the neutral theory of molecular evolution, PG got the null hypothesis that allows test the action of natural selection to genome level using neutrality tests and, also, historical-demographic events can be dated for allowing PG studies past, present, and future of genetic variability of populations. In this way, the joint analysis of contemporary phenotypic and genetic diversity, it is possible to make management decisions and design plans for the conservation of genetic resources. From studies in natural populations of *Anadenanthera colubrina* var. *cebil*, a native forest tree from South America that shows disjunct distribution, a departure for neutrality was detected for microsatellite locus *Ac41.1* in the Argentinean populations suggesting the action of diversifying selection but a specific linkage of this locus to some phenotypic trait could not be detected. Also, the phenotypic diversity analysis allowed to group the individuals regarding its geographical origin being explained by Minimum Temperature of Coldest Month while the distribution of alleles of locus *Ac41.1* showed a geographical pattern related to temperature and precipitation seasonality. These studies provide evidence about the role that climatic variables play in shaping the intraspecific distribution of phenotypic and genetic variability, being able to complement these studies with ecological studies of niche modeling to generate predictions about the possible response capacity of populations in the framework of current pressing global climate change.

CLOSING LECTURE

A20

**CHAGAS DISEASE IN MENDOZA: A CONSERVATION MEDICINE APPROACH**

*Superina M, Morales M*

*Laboratorio de Medicina y Endocrinología de la Fauna Silvestre, IMBECU – CCT CONICET Mendoza – UNCuyo, Mendoza. E-mail: msuperina@mendoza-conicet.gov.ar*

Chagas disease is one of the most important endemics of the American continent and constitutes a public health problem in several Argentine provinces. It is caused by the flagellate protozoan *Trypanosoma cruzi*, whose transmission depends on hematophagous triatomine insects that mainly feed on mammals, both wild and domestic. In order to effectively control its transmission, it is necessary to know both the domestic and sylvatic transmission cycle of the parasite. However, in Argentina, there is little knowledge of its sylvatic cycle, as well as of the overlap between both. Conservation medicine allows us to study Chagas disease in a more comprehensive way, since this discipline seeks to understand the interactions between human health, animal health, and ecosystem health. In this context, we are investigating how different levels of environmental disturbance might influence *T. cruzi* transmission cycles. We focus on the peri-urban area of Mendoza, because although historically Chagas disease is considered an important health problem in rural areas of this province, in recent years it has been shown that this infection is also a health problem in urban and peri-urban areas, where the chances of overlapping transmission cycles are higher. Urbanization causes changes in the ecological balance, reducing the diversity of native species. At the same time, it opens new niches for some wild species with a great capacity to adapt to disturbed environments, such as introduced rodents or opossums. These species are attracted to the peri-urban environment due to the increased availability of food and shelter and could act as a “nexus” between both transmission cycles. Through serological and molecular analyses, we are determining the prevalence and parasite load of *T. cruzi* in dogs, opossums, and wild rodents in areas with different degrees of disturbance. We also perform taxonomic identification of triatomine species and molecular analysis to determine if they are infected with *T. cruzi*. In combination

with the determination of *T. cruzi* subtypes (DTU) and the analysis of blood meal sources of the triatomines found in the different environments, this information will allow us to evaluate whether the domestic and sylvatic transmission cycles overlap. Ultimately, we hope this study will help improve disease control strategies.

## GENERAL, CELLULAR AND MOLECULAR BIOLOGY

### A21

#### EFFECT OF YERBA MATE ON *IN VIVO* AND *IN VITRO* MODELS OF PROSTATE CANCER

*Santiano F<sup>1</sup>, Rey L<sup>1</sup>, Zyla L<sup>1</sup>, Gómez S<sup>1</sup>, Bruna F<sup>1</sup>, Campo Verde Arboccó F<sup>1</sup>, Sasso C<sup>1</sup>, Pistone Creydt V<sup>1</sup>, Pérez Elizalde R<sup>1</sup>, López Fontana C<sup>1</sup>, Carón R<sup>1</sup>*

*<sup>1</sup>Laboratory of Hormones and Cancer Biology, Institute of Medicine and Experimental Biology of Cuyo (IMBECU), National University of Cuyo (UNCuyo), National Council for Scientific and Technical Research (CONICET), Mendoza, Argentina; Postal Address: Av. Adrián Ruiz-Leal s/n, CC855, Mendoza, Argentina.  
E-mail: fsantiano@mendoza-conicet.gob.ar*

The consumption of mate (YM) has been associated with numerous health benefits due to its antioxidant, vasodilator, anti-inflammatory, antimutagenic, and lipid-lowering properties. These protective effects can be attributed to the activity of the bioactive compounds in YM: chlorogenic acid, caffeine, rutin, and quercetin. Since YM infusion is the one most consumed in Argentina, it is necessary to expand the study of its effects on health and the development of human diseases. Prostate cancer (CaP) is the most frequent carcinoma in men and has a latency period of approximately 25 years, which provides a time long enough to carry out prevention. Therefore, the objective of this work was to expand the study of the anticancer potential of YM characterizing the influence and molecular mechanisms of YM on the development of CaP. On one hand, we established an *in vitro* model to study the direct effects of YM on the proliferation, viability, and migration of three human CaP cell lines with different aggressiveness and hormonal dependence: LNCaP, PC3, and DU145. On the other hand, we developed an *in vivo* model of CaP, through subcutaneous inoculation of TRAMP-C1 cells in C57BL/6 mice, to evaluate the influence of YM consumption on tumor development. Subsequently, the animals were divided into two study groups: YM (50 mg/mL of mate in drinking water, N = 12), and controls (only drinking water, N = 12). Our *in vitro* results showed that YM decreased the viability and proliferation of the three tumor cell lines studied ( $P < 0.001$ ), and also decreases the migration of LNCaP ( $P < 0.05$ ) and DU145 ( $P < 0.005$ ), without showing changes in the migration of PC3 cell. Our *in vivo* model showed that YM intake decreased body weight, increases the latency of the appearance of tumors ( $P < 0.01$ ), and decreases the tumor volume ( $P < 0.05$ ) with respect to controls. In agreement, the expression of PCNA, a protein related to proliferation, was lower in the tumors of animals that consumed YM ( $P < 0.05$ ). In conclusion, YM and its bioactive principles showed anticancer effects *in vitro* and *in vivo*. YM showed a more marked effect in the LNCaP line, which is characterized by expressing androgen receptors. This shows us that more studies are needed to allow us to investigate the effect of YM on circulating hormone levels.

### A22

#### HYPERTHYROIDISM AFFECTS PROSTATE CARCINOGENESIS IN MICE

*Rey LS<sup>1</sup>, Ginevro P<sup>1</sup>, Gómez S<sup>1</sup>, Cano R<sup>1</sup>, Santiano F<sup>1</sup>, López Laur JD<sup>2</sup>, Mackern JP<sup>1</sup>, Sánchez V<sup>1</sup>, López Fontana C<sup>1</sup>, Carón RW<sup>1</sup>*

*<sup>1</sup>Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)/ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Mendoza, Argentina. <sup>2</sup>Clínica Andina de Urología (CAU), Mendoza, Argentina.  
E-mail: lararey@mendoza-conicet.gob.ar*

Thyroid hormones are essential for the development and metabolism of normal and tumor prostate tissue. *In vivo* preclinical studies have found controversial effects of thyroid hormones on prostate cancer (PCa) development. The aim of this study was to determine the influence of hyperthyroidism on the incidence and progression of PCa. C57BL/6 male mice were subcutaneously inoculated with  $5 \times 10^6$  TRAMP-C1 cells/200  $\mu$ L in the left flank. Mice were randomly divided into 2 groups: hyperthyroid (Hyper, subcutaneous injection of 10  $\mu$ g/mice/day thyroxine; N = 10) and euthyroid as controls (EUT; N = 10). Animals were observed daily until the appearance of palpable tumors. Latency and progression of tumors were determined. The major (DM) and minor (dm) diameters of tumors were measured using a caliper, and tumor volume was calculated. At sacrifice, tumors were obtained for histological analysis (mitotic/apoptotic ratio) and immunohistochemistry (caspase 3 and PCNA). Statistical analysis was performed using Student's *t*-test or with Mann-Whitney's *U*-test depending on the normality of the variable. All the animals developed PCa and the latency of onset of tumors was similar in the Hyper ( $39.33 \pm 2.09$  days) and EUT group ( $37.71 \pm 5.6$  days). However, hyperthyroidism accelerated the tumor growth compared to controls ( $68.42 \pm 22.97$  vs.  $28.23 \pm 7.87$  mm<sup>3</sup>/day, respectively;  $P < 0.05$ ). Also, the tumor volume of Hyper mice was bigger than the EUT ( $P < 0.05$ ). In accordance, PCNA immunostaining was

higher and caspase 3 was lower in Hyper tumors than EUT ( $P < 0.05$ ). Furthermore, the mitotic/apoptotic ratio of Hyper tumors was greater than EUT ( $P < 0.05$ ). In conclusion, hyperthyroidism promotes tumor growth, inhibiting apoptosis and enhancing cell proliferation.

### A23

#### **EXTRA VIRGIN OLIVE OIL AMELIORATES HIGH-FAT DIET-INDUCED SEMINAL ALTERATIONS BY MODULATING THE CHOLESTEROL PATHWAY IN RABBITS**

*Funes AK<sup>1</sup>, Avena V<sup>1,2</sup>, Colombo R<sup>1</sup>, Monclus M<sup>1,3</sup>, Fornés M<sup>1,3</sup>, Saez Lancellotti E<sup>1,3</sup>*

<sup>1</sup>Laboratorio de Investigaciones Andrológicas de Mendoza (LIAM), IHEM, Universidad Nacional de Cuyo, CONICET.

<sup>2</sup>Laboratorio de Enfermedades Metabólicas (LEM), Universidad Maza. <sup>3</sup>Consejo de Investigaciones de la Universidad del Aconagua (CIUDA), Universidad del Aconagua. E-mail: abikarenina@yahoo.com.ar

Male fertility depends on cholesterol (chol) homeostasis. Chol is essential for testosterone synthesis and spermatogenesis and must be maintained in an optimal range for proper functioning of the testicles. Rabbits fed a high-fat diet (HFD) display hypercholesterolemia associated with poor seminal quality, related to cholesterol overload in seminiferous tubule cells. Sterol regulatory element-binding protein 2 (SREBP2) governs the cholesterol pathway in testis and is deregulated by the fatty diet. On the other hand, Extra Virgin Olive Oil (EVOO) supplementation improves semen parameters affected by high fat diet by reversing SREBP2 expression. The aim of this study was to explore the effects of EVOO supplementation to HFD on some molecules of the cholesterol metabolism pathway in rabbit testis. We analyzed –by indirect immunofluorescence– the expression of: INSIG1 (insulin induced gene 1), SOAT2 (sterol O-acyltransferase 2), and ABCA1 (member 1 of human transporter sub-family ABCA). Male New Zealand White rabbits were fed commercial rabbit pellet (normocholesterolemic rabbits: NCR), a high-fat diet (plus 14% bovine grease, hypercholesterolemic rabbits). At 12 months of diet, ABCA1 expression increased significantly in the HCR group compared to the control, which is consistent with previous results for SREBP2, LDLR, and HMGCoAR. The group supplemented with EVOO did not modify the expression of ABCA1 compared to HCR. However, the expression of both INSIG1 and SOAT2 decreased in the animals on fat diet and those supplemented with EVOO compared to the control. These preliminary results indicate that SREBP2 increased in animals on a fatty diet despite the fact that its regulator (INSIG) is downregulated. In addition, increased cholesterol would stimulate the expression of the ABCA1 transporter for lipid efflux, but that would inhibit the SOAT-mediated esterification pathway. All molecules in the pathway are sensitive to EVOO supplementation although further studies are needed to determine the protective mechanism.

### A24

#### **EFFECT OF CHLOROQUINE ON THE ENDO-LYSOSOMAL SYSTEM OF BREAST TUMOR CELLS**

*Pereyra L<sup>1</sup>, Peralta S<sup>1</sup>, Vargas Roig L<sup>2</sup>, Sosa MA<sup>1,3</sup>, Carvelli L<sup>1,3</sup>*

<sup>1</sup>IHEM – CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina. <sup>2</sup>IMBECU –

CONICET – Universidad Nacional de Cuyo, Mendoza, Argentina. <sup>3</sup>Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina. E-mail: lorecarvelli@gmail.com

Breast cancer is the second leading cause of cancer death in women. Estrogen Receptor (ER)-positive breast cancer are less aggressive than triple negative subtype, where patients have a higher likelihood of recurrence and poorest survival prognosis. Some tumor cells have shown an increased lysosomal biogenesis, together with an altered lysosomal integrity and/or functionality, and increased levels of lysosomal proteases such as cathepsin-D (CatD). In most cell types, lysosomal proteins are selectively transported from the trans-Golgi-network (TGN) to lysosomes by the mannose-6-phosphate receptors (CD-MPR and CI-MPR). Alterations in the lysosomal membrane permeability induce a release of CatD into the cytoplasm, triggering apoptotic processes. Thus, lysosomes are considered as potential therapeutic targets for antitumor drugs. Acidotropic amines could accumulate in lysosomes, increasing the lysosomal membrane permeability and leading to leakage of enzymes into the cytoplasm. The acidotropic amine chloroquine is known to affect lysosomal acidification, and it is used as an adjuvant for chemotherapeutic treatments. The aim of this study was to evaluate the effect of chloroquine on the cellular distribution of CatD and CD-MPR in cells derived from tumors with different grade of malignancy. MCF-7 and MDA-MB-231 cell lines were incubated with chloroquine for 4, 6, 12, and 18 h, and processed for CatD, CD-MPR, and Golgin97 (TGN marker) detection by indirect immunofluorescence and confocal microscopy. In MCF-7 cells (ER-positive), the CatD signal was decreased at 6 h of incubation with chloroquine, and a redistribution to a perinuclear area was observed, whereas the CD-MPR was mostly redistributed in the cytoplasm. This could indicate an impaired recycling of CD-MPR to TGN and an increased secretion of CatD by default. In turn, the TGN appears disorganized and co-localizes with CD-MPR. Surprisingly, after 12 h of incubation, CatD, CD-MPR, and Golgin97 returned to their initial condition denoting a loss of chloroquine effect over time. By contrast, chloroquine did not induce changes on the distribution of the proteins in MDA-MB-231 cells (triple-negative), indicating a differential response of breast tumor cells to chloroquine, which would be related to their distinct malignancy

## A25

### CULTURE OF STEM CELLS FROM PULP AND DENTAL SACK IN A MEDIUM ENRICHED WITH FETAL BOVINE SERUM

*Basal R, Butler T, Merino G, Mayocchi K, Blascetti N, Astudillo L, Pilone L, Serrano V, Suarez S, Paleo MA, Degaetano S, Paggi R, Bander M*  
*Laboratorio de Biología Molecular y Biotecnología. Facultad de Odontología. Universidad Nacional de La Plata.*

Stem cells of dental origin have a marked capacity for differentiation, especially those from the pulp and dental sac, thus constituting a fundamental pillar in scientific advances on state-of-the-art therapies. Such cells belong to the group of adult stem cells, they are characterized by having multi-differentiation potential and can give rise to cells with an osteo/odontogenic, adipogenic character. The action of suitable factors and their incidence in molecular genetics favor *in vitro* development with a long-term view of a possible use in tissue regeneration treatments. In this sense, fetal bovine serum cultures offer an optimal medium due to its content rich in nutrients, hormones, organic molecules, minerals, stabilizing factors, growth, and adhesion factors. The objective of the present work has been to analyze the growth development of pulp and dental sac stem cells using cell cultures enriched with fetal bovine serum. The biological material was obtained from dental germs from third molars and from pulps of young permanent teeth extracted at the High Complexity Center of the La Plata School of Dentistry, according to the research protocol approved by the Bioethics Committee. Each germ and pulp were immersed in a transport medium with antibiotics and taken to the Molecular Biology and Biotechnology Laboratory of the FOLP. In the first instance, the biological material was placed in two Petri dishes of 3.5 cm in diameter (two dental pulps were placed in one capsule and two dental sacs in a second capsule) with DMEM culture medium supplemented with Fetal Bovine Serum and antibiotics. The proliferation control was carried out every 48 h with an inverted microscope and the cell count with a Neubauer chamber. The cells were characterized by studying specific CM surface markers, such as CD73, CD90, CD105, and CD146. The pulp-derived cultures took an average of 22 days to reach 80% confluence, while the dental sac derivatives took 16 days to reach the same state. The analysis of cell development in the sample studied indicates that the cells from the dental sac cultured in media with fetal bovine serum allow obtaining a greater number of stem cells than those derived from dental pulp.

## A26

### APOPTOSIS AND EPITHELIAL-MESENCHYMAL TRANSITION AS INDICATORS OF KIDNEY TUMOR DEVELOPMENT

*Ferrando M<sup>1</sup>, Romeo LR<sup>1,2</sup>, Gómez SE<sup>1</sup>, Lopez-Fontana CM<sup>1</sup>, Carón RW<sup>1</sup>, Pistone Creydt V<sup>1,3</sup>, Bruna FA<sup>1,4,5</sup>*

*<sup>1</sup>Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), Centro Científico y Tecnológico Mendoza, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. <sup>2</sup>Departamento de Urología y Transplante Renal, Hospital Español de Mendoza, Argentina. <sup>3</sup>Universidad Nacional de Cuyo, Facultad de Ciencias Médicas, Departamento de Fisiología, Mendoza, Argentina. <sup>4</sup>Centro de Medicina Regenerativa, Facultad de Medicina, Clínica Alemana, Universidad del Desarrollo, Santiago, Chile. <sup>5</sup>Universidad Nacional de Cuyo, Facultad de Odontología, Mendoza, Argentina. E-mail: matiasferrando13@gmail.com*

In tumor development and maintenance of a cancerous phenotype, bidirectional communication between epithelial cells and stromal environment is necessary. We recently demonstrated that human adipose tissue around kidney tumor (hRAT) showed a differential protein expression profile respect to adipose tissue from normal kidney (hRAN). In the present work, we evaluated proliferation, apoptosis, and epithelial-mesenchymal transition (EMT) markers in histological sections of different human kidney tumors (hRT). The tissue explants were obtained from patients with tumor kidney (hRT, N = 14). Surgically removed kidney tumors presented different Fuhrman grade (from F1 to F4) depending on each patient. The Fuhrman grade is a pathological classification system and a predictor of renal cell carcinoma (RCC) prognosis. The expression of PCNA, caspase 3, Bax, Bcl-2, and vimentin was evaluated by immunohistochemistry in kidney tumors. Statistical differences among the groups were evaluated by one-way ANOVA with Tukey's *post hoc* tests. We observed a positive correlation between Fuhrman grade with Bax/Bcl-2 ratio and a negative correlation with caspase-3 expression ( $P < 0.05$ ), both findings suggest a decreased level of apoptosis in advanced cancer stages. Respect EMT, statistical differences were reported in vimentin expression along the different tumoral grades; advanced tumor showed an increased level of vimentin which allow us to propose that in these stages the EMT is enhanced. No differences were observed in PCNA expression between tumors, related to Fuhrman grade; but we did observe different levels of expression regarding tumor size, while larger size tumors express significantly higher amounts PCNA ( $P < 0.05$ ). In conclusion, the results obtained indicate a positive correlation between cell apoptosis, epithelial-mesenchymal transition, and tumor Fuhrman grade on one hand, and proliferative activity and tumor size on the other. Such findings may be useful to establish a specific molecular signature in sights of a personalized therapy.

A27

**BIOCHEMICAL ASSESSMENT OF HYPERLIPEMIC DIETS WITHOUT CARBOHYDRATE SUPPLEMENT IN ADULT NEOZEALAND RABBITS**

Avena V<sup>1,2</sup>, Heredia R<sup>1</sup>, Elias L<sup>1</sup>, Mussi J<sup>1</sup>, Funes A<sup>2</sup>, Fornés M<sup>1</sup>, Sáez T<sup>2</sup>, Boarelli P<sup>1</sup>

<sup>1</sup>LEM Laboratorio de Enfermedades Metabólicas – Universidad Juan A. Maza. <sup>2</sup>LIAM Laboratorio de Investigaciones Andrológicas de Mendoza – IHEM – CONICET. E-mail: pboarelli@umaza.edu.ar

Acquired dyslipidemias are associated with various metabolic disorders such as diabetes mellitus, obesity, and metabolic syndrome (MS). Our goal was to develop an experimental animal model (New Zealand adult male rabbits) with dyslipidemia induced by a lipid diet without added carbohydrates. Carbohydrates such as fructose are important for the development of MS. Nine animals (N = 9) were fed for 3 months with standard rabbit chow (SRC). Dietary supplementation was performed during three consecutive trimesters (I, II, III). I: F group animals (N = 5) were fed with SRC + 14% of bovine fat (F) and O group animals (N = 2) were fed with SRC + 14% of olive oil (OO). SRC group animals (N = 2) were fed only SRC. II: Three animals from F group consumed 7% of F (HF subgroup). III: HF subgroup consumed 7% of F + 7% of OO (HFO subgroup). Weight (W), glucose (G), insulin, liver enzymes (LE), and serum lipid [cholesterol (C), HDL-cholesterol (HDL), LDL-cholesterol (LDL), triglycerides (TG)] were measured at each 30 days. In the W, it was not observed significant differences with the mean age. Although the HF subgroup (4.3 ± 0.4 kg) animals did present differences with respect to the F (3.44 ± 0.4 kg) and SRC groups (3.44 ± 0.4 kg). Insulin and LE were not conclusive. For G and TG, there were not significant differences during each experimental period. However, C increased 46.7% in F group (57.32 ± 10.45 mg/dL) vs. SRC group (39.08 ± 4.9 mg/dL), with a decrease of the same magnitude HDL (5.28 ± 1.73 vs. 3.88 ± 0.57 mg/dL) and an increase in LDL (21.32 ± 5.58 vs. 36.13 ± 11.37 mg/dL). The O group did not present a significant increase in HDL (3.14 ± 0.7 mg/dL) compared to F group. The HFO subgroup presented an increase in HDL (7.9 ± 0.4 mg/dL) with OO added to the diet, but without modifying C (HF vs. HFO subgroups: 44.88 ± 7.2 vs. 46.9 ± 15.2 mg/dL). Interestingly, during I and II, the F group and HF subgroups did not experience significant changes in C and HDL. Only in III, an increase in HDL could be observed with OO added to the diet (HFO subgroup). In this study, hyperglycemia and hypertriglyceridemia were not observed. This model allows evaluating changes in the types of dietary lipids without the influence of carbohydrates, associated with experimental models of MS.

A28

**COMPARATIVE STUDY OF THE *IN-SILICO* BINDING OF MASITINIB VERSUS PLANT FLAVONOIDS TO COVID-19's PROTEASE 3CLpro**

Gomez Barroso JA<sup>1,2</sup>, Carmona Viglianco N<sup>1,2</sup>, Zárate JM<sup>1,2</sup>, Aguilar CF<sup>1,2</sup>

<sup>1</sup>Área de Biología Molecular, Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia.

<sup>2</sup>IMIBIO, Universidad Nacional de San Luis, CONICET. E-mail: jarturogomez@gmail.com

There are no specific therapies available for COVID-19 (SARS-CoV-2) patients' treatment currently. The strategies that have been implemented are limited to preventive and supportive therapies, designed to prevent further complications and affected organs damage. Chymotrypsin-like protease 3CLpro (main cysteine protease, mpro) is a protease involved in structural or non-structural protein processing for the replication and packaging of virus's new generations. Recently, it has been published the three-dimensional structure complex of 3CLpro with Masitinib. The complex has been deposited in the Protein Data Bank (7JU7). 3CLpro has three domains. Domain I (3-101), domain II (102-184), and domain III (201-303). Domains I and II are made of antiparallel  $\beta$ -barrels and constitute the catalytic domain. The  $\alpha$ -helix domain III is responsible for enzyme dimerization as the enzyme is not active as a monomer. The inhibition studies in culture human cells shows that Masitinib is a potent inhibitor of the activity of the 3CLpro or SARS-CoV-2 main protease blocking the virus replication. Masitinib binds to 3CLpro in the catalytic domain. The drug is in phase 2 clinical trials and may have collateral effects in humans. Masitinib is an orally bioavailable tyrosine kinase inhibitor that acts as a competitive inhibitor. In this work, we have compared the *in-silico* binding of Masitinib to 3CLpro versus the binding of plant flavonoids like quercetin using AUTODOCK VINA. Binding energies are calculated by classical methods in docking studies. They are relative values, and they cannot be used on their own to estimate the quality of binding. However, what it can be properly done is to compare the docking of Masitinib to 3CLpro versus the docking of plant flavonoids evaluating their place of binding and energies.

A29

**THREE-DIMENSIONAL STRUCTURAL MODEL OF *Trypanosoma cruzi* NUCLEOSIDE DIPHOSPHATE KINASE 2 PROTEIN**

Cabrera M<sup>1,2</sup>, Gomez Barroso JA<sup>1,2</sup>, Carmona Viglianco N<sup>1,2</sup>, Aguilar CF<sup>1,2</sup>

<sup>1</sup>IMIBIO, Universidad Nacional de San Luis, CONICET. <sup>2</sup>Laboratorio de Biología Molecular Estructural, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. E-mail: jarturogomez@gmail.com

Chagas's disease is caused by the flagellated protozoa *Trypanosoma cruzi* and transmitted by the insect vector *Triatoma infestans*. It has high overall prevalence (6–8 million cases), and 65–100 million people are at risk of contracting this infection. The drugs used for treatment show undesirable side effects and no vaccines are available at present. The identification of new targets for chemotherapy is a major challenge in the control of the disease. Nucleoside diphosphate kinases play a key role in the energetic metabolism acting on maintenance of intracellular ratios of NTPs and dNTPs through the catalysis of the reversible phosphorylation of nucleoside diphosphates to nucleoside triphosphates. In our laboratory, was resolved a crystallographic structure for the canonical TcNDPK1 protein. *T. cruzi* NDPK isoform 2 (TcNDPK2) is a 37 kDa protein whose primary structure suggests an N-terminal 88 residues DM10 domain and a catalytic C-terminal region. TcNDPK2 is a microtubules-associated enzyme mainly localized in the cytoskeleton and flagellum. The TcNDPK2 DM10 domain is sufficient and necessary for cytoskeleton delivery of this enzyme. In this work, was obtained a TcNDPK2 three-dimensional homology model using MODELLER program. The model was constructed from the protein templates 2Z14, 5X00, and 1ZS6. The structure shows three well-defined structural domains: N-terminal, central, and C-terminal. The N-terminal domain shows an orthogonal  $\beta$ -sandwich conformation, formed by 5 antiparallel  $\beta$ -chains and two small  $\alpha$ -helix extensions. The central domain has a  $\alpha/\beta$  fold with four  $\alpha$ -helices and 4  $\beta$ -chains beta sheets. Finally, the C-terminal domain has a ferredoxin-like fold, typical of NDPKs, with 4  $\beta$ -chains and 9  $\alpha$ -helices. The final model was validated using the RAMPAGE and PDBsum programs and complementary using I-Tasser molecular dynamics tool. The structure includes the first three-dimensional characterization for a DM10 domain. Furthermore, it is the first three-dimensional structure of a multidomain nucleoside diphosphate kinase, such as NDPKs 2, 3, and 4. The model provides the first structural data on the protein and given its role in the viability of *T. cruzi*. These results would be important in the advance towards the rational design of specific drugs for Chagas disease.

A30

**EFFECTS OF *Tessaria absinthiodes* (Hook. & Arn.) DC. (ASTERACEAE). AQUEOUS EXTRACT AGAINST *Leishmania amazonensis* ON A MURINE MODEL**

Troncoso ME<sup>1,4,6</sup>, Germanó MJ<sup>1</sup>, Garcia-Bustos MF<sup>2</sup>, Arrieta VJ<sup>3</sup>, Gamarra-Luques C<sup>1,5</sup>, Cargnelutti DE<sup>1,5</sup>, Lozano ES<sup>1,5</sup>

<sup>1</sup>Instituto de Medicina y Biología Experimental de Cuyo-IMBECU-CONICET, Mendoza-Argentina. <sup>2</sup>Consejo Nacional de Investigaciones Científicas y Tecnológicas, Instituto de Patología Experimental, Salta, Argentina. <sup>3</sup>Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia, San Luis, Argentina. <sup>4</sup>Universidad Nacional de Cuyo, Facultad de Ciencias Exactas y Naturales, Mendoza, Argentina. <sup>5</sup>Universidad Nacional de Cuyo, Facultad de Ciencias Médicas, Mendoza, Argentina. <sup>6</sup>Universidad de Mendoza, Facultad de Ciencias Médicas, Mendoza, Argentina. E-mail: elozano@mendoza-conicet.gov.ar

The leishmaniasis is a spectrum of diseases caused by infection with protozoal pathogens of the genus *Leishmania*, with an estimated two million new cases per year. Leishmania parasites are transmitted to a mammalian host through the bite of an infected sand fly. The clinical forms of the disease (cutaneous, mucocutaneous, and visceral leishmaniasis) depend on the *Leishmania* species involved. In Argentina, it affects the northern region of the country with an incidence that has increased in the last two decades. Current treatments for leishmaniasis are unsatisfactory due to the associated high toxicity, cost, complex administration, and the emergence of resistant strains. Efforts have increased considerably in the last decade to identify new compounds with anti-leishmanial properties. Therefore, a strategy in the search for new alternatives is the detection of molecules from plant sources. There are more than five hundred species of plants in the province of Mendoza, in the central west of Argentina, for which "folk medicine" has described various uses to preserve and help health. *Tessaria absinthiodes* (Ta) has been used as a hypocholesterolemic, balsamic, and expectorant to treat renal insufficiency, diabetes, and digestive disorders. Recent studies have confirmed its biological activities against different microorganisms. The aqueous extract (AE) has been shown to be non-toxic in experimental animals. We evaluated the effect of TaAE in an in vivo model of cutaneous leishmaniasis. Male BALB/c mice were infected in the right hind paw pad with  $1 \times 10^5$  *L. amazonensis* promastigotes and treated with TaAE 300 mg/animal/day administered orally in the drinking water, *ad libitum*. We observed that the treatment with the aqueous extract diminishes the swelling of the infection site compared to the mice treated with Glucantime, which was used as a positive treatment control. These results are related to the significant decrease in parasite load, splenic index, and observed IgG levels. Although other tests need to be done, TaAE may be effective to treat cutaneous leishmaniasis.

### A31

#### **EFFECTS OF EXPERIMENTAL INTRACEREBRO-VENTRICULAR INJECTION OF AMYLOID BETA PEPTIDE (1-42) ON THE DAILY BAX AND BCL-2 EXPRESSION IN THE RAT HIPPOCAMPUS**

*Alfaro M, Coria Lucero C, Ledezma C, Castro A, Mendoza G, Anzulovich AC, Navigatore Fonzo L  
Laboratorio de Cronobiología, IMIBIO-SL, CONICET-UNSL, SL. E-mail: lorenavigfz@yahoo.com.ar*

Alzheimer's disease (AD) is a neurodegenerative disorder marked by cognitive and behavioral impairment. The accumulations of amyloid- $\beta$  ( $A\beta$ ) peptides in the brain are believed to be associated with perturbations of synaptic function leading to cognitive deficits. The proto-oncogene Bax (Bcl-2-associated X protein) and related protein Bcl-2 (B-cell chronic lymphocytic leukemia/lymphoma-2) genes are triggers of apoptosis in Alzheimer's disease (AD). Besides the cognitive deficit, AD patients also show alterations in their circadian rhythms. The objective of this study was to investigate the effects of an intracerebroventricular (i.c.v.) injection of amyloid beta peptide (1-42) on daily patterns of Bax and Bcl-2 expression, as well as of clock proteins in the hippocampus and on temporal profiles of cognitive performance of four-month-old males Holtzman rats. Groups were defined as: control (CO) and  $A\beta$ -injected ( $A\beta$ ). Rats were maintained under 12 h-light:12 h-dark conditions and received food and water *ad libitum* throughout the entire experimental. Daily rhythms of Bax and Bcl-2 expression were analyzed by RT-PCR and protein levels by Western blots, in hippocampus samples isolated every 4 h during a 24 h period. Regulatory regions of Bax and Bcl-2 were scanned for E-box sites. The Novel Object Recognition (NOR) test was used to evaluate cognition, particularly recognition memory. We found E-box sites on regulatory regions of Bax and Bcl-2 genes, which display a daily oscillation of expression in the rat hippocampus. The i.c.v. injection of  $A\beta$  (1-42) modified daily variation of Bax and Bcl-2, and clock proteins. It was observed that the group injected with  $A\beta$  explored the novel object for less time compared to the control group, during the day and night periods. Thus, elevated  $A\beta$  peptide levels might affect the temporal patterns of cognitive function and apoptotic genes, probably by altering daily rhythms of clock proteins.

### A32

#### **EFFECTS OF PIOGLITAZONE-VALPROIC ACID ON DAILY RHYTHMS OF NEP EXPRESSION IN AN EXPERIMENTAL MODEL OF ALZHEIMER DISEASE**

*Navigatore Fonzo L, Cangiano B, Cortez M, Golini R, Alba R, Anzulovich A  
Laboratorio de Cronobiología, IMIBIO-SL, CONICET-UNSL, UNViMe. E-mail: lorenavigfz@yahoo.com.ar*

Alzheimer's disease (AD) is a primary cause of dementia in the elderly. AD late onset, which constitutes 90% of cases, could be mainly attributable to deficiencies in the clearance of the  $A\beta$  peptide. Direct degradation of  $A\beta$  by endopeptidases has emerged as one important pathway for clearance. Nephilysin (Nep) is one of the most important  $A\beta$ -degrading enzymes. Neurogranin (Rc3) and neuromodulin (Gap-43) play an important role in learning and memory. Recently, PPAR- $\gamma$  agonists (Pioglitazone) have shown neuroprotective effects in neurodegenerative disorders. Numerous studies have shown that the intraperitoneal administration of valproic acid (VA), an inhibitor of histone deacetylases, caused an increase in NEP activity in different areas of the brain, including the hippocampus, in an experimental model of AD. AD patients also show alterations in their circadian rhythms. Taking into account these observations, the objective of this study was to evaluate the effect of Pio/VA on the 24 h rhythms of  $A\beta$ ; Nep, Rc3, and Gap-43 expression in the hippocampus of  $A\beta$ -injected rats. Four-month-old males Holtzman rats were divided into three groups defined as: (1) control, (2)  $A\beta$ -injected, (3)  $A\beta$ -injected treated with Pio-VA. Rats were maintained under 12 h-light: 12 h-dark conditions. Tissues samples were isolated every 6 h during a 24 h period. Nep, Rc3, and Gap-43 mRNA levels were determined by RT-PCR.  $A\beta$  protein levels were analyzed by immunoblotting. We found that the treatment of Pio-VA reestablished rhythmicity of those temporal patterns. These findings might constitute, at least in part, molecular and biochemical basis of restoration of circadian rhythmicity by the administration of Pio-VA in neurodegenerative disorders.

### A33

#### **CYTOKERATIN 5 EXPRESSION IN SERTOLI CELLS OF HGSNAT KNOCKOUT MICE**

*Carvelli L<sup>1,2</sup>, Sosa MA<sup>1,2</sup>, Pshezhetsky AV<sup>3</sup>, Hermo L<sup>3</sup>, Morales CR<sup>3</sup>  
<sup>1</sup>IHEM-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina. <sup>2</sup>Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina. <sup>3</sup>Faculty of Medicine, McGill University, Montreal, Quebec, Canada. E-mail: lorecarvelli@gmail.com*

The somatic Sertoli cells of the testis are involved in secretion and endocytosis of proteins, phagocytosis of residual bodies, and in the formation of the blood-testis barrier. Sertoli cells rest on a well characterized and elaborate basement membrane (BM) benefitting cell growth, differentiation, and sperm development. The BM is a thin extracellular layer composed of glycoproteins, type IV collagen, and the proteoglycan heparan sulphate (HS). Degradation of HS occurs in the lysosome in a stepwise manner, involving heparin  $\alpha$ -glucosaminidase N-acetyltransferase (HGSNAT) enzyme. A

deficiency in HGSNAT causes a severe lysosomal storage disorder, mucopolysaccharidosis IIIC (MPS IIIC, also known as Sanfilippo syndrome), leading to a significant accumulation of HS within lysosomes and major disruption of various tissues and organs. Integrin molecules in the plasma membrane of the epithelial cell provide a strong attachment connecting the BM with the intracellular cyokeratin network. Binding of integrin to components of BM activates the cytoplasmic signaling enzyme FAK (Focal Adhesion Kinase). It has previously been shown that loss of FAK in mouse embryonic fibroblast cells led to an epithelial phenotype where cells expressed E-cadherin, cyokeratin-18, and desmoplakin. As cyokeratins 8 and 18 are expressed in Sertoli cells of testes from patients with dystopia, atrophía, and/or oligospermia, it is suggested that detection of cyokeratins in these cells could be a sensitive marker for damaged testes. Given that HS turnover is affected in MPS IIIC, we tested if the expression and distribution of cyokeratin 5 (CK5) in Sertoli cells are modified in a HGSNAT deficient mice model (HGSNAT KO). Immunohistochemical (IHC) staining and immunoblots were performed comparing HGSNAT KO and wild type testes of 7 (N = 3) and 11-month-old mice (N = 3). LM-IHC analysis of wild type testis confirmed expression of CK5 in Sertoli cells, with no apparent difference in staining intensity at the different ages. In comparison, a more intense reaction was noted in Sertoli cells of HGSNAT KO mice, with an age-dependent increased expression also noted. Immunoblot analysis showed a statistically significant increase of CK5 in KO vs. WT testes at  $P < 0.05$ . This is the first characterization of a functional link between CK5 and heparan sulfate metabolism in Sertoli cells. In HGSNAT KO mice, Sertoli cells at the EM reveal an increase in size and number of lysosomes leading to a difference in the overall shape and size of these cells. Such a situation may affect the integrity of the cell to adhere to the BM which may be altered. Hence, the Sertoli cell may produce more CK5 to compensate for this condition.

### A34

#### **EFFECT OF A BOTULINUM NEUROTOXIN FROM A NATIVE STRAIN OF *C. botulinum* ON BREAST CANCER CELLS**

Chapana A<sup>3</sup>, Guarniolo D<sup>1</sup>, Sosa E<sup>1</sup>, Fernández RA<sup>1</sup>, Sosa MA<sup>2,3</sup>, Carvelli L<sup>2,3</sup>, Caballero PA<sup>1</sup>

<sup>1</sup>Área de Microbiología, Facultad de Ciencias Médicas, UNCuyo. <sup>2</sup>IHEM-CONICET – Facultad de Ciencias Médicas, UNCuyo. <sup>3</sup>Facultad de Ciencias Exactas y Naturales, UNCuyo. E-mail: patriciacaballero7764@gmail.com

Botulism is a neuroparalytic disease caused by botulinum neurotoxins (BoNT, serotypes A-G) produced by *Clostridium botulinum*. BoNT A is currently widely used in the treatment of neuromuscular transmission disorders. Genomic and phenotypic studies showed that native strains from Cuyo region (Argentina) and their BoNTs correspond to subtype A2 and would have higher activity than A1 (Botox®), being a potential therapeutic agent. The native BoNTSu 1935 obtained from soil is known to degrade actin from rat brain homogenates, suggesting that this protein would be a target for this toxin. In this study, the action of BoNTSu 1935 on the actin and tubulin cytoskeleton was evaluated in mammary tumor cells. Both, the BoNTSu 1935 and the BoNT from the A Hall strain (subtype A1) were purified by 60% saline precipitation. MCF7 and MDA-MB-231 cells were treated with 250 LD50 of the BoNTs for 5, 10, 25, 45, and 90 min. The cells were then processed for immunoblot or immunofluorescence in order to evaluate the expression and distribution of actin and tubulin. In addition, the integrity of the Golgi complex was evaluated. In MCF-7 cells, BoNTSu 1935 induced actin degradation at higher extent than BoNT A Hall. In turn, this protein was redistributed to plasma membrane location, in a time-dependent manner. Although tubulin was affected in a similar way, this protein was redistributed to perinuclear patches. Meanwhile, the Golgi complex appears disorganized after treatment with the toxin. Interestingly, no changes were observed in either the distribution or in the expression of actin and tubulin in MDA-MB-231 cells. Regarding the cytotoxicity of BoNTSu 1935, we observed ~90% mortality of MCF-7 cells at 90 min of incubation with the toxin, while that index was reached in the MDA-MB-231 cells, as early as 25 min incubation. In contrast, BoNT A Hall only induced 50% cytotoxicity in MDA-MB-231 cells at 90 min incubation. These results would indicate a higher cytotoxicity of BoNTSu 1935, whose action mechanisms would be selective for tumor cell types. This could be related to levels of malignancy of the tumor cells.

### A35

#### **ESTROGENS MODULATE THE EXPRESSION OF LYSOSOMAL PROTEINS IN A RAT MODEL OF PARKINSON'S DISEASE**

Leiva N<sup>1,2,\*</sup>, Bonaccorso MP<sup>3,\*</sup>, Meier G<sup>1,2</sup>, Carvelli L<sup>2,4</sup>, Sosa MA<sup>1,2,4</sup>, Cabrera R<sup>3</sup>

<sup>1</sup>Laboratorio de Histología e Embriología. Facultad de Ciencias Médicas-CONICET-Universidad Nacional de Cuyo.

<sup>2</sup>Facultad de Ciencias Exactas y Naturales-Universidad Nacional de Cuyo. <sup>3</sup>IMBECU-CONICET-Universidad de Mendoza. <sup>4</sup>IHEM-CONICET- Universidad Nacional de Cuyo. Mendoza. Argentina. \* Equal contribution.

E-mail: nleiva@fcen.uncu.edu.ar

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons from Substance Nigra Compacta. A genetic study identified 24 loci that are associated with PD, 11 of the 24 genes are involved or disrupt various functions of the autophagic-lysosomal pathways. Lysosomes participate in the degradation of macromolecules from endocytosis and autophagy processes. Epidemiological and clinical studies reveal a difference in the development of PD between genders, giving sex hormones a neuroprotective function and making them an

interesting therapeutic proposal. The objective of our work was to analyze the effect of estrogens on the expression of lysosomal proteins in a rat model with the phenotype of PD. Two-month-old male Sprague-Dawley rats underwent stereotaxic surgery to administer 6-hydroxydopamine (6-OHDA) or artificial cerebrospinal fluid (V) to the left striatum. After 7 days, they received chronic treatment for 10 days with 17- $\beta$ -estradiol (E) or V. The groups were made up of: C (V lesion); E (V + E lesion); HP (6-OHDA lesion), and HPE (6-OHDA + E lesion). After the treatments, the animals were sacrificed and the left and right brain regions: substantia nigra and prefrontal cortex were extracted and homogenized. The samples were processed for immunoblotting using anti-cathepsin D (CatD) and anti-actin antibodies. Preliminary results show that chronic estrogen treatment increases CatD and actin expression both in substantia nigra and prefrontal cortex. Since the CatD protease reduces the concentration of the  $\alpha$ -synuclein protein in PD, the present results suggest that the increase in lysosomal function would exert neuroprotective effects in cells affected by the disease. Likewise, it should be mentioned that estrogens could also modulate the organization of the cytoskeleton, as a stage of neuromodulation.

### A36

#### MOLECULAR MECHANISMS UNDERLYING THE ANTIDIABETIC EFFECTS OF *Mulinum spinosum* ROOT DECOCTION

Berrueto S<sup>1</sup>, Pavarotti M<sup>2</sup>, Alvarez MS<sup>3</sup>, Scapini C<sup>1</sup>, Cremer C<sup>1</sup>, Castro C<sup>3</sup>

<sup>1</sup>Laboratorio de Fisiología FCM UNCOMA. <sup>2</sup>IHEM/CONICET-UNCUYO. <sup>3</sup>FCM UNCUIYO and IMBECU-CONICET.  
E-mail: sberrueto4@gmail.com

A bitter decoction prepared from the root of *Mulinum spinosum*, popularly known as “neneo”, it is traditionally attributed antiglycemic properties. However, the molecular mechanisms that underline this effect remain unknown. The aim of this work was to analyze whether aqueous extracts from “neneo” roots enhance insulin sensitivity in *in vivo* experiments as a possible molecular mechanism. Cytotoxicity was tested in vascular smooth muscle cells using MTT tetrazolium reduction assay, and GLUT-4 translocation, as a measure of Insulin Sensitizing, was performed in L6 myoblast cell. As a diabetic animal model, adult male C57BL/6 mice were fed with a 20% w/v fructose (Fru) in drinking water combined with 30% w/w high fat diet (HFD) over a period of 12 weeks. “Neneo” root extracts (3 or 6 g/kg/day) were administered in drinking water along with Fru-HFD. Mice were weighted weekly, glucose tolerance test was determined at the end of the treatment period, and adipose tissue was collected after sacrifice. Translocation of GLUT4 from intercellular compartment to plasma membrane as surface level of GLUT4myc, was measured by an antibody-coupled assay. “Neneo” root extract (0.5–10 mg/mL) did not affect cell viability, only higher concentrations (20 and 50 mg/mL) significantly reduced 10% fetal calf serum-induced proliferation. In the presence of 25 nM insulin, GLUT4 quantification on the membrane cell of L6 cells was higher than controls ( $P < 0.01$ ). The addition of “neneo” root extract (1 mg/mL) during 60 min, significantly enhanced translocation of insulin sensitive glucose transporters-4, in skeletal muscle cells ( $P < 0.05$ ). After 10 weeks, mice fed a Fru-HFD treated with “neneo” significant decreased body weight gain compared to the Fru-HFD group ( $P < 0.01$ ). After 120 min of intraperitoneal injection of 2 g/kg body weight of glucose, blood glucose levels in Fru-HFD fed mice treated with “neneo” extract, significantly decreased compared with Fru-HFD group ( $P < 0.01$ ). Histological examination of mesenteric adipose tissue showed a significant reduction of adipocyte perimeter and area after “neneo” intake compared with Fru-HFD group ( $P < 0.01$ ). In conclusion, our results show for the first time a possible molecular mechanism of action underlying the antidiabetic effect attributable to “neneo” extract consumption.

## BIOCHEMISTRY, PHYSIOLOGY, PATHOLOGY AND VEGETAL PRODUCTION

### A37

#### SPRING-ESTIVAL MORPHOGENESIS OF *Elionurus muticus* AT MACOLLO LEVEL

Bornand CL, Alonso C, Privitello MJL, Bacha EF, Lepotati JL

Dpto Cs. Agropecuarias - FICA –UNSL. E-mail: cynbornand@gmail.com

*Elionurus muticus* (Spreng.) Kuntze (bitter straw) is a native Poaceae of the San Luis medianosa area, perennial, with a spring–summer cycle and undesirable for cattle grazing. In order to estimate a suitable thermal time period to formulate chemical control actions according to the growth rate of the species, it was proposed to describe its morphogenic-structural behavior at the tiller level and to find a predictive model of leaf growth based on environmental factors (temperature and rainfall) during a cycle. The study was carried out on plants located in pots (15) and arranged in the Experimental Agronomy Field of the UNSL (Villa Mercedes). The start of regrowth was 9/13/2019, 8 days after a cleaning cut. When the tillers generated 2 leaves, 1–2/plant were identified. The statistical design was completely randomized. Every 7 days and in each tiller, the moment of leaf appearance was recorded, and the following structural variables were determined: number of green leaves (HV), length of green (LFV) and senescent sheet of each leaf, and the accumulated green extension of the tiller (LFVmac.: tiller LFV summation). Between the 2<sup>nd</sup> and 6<sup>th</sup> leaf, the morphogenetic variables were calculated: foliar elongation and senescence rates (TEF and TSc: mm/°Cd), phylochron

(Fc: °Cd), leaf half-life (VMF: Fc × HV; in °Cd or days) and leaf appearance rate (TAH: 1/Fc; H/d). By means of regression analysis, the leaf extension was related to two environmental variables (ST: thermal sum and PP: rainfall). The base growth temperature (Tb) was estimated by relating foliar accumulation and average temperature (average between two successive leaves). An intercept on the x-axis of 14°C was obtained and thus, an approximation to Tb. In each weekly observation, 2.5 green leaves/tiller were recorded, with a total foliar accumulation of 6–7 leaves. The maximum length of the leaf blade was reached between the 6<sup>th</sup>–7<sup>th</sup> leaf (480–530 mm), corresponding to typical bushes of the area. *E. muticus* showed important TSc. (0.38 mm/°Cd) in relation to TEF (0.57 mm/°Cd). Fc (96°Cd) indicated a high speed of leaf generation, in parallel TAH was high (0.05 H/d). The 40% of the plants and the 80% of the tillers remained in a vegetative state. For the study conditions, “bitter straw” presents low leaf density per tiller and narrow VMF (240°Cd or 50 days) before a rapid appearance of two successive leaves (Fc). By relating LFV mac. With ST and PP, a multiple regression model was obtained (LFV mac = 759 + 9.4PP – 1.6ST; R<sup>2</sup> aj = 0.76; P < 0.05). The relationships indicate that, while ST inhibits foliar extension, PP intervenes in the opposite way. Therefore, the growth of the leaves will be favored by the spring–summer PP and the early onset of senescence due to the low accumulation of average temperatures (VMF: 240°Cd) in correspondence to a rapid speed of leaf appearance (Fc: 96°Cd). These values are indicators of an accelerated growth rate and are conducive to thinking of a limited thermal time window, which does not exceed 240°Cd or 50 days from regrowth, in which the species, in an initial state of growth, may be more susceptible to control therapeutic actions. Finally, this agronomic practice could give time to organize the aerial sowing of mega-thermal species such as “weeping grass” (*Eragrostis curvula*) in spring.

### A38

#### WINTER MORPHOGENESIS OF *Nassella tenuissima* AND *Jarava ichu* AT TILLER LEVEL

*Bornand CL, Alonso C, Privitello MJL, Bacha EF, Rossi R*  
Dpto Cs. Agropecuarias - FICA – UNSL. E-mail: cynbornand@gmail.com

*Nassella tenuissima* “paja blanca” (Nt) and *Jarava ichu* “paja vizcachera” (Ji) are perennial Poaceae native species of low or no forage value, with autumn–winter–spring growth, whose control in livestock systems needs to be studied. In order to determine the appropriate thermal time period to formulate chemical control actions, according to the growth rate of species undesirable to grazing, it was proposed to determine morphogenetic and structural indicators at the tiller level and establish the relationship between foliar growth and environmental factors (temperature and rainfall) during winter. Potted plants (15/species) were located on UNSL campus (Villa Mercedes, San Luis), with completely randomized design. The regrowth began on 6/6/2019, and tillers with 2 leaves were identified to determine every 7–10 days the moment of leaf appearance and the following structural variables: number of green leaves (HV), length of green leaf (LFV), foliar senescence and the accumulated green extension of the tiller (LFVmac: sum of LFV). The thermal sum (ST) was calculated with 5°C base growth temperature in both species. The morphogenetic variables determined on 10 tillers/species were: foliar elongation and senescence rates (TEF and TSc), phylochron (Fc: °Cd), leaf half-life (VMF: Fc×HV, °Cd or days) and leaf appearance rate (TAH: 1/Fc, H/d). Predictive models of foliar growth were sought by regression analysis, in which the leaf extension was related to two environmental variables (ST: thermal sum and PP: rainfall). Both straws showed a very similar morphogenetic behavior until the end of winter, for which a limited range of variation was defined for each variable without discriminating by species. The Fc indicated a slowed speed between the appearance of the 2<sup>nd</sup> and 3<sup>rd</sup> leaf (186–192 °Cd) that then accelerated between 3<sup>rd</sup> and 4<sup>th</sup> (132–143 °Cd). In the same foliar order, TAH showed a similar trend (0.02–0.05 H/d), which confirmed the low rate of initial foliar appearance that was later activated. The relationship between Fc and HV from 2<sup>nd</sup> and 4<sup>th</sup> leaf, defined as VMF or onset of foliar senescence, was also similar in both species (VMF: 218–231°Cd or 50 days), with 150–160 °Cd of Fc and 1.5 HV/tiller. Nt and Ji showed high TSc (0.27–0.44 mm °Cd) in relation to TEF (0.53 to 0.62 mm °Cd). With the advance of the cold season, both rates tended to decrease. The species reached a moderate maximum leaf extension (200–240 mm) and low total leaf accumulation (4 leaves). Despite the limited foliar stockpiling, the low HV per tiller (1–2) implied about 3 foliar changes. LFVmac of the straws responded to independent polynomial models when related to ST and PP (P < 0.05), but ST better explained structural variable changes (0.91 R<sup>2</sup> for Nt: LFVmac: –0.001ST<sup>2</sup> + 1.92ST + 126.49 and 0.90 R<sup>2</sup> for Ji: LFVmac: –0.002ST<sup>2</sup> + 1.86ST + 153.55). It is concluded that these species show a low rate of growth, regulated mainly by winter thermal accumulation, which constitutes an important aspect to consider for control guidelines. Generally, the application of graminicides is recommended on active growth plant stage, for which it could be used as an opportune moment indicator, less than 3 green leaves/tillers, less than 200–240 mm of leaf blade extension and before 218–231 °Cd accumulated (VMF). According to FC, TAH and VMF obtained, both species show slow growth in winter, and a wide thermal time window is inferred for their control (less than 230°Cd or 50 days, from regrowth). It is proposed for further studies to validate this result empirically.

### A39

#### **EFFECT OF CUTTING MOMENT AND DEFOLIATION ON THE PRODUCTIVE BEHAVIOR OF *Thynopirum ponticum* PASTURE WITH ACCUMULATED PREVIOUS GROWTH AND INVADED WITH *Cynodon dactylon***

*Bacha EF, Privitello MJL, Rodriguez MJ, Tello IM, Montenegro MA, Vetore OS*  
Dpto. de Ciencias Agropecuarias. FICA-UNSL. Villa Mercedes, San Luis. E-mail:efbacha@gmail.com

*Thynopirum ponticum*, “agropiro”, is a species that allows to reverse the condition of low-productivity environments for livestock grazing, due to its tolerance to salinity and fluctuating groundwater nearly to surface. The previous growth accumulated on the pasture difficult to access from new shoots, so it is necessary cleaning the pasture by cutting the material. “Bermuda Grass” (*Cynodon dactylon*) advances on “agropiro” pasture in conditions of moderate salinity and competes strongly for space. On “agropiro” pasture invaded with “Bermuda”, and with accumulated previous growth, the effect of the cleaning cut at the end of summer or in autumn was evaluated. The experience evaluates the effect on biomass generated from autumn until the end of spring. A sector of the pasture was cut in February (Fc) and another contiguous sector in April (Ac). Subsequently defoliations were carried out every 30 or 60 days (3 repetitions). Dry matter accumulation (DM) for animal consumption was determined by cutting and weighing, and aerial %-coverage of “agropiro” and “Bermuda” were visually estimated. The data set was statistically analyzed with Infostat software, applying mean differences tests. In all cases, forage accumulation was low in autumn and winter period. For Fc, monthly defoliation generated a significantly higher growth in November (*t*-test,  $P < 0.10$ ), with respect to Ac. Considering the cleaning cut moment, the spring regrowth of “agropiro” began earlier with Fc, for both frequencies of defoliation. DM accumulation at the end of evaluated period did not show significant differences between treatments (*t*-test,  $P > 0.10$ ), although the highest values are obtained for Fc, independently defoliation frequency. The measurements on 60 days defoliation treatment ended earlier than monthly defoliation (November vs. December). Most of the production until December is accumulated in spring (from 61 to 100%), although the greatest effect is obtained by the moment in which the pasture was cleaned and not by the frequency of defoliation applied, because with Ac, autumn growth is substantially inhibited. Between 28 at 41% DM was accumulated at autumn–winter for Fc, while for Ac was less than 20% in this period. “Agropiro” and “Bermuda” are species with different growth cycles and habits (hulm and cespitose, respectively), although the applied treatments generated differences on their coverage (ANOVA,  $P < 0.05$ ). “Bermuda” decreases its coverage from July to Oct–Nov, approximately, because latency period. With Ac, “Bermuda” did not develop autumnal regrowth, but in spring it did so earlier than in Fc. In turn, monthly defoliation anticipated the senescence of the autumn grass due to a greater exposure to frost. The late cleaning of pasture (A) affects its growth, although less frequent defoliation (60 days) allows generating greater aerial coverage of the pasture in spring. The combination of both studied factors (cleaning time and defoliation frequency) may need further study as an alternative to control of “Bermuda” grass. The effect of cleaning cut moment should be evaluating beyond the month of December to cover the entire “agropiro” production cycle. It is recommended that, in view of the need to eliminate accumulated material from previous growths, the pasture is cleaned around February, so as not to affect its usable production.

### A40

#### **VEGETATION COVERAGE VARIATION UNDER DIFFERENT BOVINE GRAZING INTENSITIES, IN THE SANDY SOIL GRASSLANDS FROM SAN LUIS CENTER**

*Bacha EF, Privitello MJL, Ruiz OM, Vetore OS, Rossi RE*  
Dpto de Ciencias Agropecuarias. FICA-UNSL. E-mail:efbacha@gmail.com

The sandy grassland is mainly composed by perennial and annual Poaceae, mainly summer cycles. According to the condition, it will be the coverage and production of forage and undesirable species for grazing. As non-forage species, in addition to the so-called “straw”, they are also broadleaf species. The action of livestock grazing determines changes in floristic composition, and the identification of functional groups that affected vegetation as indicators is important to understand their dynamic and improve management tools. Species presence and coverage prior to grazing (November) was determined on 730 ha paddock on different intensity of disturbance sectors, associated with grazing, according to 450, 1500, and 2400 m (C, M, and L, respectively) distances from only water point, located on one of the sides of the paddock. The aim was to establish relationships between total vegetation coverage “CT”, straw coverage (*Elyonorus muticus*, “Em”); *Nasella tenuissima*, “Nt”), broadleaf coverage (LT), and different functional groups of vegetation characterized according to their utilitarian classification (with forage value: “PVF” or without “SVF”) and response to grazing (increasing “RC” or decreasing “RD”). The species identifies were recorded and the area coverage of each one was determined on 10 samplings along thirteen 10-m transects, in each sector. Finally, the relative amount of soil devoid of vegetation was estimated, according to the intensity of grazing received. Some RC species with greater coverage were: *Elyonorus muticus*, *Nasella tenuissima*, *Eustachys retusa*, *Panicum urvilleanum*, and RD species were: *Sorghastrum pellitum*, *Eustachys retusa*, *Poa ligularis*, among others. RC variable was analyzed using Kruskal–Wallis test and the others by ANOVA. There were no significant differences ( $P > 0.05$ ) in soil cover devoid of vegetation between sectors. Straw coverage varied 20–28%, mainly due to the contribution of Em, without significant differences ( $P > 0.05$ ) as a whole or individually, although Em tended to decrease in M. Nt was found at the end of the cycle and its coverage was very low compared to Em in full growth (0–2.5 % vs. 18–28 %). LT coverage did not show significant differences ( $P >$

0.05), although under high grazing pressure –C– they tended to increase by 50%. The CT did not differ between sectors ( $P > 0.05$ ), but it increased inversely with grazing pressure. Sectors differentiation arises when forage value is considered in the functional groups. The grazing intensity gradually decreases the coverage of preferred (PVF), differentiating C and L ( $P < 0.05$ ). From M towards C, a decreasing response is evidenced due to the grazing effect of the preferred species and towards L an inverse behavior. Through multivariate analysis of principal components, component 1 explains 66% of total variability and 2° component 34% of this, it can be deduced that sector C was correlated with the SVF and LT. The sectors M and L with the coverage of species with forage and decreasing value, in turn L was related to the coverage of the increasing response. Only the straws did not show a relationship with sectors. The greater grazing pressure is not evidenced by the invasion of straw, but by the loss of forage species in a decreasing way to grazing, in addition to a tendency to increase the coverage of broadleaves.

#### A41

### FREQUENCY AND DENSITY OF PRIMAVERO-SUMMER WEEDS IDENTIFIED IN THE CULTIVATION OF VICIA AND IN THE STUBBLE OF CORN AND SORGHUM, IN VILLA MERCEDES, SAN LUIS, ARGENTINA

Alonso CS, Bormand CL, Bonivardo S

Facultad de Ingenierías y Ciencias Agronómicas, Universidad Nacional de San Luis (UNSL).  
E-mail: sbonivardo@gmail.com

The study was carried out in the area surrounding the city of Villa Mercedes. The climate of Villa Mercedes is semi-arid, with concentrated rainfall in the summer (550–650 mm average annual rainfall). Weeds exhibit the common characteristic of interfering with cultivated plants by adapting to live to the different forms of cultivation carried out by man (tillage systems, species of crop to be implanted, management, among others) and to different environmental conditions. Weeds are one of the main factors of crop yield reduction. This work aims at identifying the spring–summer weeds present and calculate the frequency and density of emerged seedlings in cultivation and stubble. The sampling was carried out in the Agronomy Department of FICA–UNSL from September to December 2020. Six randomly distributed fixed land plots were established: two on a sorghum stubble, two on a vetch crop, and two on a corn stubble. The seedlings that emerged manually were not included in the sample. The frequency and density of emerged seedlings per m<sup>2</sup> were calculated. The following species was identified: *Salsola kali*, *Conyza* spp., *Portulaca oleracea*, *Chenopodium album*, *Bassia scoparia*, *Euphorbia dentata*, *Eleusine indica*, *Amaranthus* spp., and *Digitaria sanguinalis*. In vicia, the most frequent species were *Chenopodium album* (33.5%) and *Portulaca oleracea* (32.5%). In sorghum stubble: *Portulaca oleracea* (88.5%) followed by *Digitaria sanguinalis* (9.8%) and in corn stubble: *Euphorbia dentata* (44.6%). The density, in the cultivation of vetch, *Chenopodium album* (32.5 pl/m<sup>2</sup>) and *Portulaca oleracea* (31.5 pl/m<sup>2</sup>) was presented. In sorghum: *Portulaca oleracea* with 7554 pl/m<sup>2</sup> and in corn: *Euphorbia dentata* with 114.5 pl/m<sup>2</sup>. The species with the highest frequency of seedlings was *Portulaca oleracea*, and the one with the lowest frequency was *Digitaria sanguinalis*. The low rainfall in 2020 (425 mm per year) influenced their behavior; where spring births was resigned, which then appeared during late spring or early summer.

#### A42

### EFFECT OF FREEZING ON THE NUTRITIONAL QUALITY OF SORGOS SILAJES

Vetore OS, Leporati JL, Privitello MJL, Bacha EF

Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Agropacuarias, UNSL.  
E-mail: ovetore@gmail.com

Freezing (T<sup>f</sup>: temperatures ≤ 0°C at 1.5 m above the ground) can damage some annual forage crops by drying out cells and causing leaves loss, consequently less dry matter (DM), and alter their quality. The critical temperature (T<sup>°</sup>) that damage sorghum, corn and millet crops ranges from –1 to –3 °C. Sorghum is a crop that adapts to water stress and is suitable for conservation using the silage technique. Sorghum silage constitutes a very convenient forage reserve for semi-arid regions, given the adaptation of this crop to said environment, where San Luis province is located. Given this, it was proposed to evaluate changes in nutritional quality of sorghum silages due to the effect of T<sup>f</sup> on the crop to be ensiled, in Villa Mercedes. Micro-silos of whole plants of dual-purpose sweetened sorghum were made (pasty grain state) cut before and after T<sup>f</sup> (27, 28, and 29 of April: –4.1, –3.4, –2 °C, T<sup>f</sup>, respectively). The cut-off moments (CM: factors) were: 5 days before T<sup>f</sup> and 1, 5, and 12 days later. Furthermore, two treatments were applied: with and without added LactoSilo® in each CM (WL and WoL, respectively), into completely randomized design (N = 4). The quality parameters evaluated were: NDF (neutral detergent fiber: cell wall, potentially degradable fraction), ADF (acid detergent fiber: lignin content indicator), lignin (antinutritional factor, non-degradable fraction); IVDDM (in vitro digestibility of DM), DE (digestible energy) and ME (metabolic energy). In each of the mentioned variables, ANOVA was performed for repeated measures. The consecutive total values of the variables, for each CM (without discriminating by treatment) were: NDF: 50, 53, 55, 57 %; ADF: 32, 33, 33, 34 %; Lignin: 5, 5, 5, 5 %; IVDDM: 62, 64, 60, 55 %; DE: 2.7, 2.8, 2.6, 2.4 Mcal.kgMS<sup>-1</sup>; ME: 2.25, 2.33, 2.16, 1.97 Mcal.kg.MS<sup>-1</sup>. The results indicated that NDF, IVDDM, DE and ME presented normality (Shapiro Wilks test,  $P > 0.05$ ) and variance homoscedasticity (Mauchly test,  $P > 0.05$ ). Differences were detected between CM and not in the CM and treatment interaction ( $P < 0.05$  and  $P > 0.05$ , respectively). When comparing the combinations

of pairs, for the CM factor, in NDF no differences were detected between 1st–2nd and 3rd–4th (Bonferroni,  $P > 0.05$ ). In IVDDM, DE and ME, there were no differences between 1st–2nd and 1st–3rd (Bonferroni,  $P > 0.05$ ). When comparing the WL and WoL treatments, they behaved homogeneously whatever MC ( $P > 0.05$ ). In the fibrous, ADF and lignin fractions, the Mauchly sphericity assumption was fulfilled ( $P > 0.05$ ) and no differences were detected between MC ( $P > 0.05$ ) or in the MC and treatment interaction ( $P > 0.05$ ), although lignin it increased in quantitative terms as the cycle progressed. From combinations of pairs analysis (differences between 1st–4th and 2nd–4th CM), it is inferred that nutritional parameters that favor silage nutritional quality as IVDDM; DE and ME decrease at the 4th CM, whereas those that compromise the digestibility such as NDF and lignin increase, the latter at least quantitatively, with the advance of the sorghum crop cycle. It is concluded that it is not advisable to cut sorghum plants for ensiling after the 12th day of being subjected to the effect of successive freezing temperatures, because the quality of the preserved forage is negatively altered and the addition of LactoSilo® does not improve it.

### A43 EFFECT OF FREEZING ON pH, DRY MATTER AND °BRIX ON SORGHUM SILAGES

Vetore OS, Leporati JL, Privitello MJL, Bacha EF

Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Agropecuarias, UNSL.

E-mail: ovetore@gmail.com

In forage plants used in advanced stages of development, such as sorghum for ensiling, the higher organs are more exposed to damage by temperatures below 0°C ( $T^{\circ}$ ) early and consecutive freezing ( $T^{\circ}f \leq 0^{\circ}C$ , at 1.5 m from the ground) than by frost ( $T^{\circ} \leq 0^{\circ}C$ , at ground level). Knowing the alterations in the nutritional parameters of whole sorghum plant silages at the end of their cycle (pasty grain) naturally subjected to  $T^{\circ}f$  and how these affect the quality of the preserved forage, allows to know the time between the occurrence of  $T^{\circ}f$  and the convenient moment to cut-chop. It was proposed to evaluate changes in pH, DM (dry matter), and °Brix of a crop and sorghum silage affected by  $T^{\circ}f$ . For which, micro-silages of whole plants of sugary dual-purpose sorghum cut before and after  $T^{\circ}f$  (27, 28, and 29 April: -4.1, -3.4, -2 °C) were analyzed. The cut-off moments (CM: factors) were: 5 days prior to  $T^{\circ}f$ , and 1–5–12 days later. Two treatments were applied: with and without the addition of Lactosilo® (WL and WoL) for each CM, into completely randomized design ( $N = 4$ ). Correlation analysis (Pearson) was applied between DM, pH, and °Brix and subsequently, an analysis of Variance for Repeated Measures (ANOVA). There was a moderate correlation between DM and pH ( $r = 0.54$ ;  $P < 0.01$ ), not between °Brix and pH or between °Brix and DM, which were very low ( $r < 0.50$ ). In the three variables, the Mauchly sphericity condition of homoscedasticity of variance was fulfilled ( $P > 0.05$ ), there were differences between CM means (Wilks Lambda:  $P < 0.05$ ) and differences in combinations of pairs of means corresponding to CM (Bonferroni:  $P < 0.05$ ). No interactions were detected between CM and treatments (Wilks Lambda:  $P > 0.05$ ) and, except in °Brix, there were no differences between WL and WoL ( $P > 0.05$ ). The total pH values (without discriminating by treatments) and consecutive for each CM, were: 3.9, 4.2, 4.1, and 4.2. There were differences between pH means for CM, but the values remained within the optimal range for silage. Also, there were differences in pH in all combinations of CM pairs, except between the 2nd and 4th ( $P > 0.05$ ). The consecutive total DM values for each CM were: 25, 27, 30, and 31. Although there were DM differences in all combinations of CM pairs, DM of the 3rd and 4th CM correspond to the minimum value of the range recommended for ensiling (30–40 %). The consecutive total °Brix values for each CM were: 11.2, 12.4, 14.5, and 11.9. There were differences in all the combinations of CM pairs, except between the 2nd and 4th CM ( $P > 0.05$ ). It follows that sugars tend to concentrate after  $T^{\circ}f$  (3rd cut). There were statistical differences between treatments for °Brix ( $P < 0.05$ ) at 12 days of cut in favor of WoL (12.43 vs. 11.40), not so in the rest. By integrating aspects related to pH, DM, and °Brix to indicate opportune times for cutting and ensiling, before and/or after  $T^{\circ}f$ , it is inferred to wait 12 days (4th CM), after occurrence, it does not decrease the quality of the conserved forage in terms of pH and DM, but more sugars would be harvested in the plant on the 5th day (3rd CM) after said contingency. For the variables analyzed, after the occurrence of early and successive  $T^{\circ}f$ , it would not be convenient to cut-chop sorghum plants beyond the 5<sup>th</sup> day of their occurrence and that the addition of Lactosilo® does not modify this trend.

### A44 CHRONOBIOLOGICAL STUDY OF PHYSIOLOGICAL VARIABLES AND ANTIOXIDANT METABOLISM IN *Glycine max* L.

Pardo SD<sup>1</sup>, Gatica Aguilar CV<sup>1</sup>, Anzulovich AC<sup>2,3</sup>, Navigatore Fonzi L<sup>2,3</sup>, Molina AS<sup>2</sup>, Golini RS<sup>2</sup>, Pérez Chaca MV<sup>1</sup>

<sup>1</sup>Laboratorio de Morfofisiología, Facultad de Química, Bioquímica y Farmacia (FQByF-UNSL). <sup>2</sup>Área de Química Biológica. <sup>3</sup>Instituto Multidisciplinario de Investigaciones Biológicas - CONICET (IMIBIO), Argentina.  
E-mail: pardodaniela09@gmail.com

The Earth rotation exposes most of the planet's species to the environmental daily oscillations. These external oscillations synchronize the internal biological clocks that generate biological rhythms in the organisms to anticipate the environmental changes. Among these rhythms, the circadian, 24-h rhythms, are the most studied. In plants, the initiation of photosynthesis after sunrise (periodic process) triggers the accumulation of metabolites, including the production of reactive oxygen species (ROS). ROS represent a constant threat to plants, as they react with different cellular components

generating oxidative stress. However, plants have developed mechanisms to maintain redox homeostasis. The oscillations in ROS production, removal, and signaling would involve the plant biological clock. The functions of clock oscillators in agriculturally important species, such as soybean (*Glycine max* L.), are increasingly recognized. Soybean is one of the most important and cultivated legumes in the world and constitutes the main export item of Argentina, one of the main producing countries worldwide. Therefore, the objective of this work was to study the influence of the biological clock on the physiology and antioxidant metabolism of *G. max* throughout a 24-h period. For this purpose, soybean cultures were grown in hydroponics for 7 days under standard environmental conditions, with a 14 h-light:10 h-dark photoperiod. Biochemical (antioxidant enzyme activity, lipid peroxidation, and H<sub>2</sub>O<sub>2</sub> levels), physiological (photosynthetic pigment concentration), and molecular (primer design, identification of transcription factor binding sites) parameters, were determined in soybean leaves. Interestingly, we found that catalase activity ( $P < 0.05$ ), H<sub>2</sub>O<sub>2</sub> levels ( $P < 0.01$ ), and the content of chlorophyll a ( $P < 0.01$ ) and total chlorophyll ( $P < 0.05$ ) display significant daily rhythms in this tissue (Chronos-fit,  $P < 0.05$ ,  $P < 0.05$ ,  $P < 0.05$ , and  $P < 0.01$ , and % rhythm: 66.36%, 55.12%, 39.87%, 48.58%, respectively), with their acrophases occurring at ZT 04:18 ± 00:29, ZT 04:53 ± 00:59, ZT 10:41 ± 03:03 y ZT 10:41 ± 01:28, respectively. Even though carotenoid content shows a significant daily variation ( $P < 0.05$ ), it does not adjust to a cosine curve and, therefore, it cannot be defined as a rhythm. The 24-h oscillations showed here, might suggest the regulation of oxidative metabolism by the biological clock, in the leaves of *G. max*, an agriculturally important plant. These results would provide useful information for plant manipulation and crop management, taking into account how antioxidant defenses and oxidative stress vary throughout the day.

#### A45

### MORPHOMETRIC PARAMETERS DURING EARLY GROWTH OF *Jatropha curcas* L. AND *Jatropha macrocarpa* Griseb

Tavecchio N<sup>1</sup>, Olmedo Sosa L<sup>1</sup>, Terenti O<sup>1</sup>, Escudero E<sup>1</sup>, Mercado Ocampo S<sup>2</sup>, Pedranzani H<sup>3</sup>  
<sup>1</sup>Laboratorio de Fisiología Vegetal, FICA, PROICO 14-0916 UNSL. <sup>2</sup>Laboratorio de Morfología Vegetal, FICA, PROICO 14-0916 UNSL. <sup>3</sup>Laboratorio de Fisiología Vegetal, FQByF, UNSL. E-mail: n\_tavecchio@hotmail.com

*Jatropha curcas* L. and *Jatropha macrocarpa* Griseb are two species of importance for the production of biodiesel with great adaptability to marginal soils, and to arid and semi-arid environments. The aim of the work was to determine morphometric parameters during early growth in these species. Seeds without tegument of *J. curcas* and *J. macrocarpa*, were sowed in terrines with soil: perlite 50:50 and kept at field capacity. They were placed at 30°C of temperature and photoperiod 16 h-light:8 h-dark, and 60% of relative humidity. The followed parameters: length of roots (LR), hypocotyls (LH) and epicotyls (LE), and number of leaf (NL) were evaluated. Fresh weight (FW) and dry weight (DW) of roots, hypocotyls, epicotyls, true leaves, and cotyledonary leaves were measured. Samples were dried in an oven at 60°C until constant DW was obtained. FW and DW were expressed in g/plant. All parameters were recorded at once a week, for 40 days. The experiment took place in five replicates with 20 seeds per each replication. Analysis of variance (ANOVA) was applied, and data were subjected to multiple range Duncan's test using the software INFOSTAT-UNC. At 40 days, there were not significant differences in LR. On the contrary, LH, LE, and NL of *J. curcas* were significantly higher than *J. macrocarpa*. FW and DW of roots, hypocotyls, epicotyls, true leaves, and cotyledonary leaves of *J. curcas* were significantly higher than *J. macrocarpa*. The early growth seedlings study is important to investigate seed development following germination and because it determines the successful establishment of the seedling, especially against hostile environmental.

#### A46

### *Medicago sativa* L. – *Sinorhizobium meliloti* SYMBIOSIS: STUDY OF SALINE STRESS MITIGATION

Gonzalez EA, Pacheco Insausti MC, Achiary M, Zapico GM, Pedranzani HE  
Laboratorio Fisiología Vegetal. PROICO 2-3318. Ciencia y Técnica. Facultad de Química Bioquímica y Farmacia. Universidad Nacional de San Luis, Ejército de los Andes, 950, San Luis, Argentina.  
E-mail: evelynailengonzalez@gmail.com

Alfalfa is a species of great plasticity, morphologically and physiologically adapted to tolerate abiotic stresses. The objective of the work was to study the effect of the symbiotic relationship of *Medicago sativa* L. with salt-tolerant *Sinorhizobium meliloti* in relation to different salt concentrations. The trial had two factors: (1) inoculation with *S. meliloti*, and (2) application of salt stress. A vermiculite terrines system was used for the trial, in which previously germinated CW 660 alfalfa seeds were sown. Plants were grown in a culture chamber at 25°C and a photoperiod of 16 h-light and 8 h-dark for five weeks. Saline treatment was initiated one week after planting, non-inoculated plants (NIP) were irrigated with Hoagland's solution + 50, 100, and 200 mM NaCl, and (2) inoculated plants (IP) with the same nitrogen limiting solution + 50, 100, and 200 mM NaCl, no salt was added to the control plants. After four weeks of treatment, aerial and root morphological parameters, photosynthetic pigments, and proline concentration were measured. Morphological differences between NIP and IP were analyzed by Student's *t*-test ( $P \leq 0.05$ ). IP showed a significant increase in AL (aerial length) and AFW (aerial fresh weight) at 50 mM NaCl. At 200 mM, NaCl both AL and ADW (aerial

dry weight) were statistically higher in IP. RL (root length) in control plants was superior in IP with respect to NIP, RFW, and RDF (fresh weight and root dry weight) were significantly higher at 50 mM and 200 mM NaCl, respectively, in IP. In IPs, chlorophyll a level increased significantly at 50, 100, and 200 mM NaCl, with respect to their control and NIPs, chlorophyll b level also increased at 50, 100, and 200 mM NaCl with respect to their control and NIPs, but these differences were not significant; carotenoids did not show significant differences in any saline treatment. Chlorophyll a, b, and carotenoid content decreased significantly at 200 mM NaCl in NIP relative to control. In IPs, the proline level increased significantly at 200 mM NaCl relative to the control, but with lower values than NIPs. In NIP the proline level was significantly increased from 100 mM NaCl relative to the control. IP showed improved salinity tolerance in all growth parameters, increased chlorophyll a and b concentrations and reduced proline accumulation and carotenoid content. The two biochemical indicators of stress both proline and protective pigments such as carotenoids are not increased in IP as they suffer less from salt stress. On the other hand, IP can synthesize more chlorophyll a and b, which benefits their photosynthetic apparatus and increases their vigor.

#### A47

### HERBACEOUS OF THE NATIVE FLORA WITH RARE PRESENCE IN LOMA BLANCA DEL MORRO (SAN LUIS)

Gómez M. Furlan Z

*Facultad de Ingeniería y Ciencias Agropecuarias. U.N.S.L. E-mail: caldenia@gmail.com; zunifurlan@hotmail.com*

In this work, herbaceous species of the native flora that present very little or rare frequency in Loma Blanca del Morro were identified. The study area is located in Loma Blanca, a town near San José del Morro, San Luis province; it is an area of grasslands and mountain forest, with stony soil and rainfall that fluctuates between 500 and 600 mm per year. Loma Blanca occupies an area of approximately 6300 hectares, corresponding to the Chaco Serrano district. Since 2007, floristic surveys have been carried out (trees, shrubs, and herbaceous plants, without considering the Poaceae that were relevant by another research team). Of the 115 herbaceous plants identified, 80 are native, 25 endemic, 9 adventitious, and 1 naturalized. The largest families being the Asteraceae with 26 genera, and the Solanaceae and Verbenaceae, with 7 genera each. As for the species that have been registered one to three times, there are: *Calydorea pallens* (Iridaceae), endemic; *Chromolaena squarrosoramosa* (Asteraceae), native; *Cucurbitella asperata* (Cucurbitaceae), native; *Lathyrus macropus* (Fabaceae), endemic; *Austroliabum candidum* (Asteraceae), endemic; *Mirabilis ovata* (Nyctaginaceae), native; *Nama undulatum* (Boraginaceae), native; *Pelexia bonariensis* (Orchidaceae), native; *Picrosia longifolia* (Asteraceae), native; *Pluchea sagittalis* (Asteraceae), native; *Pombalia serrata* (Violaceae), endemic; *Passiflora foetida* var. *foetida* (Passifloraceae), native; *Sacoila lanceolata* (Orchidaceae), native. Like other authors, we maintain that knowledge about the plant richness of a place is of fundamental importance, both for scientific studies and for the development of conservation strategies.

#### A48

### EFFECT OF HIGH TEMPERATURE PRETREATMENT IN MAIZE SEEDS ON SEEDLING GROWTH

Eggel ML<sup>1,3</sup>, Pena LB<sup>1,2</sup>, Pérez Chaca MV<sup>3</sup>

<sup>1</sup>CONICET. Instituto de Química y Fisicoquímica Biológica (IQUIFIB-UBA). Buenos Aires. <sup>2</sup>Dpto. Química Biológica. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires (UBA). <sup>3</sup>Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis (UNSL). E mail: luz.eggel@gmail.com

Unfavorable environmental conditions can severely limit a plant's embryonic viability and growth. Stress is considered to be a significant deviation from optimal living conditions, inducing reversible and irreversible changes. Different studies show the triggering of oxidative stress, metabolism and phytohormonal disbalance by exposing plants to high temperatures. Maize seed drying is key between harvesting and seed storage; however, the effect of drying temperatures on maize seed damage is still unclear. The objective is to evaluate the pretreatment with elevated temperatures during short periods in maize seeds on the post-germinative development of seedlings. Maize seeds (*Zea mays* L.) variety NS 7818 VIP3 from Nidera were subjected to pretreatment with elevated temperatures at 40, 50, 60, and 70 °C for 72 h (N = 30). Seeds with and without heat pretreatments were superficially disinfected with 3.5% sodium hypochlorite solution for 1 min. Seeds were germinated on cotton and sterile paper towels saturated with sterile distilled water at 28°C for 96 h maximum. Length and biomass (fresh and dry weight) of coleoptile and main root of the germinated seeds with and without pretreatment were determined. Our preliminary results showed a significant increase in the length of coleoptiles ( $P < 0.0001$ ) and radicles ( $P < 0.001$ ) of the seeds subjected to pretreatment at 40°C compared to the control group. On the other hand, a significant decrease in the length was observed then of treatment at 60 and 70 °C of the coleoptile ( $P < 0.01$ ) and the radicle ( $P < 0.05$ ) with respect to control. Regarding biomass, a significant increase in fresh weight (FW) was observed in the coleoptile with the pretreatment at 40°C ( $P < 0.01$ ) while at 50, 60, and 70 °C were significantly decreased compared to the control ( $P < 0.05$ ). Dry weight (DW) showed a significant increase in the coleoptile with the pretreatment at 40°C ( $P < 0.01$ ), and significant decrease was observed at 50, 60, and 70 °C ( $P < 0.05$ ) with respect to the control. The radicle showed a significant increase in FW with the pretreatment at 40°C ( $P < 0.05$ ) and a significant decrease at 70°C ( $P < 0.001$ ) compared to the control; however, no difference was observed in DW. In summary,

increasing the pretreatment temperature of maize seeds produced significant changes in physiological parameters during the development of the seed embryo. While heat pretreatment of seeds at 40°C produced the growth induction in maize seedlings, higher temperatures between 50 and 70 °C, caused the inhibition. These results could be due to oxidative stress generation or to imbalance of primary metabolism or phytohormones. More studies are required to elucidate the mechanisms involved in the induction and the inhibition of growth after heat pretreatment of maize seeds.

#### A49

### PRELIMINARY STUDIES ON AGROCLIMATIC CHARACTERIZATION ON WALNUT CULTURE (*Juglans regia* L.) IN POTENTIAL FRUIT REGIONS, IN SAN LUIS PROVINCE

*Lucero RA<sup>1</sup>, Martínez Espeche ME<sup>1</sup>, Monti FM<sup>1</sup>, Arias A,<sup>2</sup> Pérez MB<sup>2</sup>, Monteagudo L<sup>2</sup>, Castro CS<sup>2</sup>, Cattaneo R<sup>2</sup>*

*<sup>1</sup>Facultad de Ingeniería y Ciencias Agropecuarias, Universidad Nacional de San Luis. Ruta Provincial N° 55 S/N extremo Norte, Villa Mercedes, San Luis. <sup>2</sup>Colaborador Externo. E-mail: rolandolucero1962@gmail.com*

In recent years, there has been a growing expansion of the cultivation of nuts in the province of San Luis, mainly walnut (*Juglans regia* L.), almond tree (*Prunus amygdalus* L.) and, on a smaller scale, hazelnut (*Corylus avellana* L.) and chestnut (*Castanea sativa* L.), among other species, which includes smallholder crops and other larger surface businesses by investors, given under the Provincial Fruit Development Law (Law VIII-0666-2009). The expansion of walnut cultivation with profitability should be based on the correct application of technologies and the selection of cultivars whose bioclimatic optimum is assimilated to the environmental characteristics of the proposed site. Performance and quality are genetically determined, but strongly influenced by climatic factors such as cold hours, risks of frost damage. The objective of this work was to highlight the agroclimatic feasibility and the evaluation of possible varieties of walnut in potential fruit growing areas: Quines, Candelaria, Villa de Merlo, Cortaderas, Carpintería (Valle del Conlara), and Villa Mercedes and surroundings. Records of frost, occurrence, duration and intensity, and the bioclimatic index of accumulation of cold hours were analyzed. There are new crops of medium density of plantation approximately 120 hectares with drip irrigation, grafted plants with Chandler type varieties in the coastal area. In the Concarán-Merlo area, there are family farms (approximately 60 hectares between 0.5–3 hectares per producer), vigorous plants of great size, with good nut quality, low productive level with little technical management. The Quines – Candelaria Zone records an average of 450 HF per year for the period 1998–2018, while Merlo-Cortaderas range between 760/790 HF and Villa Mercedes between 900/1100 HF. Villa Mercedes presents as limiting the occurrence of late frosts, it requires selecting late flowering varieties. The American varieties have a lower hours/cold requirement than the European varieties (300/900 HF), the varieties are recommended: Payne, Serr, Chico for Quines-Candelaria (450HF). For the Merlo – Cortaderas zone, the most demanding cold American varieties (700/800 HF) such as, Tulare and Chandler, a predominantly 3-5% pollinated variety with Cisco. For Villa Mercedes, varieties such as Chandler, Vina and Hartley (900/1100 HF) would be better adapted.

#### A50

### CHEMICAL & ORGANIC FERTILIZATION IN *Cannabis sativa* CROPS

*Isequilla Mayo R, Virginillo S, Pantuso F*

*Asociación Civil para la Investigación y Desarrollo de Cannabis sp. E-mail: fpantuso@gmail.com*

The medicinal use of *Cannabis* sp. dates from to pre-Christian times, but its use for therapeutic purpose in the 21<sup>st</sup> century is highly controversial, given the scant interest that traditional medicine gives it. However, its use is increasingly significant in people who self-medicate; in most cases, in pathologies in which allopathic medicine has been ineffective. Generally, cannabis extracts (cannabis oil) are of unknown quality and composition, making it difficult to reproduce the desired results. Taking into consideration the current legal situation of *Cannabis* and taking us an example of the countries where the medicinal crops, therapeutic, and industrial *Cannabis* crops are regulated, we consider that it is necessary to deepen the production techniques of the crops. The work's objective was to evaluate the optimal cultivation practice and nutrition techniques for obtaining high-quality medicinal plant material. As a result, *Cannabis* inflorescence were compared according to mineral fertilization (Nitrofosca) versus organic fertilization (GrowBbis®) and the control without fertilization evaluating the resin extraction according to RSO (Rip Simpson Oil) standard and filtering by heat press. Fifty seeds were sown and developed in the substrate of peat, compost, humus, and perlite. In a grow room with 18 h-light during the vegetative period and 12 h during flowering, with Full Spectrum LED panels. Of the 50 plants obtained, 10 were left as a control, 20 with chemical fertilization, and 20 with organic fertilization. Each plant was taken as an experimental unit of a Completely Randomized Design. As a result, highly significant differences were observed between the treatments with fertilizer and the control, and between organic and inorganic fertilization with a significance level ( $\alpha = 0.05\%$ ) each one. This variety takes 9 weeks to develop its cycle until full bloom. The plants with mineral fertilization present a short-stretch pre-flowering, little thickness of stems and tips with a moderate number of styles. Organic fertilization (GrowBbis®) showed greater stretching, apices, and number of pistils. Therefore, it is concluded that although mineral fertilization has greater plant development, flower whit organic fertilization shows larger glands and, for its, greater amount of resin.

### A51

## REDOX IMBALANCE EFFECTS GENERATED BY HIGH CONCENTRATIONS OF CADMIUM–ZINC IN *Glycine max* L. LEAVES

Gatica Aguilar CV, Gallardo LV, Ciminari ME, Torres Basso MB, Pérez Chaca MV

Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia - Proy2-4318.  
E-mail: caamigatica@gmail.com

Large parts of the world's soils are contaminated by heavy metals. Zinc is an essential microelement for plants, but in excessive concentrations it causes alterations in physiological, biochemical, and molecular processes. Cadmium, on the other hand, is a heavy metal without biological functions and also causes harmful effects at cellular and molecular levels. Due to the resemblance of both ions and that they share the same transporters too, toxicity symptoms are similar, such as reduction in biomass, decreased elongation and darkening of roots, chlorosis, and necrosis in leaves. Changes in oxidative stress parameters are also generated. The objective of this study was to determine stress tolerance mechanisms generated by the Cd/Zn pair, evaluating the biochemical and physiological changes *Glycine max* L. leaves. The leaves were obtained after 10 days of plant development under hydroponics in Hoagland's nutrient solution conditions and subjected to contamination with the two ions (Zn and Cd) for 6 days. The ZnCl<sub>2</sub> concentrations used in the study were: 0, 0.6, and 4.8 mM, and 40 µM of CdCl<sub>2</sub>, as a constant concentration. Morphophysiological parameters were measured: fresh weight (PF), stem length (LT), root length (LR), leaf area (AF), and photosynthetic pigments (Chlor) and carotenes (Car). MDA content was measure as a parameter of oxidative stress and antioxidant enzymes CAT and APX activities. The results showed a significant decrease ( $P < 0.001$ ) either in PF, LT, LR, and AF in all treatments with Zn, a decrease in photosynthetic pigments and carotenes is also observed in the last treatments with Zn (4.8 mM)/Cd ( $P < 0.01$ ). Regarding the stress parameters, a significant decrease ( $P < 0.01$ ) of MDA was observed in the last two treatments, with respect to the control. CAT activity decreased significantly in all treatments with respect to the control ( $P < 0.05$ , 0.01, and 0.001). In APX activity, a significant increase was observed in the treatments of Zn [4.8] with Cd and without Cd ( $P < 0.001$ ). According to these results, we can conclude that the zinc and cadmium ions presence in the nutritive medium alters not only the morphophysiological parameters but also the antioxidant and pro-oxidant activity, mainly in high zinc concentrations, which could be considered a plant defense response against the toxicity generated by the ion pair.

### A52

## EFFECT OF Cd AND Hg ON EARLY SEEDLING GROWTH AND ANTIOXIDANT SYSTEM OF *Adesmia pinnifolia*

Parera V<sup>1</sup>, Gallardo L<sup>2</sup>, Gatica CV<sup>2</sup> Pérez Chaca VM<sup>2</sup>, Parera CA<sup>3</sup>, Feresin GE<sup>1</sup>

<sup>1</sup>Instituto de Biotecnología-FI-UNSJ, Av. Libertador Gral. San Martín 1109(O), 5400 San Juan, Argentina. CONICET.

<sup>2</sup>Universidad Nacional de San Luis, Facultad de Química Bioquímica y Farmacia. <sup>3</sup>INTA- CR Mendoza-San Juan.

E-mail: victoriaparera.t@gmail.com

Phytoremediation is an effective and low-cost technique for the restoration of heavy metals contaminated soils. To improve the efficiency of such technology, a better understanding of the mechanisms underlying heavy metal (HM) tolerance in the plant is indispensable. The toxicity of HM is the leading cause of oxidative damage, resulting in the reduced growth of plants by inducing changes in membrane permeability and the production of reactive oxygen species. The aim was to evaluate the seedling establishment, the activity of ascorbate peroxidase (APX), catalase (CAT), malondialdehyde (MDA), and the concentration of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) of *Adesmia pinnifolia* under conditions of stress due to Cd and Hg. Plants were grown semi hydroponically using vermiculite as substrates for 30 days in solutions of different Cd (3, 4.5, and 6 ppm) and Hg (0.8, 1.2, and 1.6 ppm) concentrations and a control treatment (distilled water). Three replicates (25 seedlings) per level of treatment were tested in a growth chamber under control environmental conditions (T: 23°C day/night; 12 h light/dark). Statistical analysis for enzyme activity was performed using ANOVA followed by Tukey–Kramer Multiple Comparisons Test and generalized linear mixed-effects models with a binomial error distribution was used for seedling establishment. Results showed that the seedling establishment was greater than 80% in all the treatments and control. The activity of CAT (µmol H<sub>2</sub>O<sub>2</sub>/mg protein.min) and APX (µmol Ascorbate/mg protein min) was significantly higher in the control treatment compared with the three concentrations of Cd and Hg ( $P < 0.001$ ). Antioxidative enzyme activities decrease as Cd and Hg concentrations increase. Contrarily, the Cd and Hg application significantly enhanced membrane damage (MDA), which intensified with the increase in Cd and Hg concentrations ( $P < 0.01$  and  $P < 0.001$ , respectively). There were no significant differences in the production of extracellular H<sub>2</sub>O<sub>2</sub> between the control and the HM treatments. These results suggest that the exposure of *A. pinnifolia* to HM induces severe lipid peroxidation and a low response of the enzymatic defense CAT and APX. The oxidative stress could be due to another type of ROS rather H<sub>2</sub>O<sub>2</sub>. However, this specie managed to establish itself in a medium with different concentrations of heavy metals. Nevertheless, future studies on the antioxidant system should be tested to complete the performance.

### A53

#### EFFECT ON SWEET CORN'S SEEDS TREATMENT WITH Zn

Corbalan Gervasoni MB<sup>1</sup>, Virginillo S<sup>2</sup>, Pantuso F<sup>1,2</sup>

<sup>1</sup>Universidad Nacional de Lujan, Departamento de Tecnología. <sup>2</sup>Universidad el Salvador, Escuela de Agronomía.  
E-mail: fpantuso@gmail.com

Zinc (Zn) is essential for the growth and development of crops as it has an impact on vegetative development. This effect may be since it acts as a cofactor for several enzymes involved in carbohydrate, protein, and auxin metabolism, membrane integrity, flowering, and seed production. Several authors reported higher yields of different crops, such as wheat, corn, and rice, with zinc fertilization. Therefore, providing an adequate supply of Zn would appear to be crucial to ensure that crops use fertilizers efficiently. Seed treatment with Zn can not only promote germination, but also stimulate growth and subsequent metabolic processes, increasing the final crop yield. The objective was to evaluate the effect of the Zn application in seeds treated in pre-sowing on the emergence and biometric parameters such as root length and plant height in sweet corn populations. The zinc dose applied was 20 cc. (based product) dissolved in 20 cc of water. The experimental design was completely randomized *en bloc*. Eight treatments with four repetitions were evaluated. To evaluate emergence, the number of emerged plants was counted at 5, 10, and 15 days after sowing in each plot. Plant height was measured with a ruler (cm) on a sample of 20 plants chosen at random per block, 20 days after sowing, from the base of the stem to the last unfolded leaf with visible ligule. The root length was measured from the neck of the seedling to the end of the primary root. The statistical analysis was an analysis of variance, and Tukey's test was used to compare means. It is concluded that there are highly significant differences ( $P < 0.0001$ ) in the number of plants that emerged at 5, 10, and 15 days after sowing between the materials treated and not treated with Zn. When analyzing the number of seminal and lateral roots in the seedling, it is observed that all the materials responded to the application of Zn on seed in pre-sowing in a statistically significant and favorable way. Finally, when plant height was evaluated 20 days after sowing, statistically significant differences ( $P < 0.001$ ) were observed between the materials treated with Zn and those not treated. It is concluded that, in all cases, the treatment of seeds with Zn notably improved the implantation of the corn crop, which is directly related to the number of plants to be harvested and its yield per hectare.

### A54

#### CO<sub>2</sub> PRODUCTION IN A FERMENTER WITH ORANGE SHELL

Bertoluzzo MG<sup>1</sup>, Bertoluzzo SMR<sup>1,2</sup>, Tobares AI<sup>2</sup>

<sup>1</sup>Taller de Física, Facultad de Ciencias Bioquímicas y Farmacéuticas. <sup>2</sup>Facultad de Ciencias Médicas. Universidad Nacional de Rosario. E-mail: mgbertol@hotmail.com

An important application to take advantage of orange residues is to use them to combat ants that destroy citrus and rose bushes. Recent studies show that by placing small pieces of orange peel on the path made by the ants to go and return to the anthill carrying the load, they leave the cargo and take these pieces and take them to the anthill. Apparently, these pieces ferment producing a toxic element for the ants. After studying this behavior and considering the investigations about the production of citric acid from fermenters with orange peel, we aimed to study the fermentation in orange residues. Squeezed orange halves were placed under protection from light, at room temperature. After five days, a grayish-green layer was formed in the flavedo, an area rich in essential oils, and a black mold in the albedo where there is a greater amount of pectins. It is *Penicillium* that is mainly found in soils but also in the air and homes and it infects citrus fruits; in the case of black mold, it is *Aspergillus*. Beakers with agar were prepared and small pieces of orange peel were scattered on the air-agar interface and inoculated with the green powder obtained in solid-state fermenters. After four days, colonies of the fungus *Penicillium* were observed. 10-mL syringes were used, and 5% ground orange peel and water were placed. The preparation was inoculated with the green *Penicillium* powder. It was stirred, and the liquid level drop time was recorded. Bubbles were produced in a short time and the CO<sub>2</sub> production was 0.08 mL/h, and the pH dropped to 5, so it is estimated that citric acid was produced.

## BIOTECHNOLOGY AND GENETICS

### A55

#### EVALUATION OF PRODUCTIVE PARAMETERS IN THE DESCENDANCE OF PIARA IN AN INTENSIVE PORCINE PRODUCTION ESTABLISHMENT

Osses RG<sup>1</sup>, Casale JB<sup>2</sup>, Becerra CR<sup>1</sup>, Rosa ST<sup>1</sup>, Sueldo RA<sup>1</sup>, Rodríguez Rivera MF<sup>1</sup>

<sup>1</sup>Docente Dpto. Ciencias Agropecuarias Facultad de Ingeniería y Ciencias Agropecuarias (Universidad Nacional de San Luis). <sup>2</sup>Alumno Ingeniería Agronómica (FICA). E-mail: rgosses@email.unsl.edu.ar

Pork meat is the red meat most consumed in the world and the pig livestock subsector, along with poultry. This is due to the productive efficiency of the pig activity: high number of offspring (2.5 offspring per female per year), conversion of feed into meat (2.7 kg of feed/kg of live weight) and a meat yield of 80%, approximately, with a lean percentage that ranges between 54 and 57%, which makes it very attractive for the meat industry. The present work was carried out in “El Piquiyi” farm, around 30 km southwest of Villa Mercedes (San Luis), between December 11, 2020, and July 7, 2021, where an intensive pig system with 400 mothers operates in production. The main objective was to evaluate productive parameters in the offspring of the genetic material used in the farm, through the analysis and comparison of these parameters between replacement females “grandmother’s descendants” with batches of inseminated females with terminal stallions. Two batches of females were used for the study: Lot 1 (L1) and Lot 2 (L2), previously selected and identified by caravans and/or tattoos of five animals each. L1 was inseminated with semen from grandmothers to obtain the F1 (first generation), and L2 was inseminated with terminal stallion semen to obtain food animals. L2 exceeded L1 by 11.91% in the percentage of live born piglets. Likewise, L1 was 0.16 kg higher in average weight at birth and 2.54 kg in average weight at weaning. It is concluded that although there is a difference in the genetic quality between the “daughters of grandmothers” and the “terminal line”, through the comparison of the data obtained in the calculation of the productive indices, these do not show quantitative differences that affect in greater economic performance of the establishment. It was observed that the existing differences between productive indices could be mainly due to the daily handling techniques of the animals in the establishment (the same for both lines), rather than to genetic characteristics. Finally, we can say that the inclusion of genetics through the “Grandmother’s Daughters” line is very effective for obtaining the replacement of the farm, with the maternal aptitude necessary for a favorable production, but there are no quantitative differences in the productive indices. [PROIPRO 14-1420.]

### A56

#### MATHEMATICAL MODELING OF THE GROWTH AND ENZYME PRODUCTION KINETICS OF *A. NIGER* IN SOLID STATE FERMENTATION

Gil R, Santana A, Rodríguez L, Martín ML

IBT-FI-UNSJ, 5400 San Juan. E-mail: marialuciamartin79@gmail.com

In the Cuyo Region, grape pomace (GP), grape stalk, olive pomace (OP), and alperujo are the highest volume agro-industrial waste, generated mainly by wineries, grape-juice, and olive oil industries. Solid state fermentation (SSF) is a biotechnological useful for solid agro-industrial waste reutilization since it is the substrate for culture medium formulation. It is easily implementable as it requires simple equipment with low investment cost, reduces operational cost and water consumption. Obtained enzymes made through SSF are one of the most successfully obtained products and commercially spread. It reaches high biological activities, so the process economic profitability is guaranteed. In previous studies, the optimal values of the variables for *A. niger* growth and enzyme production were found in SSF with OP and GP. The objective was to obtain a mathematical model of the growth kinetics (GK) and enzymatic production (EP) of *A. niger* in SSF in a bench-scale bioreactor (BR) using a mixture of GP and OP as substrate and, thus, be able to predict the behavior to increase the scale of the process. The substrate (1.5 kg) was placed in the reactor, reaching a bed height of 7 cm. Air was supplied to the BR at a flow rate of 2 L of air per minute. The culture conditions of the BR were: glucose addition: 1.5% w/w; initial moisture content: 66% w/w; addition of micronutrient solution: 5 mL/100 g of wet substrate; temperature: 27°C; incubation time: 6 days; initial pH: 4.5; inoculum size:  $1 \times 10^7$  spores/g dry. Enzyme activities were measured in the aqueous SSF extracts. To determine the Glucosamine content, each SSF sample underwent acid hydrolysis of fungal chitin to N-acetyl glucosamine. Non-linear regression was used to model the GK and EP. The GK was fitted to a logistic growth model and the endopolygalacturonase enzyme production was adjusted to a Gaussian function. The results will allow us to advance in the search to increase the scale of the process.

**A57**

**ISOLATION AND SELECTION OF NATIVE YEAST POTENTIALLY  
USABLE IN CRAFT BREWERY**

Saavedra A, Navarta LG, Lambrese Y, Sanchez Peterle B, Juri Ayub J, Fernandez JG  
Grupo de Investigación, Desarrollo y Asistencia al Sector Cervecerero. (GIDACER). Facultad de Química, Bioquímica y Farmacia, UNSL, Ejército de los Andes 950, San Luis, Argentina. E-mail: jgastonfernandez@gmail.com

Craft beer in Argentina is an innovative sector that promotes regional productive poles. Craft production of beers gains relevance when reaches regional identity through the incorporation of local products to elaboration. The yeast is critical to beer, which involves much more than converting sugars into alcohol, it contributes to beers flavors and aroma too. With the aim to generate a technological transfer to craft breweries, we have developed an isolation and selection procedure of native yeast from grape and evaluated its potential use in beer production. The yeasts were isolated from the grape bloom. Three (3) isolates (C1, C2, and C3) strains, which were identified as *Saccharomyces cerevisiae* by biochemical tests (API 20 C BioMérieux), were selected for the study. The yeasts were inoculated in beer wort and cultivated at 20°C. In final products, there were evaluated alcohol production, transparency/turbidity, and organoleptic characteristics by sensory analysis. Commercial yeast was used as a control. The three evaluate strains show a product color similar to commercial yeast (amber yellow). But the strain named C3 showed better ability of alcohol production, with values of 3.73% alcoholic graduation, similar to control yeast (4.18%). The strains C1 and C2 showed very low values of 0.64 and 0.59%, respectively. Regarding transparency/turbidity, the strain C3 was presented limpid with low turbidity (like control), while the C1 and C2 strains showed high turbidity. Finally, the sensory analysis indicated that only C3 showed a pleasant aromatic profile and excellent taste in the mouth, with acceptable acidity. These preliminary results indicate that the C3 strain could be successfully used in craft brewery, due to its similarities to commercial yeast. Future studies are needed to adjust fermentation temperatures, batch volume, reproducibility over time, number of cells and viability necessary and conservation conditions.

**HUMAN CLINICS AND ODONTOLOGY**

**A58**

**ONYCHOMYCOSIS STUDY IN SAN LUIS CITY, ARGENTINA**

Lopresti RA<sup>2</sup>, González LE<sup>1</sup>, Lapierre AV<sup>1,3</sup>  
<sup>1</sup>Parasitología y Micología, UNSL <sup>2</sup>Hospital San Luis, Laboratorio de Análisis Clínicos Dra. Lapierre<sup>3</sup>.  
E-mail: alicia.lapierre@gmail.com

Onychomycosis accounts for up to 50% of all nail disorders. They can be caused by yeasts, dermatophytes, and non-dermatophyte molds. This study was carried out to determine the prevalence, mycological test results, etiological agents, and clinical presentation of onychomycosis. All fingernail and toenail samples taken during a two-year period at two diagnostic centers were included. A total of 532 samples were analyzed, of which 85.15% (N = 453) were from toenails and 14.85% (N = 79) from fingernails. The mean age of the patients was 38.83 ± 16.03 years, and 72% were females. In toenails, direct examination (DME) using potassium hydroxide (KOH) showed 55.63% (N = 252) positive samples, and cultures were positive in 50.55% (N = 229), similar to other studies of the same characteristics. In fingernails, the DME was positive in 44.30% (N = 35) of the samples, and 46.83% (N = 37) of the cultures was positive. Dermatophytes were prevalent in toenails of both sexes; in fingernails, yeast were prevalent in females and dermatophytes in males. Non-dermatophyte molds corresponded to 4.8% (N = 11) of toenail and 2.70% (N = 1) of fingernails isolates. As agents of onychomycosis, dermatophytes were detected in N = 210 (78.95%), yeasts in N = 43 (16.16%) and non-dermatophyte fungi in N = 12 (4.51%) patients. Among dermatophytes, *Trichophyton rubrum* was found to be the commonest etiological agent (75.24%, N = 158) followed by *T. mentagrophytes* (17.62%, N = 37). Of the samples cultured, *Candida albicans* was the most prevalent (3.72%, N = 36) yeast. The majority of fungal nail infections were characterized clinically by distal and proximal subungual onychomycosis. Early detection of onychomycosis is very important because it is more difficult to treat than most dermatophytosis due to the inherent slow growth of the nail. The prevalence is increasing, being influenced partly by its association with advanced age, lifestyle, and comorbidities. The current epidemiologic trends and knowledge of the onychomycosis causal agents demonstrate the need of new strategies for prevention and treatment of onychomycosis as health problems.

**A59**

**STUDY OF MICROSTRUCTURALLY DENTAL ENAMEL  
IN THE ACTION OF COLA BEVERAGES**

*Ingeniero MJ, Guzmán MP, Papasodaro J, Merlo D, Lazo G, Abal A, Belloni F, Barceló A, Barrasa E, Gómez Bravo F, Motta M, Ogas C, Pérez D, Pérez P, Procopio Rodríguez M, Saldías A, De Landaburu R, Tanevitch A, Doratti P, Felipe P, Loza L, Demaria V*

*Histología y Embriología. Facultad de Odontología. Universidad Nacional de La Plata (UNLP).  
Email: histofolp@gmail.com*

The frequent consumption of carbonated beverages is responsible for serious dental injuries due to its high acid content, it is a dietary habit present in the population, and the effect that they can produce on tooth enamel is of great importance in dental practice. The research about the changes that some cola drinks can produce in the morphological aspect of the adamantine tissue is undoubtedly interesting. Demineralization of tooth enamel occurs when acidity is below pH 5.5, which is the critical pH of hydroxyapatite. The objective of the work was to compare the differences in the enamel microstructure of healthy pieces that were subsequently subjected to the action of a cola drink. Healthy human dental crowns were chosen, extracted with the respective informed consent, which were cut longitudinally and later embedded in resin, worn and polished to obtain 10 samples. A commercial cola flavored drink was used to cycle for 14 days. The treatment of the samples carried out in cola was through a protocol of immersion of 3 min and then 10 min in artificial saliva, repeating the regime four times a day; at the end of each cycle, the samples were washed with running water and kept in renewed artificial saliva until the next day. The samples were metallized and observed with a scanning electron microscope (SEM). The outer enamel (radial enamel) and the inner (Hunter Schreger Band enamel) were examined and compared before and after immersion. The analysis of the images obtained was compared considering the patterns produced in the acid etching of the enamel with respect to the samples that present healthy enamel, where the characteristic arrangement of the prisms in each area is seen, being parallel to each other in the radial enamel and presenting enamel beam crossovers with BHS. The electron microphotographs of the cycled cola drink samples showed mineral loss in the prisms, translated as a depression in the center of the prism with its raised edges. Some areas were also visualized where the appearance of the prism sheath is widened. In the samples, it was also observed a thin layer of artificial saliva impregnating the adamantine surface, which masks the alterations in the enamel microstructure produced by the cola drink. The data obtained allowed us to determine the action, on the adamantine surface of the teeth, by the daily consumption of this type of beverages, illustrating clear signs of erosion. It was concluded, by means of the variations observed at the superficial level and through the microphotography of the prisms, that the considerable demineralization produced by the beverage causes irreversible alterations in the dental enamel.

**A60**

**ANALYSIS AND OBSERVATION OF THE ELECTRONIC MICROSCOPY SERVICE  
(SEM) OF TOOTH PIECES SUBJECTED TO NON-ALCOHOLIC  
SUGARED GASEOUS BEVERAGES**

*Lazo G, Abal A, Perez P, Barcelo A, Gomez Bravo F, Guzman P, Motta M, Ogas C, Saldias A, Merlo D, Barraza E, Ingeniero M, Belloni F, Procopio M, Papassodaro J, Delandaburu R, Lazo B, Tanevich A, Perez D, Loza L  
Facultad de odontología. Universidad Nacional de La Plata. E-mail: histofolp@gmail.com*

Given the increase in the daily consumption of non-alcoholic sugary beverages in recent years, teeth are affected by chemical and microstructural changes produced by the action of different beverages. Dental pieces were sectioned in the vestibule-lingual direction and were embedded in resin, abraded with sandpaper, polished to shine, and washed in ultrasound, resulting in 10 samples. Electronic Microscopy Service (scanning electron microscopy, SEM) was used to observed healthy samples and samples after being immersed for 12 min in a cola-flavored non-alcoholic carbonated drink. The samples were metallized and subjected to the corresponding protocols to be observed under SEM. The external (radial enamel) and internal (Hunter-Schreger band enamel) zones were analyzed and compared. In the radial enamel of the healthy samples, the percentage of weight (weight %) obtained by means of energy dispersive spectra (EDS), were 35.04% for elements calcium (Ca) and 18.16% phosphorus (P); and in enamel BHS 34.26% for Ca and 17.55% P. In the results obtained by EDS after treatment of the samples with the drink, the weight percentage (weight %) in the radial enamel was 33.5% Ca; 17.19% P; while in BHS enamel it was 32.62% Ca; 16.4% P. After the chemical action of the components of the drink, the cross-sectioned enamel prisms present high contours and depressed centers, while in longitudinal sections an increase in the space between rods with irregular contours is evidenced. In general, obtained results demonstrate that the acid components of the soda act by producing a demineralization of the dental tissues. We conclude that the composition of non-alcoholic sugary soft drinks has disqualifying effects on the morphological structure of radial and BHS enamel prisms.

### A61

#### ANTIBIOTIC SENSITIVITY “IN VITRO” FROM *ACTINOMYCES* SPP ADHERED TO POLIETHER-ETHER KETONE DENTAL IMPLANTS

*Butler TA, Lazo SD, Escudero E, Basal RL, Spina MS, Amaro E, Pazos F, Bentivenga N, Borrillo G, Tau F, Sararols V*

*Facultad de Odontología de la UNLP., calle 50 e/ 1 y 115. 1900 La Plata. E-mail: dikybutler@yahoo.com.ar*

The planktonic bacteria found in the oral cavity, according to various factors (presence of carbohydrates, increase oxidation-reduction potential, presence of globulin, prosthetic devices, metal implants or biomaterials, etc.), predispose mobility of other pathogenic sessile organisms that easily adhere to different substrates such as titanium, zirconium, and polyether-ether ketone (PEEK). Among them are several subspecies of the *Actinomyces* type that have been found colonizing PEEK implants, in the case of oral mucositis and/or peri-implantitis. The aim of this work was to identify the sensitivity of the most  $\beta$  lactam, cephalosporin, and macrolide antibiotics against *Streptococcus Actinomyces odontolyticus*, that frequently colonizes PEEK implants, through an antibiogram test applying the modified Kirby–Bauer technique. For this, an *in vitro* experiment, transversal work was carried out. Three different antibiotics were selected: Ampicillin 500 mg, Cephalotin 500 mg, and Erythromycin 500 mg, and a model strain *Actinomyces odontolyticus* (TCC 103). The bacterial sensitivity to the antibiotic spectrum used was performed by applying the Kirby–Bauer antibiogram technique which read-outs are the inhibition halos. The results obtained indicate that a significant difference of  $P < 0.005$  was obtained between the action of ampicillin and erythromycin or cephalothin. As conclusion, among antibiotics studied in this work, the one with the greatest inhibition halo was ampicillin 500 mg. Therefore, it would be the most indicated for the chemotherapeutic treatment of mucositis caused by the placement of a PEEK dental implant.

### A62

#### STUDY OF SPECIFIC IgE AGAINST COW’S MILK PROTEINS IN CHILDREN

*López Simón N<sup>1,2</sup>, Zéliz M<sup>2</sup>, Orellano G<sup>2</sup>, Figueroa F<sup>1</sup>, Forneris M<sup>1</sup>*

*<sup>1</sup>Cátedra de Bioquímica Clínica. Fac. Qca, Bioqca.y Fcia-UNSL. <sup>2</sup>Lab. Orellano Elorza, San Luis.*

*E-mail: mlforneris@gmail.com*

Cow’s milk protein allergy (CMPA) affects 2–3% of children, representing the most common food allergy in childhood. CMPA is defined as a reproducible adverse reaction of immune nature induced by cow’s milk protein. It may be classified into immunoglobulin E (IgE)-mediated, non-IgE-mediated, or mixed allergy, depending on the pathophysiological mechanism involved. For the diagnosis of IgE-mediated CMPA, detailed clinical history and detection of IgE specific for allergens are important tools. CMPA can affect the respiratory, gastrointestinal, and skin systems. Detection of milk-specific IgE (sIgE) indicates the presence of allergic sensitization. Our objective was to establish, in children with clinical suspicion of CMPA, the frequency of positivity of sIgE for cow’s milk protein (CMP) and if there is a correlation between the levels of total IgE (tIgE) and eIgE. This study was carried out on 256 patients aged 2 months to 12 years, who attended to a private laboratory from San Luis city between April 2018 and May 2019, with symptoms suggestive of CMPA (inclusion criteria). All patients presented with >2 symptoms suggestive of CMPA, predominantly from the digestive tract. The tIgE was determined by EQLIA (Cobas e411) and the sIgE by QLIA (Immulite 2000). Positive result for sIgE was defined as concentration  $\geq 0.35$  kUA/L. Data were expressed as a percentage, and Spearman correlation was used for non-normally distributed quantitative variables. The population was divided into three age groups: group 1 (G1: < 2 years; N = 194; 75.8%), group 2 (G2: 3–6 years; N = 45; 17.6%) and group 3 (G3: > 7 years; N = 17; 6.6%). Among the entire sample, 10.5% (27/256) of the patients were positive for sIgE: 63% (N = 17) in G1, 26% (N = 7) in G2, and 11% (N = 3) in G3. There was a male predominance among the positive group (59% males and 41% females). Levels of tIgE were increased in 70.4% (N = 19) of sensitized patients. The linear correlation between tIgE and sIgE, for positives, was low ( $r < 0.2$ ). Although the prevalence of CMPA is continuously increasing, its timely diagnosis avoids the medical, social, and economic impact on the patient and their family.

### A63

#### HORMONAL EVALUATION IN ADOLESCENTS WHO CONSULT FOR DISORDERS OF THE MENSTRUAL CYCLE

*Recabarren MV<sup>2</sup>, Oller A<sup>2</sup>, Mendoza G<sup>1</sup>, Orellano G<sup>2</sup>, Figueroa F<sup>1</sup>, Forneris M<sup>1</sup>*

*<sup>1</sup>Cátedra de Bioquímica Clínica. Fac. Qca, Bioqca y Fcia-UNSL. <sup>2</sup>Lab. Orellano Elorza, San Luis.*

*E-mail: mlforneris@gmail.com*

Menstrual disorders (MD) are common in adolescent girls. Periods can be irregular, heavy and/or painful, especially in the first few years following menarche. Although the majority of MD in adolescence are an expression of physiological changes inherent to immaturity of hypothalamic–pituitary–ovarian axis, they represent a health problem and one of the main reasons for consultation. The purpose of this study was to know the alterations in the hormonal profile of adolescents referred for menstrual complaints to a clinical laboratory from San Luis city during February 2019–2020. The study

included 141 adolescent girls aged from 12 to 19 years (mean  $15 \pm 3.1$ ). Those with a body mass index below  $20 \text{ kg/m}^2$  or greater than  $30 \text{ kg/m}^2$  were excluded. Serum levels of thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), testosterone (T), and insulin (I) were measured on days 2–4 of spontaneous menstrual cycle or gestagen-induced menstruation-like reaction by electrochemiluminescence (Cobas e-411, Roche). Concentration of androstenedione (A2) was measured by radioimmunoassay. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated (cut off value  $>2.5$ ). Descriptive statistics were used. The most prevalent menstrual problems reported were oligo-amenorrhea and dysfunctional metrorrhagia. Among teenagers, the frequency of elevated PRL, LH, A2, and TSH was 48% (N = 69), 23% (N = 33), 19% (N = 27), and 15% (N = 21), respectively. Moreover, a higher percentage of the girls (38%; N = 54) had HOMA-IR above 2.5. The lack of identification of MD affects the bio-psychosocial environment of the adolescents. In addition, they may correspond to the first manifestation of a disease or have implications for the reproductive future. In our population, the high incidence of hyperprolactinemia could explain a large part of the irregular menstrual cycles. Hyperprolactinemia should be considered in the differential diagnosis of adolescents with MD during puberty and cranial and pituitary imaging should be performed to elucidate the etiology.

#### A64

### FEASIBILITY ANALYSIS OF DIFFERENT SYSTEMS FOR THE MANUFACTURE OF DENTAL IMPLANTS WITH POLYETHER-ETHER-ACETONE

Lazo S, Butler T, Escudero E, Sararols V, Amaro E, Spina M, Bentivegna N, Borrillo G, Tau F, Basal R, Pazos F  
Facultad de Odontología de La Plata (FOLP), Universidad Nacional de La Plata (UNLP).  
E-mail: [valeriasararols@gmail.com](mailto:valeriasararols@gmail.com)

Polyether-ether-acetone (PEEK) is increasingly used in the development of dental implants, as it is a high-performance thermoplastic polymer, which has biological and mechanical properties that allow its clinical application. The objective is to evaluate whether it is possible to manufacture a PEEK implant model with the three existing production systems. For this, a descriptive, cross-sectional experimental methodology has been used. An implant has been made with 3D-modeling softwares to be manufactured by the three mentioned systems, taking into account a production process for each one: subtraction (milling machine), additive (3D printing), shaping (injection). For this purpose, a 3D modeling was generated a monobloc implant with a  $\varnothing 5\text{mm}$  thread and for each of the processes a format of the PEEK material was obtained that would allow its processing: for milling (in  $\varnothing 6\text{mm}$  bar) for roughing; in 3D printing (in  $\varnothing 1.75$  filament to be used with FDM fused deposition modeling technology) and in injection (in granules) to be melted and injected into matrices. As a result, 10 implant tests were performed with an automatic milling machine with a PEEK bar with very good definition and mechanical resistance. Then, 30 implant tests were carried out using a 3D FDM printer, which works by moving ahead in the X, Y, Z axes and deposits the filament on a layer-by-layer platform. From a software, printing parameters were configured based on the technical information of the material to start printing:  $\varnothing 1.75$  mm filament with printing  $T^\circ$ :  $390\text{--}410$  °C and  $T^\circ$  in bed of  $100\text{--}120$  °C. On the other hand, the injection process is located in the research stage for its realization. In conclusion, of the three proposed systems we have obtained tests of milled and 3D printed implants. Milled implants have good surface definition and mechanical resistance. As for the implants made with additive manufacturing, due to the crystallization characteristics of PEEK during the process, an area is generated between the nozzle and the last printed layer, where the molten material loses resolution, so it is in the process of being search for the appropriate printing parameters to reach the desired resolution.

#### A65

### HEMATOLOGICAL PROFILE AND BIOCHEMICAL MARKERS OF ADULTS WITH COVID-19 DURING THE SECOND WAVE OF THE DISEASE IN A PRIMARY HEALTH CARE CENTER

López MJ<sup>1,2</sup>; Baudo GM<sup>2</sup>, Barresi M<sup>2</sup>, Figueroa F<sup>1</sup>, Forneris M<sup>1</sup>  
<sup>1</sup>Cátedra de Bioquímica Clínica. Fac. Qca, Bioqca y Fcia-UNSL. <sup>2</sup>Hospital Atilio Luchini, San Luis, Argentina.  
E-mail: [mffigueroa@unsl.edu.ar](mailto:mffigueroa@unsl.edu.ar)

Coronavirus disease 2019 (COVID-19) has been found to be associated with the dysfunction of multiple systems in addition to respiratory failure, including hematopoietic and liver. However, abnormal hematological and hepatic findings in patients with COVID-19 have not yet been definitively explored in the local context. We retrospectively analyzed the hematological and biochemical profile of COVID-19 patients who were attending at a Public Primary Health Care Center of San Luis city, between April to June 2020 (second wave). The study included 105 unvaccinated individuals who were hospitalized between 35–65 years old ( $50.54 \pm 10.19$ ; 62% males) with a confirmed PCR diagnosis. On admission, all patients were classified into moderate/severe group and showed abnormalities on chest radiographs. Nobody received any treatment before blood sampling. Laboratory test included: complete blood count and hemoglobin (Hb) (Counter 19 Wiener), differential white cell count (peripheral blood smear), erythrocyte sedimentation rate (ESR) (Westergreen), liver biochemical parameters (AST, alanine transaminase; ALT, aspartate transaminase; TBIL, total bilirubin; DBIL, direct bilirubin and ALP, alkaline phosphatase) (InCCA analyzer), and electrolytes (Diestro analyzer). Reference values: for

white blood cells, neutrophils and lymphocytes, were 4.2–10, 1.8–7.3, and 1.5–4  $\times 10^9/L$ , respectively; ESR < 20 mm/h; AST and ALT >40 U/L, ALP >240 U/L, TBIL >10 mg/L, DBIL >2 mg/dL; Na<sup>+</sup> 135–148, K<sup>+</sup> 3.5–5.3, Cl<sup>-</sup> 98–109 mEq/L. Result: leukocytosis with neutrophilia was present in 15 males: 23.1% (12; CI: 10.7–14.4  $\times 10^9/L$ ) and 9 females: 22.5% (14.6; CI: 10.7–18.4  $\times 10^9/L$ ). The prevalence of mild anemia (Hb g/dL: men 11–12.9 and women 11–11.9) and lymphopenia were significantly higher in males than females (15.4% vs. 2.4% and 55.4% vs. 45%, respectively;  $P < 0.05$ ). The overall prevalence of mild thrombocytopenia (platelet count 100–150  $\times 10^9/L$ ) was 11.4%. Peripheral blood film showed that patients who were lymphopenic had the presence of a few reactive lymphocytes, of which a subset appeared lymphoplasmacytoid. More than 80% of patients had a high ESR (55.2  $\pm$  25mm/h). Elevated AST, ALT, ALP, DBIL, and TBIL were reported in 46.2%, 44.5%, 31.1%, 33.6%, and 12.6% of patients, respectively (negative viral hepatitis panel). Males had significantly higher median values for all liver biochemical parameters measured compared to females ( $P < 0.05$ ). Low serum concentrations of Na<sup>+</sup> and K<sup>+</sup> were detected in 28.6% (132.3  $\pm$  1.4) and 12.6% (3.2  $\pm$  0.2) of patients, respectively. For chloride, no alterations were observed. COVID-19 shows significant hematopoietic changes associated with liver dysfunction and electrolyte imbalance. Laboratory has a crucial role for the appropriate COVID-19 management since the early recognition to the assessment of disease severity and the prediction risk of evolution towards severe disease, characterized by the impairment of several organs and tissues.

### A66

#### **MOLECULAR DIAGNOSIS OF HIGH RISK-HUMAN PAPILLOMA VIRUS (HR-HPV) AS STRATEGY FOR THE PREVENTION AND DETECTION OF CERVICAL CANCER AT THE EARLY STAGES IN WOMEN OVER 30 YEARS OLD**

*Rosales E<sup>1</sup>, Bonilla J<sup>1</sup>, Cabral F<sup>1</sup>, Peñalva J<sup>1</sup>, Mastrodonato A<sup>1</sup>, Esteves B<sup>1</sup>, Molina L<sup>1</sup>, Jofre F<sup>1</sup>, Aguilera L<sup>1</sup>, Campos L<sup>1</sup>, Icazatti A<sup>1</sup>, Quijano F<sup>1</sup>, Pérez M<sup>1</sup>, Carrizo E<sup>1</sup>, Sanchez L<sup>1</sup>, Guevara A<sup>1</sup>, Rodriguez D<sup>2</sup>, Dufour R<sup>2</sup>, Lacaze A<sup>1</sup>, Talia JM<sup>1</sup>*

*<sup>1</sup>Programa Laboratorio de Salud Pública “Dr. Dalmiro Pérez Laborda” (PLSP). <sup>2</sup>Programa de Prevención de Cáncer de Cuello Uterino (CCU), Ministerio de Salud San Luis, Argentina. E-mail: lbmsanluis@gmail.com*

The human papillomavirus (HPV) is the most important etiological agent related to cervical cancer (CC). This virus is mainly sexually transmitted, and, for this reason, it appears with high prevalence in sexually active women. The most frequent oncopathogenic HPV genotypes related to cancer are 16-HPV and 18-HPV. The low-risk strains 6-HPV and 11-HPV cause genital warts, but they are not associated with cancer. The discovery of the causal correlation of CC with High Risk-HPV strains (HR-HPV), added to the long pre-neoplastic and pre-invasive period of the disease, gives us the opportunity to track down the disease through the Papanicolaou test (PAP) and molecular diagnosis. The objective of this work is to confirm the effectiveness of the molecular diagnosis for the early detection of HPV-oncopathogenic strains prior to the appearance of lesions, which are observable with PAP. Since June 2019, the PLSP began the molecular diagnosis of HPV in women older than 30 years by the Cobas® 4800 HPV Test System, approved by the FDA (Roche Molecular Systems Inc., Alameda, CA, USA), where HR-HPV DNA is detected and genotyped by real-time PCR. The PAP data was obtained from SITAM (Ministerio de Salud de la Nación). This assay simultaneously detects a total of 14 HR-HPV types: 16-HPV individually, 18-HPV individually, and 12 pooled HR-HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). In our study, 3647 endocervical cytology specimens were collected from women over 30 years old. A total of 613 samples (16.80%) were positive for the HPV test. Among these positive samples, 159 (25.94%) presented observable PAP-lesions, 339 (55.30%) did not present an observable lesion and 115 samples (18.76%) were only diagnosed for HPV using the molecular method, in the absence of PAP. In all the age-ranges evaluated (30–35 years, 36–40 years, 41–45 years, 46–50 years and >50 years) we observed a highly significant percentage of samples positive for the HPV test without observable lesion in the PAP, or only diagnosed for HPV using the molecular method. The obtained results allow us to affirm that the molecular detection of HR-HPV carried out in our laboratory is of great value for the prevention of CC in women older than 30 years. The early detection of HR-HPV strains allows us to start monitoring the patient immediately and prior to the appearance of lesions or CC, with great chances of overcoming the disease without major inconveniences.

### A67

#### **SEROLOGICAL PROFILE IN CHILDREN WITH SUSPECTED CELIAC DISEASE IN SAN LUIS POPULATION**

*Rodríguez A<sup>2</sup>, Soloa P<sup>2</sup>, Fernández K<sup>2</sup>, Orellano G<sup>2</sup>, Figueroa F<sup>1</sup>, Forneris M<sup>1</sup>*

*<sup>1</sup>Cátedra de Bioquímica Clínica. Fac. Qca, Bioqca y Fcia- UNSL. <sup>2</sup>Lab. Orellano Elorza, San Luis.*

*E-mail: mffigueroa@unsl.edu.ar*

Celiac disease (CD) is an immune-mediated, chronic systemic disease triggered by the intake of gluten-containing grains, which affects the small intestine of genetically predisposed individuals. CD in children begins with the incorporation of cereals in the diet. It is associated with growth retardation, gastrointestinal disturbances, anemia, and conduct disorders. CD is evaluated in the context of clinical signs, serologic markers, haplotypes, and intestinal biopsy. Undiagnosed and untreated disease is associated with short-term and long-term complications. The aim of the study was to determine the presence of specific autoantibodies [anti-tissue transglutaminase (tTG), anti-endomysial (EMA), or anti-deamidated

gliadin peptides (DGPs)] in children with compatible symptoms of CD. Symptoms were reported by the parents. This was a descriptive study conducted between January and December 2019. A total of 200 patients (1–10 years; mean 5 ± 2.7; 53% males) who attended in a private clinical laboratory for serological CD screening were included. Each serum was tested for total IgA (by turbidimetry) and serological markers: IgA/IgG anti-tTG were measured using an enzyme-linked immunosorbent assay (ELISA) with a cut-off value > 20 U/mL; IgA EMA by indirect immunofluorescence method, considering as positive a dilution value > 1:5; and IgA/IgG anti-DGPs using ELISA, with a cut-off value > 20 IU. Descriptive statistics were used, and continuous variables were described by median and confidence intervals (CI). IgA tTG were detected in seven (3.5%; 5 girls/2 boys) out of 200 patients (median: 48.4 U; CI: 31.6–65.2). Of them, six (85.7%) were positive for IgA EMA and four (57.1%) were positive for anti-DGPs. Four (2%) patients with normal anti-tTG levels had high IgG anti-DGPs titers, all of them corresponding to children younger than 3 years. Selective IgA deficiency was evidenced in 2 children. All cases of CD found through serologic screening were confirmed by biopsy. No alteration was observed in the serum levels of hemoglobin, glucose, cholesterol, and liver enzymes. This study highlights the importance of close monitoring of patients who present to CD-related symptoms. In addition, in well selected cases, diagnosis based on level of specific autoantibodies could avoid risks of endoscopy. It can be concluded that a greater understanding of the heterogeneous presentation of CD in childhood, together with access to serological screening studies, will lead to a reduction in morbidity and mortality associated with untreated disease.

### A68

#### IMPACT OF THE COVID-19 PANDEMIC ON THE MENTAL HEALTH OF OLDER ADULTS

*Perarnau MP, Fasulo SV  
Facultad de Psicología. Universidad Nacional de San Luis  
E-mail: veronica.fasulo@gmail.com; ariapaulaperarnau@gmail.com*

During the 2020 SARS-CoV-2 pandemic, the elderly were pointed as the most vulnerable population. An imminent question was: how would the health variables be affected in this risk group? The aim of this study was to analyze the context of the elderly in the face of the pandemic situation and expose the main prevention measures in this vulnerable group. During April 2020 and using an online form designed for this study, N = 300 adults older than ≥ 60 (68.07 SD 6.46.), 75.3% (N = 226) women and 24.7% (N = 72) men were asked to answer some questions. Demographic data such as marital status, housing, educational level, income, social work, adherence to isolation, work activities, previous pathologies and physical activity were evaluated. Regarding previous pathologies, we observed that: 27% showed no diagnosis of diseases, 38.6% had diagnosis of one disease, 21.6% with 2, 9.3% with 3, and 0.33% with 4 or more diagnoses. Prevaling arterial hypertension 46.66%, hypothyroidism 23.3%, diabetes 13.3%, cardiovascular problems 14%; obesity 10%, and to a lesser extent asthma / psychiatric disorders / cancer / neurological disorders. 52.6% reported some effect on the mood, 17% quite a lot, 3.6% a lot and 26.6% without affectation. 92% suffered damage in life in general. 73% reported between 1 and 6 symptoms / emotional states during quarantine: anxiety 36.6%, sadness 22.6% and mood changes 27.6%. Sleep routines were affected in 29.6%, daily routines 28.6%, social relationships 27.6%, more sedentary lifestyle 23.3%, psychological health 16.5%, nutrition 15.6% and health in general 14.6%. 14.66% without effects. A reevaluation and new questions would help us to have a retrospective assessment of what the greatest difficulties were faced by this age group.

### A69

#### KINETICS OF ANTI-SPIKE SARS-CoV-2 ANTIBODIES POST SPUTNIK V HOMOLOGOUS-SCHEME VACCINATION IN HEALTH PERSONNEL

*Jofre F, Bonilla J, Molina L, Campos L, Mastrodonato A, Icazatti A, Quijano F, Esteves B, Aguilera L, Carrizo E, Sanchez L, Pérez Díaz M, Rosales E, Cabral F, Peñalva J, Guevara A, Olivera M, Rivero G, Bohn J, Lacaze A, Talia JM  
Programa Laboratorio de Salud Pública (PLSP), San Luis, Argentina. E-mail: lbmsanluis@gmail.com*

In Argentina, the vaccination campaign against SARS-CoV-2 began on December 29, 2020. Sputnik V, developed by the Gamaleya National Center for Epidemiology and Microbiology in Russia, was the first vaccine to be applied in our country. This is the first vaccine registered in the world based on a platform of non-replicating human adenovirus vectors (Ad26 and Ad5) and it consists of two applications. The Phase 1 and Phase 2 clinical trials were completed on August 1, 2020, and the results of the Phase 3 were published in *The Lancet* on February 2, 2021. The vaccine is genetically modified to encode the production of SARS-CoV-2 Spike protein in the host. Around two weeks after inoculation, the immune system initiates the response by producing specific antibodies against this viral component, among others. The aim of the present study was to evaluate the kinetics of the production of specific anti-Spike SARS-CoV-2 antibodies (Anti-S), generated after receiving the complete Sputnik V homologous-scheme, in workers of the Laboratorio de Salud Pública “Dr. Dalmiro Pérez Laborda”. A longitudinal prospective study was carried out in 35 individuals from 21 to 50 years old, with prior informed consent. Serum samples were obtained at 60, 120, 180, and 240 days after the administration of the second dose of Sputnik V, excluding individuals who were infected with the virus at each point. The concentration of total anti-Spike antibodies was evaluated by using the Elecsys Anti-SARS-CoV-2 S kit, employing

the electro chemiluminescent method (EQLIA e411, Roche®). Subsequently, Anti-S and Anti-RBD IgG antibodies were determined with the ELISA COVIDAR IgG Quantitative kit. At 60 days after completing the vaccination scheme, we obtained an arithmetic mean ( $\bar{x} \pm SD$  (Min; Max) U/mL) of  $245.37 \pm 168.83$  (35.75; 863.50) for total Anti-S antibodies and  $1227.00 \pm 844.14$  (169.00; 4318.00) for IgG Anti-S. At  $t = 120$  days, we obtained similar results:  $202.45 \pm 130.60$  (48.00; 665.00) and  $986.00 \pm 670.30$  (240.00; 3325.00) for total Anti-S and IgG Anti-S, respectively. In the evaluation at 180 days, the values obtained were  $192.88 \pm 139.49$  (29.00; 685.00) for total Anti-S antibodies and  $1003.00 \pm 714.18$  (145.00; 3425.00) for IgG Anti-S antibodies. Finally, at 240 days, we determined  $184.25 \pm 131.66$  (38.00; 540.00) for total Anti-S and  $925.64 \pm 720.00$  (175.00; 3850.00) for IgG Anti-S. The results of the present study show a humoral immune response in 100% of the evaluated individuals at 60 days after completing the vaccination scheme. Furthermore, this work shows that the vaccine application produces an immune response which is maintained over time without significant differences, both for total Anti-S SARS-CoV-2 antibodies and for Anti-S IgG antibodies. Taking into account the beginning of the vaccination campaign in Argentina (275 days to 09/30/2021), this work shows almost complete coverage (240 days), being one of the most complete so far in the country.

## A70 STRATEGIES FOR EPIDEMIOLOGICAL SURVEILLANCE OF SARS-COV-2 VARIANTS IN SAN LUIS

Lacaze A, Jofre F, Bonilla J, Molina L, Icazatti A, Aguilera L, Cabral F, Campos L, Carrizo E, Esteves B, Guevara A, Mastrodonato A, Peñalva J, Pérez Díaz M, Quijano F, Rosales E, Sánchez L, Talia JM  
Programa Laboratorio de Salud Pública (PLSP) “Dr. Dalmiro Pérez Laborda”, Ministerio de Salud, Ministerio de Salud San Luis, Argentina. E-mail: lbmsanluis@gmail.com

The occurrence of mutations is a natural and expected event within the evolutionary process of viruses. The massive spread of the SARS-CoV-2 led to the accumulation of mutations within the viral genome, generating new variants. The capacity to monitor the data in real time impacts directly on the COVID-19 pandemic public health response. The knowledge of the community circulation of Variants Of Concern (VOCs), such as  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ , or Variants Of Interest (VOIs), as  $\lambda$  and  $\zeta$ , requires sampling and sequencing strategies to ensure representativeness and reliability of the results. In San Luis, the PLSP has access to COVID-19 positive samples and works closely with the *Programa de Epidemiología*. Our health institution applied two different surveillance strategies to detect the SARS-CoV-2 variants. The first strategy consisted of whole or partial genome sequencing, which were carried out at ANLIS-Malbrán and PAIS Project. On the other hand, a PCR screening strategy was developed by researchers from the UNSL and implemented for the early detection of the VOC $\delta$ . A total of 169 positive samples were analyzed applying the first strategy among epidemiological weeks (EW) 14 to 36 in 2021. 113 samples were selected by regular sampling, taking into account high transmission areas, different age ranges, geographic location and severity. Additionally, 38 immunized people, 7 samples suspected of reinfection and 7 travelers were analyzed. The main variants found were:  $\gamma$  (43%),  $\lambda$  (11%),  $\alpha$  (5%),  $\zeta$  (1%), and other Not VOC/VOI (27%). Interestingly, since EW 19, the proportion of cases associated with VOC $\gamma$  and VOI $\lambda$  increased while those associated with VOC $\alpha$  decreased. The second strategy was applied on 1367 samples from EW 25 to 40 and VOC $\delta$  was not detected as a circulating variant in San Luis. In this work we show genomic evidence of local transmission of SARS-CoV-2 VOC  $\alpha$ ,  $\gamma$  and  $\lambda$ . The  $\zeta$  variant was only found in one traveler who returned from Colombia. It should be noted that, according to the results obtained in this work, the VOC $\gamma$  and VOI $\lambda$  are the main circulating variants in the province, while VOC $\delta$  is not detected nowadays. The results were opportunely communicated to the authorities to be used for the benefit of public health.

## A71 SARS COV-2 CONFIRMATORY DUPLEX RT-PCR ASSAY BY DNA-MELTING ANALYSIS

Aguilera LJ<sup>1</sup>, Lacaze MA<sup>1</sup>, Talia JM<sup>1</sup>  
<sup>1</sup>Laboratorio de Salud Pública “Dr. Dalmiro Pérez Laborda”. E-mail: lbmsanluis@gmail.com

SARS CoV-2 had spread rapidly across the planet, with the consequent appearance of new virus variants with more aggressive behaviors both in dissemination among the population and in affecting human health. Therefore, sensitive alternatives for fast and reliable detection are needed, to confirm viral infection and help stopping the virus spread. In this study, carried out at the “Laboratorio de Salud Pública Dr. Dalmiro Pérez Laborda”, a confirmatory duplex RT-PCR assay for SARS CoV-2 was developed. The main objective of the assay is to detect the presence of two viral targets in nasopharyngeal swab specimens: A fragment of the envelope protein (E) gene and a fragment of the RNA-dependent RNA polymerase (RdRP) gene. Here we describe a different strategy for SARS CoV-2 PCR-based detection by employing E and RdRP probes labeled with the same fluorophore in a duplex reaction. RdRP signal was characterized with a probe-based fluorescence melting curve analysis in a DNA melting analysis (DMA). Likewise, the E gene was detected in a qualitative probe-based RT-PCR assay. Primers and probes (FAM labeled) were obtained from the Charité Hospital (Berlin, Germany). Experiments were performed in a real-time PCR instrument (CFX96 Touch Real-Time PCR Detection System, BioRad®) with LightCycler® Multiplex RNA Virus Master enzyme mix. The present protocol was

validated with positive samples for SARS CoV-2 from San Luis, which were obtained using different extraction methods (TIANamp Virus RNA Kit, lysis with proteinase K and heat shock, and automated system GenePure Pro). With our RT-PCR duplex E/RdRP assay, the detection of the E gene using the cycle threshold analysis (Ct) obtained in real-time RT PCR was possible. The analysis also confirmed the viral presence by detecting the melting peak signal from RdRP. The duplex analysis is faster, cheaper, more accurate and avoids false negative results that may be caused by mutations in one of the viral genes.

## MICROBIOLOGY AND IMMUNOLOGY

### A72

#### GROWTH OF THE GREEN MICROALGAE *Scenedesmus* sp. USING SYNTHETIC WASTEWATER

Saavedra A<sup>2</sup>, Daruich J<sup>1</sup>, Ferrari SG<sup>2</sup>

<sup>1</sup>Área Biología. <sup>2</sup>Área Microbiología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. E-mail: aldanasaavedra1@gmail.com

*Scenedesmus* sp. is a fast-growing photosynthesizing organism, capable of using energy from sunlight and CO<sub>2</sub> from the atmosphere to synthesize organic compounds. Domestic wastewater has been used as a substrate for both microalgae biomass production and nutrient removal. This biological treatment provides aeration and reduces operating costs. The objective of this work was to study the growth of *Scenedesmus* sp. in a synthetic wastewater (SW), for its potential use in the removal of N and P from local water bodies. *Scenedesmus* sp. grew up in static and stirred cultures at 25 ± 2 °C, with continuous illumination of 3000 lux for 12–14 days in Bold Basal Medium (BBM) and in SW (N = 1 mg/L; P = 0.5 mg/L). The growth was evaluated by optical density (OD) measurements at 580 nm and chlorophyll a and b content. The specific growth velocity ( $\mu$ ) of *Scenedesmus* sp. did not show significant differences under stirred conditions ( $P > 0.05$ ), however the biomass production was 2.78 times higher than SW. In static cultures,  $\mu$  for the culture in SW (0.0282 day<sup>-1</sup>) doubled  $\mu$  for BBM (0.0132 day<sup>-1</sup>), but the stationary phase was reached at 6 days obtaining the same final OD<sub>580</sub> for both cultures. The morphology of the cells showed differences in size in SW with respect to BBM. At the end of the culture in the static conditions, the cenobia of 4 cells (77.04%) predominated in SW, while in BBM the percentage of unicells was the majority (67.07%). For stirred cultures, the percentages were inverted with 78.40% of unicells in SW and 70.76% of four-cell cenobia in BBM. The stirring process increased total chlorophyll content of *Scenedesmus* sp. with the highest total chlorophyll concentration of 7.48 ± 0.021 µg/mL in BBM, with 77.93% corresponding to chlorophyll a. In contrast, cells grown in SW had similar proportions of the different types of chlorophyll, achieving a final composition of 44.86 Chl a and 55.14% Chl b. Therefore, this indicates that the proportion of different types of chlorophyll may be altered in different culture conditions. *Scenedesmus* sp. was able to grow in SW for 6 days, at maximal velocity, both in stirred and static conditions. These results suggest that *Scenedesmus* sp. could be used to remove N and P from wastewaters, enhancing nutrient removal, and also obtaining biomass for other purposes.

### A73

#### EVALUATION OF SANITIZING CABINS, AS A COMPLEMENTARY ELEMENT, FOR THE PREVENTION OF COVID-19

Salinas AP<sup>1</sup>, Lijteroff RE<sup>2</sup>

<sup>1</sup>Área de Biología. Departamento de Biología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. <sup>2</sup>Área de Educación. Departamento de Biología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. E-mail: asalinaskenny@gmail.com

The sanitizing cabins have been used in China, where began outbreak and more lately provoke pandemic of SARS-CoV-2. They were also used in other countries with a large number of habitants of Asian, European, and American origin. The product used to spray people was hypochlorous acid, a colorless liquid that has a slight chlorine smell with variable physical properties depending on its concentration; it is not toxic and does not attack the skin. Disinfection occurs in seconds, due to its high disinfectant spectrum. It is one of the recommended products on EPA's "N List", as an agent against emerging viral pathogens and specifically against human coronavirus (SARS-CoV-2). The product is considered suitable for medical, institutional, and residential use. The aim of the study was to test the bactericidal effect of hypochlorous acid at different concentrations in the sanitizing cabin. Bacteria and fungus were used as an experimental model. Different tests were carried out, the first one was to verify the uniformity of the dispersion of the fluid on a flat surface and it was carried out with distilled water, the vehicle used to make the subsequent dilutions of the saline compound for the generation of hypochlorous acid. Subsequently, three solutions were tested, at different concentrations (100 ppm, 250 ppm, and 500 ppm) to test the efficacy on pathogens of bacterial origin. The results of the plaque fumigation with the hypochlorous acid solution showed efficacy on *Escherichia coli* at concentrations of 100 ppm, 250

ppm, and 500 ppm and on *Staphylococcus aureus* at 500 ppm. The study was extended to the effect by flood-plate where the results showed efficacy on *Salmonella* sp., *Pseudomonas aeruginosa*, *Candida albicans*, and *E. coli*, at concentrations of 100 ppm, 250 ppm, and 500 ppm. We concluded that the cabin spray technique was not totally effective for the pathogens analyzed. Assuming that it would not be effective for SARS-CoV-2; it could be used as a complementary measure, of actions with proven efficacy, such as social distancing, hand washing, avoiding touching the face and the use of masks, for the prevention of COVID 19.

#### A74

##### ***Baccharis salicifolia* ESSENTIAL OIL EFFECT ON *Listeria monocytogenes* GROWTH**

Aliandro OE<sup>1</sup>, Centorbi HJ<sup>1</sup>, Satorres SE<sup>1</sup>, Kurina-Sanz MB<sup>2</sup>, Mattana CM<sup>1</sup>

<sup>1</sup>Área Microbiología e Inmunología, Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis.

<sup>2</sup>Instituto de Investigaciones en Tecnología Química (INTEQUI), Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, CONICET. San Luis, Argentina. E-mail: 768centorbi@gmail.com

*Listeria monocytogenes* is a Gram-positive rod-shaped facultative anaerobe widely distributed in the environment, with great ability to adapt to external stress and grow in a wide range of temperatures. This microorganism is an important human foodborne pathogen that can be isolated from raw or processed food. Manufacture of minimally processed fresh products presents great challenges for quality and safety, therefore preservative and antimicrobial agents are frequently used for the conservation of ready-to-eat food. *Baccharis salicifolia* (R.& P.) Pers. (Asteraceae), popularly known as “chilca amarga” or “jarilla de río” is a shrub that is part of the native flora of the Province of San Luis, Argentina. The objective of this work was to investigate the antibacterial activity of the essential oil (EO) of *B. salicifolia* against *L. monocytogenes*. The *L. monocytogenes* CLIP 74904 strain was grown in brain heart infusion under aerobic conditions. Overnight cultures were diluted 1:25 in different Erlenmeyer flasks containing 100 mL of basal culture medium (BM) g/L: proteose peptone, 30, yeast extract, 5; trypticase, 5; glucose, 2; pH 7.0. The EO was dissolved in 20% Tween 80, and then, dilutions were made with phosphate buffer saline (PBS) to the concentrations to be analyzed. The BM was supplemented with the following concentrations of *B. salicifolia* EO ( $\mu\text{g/mL}$ ): 0 (control), 0.24, 0.46. Cultures were incubated at 37°C for 20 h under aerobic conditions with orbital shaking (80 rpm). The cell turbidity was monitored in duplicate samples taken at various times throughout the culture period, to measure the optical density (OD) at 600 nm. The specific growth rate ( $\mu$ ), lag period (L), and the maximum biomass ( $\text{OD}_{\text{max}}$ ) were calculated from the growth curves. Under exposure to 0, 0.24, and 0.46  $\mu\text{g/mL}$  of *B. salicifolia* EO, the values obtained were as follows,  $\mu$  ( $\text{h}^{-1}$ ): 0.57, 0.39, 0.26; L (h): 0.91, 3.81, 9.87, and  $\text{OD}_{\text{max}}$ : 1.51, 1.09, and 0.70, respectively. These results demonstrated the important inhibitory effect exerted by *B. salicifolia* EO on the growth of *L. monocytogenes*, which is of interest in the development of edible films and coatings with functional properties. This new technology is environmentally friendly and offers substantial benefits to increase the shelf life of many food products, including fruits and vegetables. The addition of natural compounds to preserve minimally processed foods represents a technological challenge for the industry and a very active field of research throughout the world.

#### A75

##### **PARTIAL CHARACTERIZATION BY ULTRAVIOLET SPECTROSCOPY OF ANTI-*Yersinia* METABOLITES FROM *Bacillus velezensis* SL-6**

Cozzolino ME, Silva PG

Área Microbiología e Inmunología, Facultad de Química, Bioquímica y Farmacia, UNSL.

E-mail: marianacozzolino@gmail.com

Polyketides and non-ribosomal peptides are important antimicrobials in the control of human infections. Among the polyketides, diffidin, oxydiffidin, bacillaene, and macrolactin are the main compounds produced by members of the genus *Bacillus*. They are obtained from the supernatants by liquid-liquid extractions with different organic solvents and specific cares due to their high chemical instability. The present work describes the separation and partial characterization of potential polyketides produced by *B. velezensis* SL-6 with antibacterial activity against *Yersinia enterocolitica*, causal agent of yersiniosis. Cell-free supernatant (CFS) was obtained by batch culture, centrifugation, and filtration. Then, it was subjected to extractions with ethyl acetate, dichloromethane, chloroform and acid precipitation, followed by methanolic concentration. The antibacterial activity (AA) in the aqueous and organic fractions against *Y. enterocolitica* was estimated by inhibition zone diameters. In addition, the AA in CFS and the methanolic extract from acid precipitation (ME 10 $\times$ ) was quantified in arbitrary units per milliliter (AU/mL), and a subsequent separation of the metabolites was carried out by thin layer chromatography, using n-butanol-methanol-water (39:20:10 v/v/v) as mobile phase. The bands were visualized under ultraviolet light (UV 254 nm) and developed with ninhydrin, water and Van-Urk's reagent, using contact bioautography to detect bioactivity. Then, the bioactive band was scraped and eluted with methanol and characterized by ultraviolet spectroscopy (UV 200–400 nm). All the tested extraction systems could concentrate the active metabolites against *Y. enterocolitica* without detecting AA in the aqueous phases with values of 400 and 3200 AU/mL in the CFS and ME 10 $\times$ , respectively. The chromatogram showed several bands under UV light, recovering a single bioactive fraction (Rf 0.59–0.65). This fraction was negative with ninhydrin, positive with Van-Urk's reagent, showing a very slight reaction with water that would indicate presence of non-ribosomal lipopeptides in the bioactive

band, at Rf values referenced for bacillomycin D. Ultraviolet spectrum presented a characteristic peak of maximum absorption at 272.6 nm, including shoulders at 263 and 283 nm, which indicate the presence of a triene group similar to difficidin and oxydifficidin compounds. Therefore, *B. velezensis* SL-6 could be producing conjugated triene compounds with antagonistic activity against *Y. enterocolitica*, similar to other polyketides described, together with non-ribosomal peptides characterized in previous works. Subsequent studies should be carried out to purify and confirm the chemical structure of these trienes as responsible for anti-*Yersinia* activity.

#### A76

### ANTIMICROBIAL ACTIVITY OF REGIONAL (SAN LUIS, ARGENTINA) *Lactobacillus* AND *Lactococcus* sp. AGAINST PATHOGENIC MICROORGANISMS

Giorggi A, Mitjans N, Stagnitta PV

Área Química Biológica, Bloque I Primer piso, Ejército de los Andes 950, UNSL, 5700 San Luis.

E-mail: pvstag@unsl.edu.ar

Lactic acid bacteria (LAB) isolated from dairy products have received increased attention as potential food preservative due to their antagonistic activity against many foodborne pathogens and, also, for their action against agents causing human diseases. LAB is widely distributed throughout nature, they are typically involved in a large number of the spontaneous food fermentation, and they have been extensively studied. Some members of LAB produce bacteriocins and bacteriocin-like substances that can inhibit the growth of pathogenic and spoilage microorganisms. The purpose of this study was to determine the antimicrobial activity of LAB strains isolated from goat milk samples collected from stainless steel drums in a dairy farm (San Luis, Argentina). LAB strains were typified by API CH50 as *Lactobacillus paracasei*, *Lactobacillus fermentum*, and *Lactococcus lactis*. Antimicrobial activity of cell-free culture supernatant (CFCS) was determined by liquid medium method against *Enterococcus faecalis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, and *Candida albicans*. Organic acid production was estimated by the difference between antimicrobial activity of untreated and neutralized CFCS. Antimicrobial activity of CFCS from the three LAB strains reached values of growth inhibition between 75% and 90% against all microorganisms tested. By neutralizing CFCS from *L. paracasei* the loss of inhibitory effect against *P. aeruginosa* and *E. faecalis* was observed, as well as a decrease of inhibition against *L. monocytogenes*. Similar results were observed by studying the inhibitory effect of neutralized CFCS from *L. fermentum*. By comparing antimicrobial activity of CFCS and neutralized CFCS from *L. lactis*, similar results were observed against most of antimicrobial activity indicators. However, about 50% of the inhibitory effect of neutralized CFCS from *L. lactis* against *L. monocytogenes* was lost. These results lead us to conclude that antimicrobial activity of *Lactobacillus* strains against *E. faecalis* and *P. aeruginosa* could be due to organic acids in CFCS. In addition, antimicrobial activity against *Y. enterocolitica* and *C. albicans* remained high after neutralization which might be attributed to the production of other inhibitory substances. It remains to be clarified the chemical nature of these substances in order to establish if they are bacteriocins. It is possible to affirm that lactic acid bacteria studied in this research are able to inhibit the growth of *E. faecalis*, *L. monocytogenes*, *P. aeruginosa*, *Y. enterocolitica*, and *C. albicans*. This characteristic could promote the use of these strains as probiotics or as possible inhibitors of microorganisms that cause human diseases.

#### A77

### DIFFERENT METABOLIC RESPONSES AFTER ORAL INFECTION WITH *Yersinia enterocolitica* SEROTYPE O:8 or O:3 IN MICE

Pelegrina Cali M, Velazquez M, Di Genaro MS

Laboratorio de Inmunopatología y Citometría de Flujo (LABIP-CF), Universidad Nacional de San Luis, Instituto Multidisciplinario de Investigaciones Biológicas-San Luis (IMIBIO-SL) (CONICET-UNSL).

E-mail: sdigena@unsl.edu.ar

*Yersinia enterocolitica* (Ye) is a zoonotic pathogen that causes bacterial gastrointestinal infections in humans. Recent studies have revealed a relationship between metabolism and the immune response to infections. However, the physiologic responses to the infection with different strains of Ye are poorly known. Here, we compared the metabolic changes in response to gastrointestinal infection with *Y. enterocolitica* strains representing the highly pathogenic phylogroup 2 (serotype O:8) and moderately pathogenic phylogroup 3 (serotype O:3). In the 2 h-fasted state, male C57BL/6 mice (between 8 and 14 weeks of age) were orally infected with  $1 \times 10^8$  UFC/mouse. Non-infected mice were used as control. After 4 hours (acute phase) or 5 days (chronic phase) of infection, at the end of the light period (18 h) and in 6 h-fasted state, whole blood was harvested from mice by submandibular bleeding, and serum was isolated. Glucose and urea concentration were assayed using the corresponding commercial kit modifying the manufacturer's protocols to a micro-method. In addition, survival and weigh changes were evaluated for 21 days. The experiments were conducted with prior approval from the Animal Care and Use Committee of UNSL. We found that mice infected with Ye O:8 survived less (75%) than those infected with Ye O:3 (100%). After 24 h of infection, significant loss of weight was detected in Ye O:8-infected mice compared with control and Ye O:3-infected mice ( $P < 0.01$ ). In contrast to Ye O:3, Ye O:8 increased the serum glucose levels in acute (4 h) and chronic (5 days) phases of infection ( $P < 0.01$ ). Conversely, 4 h after infection, Ye O:3 increased serum urea level ( $P < 0.01$ , compared with control group). Our results suggest that

diverse metabolic changes in the host could be involved in the different virulence of the serotypes O:8 and O:3 of Ye. Further immune-metabolic studies will be performed to explain the mechanisms involved in the different host responses to these infections.

## A78

### BIOCONTROL OF *Penicillium* sp. PRODUCER OF BLUE ROT IN STORED APPLES

Cáceres M, Lambrese Y, Sansone G, Yamanouchi P, Sanz Ferramola MI, Calvente V  
Área de Tecnología Química y Biotecnología, Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia. E-mail: yesicalambrese@gmail.com

*Penicillium* is a filamentous fungus of which several species cause rot in fruits and vegetables, particularly *Penicillium expansum* can cause blue rot in stored apples. Synthetic fungicides are used as chemical treatment, but the existence of resistant strains that would require the use of larger amounts of these is recognized; biological control, using antagonist saprophytic microorganisms that effectively compete with the pathogen is a control alternative. The objective of the work was to study in “Red delicious” apples three Biological Control Agents (BCA) to control *Penicillium* sp. resistant to commercial fungicides. The microorganisms used were *Penicillium* sp. INTA 6 isolated from blue rot in the Alto Valle of Río Negro and the BCA: *Cryptococcus laurentii*, *Rhodospidium fluviale*, and *Kosakonia radicincitans*, isolated from healthy apples from Mendoza and San Luis. Initially, the phytopathogen was selected among 13 strains of *Penicillium* sp., challenging them against five fungicides used in Argentina. Petri dishes with Potato Dextrose Agar (PDA) medium inoculated with a suspension of the fungus were used, then holes were made that were filled with each fungicide (CAPTAN50WP, TECTO@50SC, PENBOTEC@400SC, SCHOLAR@23SC, CARBENDAZIM50SC) at the dose used in postharvest treatments. The plates were incubated at 25°C, and Mycelium Growth Inhibition (cm) was determined at 3 and 7 days. The biocontrol tests were performed on commercial apples washed and then wounded to inoculate them with each BCA and/or pathogen. Apples were stored 10 days at 25°C and the diameter of the rots was measured (mm). The rot diameters ( $\phi$ ) of the control and treatment were measured and the results were expressed as % Disease Incidence (%DI: [(rotten wounds in treatment / rotten wounds in control)  $\times$  100] and % Severity Reduction (%SR: [( $\phi$  control –  $\phi$  treatment) /  $\phi$  control]  $\times$  100) at 5 and 10. At the end of the trial, all the rotten tissue (control and treatments) was removed, weighed on an analytical balance and % SR was calculated. *Penicillium* sp. INTA 6 showed Mycelium Growth Inhibition with CAPTAN50WP (1.53 and 0.9 cm) and SCHOLAR@23SC (3.1 and 2.07) at 3 and 7 days respectively, but it was totally resistant to TECTO@50SC, PENBOTEC@400SC, and CARBENDAZIM50SC at 7 days. In apples, the three BCA controlled blue rot. After 5 days %DI for *C. laurentii* and *R. fluviale* was 0%, while for *K. radicincitans* it was 11%. At 5 days, the %SR for *C. laurentii* and *K. radicincitans* were 79% and *R. fluviale* of 80%. At 10 days %SR increased when the yeasts (*C. laurentii* or *R. fluviale*) were applied, but for the bacteria (*K. radicincitans*) the %SR was maintained over time. Finally, the rotten tissue was weighed and the %SR for *C. laurentii* was 97% and *R. fluviale* of 93% and for *K. radicincitans* was 44%. It is concluded that the implementation of BCA is a very good alternative to control *Penicillium* sp., resistant to fungicides and producer of blue rot in stored apples.

## A79

### RESEARCH AND DEVELOPMENT OF NEW IMMUNOTHERAPEUTIC STRATEGIES AGAINST AMERICAN TEGUMENTARY LEISHMANIASIS

Arrieta VJ<sup>1</sup>, Germanó MJ<sup>2</sup>, Bruna FA<sup>2</sup>, Sánchez MV<sup>2</sup>, Lozano ES<sup>2,3</sup>, Cargnelutti DE<sup>2,3</sup>

<sup>1</sup>Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia. <sup>2</sup>Instituto de Medicina y Biología Experimental de Cuyo IMBECU-CCT CONICET – Mendoza. <sup>3</sup>Universidad Nacional de Cuyo, Facultad de Ciencias Médicas, Mendoza, Argentina. E-mail: victoria.arrieta.j@gmail.com

Leishmaniasis is an expanding parasitic zoonosis in South America and is considered as one of the neglected diseases by the World Health Organization because it mainly affects the low-income population and with limited access to health services. In Argentina, the endemic area of leishmaniasis corresponds to the provinces of Salta, Jujuy, Tucumán, Catamarca, Santiago del Estero, Chaco, Formosa, Misiones, and Corrientes. *Leishmania amazonensis*, one of the etiological agents of leishmaniasis in our country, is distinguished by its ability to cause a wide spectrum of clinical manifestations: from localized cutaneous leishmaniasis, to the most serious forms (such as mutilating, incapacitating and frequently with little response to treatment). Currently, there is no vaccine for the prevention of leishmaniasis in humans and current treatments for leishmaniasis (Glucantime) are unsatisfactory due to the associated high toxicity, cost, complex administration, and the emergence of resistant strains. Therefore, we consider it necessary to develop innovative immunotherapeutic alternatives based on first generation vaccines. In this work, we evaluated the effect of a prophylactic vaccine formulated with total antigens of *L. amazonensis* (LTA) and an agonist of TLR-3 [Poly (I:C)]. It has been reported that such prophylactic vaccine formulation has generated a protective immune response of the Th1 type, characterized by high production of IgG2a and IFN- $\gamma$  with low levels of IL-4 and IL-10. Female BALB/c mice were infected in the right hind paw pad with  $1 \times 10^4$  *L. amazonensis* promastigotes and treated with LTA+Poly (I:C) formulation, that were administrated up to five subcutaneous doses with a 7-day interval. PBS, Glucantime, LTA and Poly (I:C), were used as control groups. We analyzed different parameters like the swelling of the infection site, parasite load, histopathology,

splenic index, and determination of the humoral immune response. We observed that Poly (I:C) managed to control the infection, generate less swelling, maintain the histoarchitecture, present a lower parasite load, and decrease IgG levels compared to LTA+Poly (I:C), which presented opposite results. In this study we show how the formulation of LTA+Poly (I:C) at a certain concentration generates an inadequate immune response while the formulation with Poly (I:C) alone shows a similar response to that of Glucantime, indicating a possible new alternative to treatment of leishmaniasis. As a future perspective, based on these results, we will focus on Poly (I:C) and will seek to adjust the therapeutic dose to further analyze its response to the disease.

## ECOLOGY, ETOLGY AND BIODIVERSITY

### A80

#### EVALUATION OF OVIPOSTURE AND INCIDENCE OF “CORN EARWORM” *Helicoverpa zea* (BODDIE, 1850) IN CORN WITH AND WITHOUT TRANSGENIC EVENT, IN LATE SOWING, DURING THREE PRODUCTION CYCLES, IN THE VILLA MERCEDES (SL) AREA

Bonivardo SL<sup>1</sup>, Martínez AN<sup>1</sup>, Funes MB<sup>1</sup>, Suárez AM<sup>1</sup>, Sosa MC<sup>1</sup>

<sup>1</sup>Docentes investigadores del Dpto de Cs. Agropecuarias, FICA-UNSL. PROICO N°140520. E-mail: sbonivardo@gmail.com

*Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae), is an important pest of corn in Argentina, and early detection of the species by observing eggs in stigmas is a key aspect as an integrated management strategy. In recent years, a growth of *H. zea* control was observed in late-sowing maize. During flowering, the female lays eggs singly on the fresh stigmas, when the larva hatches, it enters the spike and begins to feed, causing direct and indirect damage by favoring the entry of pathogens and other insects. The objective of the present work was to evaluate the number of ovipositions and incidence of “corn earworm” *H. zea* in maize with and without transgenic event, in late sowing, during three production cycles (2014, 2015, and 2016), in the area of Villa Mercedes (SL). The field test was in the experimental field of the Department of Agricultural Sciences (FICA-UNSL), in a randomized design of four treatments and three repetitions, with the sowing of four hybrids: one non-transgenic, two GM and one PRO triple VT, during the first half of December in direct sowing at a distance between rows of 0.52 cm. The oviposition evaluation was carried out at the R1 state of the Ritchie and Hanway Scale (1992) by direct observation and counting the number of eggs per spike. Spike damage was analyzed in R6 evaluating incidence (number of spikes affected / total spikes evaluated). The data obtained were statistically analyzed by means of the analysis of variance and test of means, using the statistical package InfoStat. The average number of eggs/spike in the three production cycles was: GM hybrids, 0.33; PRO triple Vt, 0.13; non-transgenic, 0.41; statistically, there were no significant differences ( $P > 0.05$ ) between hybrids. The average incidence for each treatment is mentioned below: MG, 71.1; PRO triple Vt, 50 and non-transgenic 64.3, being the statistically significant difference ( $P > 0.05$ ) between the MG with respect to the PRO triple Vt and non-transgenic. Behavioral studies of *H. zea* against hybrids with and without transgenic events should be continued.

### A81

#### IDENTIFICATION OF BRACKISH DIATOMS OF SALINAS DEL BEBEDERO, SAN LUIS

Muñoz Carabajal D<sup>2</sup>, Daruich GJ<sup>1</sup>, Sosa L<sup>2</sup>

<sup>1</sup>Área Biología, <sup>2</sup>Área Ecología. Universidad Nacional de San Luis. E-mail: danacarabajal1@gmail.com

Diatoms belong to the bacillariophyceae, which are a class of microscopic unicellular algae involving more than 200000 species living either in freshwater or in seawater or brackish water. The cell structure of these algae consists of a frustule that is made up of two valves filling together to enclose the cytoplasmic contents. This frustule is made of a hard siliceous skeleton, highly complex in shape, and extremely resistant to putrefaction, enzymatic or acid digestion, and to fire destruction. All the above characteristics make diatoms important as well as unique organisms. Their abundance and adaptability in a wide range of climate and geographical areas make them suitable for different applications: nutrient supplements, antibiotics, anticancerous drugs, and nanobiotechnology. In this sense, our aim was the identification of the diatoms of saline environments in order to select the appropriate species for cultivation at laboratory scale. Samples were taken during 2018, 2019, 2021 dry seasons, in three sites of Salinas del Bebedero stream. In each sampling site, five subsamples were collected from different places. Physicochemical parameters were measured. In the laboratory the samples were preserved in 4% formaldehyde and then the observation in the optical microscope (400× and 1000×). At this stage, 15 diatom samples were analyzed, in which 54 diatom taxa were identified, including genera and species. *Campylodiscus clypeus* was dominant and abundant species. It is a large benthic diatom that clearly favors shallow, brackish water environments. Frustules from all samples shows the same general structural features with the characteristic saddle-shaped

valves. The increase in knowledge about these organisms will not only help in developing a better understanding of their distribution but also for obtaining regional diatoms cultures in laboratory for biomass production to be carried out in the future.

## A82

### EFFECT OF SEASONALITY ON EPIGEAN SPIDERS ASSEMBLAGES OF SIERRA DEL GIGANTE, SAN LUIS, ARGENTINA

*Alvarez A<sup>1</sup>, Rodríguez Artigas SM<sup>2</sup>, Vega VA<sup>1</sup>*

*<sup>1</sup>Área de Zoología, Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia. <sup>2</sup>Universidad Nacional de Salta, Facultad de Ciencias Naturales. E-mail: aldana.alvarez07@gmail.com*

Spiders have been proposed as good indicators of environmental quality, ideal for biological monitoring. The study of spider assemblages and their variations throughout the year is essential to understand what factors intervene and in what way these assemblages are affected. The “Sierra del Gigante” is of interest because it is located in an ecotone between the ecoregions of “Chaco Árido” and “Monte de Llanuras y Mesetas”. The region presents important seasonal variations in rainfall and temperature. In the present study, the effect of seasonality on the composition of said assemblages was evaluated. Samples were taken in December 2018 (spring, rainy season) and June 2019 (autumn, dry season), in two study areas, the first belonging to an environment subjected to high levels of grazing (ZP) and the second corresponding to a low or no grazing environment (ZBN). In each of them, three sampling sites were selected, separated by a minimum distance of 3 km. At each site, three 100-m linear transects were drawn, and four fall traps were placed along each of them, being active for 7 days. The material was identified into families and species/morphospecies. A total of 242 spiders, belonging to 43 morphospecies and 23 families, were captured. The abundance and richness of morphospecies was higher during spring in both areas, especially in the ZBN. The range-abundance curves obtained for both zones also showed seasonal changes. This change in the structure of the communities was also detected at the family level, with Zodariidae, Lycosidae and Xenocnidae being the most abundant families in the ZBN during spring, and Amaurobiidae, Linyphiidae and Anyphaenidae during autumn. In the ZP, during spring season the most abundant families were Zodariidae, Gnaphosidae and Lycosidae, while during autumn Anyphaenidae, Orsolobidae, Lycosidae and Linyphiidae were the most abundant. The ZBN presented a total seasonal beta of 0.78, with 97% of species turnover and 87% of complementarity. In the ZP, beta diversity between stations was lower ( $\beta$ SOR: 0.68), with a higher percentage of nesting (9%) and a seasonal complementarity of 77%. Taking into account the seasonal abundance range curves and the hierarchical abundance graphs of families, we observed that the greatest differences in spider assemblages between study areas occurred during autumn. Amaurobiidae turned out to be an indicator family for the ZBN during autumn, while Orsolobidae was an indicator family for the ZP in the same season. Indicator morphospecies were also detected for each of the groups obtained in the ordering, with the exception of the ZBN in spring. It is concluded that seasonality is a key factor that acts on the diversity of spider communities, revealing changes in abundance, species richness, structure and composition of their assemblages during rainy and dry season.

## A83

### EFFECTS OF TRAFFIC REDUCTION ON COMMUNITIES OF TARDIGRADES DURING COVID-19 PANDEMIC IN URBAN HABITATS OF THE CITY OF SALTA

*Grabosky A<sup>1</sup>, Ballardini F<sup>1</sup>, González Reyes A<sup>1</sup>, Rocha M<sup>2</sup>, Doma P<sup>2</sup>, Corronca J<sup>1</sup>, Rodríguez Artigas S<sup>1</sup>, Fernández D<sup>1</sup>, Ostertag B<sup>2</sup>*

*<sup>1</sup>FCN-IEBI, Universidad Nacional de Salta. <sup>2</sup>Universidad Nacional de La Pampa, CONICET. E-mail: alfonsina.grabosky@gmail.com*

For the control and prevention of coronavirus disease (COVID-19) restrictive measures were adopted that strongly influenced social activities, this period of reduced human mobility ensured an unprecedented reduction of anthropogenic emissions, evidencing a substantial improvement in air quality in several cities. To evaluate the effects of reduced vehicular traffic on tardigrade communities in the city of Salta, we used samples previously collected during the 2016 winter season with high (H), medium (M), and low (L) vehicular traffic, and samples collected during the restrictions (R) in 2020 winter season. Data analysis was performed using PAST, iNEXT, SPADE, and R programs. Two thousand seventy-five specimens (2475) were collected belonging to fifteen species. Habitat M showed the highest abundance (N = 1088), however habitat R turned out to be 1.04, 1.40, and 1.49 times more diverse than communities L, M, and H, respectively. The assemblage of the R community showed to be like L community, represented mainly by: *Milnesium quiranae*, *Macrobotus* cf. *hufelandi*, *Viridiscus rufoviridis*, and *Paramacrobotus huziori*. Furthermore, *Mesobotus* sp. nov.1 was exclusive to the R assemblage and is new for science. The partition of the  $\beta$  diversity showed that the species turnover was higher than nesting among R and H – M – L communities. In this study, we conclude that restrictions of vehicular traffic during COVID-19 pandemic attenuated the effects of species loss by biotic homogenization previously documented, allowing colonization of new species, such as *Mesobotus* sp. nov.1. We emphasized that the diversity of tardigrades in Salta city and their assemblages dependent on several factor, primarily vehicular traffic.

**A84**

**USE OF LARVAL LENGTH AND WEIGHT OF NATIVE BLOWFLIES  
(DIPTERA: CALLIPHORIDAE) AS TOOLS TO ESTIMATE  
DEATH TIME IN FORENSIC ENTOMOLOGY**

Acosta X<sup>1,2</sup>, González-Reyes A<sup>2</sup>, Centeno N<sup>3</sup>, Mejía-Montaño E<sup>2</sup>, Palacios E<sup>2</sup>, Flores V<sup>2</sup>, Corronca J<sup>1,2</sup>

<sup>1</sup>CONICET. <sup>2</sup>Instituto para el Estudio de la Biodiversidad de Invertebrados (IEBI), Universidad Nacional de Salta, Facultad de Ciencias Naturales. <sup>3</sup>Laboratorio de Entomología Aplicada y Forense, Universidad Nacional de Quilmes, Departamento de Ciencia y Tecnología. E-mail: ximenacosta10@gmail.com

Blowflies (Diptera: Calliphoridae) are usually the first to colonize a corpse, and flies of the genus *Lucilia* are among the most important for forensic entomology for estimating the Interval Postmortem (IPM). The objective of this work was to construct forensic methods using larval body size (length and weight) of *L. ochricornis* and *L. purpurascens* that allows estimating a more precise IPM in forensic expertise. The temperature, humidity, and light were recorded in an outside environment in the four seasons of the year in the province of Salta, in 2018. With the average values of these variables the brood chamber was calibrated: autumn: 13.4°C, 74.9%, 10<sup>35</sup>:13<sup>25</sup> h (L:O) and 1916 lux; winter: 15.1°C, 63.4%, 10<sup>52</sup>:13<sup>08</sup> h (L:O) and 2292 lux; spring: 23.6°C, 69.9%, 13<sup>15</sup>:10<sup>45</sup> h (L:O) and 2276 lux; and summer: 22.3°C, 76.0%, 12<sup>25</sup>:11<sup>35</sup> h (L:O) and 1985 lux. Adult flies of *L. ochricornis* and *L. purpurascens* were captured in the town of “La Caldera” (Salta, Argentina). The larvae obtained were distributed in three replicates of 250 larvae for each species and culture temperature. Once the larvae hatched, 10 larvae were sacrificed every 12 h from each replica to which length and weight were measured at each instar (I, II, and III). With these data, MANOVA analyses were performed, and isomegalen diagrams and growth models were constructed. There are differences in the length ( $F = 29.747, P = 0$ ) and mean body weight ( $F = 30.963, P = 0$ ) between species. When analyzing the effect of the different temperatures by larval instar for each species, there were no differences in length (L) and weight (w) to *L. ochricornis* ( $F_L = 2.266, p_L = 0.053; F_w = 1.543, p_w = 0.190$ ), or *L. purpurascens* ( $F_L = 2.291, p_L = 0.051; F_w = 1.026, p_w = 0.448$ ). Studies on larval body size are scarce and less frequent are those that assess body weight. Numerous studies have considered that larval length and weight are the best estimators of the time of death while the larvae are feeding. Isomegalen diagrams are the most commonly used developmental models to represent body size data and the PMI estimates through its use is reliable. The logistic growth model is adequate and coinciding with that registered for other species of Calliphoridae. The use of information from different sources as length and weight increases the range of possibilities to obtain an accurate and reliable PMI in forensic cases.

**A85**

**SPIDERS (ARTHROPODA, ARANEAE) IN HIGH ALTITUDE ENVIRONMENTS IN  
THE PROVINCE OF SALTA (ARGENTINA): GENERATORS OF BETA DIVERSITY**

González-Reyes A<sup>1</sup>, Cruz I<sup>1</sup>, Macagno H<sup>1,2</sup>, Peñaranda A<sup>1</sup>, Rodríguez-Artigas S<sup>1</sup>, Bergeret Pacheco M<sup>1</sup>, Corronca J<sup>1,2</sup>

<sup>1</sup>Instituto para el Estudio de la Biodiversidad de Invertebrados (IEBI), Universidad Nacional de Salta, Facultad de Ciencias Naturales. <sup>2</sup>CONICET. E-mail: andyximena@gmail.com

It is vital to know the diversity patterns at high altitudes to predict how climate change might affect them since most species would be forced to change their distributions in a warmer future. In Salta, both the Puna (PU) and the High Andes (AA) are high-altitude ecoregions originating from the same geological event but that differ in altitude and different environmental variables. Each of them has ecosystem complexes defined by vegetation. The biological and ecological information known for these environments is scarce, punctual, or old. Spiders are a good indicator of variation in habitat structure, their metacommunities being controlled mainly by environmental or climatic effects. The objective of this work was to analyze the diversity patterns (alpha, beta, and gamma) of spiders in high altitude environments and examine both local and regional factors (climatic, habitat heterogeneity, and geographical distance) that can generate them. It was sampled in spring, summer, and autumn, with pitfall traps and G-Vac, in 30 widely separated sites (PU = 18 and AA = 12) distributed in the five ecosystem complexes. Different variables of heterogeneity of vegetation and soil were measured *in situ*; also, bioclimatic variables and geographical distance. A total of 695 spiders were recorded from 18 families and 87 species/morphospecies. The spider diversity of PU was 1.09 times higher than in AA. The Prepuna complex of the PU was 4.05 times more diverse than the Puna Salada. In AA, the spider community in the Sierras Orientales was 1.33 times more diverse than in Grandes Salares. The beta diversity between ecosystem complexes and between ecoregions contributed significantly to regional diversity (25 and 23%, respectively), with a high turnover of species explaining 95% of the ecoregional beta diversity. Each ecosystem complex showed its own and particular assemblage of spiders, with the heterogeneity of the vegetation and the bioclimatic variables structuring the communities of the Prepuna; while the heterogeneity of the soil to those of the Puna Salada and Grandes Salares. Geographic distance is also a key factor in the structuring of spider communities in high altitude environments, explaining that sites closer to the same complex have more similar assemblages.

**A86**

***Conyza* LESS. SPECIES PRESENT IN EASTERN SOUTH AGROECOSYSTEMS  
OF SAN LUIS (ARGENTINA)**

Aostri Amici CA<sup>1</sup>, Mercado SE<sup>1</sup>, Tavecchio N<sup>1</sup>, Bertolino JJ<sup>2</sup>, Alonso CS<sup>1</sup>, Genovese C<sup>2</sup>

<sup>1</sup>Facultad de Ingeniería y Ciencias Agropecuarias. <sup>2</sup>Facultad de Turismo y Urbanismo. Universidad Nacional de San Luis. E-mail: caaostri@gmail.com silvina.mercado@gmail.com

*Conyza* Less. belongs to the Asteraceae family, which comprises almost a 60 to 100 species worldwide, mostly of them from warm temperate regions of America. Approximately 23 are accepted botanical entities present in Argentina, 7 species, 11 taxa present in San Luis province. The species of this genus are generally annual or perennial herbs, many species of the genus *Conyza* are ruderal, and some have been found to be resistant to the herbicide glyphosate; such is the case of some biotypes of *Conyza bonariensis* and *Conyza sumatrensis*. These species particularly have increased its presence in the agricultural area of eastern central Argentina. The interest between professionals and farmers in relation to species of this genus has been subject of numerous consultations. The aim of this work is to provide a tool for an easy identification of the species of the *Conyza* genus present in eastern and south agricultural area of San Luis and generate an updated map of their distribution. Plant collections were conducted in irrigated and non-irrigated agricultural areas, documented, identified by classical botanical methods, and deposited in the Herbarium of "Facultad de Ingeniería y Ciencias Agropecuarias" (VMA). A dichotomic key was built; diagnostic characters were photographed and drawn. A visual analysis of the study area with Google Earth images was carried out, added to a field survey with *in situ* vegetation sampling. At present, only three botanic entities were identified as problematic weeds in corn and soybean crops in the study area surveyed: *Conyza bonariensis* var. *bonariensis*, *C. bonariensis* var. *angustifolia*, and *Conyza sumatrensis* var. *sumatrensis*. For this purpose, an identification key is given based on recognizable morphological characters on field. It includes *Conyza sumatrensis* var. *leiotheca* as well as other related species cited for the San Luis province and mentioned behaving as weeds in agricultural plots in other provinces. In addition, synonymy, descriptions, geographical distribution, and habitat information are provided for each species.

**DEVELOPMENT AND REPRODUCTION BIOLOGY**

**A87**

**ARP 2/3 DISTRIBUTION IN GERM CELLS OF NEW ZEALAND RABBITS FED  
A HIGH-FAT DIET: PRELIMINARY RESULTS**

Avena V<sup>1,3</sup>, Funes A<sup>1</sup>, Colombo R<sup>1</sup>, Monclus M<sup>1,2</sup>, Boarelli P<sup>3</sup>, Saez Lancellotti E<sup>1,2</sup>, Fornés M<sup>1</sup>

<sup>1</sup>Laboratorio de Investigaciones Andrológicas (LIAM), Instituto de Histología y Embriología de Mendoza (IHEM), CONICET – UNCUIYO. <sup>2</sup>Laboratorio de Investigaciones de la Universidad del Aconcagua, UDA. <sup>3</sup>Laboratorio de Enfermedades Metabólicas, UMaza. E-mail: virginiaavenaihem@gmail.com

Alterations in male fertility in association with high-fat diets (HFD), obesity, and related metabolic complications such as dyslipidemias, are the topic of extensive research. However, the impact of HFD on the testicles is still unknown. In previous studies, we observed that the elongating round spermatids of HFD-fed hypercholesterolemic rabbits display asymmetry in acrosome formation and cell elongation. In addition, an increase in cellular cholesterol and alterations in nuclear condensation were observed. All the above leads to defective seminal spermatozoa. The shape of these cells depends on a complex involving actin filaments (acroplaxome) and microtubules (manchette) anchored to membranes. Therefore, we propose that the shape alteration may be due to a modification in the distribution of cytoskeleton-associated proteins promoted by the increase in membrane cholesterol. The aim of this work was to analyze the Actin-Related Proteins 2/3 (ARP 2/3) complex distribution in germ cells of rabbits fed a high-fat diet. For this purpose, we used our animal model of New Zealand adult male rabbits fed with balanced chow (Control group) and others supplemented with 14% bovine fat (HFD group). The expression of ARP 2/3 was studied in rabbits after 12 months of diet in both groups, by indirect immunofluorescence (IFI), in both tissue (seminiferous tubules) and germ cells isolated from the tubules. We observed that the HFD group displayed a different arrangement of the protein complex under study compared to the control, both in tissue samples and in isolated cells, and mainly in round spermatids. Germ cells with abnormal ARP 2/3 distribution were quantified, and a statistically significant increase was observed in the HFD group compared to Control ( $P < 0.001$ ). In conclusion, our preliminary results indicate that there is an association between diet and the arrangement of ARP 2/3 in germ cells, which could be related to sperm malformation.

A88

**ROLE OF THYROID HORMONES IN THE UTERINE RESPONSE TO  
OVARIAN HORMONES AND DECIDUAL PROLIFERATION**

Rinaldini E<sup>1</sup>, Pietrobon EO<sup>1,3</sup>, Troncoso ME<sup>1,2</sup>, Gamarra Luques C<sup>1,3</sup>, Hapon MB<sup>1,2</sup>

<sup>1</sup>IMBECU CCT Mendoza CONICET. <sup>2</sup>Facultad de Ciencias Exactas y Naturales. UNCuyo. <sup>3</sup>Facultad de Ciencias Médicas. UNCuyo. E-mail: erinaldini@mendoza-conicet.gob.ar; bhapon@mendoza-conicet.gob.ar

The endometrium plays a fundamental role in the process of implantation and nutrition of the embryo. To enable implantation, this tissue is subjected to dynamic remodeling (decidualization) under strict hormonal regulation, mainly commanded by the ovarian hormones estradiol (E<sub>2</sub>) and progesterone (P<sub>4</sub>). During this period, E<sub>2</sub> and P<sub>4</sub> also contribute to the formation of new blood vessels, regulating the action of vascular endothelial growth factor (VEGF), the main modulator of angiogenesis and vascular permeability of the decidua. Thyroid hormones (TH) play an important role during implantation and the early stages of embryonic development, being essential in maintaining the reproductive capacity. Defects in maternal thyroid status affect the metabolism of sex steroid hormones and ovarian function that are associated with miscarriages, placental abruption, and premature labor. Our laboratory showed that hypothyroid rats present an altered sexual cycle, a lower number of pups per litter, and a delay in the onset of parturition. Therefore, we hypothesize that THs modulate ovarian hormonal secretion and their uterine response during implantation, thus altering the correct development of the uterine decidua. The aim of this work was to investigate endometrial cell response to THs signaling, through the study of the serum concentration of the E<sub>2</sub> and P<sub>4</sub> and the uterine expression of the receptors (ER $\alpha$  and PGR). In addition, the expression of TH receptor (TR) and PCNA (as an indicator of cell proliferation) in the uterus during the implantation period was analyzed. Euthyroid (Eut) and Hypothyroid (HypoT) female Wistar rats were used. Hypothyroidism was induced by daily administration of the antithyroid 6-propyl-2-thiouracil (PTU) 0.1 g/L in drinking water, while the control group only drank tap water. Groups of 6–7 rats were sacrificed on days five (G5), six (G6), and seven (G7) of gestation. Hormones were measured by radioimmunoassay and the expression of uterine ER $\alpha$ , PGR, TR and PCNA were evaluated by immunohistochemistry. Our results demonstrated that HypoT rats showed a significant increase in serum P<sub>4</sub> concentration during the G7. Hypothyroidism decreased the expression of receptors for ovarian hormones, thyroid hormones and PCNA throughout the analyzed gestation period ( $P < 0.05$ ). We conclude that, at uterine level, maternal thyroid status influences the adequate metabolism of the main ovarian hormones and their uterine response, affecting the decidualization process. This evidence shows the impact of THs for the process of implantation and the progression of embryo development.

A89

**NATIVE PLANTS *Tessaria absinthioides* AND *Prosopis strombulifera* MODULATES  
THE PROLIFERATION AND ESTRADIOL RECEPTOR EXPRESSION  
ON THE ENDOMETRIAL CELL LINE ECC-1**

Penas VA<sup>1</sup>, Castro CM<sup>2,3</sup>, Quesada MI<sup>2,3</sup>, Gamarra Luques C<sup>2,3</sup>, Hapon MB<sup>1,2</sup>

<sup>1</sup>Facultad de Ciencias Exactas y Naturales, UNCuyo. <sup>2</sup>IMBECU CCT Mendoza CONICET. <sup>3</sup>Facultad de Ciencias Médicas, UNCuyo. E-mail: bhapon@mendoza-conicet.gob.ar

Herbal medicine is regarded as a substitute or complement to occidental medicine. However, a limited number of medicinal herbs have been tested scientifically for their safety. In the province of Mendoza, folk medicine describes more than five hundred species used in the care and preservation of health. Our previous results demonstrated that the aqueous extract of the native plants *Prosopis strombulifera* (*Ps*) and *Tessaria absinthioides* (*Ta*) exhibit glycemid and lipid-lowering, antiatherosclerotic, antimutagenic, and antitumor activity. Other authors suggest that *Prosopis* sp. displays a phytoestrogenic effect resembling the isoflavones genistein and daidzein. Their administration altered the estrous cycle, uterine morphology, and sexual receptivity in female rats. On the other hand, no studies of the genus *Tessaria* have been reported in relation to reproduction. The main hypothesis of this presentation is that there are active molecules in the aqueous extracts of *Ps* and *Ta* that display phytoestrogenic effects. To test this hypothesis, we proposed to evaluate *in vitro*, on a human endometrial cancer cell line (ECC-1), the proliferation, toxicity, and modulation of steroid receptors in response to treatment with *Ps* and *Ta* extracts. Proliferation and toxicity were evaluated by MTT and the expression of steroid receptors by RTqPCR, comparing the treatment *Ps* and *Ta* extracts to the effect of estradiol on the ECC-1 cell line. *Ps* extract exerts a dual effect on proliferation, it slightly increases the proliferation of ECC-1 cells at low concentrations (< 0.5  $\mu\text{g/mL}$ ) and strongly inhibits proliferation at higher concentrations ( $\text{IC}_{50} = 2.30 \mu\text{g/mL}$ ). *Ta* extract also displays a dual effect on proliferation, it increases the proliferation of ECC-1 cells at concentrations lower than < 2  $\mu\text{g/mL}$  and hinders proliferation at higher concentrations ( $\text{IC}_{50} = 12.49 \mu\text{g/mL}$ ). We next evaluated the effect of the extracts on viability. Our results showed that the higher dose that does not affect viability is 0.5  $\mu\text{g/mL}$  for *Ps* and 6  $\mu\text{g/mL}$  for *Ta*. These doses were used to evaluate the impact of the extracts on the modulation of steroid receptors. *Ps* extract increased the expression of estradiol receptor ER2 ( $P > 0.05$ ), while *Ta* increased the expression of both estrogen receptors ER1 and 2 ( $P > 0.05$ ). We can conclude that the aqueous extract of the native plants *Ps* and *Ta*, affects the proliferation of endometrial cells and modulates the expression of estrogen receptors *in vitro*. Thus, the use of phytochemicals for desired biological effects must be handled with care due to their possible effects on reproductive physiology.

## A90

### PRELIMINAR ASSAYS OF THE ANTIOXIDANT AND ANTIAPOPTOTIC EFFECT OF PEDF ON MEPC5 CELLS

Conte MI<sup>1,2</sup>, Saez Lancellotti E<sup>1,2</sup>, López ME<sup>1</sup>, Ippoliti MC<sup>1</sup>, Manrique V<sup>1</sup>, Silva Perrelló G<sup>1</sup>, Caleri Villanueva R<sup>1</sup>, Guiñazú J<sup>1</sup>, Fornés MW<sup>1,2</sup>, Aguilera Merlo C<sup>3</sup>, Monclus MA<sup>1,2</sup>

<sup>1</sup>Facultad de Ciencias Médicas UNCuyo, Instituto de Histología y Embriología de Mendoza. <sup>2</sup>IHEM CCT CONICET Mendoza. <sup>3</sup>Facultad de Química Bioquímica y Farmacia, UNSL. E-mail: marinesconte@gmail.com

Sperm maturation is a physiological process whereby spermatozoa acquire fertilizing capacity during their transit through the epididymis. Several changes on the plasma membrane, cytoplasm and DNA compaction are necessary for the acquisition of a complete maturity. These modifications occur inside the micro environmental lumen of epididymis, where pH, O<sub>2</sub> concentrations, and temperature are lower than in the rest of the body. These particular conditions could generate reactive oxygen species (ROS), capable of affecting both maturation and proper sperm storage. In previous works, our group detected the presence of PEDF (Pigment Epithelium Derived Factor), a protein expressed with androgen dependency in male reproductive tissues of adult Wistar rats. According to its special features as an antioxidant, antiapoptotic and antiangiogenic protein, its effect on the epididymal tract are studied *in vitro*. We use MEPC5 cells, a conditionally immortalized epididymis caput cell line for cytotoxicity assays with H<sub>2</sub>O<sub>2</sub>. Hydrogen peroxide was added exogenously to the culture media at high concentration (600 and 800 μM) in absence or presence of 50 ng/mL of recombinant PEDF (rPEDF). After an incubation period of 2.5 h, the redox status was studied with resazurin assay to measure the metabolic capacity of live cells. Additionally, we use Trypan blue stain to analyze the cellular vitality. The experiments were performed in triplicate and cells were counted to analyze cell vitality by stain. We observed an increase in cellular viability (10–20 %), with higher reducing status in the group treated with rPEDF respect to the control cultures without rPEDF addition, both in presence of H<sub>2</sub>O<sub>2</sub>. Cellular vitality estimated by Trypan blue also showed an increase about 5 and 10% in treated groups. It can be initially concluded that PEDF can exert a protective antioxidant effect against deleterious substances like ROS, but further studies are required.

## A91

### HEXOSAMINIDASE FROM CAUDA EPIDIDYMAL FLUID COULD PARTICIPATE IN REMODELING CARBOHYDRATE ON THE SURFACE OF BULL SPERMATOZOA

Álvarez P<sup>2</sup>, Buttazzoni A<sup>2</sup>, Sosa MA<sup>1,2</sup>, Robina P<sup>3</sup>, Aguilera AC<sup>1,2</sup>

<sup>1</sup>Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. CONICET. Mendoza, Argentina. <sup>2</sup>Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo. Mendoza, Argentina. <sup>3</sup>Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, España. E-mail: caguilera@mendoza-conicet.gob.ar

Spermatozoa undergo biochemical and morphological changes during the epididymal transit, resulting in the gamete maturation. Among the several proteins secreted by the epididymal epithelium, hexosaminidase (HEX) is the most abundant glycosidase in bull epididymal fluid and represents upon 3% of the total secretion in the distal part of the organ. Given that HEX is a lysosomal enzyme, it is still controversial whether this enzyme is active at the pH of the epididymal lumen and whether it participates in the epididymal maturation. The aim of this study was to correlate the activity of HEX with those changes in N-acetyl-glucosamine (NAG) content observed in bull spermatozoa during epididymal maturation. By flow cytometry and fluorescence microscopy (using WGA FITC), we observed that NAG content on the sperm surface varies during epididymal maturation. Interestingly, increased activity of HEX (measured by spectrofluorometry) at the epididymal pH (6.8) was detected in the cauda lumen. In order to determine if the epididymal HEX is responsible for NAG removal from the sperm surface, we performed an assay by incubating cauda fluid with spermatozoa obtained from the caput. We observed by flow cytometry that incubation with the fluid induced a significant decrease of NAG on the sperm surface, which was partially reversed with synthetic inhibitors specific for HEX. Our results provide direct evidence that HEX from epididymal fluid may participate in removing NAG from spermatozoa, as a step of sperm maturation in bull epididymis.

**A92**

**CLIMATIC AND ENVIRONMENTAL FACTORS MODULATE THE REPRODUCTIVE CYCLE IN THE LIZARD *Liolaemus cuyanus***

*Barauna AA<sup>1</sup>, Sanabria EA<sup>2,3,4</sup>, Quiroga LB<sup>2,3</sup>, Monclús MDA<sup>1,5</sup>, Fornés MW<sup>1,5</sup>*

*<sup>1</sup>Lab. de Inv. Andrológicas, Instituto de Histología y Embriología de Mendoza (LIAM – IHEM – CONICET Mendoza), Argentina. <sup>2</sup>Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). <sup>3</sup>Facultad de Filosofía, Humanidades y Arte, Universidad Nacional de San Juan, Argentina. <sup>4</sup>Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina. <sup>5</sup>Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina. E-mail: andrea.barauna88@gmail.com*

The arid zones have a heterogeneous availability of resources in space and time as a consequence of the marked climatic seasonality. Reproductive activity in reptiles of the order Squamata is associated with climatic factors like temperature and precipitation, and environmental factors such as the photoperiod. In arid zones, these factors are limiting and cyclical, producing a seasonal variation in reproductive events, for example, gametogenesis, mating, gestation, egg laying, incubation and birth, in lizards of the Liolaemidae family. The objective of this work was to study how climatic and environmental factors modulate the reproductive cycle in *Liolaemus cuyanus*, a psammophilus and oviparous lizard that inhabits the Monte Desert. We captured adult males (N = 27) by Noosing method, from October to March in the Department of Ullúm, Province of San Juan, Argentina. After euthanasia by decapitation, we weighed (Ptest) and fixed the testes in 2% paraformaldehyde for optical microscopy (OM). Finally, we processed them by routine histological techniques. By analysis of micrographs of OM, we calculated the medium tubular surface (ST X) using the free software Image J, version 1.37v. The variation of the tubular surface and the different stages of reproductive development appreciated in the testis sections allowed us to group the months into three main periods: pre-reproductive, PrR (October), reproductive, R (November-January); and post-reproductive, pR (February-March); to later analyze the data in a grouped way. On the other hand, we took climatic data (temperature and precipitation) from the INTA Pocito station in San Juan and environmental data from light minutes (photoperiod) from the Global Monitoring Laboratory Earth System Research Laboratories (<https://gml.noaa.gov/grad/solcalc/>). Significant correlations were found between climatic and environmental factors with the morphometric variables (Ptest and ST X) for the three different periods analyzed. These preliminary results allow us to describe an involvement of the hypothalamic-pituitary-gonadal axis and infer that external factors such as temperature, precipitation and photoperiod modulate the gonadal development in the lizard *L. cuyanus* throughout its reproductive cycle.

**A93**

**DIFFERENTIAL EFFECT OF ANGIOTENSIN II TYPE 2 RECEPTOR ANTAGONIST ON CEREBELLAR CORTEX PATTERNING DURING POSTNATAL DEVELOPMENT**

*Soler García FM, Sánchez SI, Fuentes LB*

*Universidad Nacional de San Luis – Facultad de Química Bioquímica y Farmacia. E-mail: fsolergarcia@gmail.com*

The Renin Angiotensin System (RAS) inhibitors and angiotensin receptor blockers are associated with fetopathies, especially if these medications were taken during the second and third trimesters. Fetal development is dependent on RAS appropriate expression and function. Ang II AT2 receptor has been related to a neuronal differentiation during fetal and postnatal development. In the cerebellum, AT2 receptors are located only in the Purkinje cells (PC), which are topographically organized due to their molecular heterogeneity within the PC clusters, based on their early or late cohort birth. PCs regulate granule cells (GC) proliferation and migration process required for the correct folding of the cerebellum. The cerebellar cortex can be divided from rostral to caudal into transverse zones. These regions are molecularly distinct based on gene expression patterns as well as functionality. Fissure prima delimited caudal (anterior (AZ) and central (CZ) zone) from the rostral region (posterior (PZ) and nodular (NZ) zone). The aim was to investigate the effect of prenatal AT2 blockage on cerebellar Purkinje cells and the cortical patterning process from postnatal day 5 to 15 during cerebellum development. Wistar rats on the 13th day of pregnancy were subcutaneously implanted mini-osmotic pumps with AT2 antagonist (PD123319) and vehicle as control. Morphological studies by indirect immunofluorescence analysis on P5, P7, and P15 (N = 24 slices/each day) were performed. The results showed that Purkinje cell layer (PCL) width increased significantly on PZ ( $78.8 \pm 2.6 \mu\text{m}$  vs.  $64.6 \pm 2.4 \mu\text{m}$ ,  $P < 0.05$ ) and NZ ( $96.9 \pm 4.4 \mu\text{m}$  vs.  $82.2 \pm 2.7 \mu\text{m}$ ,  $P < 0.05$ ) with multilayer display at P7. At P15, NZ PCL width remained similar to the values described at P7, however layer width significantly decreased in comparison to control ( $104.3 \pm 4.9 \mu\text{m}$  vs.  $145.3 \pm 5.8 \mu\text{m}$ ,  $P < 0.05$ ), PZ achieved similar values to controls. The external granule cell layer (EGL) length was significantly increased on treated animals at P5 in all cerebellar zones ( $P < 0.01$ ). At P7, the values increased significantly only in the treated pup's rostral region. At P15, the thickness of the EGL presented no significant changes between groups in different cerebellar zones. The present study demonstrates important modifications in cerebellar Purkinje and granule cells, proliferation and migration processes in the postnatal development of treated pups. The prenatal AT2 blockage induces differential effect which could be related to the molecular heterogeneity within the PCs clusters. The results suggest a relevant participation of AT2 receptors in the cortex cerebellar organization.

**BIOCHEMISTRY, PHYSIOLOGY AND NEUROCHEMISTRY**

**A94**

**AN INTRACEREBROVENTRICULAR INJECTION OF A $\beta$  AMYLOID (1-42) AGGREGATES MODIFIES TEMPORAL PROFILES OF SPATIAL MEMORY IN RAT**

*Castro A<sup>1</sup>, Coria-Lucero C<sup>1</sup>, Ledezma C<sup>1</sup>, Sanchez M<sup>1</sup>, Garraza M<sup>2</sup>, Anzulovich A<sup>1</sup>, Delgado M<sup>1</sup>, Navigatore Fonzo L<sup>1</sup>*  
*<sup>1</sup>Laboratorio de Cronobiología, <sup>2</sup>Laboratory of Biology Reproduction, IMIBIO-SL, CONICET-UNSL, San Luis, Argentina. E-mail: lnavigatore@unsl.edu.ar*

Alzheimer's disease (AD) is the major cause of dementia in older people. AD is clinically characterized by a progressive cognitive decline followed by loss of memory. In the present study, we aimed to investigate the consequences of an intracerebroventricular (ICV) injection of aggregated  $\beta$ -amyloid peptide (1-42) on temporal patterns of cognitive functions using a modified version of the Barnes maze test. Four-month-old males Holtzman rats were divided into two groups defined as: control (CO) and A $\beta$ -injected (A $\beta$ ). Rats were maintained under 12 h-light:12 h-dark conditions and received water and food *ad libitum*. The first group received an intracerebroventricular injection of sterile saline solution (5  $\mu$ L), and the second group received an ICV injection of the solution of A $\beta$  (1-42) aggregated (5  $\mu$ L). A week after the surgery, Barnes' test modified was performed to test cognitive functions of the rats during day and night. The parameters assessed were as follows: (a) number of errors; (b) escape box latency; (c) Hole exploration frequency in the goal sector; (d) Total exploratory activity; (e) Path length. In the behavioral studies we used 23 animals from each control group and A $\beta$ -injected groups. Our results demonstrated that A $\beta$ -injected rats showed an increase in the number of errors to reach the target hole at night and exhibited a notable increase in the latent period during day and night, in comparison with control group. During the day, the A $\beta$ -injected group explored less frequently the target sector and in night showed a significantly lower exploratory activity. The total distance walked was affected by the ICV injection of the amyloid aggregates. According to these results, we could suggest that the temporal patterns of spatial memory are altered in animals injected with A $\beta$  and are correlated with the increase in A $\beta$  observed by our group.

**A95**

**EXPERIMENTAL MODEL OF NUTRITIONAL OBESITY EVALUATION OF ANTHROPOMETRIC, NUTRITIONAL AND METABOLIC PARAMETERS**

*Alfonso JO, Lopez M, Delgado SM, Perez Chaca MV, Ciminari ME, Biaggio V, Salinas E, Gomez NN, Navigatore Fonzo L, Anzulovich AC*

*Laboratorio de Cronobiología y Laboratorio Morfología, IMIBIO-SL, CONICET-UNSL.  
E-mail: javieroscaralfonso@gmail.com*

Understanding that the cause of obesity (OB) is multifactorial, including genetic, environmental, and dietary factors, among which high-calorie diets play a central role in the development of the disease, and that OB in adults increases the risk of associated neurodegenerative diseases with age, we designed a nutritional model of OB in adult rats, as part of the IMIBIO-SL institutional project (PUE-013), which studies OB as a predisposing disease to the development of chronic age-associated diseases and the search for early biomarkers with predictive potential. In this framework, the particular objective of the present work was to evaluate the effects of a rich in saturated fat diet on different anthropometric, nutritional, and metabolic parameters. For this, male Wistar rats were weaned at 21 days of age, and fed a normocaloric diet (NC), containing 366 kcal of lipids/kg diet, then, at the 2-mo-age, they were randomly separated, and fed: one group, with the NC diet (control, NC group), and the other, with a diet high in saturated fat (1570.7 kcal of margarine/kg diet, HFD group), for the next 14 weeks. The animals were kept under 12 h-light:12 h-dark and 22–24 °C, with water and food *ad libitum*. The anthropometric profile included the evaluation of body weight, body mass index (BMI), weight gain, and Lee's index; the nutritional parameters: energy intake and feeding efficiency, all of them were determined weekly, throughout the entire treatment period. The metabolic parameters included glucose (G), triglycerides (TG), total cholesterol (TC), HDLc, and LDLc + VLDLc levels, and were determined at the end of the experiment, in the serum of both, NC and HFD groups, using commercial kits. Statistical differences between groups and throughout the treatment period were analyzed by two-way ANOVA, followed by Bonferroni post-hoc test, with  $P < 0.05$ , to confirm statistical differences between groups and weeks of treatment. Our results show that feeding the HFD diet produces significant increases in the following anthropometric parameters: body weight (from week 9 to 21,  $P < 0.001$ ), BMI (from week 14 to 22,  $P < 0.05$ ), weight gain (from week 15 to 22,  $P < 0.05$ ) as well as energy intake from lipids (from week 9 to 22,  $P < 0.001$ ). We did not observe significant changes in food intake, Lee's index, feeding efficiency, or in total caloric intake. At the same time, we observe that the HFD diet increases significantly increases the circulating TG ( $P < 0.01$ ), TC ( $P < 0.001$ ), [LDLc + VLDLc] ( $P < 0.05$ ), and glucose ( $P < 0.05$ ), while decreases serum HDLc levels ( $P < 0.05$ ), compared to the NC group. Thus, we can conclude that a diet rich in saturated fat from margarine modifies anthropometric, nutritional, and metabolic parameters, thus developing a nutritional model of obesity, generated by hyper-fatty foods, in rat.

**VETERINARY, ANIMAL ANATOMY, HISTOLOGY, AND PHYSIOLOGY**

**A96**

**FORAGE RESOURCES (ALFALFA) AND EXPLOITATION SYSTEM FOR DAIRY RODEOS IN PROVINCE OF SAN LUIS, ARGENTINA**

*Becerra CR<sup>1</sup>, Cavanagh K<sup>2</sup>, Rosa ST<sup>1</sup>, Sueldo RA<sup>1</sup>, Osses RG<sup>1</sup>, Rodríguez Rivera MF<sup>1</sup>*

*<sup>1</sup>Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Agropecuaria (Universidad Nacional de San Luis). <sup>2</sup>Ingeniería Agronómica (FICA). E-mail: becear@yahoo.com*

Argentina's Dairy Production is especially with pastures and supplements related to costs; it allows to take advantage efficiently of forage resources with economic sustainable incomes for production. The present research work was realized in "Tambo San Cristobal", located 130 km south Villa Mercedes (San Luis), its dairy production counts with 480 cows, whose feeding is supplied with 472 hectares under irrigation. The main objective with this present research is to analyze the forage resource alfalfa, developed in this establishment, together with the protein quality that contribute to the dairy cow diet, information was released from its grasslands between December 2020 to June 2021. This establishment uses complemented irrigation to mitigate the alfalfa request, the forage resource is utilized in two different manners; cut and deliver (CyE) and alfalfa roll (R). According to this and to represent the research the gathered information corresponds to a survey of an irrigation circle 80 hectares wide (circle 9) in this place scissor cuts were realized at 10 cm high simulating the cut of the harvester machine with the material destined to the dairy cow feeding (CyE) or drying (R) two methods analyzed during this presentation. Transect cuts were organized in quadrants; sampling time was coincident with the phenomenological moment from 10 to 20 % of flowering in alfalfa. As a result, it was a quantified crop variable in a circle (aver. 97.98 kg – 17.421 kg Green Material by hectare) after drier by microwaves to a constant weight. Variable result is attributed to different aspects plant density, not homogeneous (12 – 28 pl/m<sup>2</sup>), some weather contingencies (poor rain and hailstorms that affected the crop), and the cut moment (flowering percentage at the cutting time). In respond to the protein quality, alfalfa showed a proportion of PB approximately in CyE between 16.64% and 17.47%, meanwhile R utilized 25 days after confection showed a PB of 15.43 %, the values obtained according NIRs DS 2500 foss. The present descriptive research concludes that "alfalfa under irrigation in San Cristobal establishment" to provide feeding for dairy production shows a high potential, variable results in production are related to weather contingencies, cut moment, and plant density, meanwhile protein quality in use by (CyE) and (R) utilized 25 days after confection does not show significant differences, it can be adopted according to the system requirement.

**A97**

**EFFECT OF SLAUGHTER WEIGHT ON OBJECTIVE AND SUBJECTIVE CHARACTERISTICS OF CARCASS AND MEAT OILING IN LAMBS**

*Ronchi FY, Bonvillani AG*

*Facultad de Agronomía y Veterinaria. Universidad Nacional de Río Cuarto.*

A carcass and meat quality criterion is fatness; this must be optimal for its conservation and transport, conferring adequate sensory characteristics. The aim of this work was to analyze, in lambs, the effect of slaughter weight on objective and subjective fattening parameters. 40 male lambs were evaluated, 20 from each category: light (21.4 ± 0.94 kg) and heavy (33.83 ± 1.98 kg). The characteristics studied were (a) Degree of subjective fattening of the carcass classifying into 5 types: 1 (very lean) to 5 (very fat), with intermediate degrees of 0.5 points; (b) Renal pelvic fattening, in 5 levels: 1 (uncovered) to 5 (covered with great thickness), and intermediate degrees of 0.5 points; (c) Relative content (%) of tissue fat in the back (subcutaneous and intermuscular); and (d) Ethereal extract (EE) that determine % of intramuscular fat at 5th–10th rib in *Longissimus dorsi* muscle, the Folch *et al.* (1957) technique was used for lipid extraction. The subjective thickening of the carcass (a) and renal pelvic (b) did not show significant differences between the slaughter weights. The carcass fattening was 1.76 ± 0.78 in light and 2.06 ± 0.94 in heavy, both categories are close to the lean category (2), although the average of the light ones was somewhat lower, towards very lean (1). Renal pelvic thickening reached means of 2.18 ± 0.83 in light carcasses and 2.45 ± 0.66 in heavy, being categorized with kidneys between little covered (2) and covered 2/3 (3). In carcass thickening deviations of up to 1 point were detected in heavy category, an important variability between individuals, having minimum and maximum values between 0.5 to 3.1 in light and 1 to 3.5 in heavy. In relation to renal pelvic thickening, the deviations and minimum and maximum values are similar in both weights. In relation to the objective results, the proportion of back and intramuscular fat showed significant differences between the weights groups. The back fat (c) in light ones showed means of 4.81 ± 3.09% and in heavy ones they reached 9.37 ± 4.56%. In the intramuscular fat percentage (d), the light carcasses obtained averages of 0.75 ± 0.86% and the heavies 2.30 ± 1.13%. It can be observed that all fat deposits are greater in heavy lambs (subcutaneous, intermuscular, and intramuscular fat), this corresponds to studies that established the order of fat deposition: first intermuscular fat, second internal deposits, third subcutaneous and finally intramuscular (Fourie *et al.* 1970, Wood *et al.* 1980, Hammond 1932). On the other hand,

these results are similar to those obtained by Campo *et al.* (2008) and are in contrast with Bianchi *et al.* (2006) who found no differences according to weight. According to these results, subjective thickening is important, quick, and simple to classify and typify carcasses, but it does not have much relationship with the objective results of fatty deposits. We concluded that the heavy ovine carcasses showed a greater amount of tissue fat in the back and intramuscular, while the average values of subjective fattening were not significant, although variability and extreme values were observed between individuals.

## A98

### ALTERATIONS OF THE ESTROGEN RECEPTOR EXPRESSION IN RETINA OF LITHIUM-TREATED VISCACHAS (*Lagostomus maximus maximus*)

*Moncho Quiroga MV<sup>1,2</sup>, Rosales GJ<sup>1,3</sup>, Perez E<sup>1</sup>, Filippa VP<sup>1,3</sup>, Mohamed FH<sup>1</sup>*

<sup>1</sup>Área de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis (UNSL), <sup>2</sup>Becaria EVC-CIN-UNSL, <sup>3</sup>UNSL/Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), San Luis, Argentina. E-mail: [vmoncho.08@gmail.com](mailto:vmoncho.08@gmail.com)

The estrogen receptors  $\alpha$  (ER $\alpha$ ) have been reported in the retinal layers and they were involved in neuroprotective functions through their ability to regulate apoptosis, phagocytosis, and antioxidant processes. Previous studies in viscacha retina have demonstrated a daily rhythm of photoreceptor renewal. Lithium (Li) modifies this process probably by inhibiting of phagocytosis in pigment epithelial cells. The aim of this work was to study the effects of lithium administration on the expression of ER $\alpha$  in retina. Adult male viscachas were divided into 2 groups, the control group and the experimental group that received lithium chloride (1 mmol/kg body weight/day) for 35 days. Retinas were obtained at 08:00, 16:00, and 24:00 h (N = 4 for each group). They were processed for light microscopy. The primary antibody anti-ER $\alpha$  (MC20): sc-542 was used by immunohistochemistry. The percentage of immunoreactive (-ir) cells was quantified by morphometric analysis. The data were analyzed statistically. Nuclear immunostaining in cells of the inner nuclear layer (INL) and in ganglion cells (GC) was observed in the retina from control and experimental groups. In the INL of the control group, the %ER $\alpha$ -ir cells was maximal at 8:00 (24.51  $\pm$  2.31). No immunostaining was detected at 16:00 h and the % ER $\alpha$ -ir presented a minimal value (6.61  $\pm$  0.38) at 24:00 h ( $P < 0.01$ ). The % ER $\alpha$ -ir cells of the experimental group was maximal at 8:00 h (42.46  $\pm$  1.33), decreased at 16:00 h (24.39  $\pm$  0.32), and it was minimal (4.03  $\pm$  1.47) at 24:00 h ( $P < 0.01$ ). Thus, in the INL the %ER $\alpha$ -ir cells were significantly higher in the experimental group than in the control group at 8:00 h and at 16:00 h ( $P < 0.001$ ). In the layer of GC, the % ER $\alpha$ -ir cells in the control group were maximal at 8:00 h (41.82  $\pm$  4.13). No immunostaining was detected at 16:00 h, and the % ER $\alpha$ -ir GC presented a minimal value (11.96  $\pm$  1.01) at 24:00 h ( $P < 0.01$ ). The % ER $\alpha$ -ir GC in the experimental group were similar at 8:00 h (43.61  $\pm$  6.91) and at 16:00 h (54.15  $\pm$  1.50), but it was not detectable at 24:00 h. Thus, the % ER $\alpha$ -ir GC did not show significant differences between experimental and control groups at 8:00 h ( $P > 0.05$ ). However, the expression of ER $\alpha$  in GC was different at 16:00 h and 24:00 h between two groups. These results show that the expression of ER $\alpha$  presents a circadian rhythm directly related to the thickness of the photoreceptor layer previously studied. The administration of Li maintains the expression of the ER $\alpha$  between 8:00 and 16:00 h. In conclusion, treatment with Li alters the expression of ER $\alpha$  in the layers of the retina and this might change the daily renewal process of photoreceptors.

## A99

### APOPTOSIS AND EXPRESSION OF ESTROGEN RECEPTORS IN PITUITARY OF MALE VISCACHAS DURING THE ANNUAL REPRODUCTIVE CYCLE

*Rosales GJ<sup>1,2</sup>, Mohamed FH<sup>1</sup>, Filippa VP<sup>1,2</sup>*

<sup>1</sup>Área de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. <sup>2</sup>Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET. E-mail: [gabirosales87@gmail.com](mailto:gabirosales87@gmail.com)

Estrogens through their specific receptors (RE $\alpha$  and  $\beta$ ) act as modulators of cell renewal in the pituitary gland, being mediators in the processes of proliferation and cell death. The aim of this work was to localize and quantify the apoptotic cells and the expression of RE $\alpha$  in different regions of pituitary pars distalis (PD) of adult male viscachas during their annual reproductive cycle. In each period of this cycle (reproductive, gonadal regression and gonadal recovery periods), four pituitary glands were collected and processed for light microscopy. Apoptotic cells were detected by TUNEL and RE $\alpha$ -immunoreactive (-ir) cells by immunohistochemistry. These cells were morphometrically quantified by image analysis. The percentage of cells per microscopic field were determined. The values were statistically analyzed and expressed as mean  $\pm$  SEM. In all groups, TUNEL-positive nuclei were scarce in the medial region. However, they were abundant in the ventral region and at the rostral and caudal ends. In gonadal regression and gonadal recovery periods, the percentage of this nuclei were significantly higher than in the reproductive period ( $P < 0.01$ ,  $P < 0.05$ ). In addition, they were frequently located close to follicular structures and blood vessels. The immunostaining pattern for ER $\alpha$  was nuclear (ER $\alpha$ n) and cytoplasmic (ER $\alpha$ c). A cytoplasmic heterogeneous pattern was observed in all groups studied. The ER $\alpha$  expression showed regional variations during the reproductive period. The RE $\alpha$ c-ir cells being higher in the medial region ( $P < 0.01$ ) and less numerous at the rostral end ( $P < 0.01$ ). The total percentage of RE $\alpha$ c-ir cells did not differ significantly among different groups, instead the total percentage of RE $\alpha$ n-ir cells was maximal in recovery period and minimum in

gonadal regression period ( $P < 0.05$ ). The regionalized expression of ER $\alpha$  receptors and apoptotic cells might be related to the different PD cell populations and their variations throughout the reproductive cycle according to hormonal demand. Besides, these results demonstrated that the minimal expression of ER $\alpha$  was related with the maximal number of apoptotic cells in the gonadal regression period, suggesting that a decreased of ER $\alpha$  expression might stimulate the apoptotic processes in the pituitary during the annual reproductive cycle.

#### A100

### PCNA EXPRESSION IN THE PINEAL GLAND OF PREGNANT AND NON-PREGNANT VISCACHAS (*Lagostomus maximus maximus*): AN IMMUNOHISTOCHEMICAL AND MORPHOMETRIC STUDY

*Busolini FI*<sup>1,3</sup>, *Gallol LE*<sup>1</sup>, *Mohamed FH*<sup>1</sup>, *Sosa-Escudero MA*<sup>2,3</sup>

<sup>1</sup>Area de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. <sup>2</sup>Cátedra de Histología, Facultad de Ciencias Médicas Universidad Nacional de Cuyo. <sup>3</sup>Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET. E-mail: [fabriciobusolini@gmail.com](mailto:fabriciobusolini@gmail.com)

The proliferating cell nuclear antigen (PCNA) is a non-histone nuclear protein that appears to play a key role in coordinating the recruitment of many proteins to sites of DNA replication and in some cases regulates their activities. PCNA acts as an auxiliary protein for DNA polymerase delta by enhancing its activity. It also works as an auxiliary factor for DNA polymerase epsilon. The pineal gland acts as a neuroendocrine interface, transforming ambient light changes into hormonal variations. Our experimental model, the viscacha, is a hystricomorph rodent with a seasonal reproductive pattern synchronized by the environmental photoperiod and modulated by the pineal melatonin. Female viscachas usually have an estrous period in early autumn, get pregnant during winter and offspring are usually born in spring. In this study, we performed an immunohistochemical and morphometrical study of PCNA expression in the pineal gland of pregnant and non-pregnant females. Four pineal glands (N = 4) per group were analyzed. The reproductive condition was assessed according to body weight and light microscope observations of ovaries. Additionally, the uterine horns were examined to evaluate the presence of embryos and fetuses. The glands were processed for light microscopy and the PCNA was immunohistochemically identified. The percentage of immunoreactive cells was determined using the formula  $(A/A + B) \times 100$ , where *A* is the number of immunoreactive cells and *B* is the number of nuclei in immunonegative cells. Statistical differences were evaluated by means of Mann–Whitney test. A value of  $P < 0.05$  was considered significant. Immunohistochemical results revealed that PCNA-immunoreactivity (ir) was detected mainly in the nuclei. Positive PCNA nuclei were frequently spherical, and some of them were oval in shape, according to the pinealocytes. The percentage of PCNA ir cells was higher in the pregnant females ( $1.99 \pm 0.09$ ) compared to the non-pregnant ones ( $0.76 \pm 0.05$ ). The higher amount of cell proliferation observed in pregnant viscachas was probably related to the increased serum levels of estradiol and progesterone reported during pregnancy. On the other hand, the percentage of PCNA ir cells decreased in non-pregnant females, in agreement to the low serum levels of gonadal hormones. However, further studies might be necessary to confirm our findings.

#### A101

### CELL PROLIFERATION AS A REGULATORY MECHANISM IN THE FUNCTIONAL ADJUSTMENT OF THE FEMALE VISCACHA ADRENAL CORTEX DURING PREGNANCY

*Gallol LE*<sup>1</sup>, *Busolini FI*<sup>1,2</sup>, *Mohamed FH*<sup>1</sup>

<sup>1</sup>Area de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. <sup>2</sup>Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET. E-mail: [gallolezequel@gmail.com](mailto:gallolezequel@gmail.com)

The hypothalamic–pituitary–adrenal axis (HPA) is essential for physiological adaptation processes and improvement of survival chances when facing homeostatic challenges through regulation of steroid hormones production in the adrenal cortex. The *Lagostomus maximus maximus* (viscacha) is a nocturnal hystricomorph rodent that inhabits the southern hemisphere from Paraguay through central Argentina. Its physiology and behavior vary according to modifications of environmental signals. In its natural habitat, this rodent is a seasonal breeder synchronized by the photoperiod. The female viscacha has a particularly long gestation period for a rodent, carrying out its pregnancy for 154 days and giving birth to well-developed offspring. In mammals, proper adjustment of the adrenal cortex functionality is necessary to achieve a healthy pregnancy. Disruptions in the HPA axis activity can lead to maternal and fetal morbidity and/or demise during gestation or puerperium. The objective of this study was to assess the proliferative activity in the adrenal cortex of non-pregnant and pregnant viscachas through immunohistochemical detection of PCNA (proliferating cell nuclear antigen). In addition, biochemical determinations of serum estradiol and progesterone were also performed. The female viscachas were captured in its natural habitat and divided into four groups (N = 4) according to the presence and sizes of the fetuses: non-pregnant, early-pregnancy, mid-pregnancy, and late-pregnancy. Non-pregnant and early-pregnancy groups showed a low number of PCNA immunopositive adrenocortical cells (PCNA-A) ( $0.67 \pm 0.13$  and  $0.76 \pm 0.07$ , respectively). On the other hand, mid-pregnancy viscachas exhibited a remarkable high number of PCNA-A ( $9.53 \pm 2.83$ ), which were observed mainly in the outer regions of the cortex. Late-pregnancy viscachas presented enlarged adrenals with few

PCNA-A ( $0.46 \pm 0.04$ ). Statically significant differences ( $P < 0,05$ ) were found when comparing mid-pregnancy viscachas with all the other groups. Serum estradiol and progesterone levels showed a pronounced peak during mid-pregnancy. Significant differences were established when comparing mid-pregnancy serum estradiol ( $75.02 \pm 2.5$  pg/mL) versus non-pregnant ( $18.01 \pm 3.19$  pg/mL), early-pregnancy ( $27.50 \pm 2.5$  pg/mL) and late-pregnancy levels ( $24.25 \pm 2.17$  pg/mL). Progesterone levels showed significant differences when comparing mid-pregnancy ( $53.75 \pm 2.39$  ng/mL) with non-pregnant ( $0.72 \pm 0.11$  ng/mL), early-pregnancy ( $4.64 \pm 0.95$  ng/mL) and late-pregnancy viscachas ( $18.61 \pm 2.25$  ng/mL). Furthermore, the comparison of serum progesterone levels between late-pregnancy and early-pregnancy also exhibited a significant difference. Our results showed that cell proliferation is an important regulatory mechanism in the functional adaptation of the female viscacha adrenal cortex to gestational demanding conditions and that this proliferative activity might be driven by female sex hormones.

### A102

#### EVALUATION OF THE IMPACT ON THE BEHAVIOR OF DIFFERENT CATEGORIES OF CATTLE WITH THE INCORPORATION OF THE SAME DIET AT THE CATTLE FINISHING IN FEEDLOT

Rosa ST<sup>1</sup>, Aguilera Merlo MS<sup>2</sup>, Becerra CR<sup>1</sup>, Sueldo RA<sup>1</sup>, Osses RG<sup>1</sup>, Rodríguez Rivera MF<sup>1</sup>

<sup>1</sup>Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Agropecuaria (Universidad Nacional de San Luis). <sup>2</sup>Ingeniería Agronómica (FICA). E-mail: sergiotuliorosa@gmail.com

The feeding of cattle in feedlot in Argentina is characterized by presenting basic structures, small scale and, in general, seasonal variants. These feedlot systems were adapted from the cattle finishing of high impact categories (price/live weight) such as steer, to others of lower economic income (cows, bulls) with different diets according to requirements, seeking in this way to make each stage of their growth and development more efficient. The hypothesis was formulated: junior bulls (MEJ) will have the higher conversion efficiency (EC) among the evaluated categories. The objective was to evaluate the productive behavior of different categories fed with the same termination diet in a feed lot. The numbers of animals according to category were: 6 cows (313 kg), 10 heifers (219.5 kg), 6 male calves (186.6 kg), 6 female calves (198 kg), and 5 junior bulls (MEJ, 169 kg). The work was carried out in the establishment "El Piquiyí", located at 30 km southwest of Villa Mercedes (San Luis), and it took place between 12/15/2020 and 02/20/2021, where a feedlot operates with hotel service regime with capacity for 2000 animals. In each category were recorded the amount of feed supplied per day and per animal and their losses by feeder bunk reading, obtaining in his way the daily feed consumption data, after which ADPV (daily live weight gain) was calculated. The performance in relation to diet/category was also determined, from the productive point of view evaluating the (EC) Conversion Efficiency, daily consumption/ADPV and, from the economic side, measuring the daily economic impact (IED) according to the difference between the daily cost of the food supplied affected by losses and economic income produced by the ADPV by category. The ration consisted of whole plant corn silo (40%), remains of soybeans (10%), corn grain (40%), nucleus (2%), and water (8%), whose formulation gives a protein content 11.44% PB. The daily cost of the food supplied was \$21.00/kg MS (dry matter). The results of EC (kg), ADPV (kg), and IED (\$) for each category were: Heifers: 6.68; 1.152; 80 – Cows: 12; 0.979; – 101 – Male calves: 6.82; 1.207; 78 – Female calves: 8.25; 0.999; 47 – Junior bulls (MEJ) 6.3; 1.418; 77; respectively. It is concluded that, by the nutritional contribution, the ration is more adapted to the category cows, but because of the high consumption and the lower ADPV from it, makes the economic result was negative, by contrast to the category MEJ, that showed a better productive performance in terms of EC, ADPV, and IED.

### A103

#### BEHAVIOR OF FOUR CATEGORIES OF WEANING CALVES SUBJECTED TO THE SAME PASTORIL REARING DIET. LAS ISLETAS (SAN LUIS)

Rosa ST<sup>1</sup>, Blasco MF<sup>2</sup>, Becerra CR<sup>1</sup>, Sueldo RA<sup>1</sup>, Osses RG<sup>1</sup>

<sup>1</sup>Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Agropecuaria (Universidad Nacional de San Luis). <sup>2</sup>Ingeniería Agronómica (FICA). E-mail: sergiotuliorosa@gmail.com

Most of bovines are traditionally fed with pasture in Argentina. This system depends on the season of the year in quality and quantity growers can get in pasture; it affects the production principal objective: the efficiency of the conversion of DM eaten, in beef produced. The aim of this work was to evaluate the difference in the performance of four categories of calves (females and males) during their growth under pasture nutrition determined by the gained weight in a system based on a differed diet with rye and corn in the four different animal categories. This work was fulfilled in an establishment called "San Antonio", placed 25 km south of Villa Mercedes city (San Luis). Food used was *Secale cereale* L. (rye) in the growing period because it is a tough country pasture which tolerates extreme weather conditions because of its deep roots which gives it relevant precociousness and has got the possibility to be used in sandy soil. It was sown from an aircraft, 20 kgs of seeds/ha on a differed corn with low results. A daily increase of alive weight (DIAW) was determined and dry material (% Alive weight: dry material related to alive weight) under the same diet of four calf categories: small females (SF), small males (SM), adult females (AF), and adult males (AM), during pasture nutrition growing. Animals were weighed each 15/25 days under pasture nutrition. Consumption of M/D. Alive weight (AW) was

measured in kilograms and percentage. It was estimated the absolute dry material consume (DMC) through the starting weight (availability) and finishing weight of each piece of land (five in total). 92 days was the period of the work (from July 20 to October 20, 2020). Statistically, we used ANOVA of repeated measure ( $P < 0.05$ ). The AW of starting and finishing average results in each category were: SF: 82.20 and 123.10 kg, SM: 76 and 116.11 kg, BF: 148.10 and 194.40 kg, BM: 155.86 and 209.21 kg. The average DIAW values in each category were: SF: 0.445 kg, SM: 0.436 kg, BF: 0.503 kg, and BM: 0.580 kg. The daily consume of DM, expressed in %AW of the pasture period was of 2.14% (as an average all over the categories) divided into: rye 0.48% AW and corn 1.66% AW. Daily consume was generally reduced but mainly in SF and SM. This result is probably related to a low nutritional quality given by the pasture (22% corresponded to rye and 78% to corn). It gives lower results of DIAW when BF and BM were separated. In conclusion, calves which require different nutrition affect their productive performance when they are under the same diet.

#### A104

### A PRELIMINARY EVALUATION OF ATRAZINE EFFECT ON THE DISACCHARIDASE ENZYMES OF EARED DOVE (*Zenaida auriculata*)

*Manzanos NA, Bach N, Cid FD, Chediack JG*

*Laboratorio de Biología Integrativa. IMIBIO-SL, Departamento de Biología, FQByF. Universidad de San Luis. CCT San Luis CONICET. San Luis – Argentina. E-mail: nazarenomanzanos@gmail.com*

The occurrence of xenobiotics in the diet of animals is a factor that can affect important functions in them. In response to these xenobiotics, organisms must rapidly perform various behavioral and physiological adjustments in order to survive. Digestive tract is the most important organ involved in coordinating the digestion and acquisition of nutrients, both vital function for organisms. This system has a high capacity to adapt to different conditions, both internal signals and external agents. In this sense, it has been observed that certain environmental pollutants can affect the enzymatic activity of the digestive system in some vertebrates. However, this effect is poorly studied in birds. Considering that atrazine is a widely used pesticide in our country and worldwide, and that negative effects on the physiology of some organisms have also been described, we propose as the general objective of this study to elucidate the long-term atrazine effects on digestive enzymes of “eared doves” (*Zenaida auriculata*). To achieve our goal, we established three independent groups of birds ( $N = 6$  each group), two groups were exposed for 15 days to 25 mg/kg and 250 mg/kg atrazine concentration in corn oil administered by intermittent gavage each day at the same time, and one control group that were administered only with corn oil without atrazine. After exposure, body weight measure, blood extraction, and subsequent removal of the intestine, stomach, liver, and pancreas were performed at the same time (8:00 am), to avoid disturbing by circadian/daily variation. The intestinal enzymatic activity of sucrose and maltase was determined in proximal, medial, and distal portions. Statistical analysis performed for enzymes was RM-ANOVA and one-way ANOVA for organ mass (Tukey post-hoc test,  $P < 0.05$ ). No difference between groups was observed in enzyme activity of maltase and saccharase ( $P > 0.05$ ), body mass, intestine, stomach, and liver mass (all  $P > 0.05$ ). The pattern found of intestinal enzymes activities were similar as those reported for other bird species, a higher activity on proximal and medial section. Although atrazine has been shown to have pathological effects in some species of birds, our results showed a non-significant inhibition of these enzymes in doves exposed to atrazine and a lack of effect in organ mass. However, further histological studies are necessary to support this observation in digestive organs. About enzyme activity, *in vitro* experiments could give more information about enzyme activity inhibition, since the presence of crop in these birds could interfere the real concentration in the gut. *Supported by CyT-UNSL PROICO 02-0820 and FONCYT PICT-201-0595.*

#### A105

### RESPONSES TO FORCED SWIM ACUTE WITH WEIGHT LOADING AND DIFFERENT MODES OF EXERCISES OF RESISTANCE WITH WEIGHTLIFTING IN WISTAR RATS

*Huck CG<sup>1,2</sup>, Biasi A<sup>1</sup>, Plaza G<sup>1</sup>, Puebla M<sup>1</sup>, Muñoz P<sup>1</sup>, Guzman V<sup>1</sup>, Becerra B<sup>2</sup>, Ruffino C<sup>2</sup>, Ferreyra M<sup>2</sup>, Sosa E<sup>2</sup>, Pedernera AM<sup>2,3</sup>*

*<sup>1</sup>Esc. Cs. de la Salud. Depto. Cs. Básicas. UNViMe. <sup>2</sup>Depto. Educ. Física. Fac. Cs. Humanas. UNRC. <sup>3</sup>Depto. Farmacia. Facultad de Química, Bioq. y Farmacia. UNSL. E-mail: ghuck@unvime.edu.ar*

The types of exercise in rats, to be able to determine the responses and adaptations to the exercise, go from the endurance modes in treadmill or forced swimming to resistance, supporting overload in suspension, or climbing a ladder with a load of weights. The aim was to determine endurance capacity to exhaustion in forced swimming modalities with an incremental weight bearing and lifting force with incremental load. A total of 24 adult male Wistar rats ( $101 \pm 2.21$  days) were housed in cages in a room maintained at  $22 \pm 1$  °C with a light-dark cycle of 12:12 h, with water and food *ad libitum*. The animals were randomly distributed into three groups ( $N = 8$ ): Forced swimming endurance exercise (ENF), ladder strength exercise (EFE), and suspension strength exercise (ESS). The swim was carried out in a swimming pool that contained water at a temperature of  $34 \pm 1$  °C. The adaptation period consisted of daily 10-min swimming efforts, performed 3 days/week for 1 week. The scheme of the effort of swimming until exhaustion was that the rat had to swim continuously for stages of 5 min in a scheme of 3%, 5%, 7%, and 10% of body weight (BW). Swimming was evaluated

from the start to the point where the rat could not hold on to the surface of the water for 10 s after sinking to the bottom of the pool. For the EFE group, a ladder with the following characteristics was used (1.1 m long, the slope of 80°). During the adaptation period carried out 3 days/week for 1 week, the animals carried a load of 5% of their individual BW. A repetition was considered successful when the animal managed to climb from the bottom of the ladder to the top. The effort scheme until exhaustion was: 30%–50%–60%–75%–85%–100%, and 110% BW × 1 repetition (1R). In the ENF group, the BW of the rats was (310.63 ± 24.27 g) With a swim time to exhaustion of (877.50 ± 270.59 s) with a load of (5.88 ± 2.1 % BW). In the EFE group the weight was (283.13 ± 16.24 g), with a load of (240.63 ± 57.6 g) attached to its tail on the ascent, representing (83.13 ± 17.92 % BW). In the EFS group, the weight was (274.38 ± 29.57 g) with a maximum grip load on its front legs of (196.13 ± 32.52 g), with a range between 145–255 g, representing a (73.13 ± 5.30 % BW). No significant differences were found between the EFE and EFS groups in any of the variables analyzed (BW – weight lifted and % BW lifted) ( $P > 0.05$ ). A significant difference was found in the BW of the ENF group vs. EFE and EFS ( $F = 4.97$ ,  $P < 0.02$ ). The types of exercise force swimming with resistance weight and lifting weights incrementally, in their two forms, represent variants of effort that can be used in murine models to study the physiological responses and adaptations to acute and chronic exercise. The form of suspension weightlifting exercise, as well as the ascent on the ladder, no they did not have significant differences in the %BW of the load and the weight lifted, can be used interchangeably, but with the consideration important that in EFE the greatest force is performed by the rear legs.

## PHARMACOLOGY

### A106

#### ORAL REPEATED DOSES STUDY OF *Tripodanthus flagellaris* IN RATS

Albornoz E<sup>1</sup>, Villegas Gabutti C<sup>1</sup>, Paredes J<sup>1</sup>, Maria A<sup>1</sup>, Petenatti E<sup>2</sup>, Wendel G<sup>1</sup>

<sup>1</sup>Farmacología, <sup>2</sup>Farmacognosia. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis.

E-mail: jdparedes@unsl.edu.ar

*Tripodanthus flagellaris* (Cham. & Schldt.) Tiegh. (Loranthaceae family) is popularly known as “liga blanca” or “liguilla”. It is used in folk medicine by their reputed hypotensive properties. The aim of this study was to evaluate the toxicity of *T. flagellaris* at 14 days in male and female Wistar rats. Infusion was prepared at 10% from the dried powdered plant material, separated by filtration, and the aqueous extract was concentrated and lyophilized to preserve it. *T. flagellaris* was administered, *p.o.*, at concentrations: 0 (control group), 125 mg/kg (low-dose group), 250 mg/kg (middle-dose group), and 1000 mg/kg (high-dose group), respectively. Routine clinical observations and body weight were measured. Peripheral blood was collected; hematology (red and white blood cells) and clinical chemical (aspartate aminotransferase [AST], alanine aminotransferase [ALT], glucose, total protein, and albumin) values were evaluated. The organs of each rat were observed grossly and weighted (lungs, heart, liver, kidneys, spleen, testes, and ovaries). Parametric ANOVA method was used. No abnormal symptoms and clinical signs or deaths had been found in rats in each group during the test. The general conditions of all rats were normal. There were no signs or symptoms of restlessness, respiratory distress, salivation, pilomotor erection, lacrimation, diarrhea, convulsions, and coma. No significant difference had been found in body weight and food consumption of rats in each test group ( $P > 0.05$ ). In addition, no significant differences were found in each hematology value, clinical chemistry value and organ/body weight ratio, either ( $P > 0.05$ ). The highest dose did not induce noticeable signs of toxicity. In conclusion, the *T. flagellaris* possesses no obvious significant toxic effects on any parameter examined.

### A107

#### HEAVY METALS DETERMINATION IN AEROBIOLOGICAL SAMPLES DURING THE COVID-19 PANDEMIC

Escudero DR<sup>1</sup>, Moyano MF<sup>2</sup>, Isaguirre AC<sup>1</sup>, Moglia MM<sup>1</sup>

<sup>1</sup>Proyecto: “Radiación solar y medio ambiente” (FCFMyN - UNSL). Lavalle s/n e/ Chacabuco y Mitre, San Luis, Argentina. <sup>2</sup>Instituto de Química de San Luis (CONICET-UNSL). Ejército de Los Andes 950, San Luis, Argentina.

E-mail: danielarocioescudero@gmail.com

Atmospheric air carries a big number of particles and environmental pollutants, to which people are exposed, coming from anthropogenic activities. Metal pollution has been increasing rapidly over the past century in relation to the growth of human population contaminants production. Thus, aero particles such as pollen grains may contain various trace elements, and their amounts vary from one species to another. They can present traces of metals such as lead (Pb), barium (Ba) and manganese (Mn), which are among the most common air and soil contaminants and are considered toxic to living beings. In the present work, we propose a simple and fast method for the multi-elemental determination of traces in aerobiological samples. The samples of aeroparticles (N = 50) were obtained with a volumetric Lanzoni sensor, which is located on the terrace of the National University of San Luis. This device allows the capture of particles suspended in

the air with a week periodicity, and with a suction flow of 10 L of air/min, similar to the volume of air inhaled by the human lung. The pollen content was analyzed for two months of 2019 (N = 25) and 2021 (N = 25) with continuous aerobiological sampling, in the atmosphere of the city of San Luis, Argentina. Aerobiological samples were observed with an optical microscope at 400×, and they were identified with the palynothèque belonging to Aerobiology's laboratory. The pollination period studied was from March to April. For multi-element determination, a mass spectrometer with inductively coupled plasma (ICP-MS) was used. The aeroparticle samples were subjected to acid digestion at 90°C for 1 h. Pb and Ba were determined in 2019 at representative levels,  $11.85 \pm 0.71$  and  $4.35 \pm 0.27$  ppb, respectively. There was a significant difference in Mn concentrations compared to 2021,  $21.75 \pm 1.24$  vs.  $12.35 \pm 0.87$  ppb,  $P < 0.01$ , respectively, determined through the non-parametric statistical test Kruskal-Wallis. Other heavy elements were not detected in aeroparticle samples in 2021. Comparatively with others pollen types, Chenopodiaceae was the most abundant in the atmosphere of San Luis, during the analysis period. Linear regression models (Spearman) were used to analyze the metals vs. pollen content. Positive associations were found between Mn with Chenopodiaceae in 2019 ( $r = 0.86$ ,  $P = 0.0015$ ). In the same sense it was observed positive associations between Pb with Chenopodiaceae ( $r = 0.78$ ,  $P = 0.0026$ ). Negative associations were found between Mn with Chenopodiaceae in 2021 ( $r = -0.26$ ,  $P = 0.002$ ). The maximum concentration values for Chenopodiaceae were 27 and 30 pollen grains/m<sup>3</sup> of air, 2019 and 2021, respectively in the month of March. In this study, during the pollination period an increase in the elemental content was observed in 2019. Therefore, the metals could be transported by aero particles such as pollen grains. The decrease in heavy metal pollution could be explained by the decrease in vehicular traffic.

### A108

#### HEPATOTOXICITY PRODUCED BY PARACETAMOL

*Moyano M, Pelzer L, Wendel G*

*Farmacología, Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis. Av. Ejército de los Andes 950, San Luis, Argentina. E-mail: mfmoyano07@gmail.com*

*Aristolochia argentina* (Aristolochiaceae) is a rhizomatous plant popularly known as “charrúa”, “charruga”, “patito”, “buche de pavo”. The roots of this plant are used in popular medicine as: diuretic, antidiarrheic, astringent and emmenagogue. In previous phytochemical tests, the presence of flavonoid compounds in its infusion was verified. The liver damage produced by paracetamol is a widely used model for the study of hepatoprotective substances that, when metabolized, produce free radicals that cause liver damage. *A. argentina* showed a high antioxidant activity of 265.8 μmol of Fe (II)/g (FRAP). *Aristolochia bracteolata* Lam. and *Aristolochia giberti* possess antioxidant activity, too. The aim of this research was to evaluate the effect of the root infusion of *A. argentina* on aspartate aminotransferase in the injury produced by paracetamol. The plant material was collected in the province of San Luis. Root infusion prepared according to the Argentine Pharmacopoeia VI edition in concentrations of 10% of *A. argentina* was used. Wistar rats of both sexes, 150–180 g, were used. Liver injury was caused by oral administration of 640 mg/kg of paracetamol. The experimental animals (N = 6–8/group) were administered via oral 10 mL/kg/day (for 3 days) of physiological solution (group 1), physiological solution plus paracetamol one hour after the last dose (group 2) and infusion of *A. argentina* (for 3 days) plus paracetamol one hour after the last dose (group 3). One day later, blood samples were obtained for the biochemical determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline-phosphatase (ALP), γ-GT (gamma glutamyl transferase), total protein, and bilirubin. Biochemical determinations were carried out with manual analysis techniques using Wiener lab reagents. Parametric ANOVA method was used. The toxic dose of paracetamol (640 mg/kg) produced liver damage, as indicated by the significant increase in AST values compared to the control group ( $62.17 \pm 3.31$  vs.  $42.24 \pm 4.03$ ,  $P < 0.01$ ). Pretreatment with the infusion of *A. argentina* produced significantly decreased AST activity ( $44.51 \pm 2.45$  vs.  $62.17 \pm 3.31$ ,  $P < 0.01$ ). No significant differences were found in the values of ALT, γ-GT, total protein, and bilirubin ( $P > 0.05$ ), (group 2 vs. group 3). In an oral repeated doses study of *A. argentina*, no significant differences were found in AST, ALT, and total protein values. The results showed that the infusion of *A. argentina* has a protective effect against paracetamol induced hepatotoxicity, evidenced by a decrease in the enzymatic activity of AST. Future studies will be oriented towards the specific study of the mechanism of action involved.

### A109

#### NEW PSYCHOACTIVE SUBSTANCES, THE CURRENT PARADIGM AND POTENTIAL EMERGING SUBSTANCES

*Ortega M, Cocco L, Pezzuchi G, Rovira E, Rainoldi F, Bianchi A, Di Loreto O, Formigo F, Llanos F*

*Facultad de Derecho y Ciencias Políticas Universidad Católica de La Plata. E-mail: laura.cocco@ucalp.edu.ar*

New Psychoactive Substances (NPS) are designer drugs that, due to their novelty, ability to evade toxicological testing, ambiguous legal status, global marketing on the Internet, and limited public awareness of their adverse effects, have become a 21st century phenomenon. These compounds are synthesized in laboratories from variations in the chemical structure of other substances such as amphetamines. The major drawback is that there is a great variability of substances, as their producers, in order to evade the law, are constantly making small modifications that allow them to design new substances with similar effects on users and which are sufficiently different to fall outside the existing regulatory

framework. The most suitable alternatives for overcoming this obstacle, within our current control system, involve (a) streamlining the procedures for incorporating compounds into the respective lists, and (b) advancing in the definition of new controlled chemical groups. In this way, efforts, both in a framework of international cooperation and at the national level, must be oriented towards promoting scientific research, in order to detect new substances that constitute a threat to public health and that are not under control. The objectives were to survey the existence of examples of New Psychoactive Substances that are not included in the current regulations (Law 23.737 and its corresponding amendments). To this end, an exploratory study was carried out focusing on the family of synthetic cathinone compounds, specifically derivatives of  $\alpha$ -pyrrolidinopropiophenone (PPP). Compounds that escape the current regulatory framework were postulated based on their synthesis feasibility and structural similarity to those compounds that are regulated. These include substitutions at carbon 4 of the benzene ring by groups such as methyl carboxylate and 2-methylpyrrole. These compounds could act in monoamine transport systems given their structural similarity, although their potential as a narcotic drug needs to be tested. A mixed approach to the search for potential non-regulated substances is also being developed, extending it to other families of compounds such as synthetic cannabinoids and phenethylamines, incorporating their physico-chemical characterization and their possible narcotic and biological effects. It also considers their efficiency in terms of production as a chemical process, with the intention of anticipating new compounds emerging on the market. It is hoped that this contribution will be suitable for the regulation of drugs and to support their incorporation into the lists annexed to Law 23.737. *Research funded by the U.C.A.L.P.*

### A110

#### EVALUATION OF HEPATOPROTECTIVE EFFECT OF *Jodina rhombifolia* (Hook. & Arn.) Reissek ON PARACETAMOL-INDUCED HEPATOTOXICITY IN RATS

Duarte SR<sup>1</sup>, Teves MR<sup>2</sup>, Wendel GH<sup>2</sup>

<sup>1</sup>Enfermería, Facultad de Ciencias de la Salud. <sup>2</sup>Farmacología, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. San Luis, Argentina. E-mail: gwendel@unsl.edu.ar

*Jodina rhombifolia* (Hook. & Arn.) Reissek (Santalaceae) is a small perennial hemiparasit tree, popularly known as “peje”, “quebrachillo”, “quebracho flojo”, “sombra de toro”. This species is utilized in Argentine folk medicine for a great diversity of health problems as anti-alcoholic, digestive, antidiarrheic, anti-inflammatory, hepatoprotective, hypotensor, among others. The major chemical constituents of leaves of *J. rhombifolia* are phenolic compounds, organic acids, tannins, flavonoids, steroids, gums, and mucilage; the extract of its leaves revealed the presence of C-glycosyl flavonoids. The present study was carried out to determine the effect of the *J. rhombifolia* lyophilized extract (JRLE) on experimental liver damage induced by paracetamol. The medicinal plant was collected in the San Luis Province, Fraga locality, “Los Chañares” establishment. Infusion to 10% was prepared following the methodology outlined in the VI Edition Argentine National Pharmacopoeia and lyophilized to preserve it. Wistar rats (180–200 g) were divided into five groups (N = 6–8). The JRLE was redissolved in distilled water just before administration. The JRLE (at doses of 500 and 1000 mg/kg) and silymarin (200 mg/kg) were given orally to respective groups once daily for 3 days. On the third day, paracetamol (640 mg/kg, v.o.) was administered to all groups except control, one hour after the respective treatment with JRLE, silymarin and vehicle. After 24 hours of paracetamol administration, blood was collected, and serum was analyzed for biochemical parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). A liver index was calculated by the following equation: Liver index (%) = (liver weight/body weight) × 100. Administration of paracetamol to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters ( $P < 0.01$  vs. control). Pretreatment of rats with JRLE exhibited significant ( $P < 0.001$ ) reduction in the paracetamol-induced increase in the levels of AST and ALT. Moreover, treatment with JRLE (1000 mg/kg) before induction of hepatotoxicity significantly lowered the ALP parameter. The liver weight and liver index in the JRLE (1000 mg/kg) + paracetamol group was lower than those in the paracetamol group ( $P < 0.01$ ). The hepatoprotective activity of JRLE (1000 mg/kg) was comparable with the standard silymarin. The rise in serum levels of transaminases has been attributed to the damaged structural integrity of the liver. Several flavonoids are reported for their hepatoprotective activities. These compounds could be responsible for the hepatoprotective activity. These results contribute to the scientific validation of the hepatoprotective indication of this botanic species in Argentine folk medicine.

### A111

#### COMPARATIVE STUDY OF CADMIUM TOXICITY IN FISH

##### (*Danio rerio* AND *Poecilia reticulata*)

Sanchez ES<sup>1,3</sup>, Pistone C<sup>2</sup>, Alvarez MA<sup>1</sup>, Alvarez SM<sup>1,3</sup>, Enriz RD<sup>2,3</sup>, Giannini FA<sup>2</sup>

<sup>1</sup>Laboratorio de Nutrición y Medioambiente, Cátedra de Fisiología, Universidad Nacional de San Luis. <sup>2</sup>Laboratorio de Bioensayos, Cátedra de Química General e Inorgánica, Universidad Nacional de San Luis. <sup>3</sup>Instituto Multidisciplinario de Investigaciones Biológicas IMIBIO-SL, CONICET San Luis. E-mail: emysilsanchez@gmail.com

The fish experimental models are ideal to evaluate toxicity as well as for research in embryology, neurobiology, endocrinology, environmental biology, and other areas. We have performed fish test for 15 years ago using bioassays designed by U.S. Fish and Wildlife Service. Columbia National Fisheries Research Laboratory based on the availability

of specimens and the substances to assay and are evaluating different biomarkers such as GOT and GTP (mainly hepatic enzymes), AChE (enzyme primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves) and creatinine (waste product formed by the kidneys). Environmental contamination of cadmium (Cd), derived from agricultural and industrial sources, has a high impact on the ecosystems, which are extremely susceptible to the effect of the toxicity of Cd. The toxicity of the different axenic is evaluated in acute assay (short time, high doses) and chronic assay (prolonged time, low doses). The acute adverse effects relate mostly to the ratio of mortality in 96 hours of exposure; meanwhile in the chronic assay the exposure last 21 days or more. In this study, we performed an acute and chronic test with juvenile specimens of *Danio rerio* (zebrafish) and *Poecilia reticulata*. The results of acute toxicity after exposure to several solutions of Cd chloride up to 100 mg/L showed no mortality in juvenile specimens of both species. Regarding chronic toxicity, we performed the analysis of different biomarkers such glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), acetylcholinesterase (AChE), and creatinine at two different doses (1 and 2 mg/L). The exposure to Cd for the long term caused a significant increase of the hepatic enzyme GOT and creatinine in both species. In contrast, we observed a remarkable decreased the levels of AChE in *D. rerio* as well as in *P. reticulata*. The analysis of these data leads us to conclude that the chronic effects observed in both species are in the same trend but in different levels and in a dose dependent manner. So that, these organic and functional effects may affect fish survival, bioavailability and physiology and impact their natural environment. The results highlight the concerns on Cd pollution and remark the use of fishes as biomarkers and its application on environmental toxicology studies for the great value of ecological protection.

### A112

#### EMBRYOTOXICITY OF CADMIUM IN ZEBRAFISH EGGS

*Pistone C<sup>1</sup>, Giannini FA<sup>1</sup>, Enriz RD<sup>1,3</sup>, Alvarez SM<sup>2,3</sup>, Sanchez ES<sup>2,3</sup>*

<sup>1</sup>Laboratorio de Bioensayos, Cátedra de Química General e Inorgánica, Universidad Nacional de San Luis.

<sup>2</sup>Laboratorio de Nutrición y Medioambiente, Cátedra de Fisiología, Universidad Nacional de San Luis. <sup>3</sup>Instituto Multidisciplinario de Investigaciones Biológicas IMIBIO-SL, Conicet San Luis. E-mail: emysil Sanchez@gmail.com

Fishes have been used in toxicology since 1950 showing formidable characteristics that make them excellent for experimental models for studies in embryology and research in other areas such as neurobiology, endocrinology, and environmental biology. Their ability to adapt to a wide variety of environments, easy and short time of reproduction, rapid development, few nutrition demands, and low cost and easy maintenance in laboratory conditions, among many other advantages, provide unique research opportunities including environmental contamination and its ecological consequences. Cadmium (Cd), a toxic element and an important environmental contaminant, have shifted with the dramatic increase of Cd industrial and agrochemical usage in the twentieth century. In this context, our lab has a large experience working with zebrafish (*Danio rerio*) that come with exceptional biological tools as a model system for vertebrate development. Cd is known as a developmental toxic. The aim of this study was to evaluate Cd effects in *D. rerio* eggs. For this purpose, zebrafish embryos were incubated for 120 h in 3, 2, 1.5, 1, 0.5, 0.25, and 0.15 mg/L of Cd chloride solution in a 24-chamber. We followed the processes to determine hatching rate, survival, and teratogenicity of the embryos. Our results indicated that exposure to Cd profoundly alters the hatching process. The survival is affected by the Cd solutions of 3 and 2 mg/L. In contrast, the other solutions did not significant affect the survival of any embryos. In addition, all tested concentration of the metal axenic has produced teratogenic malformation in all zebrafish embryos.

### A113

#### EFFECT OF THE ADMINISTRATION OF KETAMINE IN SUBANESTHETIC DOSES ON THE METABOLIC ACTIVITY OF THE HIPPOCAMPUS AT 8 MINUTES AND 24 HOURS (PRELIMINARY STUDY)

*Guevara MA<sup>1</sup>, García Menendez S<sup>1</sup>, Mazzei L<sup>4</sup>, Romanowicz E<sup>1</sup>, Barrutieta Arberas I<sup>2</sup>, Vaquero A<sup>2</sup>, Baiardi G<sup>3</sup>, Lafuente, JV<sup>2</sup>, Gargiulo PA<sup>1</sup>*

<sup>1</sup>Laboratory of Neurosciences and Experimental Psychology. Area of Pharmacology. Faculty of Medical Sciences. National University of Cuyo (CONICET). <sup>2</sup>Laboratory of Clinical and Experimental Neurosciences (LaNCE). Department of Neuroscience. University of the Basque Country, Leioa, Spain. <sup>3</sup>Laboratory of Neuropharmacology. Faculty of Chemical Sciences, Catholic University of Cordoba. <sup>4</sup>Laboratory of Basic and Translational Experimental Pharmacology. Area of Pharmacology. Faculty of Medical Sciences. National University of Cuyo and (CONICET). E- mail: vetguevara@hotmail.com

The information available on the changes in brain metabolic activity under the effect of ketamine is very varied and contradictory. Some authors report an increase in brain metabolism and other studies report a decrease in said activity. In previous works, we determined that the administration of ketamine in subanesthetic doses produces a decrease in mitochondrial metabolic activity as measured by the MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) assay, in spectrophotometer at 570 nm, in the hippocampus of male rats. This reduction occurs in the metabolism of mitochondrial oxide reduction. In the present study, we decided to investigate whether the decrease in hippocampal metabolism in rats treated with ketamine occurs temporarily or permanently. The following experiment has CICUAL protocol approval (97/2017 and 128/2018). Male Holtzman strain rats, weighing 240–290 g (N = 5 per dose),

were used. The animals were divided into two groups. The first group received the drug and 8 min later they were sacrificed by decapitation. The hippocampus was extracted and the MTT test was carried out. The second group was sacrificed at 24 h and the MTT test was applied to hippocampi. In each group, the doses used were administered intraperitoneally (1.25, 2.5, 5, 10, 15, and 20 mg/kg) and compared to saline control. Significance was studied using the ANOVA I test followed by the Student–Newman–Keuls post-test. In all cases a value of  $P < 0.05$  (two tails) was considered significant. A significant decrease was observed at 8 min with the doses of 1.25 mg/kg ( $P < 0.01$ ), 2.5 mg/kg ( $P < 0.001$ ), and 20 mg/kg ( $P < 0.05$ ). No differences were observed with other doses. At 24 h, no significant differences were found versus saline control. We conclude that the administration of ketamine produces a transient decrease in metabolic activity in the hippocampus.

#### A114

### CHRONIC ANTI-INFLAMMATORY ACTIVITY OF PLANTS FROM CUYO REGION:

#### *Larrea divaricata* AND *Parasenegalia visco*

*Pedernera AM<sup>1</sup>, Guardia T<sup>2</sup>, Pelzer LE<sup>2</sup>, Rotelli AE<sup>2</sup>, Wendel GH<sup>2</sup>*

<sup>1</sup>Laboratorio de Farmacognosia, <sup>2</sup>Laboratorio de Farmacología, Departamento de Farmacia, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis, Ejército de los Andes 980, (5700) San Luis. Argentina.

E-mail: [ampederne@gmail.com](mailto:ampederne@gmail.com)

Vegetal species have been used for therapeutic purposes from ancient times to the present day. Medicinal plants are an important source of resources, and many drugs are developed from active ingredients isolated from them. Research on this subject is of great interest to give scientific validity to the ancestral use of certain species or to discover new pharmacological activities. In this work, we compare the chronic anti-inflammatory activity of two plants found in Cuyo region: *Larrea divaricata* Cav. and *Parasenegalia visco*. *L. divaricata*, known as “jarilla” extends from the US to our country; it develops in dry and arid climates. On the other hand, *P. visco* (“aromo”, “viscote”) is used for ornamental purposes and is distributed in the Central and Northwest region of Argentina. The plants were identified by Dr. L. A. Del Vitto (UNSL), and the vouchers were deposited in the Herbarium–UNSL. The infusions and extracts were prepared according to the Argentine Pharmacopoeia. Granuloma test was performed as a model of chronic inflammation. Wistar rats (80 to 110 g) were implanted with a sterile cotton pellet (50 mg) in the dorsal area, from the next day and for 6 consecutive days each group received any of these preparations: 200 mg/kg of methanolic extract of *L. divaricata* subcutaneously (sc) or intraperitoneally; infusions of leaves or bark of *P. visco* (sc): 200 mg/kg methanolic extract of leaves or bark of *P. visco*, chloroformic or hexanic extract of leaves of *P. visco* (200 mg/kg). Dexamethasone (7 mg/kg, sc) was the reference drug. At seven days, all animals were sacrificed with CO<sub>2</sub>, and the granulomas were extracted and weighed. Granuloma weights were compared with the control group. Statistical analyses were performed by ANOVA and post-test Tukey–Kramer. The extracts and infusions tested showed anti-inflammatory activity ( $P < 0.05$  vs. control group), the highest activity (40% inflammation inhibition) was demonstrated by methanolic extract of *P. visco* leaves. The results confirm and validate the chronic anti-inflammatory activity of both plants.

#### A115

### ESTIMATION OF CONTAMINATION RISK GROUNDWATER WITH PESTICIDES IN EL MORRO BASIN (SAN LUIS, ARGENTINA)

*Martínez Espeche ME<sup>1</sup>, Garbero MM<sup>1</sup>, Hellmers MM<sup>1</sup>, Drovandi AA<sup>2</sup>*

<sup>1</sup>Facultad de Ingeniería y Ciencias Agropecuarias, Universidad Nacional de San Luis. Ruta Provincial N° 55 S/N extremo Norte, Villa Mercedes, San Luis. <sup>2</sup>Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo. Almt. Brown 52, Luján de Cuyo, Mendoza. E-mail: [mmartinezespeche@gmail.com](mailto:mmartinezespeche@gmail.com)

The expansion of agriculture in the “El Morro” basin has increased the use of pesticides, mainly associated with direct seeding. The impacts from agricultural activities are usually associated with the use of pesticides. However, pesticides are a fundamental tool in farmer’s systems, but it is a challenge to improve application efficiency and combine them with other management strategies to mitigate their impacts. The use of pesticides in a cropping system may result in surface water, groundwater, and air pollution and accumulation in soils. The purpose of this research is to estimate the possibility of groundwater pollution due to the action of pesticides and/or their metabolites. To monitor the use of pesticides in spring–summer crops, a survey was conducted in September 2020. The data was analyzed using Microsoft Excel and SPSS statistical programs. The Groundwater Ubiquity Score (GUS) is an experimentally calculated value that relates pesticide half-life and Koc (adsorption coefficient), from laboratory data. The GUS may be used to rank pesticides according to their potential to move toward groundwater. In the studies, basin farmers applied 29 pesticide molecules. The products that presented a higher frequency of use correspond to herbicides: Glyphosate, 2,4 D, Dicamba, Atrazine, and Picloran. Glyphosate and 2,4 D were used in 91% of the farms. Dicamba in 86%, and Atrazine and Picloran were used in 55% of the farms. The GUS index was calculated considering the characteristics of the soils in the basin, the agricultural history, the climatic conditions, and the physicochemical properties of molecules. According to this index, 2,4 D and Picloran have a high probability of reaching groundwater, while Atrazine and Dicamba have a moderate probability, and Glyphosate has a very low probability. Thus, the study suggests an environmental risk of groundwater

contamination due to the action of a few pesticides.

#### A116

### DIURETIC ACTIVITY VALIDATION OF AERIAL PARTS OF *Euphorbia serpens* FROM CENTRAL-WESTERN ARGENTINA AND IT'S ACTION MECANISM

Garro MF<sup>1</sup>, Garro H<sup>2</sup>, Loporati J<sup>3</sup>

<sup>1</sup>Área de Farmacognosia, <sup>2</sup>Área de Química Orgánica; Facultad de Química, Bioquímica y Farmacia. <sup>3</sup>Área de Estadística, Facultad de Ingeniería y Ciencias agropecuarias FICA. Universidad Nacional de San Luis. E-mail: mfgarro@gmail.com

*Euphorbia serpens* Kunth. var. *Mycrophylla* Müll. Arg. (Euphorbiaceae) is known as “bride’s tail”, “pigeon milk”, “stone breaker”, and popularly used as a diuretic and antilithiasic (Del Vitto *et al.* 1997). Collections were made in San Francisco del Monte de Oro, province of San Luis, on the banks of Route 2 (32°36'00" S 66°07'30" W / -32.6, 63 66.125), Voucher number, UNSL # 533. The plant material for analysis was dried at room temperature, protected from light until reaching hygroscopic humidity, and then it was ground to fine powder in a knife mill. The aim of this study was to validate the diuretic activity of aerial parts of *Euphorbia serpens* from central-western Argentina and mechanism action. Using Wistar rats in diuretic activity, Lipschitz method (1943). Each lot was treated with aerial parts (a.p.) and root (r.i.) infusions, obtained in our laboratory, furosemide (f) as standard drug, and saline solution (s.s.) as negative control. Dried, cut, and crushed aerial parts of *E. serpens* (1.310 kg) were extracted with acetone (1 × 1 L), at room temperature, yielding 9 g of crude extract (5% yield). The most important <sup>1</sup>H-NMR feature in acetone extract is the presence of chemical shifts for a triterpenoid with five-membered rings. Vinyl signal ( $\delta = 5.49$ , <sup>1</sup>H) and the hydroxymethylene group. The statistical significance of difference among means was assessed by Student’s *t*-test or  $\chi^2$  test. The present study demonstrates the diuretic activity of *Euphorbia serpens* infusions, that increases the urine volume. The diuretic pattern of the infusion at 10 % concentration was similar to that of the reference drug (furosemide), probably suggesting a similar mechanism of action. Mannitol could be the main diuretic compound in infusions and <sup>12</sup>ursene-type triterpenoids were found as principal components in acetone extract. No toxic effects were shown; likewise additional studies of *E. serpens* are necessary in order to study the possible mechanism of action.

#### A117

### HOW DEHYDROLEUCODINE INDUCES OXIDATIVE STRESS ON *Trypanosoma cruzi*?

Gomez J<sup>1</sup>, Guarise C<sup>1</sup>, Caballero P<sup>1</sup>, Cifuentes DA<sup>3</sup>, Masone D<sup>1</sup>, Sosa MA<sup>1</sup>, Barrera PA<sup>1,2</sup>

<sup>1</sup>Instituto de Histología y Embriología IHEM-CONICET, Facultad de Ciencias Médicas. UNCuyo. Mendoza. <sup>2</sup>Facultad de Ciencias Exactas y Naturales. UNCuyo. Mendoza. <sup>3</sup>Instituto de Investigación en Tecnología Química INTEQUI-CONICET. Universidad Nacional de San Luis. San Luis. E-mail: jessi.gomez.957@gmail.com

*Trypanosoma cruzi* is a parasite that causes Chagas disease (CD). The current chemotherapy is based on nitro derivatives, but their use is restricted due to the severe side effects, justifying the continuous search for alternative drugs. In this sense, natural compounds are attractive to study because its low cytotoxicity. *T. cruzi* has to evade the oxidative response of the host for successful infection and establishment of the disease. This ability is directly related to its particular antioxidant system based on a reducing molecule: trypanothione –equivalent to glutathione of mammalian cells– and reducing enzymes. Hence, the redox system of the parasite emerges as an attractive target for new antiparasitic therapies. In our laboratory, we study the mechanism of action of a natural compound called dehydroleucodine (DhL). This compound presents an  $\alpha$ -methylene group that could block the thiols groups present on trypanothione or reduce enzymes and induce oxidative stress on the parasite. In this study, we attempted to elucidate the trypanocidal mechanism of action of DhL, focusing on the parasite antioxidative defense machinery. According to our previous studies, DhL exerted an antiproliferative effect on *T. cruzi* (IC<sub>50</sub>: 4  $\mu$ M at 24 h), accompanied by a significant increase in reactive oxygen species (ROS) production. In this work, we incubated parasites with DhL, observed mitochondrial swelling, and blocked the trypanocidal effect by adding reduced glutathione. To explain these findings, we analyze the possible interaction between DhL/glutathione and DhL/trypanothione. We performed simulations of atomistic molecular dynamics with Gromacs of the order of a microsecond. The DhL, trypanothione and glutathione molecules were parameterized using LigParGen and Charmm-GUI. The radial distribution function of the distances between DhL and glutathione and DhL and trypanothione did not show significant differences. The maximum of both curves was ~0.6 nm (the most frequent distance), and neither of the simulations showed a dominant affinity between the molecules. From this analysis, we conclude that DhL exerts a trypanocidal action by the generation of oxidative stress, and this effect is independent of direct interaction with glutathione or trypanothione.

### A118

#### TOXICITY OF PLANT EXTRACTS OF *Helianthus petiolaris*: DETERMINATION OF EFFECTS ON *Daphnia magna* BY ACUTE TESTS

Durán K, Cabrera G, Cantarelli M, Pattacini S, Scoles G, Vignatti A

Universidad Nacional de La Pampa. Facultad de Ciencias Exactas y Naturales, Santa Rosa. La Pampa. Argentina.  
E-mail: katiaduran1@hotmail.com

Fungal diseases caused by plant fungi reduce crop quality and yield; control of these diseases requires research focused on reducing the use of synthetic fungicides, which generate strain resistance and environmental contamination. The alcoholic extract of false sunflower (*Helianthus petiolaris*) is a natural control alternative because it acts as a fungicide. In this work, toxicological bioassays were carried out under laboratory conditions with *Daphnia magna* to evaluate the toxicity of ethanolic extracts of *H. petiolaris* at different concentrations, using potassium dichromate as a reference toxicant. To evaluate the effect of exposure to different concentrations of ethanolic extracts, acute tests of 48 h duration were carried out. These were carried out using wide-mouth transparent plastic flasks of 20–30 mL capacity. Ten specimens of *D. magna* (less than 24 h-old neonates) were selected for each concentration, placing one specimen in each chamber, without changing the medium and without feeding. Observations were made every 12 h. The photoperiod to which the treatments were exposed was 8/16 h (dark, light), given by fluorescent tubes, controlled by a switch clock. The temperature at which the tests were performed was 22 ( $\pm 1$ ) °C and was controlled by a thermostat. Immobility or death were considered as effect indicators. For the calculation of LC<sub>50</sub> and their respective 95% confidence limits, the Probit method was used, in which the concentration belonging to Probit 0.5 corresponded to the amount of substance capable of generating the effect studied in half of the population. The ethanolic extracts of false sunflower produced the highest mortality in *D. magna* at a dose of 1.770 mg/L (LC<sub>50</sub>) compared to the reference toxicant with LC<sub>50</sub> at a concentration of 0.25 mg/L, with a percentage deviation of exposed/dead equal to 96.4486%. With the results obtained we can conclude that the extract of false sunflower could be used as a natural fungicide to replace the synthetic ones.

### A119

#### PHOTOCATALYTIC DEGRADATION OF DYES USING SILVER NANOPARTICLES FROM PLANT EXTRACT

Ancafilú Mendoza A, Durán K, Scoles G, Pattacini S

Universidad Nacional de La Pampa. Facultad de Ciencias Exactas y Naturales, Santa Rosa. La Pampa. Argentina.  
E-mail: katiaduran1@hotmail.com

Currently the dyes used in the textile industry include in their structure azo groups, anthraquinone, oxazine, thiazine. Some of the synthetic dyes, such as methylene blue, justify their use for cotton and silk dyeing due to their high solubility, brightness, and resistance. With the application of nanoparticles, water contaminated with synthetic dyes could be remediated, since they possess catalytic properties. The preparation of nanoparticles is based on the reduction of metallic salts through the action of chemical reducing agents or biological species, such as plants. Recent studies reveal that silver nanoparticles (AgNPs) have excellent photocatalytic activity under the action of sunlight, producing reactive oxygenated substances that favor the degradation of organic pollutants dissolved in water. The objective of this work was to evaluate the photocatalytic activity of AgNPs from buttercup (*Calendula officinalis*) in the degradation of methylene blue (MB) dye. A uv-vis spectrophotometer was used to evaluate the degradation by monitoring the absorption peak of the methylene blue dye at 664 nm. For the synthesis of AgNPs, 10-3 M AgNO<sub>3</sub> silver nitrate was used by mixing different volumes of the plant extracts in the presence of 0.13 mM polyvinylpyrrolidone (PVP) with constant stirring, at room temperature and at 60°C. The synthesized AgNPs were mixed with the dye, stirred in the dark for 1 h to ensure adsorption and desorption equilibrium, then irradiated with a UV lamp (black light with  $\lambda = 354$  nm, 8 W), at a distance of 10 cm. Subsequently at 1 h intervals, 5 mL aliquots of the mixtures were taken and scanned at wavelengths of 400–700 nm to determine the band of highest absorbance. The results show that the buttercup AgNPs degrade the MB dye by 72 %. It is concluded that the photocatalytic degradation process using AgNPs from plant extracts is a viable option for the treatment of water contaminated with azo dyes in the textile industry.

### A120

#### LUNG WITH ANDROGEN DEFICIENCY

Eggel ML<sup>1,2</sup>, Biaggio VS<sup>2,3</sup>, Gomez NN<sup>2,3</sup>, Ciminari ME<sup>3</sup>, Pérez Chaca MV<sup>3</sup>

<sup>1</sup>Instituto de Química y Físicoquímica Biológica (IQUIFIB-UBA), <sup>2</sup>IMIBIO-UNSL-CONICET, <sup>3</sup>Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis (UNSL). E mail: luz.eggel@gmail.com

Bleomycin (BLE) is an anti-neoplastic agent used as first-line therapy in many human cancer treatments; also, it is used in many experimental models because it induces pulmonary fibrosis. Testosterone deficiency generate morphological and biochemical alterations in lung, increasing oxidant molecules which lead to redox imbalance. Our goal is to elucidate molecular and biochemical changes produced by BLE in rat lungs with androgen deficiency (AD). Present study used Wistar male control and castrated rats (200  $\pm$  20 g) administered with two different BLE doses (BLE1 0.15 mg/kg and BLE2 10 mg/kg): (1) control (Co); (2) Co + BLE1; (3) Co + BLE2; (4) Ca (castrated); (5) Ca+BLE1; and (6) Ca + BLE2.

After 40 days of treatment, rats were sacrificed. Malondialdehyde (MDA) content was determined and Co + BLE2 ( $P < 0.001$ ), Ca ( $P < 0.01$ ), Ca + BLE2 ( $P < 0.0001$ ), and Ca + BLE1 ( $P < 0.05$ ) increased significantly with respect to Co. Also antioxidant enzymes activity were measured like catalase (CAT) activity which rise significantly in Co + BLE1, Co + BLE2, Ca, Ca + BLE1, and Ca + BLE2, compared with Co ( $P < 0.0001$ ), while Ca + BLE2 ( $P < 0.001$ ) and Co + BLE1 ( $P < 0.0001$ ) decreased significantly regarding to Ca. Superoxide dismutase (SOD) expression augmented significantly in Co + BLE2 ( $P < 0.001$ ), Ca + BLE1 ( $P < 0.0001$ ) with respect to Co whereas increased significantly in Co + BLE1, Co + BLE2, and Ca + BLE1 ( $P < 0.0001$ ) in relation to Ca. At the same time diminished significantly in Ca and Ca + BLE2 compared to Co ( $P < 0.0001$ ). Antioxidant glutathione peroxidase (GPx) gene expression decreased in Ca and Ca + BLE2 ( $P < 0.0001$ ) with respect to Co, although it rises ( $P < 0.0001$ ) Ca + BLE1 compared to Co. Finally, in all treated BLE groups, GPx increased significantly in relation to Ca ( $P < 0.0001$ ). We can conclude that, in lung, lower bleomycin dose (BLE1) activates antioxidant defense system where enzymes can respond adequately, while a higher bleomycin concentration (BLE2), increased ROS levels by lipoperoxidation, at which point antioxidant defense system is not enough to face oxidative stress. Likewise state described is exacerbated by androgen deprivation.

### A121

#### A SET-UP TO IDENTIFY NEW DRUGS TO INHIBIT SARS-COV-2 REPLICATION

*Celayes ME<sup>1</sup>, Delgui LR<sup>1,2</sup>, Mayorga LS<sup>1,2</sup>, Polo LM<sup>1</sup>*

<sup>1</sup>Instituto de Histología y Embriología de Mendoza (IHEM-CONICET), Facultad de Ciencias Médicas, y <sup>2</sup>Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo. E-mail: lpolo@mendoza-conicet.gob.ar

Severe Acute Respiratory Syndrome-CoronaVirus-2 (SARS-CoV-2) is the causative agent of the atypical pneumonia disease (COVID-19). This previously unknown virus was identified in China and has spread worldwide, causing millions of deaths globally (<https://covid19.who.int/>). To date, no specific antiviral therapies against the new SARS-CoV-2 are available. SARS-CoV-2 belongs to the *Coronaviridae* family, with a single-stranded positive-sense RNA genome. SARS-CoVs genomic sequences include the large protein named non-structural protein 3 (NSP3). That protein contains seven domains which are essential components for viral replication and transcription process. One of those domains is unique for SARS-CoVs (SARS-unique domain, SUD). That exclusiveness suggests that SUD can be a critical factor involved in the severe pathogenicity caused by these viruses. Two important features have been previously described regarding SARS-CoVs SUD. On the one hand, it was shown that SUD interacts with oligonucleotides known to form G-quadruplexes (G4), which are self-assembled secondary structures of nucleic acids composed of tetrads of poly-guanine sequences. On the other hand, the residues mediating SUD-G4 interaction are essential for SARS-CoV infection. It was recently published that SARS-CoV-2 genomes contain sequences that can form G4s. It has also been demonstrated that SUD can interact with cellular G4 RNAs, and this interaction is critical in determining the course of the viral infection. Thus, we hypothesize that drugs hampering SUD-G4 interaction would be viable therapeutic options for COVID-19. Here, we show a set-up system that allows us to find drugs impeding SUD-G4 interaction to happen, so viral replication is suppressed. To do this, we first verified that SUD is present in the SARS-CoV-2 strain, and it is highly conserved compared to SARS-CoV SUD. After analyzing genome sequences from over 7.5 thousand recently reported SARS-CoV-2 isolates, we observed very low variation frequencies of SUD residues. Later we cloned the domain and generated a mutant that is unable to bind DNA as a control. Then, we expressed those constructions using *Escherichia coli* and purified the proteins by affinity chromatography. We finally generated G4-DNA structures using custom oligonucleotides that we use to measure SUD-G4 interaction by Electrophoretic Mobility Shift Assay (EMSA). We propose to use this set-up to identify drugs interfering with SUD-G4 interactions. Those can be natural products, new or repurposed drugs. And they can constitute therapeutic options against COVID-19 and similar future diseases caused by emergent SARS-CoVs.

### A122

#### BLEOMICYN INDUCES MOLECULAR AND STRUCTURAL CHANGES IN RAT LIVER WITH ANDROGEN DEFICIENCY

*Zelarayan Sarmiento D<sup>1</sup>, Aballay F<sup>1</sup>, Pérez Chaca MV<sup>1</sup>, Razzeto G<sup>1</sup>, Gomez NN<sup>1,2</sup>, Biaggio VS<sup>1,2</sup>, Ciminari ME<sup>1</sup>*

<sup>1</sup>Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis (UNSL). <sup>2</sup>IMIBIO-UNSL-CONICET. E-mail: zelarayand@gmail.com

Sex is clearly a biological variable to consider in clinical research, as well as in medical practice. Testosterone deficiency is a relatively common condition and may negatively affect health and quality of life. Additionally, bleomycin (BLM) is a chemotherapeutic agent used in the treatment of different types of cancers (cytotoxic), however, it generates free radicals, which induce single- and double-strand breaks in DNA. Oxidative stress has been considered as a conjoint pathological mechanism, and it contributes to initiation and progression of liver injury. The present study examines potential differences in oxidative stress parameters and histological changes in control and castrated Wistar rats under two different BLM doses. Adult male rats were divided in six groups: (1) control (Co); (2) Co + BLM I (doses: 1, 5 mg/kg); (3) Co + BLM II (dose: 10 mg/kg); (4) Ca (castrated); (5) Ca + BLM I; and (6) Ca + BLM II. After 40 days of treatment, rats were sacrificed. TBARS and catalase (CAT) parameters were measured and histopathological analyses of

different sections of the liver from the six sample groups were also performed. Our results show that TBARS increase in Co + BLM II, but not significantly with respect to Co; however, within androgen deficiency treatments, together with BLM administration, TBARS decrease significantly in Ca + BLM II with respect to Ca ( $P < 0.05$ ). CAT activity reacts in the same way as TBARS do in control animals. CAT activity is higher significantly in Co + BLM II vs. Co and Co + BLM I ( $P < 0.00001$ ) and else versus all androgen deprivation treatments ( $P < 0.0001$ ), but no significant changes were observed between BLM different doses with androgen deficiency. Histopathology analysis showed hepatic alterations in Co + BLM; the liver has peripheral areas where hepatocytes have lipid vacuoles that increase with BLM concentration. Inside androgen deficiency, higher BLM dose exhibit liver inflammation, as well as bile ducts proliferation. Histological studies clearly demonstrated that bleomycin model induces pathological alterations in the liver producing partial systemic sclerosis. These data, although preliminary, could indicate that androgen deprivation and BLM administration induces an increase in oxidative parameters. The main changes would be that BLM produces different effects in control animals' liver, with respect to androgen deficient animals' liver. We can conclude that BLM responses are directly proportional to the concentration applied.

## HEALTH AND NUTRITION

### A123

#### TYPE II DIABETES RISK AND CARDIOVASCULAR RISK IN A RURAL POPULATION FROM THE PERSPECTIVE OF THE FINDRISC TEST

*De Pauw MC<sup>1</sup>, Acevedo De Pauw V<sup>1</sup>, Salinas E<sup>2</sup>*

*<sup>1</sup>Facultad de Ciencias de la Salud, <sup>2</sup>Facultad de Química, Bioquímica y Farmacia. UNSL.*

*E-mail: salinaseloy@gmail.com*

Diabetes increases the risk of cardiovascular disease (CVD). There are several scales that assess the risk of diabetes, one of the most used is the Finnish Diabetes Risk Score (FINDRISC test) that allows evaluating the risk that a person may develop type 2 diabetes mellitus (T2DM) in the next 10 years. The risk assessment of T2DM and CVD share parameters such as obesity, sedentary lifestyle, metabolic disorders, smoking. Our objective was to identify the risk of CVD in a population aged 18 or over in a rural area of the "Juan M. de Pueyrredón" department of the San Luis province from the perspective of the FINDRISC test. A descriptive, cross-sectional observational study was carried out by means of a survey, physical examination, and laboratory tests in the grouped rural population of the towns of Zanjitas, Cazador, Alto Pelado, and Beazley. The physical examination determined weight, height, waist and hip circumference, and systolic (SBP) and diastolic blood pressure (DBP) and in laboratory tests: cholesterol (COL), triglycerides (TG), high-density cholesterol (HDL<sub>c</sub>), low-density cholesterol (LDL<sub>c</sub>), and glycosylated hemoglobin (HbA<sub>1c</sub>). Body mass index (BMI) and atherogenic indices, total cholesterol/HDL<sub>c</sub> (Castelli index), LDL<sub>c</sub>/HDL<sub>c</sub> (Kannel index) and TG/HDL<sub>c</sub> and metabolic syndrome were calculated according to the NCEP ATP III (National Cholesterol Educational Criteria. Program Adult Treatment Panel III). Results: N = 244. Sex Female (F) 68% Male (M) 32%. Clinical characteristics of the population (mean): age, F 42 M 53; BMI, F 30.6 M 29.5; waist circumference, F 100.3 M 118.1; waist/height ratio, F 0.64 M 0.7; SBP, F 127.5 M 142.8; DBP, F 80.6 M 87.2; COL, F 155.4 M 155.9; HDL<sub>c</sub> F 39.2 M 37.6; LDL<sub>c</sub>, F 92.9 M 95.6; TG, F 115.1 M 108.9; mean values for both sexes of the parameters related to CVD risk according to risk of low, slightly moderate, moderate, high, and very high T2DM, respectively. **BMI F:** 23; 29; 33; 35; 37 M 24; 31; 32; 31; 32. **Waist circumference F:** 73; 95; 121; 107; 114 M 82; 138; 106; 161; 107. **Waist/height ratio F:** 0.4; 0.6; 0.7; 0.7; 0.7 M 0.5; 0.8; 0.6; 0.9; 0.6 **SBP F:** 110; 125; 133; 135; 163 M 126; 141; 148; 158; 160 **DBP F:** 72; 79; 83; 84; 101 M 76; 85; 92; 97; 107 **COL F:** 136; 156; 159; 164; 167 M 144; 162; 147; 171; 164 **HDL<sub>c</sub> F:** 39; 38; 40; 41; 36 M 35; 39; 36; 40; 40 **LDL<sub>c</sub> F:** 76; 97; 94; 98; 94 M 84; 103; 89; 111; 93 **TG F:** 75; 112; 124; 133; 206 M 77; 112; 135; 113; 164 **COL/HDL<sub>c</sub> F:** 3; 5; 4; 4; 5 M 4; 4; 4; 4; 4 **LDL<sub>c</sub>/HDL<sub>c</sub> F:** 2; 3; 2; 2; 3 M 2; 3; 2; 3; 2 **TG/HDL<sub>c</sub> F:** 2; 3; 3; 3; 6 M 2; 3; 4; 3; 5. Conclusion: The female sex was prevalent. Males outperformed females in SBP and DBP values, in waist/height ratio and LDL<sub>c</sub>. In women, BMI, HDL<sub>c</sub>, and TG were higher. In both sexes, the mean values of practically all the parameters and scales related to cardiovascular risk increased as the FINDRISK test values increased. Women at high and very high risk of T2DM had higher values than men in BMI, waist circumference, and TG. The data obtained suggest that there would be a relationship between the values obtained for the FINDRISC test and the different parameters that directly or indirectly assess cardiovascular risk. Given the easy application and the predictive value of the FINDRISC test, if other studies confirm the association, the possibility of using this test to assess the risk of CVD and improve its primary prevention is considered.

#### A124

### AN ARTISANAL *Amaranthus* DRINK: NUTRITIONAL EVALUATION AND SENSORIAL ACCEPTANCE

Angulo JH<sup>1</sup>, Riveros L<sup>1</sup>, Romero Vieyra MA<sup>2</sup>, Barcia CS<sup>1</sup>

<sup>1</sup>Área de Bromatología - Facultad de Química, Bioquímica y Farmacia - Universidad Nacional de San Luis, San Luis, CP5700, Argentina. <sup>2</sup>Facultad de Ciencias de la Salud - Universidad Nacional de San Luis, San Luis, CP5700, Argentina.

E-mail: jooaaquuiin33@gmail.com

In this work, we sought to obtain a drink based on *Amaranthus hypochondriacus* L. seeds, combining its properties with a liquid medium, resulting in a dietary product: free of lactose and gluten, with a contribution of complete proteins, fibers, and no preservatives and artificial colorants, easy to make and preserve at home, inexpensive, and with good organoleptic characteristics. A simple cross-sectional descriptive study was carried out, using an empirical observational modality to determine the nutritional composition, stability, and acceptability of the proposed drink in adults (between 19 and 59 years old) from the City of San Luis (2020). The nutritional composition, stability (pH), and titratable acidity were evaluated using Official Methodologies of AOAC). The acceptability of the product was carried out by means of an adapted panel (50 untrained judges). Along with the survey, data on organoleptic characteristics were collected. The established acceptance criteria were equal to or greater than 80%. The results obtained showed a nutritional value of 8.5 g of carbohydrates, 3.1 g of protein, 0.4 g of fat, and 0.25 g of fiber, for a daily serving of 200 mL. Regarding storage stability (10 days, 5°C), the pH values varied 5.70–4.05, the titratable acidity showed increases from its initial value between 1.10–1.61 g citric acid/L of drink. It can be concluded that the amaranth-based drink presented a good nutritional contribution, good stability at refrigeration temperature, a pleasant taste, and a slightly vegetal smell, reflecting a positive appreciation of 92%.

#### A125

### BROMATOLOGICAL CHARACTERIZATION OF *Citrullus lanatus* FRUIT

Nesteruk RI, Riveros L, Amieva MI, Barcia CS, Sturniolo HL

Área de Bromatología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. San Luis, Argentina. E-mail: rineteruk@gmail.com

*Citrullus lanatus*, commonly known as “sandilleja”, “wild watermelon”, “chayote”, “cayota” or “citron”, is a creeping plant native to Africa and is an important weed in the central region of Argentina. It has a large fruit, with an approximate size of between 20 and 50 cm long and a diameter from 10 to 20 cm. The rind of the immature fruit is dark green alternated with light green spots, and when it matures it completely turns pale yellow. The flesh of “sandilleja” is white yellowish, with olive green seeds. In this work, *C. lanatus* fruit was studied from its bromatological and physicochemical properties to promote its use in the food industry. Ripe fruits of *C. lanatus* were collected in the capital of San Luis province. Immediately, these whole fruits were washed, a sample was taken for the humidity determination, and the rest was dried, labeled, and stored for other determinations. All analysis were carried out in triplicate. Proximal analysis showed a moisture, ash, fiber, fat, protein, and carbohydrate contents of 96.7 g, 0.4 g, 0.3 g, 0.3 g, 0.2 g, and 2.2 g, respectively, and its caloric value was 12 kcal/50 kJ (Atwater factors for caloric value by difference (Proteins: 4 kcal/g – Fats: 9 kcal/g – Carbohydrates: 4 kcal/g)). Also, the fruit was evaluated in its pH, acidity, and total solids presenting pH of 5.29, acid index of 0.15 g malic acid/100 g, 1.8° Brix. The moisture content was carried out by gravimetric method in an oven at 105°C. The amount of ash was determined by incineration at 550°C in a muffle. The protein content was determined by the Kjeldahl method using the conversion factor of 6.25. Fats were determined by extraction by the Soxhlet method with petroleum ether. Crude fiber was determined by the acid and basic hydrolysis method. Carbohydrate content was calculated by difference: %Carbohydrates = 100 – (%moisture + %ash + %proteins + %lipids + %crude fiber). pH was measured using a pH meter, titratable acidity was determined by titration with sodium hydroxide, Brix degrees, soluble solids direct reading on refractometer. The results obtained are characteristic of the genus *Citrullus*: the low caloric intake comes especially from carbohydrates and the main component of this fruit is water, so it can be considered a good option for the hydration of the body. The results also allow us to infer that the fruit of *C. lanatus* presents a composition that allows it to be used in the food industry in its traditional fresh form or in the development of new food products such as jams, considering it an alternative in the population’s diet, allowing its inclusion in the Composition Tables, and preserving and revaluing its consumption.

## A126

### LIFESTYLE, EMOTIONAL ADJUSTMENT AND ATTITUDES TOWARDS NUTRITION IN INCOMING UNIVERSITY STUDENTS (PRELIMINARY STUDY)

*Galarsi MF, Don A, García AA, Doña RD*

*PROICO 12-0320: Neuropsicología del comportamiento, salud y educación. Facultad de Psicología, UNSL. E-mail: galarsimariafernanda@gmail.com*

The objective of this study is to investigate the lifestyle, emotional adjustment, and attitudes towards nutrition in incoming university students of both sexes. The sample consisted of (N = 60), 83.3% (N = 50) women and 16.6% (N = 10) men. The age span ranged from 17 to 49 years old, with M = 20.80 and SD = 6.706. The instruments used were the «FANTASTIC» Questionnaire, TMMS and the EAT-40. The «FANTASTIC» Questionnaire is used to identify and measure lifestyle through 25 items that explore ten physical, psychological and social domains related to lifestyle: Family-Friends, Physical Activity, Nutrition, Tobacco-Toxins, Alcohol, Sleep-Seatbelt-Stress, Personality Type, Introspection (Anxiety, Worry, Depression), Career (Work Activities) and Other Drugs. The final score is multiplied by 2, obtaining a final range of scores from 0 to 100. This range is classified into 5 categories: < 39 = danger; 40–59 = bad; 60–69 = regular; 70–84 = good; 85–100 = excellent. The TMMS-24 scale assesses the meta-knowledge of emotional states through 48 items. It also yields scores on three factors: Attention to emotions, Clarity, and Emotional Repair. The EAT-40 assesses common symptoms and behaviors in anorexia nervosa and provides an index of severity of the disorder. It consists of 40 items that are grouped into seven factors. The total scores can range from 0 to 120 and help in distinguishing between anorexic patients and normal population, bulimic patients, and normal population. Regarding the results on lifestyle, 90 points were obtained for the total sample, which allowed us to conclude that the general lifestyle is excellent. The TMMS-24 showed the following results: Attention, M = 25.10 and SD = 6.69; Clarity, M = 24.22 and SD = 6.992; and Repair, M = 26.83 and SD = 7.381. This would indicate that there is an adequate level in terms of the perceived ability to regulate one's emotional states correctly. The perception of one's own emotions –i.e., the ability to feel and express emotions– should be improved. Likewise, the perception of one's own emotions –i.e., the understanding of one's emotional states– should be higher. Regarding the attitude towards food, a maximum score of 55 was obtained, a mean of 16.38 was obtained with a standard deviation of 10.753. It can be inferred that there is no tendency to trigger eating disorders in this sample. Having a good lifestyle might be acting as a fundamental protective factor to maintain an optimal emotional regulation and preventing eating-oriented disorders from being triggered. [Key words: lifestyle, emotional adjustment, attitudes towards nutrition, university students]

## A127

### INFLUENCE OF SLEEP ON THE LEARNING PROCESSES OF STUDENTS FROM THE NATIONAL UNIVERSITY OF SAN LUIS

*Doña RD, Garro P, Galarsi MF, García AA, Morales P*

*PROICO 12-0320: Neuropsicología del comportamiento, salud y educación. Facultad de Psicología, UNSL. Email: roberto.dona@gmail.com*

This work aims to show the influence of sleep on the learning potential of students from the National University of San Luis. An *ad hoc* questionnaire designed for the purposes of this research was used. The questionnaire collected sociodemographic and lifestyle data, particularly sleep characteristics as quality and quantity of sleeping hours. The Rey's Auditory Verbal Learning Test and morning-evening test were also applied. The sample was made up of 79 individuals of both sexes (39 women and 40 men). All of them were first-to-fifth-year students from the National University of San Luis, with ages ranging between 17 and 52 years (M = 25.70; SD = 6.70). The exclusion criterion applied was the fact that none of them were taking medication or another factor that could affect the sleep cycle. Results revealed that the age and sex variables did not show significant mean differences, nor correlated with any of the variables under study. Ninety-six percent of the sample declared that they had a good night sleep. The range of sleep hours varied from 3 to 10 hours. The sample was divided into four statistically different groups. The comparison among mean values, correlations and the learning potential showed neither differences in mean values, nor correlations among the subjects in the sample across the different academic years. Fourth-year students showed mean differences in learning potential with respect to the rest of the analyzed students. We inferred that the energy resources of these young and healthy individuals compensate -in a few hours of sleep- the daytime energy expenditure. Therefore, the learning potential is not significantly affected by partial sleep deprivation. The cognitive flexibility of this population might be allowing them to maintain an adequate performance in the face of the learning problems posed by the environment, even if they sleep few hours.

## A128

### EATING HABITS AND LIFESTYLES IN UNIVERSITY STAFF DURING MANDATORY ISOLATION BY COVID-19 IN 2020

Ramírez MM<sup>1</sup>, Biasi AM<sup>2,3,4</sup>, Luna FG<sup>3</sup>, Pérez Chaca MV<sup>5</sup>, Gómez NN<sup>2,4,5</sup>

<sup>1</sup>Universidad Nacional de Villa Mercedes (UNVIME). <sup>2</sup>Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO)-SL/ CONICET. <sup>3</sup>Facultad de Ciencias de la Salud, Universidad Nacional de San Luis (UNSL).

<sup>4</sup>Facultad de Ciencias Médicas (UC de Cuyo). <sup>5</sup>Laboratorio de Morfofisiología, FQByF - UNSL, Argentina.

E-mail: mmramirez@evirtual.unvime.edu.ar

COVID-19 is a respiratory disease caused by the SARS-CoV-2 coronavirus, which has reached pandemic status since the beginning of year 2020. Population confinement and social distancing have been the main measures in many countries to contain virus expansion and severity. In this context, telework acquired great importance and diffusion, even in the university teaching area. This study analyzes the changes in eating habits and other lifestyles during the confinement period (March–December 2020) in a group of teachers and non-teachers from the National University of San Luis (UNSL). The data were obtained from a previously validated virtual survey of university personnel aged 40 years and over (N = 62) and were analyzed using descriptive statistics. Most of the respondents (> 60%) maintained the same protective food group's consumption. There was a notable decrease in the consumption of canned products (52%), ultra-processed foods (45%) and alcohol (40%) as well an increase in the consumption of herbal teas (65%). 50% of those surveyed presented normal nutritional status, 32.3% were overweight and 17.7% were obese. 24.2% of those surveyed reported consuming vitamin and/or mineral supplements. Most prevalent diseases were thyroid disorders (22.6%), hypertension (19.4%), hypercholesterolemia (14.5%), and hypertriglyceridemia (9.7%). 72.6% of those surveyed maintained their physical activity practice. 8% of those surveyed expressed a stress feeling. As positive aspects, the consumption of healthy foods was maintained, likewise a non-recommended foods intake reduction. In addition, the practice of physical activity was maintained, and stress was manifested in a small group. As negative aspects, excess weight stands out in 50% of cases and also the presence of chronic diseases, information that should be taken into account in health systems to promote strategies that lead to improve life quality.

## A129

### METABOLIC SYNDROME AND NUTRITIONAL STATUS IN PATIENTS FROM THE MARÍA J. BECKER HOSPITAL IN LA PUNTA CITY, SAN LUIS

Piguillem SN<sup>1,2</sup>, Kucharuk J<sup>2</sup>, Cedriani S<sup>3</sup>, Gómez NN<sup>1,4,5</sup>, Salinas E<sup>1</sup>

<sup>1</sup>Facultad de Química, Bioquímica y Farmacia. UNSL. <sup>2</sup>Facultad de Ciencias de la Salud, UNSL. <sup>3</sup>Hospital M J. Becker, La Punta SL. <sup>4</sup>Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO)-SL/ (CONICET), San Luis, Argentina.

<sup>5</sup>Facultad de Ciencias Médicas, UCCuyo. E-mail: salinaseloy@gmail.com

Metabolic Syndrome (MS) involves risk factors such as: arterial hypertension, obesity, hyperlipidemia, and constitutes one of the main causes of morbidity and mortality. National Cholesterol Education Programs Adult Treatment Panel III (NCEP ATP III) proposed an MS definition that highlights five risk factors; a subject has the MS if he or she has three or more of this five individual criteria. This classification is considered a clinical definition of MS and will be taken as a reference in this research. The five factors or indicators to take into account are: serum triglycerides  $\geq 150$  mg/dL (8.3 mmol/L), HDL cholesterol  $< 40$  mg/dL (2.2 mmol/L) in men and  $< 50$  mg/dL (2.8 mmol/L) in women, systolic blood pressure  $\geq 130$  mmHg or diastolic blood pressure  $\geq 85$  mmHg, blood glucose  $\geq 100$  mg/dL (5.6 mmol/L), abdominal obesity: waist circumference  $> 102$  cm in men and  $> 88$  cm in women. Given the impact generated by the alarming growth of chronic non-communicable diseases (CNCD) in society, and if we consider that populations in constant growth are the most affected, it is essential to carry out epidemiological studies that allow us to recognize the nutritional status and risk factors of suffering MS, which affects urban populations. The purpose of this work was to know the nutritional status and the prevalence of metabolic syndrome in patients who attended the “María J. Becker Hospital” in the city of La Punta, San Luis. A descriptive, cross-sectional study was carried out, where those older than 18 years who attended the “M J. Becker Hospital” in 2019 (N = 100) were evaluated. It consisted of two phases: a first stage that included surveys and a second stage of collecting biochemical data provided by the hospital laboratory. In addition, anthropometric parameters (height and weight) were evaluated; changes in body mass index (BMI), and waist-hip circumference (CCI). From all patients, 32% are overweight and 37% are obese, prevailing in women (55%). Regarding the lipid profile, when evaluating the triglyceride level, it was determined that 84% have normal triglyceride values ( $< 150$  mg/dL), only 14% have high triglyceride values  $> 150$  mg/dL, and 2% of the patients had values greater than 200 mg/dL. There were no significant differences between men and women. In relation to lipoproteins, 93% of the individuals presented LDLc values  $< 130$  mg/dL, and only 6% of the population group had values  $> 200$  mg/dL with risk of suffering cardiovascular diseases, such as atherosclerosis, heart attack or stroke; HDLc levels were  $< 40$  mg/dL in 28% of cases, 62% of the total had values between 40 to 60 mg/dL, and only 9% of this population had values greater than 60 mg/dL as protective factor. The risk factors with the highest incidence were high blood pressure (56%), unhealthy food intake (75%), and insufficient physical activity (32%). In this population sample, 23% of the women and 12% of the men presented MS. Considering the results obtained, we can demonstrate that the association of more than one risk factor predisposes to an unfavorable evolution towards cardiovascular diseases. Promote and encourage healthy habits that improve the life quality of the general population, will prevent the appearance of cardiovascular diseases, Diabetes Mellitus and MS, among others.

### A130

#### HYPERCALORIC DIET EFFECTS ON HYPOANDROGYNOUS RAT LUNG

*Aballay FE<sup>1</sup>, Zelarayan Sarmiento M<sup>1</sup>, Salinas M<sup>1</sup>, Piguillem SN<sup>1</sup>, Salinas E<sup>1</sup>, Razzeto GS<sup>1</sup>, Pérez Chaca MV<sup>1</sup>, Ciminari ME<sup>1</sup>, Biaggio VS<sup>1,2</sup>, Gomez NN<sup>1,2,3</sup>*

<sup>1</sup>Laboratorio de Morfofisiología, Fac. de Qca. Bioqca y Farmacia, UNSL. <sup>2</sup>Instituto Multidisciplinario de Investigaciones.

Obesity is a systemic state of inflammation and oxidative stress that affects normal respiratory physiology; however, mediators responsible for these processes are not well known yet. Even though obesity occurs more frequently in women, their impacts on men health are worse than in women health. Interest in sex hormones' role as lung function modulators has risen in the latest years. Androgen receptors' presence in lung reveals that these hormones might play a key role in their physiology. Obesity-mediated oxidative stress produced in adipose tissue, and it is one of the main factors considered as oxidant source and also inflammation mediator. The aim of this work was to study the effect of obesity diet on castrated rat lung. Wistar male rats ( $200 \pm 20$  g) were separated in four groups: Control with normal diet (CoN), castrated with normal diet (KN), control with hypercaloric diet (CoOB), and castrated with hypercaloric diet (KOB), and then they were sacrificed 30 days after castration. We analyzed different parameters: catalase activity (CAT), lipid peroxidation (TBARS), catalase (CAT) and androgen receptor (RA) expressions in lung tissue, as well as serum determinations of triglycerides (TG), cholesterol (CL), high-density lipoproteins (HDL), urea, and lactate dehydrogenase (LDH). ANOVA and Tukey tests were used for statistical analyses. The results showed that: CL increased in more proportion in CoOB ( $P < 0.001$ ) compared to control and castrated group. However, HDL increases in CoOB ( $P < 0.001$ ) and remains high in castrated with normal diet ( $P < 0.001$ ), compared to CoN, and there is no change between castrated groups. Both TG and uric acid increased significantly in KOB compared to KN ( $P < 0.001$  and  $P < 0.05$ , respectively); differences were not significant between CoN vs. CoOB groups. Only urea decreased significantly in KOB, respect to KN ( $P < 0.05$ ). TBARS levels increased in KOB group compared to CoN ( $P < 0.001$ ) and KN ( $P < 0.01$ ) groups, respectively. CAT expression and activity decreased in CoOB, KN, and KOB groups, compared to CoN ( $P < 0.01$  and  $P < 0.05$ , respectively). RA expression increased in KN group compared to control group, but it decreased in KOB vs. KN. We previously demonstrated an important oxidative stress state in a castrated animal model. Obesity plus castration alter lipid parameters and further increase lipid oxidation, added to the fact that an indispensable enzyme such as CAT is not increased by increasing the oxidative stress present in KOB. By adding obesity and castration, we would be in a situation of alteration of a greater number of biochemical parameters, including the state of oxidative stress, and this situation would produce a more severe inflammatory state in the lung.

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