

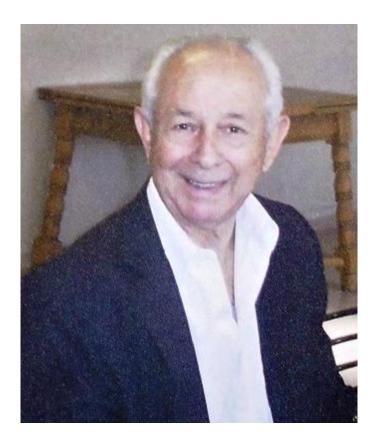
# Libro de Resúmenes

XXXV Reunión Científica Anual de la Sociedad de Biología de Cuyo



Del 06 y 07 de Diciembre de 2017 Villa de Merlo- San Luis- Argentina

### In memoriam



Dr. Egualdo Oscar Zangheri. (1931-2017)

Poco antes del inicio de la XXXV Reunión Científica Anual de la Sociedad de Biología de Cuyo, falleció en la Ciudad de Mendoza, el Dr. E. Oscar Zangheri, quien fuera miembro de la comisión directiva que refundó la Sociedad de Biología de Cuyo en el año 1973.

Oscar Zangheri fue Profesor Titular de Fisiología en la Facultad de Ciencias Médicas de la Universidad Nacional de Cuyo, realizando además, como investigador del CONICET, importantes e innumerables aportes científicos en el área de la hematología, como la demostración del origen renal de la eritropoyetina, experiencia que cristalizó como autor en varios capítulos del libro "Fisiología Humana" del Premio Nobel Bernardo A. Houssay.

Como docente y colega fue un modelo de maestro y amigo, cuya hombría de bien sembró valores éticos y morales que lo destacaron por su solidaridad ejemplar. Mendocino por adopción (ya que nació en Córdoba y vivió allí hasta los 12 años), fue un entusiasta colaborador de la Sociedad de Biología de Cuyo. Su disposición y dedicación a su trabajo, así como el compromiso con nuestra sociedad le hacen merecedor de este libro, en su memoria.

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### **CONFERENCIAS Y SIMPOSIOS**

### CONFERENCIA INAUGURAL

# A1 IMPLEMENTACIÓN DE ESTUDIOS DE FASES TEMPRANAS DE FÁRMACOS EN ARGENTINA

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Advances in translational research are expected to mitigate the recent drought in new drug development. Despite significant progress recently made in biological sciences, the results are decidedly mixed with significant breakthrough in some disease areas while extensive work remains to be completed in other areas. This conference will provide a general survey of the current landscape of translational research so as to identify progress and areas of needs and the associated strategy. While significant advances in the development of translational tools have been made in all fronts, the availability of predictive preclinical models remains critical for the success of translational research. This is directly correlated with the success of translational research as illustrated by the recent approval of targeted drug therapies. By the same logic, unexpected side effects can also be explained by laboratory findings, thus completing the translational cycle. Because of this reason, further collaboration between preclinical and clinical scientists is essential. Non-scientific issues have important influence on the future of this endeavor cannot be underestimated either. Nonetheless, with definitive commitment of private industry and public resources, the future of translational research is promising.

### Simposio: "ADVANCES IN NEUROSCIENCES"

### EXPERIMENTAL PSYCHOPATHOLOGY: VALUE OF TRANSLATIONAL MODELS

Gargiulo P

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Translational studies are experimental approaches performed on some species to extrapolate to others, more specifically to humans. These studies have experienced a marked development since the second half of the 20th century. Although illustrious antecedents are recorded, as could be mentioned the case of Pavlov, it is in this period that this discipline is strengthened. Initially Pavlovian conditioning studies and operant conditioning were developed, but then all the psychopathology was projected on various probabilities. Thus, various experimental approaches were made around the psychopathological disorders classically susceptible of pharmacological treatment. This is the case of anxiety disorders, affective psychoses and schizophrenic psychoses. These models were then used to test physiopathological conditions that are postulated as underlying these tables, but also to test possible therapeutic, both behavioral and psychopharmacological. It went from an experimental psychopathology to a systematic method of detecting possible psychotropic effects of drugs. In this area we talk about isomorphism models and similarity models. We speak of models of isomorphism when the phenomena induced in the animal do not keep the same appearance with those of man, but they respond in the same way to a drug. We speak of models of similarity when there is apparent similarity between the behaviors induced in the animal and those observed in the sick man. This has become an area of psychopharmacology of exponential growth.

### A3 SLEEP NEUROBIOLOGY

Pires GN

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Sleep biology has received increasingly attention, as attested by the growing number of scientific articles published on the field, as well as the attention sleep received by media press. This visibility is due to two factors: the new discoveries and advances in the field and the evident and ubiquitous condition of sleep deprivation experienced by the whole population. This lecture will discuss the field of sleep biology and chronobiology in a broad manner. It will encompass both topics related specifically to sleep biology and themes related to translational sleep research. It will discuss: 1. Basic concepts in sleep biology and chronobiology; 2. Sleep in normal conditions; 3. Sleep deprivation and 4. Advances in sleep neurobiology.

#### CHRONOBIOLOGICAL BASIS OF AGING AND THE ALZHEIMER'S DISEASE

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In 2005, the CEPAL and the CELADE stated that the main demographic phenomenon in this era is the population aging and urged to design specific strategies to face its undesirable consequences. Argentina is the Latin American country with the highest percentage of aged people (INDEC). Even though, there have been significant advances in the elucidation of biochemical and molecular basis undergoing aging and aging-related diseases, in the last years, just a few studies report the role of the endogenous clock and the circadian system in the cognitive functions deterioration in senile individuals or with Alzheimer's disease (AD). To date, there is not an effective treatment for AD neither. Thus, we aimed to explore the temporal synchronization and circadian organization of memory and learning-related factors in both, an aging and an experimental model of AD. Our results revealed a temporal (circadian) organization of factors involved in synaptic plasticity and long-term potentiation, in the rat hippocampus and prefrontal cortex. Such organization is lost in aged animals, or following an i.c.v. injection of aggregated beta-amyloid peptide, probably, because of the age-related alteration of the cellular redox state and deterioration of the endogenous circadian clock. The knowledge of how aging or the beta-amyloid aggregates affect the circadian rhythmicity of factors involved in cognitive functions, would contribute to design new chronotherapeutic strategies addressed to get a healthy aging and to improve the quality of life in elderly people.

### A5 PSYCHOTROPIC MEDICINES IN ELDERLY

Calderón CP

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Presence of comorbidities, polypharmacy, functional and social problems leading to accidental or inappropriate use of medications are strongly associated with aging. Elderly are very vulnerable to presenting adverse reactions, interactions, hospitalizations, mortality and poor adherence to treatment. Anxiolytics, antidepressants and antipsychotics are the psychotropic drugs more frequently prescribed. They represent at high risk in this age group, because they produce numerous serious adverse reactions and their benefits are limited. The elderly are important targets of medicalization, especially with psychotropic drugs, because it is commonly considered that old age is synonymous with disease. It is necessary to reduce the prescription and the inadequate consumption of these medicines in elderly, to promote their desprescription, to encourage patients to face their difficulties without medication and to adopt preventive measures, reducing risks and health costs.

### THE HUMAN EVOLUTION: A PROJECT IN PERMANENT CONSTRUCTION

Arcucci A

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The study of human evolution, its patterns and processes, has been interesting for many scientists even since the beginnings of the development of evolutionary theories almost two hundreds years ago. Numerous scientific studies about human ancestors have been developed since the XIX century, considering first the most modern examples of human populations and focusing in European examples. Now in the XXI century, we realize that the origin of human history is located in Africa and is at least 7 million years old, something incredible a few decades ago. Many lines of evidence contribute to build this knowledge in these days: paleontology, with classical excavations, and molecular biology with modern techniques. But the history results intrincate, has very different stages and is not linear, it bifurcates sometimes unexpectedly and give big sorprises to the specialists. This talk intended to review this complicated history to try to understand the most recent and important findings on human evolution, that media sometimes treated so superficially.

### **A7**

# NOVEL ROLES OF EXTRA-EMBRYONIC TISSUE IN DIRECTING EARLY EMBRYO MORPHOGENESIS IN ANNUAL TELEOST FISHES

Reig G.<sup>1,2</sup>, Cerda M.<sup>1,2</sup>, Sepúlveda N.<sup>3</sup>, Flores D.<sup>1,2</sup>, Castañeda V.<sup>1,2</sup>, Tada M.<sup>4</sup>, Härtel S.<sup>1,2</sup>, Concha M.L<sup>1,2,5</sup>

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We are interested in how shape emerges in the developing embryo, in particular how embryonic cells use the physical properties and mechanical stresses of their cellular environment to guide their individual and collective migration *in vivo*. In this study we focus on the process of spreading of embryonic cells over the cells, a critical event in embryo morphogenesis and tissue repair of which we still know little *in vivo*. We take advantage of unique developmental features of the non-conventional annual killifish

embryo to study the principles underlying tissue spreading in a simple cellular environment, devoid of patterning signals and major morphogenetic cell movements. Using *in vivo* experimentation and physical modelling we reveal that the extra-embryonic epithelial enveloping cell layer, thought mainly to provide protection to the embryo, directs cell migration and the spreading of embryonic tissue during early development. This function relies on the ability of embryonic cells to couple their autonomous random motility to non-autonomous signals arising from the expansion of the extra-embryonic epithelium, mediated by cell membrane adhesion and cortical tension. Thus, we present a basic mechanism regulating early embryonic morphogenesis that relies on the interaction between embryonic and extra-embryonic tissues.

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# SIMPOSIO: NEW ADVANCES IN VEGETABLE BIOTECHNOLOGY. (Simposio de Sociedades de Biología de la Argentina.)

# A8 TRANSGENIC PLANTS AS PLATFORMS FOR THE PRODUCTION OF RECOMBINANT PROTEINS

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The use of plants to produce molecules having an intrinsic commercial value (molecular farming) emerged some time ago as an alternative for the traditional production systems. Among the different projects ongoing within INDEAR, there is one called SPC (from Safflower Produced Chymosin). It consists in the production of chymosin, a bovine aspartic protease used to coagulate milk during cheese production, in seeds of safflower, an ancient oilseed adapted to semi-dry lands that has an interesting biosafety profile. During the last years, this project completed several developmental stages. From the beginning in the laboratory; functional tests of the enzyme (SPC) and their derived products; the construction and launch of a pilot plant; extensive pre-commercial evaluation of SPC, up to the construction and start-up of an industrial production plant that can supply 20% of the worldwide market. After a long and extensive period of characterization of the crop and its derived product, "SPC safflower" is now undergoing the last stages of the deregulation process within Argentina. Once this process is completed, it will be the first transgenic safflower approved worldwide, giving rise to the first molecular farming product intended for human food. This production platform can be used to produce other molecules, linking agriculture with products with a high added value that will not only allow imports substitution but also position us as a biotech benchmark.

# A9 EXPLORATION AND USE OF GENETIC VARIABILITY OF CROP WILD RELATIVES USING GENOMIC TOOLS

Muñoz N

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Genetic diversity in crop plants is severely reduced due to domestication and human selection processes. This reduction in the genetic diversity challenges proposals for plant breeding in demanding contexts of sustainable food production. There is consensus that crop wild relatives are a unique source of diversity with great potential to explore and use genetic variability in order to address current and future challenges through genomic-assisted breeding programs. The aim of this presentation is to show an approach for the exploration and use of this potential in order to improve complex agronomic traits such as, higher antioxidants content in seed. Using a recombinant inbred population resulting from a wild (Glycine soja Siebold & Zucc.) and a cultivated (Glycine max L.) soybean parent and a bin map approach (genotyping by sequencing), we have identified an overlapping genomic region containing major quantitative trait loci (QTLs) that regulate the seed contents of total antioxidants, phenolics, and flavonoids. Three putative Multidrug And Toxic Compound Extrusion (MATE) transporter genes within this QTL and one adjacent to it (GmMATE1-4) have been identified. Moreover, we have identified non-synonymous changes between GmMATE1 and GmMATE2, and GmMATE3 encodes an antisense transcript that expresses in pods. We proposed two hypotheses that could support these transporters as the major genetic determinants of the antioxidant contents in soybean seeds.

# A10 BIOTECHNOLOGICAL TOOLS APPLIED TO Cynara cardunculus BREEDING

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Cynara cardunculus L. (Asteracea), includes three botanical varieties: var. scolymus (globe artichoke), var. altilis (cultivated cardoon) and var. sylvestris (wild cardoon). In addition to its economic importance as food it is a substantive source of biopharmaceutical compounds and biomass for bioenergy production, which makes this species an object of interest for genetic improvement. A germplasm collection including accessions of different origins was developed within the framework of the

Facultad de Ciencias Agrarias (UNR) breeding program. A genetic linkage map for the species was developed using SRAP, AFLP, SSR, and SNPs markers. This map was useful to identify and locate genes and QTLs controlling traits linked to important agroeconomics traits such as headcolor, bracts and leaf spines, yield and biomass production. Currently, this map is being satured by the inclusion of new molecular markers generated by GBS. Moreover, the SRAP markers associated with QTLs of agronomic interest are being sequenced and materials with high polyphenols content are being identified by HPLC. Also, since a male sterility system is important for hybrid seed development, it is proposed to detect molecular markers associated with this character, either by identifying new SRAP markers or by transferring markers associated with the trait in other species of the Asteracea family. All this information will facilitate the species genetic improvement, through times decrease and precision increase.

#### CONFERENCIA CLAUSURA

# A11 ABOUT THE SITUATION OF SCIENTIFIC RESEARCH IN LATIN AMERICA: AN INSIDER'S PERSPECTIVE

Ciocca DR

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Although Latin America has increased the number of its scientists and research institutions in recent years, the gap between developed countries and Latin American countries is startling. In this article, a critical view of the Latin American investigator's daily life, particularly in the area of biomedicine is presented. It is evident that the prime importance of science and technology to the development of a nation remains unacknowledged. Even large and relatively rich Latin American countries do not produce a good level of science. The major factors contributing to low scientific productivity are the limited access to grant opportunities, inadequate budgets, substandard levels of laboratory infrastructure and equipment, the high cost and limited supply of reagents, and inadequate salaries and personal insecurity of scientists. The political and economic instability in several Latin America countries results in a lack of long-term goals that are essential to the development of science. In Latin America, science is not an engine of the economy. Most equipment and supplies are imported, and national industries are not given the incentives to produce these goods at home. It is a pity that Latin American society has become accustomed to expect new science and technological developments to come from developed countries rather than from their own scientists. Too many bright young minds continue to leave Latin America for developed countries, where they are very successful. However, we still have many enthusiastic young graduates who want to make a career in science and contribute to society. Governments need to improve the status of science for the sake of these young graduates who represent the intellectual and economic future of their countries.

### RESÚMENES

### ÁREA: FARMACOLOGÍA Y TOXICOLOGÍA

#### A12

### CADMIUM INTOXICATION IN AORTA, LUNG AND CEREBELLUM: IS THE EFFECT ORGAN SPECIFIC?

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Cadmium (Cd) is an environmental contaminant and affects several organs. We studied its effect on lung, prostate and aorta. Oxidative stress, apoptotic markers and histology studies were performed. 2 lots of Wistar rats were used. One group received regular water (Co) and one, 15ppm of Cd in the drinking water for 60 (Cd). Total RNA was isolated and cDNA was obtained. p47, SOD, NOX, nrf2, FAS L, Bax, Bcl 2 were determined by PCR. S28 was the internal control. The organs were fixed in Bouin and histological studies were performed. In aorta, Nox 2 increased (p<0.001) in Cd, without changes in p47. Nrf2 decreased (p<0.05), GPx did not change and SOD increased in Cd (p<0.05). p53, FAS ligand and BAX/Bcl-2 ratio increased in Cd (p<0.05). Aortas showed structural changes in tunica intima cells, exhibiting clearer and bigger cytoplasms than controls. They look more disorganized. In cerebellum, Nrf-2, Gpx, NOX, p47 and SOD decreased in Cd (p<0.05), while Bax/bcl-2 ratio increased. Purkinje cells in Cd appeared rounded and poorly differentiated. Some Purkinje cells were distorted, degenerated and deeply stained, with loss of their nuclei. Most of them were pyknotic while the granular layer showed deeply stained and pyknotic small neurons. In lung, nrf-2, NOX, p47 and SOD did not change while GPx showed a trend to increase in Cd. Bax/bcl-2 ratio increased in Cd. Lungs showed infiltration with non functional and fused alveoli. This suggests that Cd mechanisms vary in different organs although it induces apoptosis and malfunctioning in all of them.

# A13 FUNGICIDE ACTIVITY OF ESSENTIAL OILS OF LEAVES OF Schinus areira AGAINST Ascosphaera apis

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Chalkbrood is the disease caused by the heterotalic fungus Ascosphaera~apis. This disease causes a weakening of the colony and contributes to the temperature of the brood chamber descending from 34-35°C to 30°C, the latter being the optimum for the development of the fungus. In this work, the minimum fungicidal concentration (MFC) and the inhibitory concentration of the essential oils of the female and male foot of Schinus~areira~ were determined against A.~apis. The oils were obtained by steam distillation of dried leaves, harvested in Villa Mercedes, San Luis and distilled for 3 h. The Argentine breeding was collected in Villa Reynolds, San Luis and the European in Sardinia, Italy. A.~apis. strains were incubated in standard MY20 culture medium at 30°C  $\pm$  1°C. Oil concentrations were tested by dilution in the culture medium of: 20; 15; 10; 5; 2.5; 1.25; 0.6; 0.3; 0.15 and 0  $\mu$ /ml. In the center of each 9 cm diameter Petri dish, a 5 mm diameter agar disc was sown from the edge of the fungus colony at seven days of growth. The MFC of the oils of both feet, after 14 days was 5  $\mu$ /ml (p<.01) against the two strains. Inhibition halos were observed at 7 days, point at the mycelium of A.~apis. collapses in the oil-free Petri dishes. The oil of the female foot with 0.15  $\mu$ /ml inhibited the growth in both strains, the same inhibition presented the masculine foot with 0.6  $\mu$ 1 ml of oil (p<0.05).

#### A14

# CADMIUM ALTERS LEUKOCYTE POPULATION, NITRITES CONCENTRATION AND APOPTOSIS AND INFLAMMATION MARKERS IN LUNG. EFFECT OF DIFFERENT DIETS

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on inflammation and apoptosis markers, leucocytes in bronchoalveolar lavages (BAL) and nitrites concentration under different diets. 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein sources. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water for 60 days. BAL was performed, nitrites concentration was measured and cells were counted. Total RNA was isolated and cDNA was obtained. Cyclooxygenase-2 (COX-2), transforming growth factor beta (TGF-β), nuclear factor NF-κB, vascular cell adhesion molecule-1 (VCAM-1), p53, BAX and Bcl-2 were determined by PCR. S28 was used as control. Nitrites decreased in Cas-Cd and So-Co vs Cas-Co (p<0.01). Alveolar macrophages decreased in Soy-Cd vs Soy-Co (p<0.05); neutrophils augmented in Soy-Cd vs Cas-Cd and So-Co (p<0.01); lymphocytes increased in Cas-Cd vs Cas-Co (p<0.01) and in Soy-Cd vs Soy-Co (p<0.05). NF-κB decreased in Soy vs Cas groups (p<0.001) and also decreased in Soy-Cd vs its control (p<0.01). COX-2 increased in Cas-Cd vs Cas-Co (p<0.05) and decreased in Soy vs Cas groups (p<0.05). p53 decreased in Soy-Cd vs Cas-Cd vs Cas-Cd

(p<0.001). Bax/Bcl-2 ratio increased in Cas-Cd vs Cas-Co (p<0.05). This shows that Cd alters apoptotic and inflammation markers, and Soy might confer protection in lung against the metal.

#### A15

### PRESCRIPTIONS OF NON OPIOID ANALGESIC AND NON STEROIDAL ANTI-INFLAMMATORY AGENTS IN AN EMERGENCY SERVICE HOSPITAL

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Non opioid analgesic and non steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the clinical practice, mainly in primary health care, in a wide variety of indications. The aim was to identify the usage patterns of NSAIDs in emergency service of a hospital in Juana Koslay city, San Luis. An observational, transversal, retrospective, prescription- indication study was made during three months. Data of 1871 medical consultations and pharmacy records were collected. Prescribed drugs and prevalent diagnoses were classified according to ATC and ICD-10 classifications, respectively. Results (%): NSAIDs prescription 56.). For age: (15 years old 7.);  $\geq$  15 years old 92.2. For sex: F 53.8; M 46.2. Prescriptions: diclofenac 38.1, ketorolac 33.4; dipyrone 23.6. These drugs were prescribed alone 20.2 or in different combinations 79.8, with corticosteroids 47.7, metoclopramide 23.8, ranitidine 17.7. Prevalent diagnoses: Abnormal symptoms (R) 23.8; Osteomuscular (M) 22.1; Digestive (K) 21.1; injury, poisoning and other consequences of external causes (S.T) 9.7, respiratory (J) 7.6, genitourinary (N) 7.1. According to the diagnosis: dorsalgia 14.5, Odontology 9, abdominal pain 7.1; stomach flu 5.5; fever 5.2; headache 5.2; renal colic; pharyngitis 3.3; vomiting 3.1; gastritis 2.1. We found high association with glucocorticoids, administration of more than one NSAIDs and consume in pathologies that they wouldn't be indicated. The results suggest an irrational use of these drugs.

#### A16

## PRELIMINARY STUDY ON THE USE OF MOOD-ALTERING DRUGS IN OLDER ADULTS FROM THE CITIES OF SAN LUIS AND LA PUNTA

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There is a prevalence of almost 25% in psychiatric disorders and the use of mood-altering drugs among senior individuals. Pharmacodynamic and pharmacokinetic changes are evident, and pharmacologic interactions in polypharmacy are high. There are aspects that promote a lower adherence to treatments with psychotropic drugs in senior citizens, such as self-medication and memory loss. This preliminary study was aimed at investigating the use of psychotropic drugs in senior citizens from San Luis and La Punta. We used a non-random, intentional sample. It was made up of 15 individuals, 8 women and 7 men, aged between 66 and 79 (M=71.8; DE=4.28), who regularly attend retirees' centers and lead a healthy lifestyle. A semi-structured interview was used to obtain information about the use of psychotropic drugs, prescription reasons, and regular control. Our findings show that 40% of the sample lives alone, and that 60% of the individuals live with their family. Similarly, 45% have been prescribed psychotropic drugs: anxiolytic drugs, 25%; antidepressants, 17%; both types of drugs, 3%. 30% had a prescription issued by a physician, without a monthly control from either their family, or a doctor. Finally, 70% of individuals had prescriptions issued by a psychiatrist, without family intervention. While it is normal to prescribe the use of psychotropic drugs to senior citizens due to changes in mood and sleep disorders, the percentage of people under treatment is high. No monthly control on the part of doctors or families is observed, which may have negative consequences in senior citizens.

#### A17

# DRUGS PRESCRIPTION IN A LABOUR OFFICE OF A METALLURGICAL COMPANY OF SAN LUIS PROVINCE

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Work organizations constitute the social-labour context of human beings and the productive support of society and present specific characteristics related with labour health. Our objective was to analyse the most frequent diagnoses, prescription drugs and routes of administration used in a labour office of a metallurgical company of San Luis. A descriptive, cross-sectional and retrospective study was carried out between August 2013 and November 2014. Age, sex, diagnosis, prescriptions and route of administration corresponding to 1826 consultations were recorded. Drugs and diagnoses were classified according to the Anatomical Therapeutic Chemical Classification (ATC) and International Classification of Diseases (ICD-10), respectively. Results were expressed as percentages. Gender: male 73% and female 23%. Average age of workers was  $38.56 \pm 10.10$  years. The most frequent diagnoses were osteoarticular (51.37%). Among these last were found common pathologies such as: gonalgia (8.65%), lumbosciatalgia (7.61%), trauma (7.69%) and cervicobrachialgia (4.22%). The most frequently prescribed drugs were Diclofenac (31.26%), Diclofenac+Vitamin B12+Associations (10.20%), Loperamide (6.57%), Cephalexin (5.86%) and Amoxicillin (4.6%). Choice administration route was oral (88%). The most frequent

prescriptions are related to the most common diagnoses, mainly related to the selected scope of work. On the other hand, due to the importance of osteoarticular diseases, implementation of continuous training in ergonomics, body mechanics, use of personal protection elements and design of jobs is suggested. At the same time, implement of intervention measures tending to modify the prescriptive habits in the health team that favour a rational use of medicines is necessary.

### A18 GENOTOXICITY AND CYTOTOXICITY OF WATER FROM POTRERO DE LOS FUNES RIVER, SAN LUIS, ARGENTINA

Gallardo LV, Jofré M, Cangiano A.

in metabolic activity in the amygdala.

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Potrero de los Funes River in San Luis, Argentina has been extensively studied regarding water contamination, but the degree of cytotoxicity and genotoxicity of their waters is not known. The aim of this study was to assess cytotoxicity and genotoxicity of water from sites with different degree of water quality, in high waters (HW, may) and low waters (LW, august) periods, of Potrero de los Funes River, through analysis of effect biomarkers in *Allium cepa* L. apical meristems. Water samples were collected in different sites of the river. Physical-chemical water quality, toxicity, citotoxicity and genotoxicity were analyzed. Both periods shown signs of anthropogenic perturbation, in all sites. There was a positive relationship between root length and phosphate concentration in water samples (Correlation coefficient = 0.85 y p < 0.05). Mitotic index was significantly higher in HW period (p = 0.001) and was significantly different between sites in both periods (HW: p= 0.0001; LW: p = 0.003). Differences in phase index between sites were statistically significant (p = 0.0001) in the HW period and not significant in the LW period. Chromosomal aberrations were observed in the genotoxicity analysis; they were more frequent during HW period (p = 0.001). The results of this study showed the presence of substances that induce cell cycle alterations and clastogenic and aneugenic damages, indicating that Potrero de los Funes River has a potential risk of cell level contamination.

## A19 EFECT OF KETAMINE ON ANXIETY IN THE PLUS MAZE TEST AND THE METABOLIC

**ACTIVITY IN AMYGDALA** 

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Psychotogenic effects of ketamine, due to antagonism of NMDA (N-methyl-D-aspartic acid) glutamate receptors, are widely known, and they are a subject of study in our laboratory. In the present study we searched for the effect of systemic administration of ketamine at low doses in the plus maze test in Holtzman derived colony rats. Classical anxiety parameters were considered (time spent in the open arm, open and closed arm entries, time per entry and grooming). Three systemically administrated doses were compared with saline vehicle: 1.25, 2.50 and 5 mg/kg (n=20). Five rats of each group were selected for measurement of amygdala metabolic activity. It was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) through spectrophotometric wavelength measurement. Statistically significant reduction was observed in the time spent in the open arm and time per entry (p<0.01, p<0.01 and p<0.001), and in open arm entries (p<0.05, p<0.05 and p<0.01). Grooming was clearly enhanced by treatment (p<0.001, p<0.001 and p<0.01). Metabolic activity showed an evident and significant decrease in the amygdala (1.25 mg/kg, p<0.01; 2.5 mg/kg, p<0.001). We conclude that systemically administered ketamine in low doses produces anxiogenic effect. These behavioral findings are accompanied by a decrease

#### **A20**

# DIFFERENCES IN BENZODIAZEPINES PRESCRIPTION BY GENDER AND AGE IN A SAN LUIS PHARMACY OFFICE

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Benzodiazepines are a pharmacologic group very prescribed for anxiety, sleeping disorders and depression treatment. Due to their side effects, specific recommendations for its prescription exist. The objective of this study was analyzed the benzodiazepines prescription according to gender and age in a pharmacy of San Luis (Argentine) during January-December 2016. A retrospective study was carried out; data about the consumption were obtained directly from prescriptions. Diagnostics were coded according to International Statistical Classification of Diseases and Related Health Problems (ICD-10). Were registered 1144 benzodiazepines prescription during study period (1073 as mono-drugs): Clonazepam 51% (n=583; Sex: M=25.4% F=74.6%; Age:  $\leq$ 60=25.6% and  $\geq$ 60=74.4%), Alprazolam 32.2% (n=368; Sex: M=31.8% F=68.2%; Age:  $\leq$ 60=17.4% and  $\geq$ 60=82.6%), Bromazepam 8.7% (n=100; Sex: M=33% F=67%; Age:  $\leq$ 60=27.4% and  $\geq$ 60=72.6%), Lorazepam 4.5% (n=52; Sex: M=30.8% F=69.2%; Age:  $\leq$ 60=39.3% and  $\geq$ 60=60.7%), another 3.6% (n=41; Sex: M=14.1% F=85.9%). Fixed-dose combinations with Bromazepam and Alprazolam were the most prescribed (6.2%), being inappropriate combinations according to potential therapeutic value. Indications for anxiety and insomnia were predominant. A great amount of recipes without diagnostic were observed. An elevate prevalence of prescriptions for female sex was visualized, despite its major vulnerability. A high predilection for Clonazepam was found. Patients older than 60 years received more benzodiazepines than

younger patients, despite the known associated morbidity and mortality to their use, such as the psychomotor activity reduction that is responsible of falls, leading cause of mild to fatal injuries. Prescription habits of benzodiazepines must be modified, because its use is generalized by perception of a false safety.

#### A21

#### DELAYED EFFECT OF TRANYLCYPROMINE IN THE RAT FORCED SWIMMING TEST

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Major depression is a prevalent, serious, recurrent psychiatric disorder that has been ranked by the WHO as the fourth leading cause of disability worldwide. Nowadays, the mono-amine oxidase inhibitors are used for treating patients with treatment resistant depression. However, some authors refer to this group as one of the most efficient antidepressants. The aim of this investigation is to establish the doses in which tranylcypromine has an antidepressant effect to analyze pharmacological interactions in future researches. Holtzman rats were treated with saline solution, tranylcypromine 2.5 mg/kg (T 2.5), 5 mg/kg (T 5) or 10 mg/kg (T 10) and were tested in the forced swimming test 30 minutes later. T 10 rats showed an increased climbing time when compared to saline, T 5 and T 2.5 (p<0.05). Also, a significant reduction of the immobility time was seen when T 10 rats were compared to saline, T 5 (p<0.05) and T 2.5 (p<0.01). No differences were observed in the swimming time. These results indicate that tranylcypromine has an antidepressant effect when administered at the dose of 10 mg/kg. Lower doses have no such effect.

#### A22

## DESIGN OF $\epsilon$ -POLYCAPROLACTONE NANOPARTICULATED SCAFFOLDS FOR BOSENTAN MONOHYDRATE CONTROLLED RELEASE

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Electrohydrodynamic techniques refer to the dynamics of electrically charged fluids. One of its operative regimes is electrospraying, for the generation of polymeric nano and microparticles. This technique allows, to reach, a uniform dispersion of the drug within the polymer matrix achieving high encapsulation efficiency, by showing an easy operation and a good profitability. Our objective was to study the behavior of a nanostructured platform designed as scaffold of containment and prolonged/controlled drug release. Nanoparticles loaded with Bosentan monohydrate were developed by subjecting a 0.5% w/w ε-Polycaprolactone (PCL) in acetone solution to flow conditions of 0.5 ml/h and voltage of 10 kV, the drug was incorporated to the polymeric solution in a 0.8:1 (drug:PCL) ratio. The morphology and size distribution, structure, thermal and biopharmaceutical properties of these particles were determined by SEM, XRD, DSC and diffusion through dialysis bag, respectively. Heterogeneous particle sizes were observed by the SEM showing micro and nanoparticles (150 to 1150 nm) with predominance (<60%) of particles smaller than 550 nm. The obtained nanostructures presented semi crystalline properties as inferred by the XRD and DSC scanning. These properties indicate a possible interaction between the amorphous PCL and crystalline Bosentan. Additionally, *in vitro* assays during 24 and 72 hours showed prolonged release in biological media. As a conclusion, we demonstrate that the manufacture of these Bosentan controlled release nanoplatforms is an interesting contribution in the development of new therapeutic alternatives for the treatment of pathologies such as pulmonary hypertension and other related diseases.

#### **A23**

## CADMIUM INTOXICATION IN CEREBELLUM: EFFECT OF DIFFERENT EXPOSITION TIMES AND PROTEIN SOURCES

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Cadmium (Cd) is an environmental contaminant. We studied its effect on cerebellum at different exposition times and protein diets. Oxidative stress, lipid and apoptotic markers were determined. 4 lots of female Wistar rats were used (n=6): 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source, one lot received regular water (Co) and one, 15ppm of Cd in the drinking water for 30 (30d) and 60 days (60d). SOD 2, Nrf-2, NOX 2, Gpx, p47, ACC, HMGCoAR, DGAT, Bax, Bcl-2 were determined by PCR. S28 was the internal control. Nrf-2 and Gpx increased in CasCd at 30d, decreasing in Cd groups (p<0,05) at 60d. NOX 2 decreased in SoyCd at 30d (p<0,05) and in CasCd at 60d. p47 increased in CasCd, while decreasing in CasCd and increasing in SoyCd at 60d. SOD 2 decreased in SoyCd (p<0,05) at 30d, and increased in SoyCd and decreased in CasCd at 60d. At 30d ACC decreased in both Cd groups (p<0,05), without differences at 60d. HMGCoAR decreased in SoyCd (p<0,05) and increased in CasCd at 30d, increasing in both Cd groups (p<0,05) at 60d. DGAT decreased in SoyCd (p<0,05), decreasing in CasCd at 60d. Bax/bcl-2 ratio increased in both CasCd at 30 and 60d (p<0.05). At 60d it decreased in SoyCd. Cd induces oxidative stress at 30d, showing a depressed antioxidant system at 60d. Lipid markers were decreased in soy groups respect to casein groups, which showed more apoptosis. This was mildly attenuated by Soy, suggesting a potential therapeutic effect.

# REPELLENT ACTIVITY OF ESSENTIALS OILS FROM SOME AROMATIC SPECIES AGAINST RED FLOUR BEETLE, *Tribolium castaneum*

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Red flour beetle, *Tribolium castaneum* (Herbst) is a secondary plague widespread in the world. Establishment of this insect in storage sites of grain or flour decreases the quantity and quality of commercial and consumable products flour derivate. Essentials oils (EOs) are alternative way to make new ecologic friendly insecticides. The aim of present work was to design and evaluate liquid repellent formulations for *T. castaneum* control. Combinations of commercial EOs of *Citrus limon* (Cl) and EOs obtained from several aromatic species collected in pampa semiarid region, Helianthus petiolaris (Hp) and Baccharis spartioides (Bs), were used. Five repellents formulations were design containing 2% of EOs in combination: 1) Hp; 2) Bs; 3) Cl; 4) Hp-Cl; 5) Bs-Cl. Repellency of *T. castaneum* was assessed by area preference test method for five formulation tested during first 5h and 24h. The percentage of repellency after 24h exposition decrease significantly in single EOs formulations 1) Hp, 2) Bs and 3) Cl. Nevertheless, combined EOs formulations 4) Hp-Cl; 5) Bs-Cl shown the maximum repellence at 24h. Analysis of results indicate that combination of EOs formulated extend persistence of repellency at 24h more efficiently than single EOs formulations. We suggest a potential repellent activity of these essential oils from aromatic species used to the control of *T. castaneum* insect plague.

### A25 ANTIBIOTICS INITIATION-ROTATION CRITERIA IN NEONATE WITH GASTROSCHISIS DIAGNOSIS

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Gastroschisis is one the most frequent malformations in new-borns, affects 1/2000-1/5000 pregnancies. Antibiotics (ATB) use is a frequent practice in this pathology. The criteria of initiation-rotation can be Microbiological or Clinical-Laboratory. Neonates present particular conditions in relation to ATB initiation-rotation: clinical non-specificity, urgency on initiation of antibiotic therapy and lack of specific laboratory tests. Our objective was to analyse the initiation-rotation criteria of ATB in a neonate with gastroschisis diagnosis. The clinical history was used for this analysis. New-born male, gestational age: 37 weeks, weight: 2580g. 1st-scheme: Ampicillin/Gentamicin with negative blood culture (h-), Protein C Reactive (PCR-mg/L) 192, white blood cells (GB-mm3) 18400. 2nd-scheme: Cephalotin/continuous Gentamicin, h-, PCR 48, GB 23300, platelets (P/mm3) 66000. 3rd-scheme: Vancomycin/Amikacin, h-, torpid clinic. 4th-scheme: continued Vancomycin/Meropenem, h, PCR 96, GB 18400, Platelets 62000 and surgical wound with flogosis. 5th-scheme: Vancomycin/Amikacin. Positive catheter (phlebotomy) for *Staphylococcus coagulase* (-), blood, urine and faecal cultures: negative, PCR 96, GB 9100, febrile. 6th-scheme: Vancomycin/Meropenem. Blood, urine and stool cultures: negative, PCR 192, GB 45600. Initiation-rotation of ATB was mainly determined by pathological laboratory (PCR, GB, platelets) and clinical assessment (general condition, colour, temperature, wound characteristics). Future comparative studies on antibiotic schemes applied in other cases of gastroschisis at national and international level have been foreseen, their results could differ depending on the epidemiology of each health center. Education about the rational use of ATB and the protocols development with algorithms for initiation-rotation of ATB are suggested to get better results in this complicated pathology.

#### **A26**

### GASTROPROTECTIVE EFFECT OF Lithraea molleoides INFUSION AGAINST HCI/ETHANOL-INDUCED GASTRIC INJURY IN RATS

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Lithraea olleoides (Vell.) Engl. (Anacardiaceae), known in Argentina as "molle", "molle de beber", "molle blanco", is a tree which grows in South America, especially in Argentina, Brasil and Uruguay. Decoctions and infusion of the leaves are used by people of these countries for its medicinal proper (treatment of symptomatologies related to gastric ulcers). In this study, we evaluated the gastroprotective activity of L.molleoides using necrotizing agents (50% HCl/50% ethanol) to contribute to their validation of folk medicine. Gastric lesions were produced according to the method of Robert et al. (1979). Male Wistar rats, randomly assigned into groups (n=6–8), were deprived of food for 24 h prior to starting the experiments and had free access to water. All rats were housed in wire mesh-bottomed cages throughout the study to prevent coprophagy. The necrotizing agents (3 M HCl-60% ethanol) were administered orally (1 mL), and 1 h later, the animals were sacrificed. The stomachs were removed, opened along the greater curvature. Aqueous extracts and metabolites isolated of L. molleoides prevent the formation of gastric lesions. (p<0.01 y p<0.001 vs. Control -Test t de Student). The results presented indicate that L. molleoides and metabolites prevent the formation of gastric lesions.

## DRUGS DISPENSATION FOR DIABETES MELLITUS IN A CENTER OF PRIMARY HEALTH CARE OF MENDOZA

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Diabetes mellitus (DM) represents a major public health problem. The aim was to analyse its prevalence, comorbidities, dispensations and drugs consumption in a Primary Health Care Centre (CAPS) of Mendoza-Argentina. A descriptive, observational, cross-sectional, retrospective study was conducted in 700 adult patients attended the CAPS-30 pharmacy with chronic diseases in June-2015. Statistical: Chi square. Defined Daily Dose (DDD/1000 diabetic patients/day)was determined. Chronic patients: DM 35%. Sex: with DM: F 28%, M 46%, without DM: F 72%, M 54%, OR (F): 0,47, OR (M): 2.14. DM: Sex: F 47%, M 53%. Age (years): 29: 7%, 30-49: 23%, 50-69: 67%, 70: 3% (ρ<0.0001). DM1 44%, DM2 56% (DM2 Insulin-requiring: 21%) (ρ<0.0047). Comorbidities: hypertension (HT, 34%), Dyslipidaemia (16%), Anxiety (9%) (ρ<0.0001). DM + HT (85%): DM2 + HT (60%), DM1 + HT (40%). Mono-drugs: insulin-NPH (INPH, 64%), Metformin (M, 55%), Current-Insulin (CI, 17%), Atorvastatin (A, 16%), Enalapril (E, 15%) (ρ<0.0001). Frequents combinations: INPH + E 21%, INPH + IC 16%. DDD: M (416), E (234), Amlodipine (137), Ins (115), Losartan (109), A (97). DM prevalence, specifically DM2, was elevated. Significant associations between male sex and risk of suffer DM, and between DM and HT were found. The most vulnerable age was 50-69 years. Drugs frequently dispensed were insulin and metformin, but the most consumed, however, according to DDD, were metformin and enalapril. It is necessary to contribute to the acquisition of healthy habits through educational measures to prevent this pathology that decrease the life quality of the population.

#### **A28**

#### STUDY OF TERATOGENIC EFFECTS OF Aristolochia argentina IN WISTAR RATS

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Aristolochia argentina (Aristolochiaceae) is popularly known as "charrúa", "charruga", "patito", "buche de pavo". The roots of this plant are used in folk medicine. Traditionally, has been used as antirheumatic, emmenagogue, diuretic, diaphoretic, antidiarrhoeal and antihemorrhoids. We investigated the teratogenic effect of A. argentina root aqueous lyophilized extract (AALE) on gestating Wistar rats (method of Whitten), that received a daily oral dose of 250 mg/kg on day 0-7 of gestation. All rats (n=20) were weighed and observed daily to monitor toxicity throughout the treatment period. On gestation day 20, the rats were euthanized by CO<sub>2</sub> inhalation and the uterine horns removed. The placentas were weighed; the numbers of implantations, resorptions, live and dead fetuses were recorded. The fetuses were weighed and examined for external malformations. No external signs of maternal toxicity were evident (in: skin, mucous membranes, motor, normal breathing, absence of: convulsions, lethargy, tremors, diarrhea and death). The weight gain in the females AALE group (40.33±6.15) was statistically significant (p<0.05) in the last week of gestation (day 14-20) relative to the control (63.74±5.89), which did not disrupt normal gestation. There was no significant difference in mean liver, kidney, spleen and ovary weights of dams when comparing the AALE group to control group. There was no significant difference in the mean number of implantations per dam, live fetuses when comparing AALE group to control group. No external malformations found in fetuses. These findings suggest that Aristolochia argentina at the dose assayed did not have a significant adverse effect on embryo development.

#### A29

# CHECK-LIST OF CNS DEPRESSING MEDICINAL PLANTS USED IN ETHNOMEDICINE, WITH EMPHASIS IN SOUTH AMERICAN PEOPLES

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Much of the world's population satisfies their primary health care through natural products and bioactive ingredients derived from plants (MP) and animals. Among the most common conditions of our time are those related to the Central Nervous System (CNS), and MP stand out among the natural products used as remedies to combat illness or at least to alleviate their symptoms. A great number of plants are used for their attributed or verified neuroleptic, analgesic, anticonvulsant, anxiolytic and hypnotic properties. Field interviews were conducted with 25 "connoisseurs" and people referents, in Argentina, checking the botanic identity of the plant at field and/or laboratory. Semi-structured polls revealing the knowledge and popular use of native, adventitious and cultivated plants conducted in Argentina. A literature review provided data on MP ethnomedical uses for CNS conditions in the rest of South America. They were surveyed in the field and/or were found references on the use of 425 plant species (especially vascular ones as Passiflora, Melissa, Valeriana, etc.) with prevalence of those of the families Lamiaceae (7.7%); Asteraceae (7.5%); Apiaceae (5%); Solanaceae, Rosaceae and Fabaceae (c. 3.5% each); Ranunculaceae, Verbenaceae and Poaceae (c. 2.3% each). Popularly indicated as "soothing"/"to calm the nerves", "to fall asleep", "nervous tonic"/"tonic of the CNS", "against stress"/"to calm anxiety", "tranquilizer", etc., some of them are used against severe nervous conditions (Parkinson's disease, epilepsy, convulsions, neurovegetative dystonia, etc.), while others are recommended to treat conditions related to state of mind and pains, until to become analgesics (even anesthetics) of wide use.

### SELF MEDICATION IN STUDENTS OF FIRST YEAR OF THE NURSING CAREER OF NATIONAL UNIVERSITY OF SAN LUIS, ARGENTINA

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Self-medication is the use of medications on their own initiative, or by the advice of other persons, without consulting a doctor. This usual practice is widely known, brings with it serious consequences as the masking a disease, the occurrence of adverse reactions and increased antimicrobial resistance. Objective: to analyse the behaviour of self-medication in Nursing students of first year of the National University of San Luis (April-2017). A survey to 268 students of a total population of 550 was performed. An anonymous and structured questionnaire with closed questions, and multiple choices was used. Sex: F: 214 (80%), M: 5(19%), without date (WD, 1%). Age range: 17-47 years. Self-medication: 219 (82%) of the students. Reasons: speed 150(68.5%), dislike going to the physician 34 (15.5%), lower cost 2(0.9%), other reasons 32(14.6%), WD 0.5%. Drugs: analgesics 132 (60.3%), antibiotics 48 (21.9%), both 24(10.9%), others 10 (4.6%), WD 5 (2.3%). Antibiotic treatment duration days: 1: 20(41.7%), 3: 14 (29.2%), 7: 8 (16.5%), 15: 5 (1.86%).Person who recommended consumption: family 82 (39.7%), pharmacist 52(23.7%), by self-choice 53 (24.2%), by prior medical advice 25 (11.4%). Beliefs about self-medication: it is inappropriate 161 (73.5%), because drugs can have secondary effects 67(41.6%), miss the diagnosis 30(18.6%), should consult the doctor 31 (19%), WD 19%. High proportion of student believes that it is appropriate to self-medicate, because they believe they have sufficient knowledge. Self-medication in this group is elevated, even with ethical drugs; despite they know the downside of this practice. Taking measures to sensitize students about the risks involved in this common practice is of fundamental importance to the health of the population.

#### A31

## SOY PROTEIN MODULATES THE EFFECTS OF INTOXICATION WITH CADMIUN IN RAT MAMMARY GLAND

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We studied the effects of different diets at molecular, biochemical and cellular levels on rat mammary gland (MG). We looked at the potential protective outcome of those diets under exposure to Cadmiun (Cd), which is an important environmental contaminant. For this purpose, 4 female lots Wistar rats were used: 2 lots received casein and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control) and the other, 15 ppm of Cd in the drinking water for 60 days (6 animal in each experimental condition, 3 independent experiments). Lipids were extracted and total cholesterol, phospholipids and fatty acids where determined by colorimetric assay, thin-layer chromatography and gas chromatography-mass spectrometry, respectively. HMG CoA Reductase (HMGCoAR) and NFκB activation were determined by Western blot. The inflammation marker COX2 and Bax/Bcl2 were measured by PCR. MGs were subjected to hematoxylin-eosin stain. Our results show that Cd alters the lipid profile of MG and these effects are modulated by Soy based diet. The expression of HMGCoAR is also affected by Cd and Soy, showing some synergism. On the other hand, both Cd and Soy activate NFκB and this effect is also accompanied by augmented expression of COX2 and apoptosis according to the Bax/Bcl2 relation. Also Cd and Soy stimulate the development but Soy decreases the fat pad. The double treatment causes fibrosis and lost functionality. In summary, Cd affects the physiology and development of rat MGs and Soy diet may modulates the facts of Cd exposure in this tissue.

#### A32

#### GASTRIC CYTOPROTECTIVE ACTIVITY OF Ligaria cuneifolia IN RATS

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Ligaria cuneifolia (Ruiz & Pav.) Thiegh. (Loranthaceae) is a widespread hemiparasitic plant in the Argentine Republic and it is employed in folk medicine. The aim of this study was to evaluate the anti-ulcerogenic effect in rats, and role of sulfhydryl groups, seem to play a mechanistic role in gastroprotection. The infusion of L. cuneifolia 10% was prepared according to Argentinean Pharmacopoeia. We examined the effect on gastric damage induced by oral administration of absolute ethanol (EtOH). The scanned images of stomach were analyzed by using a program developed by NIH. EtOH produced gastric ulcers in all the animals treated. L. cuneifolia infusion (125, 250 and 500 mg/kg) prevents the formation of gastric lesions induced by EtOH (p<0.001 vs. EtOH). The effect elicited by L. cuneifolia at 500 mg/kg was not attenuated by pretreatment with N-ethylmaleimide (10 mg/kg, s.c.), a blocker of sulfhydryl groups. This suggests that the gastroprotective mechanism of action of L. cuneifolia does not involve sulfhydryl groups at the dose assayed. Several reports have shown that flavonoids protect against experimental ulcer. The anti-ulcerogenic effect of L cuneifolia could be due, in part, to the presence of flavonoid fraction in this plant. Moreover, acute toxicological studies have showed that an oral administration of 2000 mg/kg of L. cuneifolia did not produce any sign of acute toxicity in the animals (male and female). These findings suggest a potential beneficial use of Ligaria cuneifolia on gastric cytoprotection.

### ANATOMÍA, HISTOLOGÍA Y FISIOLOGÍA ANIMAL

#### **A33**

#### CHRONIC STRESS AND PROTHROMBOTIC STATE IN AGED HYPERTENSIVE RATS

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Hypertension per se confers a hypercoagulable state, which may explain in part why the main complications of hypertension are thrombotic rather than hemorrhagic events. Blood flow, focusing on blood rheology is considered an important factor for thrombus formation. Age, stress and oxidative status are also known to be important risk factors for thromboembolic events. We compared blood viscosity, oxidative and prothrombotic status among chronically stressed normotensive and hypertensive old rats. Control and stressed (movement restriction, 1 h/day, 3 times/week /45 days) male Wistar (W) and spontaneously hypertensive (SHR) rats (12 months of age) were used. Corticosterone, glycemia, fibrinogen, blood and plasma viscosity, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and carbonyls and MDA in erythrocytes membranal fraction and SOD and CAT in cytosolic fraction were measured. Stress increased glycemia and corticosterone, but higher glucose in SHR and higher corticosterone in W rats was found. PT and APTT decreased in both strains of stressed rats, being APTT lower in SHR. Stress increased fibrinogen in both strains and platelet count only in W. Blood viscosity, carbonyls and MDA was increased in both stressed rats. Movement restriction increased SOD in W and decreased CAT in SHR. Stress response was different in both strains, with increased adrenal-medullary activity in SHR and cortical-adrenal axis in W. Erythrocyte oxidative changes along with increased blood viscosity indicate that stress can cause significant alterations in blood rheology and hemostasis, with greater effects in hypertensive animals, indicating that stress would aggravate hypercoagulability, one of the complications of hypertension.

#### A34

# PIGMENTS IN THE PINEAL GLAND OF FEMALE VISCACHA (Lagostomus maximus): A HISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

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The presence of pigment has been demonstrated in different nervous structures such as those of retina, substantia nigra and locus coeruleus. These pigments have also been described in the pineal gland of different mammal species. The purpose of this study was to analyze the presence of pigmented cells in the pineal gland of female viscacha (*Lagostomus maximus maximus*) under natural conditions and to evaluate a probable relation between pigment content and glandular activity during pregnancy. The following techniques were applied: Hematoxylin-Eosin, Phosphotungstic Acid-Hematoxilin, Masson-Fontana Silver, DOPA Histochemistry, Schrmol's reaction and Toluidine Blue. The ultrastructural features of the pineal pigment granules were also analyzed. Estradiol and progesterone serum levels were determined by RIA. Pigment granules were evident in the female pineal gland and they were observed in a random distribution within the parenchyma, but the pigmented cells were frequently found near blood vessels. The pineal pigment was histochemically identified as melanin. Differences in the amount of pigmented cells were found between pregnant (early pregnancy  $1.73 \pm 0.18$ ; mid pregnancy  $3.52 \pm 0.35$  and late pregnancy  $2.58 \pm 0.12$ ) and non-pregnant viscachas ( $0.76 \pm 0.16$ ). The ultrastructural analysis revealed the presence of pre-melanosomes and melanosomes. Estradiol and progesterone levels vary during pregnancy, with highest values in mid pregnancy and lowest in non-pregnant viscachas. In conclusion, the changes in the amount of pineal melanin seems to indicate a direct relationship between pigment content and gonadal hormone levels during pregnancy, when estradiol and progesterone levels remain elevated for a long time.

#### A35

# EVALUATION OF LEAD EFFECTS ON THE INTESTINAL ENZYMES OF EARED DOVE (Zenaida auriculata)

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Increasing industrialization and human activities intensify the emission of pollutants; among them lead (Pb). The adverse effects of Pb have been well documented in birds and other animals (haematological damage, kidney malfunctioning, brain damage, etc.) However, strikingly, information on the Pb effects on digestive functions of bird is scarce. Recently we found a decrease of the specific activity of the intestinal enzymes (sucrase, maltase, and aminopeptidase-N) in house sparrows (*Passer domesticus*) Pb exposed. Nevertheless, there are no studies about the response of these enzymes to Pb in other bird species. The objective was to study the long-term Pb effects on digestive enzymes of eared dove (*Zenaida auriculata*). To achieve our goal, we established three independent groups of birds, two groups were exposed during 15 and 30 days to the same Pb concentration in drinking water, and one control group. The intestinal enzymatic activity of sucrase, maltase and aminopeptidase was determined and statistical analysis performed (RM-ANOVA, Tukey post-

hoc test, p<0.05). The pattern of intestinal enzymes activities found was similar to those reported for other bird species. Nevertheless, surprisingly our results showed a non-significant inhibition of these enzymes in Pb exposed doves. These results are opposed to those found previously in house sparrows. This could be because, unlike sparrows, doves have crop, and it is known that there is a lead-induced dysfunction of this organ. This could be due to a direct action of Pb on the smooth muscle or the neural elements in crop tissue. Supported by UNSL 2-0516 to FDC.

# A36 BLEOMYCIN EXACERBATES LUNG FUNCTION IMPAIRMENT AFTER ANDROGEN DEPRIVATION

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Roles of sex hormones as modulators of lung function and disease have received significant attention. Added to this Bleomycin (Ble) is an anti-neoplastic agent used as first-line therapy in many human cancers treatment; however it induces pulmonary fibrosis. The present study used bleomycin-induced pulmonary fibrosis in Wistar rats to examine potential differences in physiological and pathological outcomes in control and castrated rats under two different doses. Adult male rats were divided in six groups: 1) control (Co); 2) Co + Ble I (doses: 1, 5 mg/kg); 3) Co + Ble II (doses: 10 mg/kg); 4) Ca (castrated); 5) Ca + Ble I and 6) Ca + Ble II. After 40 days of treatment, rats were sacrificed. TBARS and catalase (CAT) were measured and histological analysis was performed in lung. Ca+Ble increased TBAR'S and CAT, with significant differences between Ca vs Ble I and Ble II (p<0.0001). Ca+Ble I showed peripheral fibrosis and nonfunctional spaces in lung stroma. Castration leads to an increment in connective tissue and inflammatory zones, what was improved in Ble I. In Ca + Ble II we observed alveolar disorganization with some non-functional spaces, fibrosis compromised up to 40% of the lobes and also intensification of non-functional spaces was observed. These data indicate that bleomycin plays an exacerbating role altering lung stroma and increasing some oxidative stress parameters, after androgen deprivation, especially in Ble II treated rats, where fibrosis may be critical in male lung function.

#### A37

# CHROMAFFIN AND SUSTENTACULAR CELLS IN THE ADRENAL MEDULLA OF THE VISCACHA (*Lagostomus maximus*): A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY

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Chromaffin and sustentacular cells are common components of the adrenal medulla of mammalian species. However, subpopulations of these cells varies among the different species. While the rat adrenal medulla is known to contain noradrenergic chromaffin cells in close relation with GFAP (glial fibrillary acidic protein)-positive sustentacular cells (SC-GFAP) other species express only chromaffin cells with an adrenergic phenotype and lack of SC-GFAPir. The aim of this study is to assess the distribution of adrenergic and noradrenergic chromaffin cells and SC-GFAPir in the adrenal medulla of the viscacha. Four male viscachas (N=4) and four male rats (N=4; technique control) were used in this study. In order to study the presence of adrenergic and noradrenergic chromaffin cells in the adrenal medulla, we used the histochemical method described by Honoré. The presence of SC-GFAPir was study by immunohistochemistry using an antibody against GFAP. In our study, the histochemical detection of noradrenergic chromaffin cells was negative throughout the adrenal medulla of the viscacha suggesting an adrenergic phenotype for this species, while the rat adrenal medulla used as control showed a strong positive reaction. The immunohistochemical detection of SC-GFAPir was also negative in the viscacha. These results showed that the viscacha adrenal medulla contains only chromaffin cells with an adrenergic phenotype and lack of SC-GFAPir. This study is in accordance and contributes with the current literature adding another observation that suggests that SC-GFAPir are only expressed in presence of chromaffin cells with a noradrenergic phenotype. However, further studies are necessary to confirm this relation.

#### **A38**

#### INFLUENCE OF BOVINE DIET ON THE PRODUCTION OF GREENHOUSE GASES (GHG)

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The fermentative process in the rumen is dependent on the substrates of the diet and the interactions between microorganisms. They generate beneficial and harmful gases for the animal and the environment where they are eliminated by eructation. These harmful gases are generically defined as greenhouse gases (GHG) and methane (CH4) is one of the main. In order to contribute to the reduction of GHG production by cattle, the relationship between the quality of the diet received and the in vivo production of CH4 from ruminal fermentation was evaluated. The in situ concentration of CH4 was evaluated in 8 steers (14 months old,  $280 \pm 0.5$  kg LW), Aberdeen Angus with micro rumen cannulas, they received 4 different diets: % dry matter (85, 60, 50, 42), % neutral and acid detergent fiber (31, 72, 60, 46/8.3, 32.5, 33, 8), % crude protein (12.6, 6.6, 15, 20) and % digestibility (DG) (80, 63, 59.1, 80). Gas measurement was

performed with a portable device (Reike Keiki RH-515) after 25 days of diet adaptation, and 5 hours after feeding. Methane level ranged from 7 to 25 % Vol. Through the least squares method we estimated the coefficients that relate CH4 and quality parameters, identifying DG as the variable that affect it the most (r = 0.89); followed in intensity by neutral fiber (r = 0.6). The prediction equation obtained will allow to predict the emission of CH4, within the domain of the values studied, through the routine chemical analysis of bovine rations.

#### A39

### POSTNATAL ACUTE STRESS BY FORCED SWIMMING IN STRESSED RATS PRENATALLY AND ITS EFFECT ON PROLIFERATION OF T LYMPHOCYTES

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Prenatal stress (PS) produces in adult offspring, a decrease in T lymphocytes functional response, at baseline and versus postnatal stress. Previously reports showed that immune changes occured during exercise by action of the Sympathetic Nervous System (SNS) and there are changes that occur in the immune response in adults. The aim of this research was to investigate the T lymphocytes proliferation under stimulation of Concavalin A (Con A), Noradrenaline (NA) and Propranolol and elucidate the effect of physical, acute, forced swimming (FS) stress in adult male rats with PS. 90 days old Wistar rats were used, stressed in uterus by chronic immobilization (IMO) and unstressed controls (CP). The FS postnatal acute stress was performed in water at  $35 \pm 2^{\circ}$ C. Corticosterone (COR), lymphocytes (L) and T cell proliferation stimulated with Con A, NA and Propranolol were determined by radioimmunoassay. The FS in male PS rats produces: increased COR levels, and decreased T lymphocyte proliferation. Data shown that the the activation of the (SNS) could be a possible cause of the decrease in T lymphocytes functionality in PS animals, due to since the use of NA in vitro decreases the proliferation of T cells. The effect of NA on T lymphocyte proliferation is mediated by  $\beta$ -adrenergic receptors.

# A40 HEMATOLOGICAL PARAMETERS VARIATION BY CORTICOSTERONE IN Passer domesticus

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It is known that stress situations (e.g. predation, climatic changes, pathogens) affect the nutritional and immunological individual state. The hematological and biochemical parameters are useful tools to test nutritional and health status in animals. Our objective was to determine the effect of corticosterone (stress hormone in birds) on hematological parameters in *Passer domesticus*. Thirty two sparrows were exposed to different concentrations of CORT in drinking water (0, 20, 40 and 80 mg/l) for 72 hours emulating a long-term stress situation. The heterophil to lymphocyte ratio (H/L) was used as an indicator of stress in birds. Hematocrit, serum protein profile by native PAGE, and plasma concentration of triglyceride, uric acid, glucose and total proteins were determined. We analyzed the data using a one-way and RM-ANOVA with Tukey post-hoc test (p<0.05). We found a proportional increase in H/L ratio and a significant decrease in the hematocrit in all the tested treatments. A significant increase in the  $\beta$  fraction of the birds treated with 40 and 80mg/l of CORT and a decrease in the concentration of uric acid to all treatments were observed, while triglycerides, total proteins and glucose remain constant through all treatments. Uric acid is known as a potent antioxidant and could be absorbed into tissues to protect against tissue damage due to increased metabolism associated to stress in birds. On the other hand, the increase in the H/L index and  $\beta$  fraction denote an immunological change associated to stress. Supported by UNSL 2-0516 to FDC and UNSL 2-0814 to ECV-JGC.

# A41 CORTICOSTERONE EFFECTS ON BODY MASS AND DIGESTIVE FUNCTION IN Passer domesticus

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It is well documented that stress produces metabolic alterations and an allostatic response (activation of the HPA axis and increase of corticosterone -CORT- levels in blood) to maintain homeostasis in organisms. The objective of this work was to study the long-term CORT effects on the corporal condition and the digestive function in *Passer domesticus*. To achieve our goal, 32 sparrows were exposed to different concentrations of CORT in drinking water (0, 20, 40 and 80 mg/l) for 72 hours emulating a long-term stress situation. The heterophil to lymphocyte ratio (H/L) was used as an indicator of stress in birds. The mass of animals and organs (heart, pancreas, stomach, intestine, shank and pectoral muscle) were measured. On the other hand, the intestinal enzymatic activity of sucrase, maltase and aminopeptidase was determined. We analyzed the data using ANOVA with Tukey post-hoc test (p<0.05). We found a proportional increase in the H/L index in concordance with CORT treatments and a significant decrease in body mass in 80 mg/l of CORT group. Differences in the mass of heart, liver, pancreas, stomach,intestine and shank muscle were not found, but a significant decrease in pectoral muscle mass was observed in birds exposed to 40 and 80 mg/l of CORT. Enzymatic activity of sucrase and maltase decreased on

the proximal section. In conclusion, long-term CORT exposure modifies the corporal condition and the digestive function (affecting carbohydrates digestion). Supported by UNSL 2-0516 to FDC and UNSL 2-0814 to ECV-JGC.

#### A42

## EXPRESSION OF ESTROGEN RECEPTOR AND PROLIFERATING CELL NUCLEAR ANTIGEN IN PITUITARY PARS DISTALIS DURING THE PREGNANCY OF VISCACHAS

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The female viscacha is a monostral animal with an unusually long gestation period for a rodent. Three stages were classified: early (EP), middle (MP), and late pregnancy (LP). In previously studies variations of lactotrope cells were reported. The aim of this work was to analyze the expression of estrogen receptor (ER $\alpha$ ) and proliferating cell nuclear antigen (PCNA) in pituitary of viscachas during pregnancy. Pituitaries of non-pregnant (NP) viscachas and pregnant from EP, MP and LP (n=4 per group) were processed for light microscopy. ER $\alpha$  and PCNA were detected by immunohistochemistry and quantified by image analysis. The colocalization of PCNA-PRL was qualitatively analyzed. Serum estradiol was determined by radioimmunoassay. The immunostaining pattern for ER $\alpha$  was nuclear and cytoplasmic. Nuclear ER immunoreactivity (ER $\alpha$ n-ir) increased significantly in LP (NP: 0.25  $\pm$  0.03%, EP: 0.11  $\pm$  0.03%, MP: 0.19  $\pm$  0.04%, LP: 0.62  $\pm$  0.19%, p<0.05). The expression of PCNA increased significantly with pregnancy (NP: 0.75  $\pm$  0.06%, EP: 1.47  $\pm$  0.25%, PM: 1.23  $\pm$  0.15%, LP: 1.60  $\pm$  0.22%, p < 0.01). Numerous PCNA-PRL positive cells were observed in MP. Serum estradiol level was significantly higher in MP compared to the other groups (NP: 20.5  $\pm$  3.36%, EP: 29  $\pm$  6.66%, PM: 80  $\pm$  3.65%, LP: 23  $\pm$  2.54%, p < 0.01). These results demonstrate that estradiol through its nuclear receptors stimulates the cellular proliferation during pregnancy. Thus, in the MP is likely that occur the most significant changes in the glandular activity in the pituitary of this rodent.

#### **A43**

### INTERPLAY BETWEEN OXIDATIVE STATUS AND ERYTHROCYTE FRAGILITY IN AGED SHR RATS SUBJECTED TO CHRONIC STRESS

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Plasma membrane fluidity modifications affect the receptors activity, enzymes and ion exchangers, altering cellular ionic homeostasis. Stress hormones and oxidative status could interact with erythrocyte membranes, impairing their elasticity and microviscosity. This can affect erythrocyte deformability and its passage through the capillaries, increasing their fragility. Oxidative status and stress reactivity rise with age. In this work we evaluate oxidative status and erythrocyte fragility in response to chronic stress in old spontaneously hypertensive rats (SHR). Control (n=6) and stressed (n=6, movement restriction, 1 h/day, 3 times/week /45 days) male SHR rats of 12 months of age were used. Erythrocyte osmotic fragility, hemogram, corticosterone, glycemia, malondialdehyde, total antioxidant capacity and bilirubin plasma levels were determined. Superoxide dismutase (SOD) and catalase (CAT) activity and total nitrites (NOx) levels were measured in the erythrocyte cytosolic fraction. In the membrane fraction, malondialdehyde, carbonyls levels and ATPase activity were determined. Increased corticosterone, glycemia, conjugated and total bilirubin and reduced plasma antioxidant capacity in stressed rats were observed. Lower CAT activity and NOx levels and increase in SOD activity in cytosolic fraction were found. In membrane fraction, stress increased carbonyls and MDA and decreased ATPase activity. These changes could affect erythrocyte deformability and be responsible for the greater erythrocyte fragility found in stressed rats. Chronic stress in hypertensive individuals would cause changes in erythrocyte functionality, ionic imbalance and increased osmotic fragility, which may contribute to the development of microvascular complications.

#### A44

# HISTOLOGICAL STUDY OF THE HEPATIC PARENCHYMA OF EARED DOVE (Zenaida auriculata) EXPOSED TO LEAD

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Birds that live in industrialized, urbanized and intensive agricultural areas has been reported having augmented Pb concentrations. The ingestion of this toxic metal can induce histological alterations in tissues. The aim was to examine the effect of chronic administration of lead on the liver histology from eared dove (Z. auriculata). Adult doves were live-trapped and acclimated to laboratory conditions before using them in experiments. They were randomly divided into two groups: control group (n=6) and experimental group (n=6) treated with lead acetate in drinking water ad libitium for 30 days. The birds were anesthetized and euthanized (CICUA-UNSL). The liver was processed for light microscopy. The following techniques were applied Hematoxylin-Eosin, Perls' Prussian Blue, and Masson's trichrome. In the experimental group, histopathological alterations were found in the liver parenchyma. Circulatory disturbances, inflammations and leukocytic infiltration, steatosis of hepatocytes and its fatty degeneration were observed. In the portal spaces proliferation of the bile ducts, dilated portal veins and more connective tissue were found. Melanomacrophage aggregations which accumulate melanin and hemosiderin pigments (positive staining with Perls' Prussian blue). In these sense, we also observed that Pb causes a decrease of delta-aminolevulinic acid dehydratase (ALAD) activity in exposed doves. It is well established that ALAD

inhibition altering the hemoglobin synthesis, which results in the release of iron from proteins. Moreover, several studies observed that the excess iron is deposited in tissues (e.g. liver) as hemosiderin. The observed histological disturbances could be due to an inflammatory response in the liver parenchyma of Pb exposed doves.

### MICROBIOLOGIA E INMUNOLOGIA

#### A45

## Listeria monocytogenes BIOFILM FORMATION IN PRESENCE OF THYMOL AND DIFFERENT CARBON SOURCES

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Biofilm formation by Listeria monocytogenes is influenced by the availability of nutrients and the presence of inhibitors among other factors. Thymol (2-isopropyl-5-methylphenol), presents a variety of pharmacological properties including antimicrobial effects. This work was carried out using *L. monocytogenes* CLIP 74902 and its biofilm formation ability tested under various carbon sources and thymol concentrations. Culture medium: trypticase soy broth (TSB) with glucose 10 g/l, trehalose 10 g/l and cellobiose 10 g/l, supplemented with thymol ( $\mu$ g/ml): 0, 250, 750. The experiments were performed in 96-well microplates. Aliquots of 150  $\mu$ l medium TSB and 10  $\mu$ l of overnight culture were added into each well. Each plate was incubated at 30°C under aerobic conditions for 24, 48, 72 and 96 h. The negative control wells contained broth only. To estimate the biofilm formed, the microplates were rinsed with sterile PBS and remaining attached bacteria fixed with methanol. Then, 200  $\mu$ l of 1% crystal violet were added into each well and left at 25°C for 30 min. After removing the dye with PBS, the crystal violet attached to biofilm was solubilized by adding glacial acetic acid. The OD 550nm was measured in a plate reader. The experiments were performed by sixfold. At 96 h, sessile biomass production was higher with of trehalose and cellobiose compared to glucose. Thymol (750  $\mu$ g/ml), completely inhibited the formation of biofilm with the three carbon sources evaluated. Results suggest that the processes of biofilm formation could be affected by metabolic pathways for energy production or carbohydrate transport system used.

#### A46

### EFFECT OF Origanum vulgare EXTRACT AGAINST Pseudomonas aeruginosa CARBAPENEMS RESISTANT STRAINS

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Pseudomonas aeruginosa is nosocomial pathogens that cause hard-to-treat infections. Carbapenems, meropenem and imipenem, are the drug of choice for the treatment of infections due to multi drug resistant P. aeruginosa. Chromosomal mechanism of imipenem resistance is the loss of porin oprD, and the over expression of efflux pumps contributing to meropenem resistance. Origanum vulgare is an aromatic herb used as flavoring agent in foods. The aim was to investigate the effect of oregano extract (OE) against P. aeruginosa carbapenems resistant strains. A total of 30 P. aeruginosa strains isolated in the Central Hospital San Luis from clinical samples and ATCC 27853 reference strain were used. Carbapenems susceptibility was determined by disk diffusion method. Molecular characterization of carbapenems resistance was determined byRT-PCR assay using MexA, MexB, MexR, OprM, OprD and housekeeping rPSL genes. The antimicrobial effect of EO on ten P. aeruginosa strains was performed by broth microdilution method according to CLSI. EO was diluted to obtain the final concentrations of 100 to 0.8 mg/mL. Minimal inhibitory concentration (MIC) was determined as the smallest amount of extract needed to inhibit the visible growth of the microorganism. EO showed MIC values ranged 6 to 12.5 mg/ml against five P. aeruginosa sensible strains tested; while 60% resistant strains showed MIC values ranges 6 to 12.5 mg/ml and over expression of efflux pumps. One resistant strain showed 1.5 MIC value and both resistance mechanism. The results suggest that EO could be used for treatment of infections caused by P. aeruginosa resistant strains.

#### A47

### ANTIMICROBIAL ACTIVITY OF VAGINAL *Lactobacillus* sp. AGAINST PATHOGENS CAUSING VAGINITIS

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Lactic acid bacteria (LAB) are the most often found inhabitants of vaginal microbiota of fertile women. LAB present antimicrobial activity due to different mechanisms. The purpose of this study was to identify a LAB strain isolated from vaginal swab, and to determine its antimicrobial activity against pathogens causing vaginitis and the nature of the antimicrobial produced. LAB strain was typified by MALDI-TOF as Lactobacillus fermentum. Antimicrobial activity of cell free culture supernatant (CFCS) was determined by liquid-medium method using Staphylococcus aureus, Escherichia coli, Listeria monocytogenes and Candida albicans as indicator

microorganisms. Organic acid production was estimated by difference between antimicrobial activity of untreated and neutralized CFCS.  $H_2O_2$  production was evaluated by treatment of CFCS with catalase. Bacteriocin presence was determined by treating of neutralized CFCS with proteinase-K, pepsin and trypsin. CFCS antimicrobial activity was higher than 85% against all pathogens tested. Antimicrobial activity against L. monocytogenes was mainly due to organic acid production (neutralized CFCS lost 64% of antimicrobial activity). By contrast, antimicrobial activity of neutralized CFCS against the other indicators decreased less than 40%. Antimicrobial activity of neutralized CFSC showed a drastic reduction by trypsin treatment while the reduction was 17% by catalase treatment. In conclusion, *L. fermentum* isolated from vaginal microbiota produces organic acids that inhibit *L. monocytogenes* growth and produces a bacteriocin able to inhibit *S. aureus*, *E. coli*, *L. monocytogenes* and *C. albicans* growth. These characteristics might promote the use of this strain as a probiotic and the produced bacteriocin on prevention and treatment of vaginitis.

#### **A48**

#### ANTIFUNGAL METABOLITES OF Bacillus amyloliquefaciens SL-6 AGAINST Candida albicans

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Several Bacillus species produce antimicrobial metabolites by ribosomal (as bacteriocins) and nonribosomal synthesis (i.e. polyketides and lipopeptides, namely surfactins, fengycins and iturins) with potential use in human health. *Candida albicans* is one of the most common opportunistic fungal pathogen for immunocompromised patients. This work studied antagonistic activity of *B. amyloliquefaciens* SL-6 against *C. albicans*. The SL-6 strain was cultured in Synthetic Mineral Broth with orbital shaking at 200 rpm for 24 h at 30°C. To separate the bioactive metabolites, the cell-free supernatants (CFS) obtained after centrifugation and filtration were subjected to: acid precipitation and methanol extraction (ME10x) for lipopeptides concentration and ultrafiltration processes with molecular weight cut-off membranes (MWCO) of 10 kDa and 3 kDa for determination of molecular size of biometabolites. The antifungal activity was tested against *C.albicans* ATCC 36801 by the agar well-diffusion method. Thin layer chromatography (TLC) was carried out to detect the presence of lipopeptides in ME10x, visualizing bands with different reagents. Active fractions were revealed by contact bioautography. Hydrophilic antifungal compounds were mainly remained in aqueous supernatant after acid precipitation, maintaining a 97.9% of initial activity. Moreover, TLC-bioautography of ME10x showed a unique inhibition zone with Rf: 0.06. The CFS separation by ultrafiltration yielded fractions bioactives with sizes ranging from 3-10 kDa to minor of 3 kDa (93.9%), indicating that small-molecule compounds were the major active metabolites. Thereby, *B. amyloliquefaciens* SL-6 secreted several anti-Candida metabolites, including mainly hydrophilic compounds with MW minor to 3 KDa and others in ME than were not the known lipopeptides.

#### A49

### FREQUENCY OF ENTEROPARASITES IN CHILDREN OF THE HOSPITAL DEL SUR, SAN LUIS – ARGENTINA

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Intestinal parasites are a frequent health problem in populations living in unfavorable social conditions affecting the growth, nutrition and cognitive ability of children. The objective of this study was to estimate the frequency of enteroparasites in children under 15 years of age who attended the Laboratory Service of the Hospital del Sur and to determine the most frequent parasitic associations. The presence of enteroparasites in the parasitological examinations performed during the period the period from March 2015 to February 2017 was evaluated using the method of concentration-centrifugation of Charles Barthelemy and brushed perianal. Enteroparasites were found in 38% of the samples. The highest frequency was observed in children aged 3 to 5 years. The most frequent species were: *Enterobius vermicularis* (62%) y *Giardia intestinalis* (26%). The frequency of monoparasitosis and polyparasitosis was 79% and 21%, respectively. The most frequent association was *Enterobius vermicularis* / *Giardia intestinalis*. The results show a high frequency of enteroparasites. The species found suggest a fecal contamination of water and foods of raw consumption and indicate deficiencies in the hygiene habits. This suggests that, at the same time to pharmacological treatment, preventive measures should be taken in relation to these factors. The low frequency of associations found in our study, compared to other authors, justifies the most exhaustive search in the analysis of the samples, incorporating fresh observation and coloring techniques.

### A50

### HOMOGENIZATION OF *Tolypothrix tenuis* FASCICULATED TRICHOMES BY MECHANICAL DISRUPTION

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Ensheathed filaments of the cyanobacterium *T. tenuis* are rarely in solitary form and grow in dense and macroscopic blackish clusters with intensely fasciculated trichomes arrangement. Due to this fact, homogenization of samples to obtain reproducible data is a real problem. Different methods to achieve this were tested. *T. tenuis* was grown in diazotrophic cultures without agitation in Petri dishes, using Watanabe medium, at 30°C under continuous illumination. The methods assayed were: vigorous vortexing during 5, 10 and 15 min, disaggregation with pestle and mortar, and sonication at 20Hz followed by Optical Density (OD580 nm) and chlorophyll a determinations in duplicate. Visual counting with Evans Blue dye was used as viability assessment on the basis of its penetration into

non-viable cells. The former procedures did not showed effectiveness. Only sonication during 4 minutes with cycles of 1 second gave homogeneous cells suspensions with an excellent correlation between OD vs chlorophyll a (R<sup>2>=1), when 14 determinations (7 individual samples in duplicate) were tested. Moreover, viability of the sonicated suspensions remained optimal with high value of viable cells (~ 95%) when Evans blue staining was applied, showing to be a rapid and easy viability assessment technique for this cyanobacterium species. We concluded that sonication was a very reliable method for homogenization of *T. tenuis* filaments, therefore allowing its application for multiple purposes, such as quantification of growth or any product of interest, as well as inoculum standardization for a wide variety of tests leading to reproducible results.

### A51 ATMOSPHERIC POLLEN OF MORACEAE IN THE CITY OF SAN LUIS, ARGENTINA

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The Moraceae family includes some of the most used trees in the cities, as ornamentals and also for their edible fruits. This family includes several genera of anemophilous plants that can cause skin reactivity and pollinosis as red (*Morus nigra*) and white (*Morus alba*) mulberries and the paper mulberry (*Broussonetia papyrifera*). In this work the Moraceae pollen content was analyzed for two years of continuous aerobiological sampling, in the atmosphere of the city of San Luis, Argentina. Pollen samples were taken with a volumetric sensor Lanzoni (VPPS 2000) and were read with an optical microscope at 400 X. The biological content was identified through palynological atlases and with the pollen library belonging to Aerobiology's laboratory. The pollination period extended from August to October. Comparatively with others pollen types Moraceae was the first most abundant in the atmosphere of San Luis. The maximum concentration values were recorded in September, with a peak of 1393 pollen grains/m<sup>3</sup> of air, while from November to June it was less than 1 grain/m<sup>3</sup> of air. Moraceae pollen exceeded the threshold considered "very high" for the whole of the trees. We recommended to gradually abandon the use of Moraceae specialty the M. alba "fruitless" and *B. papyrifera* for afforestation of the city, due to the allergy problems it causes.

### A52 BIOCHEMICAL CHARACTERIZATION OF *Yersinia* OUTER PROTEIN P (YopP)-GALECTIN-1 INTERACTIONS

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Yersinia enterocolitica (Ye) is a Gram-negative enteropathogenic bacterium. Yersinia outer proteins (Yops) are effector proteins of Ye that are injected into host cells. YopP causes suppression of pro-inflammatory cytokines and induces apoptosis in both macrophages and dendritic cells. Galectin-1 (Gal-1) is a "proto-type" β-galactoside-binding lectin widely distributed in host tissues with important immunomodulatory roles. We previously demonstrated that the Ye-induced apoptosis of macrophages depends on both YopP and Gal-1, and that Gal-1 binds only to YopP preventing its auto-degradation. The aim of this study was to characterize biochemically the YopP-Gal-1 binding. Glycosylation of YopP was confirmed by Schiff staining and lectin blots with both Gal-1 and other biotinylated plant lectins. Different glycosylation patterns were observed for the Yops. Only YopP presented permissive glycoepitopes for Gal-1 binding. Moreover, we isolated the Gal-1-YopP complex using sodium alginate beads. The presence of Gal-1 and YopP in the complex was confirmed by Western blot using anti-Gal-1 or anti-YopP antibodies. The pure complex was subjected to reducing or non-reducing conditions, and then analyzed on partially denaturing polyacrylamide gels to evaluate possible different conformations. Additionally, polyacrylamide gels using transverse urea gradient, evidenced strong interactions only inhibited by 2-4 M urea. This interaction was not affected by the oxidation of vicinal diols. We conclude that the specific binding of Gal-1 to YopP under *in vitro* conditions is protein-glycan type.

### A53

### ENTEROPARASITES IN MONEY CIRCULATING IN SAN LUIS, ARGENTINA

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Banknotes and coins are some of the most handled objects exchanged by people, thus representing a potential vehicle for the transmission of intestinal parasites. A total of one hundred banknotes and seventy coins of five and four denominations respectively were examined for the search of enteparasite contamination. The banknotes and the coins were washed with 5 and 3 ml respectively of water with 20% Tween 20 for 5 minutes each side with the aid of a sterile swab to remove the possible contaminants. These volumes were centrifuged at 3000 rpm for 10 minutes and then the sediment was observed with an optical microscopy using lugol at 10X and 40X magnification. Secondly, they were spread out and made a Kinyoun staining to visualize coccidian and observe them at 100x. A total of

5.88% (10/170) of the examined money were contaminated by parasitic/commensal taxa. *Blastocystis sp.* (4.12%), and *Giardia sp.* (1.76%) were the only the isolated species. Banknotes with less denomination were more contaminated than those with the highest denomination and 83.33% of the contaminated banknotes were dirty, while only 16.66% of the contaminated banknotes were apparently clean. No statistically significant associations were found between the coins denominations. We conclude that money circulating in San Luis, Argentina, could serve as a potential fomite for the spread of intestinal parasites. Therefore, awareness campaigns on sanitation are recommended for the proper use and handling of money or the replacement of those damaged.

# A54 DETECTION OF Yersinia enterocolitica (YE) BY 16S rDNA PCR IN ARTIFICIALLY CONTAMINATED MEAT, INCLUDING AMPLIFICATION CONTROLS

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YE is an enteropathogen that causes intestinal and immunological complications. It is transmitted by oral route, commonly by contaminated foods, with pigs as the main reservoirs. YE isolation from complex matrices such as food is complicated because of the competition between this microorganism and the accompanying microbiota. To overcome the limitations related to culture techniques, molecular methods based on polymerase chain reaction (PCR) have been developed, which are characterized by their rapidity, high specificity and sensitivity. The objective of this study was to detect YE at different dilutions in a ground beef sample by PCR targeted to the species-specific 16S rDNA gene including an internal amplification control (IAC) and an external amplification control (EAC). YE was cultured 24 h in trypticase soy broth ( $OD_{600}$  0.2) from which serial decimal dilutions corresponding to  $10^8$  to  $10^0$  CFU/ml were performed. Then, nine 10-g portions of one ground beef sample were contaminated with 0.4 ml of each inoculum dilution, submerged in 90 ml sorbitol bile peptone broth and incubated 24 h at 25°C to stimulate the YE repair in this complex matrix. DNA extraction was performed by boiling, and then, 16S rDNA PCR including IAC and EAC was performed. Amplification of the 16S rDNA gene was observed in all dilutions tested, while IAC was observed from YE concentration of  $10^4$  CFU/ml. EAC worked perfectly. The present study highlights the excellent performance of the amplification controls designed in our laboratory for the detection of YE when this bacterium is present in complex matrices.

# A55 CHARACTERIZATION OF JAWSII CELL LINE AFTER LPS-STIMULATION IN DIFFERENT CULTURE CONDITIONS

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JAWS II is an immortalized dendritic cell (DC) line established from the bone marrow cultures of p53<sup>-/-</sup> C57BL/6 mice. The characterization of this cell line from scientific literature is controversial as depend on experimental settings. Therefore, we evaluated DC surface makers and IL-12/23p40 production after LPS-stimulation in different culture conditions. JAWS II cells were cultured in presence of GM-CSF or Flt3L, as DC differentiation factors, and they were stimulated with LPS. Surface markers and cytokine production were assessed by flow cytometry and ELISA, respectively. We corroborated that the cells cultured with GM-CSF or Flt3L express surface markers of DC. After LPS stimulation, they show maturation since increased MHC-II and CD86 expressions. Interestingly, these cells displayed the two TNF receptors (TNFRp55 and TNFRp75), and reduced TNFRp75 significantly after LPS-stimulation. In addition, the JAWS II cell line secreted TNF and IL-12/23p40 in response to LPS. Human TNF (hTNF) has previously demonstrated to bind to mouse TNFRp55 but not to TNFRp75. Because of this, JAWS II cells were stimulated with LPS in presence of hTNF, which significantly reduced IL-12/23p40 secretion only in presence of GM-CSF. In culture media with GM-CSF or Flt3L, IL-12/23p40 production decreased significantly by p38, ERK and mainly JNK MAPK inhibitions. We conclude that GM-CSF or Flt3L in the culture medium impact on LPS-induced responses of JAWS II cells. This cell line could be suitable as model to gain an insight into the mechanisms underlying the regulatory effect of TNF through TNFRp55 on IL-12/23p40 secretion.

# A56 REGULATORY EFFECT OF TNF ON IL-12/23P40 PRODUCTION BY MURINE DENDRITIC CELLS

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TNF is a pleiotropic cytokine considered a major player in the initiation and orchestration of complex events in inflammation and immunity. The biological activities of TNF are mediated by two receptors, TNFRp55 and TNFRp55 is the primary signaling receptor for the majority of the pro-inflammatory and cytotoxic effects classically attributed to TNF. The purpose of the present work

was to know how TNFRp55 signaling modulates IL-12/23p40 production. Isolated splenic wild-type (WT) or TNFRp55 DC were stimulated with LPS in presence and absence of TNF or TNF inhibitors. The levels of IL-12/23p40 and IL-10 were assessed in culture supernatants by ELISA. We found that TNF significantly decreased the IL-12/23p40 secretion by LPS-stimulated WT DC. This regulatory effect was not observed in TNFRp55-/-DC. Moreover, the TNF antagonist, Etanercept, decreased IL-12/23p40 secretion in LPS-stimulated WT and TNFRp55 DC, suggesting a TNFRp75-depending IL-12/23p40 production by DC. When TNF-TNFRp55 pathway was analyzed using the specific inhibitor CAY10500, we observed that the regulatory effect of TNF on IL-12/23p40 secretion in WT DC was lost. This result mirrored the effect of TNF on LPS-stimulated TNFRp55 DC. Interesting, IL-10 amounts were not modified by Etanercept in WT as well as in TNFRp55 DC. However, IL-10 levels resulted significantly reduced by CAY10500 in LPS-stimulated WT DCs, but not in TNFRp55 DC. These data indicate that IL-10 seem to mediate the regulatory effect of the TNFRp55 pathway on IL-12/23p40 secretion by LPS-stimulated DC.

#### A57

# AEROBIOLOGICAL EVALUATION IN THREE BUILDING LEVELS OF THE LIBRARY OF THE NATIONAL UNIVERSITY OF SAN LUIS

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Studying aerobiological content inside buildings is important, because of the allergenic properties of biological particles present in these environments, especially fungal spores and pollen. Daily, hundreds of people, mainly students, utilize the facilities of the library of the National University of San Luis. In order to evaluate air quality inside the different areas of this building (underground, ground, and first floor), a continuous aerobiological sampling was performed during May 2017. Samples were taken with a volumetric sampler Lanzoni (VPPS 2000) and were read using optical microscope at 400x and 1000x. The biological content was identified through palynological atlases and with the pollen library belonging to the Aerobiology's laboratory. Most abundant particles in all three floors were spores. This result was in agreement with other works carried out indoors. In the underground floor as well as on the first floor, Cladosporium spores were the most abundant particles peaking at 456 and 480 spores/m³ of air, respectively. Meanwhile, on the ground floor, Aspergillus/Penicillium spores were the most abundant with 1910 spores/m³ of air. The pollinic content was low on all floors, recording a maximum of 2 grains of pollen/m³ of air during the sampling period. Significant differences (p=0.013) of total aerobiological content between the underground floor (620 aeroparticles/m³ of air) and the first floor (2355 aeroparticles/m³ of air) were observed, the latter containing the highest values. These differences might be explained by the higher flow of people circulating in this floor, who with their movements remove particles lying on diverse surfaces.

### A58 HYGIENIC CONTROL OF CAFETERIA'S ENVIROMENT AT NATIONAL UNIVERSITY OF RÍO CUARTO

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Environmental sampling has been identified as an effective procedure to verify the correct implementation of food safety systems. The objective of the study was to perform microbiological control of living and inert surfaces of the cafeteria of National University of Río Cuarto. Sixty-one surfaces in contact with food were sampled during 2016 and 2017. Samples were collected by swabs before and after cleaning and sanitization. Total aerobes (RAT), enterobacteria and total coliforms (TC) were counted and the presence of *Staphylococcus aureus* in the hands of operators was determined by direct contact with Baird-Parker agar. Regarding to inert surfaces, it was observed that, in 100% of the samples taken and analyzed, RAT, enterobacteria and TC values decreased between 1 and 2 logarithms after the hygiene process. As for living surfaces, 100% of the samples presented RAT values between 4x10<sup>2</sup> and 3.8x10<sup>4</sup> CFU/hand; the enterobacteria were present in 55.6%, with counts varying between 5 and 2.9 x 10<sup>4</sup> CFU/hand, and in addition, the same percentage of samples showed values of TC counts ranging from 5 to 1.3x10<sup>4</sup> CFU/hand. *S. aureus* was observed in 40% of the manipulators. The results of this study suggest that good hygiene practices aimed at minimizing bacterial counts on the preparation surfaces should be controlled as they may be deposits for the microbial contamination of ready-to-eat foods.

#### A59

# FREQUENCY OF ISOLATES OF Streptococcus pyogenes IN PATIENTS WITH CLINICAL DIAGNOSIS OF ACUTE PHARYNGOTONSILLITIS IN A PRIVATE LABORATORY IN THE CITY OF SAN LUIS

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Acute pharyngotonsillitis is one of the most common diseases in childhood. Among bacterial causes, the main responsible agent is *S. pyogenes*. Given the lack of specificity of the symptoms, it is necessary to make a correct microbiological diagnosis and avoid the overdiagnosis of streptococcal pharyngotonsillitis, with consequent unnecessary prescription of antibiotics. The objective of the study was to estimate the frequency of *S. pyogenes* in children aged 2 to 18 years with acute pharyngotonsillitis. The presence of *S. pyogenes* in the pharyngeal swabs performed during the period from May 2015 to April 2016 was evaluated using the conventional culture method and rapid test for the detection of streptococcal antigen. The microorganism was isolated in 29.8% of the cases. The highest frequency

was seen in children aged 5 to 11 years. In the 2-year group, the frequency was low (8%) and in the 3 and 4-year groups it was 27.3% and 28.1%, respectively. Due to the high frequency of *S. pyogenes* in children of 3 and 4 years, it is necessary to carry out their systematic search and to carry out an adequate treatment. In children, the particular epidemiology of each patient should be considered. Rapid tests provide a good response but must be supplemented with microbiological culture due to false positive and negative results.

#### **A60**

## COMPARISON OF RESISTANCE PROFILES TO ANTIBIOTICS OF Escherichia coli ISOLATED FROM WATER OF DIFFERENT AGROECOSYSTEMS

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The indiscriminate use of antibiotics in human and veterinary medicine has generated a growing concern in the dispersion of resistant strains. The Las Lajas stream basin and San Basilio area are agroecosystems impacted by different anthropogenic activities (livestock, agriculture, cemetery, landfill, sewage effluents, dairy industry). Although the bacteriological quality of surface and groundwater has been studied in both areas, know the dissemination of antibiotic-resistant bacteria indicating fecal contamination is relevant. In this context, the aim of this study was to compare the antibiotic resistance profiles of strains of Escherichia coli isolated from surface and groundwater in two different agroecosystems in the south of Córdoba province. The strains of *E. coli* isolated were tested by the antimicrobial susceptibility test against 10 antibiotics, using the plate diffusion method, according to standard methodology. The results showed difference in antibiotic resistance profiles between the two study areas. In the San Basilio area (n= 26), *E. coli* showed resistance to 5 antibiotics for human and animal use. The porcentages highest of resitance to cephalothin (50%) and ampicillin (58%) were observed. On the other hand, in the Las Lajas stream basin (n=33) strains showed only resistance to tetracycline or ampicillin (veterinary antibiotics) was found. The antibiotic resistance profiles of *E. coli* strains, observed in this study would be influenced by the diverse anthropic activities developed in the areas studied.

#### A61 BIOFILM AND ITS RELATIONSHIP WITH SOME FOODS

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In the food industry, biofilm is related to Foodborne Diseases (FBD). Our research approach was to learn more about the existence of bacteria in biofilms, their transfer to different foods and particularly the behavior of *E. coli* producing Shiga toxins (STEC) related to hemolytic uremic syndrome (HUS), against different inert surfaces. It consisted in allowing the production of biofilm by *E. coli* O157: H7 on different inert surfaces such as glass, plastic and stainless steel. The food under study was placed on the different surfaces and the transfer of *E. coli* O157: H7 to the different types of meats like chicken, pork and beef was evaluated. Out of the total 30 samples (10 of each), 68.20% was positive for the beef, 56.10% for the chicken and 38.20% for pork. As for the surfaces that contained the meat pool, the results were: 71.30% in plastic; 58.40% in stainless steel and 38.10% in glass. The work was carried out on 2x2 cm surfaces. The hygiene of the different surfaces should be maximized to avoid the formation of biofilm, since once developed, it is difficult to eradicate. As regards the cooking of the meat (ground meat), it must be done with sufficient time and temperature (70°C) to ensure the elimination of the bacteria and the toxin Shiga, that cause biofilm. For *E. coli* O157: H7, biofilm is the pathogenic factor.

### A62 EFFECT OF 2, 4 DICHLOROPHENOL ON THE GROWTH OF *Chlorella* sp.

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Microalgae are photoautotrophic microorganisms, although under certain conditions some species may live in environments where autotrophic metabolism is not viable. Heterotrophic cultures can be used to study the metabolic aspects of microalgae. 2,4-dichlorophenol (2,4-DCP) is a chlorinated derivative of phenol used to prepare the herbicide 2,4-dichlorophenoxyacetic acid or Agent Orange. The degradation of chlorophenols has been studied in heterotrophic organisms; however there are few bibliographic antecedents about the ability of microalgae to biodegrade these compounds. The objective of the present work was to analyze the growth of the microalga *Chlorella sp.* compared to 2,4-DCP and try to evaluate its capacity to biotransform this compound. Cultures of *Chlorella sp.* were performed in Bold Basal medium (BBM) and supplemented with 2,4-DCP in two concentrations, lang= 1 (T1) 50mg/L and treatment 2 (T2) 70mg/L. Cultures were incubated at 30°C for 6 days in the dark and continuous shaking at 75 rpm. Growth was evaluated by measuring optical density (OD 500nm) and dry weight. The experiments were carried out in triplicate. Cultures of *Chlorella sp.* in the presence of 2,4-DCP showed a higher final biomass than to the control culture, being 3.8 g/L (C), 8,83 g/L (T1) and 7,93 g/L (T2). The final OD 500nm for C, T1 and T2 was 0.527; 0.868 and 0.827, respectively. With regard to biotransformation, the production of metabolites was observed at 48 h. These results could indicate the existence of a nutritional stimulation of the growth in the presence of 2,4-DCP by using it as carbon source.

# PROTEIC FACTORS INVOLVED IN ADHERENCE OF *Pseudobutyrivibrio xylanivorans* ON HUMAN COLONIC CANCER CELLS (SW480)

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Pseudobutyrivibrio xylanivorans has shown a higher capacity to degrade hemicellulose of alfalfa hay compared to related butirivibrios species. Due to this, we started studying of the probiotic capacity of *P. xylanivorans*. Firstly, it was necessary to study the adherence of the bacteria to the digestive tract. As a main requirement of probiotic potencial selection, we started an in vitro analysis of the adherence capacity of *P. xylanivorans* using as a model human: colon cancer cells SW480, which have similar characteristics to the ruminal epithelium. SW480 cells were cultured in DMEM medium for 48 h at 37°C. *P. xylanivorans* were cultured for 48 h at 39°C until a concentration of 3 x 10 9 bacteria mL -1. Thus, we compared the bacterial adherence to the SW480 cells with a standard bacterial inoculum (MOI 200) against a inoculum submitted to two heat treatments, in order to denaturalize the proteinaceous structures of the bacterial surface: 80°C for 60 minutes and 100°C for 1 minute. *P. xylanivorans* and SW480 cells were then incubated for 1 h at 37°C. Finally, the slides obtained from each well were stained with Giemsa staining and analyzed under an optical microscope. The adherence of *P. xylanivorans* to the SW480 cells significantly decreased (p<0,05) to 38 ± 9 % after the heat treatment with 100°C for 1 minute compared to the ones treated with 80°C for 60 minutes and with untreated bacteria. In conclusion, we estimate that *P. xylanivorans* adhere to the SW480 cells using proteinaceous structures in the bacterial surface.

#### A64

# PROTECTIVE EFFECTS OF Origanum vulgare AGAINST GASTRIC DAMAGE AND ULCER INDUCED BY Helicobacter pylori IN AN ANIMAL MODEL

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Helicobacter pylori (HP) is a Gram-negative bacillus found in the gastric mucosa of the human stomach. HP infection is associated to gastroduodenal diseases. Origanum vulgare (oregano) is an aromatic plant commonly used for medical purposes. Previous studies have reported the antimicrobial activity of oregano against HP. The aim of this work was to evaluate the protective effects of oregano against gastric damage and ulcer induced by HP in a model in vivo. Four groups of five male C57BL/6 mice, were used in this study. The animals were dosed intragastrically at days 1, 3 and 5 with 300 μl of 1 mg/ml oregano extract (OE) and/or 300 μl of 108 CFU/ml of bacterial suspension each time according to the following protocol: Group 1 (HP infected), Group 2 (OE treated and HP infected), Group 3 (OE treated) and Group 4 (saline solution). After 4 days, mice were sacrificed and the stomachs were aseptically removed, fixed, stained and observed. Assays were performed in duplicate. The counts of gastric mucosa lesions by direct microscopy in the Group 1 (x=69 bleeding spots) were significantly higher than those in Group 2 (x=21), Group 3 (x=5) and Group 4 (x=6). Hematoxylin-Eosin stain revealed a less conserved mucosa in Group 1 with numerous bleeding vessels. The stomach mucosa was more conserved in Group 2, and normal in Groups 3 and 4. This is the first in vivo study that demonstrates the gastroprotective role of OE and could provide an alternative therapy for gastric diseases associated to HP infection.

#### A65

## DETERMINATION OF PARVOVIRUS HUMAN B 19 IN EXANTHEMATIC DISEASES DURING 2017 IN SAN LUIS PROVINCE

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Adiverse group of viruses causes rash and have the same symptomatology as several viruses and most of the time it is not possible to differentiate them. Among the most frequent agents are Measles, Rubella, Dengue, Varicella, Cytomegalovirus, Epstein Barr, Enterovirus and Human Parvovirus B19. Since exanthematic disease studies focus on rubella and measles surveillance, there is a deficiency of laboratory differential diagnosis of the other causative agents, although increased the sensitivity of medics to the suspicion of human parvovirus B19 in the last times. B19 is transmitted through the respiratory route, can also be transmitted vertically from the mother to the fetus, through bone marrow and organ transplantations, and via transfused blood. Laboratory diagnosis of B19 can be performed using serology, PCR, histopathologic examination and immunohistochemistry. B19 IgM is usually present 10–12 days after infection and persists for 3–4 months, sometimes longer. B19 IgG appears shortly after IgM and persists lifelong with slowly decreasing titers unless boosted by subsequent encounters with the virus. In the first half of 2017 the Public Health Laboratory of the Province received 16 samples for the differential determination of this pathology, 3 of them reactive for ELISA assay finding IgM and IgG antibodies against Parvovirus B19. This accounts for almost 20 percent of suspected cases of rashes. There are serious difficulties in the clinical differentiation of the same, and therefore it is necessary to make a confirmatory diagnosis in order to establish the causal agent involved and take the appropriate prevention and blocking measures.

# MODIFICATION OF ANTIMICROBIAL ACTIVITY OF CHALCONES AGAINST Escherichia coli STRAIN OF CLINICAL ISOLATION

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Escherichia coli is one of the most important pathogenic microorganisms in humans, as causal agent of numerous infections. Currently the indiscriminate use of antibiotics has caused the microorganisms to develop resistance to these drugs, which complicates the treatment of many diseases. In this regard, numerous previous work in our working group confirmed the antimicrobial activity of chalcones. The present work aimed to determine if the synergistic effect of the combinations of dihydroxylatedchalcones - nalidixic acid tested against the reference strain ATCC25922 of E. coli is keep on when tested with clinical strains of this microorganism, which were previously exposed to antibiotics for clinical use. Three methods were used to evaluate the antimicrobial activity of these compounds: kinetic-turdimetric, Kirby-Bauer combined and dilution in agar plates. Results obtained in recent clinical isolate strains tested, proved less efficient by the three methods, when compared with the ATCC25922 strain. This marked decrease in the antimicrobial activity of combinations mentioned could be due to the clinical strains developing different mechanisms of resistance to the antibiotics due to previous contacts during the use of the same in the treatment of infections. The easy access and inappropriate use of drugs to fight infections caused by pathogenic microorganisms promotes self-prescription, which facilitates the selection, persistence and dissemination of resistant microorganisms. Undoubtedly, a more rational use of the available antimicrobials will delay or prevent the emergence of resistant strains, but at the same time we must intensify the search for new effective antibiotic agents to deal with these microorganisms.

# A67 INFLUENCE OF QUORUM SENSING ON BIOFILM FORMATION IN Yersinia enterocolitica

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Quorum sensing (QS) can regulate bacterial gene expression in response to concentration of diffusible chemical signals called autoinducers (AI). QS modulates the biofilms formation and other essential functions for several microorganisms. Biofilms are accumulations of bacterial species that give protection to the involved cells against adverse environmental conditions. *Y. enterocolitica* is able to form biofilms on either abiotic or biotic surfaces, and synthesize AI of type II called acyl homoserin lactones (AHSL). The objective of this work was to evaluate the influence of QS on growth and biofilm formation in *Y. enterocolitica*. The *Y. enterocolitica* strain WAP-314 1B/O:8 (W) and the mutant strains: ΔyenI (deleted in yenI gene involved in the synthesis of AHSL) and ΔyenR (deleted in yenR gene which synthesizes the AHSL receptor) were used. The growth kinetics in trypticase soy broth and the biofilm formation by the violet crystal technique were determined at 25°C and 37°C. At 25°C no difference was observed in the growth of the strains whereas at 37°C the W strain showed a significantly higher growth than both mutant strains. At 25°C the biofilm formation of the ΔyenR strain was significantly lower than the W strain whereas no difference between ΔyenI and W strains was observed. At 37°C an increase in the biofilms formation in the mutant strains compared to W was demonstrated. These results suggest that QS in the *Y. enterocolitica* W strain would enhance the growth at 37°C meanwhile the biofilm formation is temperature dependent.

### FISIOLOGÍA Y NEUROQUÍMICA

#### A68

# PRESCRIPTION OF RUNNING TIMES IN MODERN PENTATLON BASED ON THE EVALUATION OF MAXIMUM OXYGEN CONSUMPTION

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In the last decade the number of pentathlon practitioners increased. The best youth represented Argentina in the World Championship in Prague 2017 and will be present in the Olympic Games of Youth Buenos Aires 2018. The discipline is complex. In a single day they must compete in fencing, riding and swimming, and thus qualify for the last two sports: shooting and races of 800 meters, repeating this 4 times. To model the career indications according to their maximum oxygen consumption (VO<sub>2</sub>) and their Maximum Aerobic Velocity (MAV). VO<sub>2</sub> was evaluated in Argentinean juvenile selection pentathlonists. A Medgraphics® VO2000 gas analyzer was used (Accuracy:  $O_2 + /-0.1\%$ ,  $CO_2 + /-0.2\%$ ). determining MAV, and applying the running system used by the elite. Final Time of Run (FTR) = [[(0.135 \* 3600 / (MAV \* 1.1)) + (0.665 \* 3600 / (MAV \* 0.98)) \* 3] + (0.8 \* 3600 / (MAV \* 1.1)). This seems to be a running scheme that less compromises the shooting performance. Pearson simple regression equation was used to study the weight of the VO<sub>2</sub> variable. Then the model was applyied and the results compared with the achievements in competitions. Regression equation FTR= 1330,464 - 10,869 \* VO<sub>2</sub>, 2=0,926. The model improved the average time by 75.05 seconds (10%). It is important to establish the VO<sub>2</sub> to give the

athletes precise indications of race. Its study also allows to establish MAV in a reliable way, and to program both competition and training systems to improve performance.

#### A69

## PRELIMINARY STUDY ON COGNITIVE RESERVE OF SENIOR CITIZENS FROM THE CITIES OF SAN LUIS AND LA PUNTA

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Cognitive Reserve (RC) is defined as the ability to progressively activate alternative brain networks in response to growing demands. It is an ability that depends on context, it contributes to the adaptation of the individual to the environment and acts as a protector against neural degeneration. CR has as a biological basis a cerebral reserve and makes reference to the cerebral characteristics such as brain size, neuronal number and density, quality and quantity of synapsis, personal traits that would endure the damage and delay the manifestation of symptoms of brain pathology. The objective of this preliminary study was to investigate cognitive reserve in senior citizens from San Luis city and La Punta. The sample was not randomized. 15 subjects (N=15) 8 women and 7 men ages 66 to 79 (M=71,8; D.E=4,28) made up the sample. The Rami, Valls-Pedrest, Batrés-Faz and Molinuevo, and CR questionnaire were used as instrument. CR of the sample was M=10,93 with DE=3,453. From the data obtained, we can conclude that the total sample of CR ranged among medium-high values. 4 subjects presented a RC lower range, 3 subjects presented a medium range and 6 subjects presented a medium-high range and 2 subjects displayed a higher RC category. We can highlight that most (11/15) senior citizens investigated have protective resources against brain ageing.

#### A70

## HEALTHY LIFESTYLE IN A SENIOR ADULT SAMPLE IN THE CITIES OF SAN LUIS AND LA PUNTA. A PRELIMINARY STUDY

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Lifestyle constitutes the major determinant of a person's health, since it integrates habits and behaviors that modulate his/her daily life activities. The aim of this study was to assess the lifestyle of senior adults from La Punta and San Luis. The sample was intentional, non-random. It consisted of 15 senior adults (7 men and 8 women) aged between 66 and 79, with an average of 71.80 + 4.28 years. Forty percent of them live alone, while 60% live with their families. We used the FANTASTIC lifestyle questionnaire, which contains 25 closed items exploring nine physical, psychological and social domains related to lifestyle. Results indicate that participants have an excellent lifestyle (47%), a good lifestyle (27%), and a regular lifestyle (27%). We can conclude that most participants (74%) lead a healthy lifestyle with regular physical activity, low consumption of alcohol, tobacco and stimulants, a diet low in sugar, fats and processed food, healthy sleep and resting habits, accident-prevention behavior (such as using the seat belt), and emotional wellbeing related to self esteem, affectionate relationships, stress and anxiety.

# A71 INFLUENCE OF PERINATAL ANDROGENIZATION ON ADULT WOMEN PERSONALITY FROM THE CUYO REGION

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The levels of prenatal androgens (mainly testosterone) cause brain sexual differentiation, and promote early and permanent male or female patterns of musculoskeletal development, higher brain functions, and cognition, among other features. The aim of this research work was to investigate the influence of perinatal androgenization on adult women's personality. The sample was intentional, and non-random. Sixty women, whose ages ranged between 18 and 42 years (M: 28; S.D: 5.08), from the Cuyo Region were studied. We used the Millon Inventory of Personality Styles, and Ratio of Digit Length (RLD) test. Particularly, the 2D:4D ratio showed the strongest difference between the sexes and the best discriminative power. Two comparison groups were formed with the anthropometric measure: one with RLD values between the minimum (0.86) and the mean value (0.995); and a second group, between the mean value and the maximum value (1.13) of the RLD of the right-hand fingers. The results obtained in the first group showed negative correlations between "individuality" (p=-0.31, z=0.05) and "intuition" (p=-0.33; z=0.04\*) scales with RLD. It was inferred that the lower the value of the index -which was promoted by a higher perinatal androgenization - the greater the value of "individuality" and "intuition" of personality scales. In the second group, with higher values of RLD, no correlations were observed. We emphasized that the biological factor studied in this sample may have a partial incidence in the development of personality because only two of the twelve bipolarities were testosterone-influenced in the less feminine group of women.

### BIOLOGÍA DEL DESARROLLO Y REPRODUCCIÓN

#### A72

## MICROVESICLES: A TOOL FOR DELIVERY OF ROTENONE AND INDUCTION OF PARKINSON DISEASE

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With the aim to develop an animal model for Parkinson Disease (PD), we selected microvesicles as a tool for rotenone administration. Rotenone is an herbicide, which is known to produce neurotoxic effects. Several methods of delivery have been explored, some of them with high mortality. The use of microvesicles allows a slow delivery of the drug an thus a long duration with a single dose administration. We assayed two different protocols for microvesicles preparation and evaluated the stability of microvesicles under different conditions. Under light microscope, microvesicles showed a homogenous size average 30 um. After liofilization, resuspension was assayed under different conditions. Resuspended microvesicles were administered by subcutaneous injection in a dose of 50 mg/kg. Animal's behavior was observed by using the bar test, grid test and rearing test. Rats were weighted every day and no difference with control animals was observed during the first month of treatment. At the dose assayed with two different protocols of microvesicle preparation, no mortality of the animals was observed. Significant changes were observed on behavior tests after 5 weeks of treatment (p< 0.01). Physiological symptoms such as rigidity and immobility are expected to appear after 3 to 5 months of treatment. An accurate experimental model of PD should reproduce the slow, progressive, and selective nigrostriatal dopaminergic degeneration seen in the disease. Although these are preliminary results, the lack of mortalitity in the treated group and the behavioral changes supports a good selection in the dose of rotenone applied in the present study.

### A73 THE ROLE OF PROSAPOSIN AND CATHEPSIN D IN MALE REPRODUCTION

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In mammalian epididymis, luminal proteins interact with the surface of in-transit spermatozoa as a requirement for maturation and acquisition of gamete fertilization capacity. The major sulfoglycolipid of the sperm plasma membrane is a sulfogalactosylglycerolipid (SGG), substrate of Arylsulphatase A (ARSA). ARSA modifies SGG in the presence of a sphingolipid activator protein, termed saposin B (SapB). Both the precursor of SapB, Prosaposin (PSAP), and ARSA are secreted by the epididymal epithelium, and they interact with the sperm plasma membrane. Moreover, the intracellular processing of prosaposin into saposins occurs in an acidic pH, and it requires the protease cathepsin D (CatD). Given that CatD is secreted by the epididymal epithelium and that the female genital tract has an acidic environment, we tested whether CatD is active for processing of PSAP at acidic pH and whether an eventual inhibition of PSAP/SapB action results in decreased fertilization. Mice epididymal fluid and spermatozoa were collected with buffers, adjusted to different pHs (5.5-6.3-7.2), with and without the presence of Pepstatin A (CatD inhibitor) or antibodies raised against PSAP/SapB. Luminal epididymal proteins were subjected to immunoblotting. Sperm motility (by CASA) and *in vitro* fertilization (IVF) were also tested. Acidification did not alter motility parameters; however, PSAP processing by CatD was enhanced at pH 6.3. Moreover, sperm motility and IVF were significantly decreased by a blockade of PSAP/SapB with a specific antibody. These results suggest that PSAP/SapB, and the hydrolases ARSA and CatD, play an important role in modifying the sperm plasma membrane during sperm capacitation and fertilization.

### A74

# HGSNAT (HEPARIN-α-GLUCOSAMINIDE N-ACETYLTRANSFERASE) GENE INACTIVATION AFFECTS THE MORPHOLOGY OF SPERMATOZOA AND THEIR FERTILIZATION CAPACITY IN ADULT MICE

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The epididymal epithelium provides a proper environment for sperm maturation, controlled in part by the secretion of proteins into the lumen and endocytosis of substances from the lumen and basal compartment. Heparan sulphate (HS) is a component of basal membranes and the apical surface of cells. HS is degraded after endocytosis by the action of several enzymes, including heparin- $\alpha$ -glucosaminide N-acetyltransferase (HGSNAT). In adult male mice, inactivation of the Hgsnat gene shows severe morphological alterations in the epididymal epithelial cells, consistent with a phenotype of a lysosomal storage disorder. In addition to this, the fact that these animals

have shown reduced litter size suggests that sperm maturation may be affected. The objectives of this investigation were to determine the effect of Hgsnat gene inactivation on the mice epididymal spermatozoa morphology (by electron and scanning microscopy), on sperm motility (by CASA) and *in vitro* fertilization. In the cauda epididymal lumen of Hgsnat-deficient mice, a significant number of spermatozoa presented abnormal tail and head shapes. Moreover, in these animals, some sperm motility parameters were affected, and their fertilization capacity was significantly decreased compared to wild types. Our results provide the first evidence that abnormal glycan catabolism, upon Hgsnat gene inactivation, could affect sperm quality.

#### A75

#### DIET INFLUENCE IN ACCESSORY SEXUAL GLANDS OF ADULT MALE RABBITS

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Mediterranean diet is a healthy diet indicated for hypercholesterolemia treatment. Its main fat component is olive oil (OO). Hypercholesterolemia is a marker of chronic non-communicable diseases in adults, mostly linked to excessive fat intake. In recent years, it is also associated with male infertility. In male rabbits fed with fat diet, it was detected a decrease in seminal volume, a reduction in sperm count and an increase in sperm morphological abnormalities. Semen volume is produced by the secretion of the accessory glands coupled to male tract. The aim of this work was to study the effect of diet in the preprostate, prostate and seminal vesicle (SV) of rabbit. Glands samples of animals under balanced rabbit diet (BD), fat diet (FD) or protective diet by addition of OO (Protective diet, PD) were obtained. They were processed for light microscopy and morphometry was performed using Image J program. We measured villi length in preprostate, prostate epithelium height and SV thickness of the mucosa. Villi length, prostate epithelium and SV mucosa decreased with FD in comparison to BD. SV epithelium in FD showed no difference vs BD. Up to now, PD has only improved villi length. These preliminary measurements suggest that accessory glands functions would be affected by a FD and could explain the reduction in semen volume.

#### A76

## PEDF (PIGMENT EPITHELIUM DERIVED FACTOR) EXPRESION IN MALE REPRODUCTIVE TRACT (WISTAR RATS) AND IN MEPC5 CELLS (MOUSE)

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Pigment epithelium derived factor (PEDF) expression has been described in many organs as showing neurotrophic, anti-angiogenic, anti-apoptotic, anti-inflammatory, anti-oxidant and pro-cell survival properties. However, references to its activity in the male reproductive system are scarce. In previous works we analyzed the role of this protein in epididymal sperm conjugation in rat epididymis. We aimed to characterize the expression of PEDF in the male reproductive tract of Wistar rats and explore their hormonal regulation. Additionally we extend the study to MEPC5 cells (mouse epididymal proximal caput cells). We found that PEDF is expressed over the epididymis, prostate and seminal vesicles by immunohistochemistry, but notably not in the testes. These results agree with those obtain by semi cuantitative RT-PCR. Androgen dependence of PEDF expression was evaluated by flutamide administration during 15 days to Wistar Rats. PEDF expression diminished along the male reproductive tract. This decreased expression was reversed after 30 days without flutamide administration. MEPC5 cells also express PEDF and proteins typical of epididymis like estrogen receptor alpha and androgen receptor in culture. The epididymis is an essential organ in sperm maturation-storage. The role of PEDF in this physiological process has not been fully elucidated. But considering that in other systems PEDF has anti-apoptotic, anti-oxidants and pro-cell survival properties, its expression along the epididymis may be related to the protection of spermatozoa while they are stored.

#### A77

### METFORMIN REGULATES THE EXPRESSION OF STEROID RECEPTORS IN UTERUS OF RATS WITH POLYCYSTIC OVARY

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Polycystic ovary syndrome (PCO) is a state of altered steroid hormone production and activity associated with decreased fertility and uterine receptivity. Metformin, a widely used insulin sensitizer, improve reproductive outcome in women with PCO. However, molecular mechanisms underlying the beneficial effects of metformin on endometrial tissue remains elusive. The aim of this work was to investigate the impact of metformin on mRNA expression of androgen (AR) and estrogen (ER $\alpha$ ) receptors and nitric oxide (NO) release in uterus (Ut) of PCO rat. The PCO condition was induced in adult rats by estradiol valerate (2 mg/rat). Uterine sections were incubated with RPMI medium (basal), metformin ( $10^3$  M) or metformin plus androstenedione ( $10^{-6}$ M) for 3 h in metabolic bath. The NO (as nitrites) was determined by the Griess reaction and mRNA expressions of AR and ER $\alpha$  were analyses by RT-PCR. The PCO-Ut expressed high levels of AR compared with Control-Ut rat (p<0.05). Metformin treatment decrease AR expression and NO release, without changes in ER $\alpha$  expression compared with PCO-Ut in basal conditions (p<0.05). In Metformin plus androstenedione group, the AR and ER $\alpha$  expression and also NO release incressed in relation to metformin-alone group (p<0.05). All this effect was reversed by flutamide treatment. The results suggest that metformin could modulate the steroid response and pro-oxidant actions induced by

androgenic environment of PCO, that altered steroid receptor expression in the rat uterus. Metformin acting synergistically with Flu would contribute with high efficacy to PCO therapy.

#### A78

### EFFECTS OF METFORMIN AND LETROZOLE ON UTERUS IN A RAT MODEL OF POLYCYSTIC OVARY

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Polycystic ovary syndrome (PCO) is a complex endocrine disorder associated with hyperandrogenism, anovulation and infertility. The chronic estrogen exposure due to ovarian dysfunction can result in endometrial carcinoma. Letrozole (Let) and metformin (Met) are used for ovulation induction in women with PCO but their effects on endometrium have not been elucidated. In this work, we investigate whether Met and Let (non-steroid aromatase inhibitor) affect the P450 aromatase (P450arom) mRNA expression in uterus of PCO rats and, if this is associated with changes in estradiol (E2) and nitric oxide (NO) release. We used a rat model of PCO induced by estradiol valerate (2mg/rat). Uterus (Ut) from PCO rat were incubated for 3 h in metabolic bath with: RPMI medium (basal), Let (10<sup>4</sup>M), androstenedione (10<sup>6</sup>M), Let plus androstenedione (10<sup>6</sup>M) or Let plus Met (10<sup>3</sup>M). The E2 release was measured by RIA, NO (as nitrites) by Griess reaction and gene expression of P450arom by RT-PCR. PCO-Ut released more E2 and expressed higher P450arom (p<0.05) than Control-Ut. Androstenedione increased E2 and NO release and P450arom expression compared to PCO-Ut in basal conditions (p<0.05). This effect was reversed in Let plus androstenedione (p<0.01). In PCO-Ut incubated with Let plus Met, the P450arom expression decrease together less E2 and NO release, compared to basal values (p<0.01). These results suggest that Met and Let induce a down-regulation on P450arom expression and reduce the local generation of E2 and NO. Also, Met and Let can improve the negative effects caused by androgens in PCO endometrium.

#### A79

#### NEPHROGENIC FACTORS PATTERN DURING THE HYPERTENSION DEVELOPMENT

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Spontaneously hypertensive rats (SHRs) develop hypertension at about 6 weeks of age without physiological, pharmacological or surgical intervention. Hypertension development amplifies the risk of onset and progression of kidney disease. In addition, the altered nephrogenic process has been recognized as a powerful risk factor for kidney disease, leading to hypertension; this makes it controversial to analyze whether hypertension is a cause or a consequence of kidney disease. Therefore, the goal of this study was to evaluate the expression of nephrogenic genes, like Wilms' tumor transcription factor 1 (WT-1) and related genes, such as heat shock protein 70 (Hsp70) and vitamin D receptors in connection with the development of hypertension. Methods: Male SHR and control rats (N=40 per group) were evaluated from birth until week 8 of life. Weekly, we took blood pressure and 5 rats per group were sacrificed. The kidneys were removed and weighed. Sections of the renal cortex were processed for histological and molecular studies. Results: The elevation of systolic blood pressure was significant since week 6. Histology showed a slight increase in deposits of collagen fibers since week 4. Additionally, in kidney cortices, the expression of WT-1, Hsp70 and vitamin D receptors decreased since week 4 (previous to the increase in blood pressure). The current results would suggest that hypertension may be a result of possible changes in renal development, evidencing the importance of nephrogenic factors in the hypertension development.

#### A80

## ANTERIOR PITUITARY-NITRIC OXIDE PRODUCTION IS MODULATED BY SPLEEN MACROPHAGE SECRETIONS IN RAT WITH POLYCYSTIC OVARY

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Polycystic ovarian syndrome (PCO) is the most common endocrinopathy in women. Previously, we showed a relationship between neuroendocrine and immune systems in a rat model of PCO, and it was observed that spleen macrophage secretions (M $\phi$ -S) in anterior pituitary (AP) modified the gonadotropins release and apoptosis. Nitric oxide (NO) and Heat Shock Protein70 (Hsp70) have been associated to PCO pathogenesis. However, is not known the interaction of those markers with immune cells on AP in PCO. Now, we studied whether PCO-M $\phi$ -S affect the NO release and, iNOS and Hsp70 mRNA and protein expressions from AP of PCO and Control (C) rats. PCO condition was induced in adult rats by estradiol valerate (2mg/rat). M $\phi$  from PCO rats were cultured (1x10<sup>6</sup> cells) for 24h in RPMI medium. Their secretions were used to stimulate PCO-AP and C-AP for 3h in metabolic bath. NO was measured by Griess reaction. iNOS and Hsp70 expression were assayed by RT-PCR and Western Blot. The PCO-M $\phi$ -S in relation to respective basal: increased the NO release from PCO-AP (p<0.001) and C-AP (p<0.05); not changed iNOS protein expression in PCO-AP but increased in C-AP (p<0.05), and increased iNOS mRNA levels from PCO-AP (p<0.01) and C-AP (p<0.05). The Hsp70 protein and mRNA

expression did not changes between groups. This finding suggests that PCO-M $\phi$ -S could modulate iNOS expression and NO production in AP. This fact would be related to high apoptotic viewed in PCO-AP. Although cannot be discards that Hsp70, as antiapoptite factor, could be involved in the citoprotection of AP.

#### **A81**

### TESTICULAR EFFICIENCY ALTERATIONS GENERATED BY HIGH-FAT DIETS ARE REVERTED BY OLIVE OIL-ENRICHED DIETS

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Hypercholesterolemia is associated with several adult chronic diseases. Meanwhile, Mediterranean diets are considered protectors against hypercholesterolemia. We have recently demonstrated that hypercholesterolemia is associated with sub/infertility in rabbits and that olive oil (OO)-enriched diets reverse that damage. The objective of this work was to evaluate the effects of hypercholesterolemia on testicular efficiency and theirs reversion by OO-enriched diet. We fed adult rabbits with commercial diet supplemented with 14% of fat (HCR). After 6 months, a group of HCR was fed with commercial diet supplemented with 7% of fat and 7% of OO (½ HCR + ½ OO) in order to reverse the hypercholesterolemia. Tubular efficiency was determined by counting the number of spermatogenic cells. Also we calculated proliferation (iep) and differentiation (ied) indexes, dividing the number of spermatozoa by the percentage of spermatogonia and spermatids respectively. Apoptosis was analyzed by TUNEL. Finally, serum testosterone levels were detected. We found lower testicular efficiency in HCR with a decrease in spermatozoa number, spermatogonia and spermatids percentages, alterations in iep and ied, increase in spermatocytes and in germ cells apoptosis. These results accord with a simil-arrest in spermatogenesis and are linked with reduced testosterone levels. On the other hand, OO diets reverse this damage increasing the spermatogenic flow with higher percentages of mature cells (spermatids and spermatozoa) at the expense of spermatogonia. In this group, apoptosis was lower and testosterone higher than HCR. In conclusion, high-fat diets could promote male fertility alterations by affecting proliferation during spermatogenesis, and OO could reverse this damage.

#### **A82**

### LITTER SIZE ADJUSTMENT IS A FACTOR INFLUENCING ON THE PHYSICAL AND NEUROBEHAVIORAL PUP'S DEVELOPMENT

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Postnatal development involves maturation of several systems. The appearance of neurological reflexes is influenced by various factors such as nutrition, maternal care and littermate interactions. The aim of this study was to evaluate alterations in physical and neurobehavioral development on normal (NL) and small litter (SL) pups. Wistar rat litters were selected as: NL (12.40±0.81 pups) or SL (gradually adjusted by sacrificed 2-3 pups in 0, 3, 5, 7 and 15 postnatal day (PND). Pups were evaluated on physical landmarks and behavioral indicators (n=18/each test). Litter size adjustment induced a significant increased in body weight (SL: 32.62±0.80 g vs NL: 23.13±0.68 g, p<0.001) and length (SL: 8.55±0.07 cm vs NL: 7.83±0.06 cm, p<0.001) at PND15. The determination of physical signs reveals a significant decrease in vibrissa growth (SL: 0.86±0.02 cm vs NL: 1.10±0.06 cm, p<0.01) and pinna detachment (SL: 46.43±3.57 % vs NL: 60.00±3.27 %, p<0.01) during first postnatal week and eye opening in PND15 (SL: 45.24±9.70 % vs NL: 73.33±9.59 %, p<0.05). Neurobehavioral tests results in increased righting reflex (SL: 5.19±1.16 sec vs NL: 10.50±0.97 sec, p<0.01) and negative geotaxis during first postnatal week (SL: 33.33±0.00 % vs NL: 13.88±7.34 %, p<0.05). Open field test shows increase motion time (SL: 48.39±4.06 % vs NL: 34.41±2.43 %, p<0.05) and decrease grooming time (SL: 4.85±1.09 % vs NL: 17.03±2.50 %, p<0.001). The results indicate that litter size adjustment modifies the physical and motor development of pups and should be take into account for an adequate application in the field of neurodevelopment.

### CLÍNICA HUMANA Y ODONTOLOGÍA

#### **A83**

# MECHANICAL ALTERATIONS OF DENTAL ENAMEL PRODUCED BY A FLAVORED DRINK. In vitro.

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There is extensive information on the effect of non-alcoholic soft drinks on tooth enamel. Flavored mineral waters are frequently consumed by children although their action on the adamantine microstructure in deciduous teeth is scarcely characterized. The objectives of the work were to determine the acidity of a flavored water and to study the mechanical modifications of the microstructure of the

enamel of deciduous teeth. Exfoliated teeth were obtained, with proper informed consent. The crowns were included in acrylic resin and were abrasioneed in a longitudinal plane. A mineral water of citrus flavor was selected and its acidity was determined with a colorimetric scale. The samples were immersed for 10 minutes in 50 ml of the beverage and nano-indentation surface hardness registers were obtained before and after treatment. The pH of the flavored water was 2. The action of the beverage produced a reduction of the surface hardness, resulting in an average value of  $1.91 \pm 0.7$  GPa, in the radial enamel and  $2.01 \pm 0.6$  in the enamel with bands. The percentage of reduction of the nano-hardness in the radial enamel was 28% and in the enamel with bands of 34%. The contact depth increased 125.48 nm in the radial enamel and 119.33 nm in the banded enamel. The beverage used contains citric acid which acts as a chelator on the hydroxyapatite causing demineralization. We conclude that organic acids produce softening of the microstructure of dental enamel

#### **A84**

### STUDY OF THE RELATIONSHIP BETWEEN VISUAL ACUITY AND THE PRESENCE OF SYMPTOMS IN CHILDREN OF 2ND GRADE OF THE CITY OF SAN LUIS

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Undiagnosed visual disabilities are a main cause of school failure. Early detection of visual problems is of paramount importance since timely treatment allows correcting these defects and preventing their progression. The aim of this studied was to study the relationship between visual acuity (VA) deficit and the presence of some symptoms or signs. We evaluated 340 children between 7-8 years belonging to the second degree of public and private schools in the city of San Luis. The VA was assessed using the Snellen optotype (normal value  $\geq 0.7$ ) and the presence of symptoms or signs were observed by: head turning or tilting to one side, wink of an eye, headache, tearing, blinking, eye pruritus and red eyes. It was observed that 23.5 % (80 children) showed decreased VA, in both eyes was 67.5% (54 children) with the left eye 20% (16 children) being the most affected vs. the right eye 12.5% (10 children). Regarding the presence of symptoms or signs, 36% (28 children) with VA deficiency had at least 1 sign or symptom at the time of examination, whereas the presence of these signs or symptoms in children with normal AV was 12 % (41 children, n=340). According to the results obtained, we can say that children with VA deficiency the 36% had symptoms or signs, so early detection would allow correcting and avoiding complications in the adult stage. For this reason, it is recommended to strengthen the strategies of early recruitment and timely treatment in children with VA deficits.

#### A85

# MEASUREMENT OF PROTEIN C IN PATIENTS WITH PERIODONTAL DISEASE AND CARDIOVASCULAR DISEASE

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C-reactive protein is an acute phase reactant in inflammatory processes, it is released by the liver in the presence of IL-1, IL-6 and TNF- $\alpha$  in the bloodstream, which are the same cytokines present in periodontitis. This protein molecule has been attributed pro-inflammatory effects and is currently classified as a cardiovascular risk factor. To determine the values of C-reactive protein in patients with chronic periodontal disease and cardiovascular alterations a study was conducted with 30 patients with periodontal and cardiovascular disease. In this first stage of the project a clinical history was made, periapical series, index plaque, dental mobility and saliva sample for the study of the photochemical degradation of C-reactive protein. In the second stage of the project the selection of the appropriate degradation product will be made for its follow-up: gas chromatography, mass spectrometry (HPLC) or spectrophotometry (UV). The plaque index was moderate in 77% and severe in 23%. The registration of tooth mobility was grade 1 in the 73% with bags of 4 mm and bleeding on probing and grade 2 in the 27% with bags larger than 4 mm and bleeding on probing. The initial inflammatory process of periodontal disease is characterized by an increase in the levels of C-reactive protein and other inflammatory markers that are also found in the cardiovascular diseases an association could be established between periodontitis and the episodes where they exacerbate the cardiovascular diseases.

#### **A86**

# NUTRITIONAL STATUS OF SCHOOL-AGE CHILDREN ATTENDING AT DINING COMMUNITY IN SAN LUIS CITY

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The number of school-age users of collective food services is increasing. The growth and development of the child are cornerstones in their health care. We conducted a quantitative, descriptive and transversal study in order to assess the nutritional status of school-age children who attend a community dining room ("Mother Teresa of Calcutta") in the suburban areas of San Luis city. We weighed and measured children between 6 and 13 years old and a nutritional diagnosis was made by applying BMI/age and size/age using curves charts of BMI/age according to sex and age. According to BMI/age 25% of the children present overweight and obesity and 14.3%

showed low weight; the remaining 60.7% presented normal nutritional status. According to size/age 17.85% of children presented low size and 82.14% normal size. Analysis of the anthropometric assessment indicates the coexistence of malnutrition, both in excess and deficit. The low BMI/age is associated with acute malnutrition; instead the deficiencies in height/age indicated cumulative health deficiencies and this situation is associated with chronic malnutrition. The health of the school-age population is deeply conditioned by the type of alimentation and the maintenance of a healthy size and weight. This kind of identification allows us to select the individuals at risk, reflecting present or past situations, that would be candidates for the implementation of interventions, as well as to assess the impact of such interventions.

# A87 RENIN-ANGIOTENSIN SYSTEM GENE POLYMORPHISMS, BLOOD PRESSURE AND DYSLIPIDEMIA IN SAN LUIS POPULATION

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Hypertension (HTA) is a major risk factor for cerebrovascular and coronary heart disease, particularly when associated with diabetes, dyslipidemia and obesity. The renin-angiotensin system (RAS) helps maintain blood pressure and salt homeostasis and may play a role in the pathogenesis of aspects of the metabolic syndrome. We investigated two RAS gene polymorphisms, angiotensin converting enzyme insertion/deletion (I/D) and angiotensin II type 1 receptor (A1166 C), for a possible role in modulating dyslipidemia disorders. 200 subjects were divided in two groups: Hypertension (HTA) and control (C), 98 HTA (53% women) / 102 C (69% women), body mass index HTA/C: 31.8±4.2 / 26.9±4.7 (p<0.001), systolic blood pressure HTA/C: 152.3±16.3 / 117.3±11.4 and diastolic 89.2±10.4 / 69.5±9.6 mmHg (p<0.001). Lipid profile HTA/C (mg/dl) Cholesterol: 205.1±37.5 / 188.3±40.5 (p<0.003), HDL: 49.6±10.3 / 50.4±11.3, LDL: 125.1±36.1 / 115.4±34.8 (p<0.03), Triglycerides: 183.7±95.7 / 136.1±78.5 (p<0.0001). Significant correlation between PAS/PAD and lipid profiles was found (p<0.01). Polymorphisms were analyzed by PCR and PCR-RFLP. Genotypes A1166C distribution AA, AC, CC: 49%, 41%, 10% HTA and 57%, 33%, 10% C. Frequencies I/D genotypes II, ID, DD: 21%, 51%, 28% HTA and 23%, 31%, 46% C. Allele frequencies HTA/C: 0.69/0.74 A, 0.31/0.26 C, 0.54/0.39 I and 0.46/0.61 D. Polymorphism DD was associated with dyslipidemia in HTA (p<0.03). The CC genotypes shown significant difference in HDL levels in both group (p<0.004). Significant relationship was identified between RAS polymorphisms and dyslipidemia in this population. These findings suggest that these polymorphisms could be involved in the pathogenesis of hypertension.

# A88 IMPORTANCE OF THE VAGINAL CONTENT BALANCE STUDY IN ADOLESCENTS IN PRIMARY HEALTH CARE

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Vaginal dysfunction (VD), provoked by an imbalance in microbiota is a high-prevalence pathology that can appear since adolescence and entails a risk factor for sexually transmitted infections. The standardized microscopic study of the balance of vaginal content (BAVACO) allows defining five basic vaginal states (BVS) by the characterization of vaginal microbiota composition and presence or absence of vaginal inflammatory response (VIR). The aim of this work was to determine the frequency of each BVS in adolescents by BAVACO procedure. This study included 40 girls aged 13-19 years  $(17.4 \pm 1.50)$  who attended to primary care center of San Luis city (Hospital A. Luchini), between April-July 2017. Samples of cervico-vaginal smears were analyzed by wet mount, Gram and Giemsa stains. In asymptomatic (A) (N: 19) and symptomatic adolescents (S) (N: 21) BVS I was 52.6% (N:10) and 19% (N:4), respectively. BVS II with VIR was observed in 15.78% (N:3) of A and 28.6% (N:6) of S. BVS IV was detected in 15.78% (N:3) of A and 28.6% (N:6) of S while BVS V was identified in 15.78% (N:3) A and 23.8% (N:5) in S. Candidiasis were observed in 66.6% of BVS II and Trichomonas vaginalis were detected in 37.5% of BVS V woman. A high frecuency of DV is observed in girls, even in the asyntomatic ones, that requires an occupation of the subject. The use of BAVACO methodology, a simple and low cost procedure, is suggested as an diagnostic tool in primary health care.

#### A89

# ELEVATED SERUM PROLACTIN IS ASSOCIATED WITH DECREASED ANTI-MÜLLERIAN HORMONE IN WOMEN OF REPRODUCTIVE AGE

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Female reproductive function decline with age due to the reduction of follicular reserve, for that is necessary to evaluate ovarian sufficiency thought the anti-Müllerian hormone (AMH), a biomarker of ovarian function. The aim of this work was evaluated the relationship between AMH and prolactin (PRL) levels by comparing them in patients who consulted for infertility. A total of 66 women (37.91 ±4.41; 26-43 years), who attended to a private clinical laboratory (February-December, 2016) formed the study subjects. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, PRL and AMH were measured by electrochemiluminescence (Elecsys Roche®). Women with polycystic ovary syndrome were excluded. Median AMH, FSH and PRL levels for all participants were

1.9 ng/mL (CI: 1.26–2.6), 9.03 U/L (CI: 7.45-10.46) and 23.41 ng/mL (CI: 17.25–27.0), respectively. Median AMH values decreased in a manner correlated with advancing age (r= -0.4; p<0.05). Considering FSH cut-off level of 12 U/L, 2 groups were analysed: group 1, FSH<12 U/L (80%; N=32); group 2, FSH>12 U/L (20%; N=8). The 48% of patients with FSH<12 U/L showed AMH<1 ng/ml. Among women with FSH>12 U/L, 87.5% had AMH<1 ng/mL. Concentrations AMH<1 ng/ml were negatively correlated with PRL levels (r=-0.32; p<0.05). There were no differences in estradiol or TSH levels between groups. In women with PRL levels upper reference limit it would be useful to determine AMH. Considering discordant values observed between AMH, FSH and estradiol levels, these hormones would be assayed in parallel to have the greatest likelihood of detecting reduced ovarian reserve.

#### A90

#### PROLIFERATION OF MESENCHYMAL CELLS OF GROWING DENTAL PULP

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Dental stem cells constitute a way to be able to regenerate pathologies of the complex dentino-pulp and periapical and the totality of a lost tooth. The pulp cells represent mesenchymal cells from an adult tissue capable of self-renewal and differentiation into other phenotypes. The pulp cells would be an alternative in the engineering of dental tissues by easy surgical access, sample collection, the preservation of the viability and low morbidity, issues that make them competent to create in vitro. In order to establish the dental pulp cells morphologically was staining routine (hematoxylin-eosin) and inverted microscope Olympus I phase-contrast observations x 100 coupled to a computer (Biotechnology Laboratory FOLP). Also, we use MET by setting glutaraldehide and contrast with uranyl acetate (electron microscopy) with osmium tetroxide. Worked with third parties retained molar (indication - informed consent). The explants were transferred to the Eagle's medium amended by Dulbecco (DMEM), adding SFB (15% and gentamicin (50  $\mu$ g /ml) with collagenase (3%) at 37 ° C, for a week.) Cells with morphology suggestive of post-natal stem cells, which are similar to fibroblasts, were obtained. The same are elongated and flattened in colonies clonogenic. These trials coincide with others before and allow us to infer that the clonogenic colonies constitute an essential characteristic of post-natal stem cells. To validate this type of study the application of different marker proteins is required.

#### A91

#### PRIMARY EXTRAOSSEOUS EWING SARCOMA OF THE LUNG: CASE REPORT AND REVIEW

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Ewing sarcoma, a branch of neuroectodermal tumor (PNET), is the second most common primary osseous malignancy in children and young adults. Most cases of Ewing's sarcoma are reported in the bone, and extraosseous Ewing's sarcoma is an extremely rare disease. Approximately 53 cases of lung affection have been reported so far. In this case report, we describe such a case in a 8-year-old female. The patient was admitted with fever, dry cough, decay and respiratory distress. She was initially treated with antibiotics under suspicion of pneumonia. Radiological investigations were performed including ultrasonography, computed tomography and PET-SCAN and they revealed solid expansive lesion within the left hemitorax and pleural efussion. A biopsy was taken and histopatological features were analized. Immunohistochemical stainings for CD99 and synaptophysin were positive. Translocation t (11, 22) (q24; q12); wich gives rise to the formation of the EWS-FLI 1 fusion gene, was positively tested with RT-PCR. Thus, the histological, immunohistochemical, and molecular findings were compatible with Ewings sarcoma PNET. She was started on chemotherapy but unfortunately the patient died. In addition to the case report we expose a brief resume of the new literature about the molecular cell biology of this neoplasm.

#### A92

# STAGES OF CHRONIC KIDNEY DISEASE IN PATIENTS WITH HYPERTENSION AND TYPE 2 DIABETES. A SITUATIONAL ANALYSIS IN PRIMARY HEALTH CENTRE FROM SAN LUIS, ARGENTINA

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The key risk factors for chronic kidney disease (CKD) are arterial hypertension (AH) and type 2 diabetes (T2DM). Primary health care (PHC) is essential in the early detection and monitoring of CKD. Our objective was estimate the frequency and stage of CKD in patients with T2DM or HT attending at Juana Koslay Hospital (San Luis, city), between December 2015-August 2017. A total of 194 ambulatory patients aged 24-70 years ( $60 \pm 8$  years) were studied; 92 were diabetic (60 males, 32 females) and 102 were hypertensive (52 males, 50 females). Exclusion criteria: body mass index <19 kg/m2 or >35 kg/m2, amputations, liver disease and acute renal failure. Data from the Integrated System of Sanitary Information (SISA) were analyzed: age, sex, plasma creatinine, albumin/creatinine ratio and glomerular filtration rate (eGFR) estimated by Modification of Diet in Renal Disease (MDRD4) equation. Mean eGFR was 65 (range, 44–86) and 54 (range, 44–64) ml/min/1.73m2 in T2DM and AH patients, respectively (p<0.05). The frequency of patients with eGFR <60 ml/min/1.73m2 increased with age in male. Stages AH group: stage 2 (eGFR 89-60) 3.92%, stage 3 (eGFR 59-30) 89.21%, stage 4 (eGFR 29-15) 3.92%. Stages T2DM group: stage 2 (26.1%), stage 3 (53.26%), stage 4 (1.1%). In this group the prevalence of

albuminuria was 54.34%. Since 72% of subjects were classified in stage 3 and only 12.38% in stage 1, CKD screening would probably be suboptimal. CKD must be approached from the PHC, unifying biochemical markers for early diagnosis and management to reduce cardiovascular mortality associated.

#### A93

# GENDER DIFFERENCES IN PHYSICAL, CHEMICAL AND METABOLIC FACTORS INVOLVED IN NEPHROLITHIASIS

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Kidney stones forming require investigations to detect predisposing metabolic abnormalities. Some metabolic causes are hypercalciuria, hypocitraturia, gout, hyperoxaluria, and hyperuricosuria. The aim was to investigate physical, chemical and metabolic abnormalities involved in the risk of lithogenesis in both sex. Observational and cross-sectional retrospective study was undertaken. Total metabolic variables were assessed in the data base, including serum and urine chemistries. 421 patients (20-70 years), 57.5% women (W) and 42.5% men (M), Age (years):  $44.6 \pm 13.8$ (W)  $43.9 \pm 13.9$  (M), weight (Kg):  $64.4 \pm 13.1$ (W)  $85.5 \pm 14.5$  (M), p< 0.0001. BMI:  $24.9 \pm 4.7$ (W)  $28.2 \pm 4.3$  (M), p< 0.0001. Serum uric acid, creatinine, urea (mg/dl) increased significantly in M (p<0.0001) and potassium (mEq/l) p<0.01. Urinary excretion 24 h (mg/dl): phosphorus, uric acid, creatinine, magnesium, sodium (mEq/l), potassium (mM/l) increased significantly in M (p<0.0001). Creatinine clearance:  $111.0 \pm 28.4$  M and  $88.1 \pm 24.0$  W, p<0.001. Calcium/creatinine:  $0.20 \pm 0.09$  W and  $0.13 \pm 0.06$  M, p<0.001. Citrate/creatinine:  $0.51 \pm 0.37$  W and  $0.28 \pm 0.16$  M, p<0.001. Uric acid/creatinine:  $0.43 \pm 0.15$  W and  $0.39 \pm 0.11$  M, p<0.05. Significantly differences in PTH compared W respect to M, p<0.001. No significant differences were found in other parameters studied. The risk of renal stone, forming and some metabolic gender differences were found. The incidence and prevalence of nephrolithiasis were similar in women and men (26%). The results of these investigations can also be used to help guide therapy to prevent future stone formation.

#### A94

## PREVALENCE OF HYPERTENSION AND RISK FACTORS IN PEOPLE ATTENDING TO THE ANNUAL MEETING OF THE BIOLOGY SOCIETY OF CUYO

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Arterial hypertension (HTA) is an important problem of health, main factor of risk of the cardiovascular disease (CVD), constitutes the first cause of death. Industrialized countries show a major trend to sedentarism and obesity, increasing prevalence of HTA and cardiovascular complications. We aimed at evaluating the association of physical, family history and lifestyle risk factors with HTA in the adult population attending to the Annual Meeting of the Biology Society of Cuyo. A structured survey was used. 112 subjects (20-80 years), 33% men (M) and 67% women (W) were tested. Mean age M/W: 40.47±14.14/44.35±13.95, weight M/W (kg): 82.66±17.34/65.57±14.05 (p<0.0001), BMI M/W (kg/m2): 27.48±4.59/24.68±5.42 (p<0.009),waist circumference M/W (cm): 96.31±17.87/86.07±17.02 (p<0.004), mean systolic and diastolic blood pressure M/W (mm Hg): 124.29±13.99/114.72±12.56 (p<0.0006) and 81.11±10.9/72.08±10.87 (p<0.0001). The prevalence of HTA was 26% (9% M ,17%W), unknown 9% (4%W). Diabetes: 13% (8%W), dyslipemia: 6% (4%W), hyperuricemia: 7% (4%W), admission for chest pain: 4% (3%W), IAM: 3% W, IC: 6% (4%W), ACV: 7% (4%W), coronary disease: 7% (4%W). Nicotinism 26% (14%W); alcoholic drinks frequently 8% (4%W), occasionally 75% (48%), never 17% (14%W); physical activity frequently 42% (30%W), occasionally 45%(25%W), never 14% (12%W), dietary fat frequently 24% (13%W), occasionally 57% (38%W), never 20% (17%W), family history of CVD: 57% and 87% know about risk factors. The OMS estimate 30% of HTA in adult population. 26 % in the population evaluated, particularly in women. We found association between hypertension and gender, and with other variables studied. To emphasize need promoting prevention campaigns for hypertension and risk factors involved in cardiovascular diseases.

# A95 PREVALENCE OF HYPERTENSION AND RISK FACTORS ASSOCIATED IN NURSING STUDENTS

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Over recent decades, the prevalence of arterial hypertension (HTA) has increased in young people. The CARMELA study in Buenos Aires reported 29% of prevalence. Several risk factors are involved in the genesis of HTA, and their early identification can prevent the development of that disease. We aimed at determining the risk of hypertension and associated factors in freshman university nursing students from 2014 to 2016. A systematic survey of controlled studies was carried out in 880 adults, 19% men (M) and 81

% women (W)( 2014: 7%M and 23%W, 2015: 6%M and 33%W, 2016: 6%M and 25%W). The variables studied in both genders: age (years)  $23.3\pm5.6$  M and  $24.9\pm6.5$  W; weight (kg):  $75.1\pm17.8$  M,  $65.9\pm14.9$  W, p<0.001; waist circumference (cm):92.4  $\pm12.4$ M, 90.6 $\pm11.9$ W; BMI (kg/m2):  $25.4\pm3.9$ M,  $25.8\pm5.9$ W. Systolic and diastolic blood pressure (SBP/DBP) was measured with the auscultator method (mm Hg), SBP M/W:  $117.4\pm11.0$ /  $110.2\pm10.4$ , p<0.001, DBP M/W: $75.4\pm10.8$ /70.9 $\pm8.8$ , p<0.01. The highest percentage of HTA in 2014: 18% in M and 15% in W. Other risk factors, diabetes:13%M, 17%W; dyslipemia: 5%M, 4%W, IAM: 3%M, 5%W, ACV: 7%M, 3%W; coronary disease: 2%M, 3%W. Family history of hypertension in 2015:40%M, 52%W and other related habits, such as smoking: 26%M, 34%W, consuming alcohol frequently:13%M, 5%W and fat- rich foods 30%M 35%W. Physical exercise: 47%M,

32%W and 98% know about of risk factors. Higher HTA prevalence and DBT in 2014 and decreased in 2015 and 2016. Early identification of younger people with high risk of HTA is relevent to reduce the incidence of cardiovascular disease.

#### A96

# PREVALENCE STUDY BETWEEN PATIENTS WITH PSORIASIS TYPE I-II AND THE ARTERIAL HYPERTENSION

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Psoriasis is an inflammatory, chronic, systemic disease that primarily affects the skin. Its prevalence ranging between 1 and 3% of the general population. There are two clinical forms, according to age-at-onset, type I and type II psoriasis, before and after the age of 40, respectively. Patients with psoriasis have a higher incidence in cardiovascular diseases, including arterial hypertension. The aim of this studied was to evaluate the relationship between patients with psoriasis type I-II and the prevalence of arterial hypertension in each study group. We evaluated 33 patients with psoriasis; the data were collected from the clinical history obtained from a dermatological institute of the Province of San Luis between May and June 2017. The mean age of patients was 58.87 +/- 16.10. The 55% were male and 45% female. The 58% of the patients presented type II psoriasis, while 42%, type I psoriasis. Regarding arterial hypertension, 47.6% of patients with type II psoriasis presented hypertension, whereas patients with type psoriasis I, only 7.14%. In this way it is assumed that patients with type II psoriasis are more likely to have arterial hypertension than patients with type I psoriasis.

#### A97

### POSTURAL CHANGES AND FLEXIBILITY IN ADOLESCENT STUDENTS IN SAN LUIS CITY

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This study aimed to identify and analyse postural changes and flexibility in schoolchildren between 11 and 17 years old in San Luis city, because different studies suggest that postural alterations in global students has been increased. One hundred and six students authorized by their parents, were photographed in the orthostatic position in the front, back and side views and evaluated flexibility back muscles though Schober and toe touch test. Afterward the following items were analysed in photographs en each view: type of posture, lateral deviation, shoulder alignment, head and shoulders position, it implies meticulous analysis of different anatomical structure in each photography. Only 4% had a normal posture. 46% was type back posture, most of them were male, and 38% was type anterior posture, most female (p< 0,0001). 80% had lateral deviations and 84% had shoulder no alignment. 66% had head protraction. 27% had a lower Schober test, and the 36% did not reach the foot with the fingers hands. The results suggest that there is a high prevalence of postural alterations in adolescent students in San Luis city, but the most has a regular flexibility back muscles.

# A98 PREVALENT RISK FACTORS IN POSTMENOPAUSAL WOMEN WITH ALTERED MINERAL BONE DENSITY

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Menopause has a negative effect on bone health because it produces a reduction in the efficiency of calcium absorption in the gut, and loss of calcium increase by urine system. The greatest challenge of this work is to determine the prevalent risk factors in postmenopausal women who provide altered bone density (osteopenia or osteoporosis) conducted a descriptive correlational cross where they were interviewed 200 postmenopausal women, over the age of 45. We use a quali-quantitative food frequency questionnaire, and I search over sun exposure. Data were obtained from the bone mineral density through the T-score of lumbar spine, neck of femur and total hip, according to densitometric criteria (-1 to -2,5 DS as osteopenia and to -2,5 DS as osteoporosis). HOLIG Discovery QDR model 0101549 was used for densitometry. It was established that 80% of women with altered bone mineral density had in common an insufficient intake of Ca, vitamin D and inadequate sun exposure; but when compared with the group with normal DMO, no significant difference could be established between them (p<0.05). This suggests that there are other factors such as: absorption efficiency, calcium fixation, active levels of D vitamin, hormone levels, etc. In addition to the consumption of calcium, vitamin D and sun exposure that

influences the development of osteopenia and osteopenosis. These negative factors can be remedied in part with a consumption of calcium and vitamin D suitable, as well as, doing aerobic exercise of moderate intensity with a proper sun exposure.

### ECOLOGÍA

#### A99

# RICHNESS, ABUNDANCE AND DIVERSITY OF BENTHIC MACROINVERTEBRATES FROM WETLANDS OF THE SOUTH AREA OF LAGUNAS DE GUANACACHE SYSTEM, SAN LUIS, ARGENTINA

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The wetland system Guanacache-Desaguadero-Salinas del Bebedero is part of the RAMSAR site "Lagunas de Guanacache", and its southern part suffers from growing anthropogenic impacts and risk of desertification. The objective of this study was to measure richness, abundance and diversity of benthic macroinvertebrates, in sampling points located in Desaguadero River, and at the lagoon and marshlands of Bebedero, which are included in the south portion of this wetland in San Luis. Samples of macroinvertebrates were collected using a 300 µm D net, in six points, during high water (HW) and low water (LW) periods. Collected samples were fixed in 70% ethanol and analyzed until reaching the highest possible taxonomic level, using a stereoscopic magnifier. Richness, abundance and Shannon-Wielner's (SW) diversity were calculated. A total of 199020 individuals were quantified, and richness varied between 9 and 20 taxa. Most abundant families were *Hydrobiidae* (62.6% both periods) and *Ceratopogonidae* (27.1% HW, 16% LW). Significant differences in abundance were shown between both, sites and periods: site 4 in Desaguadero showed the highest abundance (62238 organisms during LW) and the highest variation between periods, in both, abundance (80% reduction in HW compared to LW) and richness. All the sampling points showed variations in diversity between periods; sites with the highest diversity were Desaguadero during HW (SW=1.98) and Bebedero stream during LW (SW=1.64). These results contribute to the knowledge of this poorly studied ecosystem's fauna, and will allow, along with physical-chemical variables and evaluation of riparian state, an assessment of its environmental quality.

#### A100

# THERMOPHILIC BEHAVIOR DURING POSTPANDRIAL STATE, REGULATED BY THE ENDOGENOUS CYCLE IN THE LIZARD *Liolaemus darwinii*

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The thermophilic behavior during the postpandrial state is common in Squamata order. However, in species with seasonal activity such as lacerides of arid zones, this pattern in relation to food intake will be different throughout the period of activity. The aim of this study was to compare the selected body temperature (Tsel) in two different conditions of ingest in the psammophilus lizard *Liolaemus darwinii*. We captured 30 specimens and divided them in two groups, to receive two simultaneous treatments ("feeding"/"fasting") per 4 days. The Tsel were measured every 30 minutes from 08:00 to 20:00, in a terrarium with linear thermal gradient (25-55°C). Significant differences in Tsel were found in relation to their ingestion state (gl=722)=18.51, p<0.00002), being lower in "feeding" lizards (30.9 $\pm$ 0.2°C) than in "fasting" (32.3 $\pm$ 0.2°C). In both groups, "feeding" (ANCOVA:  $F_{(12, 169)}$ =0.59, p=0.84, Cov.: Body weight) and "fasting" (ANCOVA:  $F_{(12, 182)}$ =1.53, p=0.11, Cov.: Body weight) there were no significant differences in Tsel during their daily activity. These results contradict the optimal thermoregulation models, which predict that the animals will decrease their Tsel when food availability is restricted. This could be due to the fact that the presence of this pattern is associated to its endogenous cycle, and, at this time of the year, lizards are coming out of lethargy. As a consequence, "fasting" lizards would select elevated body temperatures to make their capture of potential preys more efficient. The "feeding" group would select lower temperatures to retain food longer in the digestive tract and to ensure a good digestion.

#### A101

### RELATIONSHIP BETWEEN WATER QUALITY AND AMPHIBIANS IN URBAN RIVERS

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Water contamination has increased in recent years. Species losses are due primarily to habitat destruction and alteration for urban purposes. Many amphibian species are exhibiting population declines and the contaminant concentrations in those areas have a role in these declines. Water quality needs for amphibians are still undetermined. The objectives of this study were: (i) determine water quality parameters and amphibian community metrics in urban rivers and (ii) apply multivariate analysis in order to understand the interrelationships between amphibian community metrics and water quality parameters (Principal Component Analysis) and, to group the

rivers according to its similarities (Cluster Analysis). The study focused on 4 rivers with similar geomorphological and hydrological features: Chorrillos, Potrero, Trapiche and Volcan rivers. The amphibian surveys were performed during the rainy season between 2009 and 2013. The physical-chemical samples were taken at the same time. Results showed a significant difference between community metrics among rivers ( $\alpha$ =0.05). Among all physical-chemical parameters analyzed, phosphate was the most strongly correlated with species richness (r=-0.69; p<0.01) and moderately correlated with relative abundance (r=-0.39; p<0.05). The first two components of the PCA explained the 90%. Cluster Analysis resulted in Cluster 1: Potrero and Trapiche River and Cluster 2: Chorrillos and Volcan River. Cluster 1 represented rivers with lower values in community metrics and higher levels of nutrients, while Cluster 2 were rivers with higher community metrics and lower levels of nutrient load. These results agree with others reported for other areas. Water quality requirements for amphibians are yet to be determined.

#### A102

# NATURAL REGENERATION OF Aspidosperma quebracho-blanco Schltdl IN NATURAL FORESTS OF DRY CHACO (SAN LUIS, ARGENTINA)

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The natural regeneration of *A. quebracho-blanco* in Dry Chaco natural forest is based on two strategies, vegetative and sexual. We evaluated the genetic structure of the population and relatedness between adults and young with the aim to estimate if *A. quebracho-blanco* reproduces from sexual or vegetative strategy. Regeneration was defined as trees with a height equal to or greater than 25 cm and a DAP less than 7 cm distant maximum 10 m from the adult tree. The genetic structure was analyzed by molecular markers RAPDs. Exemplars of *A. quebracho-blanco* from two fragments of *Prosopis flexuosa* forest, located in San Jerónimo (SJ) and Las Chacras (LCH), Dry Chaco, were studied. Regarding the population genetic structure, we observed higher polymorphism in SJ (72.29%) than in LCH (61.45%). AMOVA analysis of the RAPDs pattern evidenced a high intra-population (82%) and lowers inter-population (18%) variation. Three different analysis were performed: UPGMA cluster analysis by using DICE coefficient; principal coordinate analysis; and cluster analysis using Nei's genetic distance. All of them support the presence of two populations genetically different, each of them with subpopulations. The coefficient of genetic differentiation (G<sub>ST</sub>) provides similar results. In SJ population predominates the genetic flow (Nm), while for LCH (Nm=0.51) the genetic drift predominates. Considering the reproductive mechanism, the present results support the hypothesis that the youngs comes from seeds (sexual reproduction), since from the 29 individuals evaluated in the two fragments, at none case the genetic distance was equal to 0 (zero) with the adult individual.

#### A103

#### QUALITY OF RIPARIAN FOREST IN AN URBAN RIVER OF SAN LUIS PROVINCE, ARGENTINA

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Riparian systems are important elements of landscape diversification and a key biotic and structural component for fluvial ecosystems. They characterize for being a transition zone, having functions of depuration and filtration of nutrients and contaminants. The development and application of riparian quality indexes enables to detect, monitor and manage alterations caused by anthropogenic activities. The index of Riparian Forest Quality (RFQ) is a simple and rapid tool to recognize and compare present and reference conditions, regarding composition and dynamics of plant formations. The objective of this study was to establish the quality of river banks of Chorrillos River through the application of the RFQ index. Five sites with different degree of impact were studied. Two parcels (sampling unit) of 50 meters in length and variable width (due to geomorphologic characteristic of riparian areas), located over the first herbal vegetation strip in opposite direction to the river current, were analyzed on each site. An adjustment of RFQ index to the ecoregion and changes observed in the study area was performed before application. For this adaptation and further application the Freshwater Ecology and Management (F.E.M.) protocol was used. RFQ index values varied from 10 to 55 points, and determined surroundings with extreme degradation, with strong alteration and with incipient disruption. RFQ results were integrated to physical-chemical (temperature, pH, conductivity, dissolved oxygen) and other biological data (benthic macroinvertebrates metrics), showing a contamination gradient and ecological degradation on the river. This study represent the first diagnostic using riparian quality index in the area.

#### A104

# WATER-VEGETATION INTERACTION IN THE ARID: INFLUENCE OF Bulnesia retama (ZYGOPHYLLACEAE) ON THE INFILTRATION RATE, IN THE SOUTHERN ZONE OF THE BERMEJOBASIN, SAN JUAN (ARGENTINA)

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The influence of *Bulnesia retama* on the infiltration rate in a pilot site of the basin Bermejo (San Juan) was studied. Infiltration is related to surface runoff, recharge of aquifers and its vulnerability to contamination and is affected by soil texture and structure, organic matter

content, initial moisture, and vegetation cover. The field data were taken by homogeneous units defined on the basis of digital processing of an elevation model and a satellite image visual interpretation. Morphometry on the basin was performed, defining microbasins on a scale of 50,000 cells. Five linear transects of 50 meters were made, measuring coverage with the technique of intersection per line. The infiltration tests were performed inside and outside the canopy, with a simple ring infiltrometer (21 cm diameter) and variable load. The data were adjusted according to the diameter of the infiltrator. Soil samples were taken, determining: organic matter content, texture, total nitrogen, conductivity, and pH. The average coverage was: *B. retama* 34% and bare soil 44%. The rate of infiltration was higher below the canopy of *B. retama* (slope 6.5%) compared to bare soil (4%). The results suggest that the presence of *B. retama* favors the velocity of infiltration in the soil.

#### A105

# IDENTIFYING PRIORITY AREAS FOR CONSERVATION OF Aspidosperma quebracho-blanco Schltdl. IN ARGENTINE DRY CHACO

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Little is known about the population genetic structure of *A. quebracho-blanco*. Know the effects of spatial isolation on genetic diversity and gene flow is very important for providing recommendations for in situ and ex situ conservation efforts. We assessment variability and differentiation genetic in five populations of Dry Chaco: Quebracho de la Legua (QL), Bajo de Véliz (BV), Chancaní (C), INTA La María (SG) and San Jerónimo (SJ) by RAPDs molecular markers. At a population level, percentage of polymorphism (P), varied between 48.9% in SG to 65.4% in QL and SJ. The highest values of Nei's genetic diversity were observed for QL and SJ (0.183 and 0.214 respectively). Shannon-Weaver evidence a 75% and 25% of genetic diversity intra and inter-population respectively. The analysis of RAPD patterns using AMOVA showed that 63% of intrapopulation variability and 37% the interpopulation variability. Genetic differentiation between populations estimated by  $\Phi$  (0.372, p<0.001). According to AMOVA, Gst (0.348) was lower for interpopulation variation, and gene flow among populations was Nm = 0.468 indicate genetic drift. The UPGMA dendrogram suggests that individuals may be grouped in five populations and two subregions. Mantel test (r = 0.311, p = 0.001) indicate a significant relationship between geographic distance and genetic distance. The geographical distribution of populations and their genetic relationships were consistent and most likely due to the natural geographic fragmentation of the *A. quebracho-blanco* forest in Dry Chaco. The populations studied contain an interesting genetic diversity, in special QL and SJ populations are priority conservation areas.

### ETOLOGÍA Y BIODIVERSIDAD

#### A106

#### USE OF GIS FOR OBTAINING MAPS OF ISOSALINITY

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Wetlands are flooded with saline groundwater levels close to the surface. Our objective was to study the salinity of the water table of the "Bajo La Salada" from May 2015 to May 2016 through GIS maps. It was determined the location of the phreatimeter with GPS and probes were extracted water samples. Isoline maps were made of each of the parameters measured in the physiognomy types: conveys something of the halophyte scrub (*Atriplex spp.*), scrubland patches of halophyte crawling (*Sarcocornia neei*), prairies halophytes (*Distichlis spicata*) and saline beach. The ANOVA of pH, electrical conductivity (EC), dry residue (RS) and sodium absorption ratio showed significant differences (p<0.05) to date of sampling and physiognomy types. The same thing happened to the anions and cations, except for CO<sub>3</sub> and Ca<sup>2+</sup>. In the comparison of the maps produced a sharp decline in the EC, RS, anions and cations. This reduction in the parameters of salinity over time would indicate the beginning of the desalination by increased rainfall to be a year with ENSO (El Niño Southern Oscillation). The classification of waters indicates in general, which are sodium sulfate. The most important relationship was found between the Na<sup>+</sup> with the SO<sub>4</sub> whose regression equation has an r<sub>2</sub> of 0.94. It is concluded that: a) there is a good efficiency and usefulness of the maps of isosalinity for the observation of the spatial distribution of the measured parameters; b) groundwater have sodium sulfate characteristics and c) the salinity decreases as the sampling in the period considered.

#### A107

# FLORA OF APICULTURAL INTEREST IN THE IRRIGATION ZONE OF THE SILIPICA DEPARTMENT (SANTIAGO DEL ESTERO, ARGENTINA)

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The Silípica department is located within the Irrigation Area of the Rio Dulce, being the agricultural activity the most important. Beekeeping is also developed in this department; the plant resources present around apiaries include native forest species, crops and weeds. The objective of the work was to determine the flora of apicultural interest present in the irrigated area of the Silípica department.

Semi-structured interviews were conducted with beekeepers; we consulted on the species visited by the bees in their apiaries, the flowering season and the type of contribution (nectar, pollen). In addition the present species were verified and material was collected for the herbarium and to determine. Of the 41 species mentioned, most belong to native flora (63%) compared to exotic plants (37%). There is a notable predominance of herbs (46%) and trees (41%) on shrubs (11.8%) and succulent sub-bushes (2%). In relation to the botanical families 17 were recorded, the best represented were Fabaceae (20%), Asteraceae (15%) and Cucurbitaceae (12%). The most mentioned species was *Medicago sativa* L., *Schinus bumelioides* I.M. Johnst. and *Melilotus albus* Medik. These results indicate that for the beekeepers of this zone the species that play an important role in the development of the apicultural activity belong both to the native flora and to the cultivated plants.

#### A108

# PRELIMINARY STUDY OF THE TARDIGRADES FAUNA OF SANTA ROSA CITY (LA PAMPA, ARGENTINA)

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The distribution of urban tardigrades worldwide is poorly understood. Sites with varying vehicular traffic intensity (high, medium, low) and rural areas near the capital city of the province of La Pampa were sampled. This work is part of a project whose general objective is to identify the tardigrade assemblages present in the rural urban gradient and to relate these assemblages with the conditions that prevail in the anthropized ecosystems. Here we present a preliminary list of species present in sites of medium vehicular traffic of the capital city. The tardigrades were extracted from samples of mosses and / or lichens developed on sidewalk trees. In the laboratory the specimens were anesthetized, fixed and later mounted in polyvinyl lactophenol for later identification. Until now 22 samples were analyzed and 353 individuals and 40 eggs were extracted. The tardigrades collected correspond to heterotardigrades of the family Echiniscidae and eutardígrades of the family Milnesidae, Macrobiotidae and Ramazzottidae. The species richness was represented by: *Echiniscus rufoviridis, Ramazzottius oberhaeuseri, Paramacrobiotus areaolatus, Milnesium sp.* and *Macrobiotus kristenseni*. The number of species of tardigrades per sample was 2 to 3. The species that most frequently appeared was *R. oberhaeuseri* (89), followed by *Milnesium sp* (61) and *M. kristenseni* (53). With these findings it is hoped to contribute to the knowledge of the fauna of urban tardigrades poor studied of the neotropical region.

#### A109

### NEW RECORDS OF EPHEMEROPTERA FOR THE RÍO CONLARA (SAN LUIS, ARGENTINA)

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The order Ephemeroptera is an abundant and diverse group in freshwater ecosystems, in which they are important because they are considered indicators of water quality. Despite its importance, specific information about this group is limited in the province of San Luis, where only 6 species are known. This number is low given the richness described for the central region of the country, so it is necessary to deepen the knowledge of the group. Recently, new citations have been recorded extending the distribution of known species in the northeast of the province, more precisely in the Río Conlara watershed, where macroinvertebrate are generally barely studied. Therefore, the objective of the present work is to continue the inventory of Ephemeroptera of San Luis, presenting new records of the genera Americabaetis, Camelobaetidius, Varipes, Baetodes, Leptohyphes, Meridialaris and Caenis. Specimens were collected in periods of high and low waters between the years 2011 and 2013 in four stations located along the longitudinal gradient, using Surber network with 200 µm of mesh opening. Identification was made through an optical microscope with a magnification of 400x, and with the use of dichotomous keys, it was possible to reach the specific level of *Camelobaetidius penai*, *Varipes singuil*, *Baetodes huaico*, and *Leptohyphes eximius*. The results obtained are an important contribution to the taxonomic knowledge of the group for the province, and for the Río Conlara which in recent years has suffered constant anthropic alterations.

### BIOLOGÍA GENERAL, CELULAR Y MOLECULAR

#### A110

### 5-O-METHYLEMBELIN INDUCES ANTIPROLIFERATIVE EFFECT ON Trypanosoma cruzi

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Chagas' disease is a zoonotic pathology, caused by *Trypanosoma cruzi*. In San Juan Province, decoctions of *Oxalis erythrorhiza* Gillies ex Hooker et Arnott (Oxalidaceae), are recommended for hepatic and heart complains. Its main compound embelin was active against *T. cruzi*, a possible link between the traditional use and the trypanocidal effect was suggested. Additionally, activity against protozoan of its

methylated derivative 50-methylembelin (ME) was assayed. ME showed that is more active against T. cruzi epimastigotes than embelin. To elucidate the mechanism of action of ME, its effect by transmission electron microscopy was evaluated. Results indicate a mitochondrial swelling, in line with alterations in mitochondrial activity, observed by MTT assay. Additionally, an increase in ROS generation was detected with  $H_2DCFDA$  probe. In order to evaluate the influence of ROS in epimastigote proliferation, parasites were incubated in Diamond medium for 3 days in presence of ME or with ME and GSH. The addition of GSH reversed partially the antiproliferative effects. To extend this information to other parasite stages, Vero cells were infected with T. cruzi (48 h) and subsequently, the medium was removed and cells were treated with  $16\mu M$  ME (24h). Afterwards, the cells were fixed and stained with Giemsa to evaluate the infection percentage and intracellular amastigotes. The number of treated cells containing more than 20 amastigotes, compared to untreated cells present significant differences. In conclusion the ability of ME to reduce the parasite load becomes it in a special candidate for the treatment of chronic Chagas disease.

#### A111

#### HIGH FAT DIETS TO OBTAIN AN EXPERIMENTAL MODEL OF OBESITY IN RAT

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Obesity is one of the major risk factors for diabetes, hypertension and atherosclerosis. In order to design a diet on a chronobiological basis for obesity treatment in the future, we first worked on obtaining an animal experimental model of obesity, as a consequence of a high-fat diet exposure. The objective of this work was to investigate the effects of high unsaturated and saturated fat diets on different anthropometric parameters in Holtzman rats. The animals weaned at 21d of age were randomly separated and fed two types of diet for 4 weeks: one group was fed with a normocaloric diet (ND group) and another group with a high unsaturated fat diet (F1D group). From the fifth week, the F1D group received a diet based on high saturated fats (F2D group) for 4 more weeks. Rats were maintained under 12h-light:12h-dark conditions. The anthropometric profile was evaluated according to the following parameters: food intake, body and fat weight, size and body mass index, final weight/initial weight and weight gain/dietary consumption indexes. Food intake, body weight and height were measured daily. Our results show that ND and F1D groups did not present significant differences in any of the studied parameters. However, modification of the F1D to the F2D diet resulted in significant differences in the final weight/initial weight index as well as in the weight gain/dietary consumption index, in comparison to the ND. Thus, a diet based on saturated fats is more effective for the development of obesity in this animal model.

#### A112

# Chlamydia trachomatis MANIPULATES AKT/AS160/RAB14 PATHWAY FOR SPHINGOLIPIDS ACQUISITION FROM INFECTED CELLS

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Chlamydia trachomatis (CT) is an obligate intracellular bacterium that replicates in a bacterial-modified phagosome called inclusion. By hijacking Rab functioning, CT exploits different trafficking pathways to acquire nutrients essential for its survival. We have demonstrated that Rab14 is recruited to the inclusion and drives sphingolipids delivery from the Golgi to the inclusion. In addition, Akt, a Serine/Threonine kinase, phosphorylates AS160 (Akt Substrate 160), a GAP (GTPase Activating Protein) for several Rab proteins, including Rab14. The phosphorylation of AS160 results in the inhibition of its GAP activity, thereby the Rabs remain associated to GTP, thus, in their active state. In this work, we demonstrate that CT, through the manipulation of the Akt/AS160 pathway, ensures the capture of Golgi-derived sphingolipids delivered by Rab14-positive vesicles. The blockage of Akt activation reduces not only Rab14 association with the inclusion but also decreases chlamydial inclusion size in a dose-dependent manner. Moreover, abnormal bacterial forms are likely produced during Akt inhibition. Likewise, bacterial multiplication and infectivity measured by inclusion forming unit analysis is clearly diminished by iAkt treatment and AS160 interference. These data suggest that CT selectively usurps Akt/AS160 pathway to ensure the delivery of sphingolipids necessary for its survival and development.

#### A113

# SPECIFIC CpG METHYLATION OF THE KLF14 GENE PROMOTER COULD BE ASSOCIATED WITH TYPE 2 DIABETES MELLITUS

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Kruppel like factor 14 (KLF14) has been associated with type 2 diabetes mellitus (T2DM). However, the mechanisms by which KLF14 contributes to the disease remain largely unknown. In addition, epigenetic mechanisms, such as changes in DNA methylation patterns, might have a role in the pathophysiology of T2DM. The aim of this study was to compare the epigenetic profile (defined here as the CpG methylation pattern of the KLF14 gene promoter in DNA from peripheral blood) between type 2 diabetic patients and age-matched controls. DNA was treated with sodium bisulfite using EpiTect Bisulfite kit (Qiagen). The bisulfite-modified DNAs were amplified with primers specific to human KLF14 promoter region (designed using Methyl Primer software). The PCR products were sequenced to determine the status of CpG methylation. The sequenced data of each sample was analyzed by Chromas Lite 2.1.1 software. CpG

methylation levels in DNA were obtained for 35 sites covering the region between -409 and -142bp according to the ATG position for the KLF14 gene. Results showed that of 35 analyzed sites, 26 CpGs (73.3%) were methylated, 17 out of the 26 CpGs (65.4%) showed significant differences in CpG methylation values between T2DM and controls (p<0.05), 7(26.9%) and 2(7.7%) out of the 26 CpGs were only methylated in T2DM and controls, respectively. In conclusion, the epigenetic analysis identified differences in specific sites of the KLF14 promoter that could be associated with T2DM. These results could help to highlight about the physiopathology of T2DM in future researches.

### A114 THREE-DIMENSIONAL HOMOLOGY MODELS FOR *Trypanosoma cruzi* RIBOSOMAL STALK P PROTEINS

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Trypanosoma cruzi is the etiologic agent of the Chagas´ disease. This work intends to study the three-dimensional structure of T. cruzi ribosomal complex stalk in the large subunit of the ribosomes. Ribosomal proteins are involved in important metabolic processes and in the development of Chagas´ disease pathology. The stalk is formed by the P proteins: TcP0, TcP1a, TcP1β, TcP2a and TcP2β. TcP0 protein has 34 kDa, TcP1 and TcP2 proteins are smaller with a molecular weight of 10 kDa. The crystallographic structure of T. cruzi P0 and the stalk complex TcP0-TcP1α-TcP1β-TcP2α-TcP2β have not been solved to date. A homology model for TcP0 has been obtained in our laboratory. Additionally, we have made three dimensional homology molecular models for the four small P proteins using the Modeller program. These proteins are formed by three structural domains: an N-terminal α-domain, an inherently unstructured coiled A-rich domain and a C-terminal negatively charged domain. TcP0 is formed by a N-terminal globular domain, an alpha domain, a disordered region and a C-terminal negative tail. Observations of both the molecular electrostatic potential and the hydrophobic surfaces for the P proteins suggest a model for the formation of a pentameric complex. We have explored and identified protein interactions that may be involved in conformational stability. This work represents an important three dimensional characterization for these T. cruzi proteins and provide clues for understand its functional properties.

#### A115

### THE RENIN ANGIOTENSIN SYSTEM IN THE AUDITORY PATHWAY DURING DEVELOPMENT

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The central auditory pathway involves several nuclei located at the midbrain: ventral coclear (VC), locus coeruleus (LC), superior olivary complex (SOC) and the inferior colliculus (IC). In order to evaluate the role of the RAS, we studied Ang II receptor localization at the different nuclei, by means of binding autoradiography, *in situ* hybridization (ISH) and immunofluorescence during brain development. Coronal sections were obtained at the different ages: postnatal days, PND0, PND8, PND15 in a cryostat. Ang II AT₁ receptors were absent at early developmental ages (PND0) at all analyzed nuclei and a low level was observed at the IC at the age PND8. AT₂ was present at all ages in IC, LC, VC. Besides, at PND8, AT₂ receptors were observed by binding autoradiography at the SOC. The IC is a large midbrain structure which serves as an obligatory synaptic station in both the ascending and descending auditory pathways. We further explored Ang II AT₂ receptors at the IC by *in situ* hybridization and immunofluorescence at PND15, since at this age the highest expression level was present. We demonstrated by ISH that AT₂ receptor mRNA localized at the same area recognized by AT₂ antibodies at the IC. Immunofluorescence staining with anti-AT₂ receptor antibody and anti β III—Tubulin antibody evidence the presence of immunoreactive neurons, confirming previous data obtained by binding autoradiography. AT₂ receptors might play a role in organogenesis and the establishment of neuronal circuits of the audition pathway.

#### A116

#### SESQUITERPENE LACTONES INDUCE OXIDATIVE STRESS ON Trypanosoma cruzi

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Chagas disease is a parasitic infection caused by the protozoan *Trypanosoma cruzi(T. cruzi)* that affects about 6 million people in Latin America. Despite its sanitary importance, there are currently only two drugs available for its treatment: benznidazole and nifurtimox, both exhibiting serious adverse effects on patients. To complete its life cycle, *T. cruzi* should alternate between mammalian and invertebrate hosts, and face extreme fluctuations such as oxidative environment. It is known that antioxidant defense system in the trypanosomatids is different from mammalian cells, since the parasites have exclusive molecules and reducing enzymes. Due this, parasite redox pathway is an attractive target for antiparasitic therapies. Our research is focused on the action mechanisms of the natural sesquiterpene lactones (STLs) dehydroleucodine (DhL) and some derivatives such as DC-X-1, DC-X-2, DC-X3 and DC-X4 obtained by chemical substitutions. We have previously described to DhL as a leishmanicida drug by oxidative stress generation. In this work, it is

shown an antiproliferative effect of DhL, and its chemical derivatives on *T. cruzi* epimastigotes. This effect was blocked by 3 mM reduced glutathione, suggesting that compounds are reactive upon intracellular sulfhydryl groups. Moreover, *T. cruzi* overexpressing reducing enzymes, showed a protective effect against these compounds. Consistent with these results, the active STLs induced ROS generation in the wild type parasites, and this effect was at lesser extent in *T. cruzi* overexpressing reducing enzymes. These results indicate that the induction of oxidative stress is, at least, one of the mechanisms of STLs antiparasitic action.

### A117 PREDICTIVE PROKARYOTIC FUNCTIONAL METAGENOMICS APPLIED ON AN ACID MINE DRAINAGE AFFECTED ENVIRONMENT

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In San Luis, Argentina, the acid mine drainage of an abandoned gold mine is released into La Carolina stream. In previous studies, we determined that physicochemical parameters and both eukaryotic and prokaryotic diversity are affected by the presence of this mine in the study area. Likewise, we stablished specific relations between physicochemical parameters and microbial taxonomic groups. The aim of the present study is to determine the prokaryotic functional differences among samples according to the location in the study area. Twelve sediment samples from the mine (7) and from the stream (5), before and after receiving the drainage, were selected to 16S rRNA gene amplicon sequencing (MR DNA, TX, USA) on MiSeq platform (Illumina). PICRUSt software (www.picrust.github.io) was used to predict metagenome functional content from 16S rRNA marker gene using KEGG Orthology (KO) database. Principal Component Analysis was performed to visualize the distribution of functions and samples. This analysis showed that functions such as environmental adaptation, biosynthesis of secondary metabolites, cellular processes and signaling, protein biosynthesis, energy metabolism, cell motility and genetic information processing were present to a greater extent in the samples taken from the south and lateral galleries of the mine, characterized by high heavy metal concentrations and low pH values. Cell communication function was associated to stream and main gallery samples, which were characterized by normal parameters. These results indicate that extreme conditions make to microorganisms to maintain an active metabolism, while cell communication is associated to normal conditions, where it is expected to find stable consortia.

# A118 PARTICIPATION OF HUMAN ADIPOSE TISSUE IN THE REGULATION OF TUMOR PROGRESSION IN KIDNEY CANCER

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We have recently demonstrated that human adipose tissue of tumor kidney [hRAT], showed leptin and versican overexpressed compare to adipose tissue of normal kidney [hRAN]. Meanwhile, adiponectin and perilipin were significantly increased in hRAN. Conditioned media (CMs) of hRAN significantly increased proliferation of tumor [ACHN] and non-tumor [NK-2] renal epithelial cells. Now, we evaluated the expression of receptors (R): adiponectin -1, -2 (AdipoR1/R2) and leptin (ObR) in hRAN vs. hRAT and ACHN vs. HK-2. Additionally, CD44 expression (homing cell adhesion molecule) was assessed in cells. Human renal adipose tissues were obtained from patients with renal cell carcinoma [hRAT, n=6] and kidney donors [hRAN, n=8]. The CMs of hRAN and hRAT were collected 24h post incubation and cells were treated with CMs by 24 h. Protein expression was evaluated in tissue by IHQ, and WB in cells. Statistical differences among experimental conditions were evaluated by one-way ANOVA or t test, with Tukey's post hoc tests. We found AdipoR1 increased and ObR decreased in hRAN vs. hRAT (p<.05). In addition, ACHN and NK-2 incubated with hRAT- showed CD44 and ObR increased vs. hRAN- and control-CMs (p<0.05). Further, AdipoR1 was significantly lower in ACHN incubated with hRAT-CMs compared to hRAN-CMs (p<0.05). And AdipoR1 decreased in hRAT- and hRAN-CMs vs. control-CMs (p<0.001). No significant changes were observed in AdipoR2 between the cell lines with different CMs. In conclusion, changes observed in the adipose microenvironment could be favoring tumor progression and therefore, the tumor stroma should be taken into consideration when dealing with a malignancy.

### THE FLAVONOID SALVIGENIN IS ACTIVE AGAINST Trypanosoma cruzi EPIMASTIGOTES

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Trypanosoma cruzi is the etiological agent of Chagas' disease. In cultures, these parasites cycle between the flagellate epimastigote form, and scarcely they differentiate into the infective trypomastigote. Natural compounds extracted from plants have shown to be effective against the parasite. Among them, flavonoids are an important family of natural compounds largerly studied. In this study, a novel flavone, Salvigenin (SVG), purified from aerial parts of Baccharis scandens, has been evaluated on T. cruzi growth. We used epimastigotes of T cruzi (Dm28c strain) in axenic cultured conditions and treated with different concentrations of the SVG dissolved in DMSO. SVG exhibited an antiproliferative effect on epimastigotes, even at very low concentrations. This effect was irreversible even at short time (1 h) of exposure to the drug. Also, the drug affects the mitochondrial activity of the parasites, as evaluated with MTT. Based on this results and the SVG structure, a possible mechanism of action of the compound would be throughout of reactive oxygen species (ROS). By using the probe 2',7'-Diclocrofluorescein diacetate (H2DCFDA) we measured ROS in parasites and observed that SVG induces increase of ROS production, which was proportional to the drug concentration. We concluded that SVG affects T. cruzi proliferation by multiple mechanisms during the cell cycle and through mitochondrial dysfunction. Further studies should be done to identify the molecular targets of the parasites.

### A120 HOMOLOGY MODELING OF THE CONTACT SURFACE OF TcCPSF30 AND TcFIP1- LIKE PROTEINS

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In trypanosomes transcription is polycistronic and individual mRNAs are generated by a trans-splicing/ polyadenylation coupled reaction. Both, TcCPSF30 and TcFIP1- LIKE are nuclear proteins that are involved in mRNA cleavaje and polyadenylation processes which are essential for cell viability. Previous reports have described the specific amino acids in these proteins that are involved in the protein-protein interaction. Studies carried out in our laboratory have showed that TcCPSF30 and TcFIP1- LIKE are intrinsically disordered proteins (IDPs). These type of proteins are very difficult to crystallize and therefore to be solved by x-ray crystallography. We have modelled by homology the structural molecular contact surface of TcCPSF30 and TcFIP1- LIKE proteins from *Trypanosoma cruzi* using the software MODELLER (9v10). Proteins of known structure that share sequence similarity with TcCPSF30 and TcFIP1- LIKE were chosen from the protein databank (PDB) using BLASTp. Models were validated using the PROCHECK and ERRAT software. The structural elements observed from homology modeling would indicate that the interaction region is ordered and would correspond to alpha helices. This study is the first step to characterize components of the large 3'end-RNA processing complex and may provide new targets for the control of this important group of protozoan pathogens.

# A121 IS GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AN ADEQUATE NORMALIZER GENE DURING CEREBELLAR DEVELOPMENT?

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been referred as a "housekeeping" gene by remains constant under changing cellular conditions. Recently, GAPDH has been considered as a pro-apoptotic agent in cerebellar granule cells. Angiotensin II exerts its physiological effects through binding to two receptor subtypes: AT<sub>1</sub> and AT<sub>2</sub> receptors. AT<sub>2</sub> is located in the Purkinje cells membrane and its expression is highly modulated during cerebellum development. In view of the emerging evidence, the aim of this study was to analyze GAPDH mRNA levels in neonatal cerebellum in control and AT<sub>2</sub> receptor antagonist-treated animals. Mini-osmotic pumps with PD123319 (AT<sub>2</sub> antagonist, 1.0 mg/kg/day) or saline solution were implanted in pregnant Wistar rats during the last week of pregnancy. GAPDH expression was evaluated in cerebellum of both groups by RT-PCR at different postnatal ages: P3, P5, P8 and P15. In control animals, GAPDH expression gradually decreased during cerebellar development (P3 vs P5, P8; P5 vs P8, p<0.001). In PD123319-treated animals, GAPDH expression decreased significantly at P3 (<0.05) and P5 (p<0.01), and then increase at P8 (<0.001) and P15 (<0.05), respect to control. Accordingly, we observed a significantly enlarged external granular layer (EGL) in PD123319-treated P15 pups (base of fissure: <0.001; crown of folia: p<0.01). These results suggest that, despite its wide use, GAPDH would not be always an adequate normalizer. In addition, our findings indicate that AT<sub>2</sub> is involved in granule cell apoptosis mediated by GAPDH, probably due to crosstalk between these cells and Purkinje cells in developing cerebellum.

# DAILY PATTERNS OF APO E ARE MODIFIED BY AN I.C.V. INJECTION OF AMYLOID BETA PEPTIDE IN THE RAT TEMPORAL CORTEX

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Alzheimer's disease (AD) is a devastating disease characterized by loss of synaptic and neural cells in the elderly. Accumulation of the  $\beta$ -amyloid peptide (A $\beta$ ) in the brain is thought to be central to the pathogenesis of AD. ApoE plays a key role in normal and physiological clearance of A $\beta$ , since it facilitates the peptide intra- and extracellular proteolytic degradation. 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 i.c.v. injection of A $\beta$  (1-42) peptide on the 24h rhythms of Apo E, BMAL1, RORa and A $\beta$  in the rat temporal cortex. Four-month-old males Holtzman rats were used in this study. Groups were defined as: control (CO) and A $\beta$ -injected (A $\beta$ ). Rats were maintained under 12h-light:12h-dark conditions before the sacrifice. Apo E, BMAL, RORa, and A $\beta$  protein were analyzed by immunoblotting, in temporal cortex samples isolated every 6 h throughout a 24h period. We found that i.c.v. injection of A $\beta$  (1-42) phase shifted ApoE, BMAL 1, RORa and A $\beta$  daily rhythms in the rat cortex. These findings might constitute, at least in part, molecular and biochemical basis of altered daily beta clearance in AD.

#### A123

#### MORPHOFUNCTIONAL ANALYSIS OF CREB HETEROGENEITY IN THE RAT PINEAL GLAND

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Adaptation to environmental changes is facilitated by the endogenous circadian clock. The pineal gland (PG) via the nocturnal melatonin production is a key effector and regulator of the mammalian circadian timing system. The PG is under sympathetic regulation by local norepinephrine (NE) release from the conary nerves at night. In rat, the NE-dependent phosphorylation of the transcription factor CREB initiates the expression of the aa-nat gene, which encodes for one of the pivotal enzymes in the melatonin synthesis. To challenge the well-accepted concept of pineal homogeneity and to determine if a spatiotemporal dynamism of CREB occurs within the PG, we analyzed and quantified the protein levels at different ZTs (Zeitgeber time; L:D 12:12) in adult naive, ganglionectomized (GCSx) and sham-operated rats. We performed immunohistochemistry (IHC) in PG sections followed by confocal microscopy, and morphometric and statistical analyses. CREB was found in pinealocyte nuclei at ZT6, 10, 14, 18 and 22. Immunoreactive granules of variable size and different nuclear distribution patterns were observed. The fluorescence intensity of CREB varied among the ZTs, with higher values during the night-time (ZT14 and ZT18). Although the nuclear area was significantly higher at ZT14, the relative area occupied by CREB within the pinealocyte nuclei increased at night. The disruption of the circadian circuit by GCSx reduced both the abundance and the area occupied by CREB at ZT14. These findings suggest a NE effect over CREB availability and distribution in the pinealocyte nuclei and therefore over its transcriptional capacity.

#### A124

# STUDY OF CELL ALTERATIONS AND THE INTRACELLULAR CHOLESTEROL PATHWAY IN THE TESTIS OF ADULT RABBITS UNDER ACUTE HYPERCHOLESTEROLEMIA BY FAT DIET. THE REVERSE EFFECT OF OLIVE OIL

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The relationship between hypercholesterolemia and reduced male fertility has been reported previously, but the mechanism involved remains unknown. Olive oil (OO) supplementation improved semen parameters affected by high fat diet. Light and electron microscopy were performed to study cell alterations. Cholesterol pathway regulation was studied by PCR and Western blot of SREBPs (Sterol-Regulatory-Binding-Proteins) and its target molecules. Light microscopy showed lipid vacuoles in liver cells as well as in testis from rabbits under fat diet, corroborated by electron microscopy. Within molecular regulation, we investigated the intracellular pathway of cholesterol in rabbit liver and testis under different diets (control; 0.05% cholesterol and protected by 7% OO). After 3 and 6 months of diet the expression of mRNA encoding SREBP-2 and its target molecules showed the expected behavior in liver. Olive oil recovered the level of the main molecules regulating cholesterol pathway (not reported previously). Instead, in testis both protein and mRNA expression for the same molecules, did not significantly change at 3 and 6 months of diet. These results suggest that the poor semen qualities found in HCR are supported by morphological alterations. However, in short term studies molecular pathway remains intact in testis even though the liver already displays changes.

Mycobacterium tuberculosis MODULATES RAB24 IN MACROPHAGES

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*M. tuberculosis* is a pathogen that causes tuberculosis, a health threat worldwide infection disease that affects a high percentage of Argentinian population. After internalization, M. tuberculosis resides in a phagosome labeled by the GTPase Rab7. A previous publication from our laboratory shows that Rab7 interacts with Rab24, forming a multi-protein complex (Amaya et al., 2016). Rab24 is an atypical protein of the Rab GTPase family which has been described that participates in late endosomal traffic and in autophagosomal maturation. Our present results indicate that M. tuberculosis recruits Rab7, but not Rab24, to the phagosomal membrane. Furthermore, we demonstrate, that the levels of Rab24 in M. tuberculosis infected macrophages were modified compared to control conditions (uninfected cells). These results suggest that M. tuberculosis likely modulates Rab24, probably to avoid lysosomal degradation. Taken together, these results contribute to the understanding of M. tuberculosis survival in macrophages.

#### A126

# NOVEL SYSTEM OF TWO PERPENDICULAR COILS TO GENERATE MAGNETIC FIELDS OF EXTREMELY LOW FREQUENCIES FOR in vitro APPLICATION TO TUMOR CELLS

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The possibility of using extremely low frequency magnetic fields (ELF-MFs) as therapeutic agents for cancer has received increasing attention during the last years. The general approach for *in vitro* studies is to expose cancer cells to different ELF-MFs configurations (intensity, frequency, waveforms, etc.), to found the best condition that reduces cell viability compared to untreated cells used as controls. It is usual in the literature to test only one configuration per experiment. We present here, for the first time, the use of a novel system of coils that screens 96 different field conditions in a single experiment. The coils generate inhomogeneous fields that span throughout a 96-well plate exposing each well to a different field configuration. In the present study, we aimed to measure the spatial distribution of these inhomogeneous fields, and to test their in vitro anti-tumoral effect on the B16 melanoma cell line. We found good agreement between theoretical predictions and the field distribution of our system measured with a tri-axial gaussmeter. TheB16 melanoma cells were exposed to two different ranges of intensities of ELF-MFs, which were modulated according to the forward Thomas-EMF pattern. The time exposure was 6 h/day for 2 days. Then, the cellular viability was measured by MTT assay. In our experimental conditions, stronger ELF-MFs decreased the cellular viability of the melanoma cells. This result agrees with other reports that used one configuration per experiment. We conclude that our system is appropriate to conduct a fast screening of the effects of different ELF-MFs on cellular cultures.

### A127 EXPRESSION OF GENES RELATED TO APOPTOSIS IN THE COLON OF CADMIUM-TREATED RATS

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Cadmium (Cd) is a recognized carcinogen, but information about the changes that induces in intestinal epithelium and molecular response mechanisms involved are missing. The normal murine intestinal epithelium presents differences in the apoptotic response in the diverse regions. In particular, the colon shows greater probability of cellular transformation. Previous results in our laboratory showed a significant increase in Bcl2 expression in the small intestine, whereas TNF $\alpha$ , TGF $\beta$  and Bax showed no difference in expression. The objective of this study was to analyze the apoptotic response of colon to exposure of Cd. Adult male Wistar rats, 180 to 200g body weight, were exposed to CdCl2 (15 ppm) in drinking water. Feeding was *ad libitum*. After 2 months of treatment, the animals were sacrificed and the colon was extracted and stored at -80 ° C for further study. To evaluate the apoptosis, the expression of genes related to the extrinsic pathway, FasL and TNF $\alpha$ , and to the intrinsic pathway, Bax and p53, were measured by RT-PCR. The results obtained showed a tendency to decrease in the expression of p53 (P<0.1) and Bax (P<0.1), without modifications in the other genes. These results suggest that cadmium does not affect the apoptotic response in the colon. However, more studies are necessary to elucidate the role of p53 and Bax pathways in the colon of Cd-treated rats.

# DAILY RHYTHM OF TNFalpha IS MODIFIED IN THE PREFRONTAL CORTEX OF AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of elderly dementia. Recent research has shown that inflammatory mechanisms have a key role in the pathogenesis of AD. TNF- $\alpha$  is a proinflammatory cytokine implicated in the pathogenesis of neurodegenerative diseases, such as AD. Besides cognitive deficit, AD patients show alterations in their circadian rhythms. The objective of this work was to investigate the effects of an i.c.v. injection of agregated  $\beta$ -amyloid (1-42), on temporal patterns of TNF $\alpha$ , BMAL1, RORa, and A $\beta$  protein levels, in the prefrontal cortex of four-month-old males Holtzman rats. Groups were defined as: control (CO) and A $\beta$ -injected (A $\beta$ ). Rats were maintained under 12h-light:12h-dark conditions throughout the entire experimental period. Prefrontal cortex samples were isolated every 4 h during a 24h period. Protein levels were analyzed by Western blots. We found an i.c.v. injection of A $\beta$  (1-42) modified the daily variation of TNF $\alpha$ , BMAL1, RORa, and A $\beta$  protein in the rat prefrontal cortex. A $\beta$  peptide aggregates caused a phase shift specifically, an advance of the rhythm's acrophase (p<0.05). These findings might contribute to the knowledge on the relationship between TNF-mediated neuroinflammation and altered circadian rhythms in the AD.

### A129

### ESTROGEN-MEDIATED MODULATION OF Chamydia trachomatis INFECTION OF ENDOMETRIAL CELLS

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Sexually Transmitted Diseases (STD) are highly prevalent pathologies. There are more than 30 bacteria, viruses and parasites that can cause sexually transmitted infections. Among them, *Chlamydia trachomatis* (CT) is the most frequent bacterial STD causing agent worldwide. It is an obligate intracellular Gram negative bacterium that multiplies in a non-acidic, non degradative, single vacuole called the inclusión. This bacterium avoids degradation in the phagolysosomal pathway, and at the same time, it hijacks the biosynthetic pathway to obtain nutrients from the host cell. CT alters vesicular transport by the sequestration of certain host traffic controllers such as Rab proteins. In addition, chlamydial infections mainly affect women of reproductive age. Thus, sexual hormones may play a role in CT infection course. At present, it is unknown whether estrogen could regulate the expression and/or function of Rab proteins and their effectors in CT-infected cells. Using advanced confocal microscopy and Western blot techniques, we have analyzed whether the expression and/or localization of Rab14 and its interacting protein FIP2 could be affected by sexual hormones in CT-infected cells. Our results show that hormonal microenvironment impacts on chlamydial infection course by modulating Rab functioning. These findings could open new scenarios that justify novel therapeutical options for the treatment of chlamydial infections.

#### A130

# EFFECT OF AN I.C.V. INJECTION OF AGREGATED BETA-AMYLOID (1-42) ON THE CIRCADIAN EXPRESSION OF REV-ERB $\beta$ and ROR $\alpha$ IN THE RAT HIPPOCAMPUS

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Alzheimer disease (AD) is the most frequent form of dementia in the elderly. It is characterized by a progressive cognitive decline and circadian rhythms alterations. At the cellular level, circadian rhythms are generated by two interacting transcription/translation feedback loops, a positive loop constituted by the transcriptional activator BMAL1:CLOCK and a negative loop established by phosphorylated PER-CRY repressor complexes. The REV-ERB/ROR orphan nuclear receptor family regulates the expression of Bmal1 gene and contributes to the robustness of the clock mechanism. The objective of this work was to investigate the effects of an i.c.v. injection of aggregated beta amyloid (1-42) on the circadian patterns of REV-ERB $\beta$  and ROR $\alpha$  expression, as well as on oscillating BMAL1 and A $\beta$  protein levels, throughout a 24 h period, in the rat hippocampus (CICUA:B-263/17). Four-month-old males Holtzman rats were divided into two groups defined as: control (CO) and A $\beta$ -injected (A $\beta$ ) groups (Zhang 2013). Rats were maintained under 12h-light:12h-dark lighting conditions and received water and food *ad libitum*. Hippocampus samples were isolated every 4 h during a 24h. REV-ERB $\beta$  and ROR $\alpha$  mRNA levels were determined by RT-PCR. A $\beta$  and BMAL1 proteins were assessed by immunoblotting. We found that an i.c.v. injection of aggregated A $\beta$ (1-42) increased A $\beta$  peptide content in the rat hippocampus and phase shifted daily rhythms of A $\beta$ , BMAL1, Rev-Erb $\beta$  and Ror $\alpha$  expression. Therefore, elevated levels of A $\beta$  peptides could modify the temporal patterns of clock genes in the hippocampus. These observations would contribute to a better understanding of clock involvement in the pathogenesis of the AD.

### STRUCTURAL STUDY OF THE Trypanosoma cruzi TcADK1 PROTEIN

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The flagellated protozoan parasite *Trypanosoma cruzi* is the etiological agent of Chagas' disease which constitutes a serious health problem in the Americas. This parasite presents a complex life cycle which involves major morphological and gene expression modifications as they encounter different environments. Due to the extremely variable nutrient availability conditions, they need a coordinated regulatory response to maintain ATP homeostasis. Adenylate kinases (ADKs) are key enzymes in that process since they catalyze the interconversion of constituents of the adenine nucleotide pool, duplicating the ATP energetic potential. Most organisms express one to three ADK variants while the presence of an expanded ADK family seems to be a common feature of trypanosomatids. In the case of *T. cruzi*, there were recently characterized seven ADK variants in different subcellular compartments. The aim of this work was to study the structure of the *Trypanosoma cruzi* TcADK1 isoform. We are working on obtaining the protein in high concentration using recombinant DNA technology in *E. coli*, and thus generate crystals of suitable quality for X-ray crystallography diffraction. The purification will be carried out by affinity and size exclusion chromatography. Complementary, we have obtained a three-dimensional homology model of the protein. They will provide information to the structural study of TcADK1. The results will be useful in the rational design of drugs for the treatment of Chagas' disease.

#### A132

# SYNERGISTIC ANTITUMOR ACTIVITY AGAINST THE HER-2 POSITIVE HUMAN BREAST CANCERS CELLS BY COMBINING TRASTUZUMAB WITH RETINOIC ACID

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Breast cancer is classified in molecular subtypes. Tumors overexpressing HER-2 are more aggressive and the patients have a poor prognosis. Therefore, the use of anti-HER-2 monoclonal antibody Trastuzumab (Tz) has been adopted. Unfortunately, some patients are unresponsive to this therapy. Retinoids, mainly retinoic acid (RA), have been suggested as adjuvant treatment of breast carcinoma because of their ability to inhibit cell growth. Moesin and FAK are proteins involved in cell adhesion and migration. We propose to evaluate the effect of a combined therapy (anti-HER-2 plus RA) on the viability, adhesion, migration, invasion and expression of moesin, FAK and HER-2 receptor in human breast cancer cell lines SKBR-3 and BT-474. MTT assays, pharmacological interaction analysis, immunofluorescence, adhesion, migration and invasion assays, and western blot were performed. The coadministration of both drugs synergistically decreased cell proliferation. Moreover, simultaneous administration of the two drugs significantly decreased adhesion, migration and invasion in both cell lines. By immunofluorescence, we determined that Tz+RA induced FAK translocation from the cytoplasm to the nucleus. In addition, a granular distribution of HER-2 receptor was observed after the combined treatments. RA and Tz combined treatments strongly decrease FAK, Moesin and HER-2 expression. In conclusion, the coadministration of both drugs in patients with this type of cancer could contribute to improve their prognosis and reduce the adverse effects of therapy because the Tz doses applied would be lower due to the adjuvant effect of RA.

## A133 GABA<sub>B2</sub> IN THE POSTNATAL RAT CEREBELLUM

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GABA is currently known for its dual action, inhibitory or excitatory, in the developing brain. Two types of GABA receptors mediate these actions: GABA<sub>A</sub>R and GABA<sub>B</sub>R. The functional metabotropic GABA<sub>B</sub>R is composed of two subunits, B1 and B2. GABA binding to B1 triggers the subsequent cellular responses via B2. Cerebellar development starts during late embryogenesis and continues postnatally (P). Previous data from our group showed a postnatal dynamism of GABA<sub>B1</sub> in the rat cerebellum. To define the functionality of GABA<sub>B</sub>R, we studied GABA<sub>B2</sub> in cerebellum at P5, 15 and 90, performing RT-PCR, Western blot (WB) and immunohistochemistry (IHC) followed by confocal microscopy. Several cell type-specific markers were included (calbindin, NeuroD1, S100β, Iba1, GM130, calnexin and actin as internal control). Statistical analysis showed increasing levels in Gabbr2 and GABA<sub>B2</sub> from P5 to P15, and differences in mRNA and protein expression trends at P90. GABA<sub>B2</sub> was widely distributed in the postnatal cerebellum however the developing Purkinje cells (PkC) showed the most dynamic expression pattern in accordance with the process of terminal differentiation and maturation of these cells. GABA<sub>B2</sub> at P5 was mainly cytoplasmic, co-localizing with calnexin and GM130, markers of endoplasmic reticulum and Golgi, respectively. From P15 onwards GABA<sub>B2</sub> was observed as discrete granules on the surface membrane of PkC, at both the soma and dendritic tree levels. GABA<sub>B2</sub> was also present in the granular cell lineage whereas the Begmann glia was negative. Our data suggest that GABA<sub>B</sub>R mediates the postnatal development of the cerebellar cortex in a cell type-specific manner.

# 6 MONTHS OF VITAMIN A DEFICIENCY ALTERS THE TOTAL LIPIDS FATTY ACIDS INDEX IN VIRGIN MAMMARY GLAND

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The mammary gland is unique in its association with a deposit of adipose tissue. Our objective was to determine the levels of saturated (SFA), monounsaturated (MFA) and polyunsaturated fatty acids (PFA),  $\omega$ 3 and  $\omega$ 6 of total lipids; and to analyze the expression levels of PPAR- $\alpha$  mRNAs, and SERBP1c, in virgin mammary glands with vitamin A deficiency (VAD). Virgin female rats of the Wistar strain were separated at weaning into 3 groups: fed with VA sufficient diet for 6 months (A+), VAD diet for 6 months (A-) and VAD diet for 150 days followed by refeding with VA sufficient for 30 days (R). FA was determined by c-GLC-FAMEs. Analysis of the gene expression of SREBP1c, PPAR $\alpha$  was performed by RT-PCR. Data were analyzed by one-way ANOVA, Tukey's post hoc test. It was observed that the PFA and the levels of SREBP-1c did not change with the diet. The level of MFA,  $\omega$ 6 FA,  $\omega$ 3/ $\omega$ 6 ratio, index of unsaturation (IU) and PPAR- $\alpha$  expression increased (P<0.05) and SFA decreased (p<0.05) in A-, respect to the A+; in the R, the SFA,  $\omega$ 6 FA,  $\omega$ 3/ $\omega$ 6 ratio, IU and PPAR- $\alpha$ 6 expression returned to the values of the A+, but not in MFA. The percentage  $\omega$ 3 FA decreases in A-(P<0.05) compared to A+, while R recovered but not reached the value of A+ (P<0.05). VAD could cause these changes in FA and an increase in  $\omega$ 6/ $\omega$ 3, generating an inflammatory state that could increase PPAR- $\alpha$ 6 expression.

### A135 KCTD15 INDUCES GIANT VACUOLES FORMATION IN MAMMALIAN CELLS

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Kctd15 belongs to the potassium channel tetramerization domain (KCTD) proteins that contain bric-a-brac, tramtrak and broad complex (BTB) domain. The BTB domain is a protein-protein interaction motif that is found throughout eukaryotes. Kctd15 homologs are found in different vertebrates, representing a well -conserved family. It has been demonstrated that Kctd15 affect Wnt signaling and AP-2 transcription factor function during embryonic development in zebrafish. The precise molecular mechanism of these processes remains unknown. To better understand the role of Kctd15 we transfected CHO, MEF and HeLa cells with Kctd15-FOS plasmid and analyzed the protein localization by immunofluorescence and confocal microscopy. We observed the formation of giant vacuoles when we overexpressed Kctd15 in mammalian cells. In order to characterize these structures we used endosomal markers, such as Rab5, Rab7, CD63 and lysosomal markers (Lysotraker). Our recent results indicate that these vacuoles do not acquired Rab5, Rab7 neither CD63. In addition, Lysotraker was not observed inside the vacuoles. These results suggest that the vacuoles induced by Kctd15 are not endosomal compartments. However, we did observe accumulation of GFP protein inside the vacuoles, indicating that the membrane of the vacuole may be permeable. We believe that the formation of these giant vacuoles may be involved in the secretory pathway. To confirm our hypothesis we will test Rab27, Arl8, actin and β-catenin as markers to begin with. We consider that the particular phenomenon induced by Kctd15 overexpression might be part of the regulation of some of the signaling pathways it modulates.

### A136 ADIPOSE TISSUE OF HYPERTHYROID RATS REDUCES SURVIVAL AND MIGRATION OF MAMMARY TUMOR CELLS

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We evaluated changes in the viability, adhesion and migration in ex vivo cultures of mammary tumors and in tumoral and non-tumoral mammary epithelial cell lines incubated with conditioned media (CMs) from mammary adipose tissue explants (TAM) of hyperthyroid (Hyper) and euthyroid rats (EUT). Female Sprague-Dawley rats were treated with dimethylbenzathracene (15 mg/rat) at 55 days of age and divided into two groups: Hyper (T4, 0.25 mg/ml/kg injected s.c. daily, n=10) and EUT (untreated control, n=10). At sacrifice, TAM was obtained and the CMs were collected after 24 h of incubation with M199. Ex vivo cultures of mammary tumors from EUT, MCF-10A, MCF-7 and MDA-MB-231 cells were incubated with TAM-CMs of Hyper or EUT rats. Viability, adhesion and migration ability of cells were quantified. Soluble components present in CMs were identified by proteomics. Proteins were separated in polyacrylamide gels. The identification of proteins from complex samples was performed by two-dimensional nano-liquid chromatography (2D-nanoLC)-mass spectrometry (MS/MS) and analyzed with ProteoIQ (Premier Biosoft) software. Ex vivo cultures and tumor and non-tumor cell lines incubated with MCs-TAM-Hyper survived and migrated significantly less than those incubated with MCs-TAM-EUT (p <0.05) rats. MCs-TAM-Hyper had a greater diversity of proteins (at equal total protein) (p<0.05) than MCs-TAM-EUT. The expression of proteins related to anti-metastatic processes, like Lumican; Gelsolin; Netrin-1; fibrinogen  $\beta$ ,  $\alpha$  and  $\gamma$  were augmented in MCs-TAM-Hyper (p<0.05). In conclusion, hyperthyroidism produces changes in the ability of adipose tissue to secrete soluble factors that regulate the survival and migration of normal and tumor mammary cells.

### BIOQUÍMICA

#### A137

# ANDROGEN DEFICIENCY AND BLEOMYCIN TREATMENT DISRUPT THE HOMEOSTASIS OF HEMATOLOGICAL PARAMETERS AND BLOOD PRESSURE

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Influence of sex hormones in modulating health and disease, are increasingly relevant in many clinical areas. Testosterone deficiency (TD) is a relatively common condition and may negatively affect health and quality of life. Additionally, bleomycin an anti-neoplastic agent is used as first-line therapy in many human cancers. The aim was to study the bleomycin-induced pulmonary fibrosis and potential differences in cardiovascular system and blood outcomes. Adult male Wistar rats control and castrated using two different doses, were divided in six groups: 1) control (Co); 2) Co + Ble I (1,5 mg/kg); 3) Co + Ble II (10 mg/kg); 4) Ca (castrated); 5) Ca + Ble I and 6) Ca + Ble II. After 40 days of treatment, rats were sacrificed. Blood pressure (BP) was measured and hematological parameters were quantified. Concerning to BP, there was a gradual and significantly increase during time of treatment between Ca and Ca + Ble II (p<0,001), previously day after slaughter. Hematological and biochemical profile showed an increase in the levels of RBCs, a decrease in WBC, a rise in cholesterol level, glycaemia and triglycerides in Ca + Ble II vs Ca, in all case the difference are significantly (p<0.05). We can conclude that castration and bleomycin treatment combined increase BP and some blood parameters, so that cardiovascular system homeostasis is disrupted during androgen deficiency and Ble treatment. Added to this, the changes produced by Ble are dose dependent.

#### A138

# RELATION OF HEPATIC OXIDATIVE STRESS WITH LIPID LEVELS FROM SERUM AND LIVER IN A VITAMIN A DEFICIENCY MODEL

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Vitamin A (VA) plays an important role in growth and cellular development. Previous works in our laboratory have shown that VA deficiency (VAD) modifies oxidative parameters in liver. The objective of this study was to determine the relation between pro-oxidant and antioxidant genes expression in liver with lipidic levels of hepatic and serum in VAD rats. Methodology: Virgin female rats of the Wistar strain were separated at weaning into 3 groups: fed with VA sufficient diet for 6 months (A+), VA deficient diet for 6 months (A-) and VA deficient diet for 150 days followed by refeeding with VA sufficient for 30 days (R). Serum and liver were extracted for lipid assessment of total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDLcol) and glucose (GL) in serum; and HDLcol in liver (hHDLcol). Expressions of eNOS, iNOS, and (SOD) were measured by RT-PCR. Statistical analyzes: Regression Analysis with Graphpad Prism, p<0.05 was used. Results: A positive correlation was found between eNOS versus TG ( $R^2 = 0.8513$ ; p<0.0031), HDLcol ( $R^2 = 0.6609$ ; p<0.0262) and hHDLcol ( $R^2 = 0.8513$ ; G p<0.0031); between iNOS versus GL ( $R^2 = 0.5487$ ; p<0.0059), CT ( $R^2 = 0.8149$ ; p<0.0001), TG ( $R^2 = 0.7805$ ; p<0.0001), HDLcol ( $R^2 = 0.6559$ ; p<0.0014) and hHDLcol ( $R^2 = 0.5913$ ; p<0.0257); and SOD versus GL ( $R^2 = 0.7938$ ; p<0.003), CT ( $R^2 = 0.8863$ ; p<0.0268), TG ( $R^2 = 0.8891$ ; p<0.0004), HDLcol ( $R^2 = 0.5396$ ; p<0.0379) and hHDLcol ( $R^2 = 0.5935$ ; p<0.0253). We demonstrated the high relation between the hepatic and serum lipid levels and the expression of pro-oxidant and antioxidant genes in a long term VAD model.

### FISIOLOGÍA, PATOLOGÍA Y PRODUCCIÓN VEGETAL

#### A139

# MULTIVARIATE ANALYSIS OF SEED NUTRIENTS OF MAIZE (Zea mays) INDICATE DIFFERENCES BETWEEN MAIZE VARIETIES AND ITS TECHNOLOGIES

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Present work determined the profile of nine elements (Cr, Co, Mo, Ni, Mn, Ca, Mg, P, and Fe) by ICP-AES on digested seeds with HNO<sub>3</sub> in thermostatic water bath at 100°C for 30 minutes. Samples were collect in 27 sites where it was identified 22 maize varieties from six marcs (Don Mario, Dekalb, Syngenta, KWS, Pioneer and DOW) with two technologies, Monsanto and Syngenta. Data was analyzed with Uni and Multivariate statisticals. Univariate statistical indicate significant differences for three elements, P, Ni and Mg. The most interesting result was for P content due that six marcs can be divided in two groups according to P content: DOW, Dekalb and Syngenta contain on average 6700 ppm of P while Don Mario, KWS and Pioneer contain on average 3200 ppm of P. Multivariate analysis was performed using a model with the content of elements Cr, Co, Mo, Ni, and Mn as variables. We consider the technology

from each variety looking for differences between Monsanto and Syngenta technologies. Scores plot show two groups with the expected differentiation and loadings plot indicates that such difference are given by the influence of variables Mn and Co where high contents ( $Co \sim 2.35$  ppm; Mn  $\sim 5.5$  ppm) correspond to Monsanto technology and low contents to Syngenta ( $Co \sim 1$  ppm; Mn  $\sim 4.5$  ppm). Although the present work does not intent to compare physiological quality of two technologies, it was possible to determine differences between them using a multivariate model with the content of micronutrients.

#### A140

### IMPACT OF OXIDATIVE STRESS ON THE SUNFLOWER SEEDLING RESPONSES TO WATER DEFICIT

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One of the most important effects of environmental stresses on plants is overproduction and generating of reactive oxygen species (ROS). Our aim was to evaluate the content of hydrogen peroxide ( $H_2O_2$ ) and the activity of three antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) in shoots and roots of seedlings of two sunflower inbred lines (B59 and B71, respectively sensitive and tolerant to water stress) in response to moderate water stress and subsequent re-watering. The  $H_2O_2$  content was determined according to Sergiev et al. (1997) and SOD by Beauchamp y Fridovich, (1973). CAT activity was measured according to Aebi (1984) and APX activity according to Chen and Asada (1989). In response to water stress, higher content of  $H_2O_2$  were detected in both lines. However, this increase was superior in shoot and roots of B59 seedlings. During re-watering period a decrease in  $H_2O_2$  was observed in shoots of both lines. In response to water stress, CAT activity showed a high level in shoots of B59 respect to B71, SOD activity decreased in shoots and roots of B71 line, and the activity of APX was high in both shoots and roots of B71 tolerant line. In B71 tolerant line, the increase of SOD activity during re-watering would reduce the oxidative stress caused by water stress, which was evidenced as  $H_2O_2$  content decrease. This finding could be used to choose hybrids from B71 line with better performance against water stress conditions in the Pampa Central-West.

### A141 ACCUMULATED PRODUCTION OF TWO MULTI-ANNUAL SORGHUM IN SAN LUIS, ARGENTINA

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Many farmlands in San Luis find it difficult to access technologies such as silage, and require alternatives within their reach. We hypothesized that crops such as Silk sorghum (*Sorghum sp* hybrid cv."Silk") and Black sorghum (*Sorghum x almum* Parodi), with ability to differ in standing, good productivity, persistence, and reduced management costs, constitute a valid option to adress this limitation. The objective was to establish the productivity, the cumulative yield of two multi-annual Sorghum (Black sorghum, S. x almum) accession 'Santa Rosa del Conlara' and Silk sorghum (S. sp hybrid cv. "Silk") accession 'Berrotarán'. The study was carried in 2015-2016 in field plots in the INTA experimental station, near Villa Mercedes. Two treatments were applied: T1 (Black sorghum) and T2 (Silk sorghum). A completely randomized design with 3 replications was used. All plots were fertilized with the equivalent of 60 kg N ha-1 and 50 kg P ha-1 using diammonium phosphate (46% P and 18% N) and urea (46% N). The sample processing included manual separation of the leaf (L) and stem + sheath + inflorescence (T), converting yields to kg DM.ha-1.year-1 for whole plant (PE - includes T + L) and sheet (L). The samples were dried (65 ° C to constant weight). Yields in both treatments were compared. Dry matter production differed among treatments (p <0.05), with values of 7,857 and 11,960 kg DM for T1 and T2, respectively. It is concluded that the sorghum Silk is presented as a great productive alternative for the farmlands.

#### A142

# EVALUATION OF THE GENETIC DIVERSITY OF THE OLIVE COLLECTION (INTA SAN JUAN, ARGENTINA) BY MORPHOLOGICAL AND MICROSATELLITE MARKERS

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The principal problem in plant collections is the correct identification of existing material, as well as that of new entries. The correct identification is crucial to achieve the goals of genetic improvement programs by crossbreeding as well as to identify varieties in nursery plants. The INTA-EEA-San Juan-Argentina, possesses a germplasm bank of 38 olive cultivars. The aim was the morphological and molecular characterization of 12 olive cultivars, destined mainly for oil production, was carried out. Six morphological descriptors and 6 microsatellite markers were used. Total DNA was extracted from young leaves using the GeneElute Plant Genomic DNA Miniprep Kit (SIGMA). Subsequently, amplification was performed by PCR using those microsatellites. The morphological and molecular data obtained were compared with the "OLEA databases" database and that the CNR-IBBR Institute (Perugia, Italy), respectively. The results

(morphological and molecular) indicated a correct identification of the 'Arbequina', 'Blanqueta', 'Empeltre', 'Leccino' and 'Maurino' cultivars. Additionally, it was verified that some cultivars were not well identified, the following correspondences were determinate: 'Grappollo' with 'Leccio del Corno'; 'Manzanilla' with 'Racioppa lucana'; 'Dritta' with 'Frantoio'; and 'Carboncella' with 'Canino'. Finally three cultivars, according to the molecular data, do not correspond to the genotypes of the reference database, while the morphological analysis agrees with the reference data for these cultivars. The knowledge and final evaluation of such genetic diversity will allow an optimal management of the collection, and implementation of future breeding programs in the crop.

## A143 PHARMACOBOTANICAL CHARACTERIZATION OF Annona emarginata (Schltdl.)

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Annona emarginata (Schltdl.) H. Rainer(Annonaceae) is predominantly a shrub or tree (1-5 m tall). It grows in the South America tropical region (eastern Paraguay, extreme northwestern Uruguay, central and southern Brazil and northeastern Argentina). Its vulgar name is "Aratikú" imposed by the aboriginal Guarani, which means "fruit of the sky". It is appreciated for its edible fruits as antiscorbutic. The leaves decoction is use in folkmedicine in gargle against sore throat (tonsillitis) and swallows to quench toothache. The stems bark of is used, along with *Ilex paraguariensis*, in "mate" infusions to treat migraine and mildly sedating. The aim was to carry out the botanical characterization of this species to establish bases for its quality control in pharmacological applications. The leaves, petioles and stems were fixed and preserved in formaldehyde: acetic acid: alcohol (1:1:1), cut and mounted in DPX<sup>TM</sup>. The leaves show a bifacial structure. The epidermal characters are essential to distinguish this species. The hypostomatic leaves presents thick cuticle characteristic of xeromorphic plants; the stomata is paracytic. Indument is performed by scarce eglandular trichomes. In this tissue brownish-yellow glands with large vacuoles are observed, which, when compared to histochemical techniques with Sudan IV, acquire a reddish coloration which reveals the presence of compounds of lipid nature. The primary petiole and stem structure presents collateral vascular bundles, cortical parenchyma with sclerified idioblasts and oils secretors glands.

# A144 RESPONSE OF Jatropha curcas L. TO SALINE STRESS

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J. curcas L. grows in tropical and subtropical regions of the world, and shows tolerance to abiotic and biotic stress. The main importance of J. curcas seeds is their high oil content, suitable as material for biodiesel manufacturing. The aim of this study is to evaluate and measure saline stress tolerance of this species. Plants used in this experiment were germinated in sterile conditions and grown in hydroponic system with vermiculite as growing media and irrigated with Hoagland solution. The plants grew in a chamber at 30°C, 60% of relative humidity and a photoperiod of 16:8 (light: darkness), during 70 days. Saline stress was provided along 4 weeks, through irrigation with nutritive solution with addition of NaCl at concentrations 0 mM (Control), 100 mM, 250 mM and 400 mM, respectively. At the end of saline stress period, physico-chemical parameters were analyzed: roots and aerial length, fresh and dry weight, proline (Bates, 1973) and chlorophyll contents. Results obtained showed that J. curcas wouldn't suffer measurable stress under saline conditions up to NaCl 400mM concentrations. J. curcas is a tolerant species with possibility of bioremediation in salinity soils with 50 dSm.

#### A145

### GERMINATION OF Tetrachne degrei Ness UNDER SALINITY STRESS

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Tetrachne degrei is a native species from South Africa and Pakistan, belong to the family of Poaceas, introduced in Argentina, in the process of domestication. Its small seeds do not have dormancy and germinate under normal conditions. The objective of this study was to study its response to osmotic potentials by irrigation with saline solutions. Seeds were seeded in Petri dishes on wet paper and watered with solutions of 0, 50, 100, 150, 200 and 250 mM NaCl (T0, T1, T2, T3, T4 and T5 respectly) at 25° C under light and dark conditions. Germination energy (GE) was evaluated at three days and germinative power (GP) at the seven days. In the dark the seeds germinated normally and its GE and GP decreased as the salinity levels advanced; in the control (T0) GE was 79% of GE and 85% of GP, in the T4 the levels descended to13%: 42%. The light caused an inhibition in the germination of Tetrachne degrei, observing a significant difference with respect to the control (T0) without light where the GE was 59.6 % and 64.4% of GP. Under light and salt the germination decreased drastically reaching 5% of GE and 18,6 % of GP (T4). In conclusion it can be said that light negatively affects germination and that Tetrachne degrei species does not support salinity above 250 mM NaCl.

#### A-46

# NUTRITIONAL PARAMETERS OF "MATE COCIDO" WITH MILK PREPARED FROM TWO SPECIES OF *Ilex* AND ITS COMMERCIAL MIXTURE, FOR THE SCHOOL AGE POPULATION

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The 1.5% infusion of "yerba mate" (*Ilex paraguariensis*, Aquifoliaceae) (Ip) added with whole milk (50:50 v/v) (M) and refined sugar (S) is the "mate cocido con leche" (MC+M+S), a popular drink in South America and the dietary supplement offered to school children (3-13 y/o) in Argentina. Our aim was to evaluate the nutritional value of partial substitution of Ip by low-caffeined. *Ilex dumosa* Reissek (Id), that presented in mix (Id: Ip 70:30 w/w). The applied methods were HPLC, ICP-OES and IRAM. The ingestion of a double daily serving of MC+M+S provides high energy and carbohydrates, proteins, vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>5</sub>, B<sub>6</sub>, B<sub>12</sub>, biotin and essential minerals (Mn, P, Zn, Cu, Ca, Mg, Na, Cl, K, Fe and Al), with low cholesterol. The Ip MC+M+S contains caffeine in a quantity similar to that of the tea infusion (*Camellia sinensis*) and 1/2 to 1/3 of the coffee. The caffeine content of the Id: Ip mix is 3 times lower than that of Ip and tea, and 10-20 times lower than that of the coffee; the substitution of Ip by the aforementioned mix maintains nutritional values in the MC+M+S and lowers the caffeine levels. This allows a greater consumption throughout the day, without reaching the daily limits of xanthine intake for the age group (40-100mg day), favoring intellectual and physical performance of schoolchildren without harmful effects.

# A147 EFFECT OF EXOGENOUS ABSCISIC ACID APPLICATIONS ON ANTHOCYANIN CONCENTRATION IN PURPLE CARROTS

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Anthocyanins are among the most abundant natural pigments in the plant kingdom. The consumption of these pigments is associated with health-promoting effects, due to their antioxidant and anticancer properties. Purple carrots accumulate anthocyanins in their roots, exhibiting a broad range of pigment concentration, depending mainly on the genotype and growing conditions. The phytohormone abscisic acid (ABA) modulates multiple physiological and biochemical processes of plants. In some species, exogenous application of ABA increased anthocyanin concentration and composition in fruits and leaves. The effect of exogenous ABA on the pigment content in underground organs of the plant has not been evaluated. In this study, we examined the effect of ABA -applied exogenously to the plant foliage- on root anthocyanin content in four carrot cultivars. ABA sprays using four different concentrations [0 (control), 100, 200, and 300 ppm) were applied every 10 days, beginning 65 days after plant emergence. Total anthocyanin content was estimated in the root phloem and xylem, independently, by spectrophotometry. Preliminary results revealed genotype-dependent and tissue-specific increase in anthocyanin concentration for the highest ABA doses applied (200-300 ppm). At these doses, cultivar Ping Ding exhibited a significant (p<0.05) 3.5-fold increase in phloem anthocyanin concentration, relative to ABA-untreated controls, whereas no significant variation was observed in the xylem tissues of this cultivar. Analyses of pigment content and pigment composition (by HPLC) in other carrot cultivars treated with ABA are ongoing. These data will be important for assessing the potential of ABA applications for increasing carrot pigment content and functional value.

# A148 PRELIMINARY LIST OF MACROPHYTES PRESENT AT THE RIO QUINTO NEARBY VILLA MERCEDES ( SAN LUIS)

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Villa Mercedes city is located on the left bank of the Quinto River, approximately 100 km east of the city of San Luis in the Midwest region of Argentina. Upstream of the city the Vulpiani dam was built in 1904 where the water is collected for urban supply. Downstream the reservoir Villa Mercedes was built for recreational purposes, in 2016. The water regime of the river depends on the rainfall, is characterized by a high summer flow and a low winter one. The climate of the area is classified as dry continental. San Luis comprises two well-defined physiographic provinces: the Chaco-Pampean Plain and the Pampean hills. Quinto River is born in the San Luis hills and its endorheic basin covers 34.360 km2. The aim of this work was to investigate the taxonomic identity of the macrophytes growing in the course and riversides Quinto River at the dam and the reservoir in Villa Mercedes. The specimens collected since February of 2016 to September 2017 were identified following the classic botanical methods and incorporated to the VMA Herbarium. As a result of an extensive fieldwork and herbaria study, 54 species belonging to 21 families were documented and, 16 of them, were aquatic and helophyte species. The dam and the recently built reservoir provide the conditions for a generous marsh vegetation of ecological interest.

# PRESENCE OF INCLUDED TREE BARK AND DOMINATED TREES IN A SECTOR OF THE VILLAGE OF VILLA MERCEDES (SAN LUIS, ARGENTINA)

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The objective of this work was to quantify the presence in urban woodland of dominated trees and specimens that have included tree bark, because both problems must be taken into account in a management plan. Dominated trees are those that grow between two larger trees and can't fully develop. The "included bark" take place when the bark is compressed between two codominant stems (weak horqueta) that can cause the fall of one of them in seasons of strong winds. This survey was conducted in twenty urban blocks in a traditional neighborhood of Villa Mercedes, whose wooded areas were planted in the 1980s with selected species. The survey also included the number of trees per block and identification of the species. Of the seven hundred and ten trees surveyed, 7 % are dominated and 5 % have included tree bark. As expected, the dominated trees are in the blocks which present a higher number of specimens. The included tree bark is a defect that can be solved in the nursery, and therefore the good urban trees should not present these problem at all.

#### A150

#### THE EFFECTS OF BIOFERTILIZATION ON THE INITIAL GROWTH OF Glycine max"SOYA"

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Cyanobacterias are photosynthetic microorganisms that promote the development of the soil aggregates, increase the total organic carbon and they are fixers free of nitrogen. Because of this, they are used for biofertilization since ancient times. The aim of this work was to evaluate the effects of biofertilization and inoculation with native edaphic cyanobacteria and Bradyrhizobium japonicum on the initial growth of the soybean crop. Four treatments were evaluated: 1. Consortium of Cyanobacteria, 2. Bradyrhizobium japonicum, 3. Consortium Cyanobacteria / Bradyrhizobium japonicum, 4. Witness. With the algal biomass obtained from the consortium of nitrogen fixers, soybean seeds were inoculated for 48 hours and 5 seeds were seeded in the 4 treatments with 7 replicates. After planting the treatments 1 and 3, they were watered with a solution of cyanobacteria in a concentration of 1x109 CFU. For treatment 2 and 3 the soybean seeds were inoculated with Bradyrhizobium japonicum at a concentration of 1x1010 rhizobia per ml of commercial product and treated with fungicide curasemilla Metalaxil to avoid fungal diseases in seedlings. Once the plants were emerged, they were thinned leaving three plats per pot. Phenological states were recorded according to the Fehr, from VE to V5. The following variables were analyzed: plant height from V1 to V5, and aerial biomass. No differences were detected regarding growth and phenological stages of plants, although it should be taken into account that they are early results.

#### A151

# MORPHO-ANATOMICAL CHARACTERS AND PHOTODYNAMIC POTENTIAL OF ARGENTINIAN SPECIES OF *Bidens*(ASTERACEAE: HELIANTHEAE)

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The study of native plants is essential to correlate the popular use, pharmacological activity and discovery of new photoactive metabolites. Some native species of *Bidens* are useful in popular medicine and are frequently sold in local markets, thus being a promising objects of study. This work aims to find diacritical characters that enable their differentiation and to analyze their photoactivity. Macro- and micrographic parameters, both quantitative and qualitative, were established *for Bidens triplinervia* var. *macrantha*, *B. pilosa*, *B. subalternans* var. subalternans and *B. subalternans* var. *simulans* by applying macro- and micromorphological and taxonomic methods. The extracts were obtained from successive extraction with solvents of different kinds of polarity, and the typical fluorescence of the photosensitizer compounds was subsequently determinated. Both extracts and photosensitizer metabolites were characterized with GC-MS, HPLC and UV-Vis. The foliar anatomy shows an unistrate epidermis, anisositic stomata and multicellular trichomes. The structure is dorsiventral, and the vascular bundle is collateral with angular collenchyma towards both epidermis and a bundle sheath. The stem is tetragonal or hexagonal and costate; it has an unistrate epidermis with thick cuticle and multicellular trichomes with rought walls; the cortex is mainly represented by spongy parenchyma. Each vascular bundle is protected with a sclerenchyma fiber cup and a parenchymous sheath. In the cortical parenchyma, the stem has calcium oxalate druses. The pith tissue is a fundamental parenchyma and it is made of amiliferous large cells. The results revealed that the extracts presents the same spectral regions as the compounds of interest.

### A152 PHARMACOBOTANICAL CHARACTERIZATION OF COMMERCIAL SAMPLES BASED ON "SPIRULINA"

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Dietary supplements are products formulated to improve nutritional intake unsatisfied in the diet of healthy people. In recent decades there has been an increase in its use, thus increasing the number of reports on patients who have suffered harmful effects on their health. World Health Organization (WHO) has established the need to determine quality control for crude vegetable drugs by virtue of the influence of variability factors. The market for these products shows great complexity, which makes it essential to determine their authenticity and confirm their quality. It is proposed to taxonomically characterize commercial samples of "Spirulina" in order to contribute to an effective quality control of these products. The samples were obtained in pharmacy and commercial herbal shop, conditioned, pulverized and observed in Optical Microscope (light / dark field), with interferential phase contrast (DIC-Normansky) and Scanning Electron Microscopy. The sampling procedure was carried out according to the Argentine Pharmacopoeia (2010). From the study of 20 commercial samples that have been able to determine that correspond to both microalgae and cyanobacteria. Sixty % is commercialized as tablets, 25% capsules, and 15% powder. Ninety percent of the samples corresponded to Arthrospira máxima Setchell et N.L. Gardner, 4% to Arthrospira platensis Gomont (Microcoleaceae Family) and 5% to Chlorella vulgaris Beyerinck (Chorellaceae Family) and 1% to a mixture, added or not added with excipients. It is concluded that most of the products exposed very good quality.

#### A153

# EFFECT OF HEAT TREATMENT ON ANTIOXIDANT CAPACITY AND BIOACTIVITY IN AN ADVANCED LINE OF Amaranthus cruentus L.

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The Amaranth grain is a pseudocereal that stands out for the contribution of natural antioxidants. These play an important role in the inhibition of free radicals, preventing or decreasing oxidative deterioration. In this work, total phenols, antioxidant capacity, and some antinutrients were evaluated. We worked with seed flour of the advanced line ACRUs-G10/13 II of Amaranthus cruentus L. A portion was used without treatment (ACRU) and another portion was exposed to 90 ° C for 1 hour (ACRU treated). Total phenols (TF) were determined, as well as antioxidant activity by means of tests of Inhibition% DPPH (%DPPH), Nitric Oxide (%NO), % Inhibition  $\beta$ -carotene, Nitrates, Phytic Acid, Antitriptic activity, and Oxalic acid. The Hemolytic activity by Saponins were determined, too. The results were: TF 16.23-39.23 mg gallic acid/100 g; % of DPPH: 88.34-90.64; % NO: 64.58-66.89; % Inhibition  $\beta$ -carotene: 26.37-23.70; Nitrates: 206.44-316.27 mg/100 g; Hemolytic Activity: not observed; Antitrypsin activity: 3.49-2.97 TIU/mg; Oxalic Acid: 156-144.32 mg oxalic acid/100g; Phytic Acid: 0.36-1.34 mg P/100 g in ACRU, and ACRU treated, respectively. From these results, it follows that the heat treatment did not significantly affect bioactive compounds evaluated. Antinutrients are within acceptable values without risk to human health and this Amaranth line it could be considered a natural source of antioxidants.

#### A154

### BIOACTIVITY AND IN VITRO ANTIOXIDANT ACTIVITY OF Amaranthus hybridus G16/9 SEEDS

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Oxidative stress caused by exposure to environmental pollutants generates the production of reactive oxygen species and reduction in cellular antioxidant systems. Amaranths have relevant nutritional properties and antioxidant capacity. The objective was to evaluate in *Amaranthus hybridus* G16/9 line the content of antioxidant and mineral compounds associated with the defense system, as well as some antinutrients. Total Phenols (TF), antioxidant capacity by DPPH free radical-scavenging assay (% DPPH), Nitric Oxide test (% NO),  $\beta$ -carotene-linoleic acid assay, chemical elements, Oxalates, Nitrates and Trypsin inhibitors (TI) were determined. The results were: TF:  $32.83 \pm 0.05$  mg/100g of gallic acid equivalent; % DPPH:  $89.14 \pm 1.32$ ; % NO:  $79.05 \pm 2.94$  and % Inhibitión  $\beta$ -carotene:  $27.26 \pm 2.48$ . The minerals expressed in  $\mu$ g/g of flour, were as follows: Co: 0.084, Cr: 0.18, Cu: 6.35, Mn: 34.90, Mo: 0.65, Ni: 0.08, Zn: 37.96. Antinutrients, Oxalates: 147 mg oxalic acid/100g; Nitrates:  $209.82 \pm 9.87$  mg/100g and TI:  $2.41 \pm 0.9$  TIU/mg. The results show that *A. hybridus* G16/9 is a good alternative to prevent the pathologies associated with oxidative stress because it has a protective effect against the formation of free radicals, while the antinutrient values are in a range that does not affect health.

## A155 PREDICTION OF THE GROWTH BASE TEMPERATURE OF Digitaria eriantha Steud IN POTS

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Digitaria eriantha is a perennial megatermal pasture of relevance in the cattle systems of San Luis. The knowledge of morphogenesis and the structure of the species expressed in thermal time are important to establish management guidelines that allow optimizing the utilization of the pasture. Determining the growth base temperature of the species is necessary to be able to establish predictive models in relation to the thermal time. Therefore, it was proposed to determine the base temperature of digitaria growth in Villa Mercedes (San Luis) from a regression between the number of leaves and the average daily temperature. Plants (1/pot) were placed in pots (30 liters) and 3 tillers/plant were identified. The study was carried out in a completely randomized design with 4 replicates. During the spring of 2015 the appearance of leaves was recorded twice a week. The average date of appearance of each leaf allowed determining the average temperature that occurred during the period in which it grew. A simple linear regression model was generated between the number of leaves and the average daily temperature. The mathematical model obtained was: y = 0.6732x - 4.722. In addition, it presented a good fit and statistical significance (adjusted R2 = 0.86, for model p <0.0001, statistical coefficients p<0.0001). From the mathematical model the temperature (x) was calculated when the sheets start to appear (y = 0), that is, when the line intersects with the x-axis. The base temperature determined for the year of study was 7.01 °C.

### A156 EFFECT OF FERTILIZATION AND IRRIGATION ON THE PHYLLOCHRON OF PANGOLA-GRASS (Digitaria eriantha, POACEAE) IN POTS

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Knowing the morphogenesis of a forage species and the response to different situations allows to adjust management guidelines that adequate to the growth of the pasture. It was proposed to determinate the changes in the phyllochron of *Digitaria eriantha* Steud. against different levels of irrigation and nitrogen fertilization. One plant/pot was placed and growth stabilization was expected for one year. Next, 3 tillers (subsamples) per plant were marked in a Split-plot design with a 2 x 2 factorial arrangement (combination of irrigation and fertilization, 4 treatments, with 5 replicates) in 30 liters pots. Irrigation: eventual (Re, when the plant had symptoms of wilting), and frequent irrigation (Rf, 2-4 times a week as needed). Fertilization: unfertilized (N0) and equivalent to 200 kg N/Ha (N200) seeking to reach the growth potential. The appearance of leaves was recorded twice a week. The phyllochron was expressed as the leaf appearance interval in days. The ANOVA showed that there was interaction between the factors (fertilization \* irrigation) (p<0.05). RfN200 was significantly lower than ReN0 and RfN0 (p<0.05) (9.03±0.21 vs 10.96±0.63 and 13.72±1.8 days, respectively), while it did not maintain statistical differences with ReN200 (9.35 days). Fertilized plants emitted their leaves faster, with a tendency to increase with greater availability of water. The lower rate of leaf turnover due to irrigation in unfertilized treatments could be associated with nitrogen washing. Fertilization (both Re or Rf) accelerated leaves appearance, which implies a defoliation model according morphogenetic changes following the application of the described technologies.

### A157 DETERMINATION OF ENDOPHYTIC MICROBIAL IN *Jatropha curcas* L. AND *J. macrocarpa* Griseb. SEEDS

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Jatropha curcas and Jatropha macrocarpa are useful for restoring marginal soils and have great adaptability to arid and semi-arid environments. The aim of the work was to determine the endophytic microbial of J. curcas and J. macrocarpa seeds. Seed samples were washed in running tap water to remove adhered epiphytes and the seeds were then surface sterilized. Genomic DNA extractions, PCR amplification of the 16S rRNA gene using specific primers were carried out. The products were detected by electrophoresis. Microbial community analysis was performed using terminal restriction fragment length polymorphism (TRFLP) and denaturing gradient gel electrophoresis (DGGE). TRFLP: marker genes are amplified followed by restriction digestion, only labeled terminal restriction fragments (TRFs) are detected. For analysis of TRFs profiles web-based phylogenetic assignment tool (PAT) was used. DGGE: The gel was made with denaturing gradient in the presence of urea. The bands were compared with known nucleotide sequences contained in the genbank database. The determinations by t-RFLP showed the presence of non-cultivable microorganisms of the genus Streptomyces notorious in both species DGGE analysis indicated the presence of Chthonomonas in J. curcas and Pseudanabaena in both species. These species grow in degraded areas and have high nutritional requirements, hence the importance of native microbes to improve plant growth and nutrition.

# LANDRACES AND COMMERCIAL TOMATO CULTIVARS: HEALTHY PROPERTIES OF FRUITS AND PROCESSING PRODUCTS

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Tomato is one of the vegetables of greater production and importance in feeding world-wide. Its fruits, both fresh and processed, are a valuable source of nutrients and beneficial compounds, mainly antioxidants with positive effects in the prevention of various tumor and cardiovascular pathologies. The objective was to evaluate variability in recently recovered local landraces or "criollos", and commercial cultivars for fruit quality characteristics and their nutraceutical properties. Organoleptic and physicochemical characteristics (pH, acidity, moisture, total and soluble solids) were evaluated in 4 landraces and 2 commercial cultivars for fresh consumption. Additionally, the same characteristics were evaluated in four industrial type cultivars, both in fresh fruits and processed products. Nutraceutical properties were assessed taking in account the main antioxidant compounds: lycopene,  $\beta$ -carotene and polyphenols, and antioxidant capacity, as well as the influence of four processing methods (crushed with skin and seeds, whole peeled tomato, crushed, dehydrated at 70 ° C and marmalade) on antioxidant compounds. The analysis of fruit organoleptic and physico-chemical characteristics revealed the importance of the cultivar, being landraces or "criollos" preferred by their fruit taste and quality. Processing methods affected antioxidants contens: dehydration reduced the amount of lycopene (66-80%) but in whole peeled tomato increased. Dehydration preserved the total polyphenols, while in crushed tomatoes produced a greater reduction. The amount of  $\beta$ -carotene also depends on the cultivar, being significantly elevated in orange fruit cultivars used for the production of a healthy marmalade.

### A159 STUDY OF DRYING TECHNIQUES AS A CONSERVATION METHOD OF PUMPKIN (Cucurbita moschata)

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The work carried out was based on laboratory studies on the application of different drying methods with the purpose of prolonging the life span of the processed samples of pumpkin Anco variety (Cucurbita moschata), minimizing the deterioration of physical, nutritional and organoleptic characteristics. We used favorable drying techniques and production cost accessible to all types of enterprises. The behavior of the samples was analyzed in three presentations: crushed, grated in irregular strands and in pieces of cylindrical form. The drying of the samples was done by two methods: a) Drying by natural convection with hot air at 65°C in stove and weighing of the samples in electronic scale and b) Radiation drying at 65°C in thermobalance. The drying process was carried out up to constant weight of the sample. The behavior of the pumpkin was evaluated and compared under hot air drying conditions. By applying these techniques, a product with a water activity (wa), less than 0.40 corresponding to a value below the minimum required for the growth of microorganisms, was obtained, thus obtaining a microbiologically stable food. A good color conservation of the final product was achieved in the three preparations, with a significant reduction in weight and original volume, valuable attributes for the processing and handling of the product. The drying times were similar for the different methodologies studied. With the data obtained, the drying curves were constructed, which showed a good fit to three proposed mathematical models, Page, Midili and Parabolic.

#### A160

# USE OF BASAL DISKS FOR PROPAGATION SUSTAINABLE OF Habranthus cardenasianus AND Rhodophiala mendocina SPECIES

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Plants are widely used to produce active compounds for pharmaceutical industries in all over the world. Amaryllidaceae have floriculture and pharmaceutical potential. Some have alkaloids used to treat Alzheimer's disease. The aim was know if possible to propagate *H. cardenasianus* and *R. mendocina* species, by the use of basal disks, to allow the sustainable use and preservation of the specie. Natural Bulbs of *H. cardenasianus* (april 2016) and *R. mendocina* (October 2016) were collected. The external cataphiles were removed and the bulbs were disinfected with 0.03% sodium hypochlorite, and then Captan 1g/l. They were cut according to 4 treatments: segments, twin scales, root discs and complete bulb (Control). The bulbs were grown in individual pots with 1: 1: 1: 1 substrate (1 soil, 1 sand, 1 perlite and 1 peat), and placed in a germination chamber for 120 days for alternating temperatures 25°C for 16 hours (light) and 15°C for 8 hours (dark). After 120 days, survival (%S), bulb multiplication rate (TM) and biomass gain (GB) were analyzed. A randomized design with four repetitions per each treatment was applied. The results were different between species. The treatment of root discs for *H. cardenasianus* had the highest %S (64%), TM (3.72 bulbils/bulb) and GB (30.85%). Nevertheless, *R. mendocina* no presented a good performance under any treatment, shown a %S lower than 50%, a TM below 1.5 and negative GM. In conclusion, propagation by basal disc allows a sustainable use and preservation of the species when bioactive metabolites are extracted. (CICITCA UNSJ-PICT2014-3425/BIFRENES-CYTED416RT0511).

# VIABILITY AND IMBIBITION TIME EFFECT ON Rhodophiala mendocina GERMINATION AND MORPHOPHYSIOLOGICAL DORMANCY

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The interest in native ornamental plants promotes prospecting and domestication studies. Amaryllidaceae family multiplication rate is low. Seeds are a good propagation material to preserve genetic diversity. In Argentina, seed propagation of other Amaryllidaceae species (*Zephyranthes mesochloa, Rhodophiala bifida* and *Habranthus tubispathus*) was studied. Previous studies with *R. mendocina* seeds showed that the best conditions to germination were 25-15 °C with imbibition (24 hs). The aim was to evaluate viability and imbibition times effects, in *R. mendocina* (Phil.) Ravenna morphophysiological dormancy. In December 2016, *R. mendocina* seeds (after ripening fruits) were collected of natural populations from Caucete dunes, San Juan, Argentina. Then, they were disinfected (70% ethanol), washed with sterile distilled water, and put on germination paper, and conducted to germination chamber (20 days, alternanting temperatures 25°C/16 hours (light) and 15°C/8 hours (dark). The percentage (% G), velocity (VG), and germination energy (EG) were analyzed. A randomized design with five replicates and two treatments: viability (1, 15, 30 and 45 days after collection), and imbibition time (0, 1, 3, 5 and 7 days) were applied. The results were analyzed by ANOVA and Tuckey test. Significative (p<0.05) difference were presented between treatments. *R. mendocina* seeds had the highest %G (96%), VG (0.48 seeds/day) and EG (70%) in following treatment conditions: on the third day after collected, and three days imbibition. In conclusion is possible improve the viability and remove the morphophysiological dormancy in *R. mendocina* respecting the conditions established herein. (CICITCA UNSJ-PICT2014-3425/BIFRENES-CYTED416RT0511).

### BIOTECNOLOGÍA Y GENÉTICA

#### A162

# DETERMINATION OF THE PERCENTAGE OF WATER EXTRACTION IN VEGETABLE TISSUE SAMPLES SUBJECT TO THE OSMOTIC ACTION OF SUGAR SOLUTIONS AND ITS RELATION TO THE INHIBITION OF GROWTH OF MICROORGANISMS

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The sugar (sucrose) is used in wound healing due to its high osmolarity that generates migration of water and lymph, inhibiting the growth of microorganisms by the decrease in the activity of the water of the substrate. The objective was to determine the osmotic power of sucrose, glucose, fructose and inverted sugar as a function of time. The inverted sugar was obtained by enzymatic hydrolysis of sucrose solutions. The enzyme was isolated in areas obtained by dripping a sodium alginate homogenate and a yeast suspensión of Saccharomyces cerevisiae in a calcium chloride solution. The spheres were placed in a sucrose solution and the angle of rotation of the plane of polarization of the light was recorded with a polarimeter until obtaining an angle of negative rotation, that indicate the hydrolysis completion. And then, different potatoes samples (equal size) were immersed in solutions of sucrose, glucose, fructose and inverted sugar to a same concentration and period of time. The initial and final weights of each sample, as well as the percentage of water extraction in the vegetal tissue were determined. The preliminary results indicate that the highest osmotic effect corresponds to glucose, followed by inverted sugar, fructose, and sucrose. Then, we could infer that glucose and invert sugar would inhibit the growth of microorganisms more than sucrose.

# A163 PEANUT SMUT DISEASE: PERFORMANCE OF Arachis hypogaea L. GENOTYPES

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Peanut smut caused by *Thecaphora frezii* is a fungal and monocyclic disease in peanut crops (*Arachis hypogaea* L.). Cultural and chemical controls are suggested to reduce initial inoculum, without constituting the definitive disease management. Genetic resistance emerges as an alternative tool to decrease the intensity of the disease. The objective of this study was to describe the performance of a collection of peanut genotypes against the disease. The collection constituted by 211 genotypes, owned by Criadero El Carmen, was evaluated in the area of General Cabrera, Córdoba, during the agriculture season 2015/16. The measured characters were mean fruit number in 200 grams (FN) and mean node number (NN). For each genotype were estimated: disease incidence (INC), disease mean severity index in total fruits (SIT), disease mean severity index in affected fruits (SIA) and disease severity index (DSI). A multivariate approach, principal component analysis, was used to describe the relationship between genotypes, characters and between genotypes and characters. The first components of the biplot graph explained 86% of the variation. In this study were observed positive correlations

between FN, INC, SIT, SIA and DSI and negative correlations between NN and FN, INC, SIT, SIA and DSI. Only one genotype did not present peanut smut disease and forty genotypes manifested INC and SIT values above 30% and 20%, respectively. Although the results correspond to a single evaluation environment, these allow identifying promising genotypes to obtain peanut varieties with good behavior against the peanut smut disease.

#### A164

## NOVEL PAPER-BASED METHOD FOR DETERMINATION OF ZINC IN WATER SAMPLES USING SOLID STATE FLUORESCENCE

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Zinc is an essential trace element with great nutritional importance; it is indispensable for the normal growth and the reproduction of humans. In addition, it plays a key role in immune function. The determination of zinc in foods and beverages at trace levels is one of the most difficult and complex analytical tasks. The purpose of this work was to propose an alternative method for the quantitative determination of zinc in water using a paper-based pre-concentration/separation method coupled to detection by solid state fluorescence (SSF). Zinc present in water samples and standards was complexed with a mixture of o-phenanthroline 5.0 x  $10^{-7}$ M and eosin 5 x  $10^{-8}$ M at pH 7.5 in Tris buffer 5.0 x  $10^{-4}$ M. Pieces of Blue Ribbon filter paper (1 cm) were impregnated through contact with each mixture for 1 min (n = 5). Then, the filter papers were dried at room temperature and finally, they were arranged in a conventional quartz cell adapted for solid support. The samples were determined at  $\lambda$ em = 445 nm (emission) and  $\lambda$ ext = 380 nm (excitation). A calibration curve for different Zn(II) concentrations from 1.5 x  $10^{-3}$  to 5.0  $\mu$ g L<sup>-1</sup> was performed by applying the proposed method. The Zn(II) determination was possible with a detection limit of 1.5 x  $10^{-3}\mu$ g L<sup>-1</sup>. The non-sophisticated equipment required, the sensitivity, accuracy and the appropriate detection limit achieved by our method, represent relevant parameters, particularly when the determination of zinc at trace levels is considered.

### A165 PHENOLOGICAL CHARACTERIZATION OF 48 SOYBEAN GENOTYPES IN SAN LUIS PROVINCE (ARGENTINA)

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In order to characterize phenologically the germplasm of soybean corresponding to the program of genetic improvement of the industrial quality of the grain (UNSL-INTA Agreement), 48 genotypes that were planted in the 2016/17 season in the province of San Luis were introduced. The Hill Plot design was used with 3 replicates and the following variables were evaluated: emergence (VE), beginning bloom (R1), beginning pod (R3), full seed (R6), full maturity (R8). For the phenological characterization, the following periods were determined: R1-R3, R3-R6, R1-R8 and VE-R8. An analysis of conglomerates was performed using the Euclidean distance. The resulting dendrogram made it possible to distinguish 5 conglomerates in terms of the duration of their phenological states. One conglomerate (C4) that combines the genotypes with the longest cycle (VE-R8: 148 days average), two that join those of the intermediate cycle (C5 and C3) and the conglomerates C1 and C2 that group the smaller cycle ones (VE-R8: 111 and 117 days average) were observed. The genotypes grouped in C1 have the longest full seed period R3-R6 with an average of 38.5 days. The germplasm characterization allowed to differentiate genotypes based on the duration of their phenological states which will permit the selection according to Maturity Group.

### A166 LED VS. FLUORESCENT LIGHTS: IN VITRO MORPHOGENESIS AND COSTS

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Light is an indispensable source of energy for the growth and development of plants. But, in *in vitro* culture technique, the lighting system is generally the most expensive component of an installation in terms of initial costs, such as current expenses (electricity use and replacement lamps). In the actually, LED lamps are the latest technology to get into these environments. LEDs have technical and operational advantages over current lighting (fluorescent tubes). The present work had as objective to compare the cost, consumption, and effect of the different lighting systems in the micropropagation of *Salvia hispanica* L. Four treatments were performed, three under LED lights: TI: 40% red, 30% blue, 30% white; TII: 60% red, 20% blue, 20% white; TIII: 80% red, 10% blue; 10% white, and TIV: fluorescent tubes. The parameter to be evaluated was the number of shoots, by which the different lighting systems were monitored and weekly evaluated for 90 days. It was measured the electricity consummation and estimated its monthly cost. With this work it was possible to evidence that the treatments TII y TIII achieved higher shoot production by increasing the multiplication rate *in vitro* and decreased energy consumption by 41%, giving a decrease in fixed costs. For this species, the use of LEDs is justified by their profitability in relation to fluorescent lighting.

## A167 ACCLIMATION OF Hedeoma multiflora Benth

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Hedeoma multiflora Benth is a native species that has flavoring and medicinal properties for digestion and rheumatic pains. Because of their multiple uses, their populations are overexploited and in danger of extinction, for that reason it is interesting to implement efficient propagation methodologies to achieve the sustainability of this vegetal resource. The methodology of *in vitro* culture is effective from the culture of nodal segments. In the present work the results obtained in the acclimatization of micropropagated plants are reported. Four different substrates for acclimatization were evaluated: perlite, perlite: fertile soil (1: 1), perlite: peat (1.1) and soil (obtained at sampling sites, Haplustol udorténtico French, mixed, thermal, Cortaderas series). Survival was evaluated at week 1 and at week 5 of acclimatization, on all substrates tested. The average length of shoots and roots of each acclimatized plant was measured, determining the ratio of shoot: root. There were no significant differences in survival at both week 1 and week 5 in the different substrates tested. To determine the effect of the shoot: root relationship on survival, a one-factor ANCOVA was performed, with a p<0.05 at week 1; demonstrating that the relationship shoot: root influences the early stages of acclimatization on survival. The composition of the substrate did not show significant differences for survival. It is expected that this contribution will contribute to the multiplication of chemo-types in programs of genetic improvement, conservation and domestication of native germplasm with added value.

#### A168

### MICRONUCLEUS TEST ON AMPHIBIANS AS INDICATORS OF ENVIRONMENTAL QUALITY

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The micronucleus test is an indirect indicator of chromosomal damage originated during cell division, by fragments that were not incorporated in the nuclei during the cytokinesis. The nucleated red cells of amphibians incorporate or not alterations. Micronucleus frequency is a biomarker that reflects early exposure to a contaminant. Studies in populations inhabiting environments with different types of alterations are useful to know the relationship between organisms, their environment and the health of the environment. The Sierras of Valle Fértil (San Juan) present mineral exploitation quarries such as quartz and calcite in the sources of the rivers that make up La Majadita and surroundings. It is important to monitor populations at sites downstream and compare to sites without influence of these disturbances. This trial is proposed to Pleurodema tucumanum, a species of frog, as environmental quality monitoring and diagnostic model. After the blood extraction were performed two extended for each individual. They were dried in the air, fixed and stained with the coloration of May Grünwald Giemsa. 4000 cells were quantified per individual. To recognize them, the micronucleus diameter and other parameters were taken. Statistical analysis of the data was performed. The specimens observed in the region of La Majadita, lack micronuclei, a situation expected due to the analysis of individuals outside the area of influence of the quarries. The test serves as a bioindicator of healthy environments. It remains to analyze in a second stage, individuals located in areas with mining activity.

## A169 PRETREATMENT OF ALPERUJO FOR BIOGAS PRODUCTION

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Alperujo (AL) is the semi-solid waste from the extraction of olive-oil in a two phase decanter process. AL is a mixture of olive pulp, husk, stone and vegetation water, with high concentration of phenolic compounds (PC) and acidic pH what makes difficult the application of some biological treatments such as the anaerobic digestion (AD) for biogas production. Pretreatment that reduces PC should be applied. The aim of this work was to pretreat AL with  $H_2O_2$  at different pH values, and to assess its effect on PC concentration and biogas production. The oxidation tests were made with AL-  $H_2O_2$  at 0.25 g  $H_2O_2$ /g COD ratio; the initial pH was set at values of 5 and 8, and assays were performed at 25°C for 3 h. The influence of the NaOH addition (for pH setting) on PC concentration and the mode of  $H_2O_2$  dosage (the whole  $H_2O_2$  volume once, or by adding in aliquots at 30 minute intervals) were also determined. The AD was set up in 60 ml syringes containing 20 ml of AL which was inoculated with 10</br>
set up in 60 ml syringes containing 20 ml of AL which was inoculated with 10set/sup>
methanogenic bacteria and incubated at 37°C for 31 days. After the oxidation step, PC depletion was almost negligible; nevertheless, a reduction of PC concentration about 50% was obtained after the AD for both pH values in initial oxidation. Methane production increased 68% as regards to untreated AL. Alkalization produced decreases in PC concentration and the sequential  $H_2O_2$  dosage was not relevant in this case.

# PROMISING PERFORMANCE OF NEW Arachis hypogaea GENOTYPES FOR PEANUT SMUT (Thecaphora frezii)

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Peanut smut disease caused by *Thecaphora frezii* affects the peanut crop (*Arachis hypogaea* L.), turning its grains into a mass of teliospores. At present there are no tolerant commercial cultivars and the entire crop area in Córdoba province exhibits contamination. Considering the genetic resistance as a basic tool for the crops health management, we proceeded to evaluate the performance of new genotypes against the disease. Varieties and advanced lines were evaluated during the crop season 2016/17 under two environments conditions: General Deheza and Las Acequias (Córdoba province). The field trials were planted 11/4 and 11/26, respectively. The genotypes evaluated were provided by FAV-UNRC (National University Rio Cuarto) and INTA Ascasubi. In addition, a susceptible variety from a Seed company was used. The experimental trials were a randomized complete blocks design with three replicates. The genotypes were evaluated in three-row plots (6 m long and 0.70 m apart). The disease incidence and final severity of peanut smut were measured in the middle row in each plot. The results were analyzed using ANAVA and the comparison of means of LSD ( $\alpha$ =0.05). Significant differences were observed between genotypes in both environments, for the two variables. The resistant genotypes shown incidence values from 0.7 to 1,8 %, under conditions of low and high inoculum density, respectively. FAVar-2 of UNRC and Ascasubi Hispano of INTA are varieties that stand out against peanut smut, as well as some lines that, in addition, show aptitude to become new varieties, or constitute source of genetic resistance.

#### A171

### USE OF CELL-FREE SUPERNATANTS FOR INHIBITION OF WINE SPOILAGE YEASTS

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The transformation of grapes into wine results from the metabolism and the sequential development of several species and strains of yeasts. *Dekkera sp.* and *Zygosaccharomyces sp.* present on grapes, are responsible of spoilage in wines by production of acetic acid, volatile phenols, turbidity and CO<sub>2</sub>, with important economic losses. Traditionally, enologists have used SO<sub>2</sub> as antiseptic, which nowadays is considered a potential toxic to human health. The use of killer yeasts (KY) has been reported to reduce/eliminate spoilage yeasts. Nevertheless, there are risks due to introduction of foreign microbiota into the must. We propose to use cell-free supernatants derived from KY to control wine spoilage yeasts. Assays were done using *Wickerhamomyces anomalus* (BWa156), a toxin-producing KY, and two wine spoilage yeasts, *Zygosaccharomyces rouxii* (BZr6) and *Dekkera bruxellensis* (BDb20). The toxin was produced in a parallel bioreactor and added in concentrated (20X) and unconcentrated forms at two volumes: 1/5 and 1/6 over BZr6 and BDb20 liquid cultures initially containing 1\*10<sup>6</sup> cell/mL, in a microtiter plate (300 μL) (n=3). Unconcentrated toxin had no effect over BZr6; nevertheless, the 20X form restricted the beginning of the exponential growth phase for 24h and 72h at increased toxin volumes. Over BDb20, the toxin delayed the beginning of exponential growth phase for 24h and 72h by increasing toxin volumes, and 20X toxin avoided the BDb20 development in the time period evaluated. The toxin elaborated in a parallel bioreactor to the fermentation was successful in the control of spoilage yeasts which matched results reported in bibliography.

### A172

# DOPC LIPOSOMES AS POTENTIAL LABELS FOR GENETICALLY MODIFIED ORGANISMS DETECTION ASSAY DEVELOPMENT

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Genetically modified organisms (GMO) detection has acquired great interest in food safety studies. In this regard, immunosenors, based on antigen-antibody interaction, have aroused interest due to their specificity and low detection limit. To improve sensitivity, liposomes are being used as labels because of their ability to encapsulate different molecules, allowing to amplified detection signals. In this work, we studied the 1,2-di-oleoyl-sn-glycero-3-phosphatidylcholine (DOPC) vesicles ability to encapsulate potassium ferrocyanide  $(K_4Fe(CN)_6)$  to be used as labels in GMO immunosensors development. Vesicles of a size of  $193.2 \pm 0.75$  nm were obtained.  $K_4Fe(CN)_6$  was released for electrochemical detection by adding Triton X-100. The average electrical charge from different vesicles aliquots was  $2.79 \pm 0.1 \times 10^{-6}$  C, which demonstrated that all vesicles incorporated approximately the same molecules number. On the other hand, the leakage percent was determined to be 25% after storage for 30 days. In order to increase stability, the vesicles lyophilization was probed, using cholesterol and disaccharides as lyoprotectants. Transmission electron microscopy images did not show structural differences between lyophilized and non-liophylized vesicles. In addition, lyophilized vesicles maintained the electrochemical response, these results suggest that lyophilization could be used for increasing vesicles stability in time. A calibration curve was performed using differents concentrations of these vesicles. The peaks currents obtained showed linear dependence (R=0.992) with the increase of the vesicles concentration. On the other hand, the vesicles were successfully attached to proteins. According to these results, DOPC vesicles could be utilized as signal amplifiers in electrochemical immunosensors for GMO detection in food.

### A173 CATANIONIC UNILAMELLAR SYSTEM AS POTENTIAL INSULIN NANOCARRIER

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Catanionic surfactants arise from binding anionic and cationic surfactants. They have the ability to form unilamellar vesicles spontaneously, which is advantageous in drug vehiculization. Previously we have demonstrated the biocompatibility of the vesicles either *in vitro* or *in vivo*. The goal of this work was to evaluate the vesicle stability in different media, its ability to encapsulate insulin and the efectivity of vesicle with insuline in the mouse. Catanionic vesicles were exposed to different pH and solutions: physiological solution, albumin, cultive media (DMEM) and PBS. Vesicles were prepared with a  $5 \times 10^{-5}$  M insuline and were administered via intraperitoneal or sucutaneous to BALB-C mouses. Vesicles maintained their size and polydispersity at pH =7, pH 8 and in albumin and DMEM solutions. At pH = 2 were stable for 90 min. In physiological solution and PBS, an increase in size, but equal polydispersity was observed. Catanionic vesicles at 2 and 5 mg/ml encapsulated about 55% and 73% of insulin respectively. An increase in size, thickness, roughness and a decrease in electronegativity, with a low polydispersity of the V-In surface were observed, demonstrated the insulin incorporation. Electron microscopy showed that V-In maintained the morphology and integrity at pH 2 and pH 8. Insuline incorporation inside to vesicle was detected. At 30 min of intraperitoneal or subcutaneous administration a glycemia decrease of 49% and 30% respectively was found. These results show that this catanionic vesicle is stable in biological media, has a high efficiency, resistance and stability as a vehicle of insulin.

#### A174

# OPTIMIZATION OF THE PRODUCTION OF LACCASES AND LIGNIN PEROXIDASES, IN SOLID STATE FERMENTATION FROM "ALPERUJO"

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In San Juan city the alperujo is a very abundant agroindustrial waste coming from the industrialization of olives. The elimination of this residue is very difficult due to the presence of polyphenols mainly. In many places that produce it, efforts are being made to find ways to decontaminate or revalue this waste. One way is to digest it, using solid-state fermentation processes, an environmentally friendly technology. In previous studies, filamentous fungi capable of growing and degrading this residue were isolated from the alperujo. Using statistical Plackett Burman method were found the relevant variables for the production of laccases and lignin peroxidases by action of a fungi previouly isolated, called LA2. In this work, using optimization methods we determined the optimum conditions for obtaining these enzymes, as a contribution to the possibility of revaluation of the alperujo; using an experimental design with Box Benkhen. The variables tested were initial moisture, temperature and agitation. The optimum values were: initial moisture, 70%, temperature, 34°C and agitation twice a day. These values were satisfactorily validated. We can conclude that it is feasible to obtain the enzymes, thus achieving the valorization of a solid residue of the olive industry, through an ecological technology.

#### A175

# APPLICATION OF AN ENZYMATIC COMPLEX, OBTAINED BY FERMENTATION IN SOLID STATE OF GRAPE POMACE, IN MACERATION MALBEC WINE

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The wine is a traditional drink of the gastronomy of Argentina and the province of San Juan is the second argentinian producer. During its elaboration the grape pomace (GP) is obtained, an abundant agroindustrial residue. Their safe disposal and/or revaluation become a problem. In previous works, a complex of hydrolytic enzymes (CE) was obtained from the GP by solid state fermentation. The objective of the present work is to evaluate its applicability in maceration processes of grape musts of the variety Malbec for vinification. Two treatments were done in parallel: one with the CE and the other with a commercial enzymatic preparation (CEP). As a control, wort was used without addition of enzymes. The test had duration of 4 days of maceration and 4 days of fermentation, daily samples were taken for the different determinations. The parameters analyzed were: color intensity and hue, total anthocyanins, total polyphenols index and total tannins. The results show that, at 2 days, 86% more color was extracted than the control. Also, the application of CE was effective to provide a higher phenolic content (by the fifth day 25%). These experiments reflect that a similar behavior is obtained if the CE is used or if a CEP is used. This will reduce the costs and logistics associated with the import of inputs, valuing the solid waste of the wine industry, through the use of an environmentally friendly technology.

### A176 MULTIENVIRONMENTAL YIELD FROM AMARANTH GENOTYPES

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Amaranth requires respectable grain yields and high quality levels of the grain to be economically competitive with other grain crops. The aim was carried out to evaluate the genotype-environment interaction of different materials of grain amaranth (*Amaranthus sp*) to identify the best genotypes for yield. Eighteen genotypes were evaluated in three environments (sowing dates) in Río Cuarto-Córdoba, during the 2016/2017 cropping season, using a randomized complete block design with three replications. Combined variance analysis showed that grain yield was significantly affected by environments (E), genotypes (G) and GE interactions. The first two principal components (PC1 and PC2) were used to create a two-dimensional GGE biplot and explained 57.2 and 27.7% GE interaction squares sums, respectively. The biplot GGE showed two groups of environments in Río Cuarto. Between test environments, the first sowing date is included in environment one (E1), while the remaining two sowing dates were included in environment two (E2). The GGE biplot also identified that Candil and H20a were the best genotypes in E1, whereas H21II and H17a were identified as high yielding genotypes in E2. According to the average environment coordinate (AEC) of the GGE-biplot, H17a was identify as the genotype with most stability and yield, while 7/L9 was identified as the low yielding genotype and the H30I as the genotype with worst stability. Taking into account the representativeness and discriminative capacity of the environments, the later sowing date was the most representative and the earliest sowing date the most discriminating, with respect to the mean environment.

### A177 LYSOZYME SEPARATION USING AFFINITY CHROMATOGRAPHY

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Protein purification is an essential process in scientific research. Considering its final application these biomolecules need to be isolated and purified. The most effective affinity purification technique has been affinity chromatography, which combines conventional column chromatography with affinity interactions. The aim of this project is to develop suitable methodologies for technological innovation in the field of protein purification performing affinity chromatography based on the use of macroligand Cell-Cibacron immobilized in solid gels. Affinity macroligand was prepared from yeast cells modified by chemicals and with the Cibacron Blue F3GA ligand molecule immobilized to the wall cell by covalent bond. An fixed-bed column system with immobilized agarose-macroligand cubes was prepared. Lysozyme (Lys) adsorption from hen egg white was studied. The degree of recovery and purity of Lys was analyzed. Supernatants from each adsorption sample as elution were analyzed by SDS-PAGE. Protein concentration was determined by spectrophotometry. Lys was purified with high purity (more than 90%) with the affinity chromatography column system using agarose-macroligand cubes. Using this model, we obtained 190 mg of Lys/g of adsorbent. It is an easily reproducible system, which also demonstrated an adequate sample processing speed. There was no occlusion or blockage of the affinity column during the separation process.

# A178 DEVELOPMENT OF AN AFFINITY TECHNOLOGY FOR THE ISOLATION AND PURIFICATION OF PROTEINS

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Adsorption Isotherm represents an experimental methodology that describes the distribution of the adsorbed molecules between the solid and liquid phases system in equilibrium at constant temperature. The objective of this work is the study of the adsorption parameters and characterization of Lysozyme (Lys) adsorption to agarose-macroligand adsorbent to be applied in separative systems such as the chromatographic column. Affinity macroligand was prepared using yeast cells that were modified by chemicals and the Cibacron Blue ligand molecule was immobilized to the wall cell by covalent bond. The amount of ligand immobilized on the wall cell was determined by spectrophotometric method. Agarose-macroligand cubes were prepared suspending the Cell-Cibacron macroligand in 4% agarose and 2x2x2 mm cubes. Adsorption experiments were carried out in batch. The adsorbent was characterized by determination of the dissociation constant ( $K_d$ ) and adsorption capacity ( $q_m$ ) by means of adsorption isotherms (Langmuir) using Lys as target protein. Results indicate that the macroligand have high capacity of immobilization of ligand (240  $\mu$ mol ligand/g cell). Values of adsorption such as  $K_d = 6,18x10^{-6}M$  and  $q_m = 88,5$  mg/ml adsorbent were calculated by linear transformation of the Langmuir equation. To determine the time required to reach equilibrium of Lys on the agarose-macroligand cubes, a kinetic study of the adsorption was carried out. The adsorption equilibrium was research at 2h with continuous stirring. According to these results, we propose to improve the separative process of Lys using the adsorbent agarose-macroligand in chromatographic column system.

# PHENOLIC COMPOUNDS DEGRADATION IN SOLID-STATE FERMENTATIONS OF ALPERUJO AT PILOT SCALE

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During Olive-Oil extraction by two-phase process, a by-product called "Alperujo" (AL) is obtained. AL is composed by olive pulp, husk, stone and vegetation water. The AL contains, among other organic substances, phenolic compounds (PC's) often reported as responsible of the AL toxicity. Biological treatments, such as co-composting and solid-state fermentation (SSF), have been proposed for AL detoxification. In a previous work, the PC's depletion during a fungal SSF of AL in a static lab-scale bioreactor. The present work reports a comparison of PC's degradation during a SSF and a Co-composting process, both using AL as main substrate. The SSF was carried out in a rotary-drum bioreactor (400-liter capacity) containing 50 Kg of substrate (AL-grape marc, pH=5.0 and water content 63%), inoculated with *Aspergillus sp* M9-IBT (IBT-UNSJ Collection) (1\*10^7 conidia/gram), during 30 days. Mixing (2 minutes each 12h) and temperature sensing were set. The Co-Composting was carried out in pyramidal piles (1x1.5x6 m³) containing AL and olive leaf, making periodicals mixing and water restitutions, during 150 days. PC's degradation at bioreactor was faster and larger than in co-composting piles (65% up to day 10 vs 15% for the same period in piles). PC's degradation stabilized since day 10 at bioreactor and near day 120 in piles). Enzymes activities Lacasse (Lac) and Lignin-Peroxidase (LiP) found in both fermentations were related with PC's degradation. In bioreactor, maximum Lac (4.182 U/g) and LiP (3.5 U/g) were found at 10 and 14 day, respectively. In piles, maximum activities were found between days 60 and 90.

### A180

# VARIABILITY EVALUATION OF SOYBEAN GENOTYPES FOR PHENOLOGICAL AND YIELD CHARACTERS IN SAN LUIS PROVINCE (ARGENTINA)

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In the framework of the technological linkage agreement between the Universidad Nacional de San Luis (UNSL) and the Instituto Nacional de Tecnología Agropecuaria (INTA) for the development of soybean genotypes with nutritional and industrial quality, germplasm was evaluated. With the objective of exploring the phenotypic variability of phenological and yield characters, 52 genotypes were sown in the province of San Luis in a hill plot design with three replicates during the 2016/17 crop season. The following variables were registered: emergence (VE), beginning bloom (R1), beginning pod (R3), full seed (R6), full maturity (R8), number of nodes per plant (NN), number of pods per plant (NV) and weight of 100 seeds (PS). R1-R3, R3-R6, R1-R8 and VE-R8 phenological periods were determined. An analysis of main components was performed. The results visualized at biplot graph explain 60.5% of the total variability. At the CP1 the 109 and 94 genotypes differ from 151 genotype. The first two genotypes were associated with R3-R6 and PS variables, positively correlated and the second genotype was associated with VE-R1 and VE-R8, also both positively correlated. The NV variable was negatively associated with PS. Likewise, the VE-R1 and VE-R8 variables showed negative correlation with R3-R6 and PS. At the CP2, they separated NN and NV variables, positively correlated and associated with the 1 genotype. The germplasm evaluation allowed to differentiate genotypes from their phenotypic variability for phenological and yield characteristics.

#### A181

# ANALYSIS OF GENOTYPE-BY-ENVIRONMENT INTERACTION FOR DISEASE SEVERITY INDEX OF MAL DE RÍO CUARTO VIRUS IN MAIZE INBRED LINES BY USING THE AMMI MODEL

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Plant germplasm collections are a valuable resource in genetic plant improvement. The germplasm that will be utilized for association mapping should have the greatest possible phenotypic variation. The objective of this work was determining the genotype-by-environment interaction (GEI) in maize inbred lines evaluated for disease severity index to Mal de Río Cuarto Virus (MRCV). A diverse population of 291 maize inbred lines developed and provided by the International Maize and Wheat Improvement Center (CIMMYT) were evaluated. Multienvironment trials included two years and three locations in province of Córdoba where the MRCV is endemic. Environment combinations were Chaján 15/16 (E1), Chaján 15/16 (E2), Chaján 16/17 (E3), Río Cuarto 15/16 (E4), Río Cuarto 16/17 (E5), Rodeo Viejo 16/17 (E6) and Rodeo Viejo 16/17 (E7). In Chaján 15/16 and Rodeo Viejo 16/17 the sowing dates were two. For each maize inbred line, disease severity index (DSI) was evaluated by a partially replicated experiment (p-rep) with a 12% of test lines in three replications. Data was analyzed using the Additive Main effect and Multiplicative Interaction (AMMI) model to establish the genotype-by-environment interaction. Given that in some environments the disease presence was very low, the AMMI analysis was performed only with the environments E1, E3, E6 and E7, where the disease presence allowed differentiate the genotypes. The AMMI model allowed us to identify genotypes with high DSI values in each environment, as well as, genotypes that showed stability through environments.

# VARIABILITY, GENETIC STRUCTURE AND LINKAGE DISEQUILIBRIUM IN A DIVERSE GROUP OF MAIZE LINES

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The CIMMYT (International Maize and Wheat Improvement Center) Maize Lines (CMLs) have been developed over the last 25 years. The CMLs represent one of the most widely distributed sources of publically generated elite lines, which are freely available to both public and private sector breeders, research and growers, worldwide. Germplasm resources allow utilizes ancestral recombination events in association mapping studies. The presence of population stratification and an unequal distribution of alleles within these groups can result in nonfunctional, spurious associations The aim of this study was assess the variability, genetic structure and linkage disequilibrium (LD) in a diverse group of maize inbred lines. A diverse group of 279 maize inbred lines development by CIMMYT was characterized for 2498 SNP. Gene diversity and polymorphic information content means were 0.33 and 0.30, respectively. Bayesian model of STRUCTURE software was used to describe the population genetic structure. The ad hoc statistic ( $\Delta$ k) based on the rate of change in the log probability of data between successive K values showed the presence of three sub-populations. The distributions of  $r^2$  with respect to the physical distance for all chromosomes showed that LD decayed in 500-1000 kb. The presence of population stratification should be consider in further studies with this population. The LD rapid decay suggests that the diverse group of 279 maize inbred lines is interest for use in mapping association studies.

#### A183

### PEANUT LEAFSPOT DISEASE: PERFORMANCE OF Arachis hypogaea L. GENOTYPES

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Peanut leafspot, caused by *Cercospora arachidicola* and *Cercosporidium personatum*, is the main foliar disease of the crop in producing countries. Chemical control is the management tool more used. Genetic resistance to control of diseases in extensive crops emerges as the most sustainable control strategy. The aim of this work was to describe the performance of a collection of *Arachis hypogaea* L. genotypes against peanut leafspot. The collection constituted by 202 genotypes by Criadero El Carmen was evaluated in the area of General Cabrera, Córdoba during the agricultural season 2015/16. The measured characters were total leaflets (TL), fallen leaflets (FL), spotted leaflets (SL), nodes number (NN) and fruits number in 200 grams (FN). For each genotype of the collection were estimated: disease incidence (INC) or percentage of diseased leaflets and disease severity (SEV) or affected leaf area percentage. Principal component analysis, a multivariate approach, was used to describe the relationship between genotypes, characters and between genotypes and characters. The first components of the biplot graph explained 70% of the variation. Positive correlations were observed between FL, SL, FN, INC and SEV. A group of nine genotypes presented values below 60% of INC and 10% of SEV. Eight genotypes showed INC and SEV values above 60% and 25%, respectively. Although the results correspond to a single evaluation environment, these allow identifing promising genotypes to obtain peanut varieties with good behavior against the peanut leafspot disease.

# A184 In vitro MORPHOGENESIS OF Gomphrena pulchella Mart.

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Gomphrena pulchella Mart. ssp pulchella is a species with ornamental attributes for its colorful inflorescence, suitable to form part of flower beds. In addition, its inflorescence is much appreciated as a cut and dried flower, since its chapters retain their texture and color still dry. It also has medicinal properties because of its 5-lipoxygenase content, a key enzyme in the biosynthesis of leukotrins that play a role of mediator in allergic and inflammatory reactions. In the present study, the *in vitro* organogenic capacity of this species was evaluated, in order to establish alternative methodologies and greater efficiency of propagation of induced polyploid individuals. Direct organ formation was evaluated from leaf explants cultured in MS culture medium with different combinations of growth regulators: naphthalene acetic acid and kinetin. The cultures were incubated at  $24 \pm 2$  °C with photoperiod of 16 hours and a light intensity of 48 mmol.s<sup>-1</sup>.m<sup>-1</sup>. Weekly it was evaluated the formation of shoots and roots and the data were analyzed using Kruskal Wallis statistics for non-parametric samples. The formation of shoots was observed on the adaxial side of the leaf explant in MS medium with 0.46  $\mu$ M kinetin and 2.69  $\mu$ M naphthalene acetic acid. While root formation was observed in explants cultivated with 0.46  $\mu$ M kinetin and 5.63  $\mu$ M naphthalene acetic acid. This alternative methodology, by using foliar tissue as an explant, allows the regeneration of selected individuals efficiently and on a large scale.

Hedeoma multiflora Benth AND Clinopodium odorum (Griseb) Harley: PROPAGATION OF STAKES

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The aromatic species are the distinctive characteristic of the mountainous areas and are part of its tourist attraction, as well as the commercial value for people living from the collection and collection of aromatic plants. Both *Hedeoma multiflora* and *Clinopodium odorum* are aromatic / medicinal species of interest in the pharmaceutical and food industries, in the saws of the Comechingones (San Luis, Argentina). In the development and strengthening of the early links of the economic and medicinal production chain of aromatic and medicinal plants, the clonal propagation of selected chemotypes is an essential factor. The present work evaluates different methodologies of propagation by cuttings in the mentioned species. A multifactorial design consisting of different combinations of substrates (perlite, worm humus, compost, wood chips, peat, sand, soil) and growth regulators (naphthalene acetic acid and indole butyric acid) with different concentration and exposure time were established. The evaluated cuttings were both herbaceous and lignified, from 8 to 10 cm long and were cultivated in a humid chamber and in a greenhouse. At 15 days, herbaceous cuttings of *Hedeoma multiflora* showed viability and formation of abundant roots with significant differences in naphthalene acetic acid (200 mg/l, immersion 10 minutes), and without significant difference with respect to the substrates. While in *C. odorum* the treatment with the longest shoots and roots of the cuttings was with indole butyric acid (500 mg/l, immersion 5 seconds) in perlite. These preliminary tests contribute knowledge to achieve technological transfer to the productive sector.

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