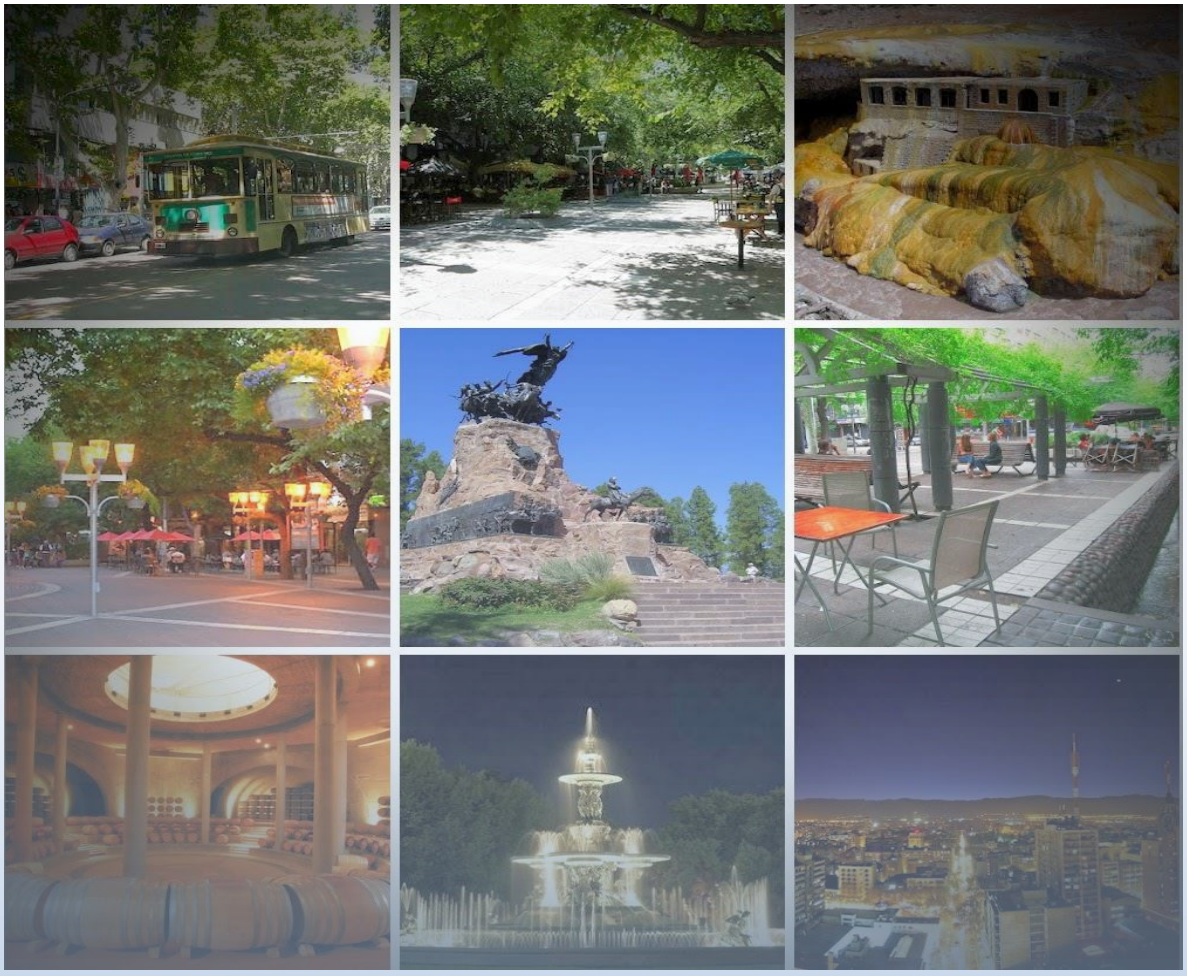
XXXIV Reunión Científica Anual De La Sociedad De Biología De Cuyo



01 al 03 de Diciembre – Mendoza- Argentina

Libro de Resúmenes

XXXIV Reunión Científica Anual de la Sociedad de Biología de Cuyo

Del 01 al 03 de Diciembre de 2016
Mendoza-Argentina

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Lectures and Symposia

A1
TODAY SCIENTIFIC SYSTEM AND SOCIAL IMPACT

Bottini, R.
Investigador Superior CONICET y Director IBAM

Scientists feel a social responsibility coming from our own notion of public officials that work in governmental organisms, in addition to that imposed by people' opinion that fund the scientific activity through taxes. This generates tension between the duties of generating knowledge in a frame of creative freedom, with the "moral obligation" to solve ordinary people's problems. In this talk I shall expose some ideas and examples of integration of "pure" scientific activity with practical application, with the aim to open a space of reflection and debate

A2
ALTERNATIVE METHODS FOR MEASURING KIDNEY FUNCTION IN THE CURRENT TIME

Rabito, C.
Massachusetts General Hospital/Harvard University, Boston, USA

The high mortality rate among patients with acute renal failure (ARF) remains unresolved. An often-overlooked critical issue is that most of the proposed therapeutic approaches are applied usually hours or even days after the initial renal insult. This common shortcoming is the result of the limited ability of the current techniques to provide real-time data of the renal function particularly of the glomerular filtration rate (GFR)**.** Consequently, two novel approaches were developed to perform actual estimate of GFR on patients at risk of ARF.

We use specially designed external radioactivity or fluorescence detection system to determine the rate of disappearance from the extracellular space of a radioactive or fluorescence-labeled agent that is cleared exclusively by glomerular filtration. This novel technology should allow the health care provider to observed the functioning kidney more closely and adjust therapy sooner than currently possible and, therefore, avoid or limited irreversible renal damage

A3
METABOLIC SYNDROME (MS) AND ITS COMPLICATIONS

Filippini, F
President SAHA

MS is a complex disease in which genetic and environmental factors determine central obesity and the expression of several risk factors. Its origin was considered for years a mutation in genes (Thrifty Gene Hypothesis), which determined insulin resistance (IR) as a mechanism to generate intra-abdominal fat that serves as an energy reserve during periods of hunger. This gene could not be identified in the human genome.

The original hypothesis was modified, assuming IR generates an inflamed fat, which can release large numbers of pro-inflammatory cytokines and persistent and elevated circulation of Free Fatty acids.

This leads to endothelial dysfunction, pro-inflammatory and pro-trombotic states, arterial hypertension, aterogenic dyslipidemia, ectopic fat deposit in liver, muscles, kidneys, vessels and epicardium. Also, sarcopenia and eventual tumor appearance.

In all these situations we can find an activation of the RAA and Sympathetic systems, fibrosis and organ damage, endothelial dysfunction, clinical atherosclerosis (AMI and stroke).

A change in lifestyle and specific drugs is reccomended as treatment.

A4
EFFECTS OF GRAPES DERIVATES IN THE HEART

Diez, EA
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Cardiovascular diseases (CVD) are a major cause of morbidity and mortality worldwide due to malignant arrhythmias and heart failure. Patients suffering from metabolic syndrome, a combination of hypertension, dyslipidemia and obesity, are in greater risk for CVD. Therefore, it is extremely important to develop preventive treatments for those exposed to risk factors to reduce the progression to CVD and/or to decrease the severity of acute events, particularly lethal arrhythmias. Grape-derived compounds like wine and more recently grape pomace are promising agents for CVD reduction. Among the potential cardioprotective candidates derived from grapes, resveratrol catechin, epicatechin and quercetin are the most promising ones. In addition,

melatonin was recently included in the list of grape-derived protective agents. However, the effects of the by-products of grapes in animal models that resemble the exposure to risk factor are still scarce. The general assumption is that grape-derived products are a good source of antioxidants, free radicals scavengers with anti-inflammatory effects. However, other biological effects should explain its beneficial effects because other antioxidants with similar potency fail to demonstrate protection in clinical scenarios. We tested some grape related compound and some of them (dealcoholized wine, grape pomace, resveratrol and melatonin showed protection against arrhythmic complications' of metabolic syndromes in rat models of risk factors exposure with increased arrhythmogenesis. Since Mendoza is wine producing area, our findings could contribute to adding value to grape-related products and serve as fundamental to use more of the constituents of grapes for human health.

A5

WHEN IMAGING STUDIES HELP TO THE BEST TREATMENT OF HIGH BLOOD PRESSURE

Farez, BG
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Currently the study on the rational use of drugs for the treatment of high blood pressure is of particular interest. We think that not all medication can be useful for all patients. There are many differences between our patients like age, sex, hemodynamics states, comorbidities, and many times we start the treatment with wrong election. This produces not getting the goal of treatment and the addition of another drug will result in poly medicated patients. For the other hand the guide treatment with cardiac impedance, wave pulse velocity or Doppler echocardiography can helps us to choose the best treatment and to check the treatment's result. Furthermore not few times we can see patients with normal blood pressure in the office but they could have high blood hypertension in their real life. A lot of papers show us pseudo normal patient, generally they belong to hyperdynamic state. If the medical doctors just take the traditional date they are losing information that could help them therefore their patients. I think that the information is in the report but the doctor, don't understand it at all, and we should to teach them to use that tips. Now we are starting to see a bridge between the molecular base of arterial stiffness or left ventricular hypertrophy and the images, and this allows us to treat our patients more rationally

A6

FUNCTIONALITY STUDIES OF ORGANOSULPHUR COMPOUNDS (OSCS)

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Laboratorio de Cromatografía para Agroalimentos. IBAM, UNCuyo, CONICET-Mza.
There are two major sources of sulphur-containing plant compounds in the diet; those derived from the glucosinolate–myrosinase (substrate–enzyme) system found in cruciferous crops, such as cabbages, broccoli and watercress, and those derived from the alliin-alliinase system found within Allium crops, such as garlic, onions and leeks. Currently, there are abundant evidence to suggest that OSCs may have a protective role against a variety of chronic diseases such as cancer and cardiovascular disease. Consequently, the studies focusing in the utilization of those compounds to improve the consumer health are of interest. According to that, the new concept of functional food is becoming more widespread and it is related to compounds that offers additional physiological benefits and /or reduces the risk of chronic disease beyond basic nutrition. Nowadays the functionality claims for foods must be supported by scientific evidence. Functionality studies require a multidisciplinary approach that involves a sequence of various aspects such as the identification of bioactive compounds, the use of extraction and separation techniques, component stability measurements, bioaccessibility and bioavailability studies, and of course, the study of biological properties. In the present conference, the organosulphurs compounds functionality studies and recent results, were reviewed.

A7

ANDEAN MEDICINAL PLANTS OF THE GENUS *Senecio*, VOLATILE, NON VOLATILE CONSTITUENTS AND VALIDATION OF THEIR PROPERTIES IN TRADITIONAL MEDICINE

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In Argentina the genus *Senecio* is represented almost by 270 species, some of them used in traditional medicine, while others are toxic because of their pyrrolizidine alkaloids content. *S. nutans* ("chachacoma"), *S. spegazzinii* ("salvia de la puna") and *S. viridis* var. *viridis* ("mocora") are some of the species used in the folk medicine of Northwestern Argentine for the treatment of different diseasses.
The essential oil from *S. nutans* is highly homogeneous, while the essential oil from *S. viridis* presented a dissimilar composition. Extracts of different polarity of the aerial parts of the three species were also obtained and characterized by the content of

different phenolic compounds. The antioxidant, antibacterial, antifungal and cytotoxic activities were evaluated. The *S. nutans* extracts were the most active, particulary the infusion and decocction exhibited higher antioxidant activities and low cytotoxicity. The mayor secondary metabolite of *S. nutans* (4-hydroxy-3-(3-methyl-2-butenyl)-acetophenone) was isolated and other sixty two compounds of low and medium polarity were indentified in the organic extracts, infusion and decoction. The mayor secondary metabolite of *S. nutans* presented antibacterial, antomicotic and tripanocidal activities.

A8
IN VIVO EVALUATION OF NEW POSSIBLE THERAPIES OF NATURAL ORIGIN FOR THE ENDOMETRIOSIS

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Endometriosis is defined as to the implantation and benign growth of endometrial tissue out of the uterus, being them locations more frequent the peritoneum pelvic and the ovaries, while, occasionally can find is foci endometriotic in others many parts as the bowel, bladder, stomach, lung, etc. It affects around 10% of the female population, whereas the prevalence among women with infertility varies between 35 and 50%. For many patients, the endometriosis is a process chronic and recurrent, which affects their quality of life, from their relations to their activities daily. One of the most common symptoms is acute pelvic pain, being this and the infertility the main reasons for consultation and the diagnostic approach. Is a disease benign, inflammatory, estrogen-dependent and multifactorial. Sex hormones stimulate the foci of ectopic tissue (called implants or lesions) in the same way that stimulates the eutopic tissue. However, the blood and the tissues not have way of exit of the body causing a constant focus inflammatory. The therapeutic conventional for the endometriosis is focused in remove injury through a procedure surgical and decrease them levels of estrogens in blood. In the last years is began to evaluate the use of medicinal herbs and botanical products to control them symptoms of various disorders gynecological. Many of these phytochemicals have shown experimentally that have effects anticancer and anti-angiogenic. In our group we have evaluated two products from of them fruit red and of the tea green, resveratrol and EGCG respectively, and we have retrieved clear effects inhibitory on the development of the Endometriosis. Also we have observed that the suppression of the activation of NF-κB by the resveratrol and EGCG can be a possible mechanism that explains the inhibition of the proliferation endometrial. Currently, we have begun to study in vitro action of other compounds such as ellagic acid (AE) which is a polyphenol present in red fruit including blueberries, strawberries, raspberries, blackberries, cherries and pomegranates and Retinoic acid (RA). We are evaluating its effect on one of the aspects more relevant in the development of the endometriotic injury as is the high rate of proliferation of the woven endometrial ectopic. The integration of these data we encouraged to investigate these compounds as new strategies for the treatment of the endometriosis.

A9
IMPROVEMENT OF Cynara cardunculus L.: TOWARD A PRODUCTION OF FUNCTIONAL AND NUTRACEUTICAL FOODS

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Since 1993, a research group belonging to the Universidad Nacional de Rosario (Argentina) has conducted different scientific studies concerning globe artichoke (Cynara cardunculus var. scolymus L.), including the development of new varieties adapted to the Argentine productive regions and new management practices, as well as the incorporation of germplasm with different genetic origins. A germplasm collection was developed including not only globe artichoke artichoke accessions but also cardoons (wild and cultivated) which belong to the same species. The genetic diversity available in the specie was evaluated and a core collection was constructed. A genetic linkage map for the species was developed from a mapping population generated from a cross between a wild cardoon (var. sylvestris) accession and the local globe artichoke (var. scolymus) accession “Estrella del Sur FCA”. Although the main use of artichoke is in human food, several studies had demonstrated health-promoting properties of their extracts, which are related to a high content of phenolic compounds (flavones and caffeoylquinic acids). The polyphenol content and profile in the different plant tissues is genetically determined, but its expression has a high environmental influence. An appropriate phenotypic evaluation of the mapping population will allow the identification of QTLs (Quantitative Trait Loci) controlling traits linked to the polyphenol synthesis. These advances would facilitate the development of molecular-assisted breeding strategies with pharmacological and nutraceutical purposes

A10
NANOFIBERS AS POTENTIAL DRUG-CARRIER SYSTEMS FOR POORLY WATER-SOLUBLE DRUGS

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Poorly water-soluble compounds are difficult to develop as drug products using conventional formulation techniques and are frequently abandoned early in the drug development process. Technologies to reduce drug particle size to sub-micrometer range are being applied to product development more frequently. Electrospinning is being considered as one of the technologies which can produce nanosized drugs incorporated in polymeric nanofibers. The application of nanotechnology to drug delivery is widely expected to create novel therapeutics capable of changing the landscape for the pharmaceutical and biotechnology industries allowing the developing of more effective and safer therapeutics. The main goal of our project is to design and fabricate amorphous electrospun nanofibers for synergistically improving the dissolution rate and permeation profiles of poorly water-soluble drugs. The nanofibers obtained are characterized using solid state conventional techniques and in vitro dissolution and permeation tests.

A11
QUANTITATIVE EVALUATION OF TRIBOELECTRIC PHENOMENA IN BIOLOGICAL MODELS: A STUDY OF THE BIOPHYSICAL MECHANISMS RESPONSIBLE FOR THE INSECTICIDAL EFFECT OF NANOMATERIALS

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Nanotechnology involves science and engineering on the nanometer scale, generally less than or of order 100nm. It involves the design, synthesis, and processing of nanoscale structures for engineering applications. In some cases this can entail the assembly of materials one molecule or even one atom at a time. At these small length scales, materials often display novel behavior that can be exploited technologically. Nanomaterials also display enhanced mechanical, optical, magnetic, and chemical properties that offer a wide variety of technological uses. Recently nanotechnology has also become extremely important in the area of biotechnology, allowing for the study of the science and engineering of biological materials for a variety of medical applications. Agriculture has also benefited significantly from nanotech-based materials in terms of sustainable food production by reducing the environmental impact of agricultural practices throughout nanoencapsulation of fertilizers and herbicides. Moreover, nanotechnology is a new frontier for the design and development of alternative insecticidal products, which instead of acting through biochemical-toxicological mechanisms, perform through physical phenomena. The development of this new generation of insecticides is based on information acquired by ultra-sensitive electronic measurements of the electric charge of nanoparticles and of the insect body surface. These electronic measurement techniques are the starting point for the design of novel nanoinsecticides for pest management with low impact on human and environmental health.
health.

A12
GENOMIC EFFECTS OF THE ALUMINA NANOINSECTICIDE “NSA” IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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In the last few years, there was an increasing interest in exploring new biorational pesticide products in agriculture. The nanostructured alumina insecticide "NSA" seems to be an ideal candidate for organic agriculture because of its high efficacy and reduced potential to induce resistance. However, toxicity studies are required to assess potential hazards of NSA to humans and to the environment. **Objectives:** To determine whether NSA induces DNA damage in human peripheral blood mononuclear cells, in contrast with natural products and conventional pesticides. **Methodology:** Peripheral blood lymphocytes (PBL) were isolated from a healthy donor venous blood and cultured at 37°C in RPMI 1640 medium supplemented with: 10% fetal bovine serum, 5 µg/mL phytohemagglutinin, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. PBL were exposed for 24 hours to increasing concentrations of NSA, Al_2O_3 and SiO_2 (50, 100 and 200 µg/mL) and then collected. To study DNA damage and chromosomal damage we used alkaline comet assay and micronuclei (MIN) test, respectively. Cell viability was tested with resazurin assay. **Results:** Comet assay revealed no significant increase of DNA damage by NSA compared with a natural insecticide. As expected, DNA breaks were significantly higher in cells exposed to an organophosphate pesticide, (OP´s) ($P<0.05$). No statistical significant differences were found in terms of cellular viability, but a lowering was noted in PBL treated with OP´s. NSA had no significant effect on MIN induction. **Conclusion:** Our preliminary results indicate that NSA is non cytotoxic and genotoxic for human PBL.

A13
ASSESSMENT OF NANOMATERIALS FOR THE CONTROL OF INSECT PESTS AND DISEASE VECTORS

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This study assessed the acute exposure toxicity, the intake toxicity, and sub-lethal effects, as repellency, feeding deterrence and progeny (F1) reduction caused by the nanostructured alumina insecticide (NSA) on *Sitophilus oryzae* adults under laboratory conditions. Acute toxicity of NSA was higher to that of commercially available products as diatomaceous earth (DE) Protect-It® and DiatomiD®, demonstrating its insecticidal potential. NSA resulted in significant adult mortality of *S. oryzae* in laboratory exposure bioassays. Median lethal concentration values (LC_{50}) obtained were LC_{50}= 97 (90-104) ppm for NSA, LC_{50}= 152 (140-165) ppm for Protect-It® and LC_{50}= 324 (289-371) ppm for DiatomiD®. NSA as well as other inert dusts resulted toxic by ingestion, after 35 days of feeding. CL_{50}= 200 (186-216) ppm for NSA, Protect-It® CL_{50}= 232 (214-251) and DiatomiD® CL_{50}= 535 ppm (480-615) ppm. Efficacy tests of NSA were conducted in small silos (400mL), using untreated and treated wheat kernels. Grain damage and parental survival was highest in the untreated controls, followed in decreasing order by DiatomiD®, Protect-It® and NSA. Progeny (F1) was significantly suppressed by NSA in wheat, followed by DE. Results of repellence bioassays indicate that NSA and DE are not repellent. Feeding deterrence bioassays showed that NSA as DE has strong anti-feedant action. Besides, we recommend evaluating nanoinsecticides as innovative vector and disease control tools.

A14
MITIGATION: HELP ROOTED IN SOIL

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Considering global change, technological changes and substitutions trending to reduce energy inputs and greenhouse-gas-emissions (GHG) per unit of ecosystem output are called mitigation. Strategies that conduce to mitigation include reduced use of fossil fuels, capture of CH_4 and C sequestration in agriculture and forestry, reduced inputs of chemical fertilizers, and efficient use of water resources. Land uses contribute almost 1/3 of global GHG, mostly from deforestation and agriculture. Soil concept has been changed from a simple physical and nutritional substrate for plant growth to a complex ecosystem, harboring diverse microorganism communities interrelated between them and with the plants roots. Soil biodiversity is huge, including bacteria and fungi directly related to mitigation purposes. Arbuscular mycorrhizal fungi (AMF, Glomeromycota) are cosmopolitan and important components of soil microbial communities of terrestrial ecosystems, including agroecosystems. These mutualistic symbionts colonize the roots of 80% of terrestrial plants, including most crops, and increase host resistance to abiotic and biotic stress factors (drought, heavy metals, salinity, and pathogens). AMF improve plant growth by supply of essential nutrients (P, N, K) with low availability from soil, through a network of hyphae that captures and transports them interconnecting different hosts species and translocating nutrients in the plant community and increase soil fertility, also aggregated soil particles and reducing soil erosion by production of glomalin. Different agricultural practices / land use affect them, influencing their diversity and, therefore, the productivity of agroecosystems. AMF play key roles in soil ecosystems, which allow them to provide ecosystem services suppliers as plant productivity, increase tolerance of crops to abiotic stress, decrease the use of chemical fertilizers, increase the quality of the plant product for human health, bioaccumulate heavy metals from phosphorus fertilization, prevent erosion, conserve biodiversity, sequester carbon, reduce nitrous oxide emission, and increase water use efficiency. Therefore, AMF are cosmopolitan inhabitants of the soil, a nonrenewable resource / ecosystem, colonizing the roots of the most plant species around the world constituting by themselves as key microorganisms for mitigation. Thus, mitigation help is rooted in the soil

A15
MITIGATION OF ABIOTIC STRESS IN CULTIVATED PLANTS BY SYMBIOTIC ASSOCIATION WITH MYCORRHIZAE

Pedranzani H

Plants are in contact with an immense variety of microorganisms in constant symbiosis and we often study them from an absolutely independent perspective, thinking that the plantlives in isolation and not in symbioticassociationwith living organisms. Mycorrhizae and bacteria are the most common organisms in symbiotic association with plants and help maintain homeostasis especially in times of environmental stress. Mycorrhizal plants are located in different soils and terrestrial ecosystems, from the Andes to the more arid desert. The symbiosis is non-specifical though there is a "functional compatibility" between ecotype of fungus and the vegetal species. Mycorrhizae are therefore also present in agricultural systems and associated with many cultivable species as they have morphological and physiological plasticity, possess great genetic diversity and are of great interest in agroecology.

They are classified as endomycorrhizae, ectomycorrhizae and ectendomycorrhizae, according to the colonization of the fungus in the roots of the plants. Endomycorrhizae include Arbuscular Mycorrhizae (AM) which play a key role in the physiology of plants: promoting rooting, favoring their nutrition (P and N); protecting it from biotic and abiotic stresses. On the other hand, AM promotes soil conservation, diversity and plant succession. It has been shown that the symbiotic association of mycorrhiza with cultivated plants brings numerous benefits in establishing an "interface" between the hyphae of the fungus and the cells of the root, which allows the capture of numerous nutrients. P is the main nutrient involved in the AM effect, because of its low mobility and availability. The uptake of N, in the ammoniacal form, and of K are also the usual AM effect. The association of plant-AM increases the biomass of the host plant until a rise in photosynthesis and compensates the drainage of C from the plant to the fungus maintaining a control through a feed-back mechanism. Under abiotic stress mycorrhizal plants increase stomatal conductance, photosynthetic efficiency, levels of osmocompatible substances in order to mitigate it. The different enzymatic and non-enzymatic antioxidant defense mechanisms are induced byAM promoting an increase in defense. Stress hormones such as Abscisic acid and Jasmonic acid increase against abiotic stress and is a chemical signal released by the plant in order to stimulate defense mechanisms.

A16

EMISSION OF GREENHOUSE GASES AND *Bradyrhizobium*-SOYBEAN INTERACTION

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The progressive ascent of greenhouse effect gases in the atmosphere is contributing significantly to global warming. Agriculture is a major sources emissions of these gases, especially nitrous oxide (N_2O), which is mostly is produced. The application of nitrogen fertilizers in agricultural soils increases the emission of these gases since they mainly originate in the nitrogen cycle. Argentina and Brasil are second and third largest soybean producer in the world with an 2015 season crop yield of about 60 million tons. The inoculation of this legume with *Bradyrhizobium* genus is a common practice in South America, and some regions have used this practice for more than 40 years. However, only a small percentage of isolated *Bradyrhizobium* strains from Argentinian soils are true denitryfing. Due to the fact that N_2O emissions in soybean nodules have been proved, and if we consider large extensions de cropped soils with that legume, soybean could become a main source of emission of greenhouse effect gasses to the atmosphere. Since denitrifying activity of these strains is still unknown, the main aim of this study is to test NO and N_2O emissions from *Bradyrhizobium* strains currently used as soybean biofertilizers in order to make it possible to formulate future environment-friendly inoculants. The information on the emission inventory derived from the legume-rhizobium interaction is incipient and available reports confirm that important N_2O values occur in this symbiotic process. Recent studies indicate that the production of N_2O in both the symbiotic interaction and in soils cultivated with legumes shows a higher production of this gas compared to soils cultivated with non-legume species. In the case our country, denitrifying activity from the most used strains for legume inoculation in Brasil [SEMIA 5019 y 5080 strains (*B. diazoefficiens*); SEMIA 5019 y 587 strains (*B. elkanii*)] and Argentina [E109 strain (*B. japonicum*)] was analyzed. These strains present incomplete denitrification and therefore, they are emitters of nitrous oxide in free living. In addition, N_2O production was determined in symbiosis with soybean roots. The emission of this gas presents significant differences when these strains were inoculated in comparison with the low emission from USDA110 and SEMIA 5080 strains (denitrification complete rhizobia). As an alternative to N_2O mitigation, it was found that the possible mixed inoculations between rhizobia with complete and incomplete denitrification decrease the N_2O production in nodulated soybean roots, with the combinations between E109, SEMIA 5019 and the complete denitrification strains being the most effective to reduce the emission of this gas. According to this, it is necessary to continue with future investigations that allow knowing potentially effective alternatives that contribute with the mitigation of the emission of nitrous oxide product of the interaction *Bradyrhizobium* - Soybean.

A17

CANCER: MOLECULES IMPLICATED IN DNA REPAIR AND CELLULAR RESISTANCE

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Heat shock protein 27 (HSP27) is a molecular chaperone with numerous roles in cell functions. There is evidence pointing HSP27 as a key regulator in apoptosis, DNA repair and metastases, between others. In several cases, the overexpression of this protein is associated with drug resistance. This fact suggests that the downregulation of HSP27 could be a novel strategy for optimization of cancer treatment. Recently, we described an association between DNA repair proteins MLH1 and MSH2 and HSP27, but the implication of this interaction remains unclear. The DNA mismatch repair (MMR) system is necessary for the maintenance of the genomic stability which is important to avoid further transformation in cancer cells. Here we use database meta-analyses in uterine cervix carcinomas, and in HeLa tumor cells (wild type or downregulated for HSP27 expression), exposed to different cadmium ($CdCl_2$) concentrations to unravel some of the biological function of HSP27 in the DNA repair. We evaluated the expression of MLH1, MSH2, MSH6 by immunoblotting in HeLa cells and by in-silico meta-analysis in TCGA database. Several biological parameters as viability, cellular death, DNA damage/repair and drug resistance were analyzed. The

expression of MSH2 and MSH6 was strongly affected by HSP27 levels, and both were inversely related to HSP27 Additionally, database meta-analysis supported those findings for MSH2 mRNA (Pearson correlation -0,41; p value= $8.83x10^{-12}$). **Conclusion**: Our data indicate that the modulation of HSP27 affects the cell survival, but it might not affect DNA repair even when in cells were exposed to high Cd-concentrations. This could be explained by the interplay between DNA repair systems. In addition, we found an inverse correlation between HSP27 and MSH2, suggesting that HSP27 may affect the DNA damage recognition by MMR or its regulation.

A18
EMERGING ROLE OF SPHINGOSINE-1-PHOSPHATE AS MODULATOR OF THE TUMOR MICROENVIRONMENT

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The observation that leukocytes infiltrate tumors led Virchow in 1863 to propose the existence of a link between chronic inflammation and cancer. The tumor microenvironment is populated by different cell types that can interact by direct cell-cell contact or may communicate through soluble factors. This bidirectional communication critically influences tumor progression and recent insights suggest that has a crucial role in the resistance to chemotherapy observed in many cancer types. In that regard, increasing evidences in the last few years demonstrate that sphingosine-1-phosphate (S1P), a bioactive sphingolipid with many functions in cancer and inflammation, displays important roles in the tumor microenvironment. Indeed, numerous studies show that augmented tissue levels of this sphingolipid metabolite affect survival, proliferation, angiogenesis and metastatic spread of many cancers. We and others have shown that S1P recruit monocytes toward the tumor tissue and induces their differentiation to M2-like tumor associated macrophages (TAM). Moreover, our results uncovered that S1P is involved in the activation of NF-κB, a transcription factor that has been pointed as critical for inflammation and tumorigenesis. In turn, activation of NF-κB reduces apoptosis while induces cytokine and chemokine release that may enhance tumor growth. In addition, our preliminary results indicate that S1P modulates the viability of murine melanoma cells and enhances the migratory ability of human $BRAF^{V600E}$ melanoma cells in hypoxia, a main hallmark of the tumor microenvironment. Altogether, these results highlight the many functions of this sphingolipid in cancer progression through modulation of distinct and important features of the disease, including phenotypic plasticity of immune cells, survival and metastatic potential of tumor cells and hypoxic response.

A19
ADIPOSE TISSUE AND EPITHELIAL CELL: A DANGEROUS DYNAMIC DUO IN BREAST AND KIDNEY CANCER

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Normal morphogeneis and functionality, as well as cancer development, require essential information exchange between epithelial tissue and fibroblastic/adipose stroma. Adipose tissue (AT) is a bioactive endocrine organ that secretes soluble factors and contributes significantly to the composition of the extracellular matrix. Adipose microenvironment is involved in signaling pathways that influence breast and kidney cancer. We showed that conditioned media (CMs) of human adipose tissue from tumor breast (hATT) differentially regulate proliferation, adhesion and migration of breast cancer epithelial cell lines (MCF-7 and IBH-7), as opposed to hATN-CMs (human adipose tissue from normal breast-CMs). Therefore, our results show that proteoglycan versican, membrane protein HCAM and adiponectin receptor type 1 (AdipoR1) are possibly involved in the biological effects observed. Additionally, demonstrated that CMs from human AT explants from renal cell carcinoma near the tumor (hRATnT) regulate adhesion and migration of tumor (786-O, ACHN) and non tumor (NK-2) renal epithelial cells, contrary to CMs from human AT explants from renal cell carcinoma farther away from the tumor (hRATfT). We observed a higher expression of versican, leptin and adiponectin, as well as a lower expression of ADAMTS1 in hRATnT-CMs compared to the expression in hRATfT-CM. We developed a model in which we obtained CMs from AT explants completely, from both normal and tumor breast or kidney. Also, we showed hypothyroidism produces changes in the ability of AT to secrete soluble factors that regulate the survival and migration of tumor and non-tumor mammary cells. The identification of these factors, both in AT and epithelial cells might help develop new strategies to prevent and/or treat cancer.

A20

PLANTS VS CANCER: ANTI-ONCOLOGICAL PROPERTIES OF ACTIVE CONSTITUENTS OF *Tessaria absinthioides* (Hook & Arn.) DC. (ASTERACEAE). IN VITRO AND IN VIVO STUDIES

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Natural products represent the most abundant source for identification of novel chemical structures, making them suitable as basis for design novel drugs; particularly in certain areas of medicine such as a cancer research. From the research of native plants with popular reports of medicinal activity, our group demonstrated the cytotoxic and antitumor activity of the aqueous extract of T. absinthiodes (TA), so called "pájaro bobo".

The aim of this work is to evidence the antitumoral properties and compounds of native plants with ethnopharmacological reports of activity.

TA aqueous extract (EA) in vivo studies were performed to evaluate, oral toxicity and colorectal antitumoral effects; in vitro assays were carried out to evidence selective cytotoxicity against tumoral and non tumoral human cell lines, and to select different fractions of EA to trace the cytotoxic activity destined to purify the active principle by different chromatographic methods.

The results allowed us to identify a single molecule with biological activity, which represents a promising basis to develop new research and therapeutic alternative in oncology

A21

PROTEOMICS APPLIED TO PEPTIDES AND PROTEINS IDENTIFICATION AND CHARACTERIZATION FROM BIOLOGICAL SAMPLES

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Proteomics is the study of the proteome and its variation in space and time. Mass spectrometry-based proteomics- has been successful through the use of a combination of analytical and spectroscopy techniques such as Liquid Chromatography coupled with Mass Spectrometry. The applications of these technologies have begun to be powerful tools in the proteomics field for the identification, characterization and quantification of biomolecules such as proteins and peptides, which may be associated with diseases and their therapeutics, as well as protocols for early diagnosis and prognosis. Bioinformatics tools are used as a complementary approach to create a bridge in the process of "translation" of the data coming from mass spectrometry (peptide mass fingerprinting) into peptide sequences which potentially could match the protein identity. This kind of technology contributes to understanding more about the different factors and processes that can affect human health (microbiology of pathogens, viruses, cancer, cardiovascular and neuroscience conditions). Several proteomics protocols such as in gel and/or gel "free" combined with MS and tandem mass spectrometry (MS/MD) are available to perform protein analysis from simple and complex mixtures. Today, many high resolution/mass accuracy (HR/MA) mass spectrometers are on the market to handle two main levels of analysis: "discovery" (global) and "targeted" or quantitative proteomics. All of the protocols as well as technologies mentioned before, are available at the South Dakota Proteomics Core Facility. The Facility was created in 2002 by support of SD BRIN and has been working in a collaborative research manner to enhance research capabilities and lead to further strong collaborations among researchers.

Resúmenes

Clinical Medicine, Veterinary and Odontology

A22
STUDY OF TWO SALIVARY BIOMARKERS OF THE PERIODONTAL PROGRESSIVE DISEASE

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Periodontal disease is a chronic infection of bacterial origin. The IL-1 and the TNF-α are potent stimulators of resorption bone. The objective of the study is the identification of IL-1 and TNF-α in saliva of patients with periodontal disease, postoperative phase and maintenance and correlating its concentration with clinical parameters. Material and Method: it took a display of 30 individuals sick periodontal. Is made history clinical, serial periapical, index of plate and measuring of the depth of the bag periodontal. Samples of saliva were collected, which were studied by gas chromatography identifying levels of IL-1 and TNF-α. Results: In the operation stage, plaque index was 68% mild and 32% moderate. 620 pg/mL levels of TNF-α, IL-1 levels were 51, 14 pg/mL. In the maintenance phase plaque index was 77% mild and 23% moderately. In 23 patients with mild plate and without bleeding on probing the levels of IL-1 were 582 pg/mL and TNF-α were 45,44 pg/mL. In 6 patients with plaque index moderated and bleeding on probing the IL-1 level was 889 and those of the TNF-α 106 pg/mL. Conclusions**:** In the maintenance phase 7 patients had high levels of biomarkers and recurrence of the disease. The results demonstrate the value of biomarkers as determinant to set its incidence in the severity of periodontal disease.

A23
UNCOMMON PANCREATIC NEOPLASMS: CASE REPORT OF A MICROCYSTIC SEROUS CYSTADENOMA

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Serous cystic neoplasms comprise approximately 25% of all cystic neoplasms of the pancreas and they can be categorized into microcystic, honeycomb, oligocystic, and solid patterns based on imaging appearance. Serous microcystic adenoma (SMCA) is an uncommon condition, with an incidence of 1% to 2% of all exocrine pancreatic tumors. It is a benign neoplasm composed of uniform cuboidal glycogen-rich epithelial cells, presumably originating from centroacinar cell/intercalated duct system and typically forms innumerable small cysts containing serous fluid. They can be located anywhere in the pancreas and are rarely malignant, in contrast with intraductal papillary mucinous neoplasms (IPMN) and other mucinous cysts. Here we report the case of a 59 years-old female with abdominal pain and a mass in the body of the pancreas. The patient was initially treated with abendazol under suspicion of an echinococcal cyst. Other differential diagnoses were: pseudocysts, congenital cyst, retention cyst, mucinous pseudocyst and solid pseudopapillary tumor. Radiological investigations were performed with computed tomography. Pancreatectomy and splenectomy were the treatments of choice. The accurate anatomopathological diagnosis was performed analyzing the macroscopic, histological and immunohistochemical features of the tumor. We also discuss the current management guidelines for pancreatic cysts, their underlying genetics, and the integration of molecular testing in cyst classification and prognostication.

A24
ASSOCIATION BETWEEN POLYMORPHISM OF ANGIOTENSIN II TYPE 1 RECEPTOR GENE AND METABOLIC SYNDROME IN SAN LUIS POPULATION

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Correlation between angiotensin II type I receptor gene polymorphism with hypertension, myocardial infarction, insulin resistance and cardiovascular disease risk has been reported. The study of RAS regulation sustained that this system could be a fundamental participant in metabolic processes and could explain the origin and complications of Metabolic Syndrome (MS). There are limited data available about the genetic MS susceptibility in Argentina population. The aim of this work was to analyze the association between $A^{1166}C$ polymorphism and MS in patients from San Luis. Adult Treatment Panel III criteria was used to classified 156

patients: 80 MS (46.2% women)/76 control (72.3% women), body mass index MS/C 32.6 ± 3.6/27.1 ± 4,8 ($p<0.0001$), systolic blood pressure MS/C 149.7 ± 18.7/126.4 ± 20.2 mmHg and diastolic 89,2 ± 11.5/72.8 ± 11.6 mmHg ($p<0.0001$), triglyceride MS/C 213.1 ± 99.2/127.1 ± 71.0 mg/dl ($p<0.0001$). The $A^{1166}C$ polymorphism was analyzed by PCR-RFLP and biochemical parameters were determined. Frequencies of the AA, AC and CC genotypes were 47.5%, 41.2% and 11,2% MS patients and 57.9%, 35.5% and 6.6% in control. The allele frequency in MS/control was A: 0.68/0.76 and C: 0.32/0.24, according to Hardy-Weinberg equilibrium. There was no significant difference in genotype or allele frequencies between groups. The MS was higher in men compared with women in the population studied ($p<0.001$).The genotype CC was associated with MS in women ($p<0.04$). We suggest a correlation between $A^{1166}C$ polymorphism and MS in women. Further studies in a larger population are needed to clarify the genetic association between candidate genes and MS.

A25
USING THE EMERGENCY SERVICE IN DAY HOSPITAL OF SAN LUIS

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Services emergency hospitalaries have become a key part of health systems attending both high and low complexity health problems. Our objetive were to identify the prevalent diagnoses in outpatients receiving pharmacological treatment in an emergency room (ER). An observational, cross-sectional, retrospective study was performed on Hospital of day for outpatients, assessing prevalent diagnoses. The data were collected from 1871 medical care sheets and medical prescriptions during three months (october- december 2014). Health problems were classified by ICD-10 (International Classification of Diseases 10th review). Distribution (%): for age: <15 years old 13.7; >15 years old 86.3. For sex: F 56.7 M 43-3. Diagnoses according to categories ICD: Abnormal symptoms (R) 25.3; Digestive (K) 21.7; Respiratory (J) 15.4; Osteomuscular (M) 12.7; Injury, poisoning and certain other consequences of external causes (S-T) 9.9; Genitourinary (N) 4.5; Skin and subcutaneous tissue (L) 2.6. Prevalent diagnoses (<15): vomiting 28.9, gastroenteritis 10.5, fever 8.6, bronchospasm 4.9, allergy unspecified 4.9, bronchiolitis 4.1, pharyngitis 4.1, acute tonsillitis 4.1, toothache 3.8, laryngitis 2.6, cough 2.3, otitis 1.9. Prevalent diagnoses (>15): dorsalgia 9.6, gastroenteritis 7.6, abdominal pain 7.0, toothache 5.6, vomiting 5.6, gastritis 5.2, pharyngitis 3.6, allergy unspecified 3.5, headache 3.4, tonsilitis 2.8, bronchospasm 2.7, renal colic 2.7, fever 2.6, bile colic 1.8. Patients were derived to a more complexity service 1,01. Most diagnoses have corresponded to health problems of low complexity. The emergency service was inappropriately used. Saturation of emergency services affects a greater or lesser extent attention of seriously ill patients because it creates delays in the cares and increase errors in decisions and procedures to be performed. It is necessary to create a health culture that should lead to an appropiate use of health services.

A26
CERVICITIS CAUSED BY *Trichomonas vaginalis* IN ASSOCIATION WITH *Leptothrix.* DIAGNOSIS THROUGH VAGINAL CONTENT BALANCE

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Vaginal dysfunction (VD) is high-prevalence pathology in women of reproductive age. It is induced by an imbalance in normal microflora. Given the gravity of associated morbidity, an opportune diagnosis and treatment is critical. Balance of the vaginal content (BAVACO) is the most widely accepted clinical method to identify VD for its high predictive value. The aim of this work was to show an unusual case of cervicitis associated to *T. vaginalis* and *Leptothrix* detected by BAVACO procedure. A 26-year-old woman was referred to Juana Koslay Hospital (San Luis, city), with complaints of heavy vaginal discharge, pruritus and dyspareunia for one month. Intrauterine device was present. Samples of cervico-vaginal smears were analyzed by wet mount, Gram and Giemsa stains. Smear examination showed absence of *Lactobacilli spp* with significant vaginal inflammatory response in association with *Leptothrix* and *T. vaginalis*. The result obtained is associated a non-specific microbial vaginitis, according BACOVA. *Leptothrix* was identified as long, curving, filamentous bacteria, ranging between 40-75 μm in length. Despite of pathologic significance of *Leptothrix* in the female genital tract is controversial, its diagnosis, although rare, should alert the laboratory to search the co-existing trichomoniasis, considered a risk factor for several obstetric and gynecologic diseases. Since clinical presentation cervicitis may be suggestive of vulvovaginal candidiasis, recognition of cytomorphological *Leptothrix* features is essential for appropriate treatment and resolution of chronic vaginal subjective complaints. In this sense, BAVACO represents the most cost-effective procedures available for laboratories in primary care health.

A27
DIETARY CONCERNS FOR VEGANS AND STRICT VEGETARIANS

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The number of people, who for various reasons adopt a vegetarian diet, is increasing. To determine consumption of foods which are sources of key nutrients and assess Body Mass Index as well as lifestyles in vegans and strict vegetarians of both sexes between the ages of 20 and 50 in the city of San Luis. In this study the nutritional requirements according to sex, age and sports were not evaluated. The sample was non-probabilistic intentional, a selection was performed desirability of 41 vegans and strict vegetarians 20-50 years old. According to the Nutrition Society Argentina, strict vegetarians and vegans are the "True Vegetarians", this diet rules out any kind of meat or animals (derived milk and eggs). Vegans also avoid honey and silk dress with leather of animal origin. Strict vegetarian and vegan to practice a vegetarian diet less than a year ago, to submit any disease or condition, such as diabetes and pregnant women or lactating were not included because their nutritional requirements as well as BMI, they are different. We inquired about how many times per week the participants consumed food sources with key nutrients, the BMI was assessed using anthropometric indicators and data pertaining lifestyle factors obtained through self-evaluation in the questionnaire, Fantastic Life Styles. It was observed that, on a daily basis, the number of research participant who get enough protein from vegetable sources was 43.9%. Those with a sufficient iron intake from non-heme sources amounted to 36.6%. 34.2% had diets which included significant sources of omega 3 fatty acids. The percentage of participants who were conscious of choosing food sources with adequate levels of calcium was 34.2%; 43.9% for zinc, and 22% for B12. 95.2% of participants have a normal BMI; no difference in BMI was found between number of underweight participants (2.4%) and overweight participants (2.4%). The 48.7% of participants describe their lifestyles “good”, 39% practice a lifestyles of “excellent”, 9.8% consider theirs to be “regular” and 2.5% “bad”. Keywords: vegans, strict vegetarians, food, key nutrients, Body Mass Index and lifestyles. It can be concluded that less than half (43.9%) of the sample consumes food source of key nutrients daily. In relation to BMI and lifestyle numbers show that most vegetarians have 95.2% of suitable weight and 87.7% a healthy lifestyle.

A28
DETECTION OF PATIENTS WITH RISK FACTORS FOR DEVELOPMENT OF METABOLIC SYNDROME IN A PRIMARY CARE CENTER OF SAN LUIS

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Metabolic syndrome (MS) is a set of metabolic abnormalities caused by the combination of genetic and environmental factors associated with lifestyle, which involves *per se* an increased cardiovascular morbidity and mortality. The aim of this work was to identify individuals who may be developing MS from the evaluation of various risk factors preset (Criteria NCEP-ATP III). This study included people who attended to primary care center located in the west of San Luis city (Hospital A. Luchini), between April and September 2016. The participation of the subjects was voluntary and confidentiality of data is guaranteed. The anthropometric data (weight, height, waist circumference, blood pressure) and laboratory data were requested. It enlists the habits (smoking, physical exercise) and socio-demographic variables (age, gender, education). Subjects diagnosed with diabetes, MS or chronic kidney diseases were excluded. The serum levels of glucose (Glu), uric acid (UA), colesterol (Chol) and triglycerides (TG) were determined by enzymatic colorimetric methods. Blood pressure was measured with mercury sphygmomanometer. A total of 89 surveys of adults between 18 and 60 years (44 ± 14), 68 women and 21 men were obtained. Of all patients: 38.2% were smokers, 48.2% sedentary, 42.7% showed blood pressure values ≥130/85 mmHg, 15.7% Glu >110 mg/dl, 16% UA >6mg/dl, 37.1%, Chol >200mg/dl and 31.7% TG >150 md/dl. Body mass index (BMI): 22.5% had overweight (BMI ≥25 kg/m^2) and 46.1% obesity (BMI ≥30 kg/m^2). From the results it is evident that the 34.83% of patients had 3 or more MS diagnostic criteria established by NCEP-ATP III. The 74.80% of patients with MS had no/little, primary, secondary and graduation level education. Whereas the SM is associated with modifiable factors, it is important to deepen and make effective public policies that allow timely diagnosis and promote health at the primary care level.

A29
RELATIONSHIP BETWEEN VITAMIN D STATUS AND BLOOD LIPID PARAMETERS IN ADULTS OF SAN LUIS (ARGENTINA)

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The 25-hydroxyvitamin D (Vit D) play an important role in human health and its deficiency is often underdiagnosed in clinical practice. Participates in bone mineral metabolism, regulation of cardiovascular system, neurological development and immune modulation. The aim of study was to examine the relationship between serum Vit D levels and lipid profiles among adult residents in San Luis city (33°17'42"S; 66°20'08"W). A total of 449 patients (53±15 years), 370 women and 79 men, who attended to a private clinical laboratory (September 2015-February 2016) formed the study subjects. Patients with BMI>25kg/m^2, serum creatinine>2mg%, malabsorption syndrome, pregnancy or treatment with vitamin D were excluded. The stratification of 25[OH]D

level with age was analyzed. Serum total Vit D [Reference Value (VR) ng/ml: Sufficiency >30; Failure 10-30; Deficiency <10] and Thyrotropin (TSH; VR: 0.27-4.20 mUI/ml) by electrochemiluminescence, Triglycerides (VR: 35-150 mg/dl) and Cholesterol (VR <200 mg/dl) by spectrophotometry, were measured. Vit D deficiency and insufficiency were reported in 17.6% [6.98 ng/ml (CI: 6.25-7.72)] and 57.9% of the study sample, respectively. People with Vit D levels lower than 30 ng/ml showed increased triglycerides levels compared to those with Vit D levels higher than 30 ng/ml [107.30 mg/dl (CI: 101.02-114.97) *vs* 85.81 mg/dl (CI: 72.06-101.02)], ($p < 0.03$), without changes in cholesterol between the two groups. In patients with Vit D deficiency, serum TSH levels were higher [4.72 mUI/ml (IC: 4.15-6.14)] compared to insufficient [1.99 mUI/ml (IC: 1.65-2.28)] and sufficient groups 1.9 mUI/ml [(IC: 1.72-2.26)], ($p < 0.05$). Poor Vit D status was related to unfavorable lipid metabolism and thyroid homeostasis. Since low Vit D levels are associated with dyslipidemia and increased risk of cardiovascular, it would be appropriate in those patients a laboratory long-term follow-up.

A30
PREVALENCE AND DIAGNOSIS OF KIDNEY DISEASE IN ADULT PEOPLE OF OUR COMMUNITY

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Chronic kidney disease is a worldwide public health problem with an increasing incidence and prevalence, affect 10% of adult population in different parts of the world. Kidney disease is defined as a decrease in renal function, equations to estimate glomerular filtration rate (GFR) are routinely used, expressed by a GFR <60 mL/ min/1.73m^2. The aim of this study was to determine the prevalence and the importance of diagnosis of early kidney function in adult patients of our community.

We studied a sample of 421 individuals (both sexes, aged 20-70 years old) to which they made a series of metabolic biochemical studies necessary to evaluate overall condition as well as identification and classification of GFR. Equations Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD) and National Kidney Disease Education Program's (NKDEP) were used to estimate kidney function. The anthropometric variables were evaluated. The total sample represented 57% women (W) and 43% men (M). Characteristics of the population: mean age M/W 43.9±13.9% / 44.6±13.8 years, mean weight M/W: 85.5±14.5 / 64.4±13.1 Kg, IMC M/W: 28.2±4.3/24.9±4.7, serum creatinine (SC) concentration M/W: 1.1±0.2/ 0.8±0.2 mg/dL. We determined the prevalence of the sample according to the classification (K/DOQI), 50.2% M and 62.9 % W by MDRD 57.4 %M and 70.4%W by NKEDP within the stage 2, 14.9%M and 8.3%W stage 3 having only 1.65% M and 1.25 % W stages 4 of the failure disease. Positive correlation IMC vs SC ($r=0.18$, $p<0.01$) and age vs SC ($r=0.33$, $p<0.001$) in M. Is very important to determine the prevalence and diagnosis of early renal disease, the pathological process is a major cause of death and severe disability.

A31
DIETARY HABITS IN SUBJECTS WITH METABOLIC SYNDROME IN SAN LUIS

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Metabolic syndrome (MS) is the association of health problems with polygenic and multifactorial origin. It involves metabolic disorders that are associated to diabetes mellitus type 2 and increased cardiovascular morbidity, both directly related to the current epidemic on these pathologies. The goal of this study was to assess eating habits, in adult patients which were diagnosed with metabolic syndrome in the Nutrition Service from Policlinico Regional San Luis. A cross-sectional descriptive study was made in both sexes patients with MS (n= 30) aged 22-45 years. To assess dietary habits, a food frequency questionnaire and questions about visiting to a fast food restaurant, habit of skipping meals, was incorporated into the questionnaire, considering them as possible risk indicators for SM.

A low consumption of foods of high nutritional density (6,66 %- 33%) and a high consumption of those with poor nutritional and high density empty calories (60%- 96%) was shown. 70% reported to have 4 meals daily, giving greater importance to lunch and dinner 53.5% and 46.6% respectively. 80% regularly eat outside the home and 20% only in their homes. Participants feel that their lifestyle is bad (63.3%); Regular (26.66%) and Good (10%). 66.6% believe that their food "almost never" is balanced, 30% "sometimes" and 3,33% "almost always" balanced. It is concluded that in these patients with MS, dietary habits with high consumption of empty calories and some aspects of lifestyle would cause this condition.

A32
PARTICIPATION OF ANGIOTENSIN RENIN SYSTEM IN VASCULAR DISEASE OF PATIENTS WITH X CHROMOSOME ABERRATIONS

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Patients with cytogenetic abnormalities of X chromosome have an increased cardiovascular mortality, making a necessity to stratify their risk. The aim of this study was: to analyze the relationship between the patients´ karyotype and cardiovascular phenotype in order to assess their cardiovascular risk by vascular Doppler ultrasound and dosage of angiotensin converting enzyme 2 (ACE2) using ELISA. A longitudinal descriptive study of clinical research in genetics and cardiology was designed, including 36 patients with X chromosome aberrations who consulted the Institute of Genetics clinic, UNCuyo. Data concerning their cytogenetic findings, clinical and laboratory variants were analyzed. Carotid and brachial arteries Doppler ultrasound was performed and ACE2 levels were measured in urine. Seventeen per cent of patients had high blood pressure, 46% hypercholesterolemia, 27% hypertriglyceridemia, 34% hypothyroidism and 63% showed abdominal circumference > 88 cm. Echo Doppler studies demonstrated that 63% of patients had atherosclerotic carotid disease, including the presence of increased intima-media thickness or atherogenic plaque, and 85% of patients had endothelial dysfunction in the brachial artery study. ACE2 levels were decreased in women with X chromosome abnormality, indicating that it affects the protein synthesis. There is a low but statically significant regression/correlation between ACE2 and brachial dilation.

Microbiology and Immunology

A33
CO-ADMINISTRATION OF rNP INFLUENZA WITH NANOPARTICLES BY SUBCUTANEOUS ROUTE, INDUCE INTENSE HUMORAL AND CELLULAR IMMUNE RESPONSES IN BALB/C MICE

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Currently, the immune response induced by actual influenza vaccines is based on the induction of antibodies against surface viral proteins, which can neutralize the virus. These antibodies are specific of vaccine strains and do not protect against antigenic variants or new subtypes. The induction of strong T cell responses to conserved internal viral proteins, as the viral nucleoprotein, is associated with reduced disease severity as well as broad cross-reactivity protection. In recent days, the evaluate the immune responses of a new vaccine based on recombinant influenza nucleoprotein (rNP), co-administered with non-porous silica or alumin oxide nanoparticles. To achieve this goal, groups of 5 female BALB/c mice were immunized subcutaneously with 2 doses of 10 µg of rNP alone or co-administered with 250 µg of the different nanoparticles, on day 1 and 21. Mice were sacrificed on day 42, and sera and spleens were recollected. The humoral immune response was evaluated by determination of antigen specific IgG and IgG subtypes antibodies titers by ELISA. The cellular immune response was evaluated by determination of INF-γ and IL-4 in supernatants of splenocytes re-stimulated with rNP after 72 h, by ELISA. The results showed a intense humoral immune response by high IgG and IgG1subtype specific titers from mice immunized with rNP/silica (1/715680 and 1/6400 respectively) and rNP/alumin oxide (1/5440 and 1/500000 respectively) compared with rNP alone. A significant increase in production of INF-γ but not IL-4, was elicited by splenocytes from mice immunized with rNP/silica and rNP/alumina ($P < 0.05$). The obtained results showed that the formulations of rNP/ silica as well as rNP/alumin oxide confer a Th1 bias response. In conclusion, the use of nanoparticles combinated with NP could be taken into account for the development of innovative vaccines against influenza.

A34
PREVALENCE OF *Chlamydia trachomatis* INFECTION IN URBAN POPULATION OF SAN LUIS, ARGENTINA

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Infection with *Chlamydia trachomatis* (CT) is an important public health concern because it primarily affects women in reproductive age and has been associated with pregnancy complications, including decreased fertility and chronic pelvic pain.To date, there have been few reports on the epidemiology of CT in Latin America. The aim of this study was to determine the prevalence of CT infection among urban population in San Luis, Argentina. For the study, 315 women, age range 17-60 years were attended a private laboratory of clinical analysis in San Luis between May 2014 and July 2016. Vaginal swabs collected were evaluated using one-step Immununoassay based on the immunochromatographic sandwich principle. Among the 315 women, the overall prevalence of chlamydial infection was 142/315 or45.08 %.The average age of prevalence of infection in

women was 32.13±7.26 years and CT infection varied according to age of participants. The highest prevalence was in the age group (30–40 years), which comprised 52.11%, between 17-29 years old: 35.91%, and between 41-60 years old was11.97%. The majority of participants with laboratory-diagnosed CT infection were asymptomatic. Among the 142 women infected with CT, 73/142 (51.41%), reported "no genitourinary symptoms during last six months". In this study, the association between CT, vaginal candidiasis and GAMM complex was 42/142 (29.58%).The prevalence among young women was especially high and deserves attention, given the amount of asymptomatic infection and the adverse health effects known to be associated with untreated CT infection.

A35
INFLAMMATORY EFFECTS OF *Helicobacter pylori* ON LUNG

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Helicobacter pylori infection has been associated with respiratory illness; however, the impact of this bacterium on lung is not well understood. Inflammatory response is mediated by the release of chemokines, cytokines, interferon, and enzymes such as metalloproteinases (MMPs). Interleukin-1β (IL-1β) and tumor necrosis factor (TNF-α) stimulate the fibroblast proliferation, and ROS and the nitrogen metabolites modulate fibronectin-induced fibroblast migration. This may contribute to collagen accumulation during the early phase of infection. MMPs are proteolytic enzymes involved in degradation of extracellular matrix and MMP9 is a majority in lung. This work was aimed to study the inflammatory effects of *H. pylori* on lung. Balb/c mice were infected by orotracheal instillation with 20 µl of a *H. pylori* reference strain 1×10^8 suspension once per day for 3 days. Infected animals and controls were sacrificed at 3, 7, 14, 21 and 30 days. Expression level of multiple markers implicated in inflammation TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-10, MMP9 and endothelial dysfunction markers (I-CAM and V-CAM) were determined from mRNA of lung tissues by RT-PCR. Gene relative expression was calibrated by the β-actin housekeeping gene expression. Results showed that mRNA of IL-1β and TNF-α, MMP9, I-CAM and V-CAM increased at 3-7 days of infection. Also, iNOS, IL8 and phosphocholine cytidylyltransferase (CT) increased with lung injury. Anti-inflammatory IL-4 and IL-10 increased at 7 days of infection. We demonstrated previously that *H. pylori* induced morphological changes in the lung tissue with recruitment of inflammatory cells and lung parenchymal dysfunction. Results obtained in this study suggest that the pathophysiological mechanism of *H. pylori* on lung might be strongly associated with lung injury as measured by elevation in the expression of inflammatory mediators and endothelial dysfunction markers.

A36
EXPERIMENTAL INFECTION OF THE APPLE SNAIL *Pomacea canaliculata* WITH *Mycobacterium marinum*

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Previous studies in this snail have shown the existence of phagocytic hemocytes in the circulation and renal islets, as well as the formation of hemocyte spheroidal aggregates in the kidney and lung after *in vivo* injection of yeast cells. These aggregates remind the granulomata of human tuberculosis, however lacking the typical lymphocytic rim, which is not surprising since there are no lymphocytes in *P. canaliculata*, as well as no other evidence of an adaptive immune system. In a preliminary experiment, we have injected 9 adult male *P. canaliculata* in the visceral mass with 100 cfu of *Mycobacterium marinum* and have recorded snail mortality and the histopathological changes in the kidney, gill and lung of both surviving and dead snails. Three surviving snails were sacrificed 4 days after injection, while 3 other snails died on days 5-19 after injection. Surviving snails showed hypertrophy of the renal islets and spheroid formation within them, accompanied with extensive loss of epithelial cells of the renal cryptae, particularly in the pallial region of the kidney. The gills showed numerous multinuclear cells infiltrating the epithelium of the lamellae, thickening and granulocyte infiltration of the underlying connective tissue and obliteration of the hemocoelic spaces. The lungs were apparently unaffected in surviving snails, but dead snails showed atrophic lung walls, with apparent depletion of the normal urate tissue. It is suggested that this snail may become a useful animal model for studying the formation of tuberculosis-like granulomata with the participation of the innate immune system only.

A37

FORMATION OF BIOFILM BY *Listeria monocytogenes* AT DIFFERENT INCUBATION TEMPERATURES IN PRESENCE OF THYMOL

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L. monocytogenes adhesion and biofilms are of great importance for the food industry and occur on a high variety of food contact surfaces. Biofilm production may be affected by environmental conditions and the presence of inhibitors. Thymol (2-isopropyl-5-methylphenol) presents a variety of pharmacological properties including antimicrobial effects. This study was carried out using *L. monocytogenes* CLIP 74902 and its biofilm formation ability was tested under various concentrations of thymol and two growth temperatures. Trypticase soy broth (TSB) with glucose 10 g/l supplemented with thymol at 0 (control), 250 and 750 µg/ml was used. The experiments were performed in 96 well microplates. Aliquots of 150 µl TSB and 10 µl of overnight bacterial culture in brain heart infusion broth were added into each well. Each plate was incubated at 30ºC and 37ºC 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 to remove the suspended cells and the attached bacteria were fixed with methanol. Then, 200 µl of 1% (w/v) crystal violet were added into each well and left at 25°C for 30 min. After removing the dye with sterile PBS, the crystal violet attached to biofilm was solubilized by adding 33 % (v/v) glacial acetic acid. The OD_{550nm} was measured in a plate reader. The experiments were performed by sixfold. At 72 h, the sessile biomass (OD_{550nm}) obtained in TSB under exposure to 0, 250 and 750 µg/ml of thymol at 30ºC was 1.140, 0.690 and 0.020; while at 37ºC, it was 0.801, 0.504 and 0.010, respectively. Biofilm formation was significantly influenced by thymol concentration and incubation temperatures, probably by modifying cell surface hydrophobicity and the expression of flagella, important first steps in the adhesion, which precedes the formation of the biofilm polysaccharide matrix.

A38

CO-CULTURES OF *Tolypothrix tenuis* - *Bacillus* sp.

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Phosphate-solubilizing bacteria (PSB) and nitrogen-fixing bacteria (NFB) are considered prospective biofertilizers. Most cyanobacteria accumulate polyphosphate (polyP) granules as the main P reserve, which plays a role in the regulation of enzymatic activities, the expression of many genes and the stress adaptation. The aim of this work was to study PSB and NFB co-cultures under insoluble P (iP) conditions. *Tolypothrix tenuis* as NFB was co-inoculated with different spore concentrations (10^3-10^6) of two PSB, *Bacillus* sp. SL7 (native isolate) and *B. amyloliquefaciens* FZB42 (reference strain) in Watanabe medium without (W) or with insoluble P (W-iP), and incubated at 30°C for 10 days with continuous illumination (2300 lux). Final biomass was obtained by dry weight prior iP solubilization. PolyP granules of *T. tenuis* were observed in brightfield microscopy with toluidine blue stain and fluorescence microscopy with DAPI. *T. tenuis* maximal biomass value was 2.74 g/L in W; however, it decreased by 68.61% in W-iP. Co-cultures with the SL7 strain showed a recovery of the photoautotrophic growth (1.37-1.54 g/L) while biomass values obtained with the FZB 42 strain ranged between 1.19-1.34 g/L. Significant differences ($P<0.05$) were observed in the former of co-cultures. Growth improvement was independent of the PSB spore initial inoculum. PolyP granules of *T. tenuis* filaments in W-iP showed degranulation (23,95%) and size decrease in the remaining ones (≤1 µm), while the presence of polyP granules ≥1µm was observed in all the cells grown in W. Co-inoculation with the PSB native strain showed a beneficial effect in W-iP. Hence, PSB and NFB mixed inoculation could be an option of ideal self-sustaining biological systems for overcoming limitations by poorly soluble phosphorous compounds in soil and nitrogen fixation from atmospheric nitrogen.

A39

SEARCH FOR A *Larrea divaricata* PURIFIED ANTIGENIC PROTEIN FOR OBTAINING ANTI-*Pseudomonas aeruginosa* IGG ANTIBODIES

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Larrea divaricata Cav. (Jarilla) is a shrub widely distributed in North and South America and used in folk medicine. Works in our laboratory have shown that proteins from *L. divaricata* aqueous crude extracts (JPCE) can induce a specific immune response that exhibits cross-reaction with *Pseudomonas aeruginosa* proteins. Considering that this bacterium is an extracellular pathogen, the induction of a humoral response with specific antibodies that can target virulence factors is important to prevent infections in susceptible patients. Innate immune cells also participate in the eradication of bacterial infections through mechanisms such as phagocytosis of IgG-opsonized microbes. The aim of the present work was the search for a "jarilla" purified antigenic protein that show cross reaction with *P. aeruginosa* proteins involved in the pathogenicity of this bacterium. JPCE proteins were partially purified by using membranes with 10, 30, 50 y 100 kDa cutoffs (J10, J30, J50 and J100 kDa); then, mice were immunized with these JPCE fractions and sera were obtained. *P. aeruginosa* ATCC 27853 total membrane proteins (TMP) were challenged with these sera. Additionally, JPCE proteins were purified by SDS-PAGE and the bands obtained were confronted with the immune sera by using enzyme immunoassays (EIA). Nine bands of JPCE were tested (MM: 22-71 kDa). Besides, different specific IgG levels for these bands were compared with results of cross-reaction of anti-JPCEs with TMP. From the analysis obtained it was

observed that sera involved in the cross-recognition of *P. aeruginosa* membrane proteins such as chaperonins, porins and secretion system proteins reacted with antigenic determinants present in most of the JPCE bands. Future studies on the immunogenicity and protection of each JPCE band will help us to find the *L. divaricata* specific protein or proteins to be proposed as candidate for the formulation of vaccines against *P. aeruginosa*.

A40

EVALUATION BY FLOW CITOMETER OF *Brucella abortus* SURVIVAL

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The strategies implemented by facultative intracellular parasites that allow you to evade host responses have been observed in *Brucella abortus.* This bacterium can survive and even multiply within the macrophages. To elucidate the mechanism by which this phenomenon occurs, it was proposed to determine the involvement of Akt1 kinase of the infected cell, intracellular replication and survival of different *Brucella abortus* strains. Akt kinase phosphorylates AS160 (AKT substrate) and inactive, this promotes the Rab 11stay bound to GTP (active form) so allows nutrient inputs and gives an appropriate environment to brucella multiply. The Akt inhibitor causes the opposite effect, promotes GTP hydrolysis, inactivating Rab 11 (GDP-bound form), whereby the environment is unfavorable and bacteria die from lack of nutrients. Macrophages were infected with the virulent strain of *Brucella abortus* 2308 and virulence attenuated vaccine strains S19 and RB51. Intracellular bacterial multiplication and survival of the bacteria in control cells and in cells treated with Akt1 kinase inhibitor (Akti) was studied. Survival and bacterial replication capacity was quantified by counting events in flow cytometer. Treatment with a specific inhibitor of eukaryotic kinase Akt1 significantly reduced ($p < 0.05$) the progeny of the virulent bacterial strain 2308, and the vaccine strains lesser proportion which was evident with flow cytometry, trying to cells with a DNA marker as propidium iodide (PI) and compared to a control. Count viability of *Brucella abortus* was performed with and without Akti outside macrophages to confirm the action of this inhibitor to the level of Rab and discard direct action on bacteria. These results confirm that one of the intracellular mechanisms used by virulent *B. abortus* strain 2308 for survival involving kinase Akt1 pathway. The behavior in vaccine strains of attenuated virulence is different.

A41

ATMOSPHERIC POLLEN OF CUPRESSACEAE IN SAN LUIS CITY, ARGENTINA

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Many species of the Cupressaceae family are used for ornamental purposes in cities. These plants produce a lot of pollen that can be allergenic in persons with hypersensitivity. In San Luis urban afforestation, species of this family (genera *Cupressus*, *Platycladus*, *Juniperus*, *Chamaecyparis*, among others) are frequently cultivated. In this work, the Cupressaceae pollen content was analyzed during two years of aerobiological sampling in the atmosphere of San Luis city, Argentina. Pollen samples were taken with a Lanzoni (VPPS 2000) volumetric sensor and were read with an optical microscope at 400X. In addition, a Cupressaceae tree census was conducted in 1 km^2 around the sensor. One hundred and twenty-two individuals were recorded in the urban afforestation. *Cupressaceae* pollen type was the second most abundant in the San Luis atmosphere, after Moraceae. The pollination period extended from July to October. The maximum concentration values were recorded in August, with a peak of 486 pollen grains/m^3 of air, while from November to June it was less than 1 pollen grain/m^3 of air. The values of Cupressaceae pollen content during August doubled the high threshold, with the consequent risk to the health of sensitive individuals. It is recommended to reduce the ornamental use of these species in the city.

A42

ANIMAL MODEL TO STUDY ENDOGENOUS *Clostridium chauvoei* INFECTION

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Clostridium chauvoei is a Gram positive spore forming bacterium, causative agent of blackleg, a myonecrotic disease that affects leg muscles. The disease affecting livestock, is widely spread in Argentina and the world. The definition of the term "Sudden Death Syndrome" (SDS) often includes feedlot cattle found dead unexpectedly. The specific causes of deaths frequently can be determined by necropsy. Etiology and preventive measures are poorly defined. The current literature indicates that sudden death is often associated with digestive upsets. Death is thought to be the result of interactions between factors such as acidosis, bloat, and endotoxemia. Recently, SDS has been associated with specific *Clostridium perfringens* strain infections. The objective of this work was to study an animal model to determine endogenous *C. chauvoei* infection. *C. chauvoei* ATCC 10092 spores were obtained in a clostridial modified medium at 37°C under anaerobic conditions. Groups of two Rockland mice were orally administered with 10^6 and 10^4 spores in jelly-skim milk support during one month. The mice showed sudden clinical manifestations and euthanasia was performed. The manifestations included incoordination and weakness. Postmortem examinations were carried out immediately after death on each animal group. The gastrointestinal tract was collected and fixed.

Peritoneal fluid had 2 x 10^6 leukocytes/ml and presence of a large number of erythrocytes. Macroscopic observation indicated necrotic areas in the small and large intestine, some of them bloated. Our results suggest that *C. chauvoei* spores can germinate and produce gut colonization inducing mucosal necrotic damage. Further studies will determine if *C. chauvoei* could be associated with SDS in animals.

A43

ANTIMICROBIAL ACTIVITY OF *Annona emarginata* (SCHLTDL.) H. RAINER

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Infusions and decoctions of the leaves from *Annona emarginata* (Schltdl.) H. Rainer are commonly known as "arachichú", "araticú", "aratigú" and "yerba mora". In folk medicine, the juice produced by the leaves is used to treat throat pain and toothache. The extracts from *A. emarginata* flowers, fruits, leaves and stem barks were assayed by their antimicrobial properties against opportunistic pathogenic bacteria. Minimum inhibitory concentration (MIC) of each extract or compound was determined using broth microdilution techniques according to the Clinical and Laboratory Standards Institute guidelines. The flower extract (H, DCM) and the fruit global methanolic extract (MGEF) showed high antimicrobial activity against *Escherichia coli* ATCC 25922, PI-*Yersinia enterocolitica* and MI-*Salmonella enteritidis* (MIC values were in the range of 16-32.5 µg/mL). MGEF was subjected to bioassay-guided fractionation by successive columns (Sephadex LH 20 and silica gel column) which led to 8-transp-coumaroyloxy-α-terpineol (1) as the compound responsible for the elevated antimicrobial activity (MIC values were in the range of 3-4 µg/mL) against enterobacteria as *E. coli*, *Y. enterocolitica* and *S. enteritidis*. In addition, the compound **1** showed a strong antimicrobial activity (MICs = 4-8 µg/ml) against Gram positive strains such as methicillin-sensitive *Staphyloccocus aureus* ATCC 25923 and methicillin-resistant *S. aureus* ATCC 43300. Compound **1** was chemically elucidated using 1D and 2D NMR, IR, UV, and HRMS experiments.

A44

ANTIGENIC SIMILARITY BETWEEN *Larrea divaricata* CAV. AND *Origanum vulgare* PROTEINS WITH BACTERIA AND PROTOZOA PROTEINS

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Larrea divaricata Cav. (Jarilla) and *Origanum vulgare* (Oregano) are known in folklore medicine. Previously, we demonstrated that aqueous extracts of both plants have immunomodulatory effects and antimicrobial activity. Due to antibiotic resistance, toxic effects of antiparasitic drugs, side effects and high costs, there is great interest in the search for new therapies based on phytoextracts. The purpose of this study was to evaluate the extent of antigenic cross-reactivity among vegetable proteins and bacterial and protozoal proteins to identify shared antigenic components. *Helicobacter pylori* is a gram-negative bacillus that colonizes the human stomach and it is the cause of peptic ulcer and gastric cancer. *Trypanosoma brucei* is a parasite that causes african trypanosomiasis (or sleeping sickness) in humans a nd animals in Africa. *H. pylori* 146128 reference strain and the *T. brucei* 29-13 strain were used. Oregano (Op) and Jarilla (Jp) proteins were partially purified by 10 kDa cutoff concentrators. *H. pylori* and *T. brucei* proteins were obtained by sonication. Mice were immunized with Op and Jp. The cross reactivity of antibodies anti-Jp and anti-Op was evaluated by qualitative and semiquantitative ELISA. IgG levels were expressed as ELISA Index (EI) and antibody titers ($\log_{10}$). Homologous EIs were 1.74 and 1.21 with anti-Jp10 and anti-Op10, respectively. Anti-Jp10 and anti-Op10 reacted with *H. pylori* and *T. brucei* proteins. Anti-Jp10 IgG cross-reaction was significantly higher for *T. brucei* ($p= 0.03$). However, no significant difference between IgG titers was observed in anti-Jp10 and anti-Op10 sera. Jp and Op are immunogenic proteins and showed antigenic similarity with *H. pylori* and *T. brucei* proteins. They appear to have potential as vaccine candidate antigens against diseases caused not only by *H. pylori* but also by protozoa as *T. brucei*.

A45

MOLECULAR ANALYSIS (NGS and PCR/DGGE) OF RUMINAL BACTERIA OF GOATS DURING SHIFT FROM FORAGE TO CONCENTRATE DIET.

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High-grain feeding used in the animal production is known to affect the host ruminal bacteria, but our understanding of consequent changes in goats is limited. Considering this, the goal of this study was to evaluate the ruminal bacteria dynamics during adaptation to a high concentrate diet in 4 fistulated goats, which were fed for 30 days with alfalfahay (AH). Later on, 40% of corn (C) was joined, maintaining this diet (AH/C) for 20 days. Samples of rumen contents were taken on days 2, 10 and 20 for both diets, with their respective pH measurement. The sequence of16S rDNA for the analysis of bacterial populations was

amplified by two culture independent methods (PCR/DGGE and NGS). In the beginning, the measurement of the pH was taken, which showed a significant decrease, but without exceeding the limit established for acute and subacute ruminal acidosis. PCR/DGGE analysis showed that bacterial population were grouped considering the diet but not strictly reflect the sampling day, indicating some stability in the ruminal bacteria core, despite high individual variation. The NGS analysis showed a significant decrease ($p<0.05$) of *Butyribibri* and *Prevotella* in AH/C-diet fed goats (4.6±1.3 and 7.6±1.1%, respectively) compared to AH diet (7.0±0.6 and 12.4±3.2%, respectively).While a significant increase ($p<0.05$) of unclassified genus of groups of Bacteroidales and Ruminococcaceae was observed in AH/C-diet fed goats (25.7±3.2% and 8.6±0.9, respectively) compared to AH diet (15.1±4.7% and 6.1±1.6, respectively).NGS provides valuable information of the ruminal bacteria dynamics, which appears to be different from.

A46

Yersinia OUTER PROTEIN P (YOPP) INTERACTS WITH THE ENDOGENOUS LECTIN GALECTIN-1.

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Yersinia enterocolitica (Ye) is a Gram-negative enteropathogenic bacterium. Ye injects the effector proteins called Yersinia outer proteins (Yops) into the cytosol of host cells. YopP induces apoptosis. Galectin-1 (Gal-1) is a "proto-type" β-galactoside-binding lectin widely distributed in host tissues. We previously demonstrated that the Ye-induced apoptosis of macrophages depends on both YopP and Gal-1. The aim was to demonstrate that Gal-1 binds to YopP, and that this interaction prevents YopP degradation. Secretion of Yops was induced in Ye wild-type (WT) or Ye yopP cultures. Binding of Gal-1 to YopP was evaluated by Western blot (WB) and ELISA using rhGal-1, and anti-Gal-1 or anti-YopP antibodies. Clustal Modeller 9v12v and 3Drefine were used for YopP modeling; and YopP-Gal-1 interaction was assayed using the server Dock / Pierr. Moreover, Yops were incubated with rhGal-1, and the stability of YopP was studied by WB at different times. The Gal-1 binding was observed to a band of Yops of Ye WT, whereas non binding was detected with Yops of Ye yopP. Glycosylation sites were found in YopP. The YopP band was conserved in samples pre-incubated with rhGal-1. We conclude that Gal-1 binds specifically to YopP and this interaction has a protective role against rapid YopP auto-degradation.

A47

TNF RECEPTOR DEFICIENCY DECREASES NITRIC OXIDE PRODUCTION AND DECREASES LOCAL INFLAMMATION DURING CUTANEOUS LEISHMANIASIS

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Leishmaniasis caused by *Leishmania (Leishmania) amazonensis* (LA) is a tropical infectious disease affecting different countries in South America. Due to LA infects macrophages, development of a Th1 immune response with production of TNF-α and IFN-γ is essential to eliminate this pathogens. The aim of our work was to evaluate the role of TNF receptor p55 subunit (TNF-R) in IFN-γ-induced nitric oxide (NO) production by peritoneal macrophages and their impact in cutaneous leishmaniasis. To this end we obtained peritoneal macrophages from TNF-R KO and WT mice and cultured for 24 hs with LPS (1µg/ml) and IFN-γ (10 UI/ml). NO production was determined by the Griess Reaction. Cutaneous leishmaniasis were performed by the infection of $1x10^5$ LA promastigotes at mice left hinds pads. After 7 weeks, cutaneous lesions were surgery obtained, minced, stained with CD45 and analyzed by flow cytometry. We found that TNF-R KO macrophages showed a decreased production of NO after LPS+IFNγ compared to WT (WT,untreated UT, 2,1±0,9µM;WT,LPS+IFNγ,19,0±1,5µM;TNF-R KO,UT 1,1±0,5 µM;TNF-R KO, LPS+IFNγ, 3,±0,5 µM; $p<0,05$). In addition, we found that infected TNF-R KO mice displayed a reduced percentage of $CD45^+$ leukocyte infiltrating infection site compared to WT (WT,UT,1,0±0,5%;WT,LA, 23±3%; TNF-R KO, UT 1±0,5%; TNF-R KO, LA, 11±2 %; $p<0,05$). In conclusion, these results suggest that TNF-R is crucial to produceg NO. In addition, our *in vivo* data suggest that TNF-R would also be important in the recruitment of inflammatory cells promoting TNF as a potential target in designing future vaccines to leishmaniasis.

A48

POSSIBLE VEHICLES OF *Yersinia enterocolitica* (YE) TRANSMISSION IN SAN LUIS, ARGENTINA, AND YE DETECTION LIMIT IN PURE CULTURE.

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Yersinia enterocolitica (YE) is a human enteric pathogen that causes enterocolitis, extraintestinal symptoms and immunological sequelae. Its transmission is through oral route, usually by contaminated foods, and its main reservoir is the pig. YE includes 6 biotypes and over 60 serotypes, and its pathogenicity is attributed to plasmidial and chromosomal virulence factors. The objectives of this work were: a) to detect and to characterize YE strains isolated from foods, and b) to determine the YE detection limit (DL) in plate culture and PCR directed to the housekeeping gene *ADNr16S*. For a), 80 samples of raw foods from bovine, porcine and poultry origin were processed. Samples were seeded in enrichment and selective culture media, and isolates were characterized by standard biochemical tests. For b), serial dilutions of a YE pure culture were made, then: i) each dilution was cultured on MacConkey agar, and ii) DNA extraction and subsequent PCR directed *ADNr 16S* gene were performed for each one. Among the total samples analyzed, four strains were isolated (5% positive samples) and characterized as belonging to *Yersinia* genus. The YE DL in culture corresponded to the 10^{-5} dilution with 45 cfu, while the DL obtained by *ADNr16S* PCR corresponded to the 10^{-6} dilution with 6 cfu. This study highlights the importance of the studied foods as possible vehicles for YE transmission in our region, and it also demonstrated that the PCR technique was 10 times more sensitive than the plating technique for YE detection from pure cultures.

A49

DESIGN OF A COMPETITIVE INTERNAL AMPLIFICATION CONTROL (cIAC) AND AN EXTERNAL AMPLIFICATION CONTROL (EAC) FOR *16s rDNA* PCR OF *Yersinia enterocolitica*

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Yersinia enterocolitica, an enterobacterium present in certain foods, can cause infections in humans. Its detection is possible by PCR targeted to the *16S rDNA* gene using the primer pair 16SYerF-16SYerR. However, food samples are complex matrices and they might contain PCR inhibitors. To avoid false negative results, it is advisable to introduce a competitive internal amplification control (cIAC) which is a DNA fragment usually cloned into a plasmid, that is co-amplified with the target DNA sequence by the same primer pair in PCR. The cIAC and target DNA products can be identified by their different molecular sizes. Thus, the cIAC is incorporated into the PCR reaction mixture where will compete for primers with the target gene. In contrast with positive samples where two bands (*16S rDNA* gene and cIAC) will be observed, negative samples will show only one band corresponding to cIAC. Furthermore, an external amplification control (EAC) which has the same size as *16S rDNA* amplicon (300 bp) is amplified in parallel as a positive control regarding the unknown sample. In this study, cIAC was designed by using bioinformatic tools, based on a 624 bp exogenous sequence from another organism, which was analyzed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to check absence of complementary regions to the primer pair used. Flanking fragments complementary to the primers were added to this sequence. cIAC was amplified by PCR using Taq polymerase and subsequently cloned into pGEM-T Easy® vector (pG, Invitrogen), yielding a 710 bp product. Besides, the *16S rDNA* amplicon was purified, inserted and cloned into the pG vector, generating a 300 bp fragment corresponding to EAC. Both controls will allow check the *16S rDNA* PCR efficiency in the detection of *Y. enterocolitica* from food samples.

A50

ANTIMICROBIAL EFFECT OF BACTERIOCIN FROM *Lactobacillus fermentum* ISOLATED FROM GOAT MILK ON PERISHABLES FOODS. SAN LUIS. ARGENTINA.

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In the last decades, food industry has been focused on the reduction of the use of chemical preservatives. Numerous investigations are aimed to discover new bacterial strains able to produce bacteriocins, natural antimicrobials, to be used as food preservatives. The purposes of this study were to evaluate the spectrum of antimicrobial activity of a strain of *Lactobacillus fermentum* isolated from raw goat milk, determine the nature of the inhibitory substance and study its effect on the conservation of perishable foods. The antimicrobial activity of cell-free supernatant (CFS) of a culture of the strain under study was evaluated against *Enterococcus faecalis, Listeria monocytogenes, L. innocua, Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* (liquid medium method). The peptide nature of the antimicrobial agent was studied by treatment of CFS with proteases. By Tricine-SDS PAGE the presence in the CFS of low molecular weight peptides was investigated. To study its possible application as a food preservative, aliquots of cooked pork shoulder, semi-hard cheese and cream were immersed in CFS, and then incubated in suspensions (10^7 CFU / ml) of *S. aureus* and *E. faecalis* which are indicators of inhibitory activity. *L. fermentum* showed inhibitory activity superior to 70% against all the indicators tested. The peptide nature of the inhibitory substance was demonstrated by loss of antimicrobial activity of CFS after proteases treatment and the low molecular weight peptide obtained by electrophoresis. These results provide strong evidence that inhibitory substance is a bacteriocin. Bacterial counts in food treated

with CFS were between 10 and 100 times lower than in controls, according to the food, demonstrating that bacteriocin inhibited the growth of selected indicators. These studies confirm that *L. fermentum* produce a broad-spectrum bacteriocin which might be used to prevent spoilage of perishable foods.

A51
CHRONIC STRESS ENHANCES AUTOIMMUNITY EXPANDING IMMUNE CELLS AND INCREASING THE RECRUITMENT OF CD4$^+$ T CELLS TO TARGET ORGANS

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Immune cells can be modulated by several neuroendocrine mediators. It is known that stress may influence the immune system and even be associated to relapses of autoimmune diseases. The aim of this work was to evaluate whether exposure to stress worsens autoimmunity in the NOD (non-obese diabetic) mice which develops spontaneous Type 1 diabetes. To this end, twelve weeks old female NOD mice (n=6; two experiments) were subjected to 60 days of chronic variable stress (CVS) while female NOD control group remained untreated (UT, n=10; two experiments). CVS protocol consists in 5 stressful stimuli, isolation, restraint, illumination, 45° inclination and forced swim test. Disease severity was evaluated by the assessment splenomegaly and leukocyte infiltration of the pancreas. At the end of the protocol, mice were euthanized and spleens were weighed while pancreas were minced and stained by monoclonal antibodies against CD45 (pan-leukocyte marker) and CD4 (T cell marker). We found that CVS group showed an increase in the spleen weight compared to UT (UT,119±7 vs CVS 166±35; *t* test; $p< 0,05$). In addition, CVS group showed an increase in the CD45$^+$ cell population in the pancreas compared to the UT group (UT 0,779±0,122 vs CVS 2,169±0,50; *t* test; $p< 0,05$). Furthermore, the CVS group displayed a 3,2 fold increase in CD4$^+$ T cell infiltration of the pancreas compared to the UT group (UT 0,207±0,039 vs CVS 0,673±0,196; *t* test; $p< 0,05$). In conclusion, these results suggest that CVS increases leukocytes and T helper cells recruitment to the pancreas as well as expands immune cells as measured by spleen increase. Our data support the notion that stress plays a crucial role in autoimmune progression, promoting the study of hypothalamic-pituitary-adrenal axis as a target for new immunotherapeutic designs.

A52
Cryptococcus neoformans/gatti ISOLATES FROM ENVIRONMENT: CHARACTERIZATION AND BIOFILM ASSAYS

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The Cryptococcus neoformans / gatti complex consists of yeast environmental capped that by entering through inhalation and in immunosuppressed patients (mainly C. neoformans) trigger a systemic infection marked course and neurotropic. It was observed that the epidemiological distribution of the different species is not the same or uniform for each. From the emergence of AIDS was a significant increase in the number of cases, transforming this condition in the most important predisposing cause of this mycosis. Most AIDS patients with histoplasmosis have signs and symptoms of meningitis and subacute meningoencephalitis. The objectives for this second stage of labor were typing of strains isolated in the above, from public places in Mendoza stage and assess the ability of biofilm formation and susceptibility to antifungal agents commonly used. Colonies considered suspicious, were made basic identification tests (ink and urea) and then typed based on the presence or absence of different enzymes. Moreover, we worked on the development of the technical of biofilm production by a semiquantitative measurement for reduction assay hydroxide tetrazolium incorporated in a special medium, formazan tetrazolium, using a microplate reader to 492 nm. Positive are preserved for study of antifungal susceptibility. For all the positive isolates, based on the basic identification tests for the genus Cryptococcus, which corresponded to 27.8% (64/230) of all samples studied, were completed typing. Regarding the development of the technique for the production of biofilm, the results are promising. The results are comparable with those reported by other researchers in different areas of the country. Moreover, it is necessary to continue with antifungal susceptibility test and biofilm production, to complete the epidemiological data.

A53
STUDY AND COMPARISON OF BIOFILM FORMATION BY REGIONAL *Clostridium* spp. STRAINS

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Clostridium perfringens is an anaerobic gram-positive pathogen, causative agent of numerous diseases, biofilm with ability to form biofilm, recently informed. *C. septicum*, like *C. perfringens* is an opportunistic pathogen causative agent of myonecrosis. *C. chauvoei* is the causative agent of the stain, an infectious disease considered endemic in San Luis province. Bacterial biofilms are communities of cells adhered to surfaces, immersed in a extracellular matrix of that protects the antibiotic attack and increases its

resistance. The aim of this work was characterize phenotypically the biofilm formation of *C. perfringens*, *C. septicum* and *C. chauvoei* and compare with our preliminary studies on different surface. Strains used: *C. perfringens ATCC 3624*, *C. septicum ATCC 12464* and *C. chauvoei ATCC 10092*. For the biofilm formation, Petri dishes with OMA (optimized medium for anaerobes) containing coverslips were cultured at 37°C in anaerobic conditions for 24, 48 and 72 h. For the planktonic and sessile cells analysis, Gram, 0,1% crystal violet colorations and scanning electron microscopy (SEM) were used. Quantification of biofilm and planktonic cells was performed by Abs 570/DO580 relationship. For statistical analysis Student's test was used. At 24 h a good production of biofilm was obtained, their proportion increase to 48 and 72 h for 3 strains. The Abs570/DO580 at 48 h of culture showed that the biofilm formation efficiency in glass surface was *C. perfringens*> *C. septicum*> *C. chauvoei* ($p \leq 0.05$). The same was observed with optical microscopy and similar results were observed with SEM. The biofilm formation on polystyrene plastic was as following: *C. septicum*> *C. perfringens*> *C. chauvoei* ($p \leq 0.05$). Influence of different surfaces on biofilm formation and affinity for bacterial adhesion to various materials was evidenced. The ability to form biofilm is very important in the pathogenesis and antimicrobial resistance because difficult the diseases treatment caused by these microorganisms.

A54
BIOFILM FORMATION BY *Escherichia coli* O157:H7 ON STAINLESS STEEL AND GLASS

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Escherichia coli O157: H7 is a common bacteria in the gastrointestinal tract of warm blooded animals via manure and contaminated irrigation water reaches plants. *E. coli* O157: H7 form a best biofilm on stainless steel that on glass. The biofilm formation also depends on the characteristics of the bacterial and the surface to which they will adhere, as hydrofobicity, electric charges of the bacteria and the surface. Biofilm formation contributes to the pathogenicity of *E.coli* O157: H7; microbial adhesion is increased in the presence of organic substrates. The biofilms are most productive in chicken between the meat and in the lettuce among the vegetables.

The culture media used were prepared with broths lettuce, spinach and bean sprouts and beef based on chicken, pork and beef Biofilm production on the two materials tested (glass and stainless steel) is higher on stainless steel, hence the importance of hygiene constantly and with suitable products to eradicate the microorganisms in the first stage that is reversible.

A55
SEROTYPES, ANTIMICROBIAL SUSCEPTIBILITY AND CLONAL RELATEDNESSS OF SHIGA TOXIN-PRODUCING *Escherichia coli* (STEC) STRAINS RECOVERED FROM BOVINE SAUSAGES

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Shiga toxin-producing *Escherichia coli* (STEC) can produce hemorrhagic colitis and hemolytic uremic syndrome (HUS) mainly after consumption of contaminated food of bovine origin. The O157:H7 serotype is frequently implicated in clinical manifestations, and some non-O157 serotypes can also be associated to human infections. Even though the antimicrobial therapy is not recommended, the study of antimicrobial susceptibility of STEC strains has epidemiological relevance. In this work, we studied serotypes, antimicrobial susceptibility and clonal relatedness of one strain (STEC 1) isolated from 52 cooked sausages (one positive sample, 1.9%) and three strains (STEC 2, 3 and 4) isolated from 58 fresh sausages (one positive sample, 1.7%) in San Luis, Argentina. Samples were purchased in different retail stores from August 2012 to July 2013, and subjected to molecular methods and culture for STEC detection. PCR for $rfbE_{O157}$ and *fliCh7* genes and serological reactions to assess serotypes were performed. Antimicrobial susceptibility was assayed by Kirby-Bauer method, and the clonal relatedness between strains was determined by *XbaI*-PFGE. STEC 1 was serotyped as ONT:H7 (NT: not typeable), STEC 2 was ONT:HNT, whereas STEC 3 and STEC 4 were O112:H19. All the strains showed resistance only to erythromycin. A 72.10% similarity was demonstrated by *Xba*I-PFGE, and the two STEC O112:H19 strains showed an identical DNA profile. At the present, the O112 serotype has not been involved in STEC outbreaks or sporadic cases in humans in our region. However, STEC O112 has been previously identified in cattle from Argentina and the presence of this pathogen in

A56

ANTIMICROBIAL ACTIVITY OF CHALCONES: COMPARISON BETWEEN E*scherichia coli* CLINICAL AND ATCC STRAINS

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E. coli is an important pathogen associated with a large number of human infections. The present work aimed to determine the synergistic effect of di-hydroxychalcones-nalidíxic acid combinations against strains of this microorganism. Using a kinetic turbidimetric method developed previously, the antimicrobial activity of 2´,3-$(OH)_2$-chalcone and 2´,4´-$(OH)_2$-chalcone (the final concentrations of Ch were 5, 10, 15, 20 $\mu g.mL^{-1}$ without exceeding 2 % ethanol) and its combinations with nalidíxic acid (constant concentration: 2 µg/mL) were assayed. Growth curves were obtained for both microorganisms in culture media added of di-hydroxychalcone increasing amounts. According to a bacteriostatic inhibition mechanism proposed above, the specific growth rate (µ) varied linearly with the concentration of the antimicrobial in the form: $\mu = \mu_T - k_I \times C$, where µ: specific growth rate in bacteriostatic agent presence (min^{-1}); μ_T: specific growth rate (control), k_I: specific inhibition rate (µg.min) and C: antibacterial compound concentration (µg/mL). The application of this action mechanism allowed the evaluation of minimal inhibitory concentrations (MICs). The quantitative determination of antimicrobial activity of these compounds was evaluated in clinic strains isolated from urinary tract infections in patients of hospitals in San Luis city, and in the ATCC 25922 strain. To facilitate comparison of the inhibitory action of dihydroxichalcones and their combinations with nalidixic acid against *E. coli*, the percentage bacteriostatic efficacy (PBE) it was also determined. All combinations assayed showed synergism. However, the results achieved in the ATCC strain proved to be much more efficient than those obtained in clinical isolates. This occurs because clinical strains were previously in contact with some antibiotic and probably developed antimicrobial resistance mechanisms. This was not observed in the ATCC strain. It is necessary to consider new strategies for the development of novel therapies for infections caused by this microorganism.

A57

INFLUENCE OF STIRRING ON GROWTH AND EXOPOLYSACCHARIDES (EPS) PRODUCTION BY *Nostoc minutum*

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EPS have found applications in many industrial sectors because of their interesting physical and chemical properties. Among the microorganisms that produce EPS, cyanobacteria can be regarded as a very abundant source of structurally diverse polysaccharides. Cyanobacterial EPS possess unique properties for specific applications to currently available polymers are unsuitable. *Nostoc minutum*, a diazotrophic cyanobacterium locally isolated, has been investigated for its EPS production capabilities. The culture medium used was Watanabe (W), a simple culture medium without nitrogen source added. Cultures were incubated at 30°C in a temperature-controlled room for 14 days under continuous illumination of 9.860 lux with or without stirring conditions. Growth was determined by optical density (OD) at 580nm and by dry weight determinations at different time intervals. The EPS was estimated by dry weight determinations following the Mondal technique. Alcian Blue and India ink staining were used for qualitative determinations. It was observed that biomass production at the end of the culture (14 days) was higher in stirred conditions (0.88 g/L) and EPS production was stimulated in the same conditions (2.67g/L). Stained preparations, with both Alcian blue and India ink, showed an EPS production according to that determined by dry weight technique when they were observed at light microscope. Diazotrophic conditions may be a stress factor that enhances EPS production. We concluded that the stirring conditions were the best both for growth and EPS production for *N. minutum* growing in W medium.

A58

PROLACTIN IS A NEW SELF-ANTIGEN OF THE AUTOIMMUNE RESPONSE IN THE TYPE 1 DIABETES NOD MURINE MODEL.

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Autoimmune diseases are chronic and difficult to treat pathologies that produce a negative impact on the community. NOD (non-obese diabetic) mice are an animal model used for the study of Type 1 diabetes displaying autoimmunity against pancreas leading to a reduced fertility with advancing age. The aim of this work was to evaluate the presence of autoantibodies against hypophysis which in turn, may impair fertility. Serum samples from adult female and male NOD and female C57BL/6 mice were collected. Hypophysis and pancreas total antigens were obtained from Sprague-Dawley rats and C57BL/6 mice respectively, while purified ovine prolactin was purchased. The presence of IgG autoantibodies against pancreas, hypophysis and prolactin were evaluated by ELISA. As expected, we found that female NOD mice developed diabetes measured by the presence glucosuria and

autoantibodies against the pancreas (p< 0,05). In contrast, autoantibodies against the pancreas were absent in male NOD and female C57BL/6 mice. Interestingly, we found high levels of IgG against hypophysis only in female NOD mice(p< 0,05) compared to male NOD and female C57BL/6 mice. Furthermore, when we evaluated prolactin as a new target of the autoimmune response, female NOD mice showed increased levels of anti prolactin IgG compared to male NOD and female C57BL/6 mice (p< 0,01). In conclusion, we found that female NOD mice displayed a significant autorreactive response of IgG specific to hypophysis and prolactin, suggesting that an imbalance in hormonal status provoked by autoimmunity may promote diabetes progression and infertility. The screening for these new autoantibodies could be a novel contribution to an accurate diagnosis of endocrine pathologies.

Biotechnology and Genetics

A59
FUNGI FROM ALPERUJO: ISOLATION OF PHENOLIC DEGRADING STRAINS

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Alperujo (AL) is a semi solid agro-industrial waste from olive oil extraction in two-phase systems, composed by olive pomace, olive husk and water. If a natural decantation process occurs, a liquid fraction is obtained, of dark brown color, pH 3-6 and organic matter, including highly contaminant phenolic compounds (PC), sometimes reported as responsible for the toxicity attributed to the AL. Biological treatments involving filamentous fungi have been proposed for the detoxification of AL. The aim of this work is to isolate fungi growing in culture media containing AL and to evaluate its ability for PC degradation. At first, several fungi naturally grew in AL were cultivated in Petri dishes containing potato-dextrose agar medium. By using T-Method spreading and after several isolating procedures, four fungi morphologically different were isolated and named as LA1, LA2, LA3 and LA4. Then for studying PP degrading ability, each of 4 fungus isolated were cultivate in 4 different culture mediums containing Czapeck Salts in AL liquid fraction dilutions %v/v (30, 50, 80, 100) and agar. The fungi were incubated for 7 days at 28°C. Results show that LA1 and LA3 only grow in the medium containing AL liquid fraction 30% v/v dilution. LA4 did not grow at all and LA2 grew in all the culture mediums. The conclusion is that fungus AL2 shows the best ability for growing in the high phenolic content conditions. Experiences about PC degradation and enzyme activities using the LA2 isolation, are in progress.

A60
STUDY OF ANTIFUNGAL ACTIVITY IN VEGETABLE TISSUE EXTRACTS

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Bioactive compounds of vegetable tissue are a natural source of fungicides. The aim of this paper is to describe in vitro antifungal activity of ethanol extracts of papaya leaves and perform identification tests flavonoids, which are responsible for the inhibition of fungal growth. Extraction of bioactive compounds was carried out by using ethanol under constant stirring. The presence of flavones, flavonols and flavanones by Shinoda test was analyzed; by adding a trace of magnesium and 1ml of concentrated HCl to 100 µl of extract. The presence of flavones and flavonols was evaluated by dissolving 100 µl of the extract in 1 ml 2M NaOH or H2SO4 1ml concentrated. Positive reactions were considered those that gave yellow solutions. The presence of flavonoids with a carbonyl group and a hydroxyl group in position 5 was determined by dissolving 100 µl of the extract in 1ml of concentrated H2SO4 and adding 2mg of boric acid. Positive reactions formed yellow complexes with yellow-green fluorescence. Flavonoids of vegetable tissue were extracted and purified by aqueous biphasic systems consisting of ammonium sulfate and ethanol. The phases were separated and the top phase was used to analyze the antifungal activity as this is the one containing most flavonoids. Fungi from decaying fruit were used, which were cultured on potato dextrose agar for four to seven days at room temperature. Inhibition of mycelial growth was determined by cutting discs of 5mm diameter from the end of a colony of fungi and placing them in the center of a Petri dish with potato dextrose agar, containing aliquots of extract papaya leaves. The minimum amount to inhibit fungal growth was 400 microliters of extract 0.1 g/ml concentration. For 100, 200 and 300 microliters a delay in the growth of colonies was observed compared to the control.

A61
IN VITRO ESTABLISHMENT OF *Salvia hispanica* L.

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Salvia hispanica L (Lamiaceae), known as "chia" is an herbaceous plant native to central and southern Mexico and northern Guatemala. It contains among its main components acids and alpha-linoleic and linoneic, representing the largest natural source of essential omega-6 and omega-3 important in human nutrition since that reduce the risk of cardiovascular disease. The establishment of this species in vitro allowed to use the potential of various biotechnical suitable for clonal propagation and breeding. In that sense, this paper reports the results of disinfection treatments most appropriate initial explant. The culture medium used was Murashige Skoog complete and without growth regulators with agar (6g) and sucrose (20g). The seeds subjected to five disinfection treatments with different combinations of detergent, 70% ethanol, 3% sodium hypochlorite, hydrogen peroxide 20% and cefotaxime 500ppm were used. In all cases three washes with distilled water were conducted. For each treatment the sample size was 60 seeds. The detergent (5') treatment, 3% sodium hypochlorite (6') and alcohol 70% (3') were the most effective for pollution control and germination rate. The seeds showed contamination, and also presented low percentage of germination in both treatments with 20% hydrogen peroxide (10') and cefotaxime 500ppm (10'); these results demonstrate that they can cause inhibition in seed germination. The growth rate was 0.11cm /day to 72 days with an average of 8 axillary buds by each plant. It continues with the next stages of multiplication to complete the process of micropropagation.

A62
SYMBIOTIC EFFICIENCY OF *Vicia* spp. RHIZOBIA FROM PAMPEAN SEMIARID REGION

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Biological N2-fixation through symbiotic interaction between rhizobia and legumes is a major source of N input into the terrestrial ecosystem and therefore of great agricultural and ecological significance. However, an efficient symbiotic association is required for the successful use of rhizobia as inoculants. Thus, the purpose of this study was to select native rhizobial strains nodulating *Vicia* spp. adapted to the environmental conditions of the Pampean semiarid region for inoculant production. A collection of 160 rhizobial isolates from field-grown *Vicia* spp. plants were characterized by BOX-PCR genomic fingerprinting to distinguish isolates at the strain level and the assessment of genotypic diversity. Cluster analysis of fingerprints revealed 4 groups among isolates at 70% similarity level related with their geographical origin. Within each cluster, isolates showed highly similar fingerprints, thus, we chose one representative strain of each group to evaluate their symbiotic efficiency on *Vicia* sp. In greenhouse inoculation assays, the native strain named 25b and *Rhizobium leguminosaru*m D70, the strain commonly used as seed inoculant, showed the best performance among all treatments. Plants inoculated with 25b or D70 strains showed a significantly increase in N content (53 and 61% respectively) than those from N-fertilized treatments. The use of native strain 25b as inoculant, adapted to the soil characteristics and environmental conditions of Pampean semiarid region, will maximize the benefits of symbiotic N2-fixation and contribute to the success of inoculants for *Vicia* spp. for this region.

A63
ANALYSIS OF RATE IN VITRO MULTIPLICATION OF *Hedeoma multiflora* BENTH

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Hedeoma multiflora Benth. (Lamiaceae) is aromatic and medicinal perennial plant. The ethnobotanical use attributes flavoring properties, digestive and rheumatic pains. Has glandular hairs secreting essential oils mainly composed of a mixture of terpenes; monoterpenes being the main constituents. Because of its many uses, this aromatic and medicinal species is very depressed and endangered, making it necessary to implement efficient production methods to achieve sustainability of plant resources. The aim of this work was to study the *in vitro* multiplication rate of *H. multiflora* evaluating different nutritional conditions. Binodal segments (explants) of *H. multiflora* were cultivated on Murashige and Skoog (MS) Medium 50% and Woody Plant Medium (WPM) supplemented with naphthalene acetic acid (NAA) and benzyl amino purine (BA). Were studied the following treatments: 0.01 mg/l NAA and 0.25 mg/l BA (T_1); 0.01 mg/l NAA and 0.1 mg/l BA (T_2); 0.1 mg/l NAA and 0.5 mg/l BA (T_3); 0.5 mg/l NAA and 0.5 mg/l BA (T_4); 0.05 mg/l NAA and 0.5 mg/l BA (T_5). The MS 50% treatments (T_1, T_4 and T_5) and WPM treatments (T_2, T_3 and T_4) were studied. The explants were cultivated in 50 ml of sterile nutrient medium, and cultured at 24 ± 2 °C with 16 hours photoperiod and an intensity of 48 $mmol.s^{-1}.m^{-1}$. To determine the multiplication rate, the average number of sprouts obtained by explant was calculated at twelve weeks of culture. In MS 50% the following averages of these measurements were obtained: 40.6 for T_1; 119.2 for T_4; and 129 for T_5. While for WPM medium it was obtained: 64.4 for T_2; 130.5 and 135.5 for the T_3 to T_4, respectively. These essays demonstrated that the T_4 has a high multiplication rate beyond the salt concentration of the medium. These results will contribute to the multiplication of quimiotypes in breeding programs and germoplasm conservation of this specie.

A64

REDUCTION OF PHENOLIC COMPOUNDS OF ALPERUJO BY USING FILAMENTOUS FUNGI IN SOLID STATE FERMENTATION PROCESS

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San Juan is among the four provinces with the highest production of olive oil from Argentina. Most factories have replaced your traditional methods of extraction of oil by continuous two-phase systems. With this technology are obtained, in addition to oil, a semi-solid residue called "alperujo". This residue, presents difficulties related to final disposal, because of its contaminating action derived from its high content of polyphenols. The annual amount of alperujo generated in San Juan is estimated to be 45,000 tons. Of this residue, 30% is transferred to the only dehydration plant that exists in the province; a small percentage is composted or used as animal feed, fuel boiler, etc. The rest is applied directly and often uncontrolled in soils. Given this scenario in this work the possibility of using a laboratory scale solid state fermentation by inoculating filamentous fungi is evaluate; and the ability to reduce phenolic compounds of alperujo is determined, to make their safe disposal or use in processes that add value to the residue. Four filamentous fungi isolated from alperujo of San Juan factories, were inoculated on a mixture of alperujo (80%) and grape stalks as support (20%). Samples were taken every other day for two weeks, and the polyphenol content and percentage discoloration were measured. It was observed that the four filamentous fungi had approximately the same behavior until the eighth day (59% decrease of polyphenols), but one of them (LA2) achieved a greater reduction in phenolic compounds (90%) to the thirteen days of culture. Discoloration profiles had correspondence with decreasing phenolic compounds. These results encourage us to perform optimization of culture conditions using the microorganism selected for its ability to detoxify the alperujo, including the study of enzymes responsible for detoxification.

A65

SCREENING RELEVANT CULTURE CONDITIONS FOR GROWTH OF *Pleurotus ostreatus* AND PHENOLICS BIODEGRADATION IN ALPERUJO, BY PLACKETT–BURMAN DESIGN.

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Alperujo (AL) is the semi-solid, strong-smelling waste –containing bone, pulp and vegetation water– from the production of olive-oil by the two-phase extraction system. Its deposition into waterways and soil are not allowed, because AL contains pollutants (fatty acids, water soluble phenols, etc.) which produce a phytotoxic effect. On the other hand, the organic matter of AL includes lignocellulosic materials, of difficult biodegradation. About 45,000 tons/year of AL are generated in San Juan. In this work, it is proposed the cultivation of the edible mushroom *Pleurotus ostreatus*, as an alternative for detoxification and valorization of AL. Therefore, it is necessary to find the variables that affect significantly on the growth of the microorganism, and phenolics content. Solid state fermentations were done according the Placket-Burman design, in bags containing 400 g substrate (50% AL, 33% oat and 17% poplar chip), for fifteen days of culture. The variables studied were: initial water content, temperature, added grape stalks, added cotton, added grape pomace, added CO_3Ca, addition of urea, initial pH, lighting, and sterilization. As Response of the system, the microorganism growth was considered. Results show that increasing the initial water content and initial pH values were significant for good growth; the addition of grape pomace was significantly counterproductive; the rest of the variables had no significant effect, at the confidence level 99% and for the ranges of the variables stated in this opportunity. About the evolution of phenolic compounds, a decrease between 70% and 90% was observed in some experiments. Further optimization, fructification and scale-up study are in progress.

A66

EVALUATION OF THE GENOTOXIC POTENTIAL OF AFLATOXIN B1 IN DIET POST WEANING PIGS BY COUNTING CHROMOSOME ABERRATIONS

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Mycotoxins are secondary metabolic products of fungi that cause adverse effects on human and animal populations.
Causes a decrease in growth rate, reproductive capacity and immune function. Aflatoxin B1 (AFB1) is produced by certain strains of A. flavus and A. parasiticus, presents a marked hepatotoxic, mutagenic and carcinogenic effect. It is the most toxic and has a high impact on pig production. In this study *in vivo* genotoxic potential of AFB1 was evaluated by counting chromosome aberrations. Pigs from the Río Cuarto University Experimental Farm were used. Two groups of pigs after weaning were evaluated, one control and another fed *ad libitum* with contaminated provisions containing 48 ppb of AFB1. Genotoxicity was analyzed by cytogenetic analysis of peripheral blood lymphocyte cultures. *In vivo* test was made with post weaning pigs after ingestion of a dose of AFB1. Blood samples were taken on days 0 (before ingestion), 45 (post ingestion) and 63 (AFB1 free food) in order to assess whether the detected genotoxic effect is reversible. Chromatid and chromosome breaks, polyploidy, multiple aberrations cells and chromosome pulverization were counted. Among control and treated pigs were found difference,

but not statistically significant in the frequency of chromosomal alterations cells ($p<0.05$). No significant differences in the frequency of chromosomal aberrations and chromosome pulverization in the treated sows post ingestion AFB1. Post period mycotoxin intake in the diet indicates that AFB1 maintains its mutagenic potentiality in lymphocytes being able to induce chromosomal damage 45 days by feeding pigs with food with AFB1 a concentration evaluated in this work.

Reproductive and Developmental Biology

A67
ESTRADIOL PARTICIPATES IN OVARIAN GnRH SYNTHESIS AND RELEASE IN DIOESTRUS II.

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Estradiol (E_2) and Gonadotropin release hormone (GnRH) are involved in the regulation of reproductive processes, acting on peripheral organs and sympathetic neurons associated with reproduction. The objective was to evaluate the effect of E_2 (10^{-8}M) with and without the presence of GnRH antagonist receptor, Cetrorelix (Cx), in two different experimental schemes in Dioestrus II (DII). We used a)- the celiac ganglion-superior ovarian nerve- ovary system (CG-SON-OV) and b)- incubations of ovaries alone (OV) in DII. Both experimental schemes were incubated in Krebs Ringer at 37ºC, in specific cuvettes designed for this purpose. Periodic extractions of the ovarian incubation liquid were carried out at 120 min. GnRH was measured by RIA. ANOVA 1 followed by Tuckey test with a statistical significance of $p<0.05$ was used. In CG-SON-OV system, E_2 and Cx in CG significantly increased ovarian GnRH release (**$p<0,05$), E_2 in GC and Cx in ovary increased ovarian GnRH release (**$p<0,05$), E_2 and Cx in ovary of the system increased GnRH release (*$p<0.001$). In incubations of ovary alone, E_2 and Cx increased GnRH release (*$p<0.001$). This work confirms that our experimental scheme can demonstrate that GnRH and E_2 are interrelated with the peripheral nervous system to modulate the ovarian physiology. We conclude that these results and advances in knowledge about the presence of GnRH have utility in the clinic as GnRH analogues are used in treatments of precocious puberty, polycystic ovaries, endometriosis and cancer.

A68
NOVEL FINDING OF A TESTICULAR PROTEIN IN KIDNEY DUCTS

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During sperm maturation, tail structures are stabilized, through oxidation of thiol groups. It was proposed that sperm thiol oxidation is necessary for sperm motility. This oxidation occurs in many proteins of the sperm, like ODF1. ODF1 is a cytoskeleton protein which function in the sperm has not been clarified at all. ODF1 was described as a testis and sperm protein exclusively, and was involved in the development of the flagellum. The aim of this work was to evaluate the presence of this protein in other tissues. We applied western blot, RT-PCR, and inmunofluorescence techniques using an antibody against ODF1. Surprisingly, the strongest signal was found in the proteins obtained from marrow kidney and cells from collecting duct. Also some cortical ducts presented positive mark. Extracts from brain, liver, skin, and lung did not show positive signal to ODF1 antibody. By RT-PCR we identified the mARN of this protein in kidney and testis extracts. This was the first time that ODF1 was described in other tissue. In the future we will analyze if ODF1 participates in the development of the primary cilium in collecting ducts.

A69
EFFECT OF HYPERCHOLESTEROLEMIA IN PROSTATE OF ADULT MALE RABBITS: REVERSAL OF ADVERSE EFFECTS BY OLIVE OIL ADDED TO FAT DIET

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Hypercholesterolemia (HC) is a biochemist and serum 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 (FD), it was detected a decrease in seminal volume (Control 759.8 µl ± 68.66; FD: 432.2 µl ± 45.6), 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, as prostate. Therefore, the decrease in seminal volume could be explained by alterations in one or all of them. Mediterranean diet is a healthy diet indicated for HC treatment. Its main fat component is olive oil (OO). The addition of OO to the diet could reverse the glandular disorders. Prostate samples of animals under balanced rabbit diet (control), 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. Histology showed a decrease in villi area in FD and an increase in PD in comparison to control prostates (Control: 99621 ± 19727 μm^2; FD: 49272 ± 5228 μm^2; PD: 131808 ± 17931 μm^2, $p<0.05$). Moreover, images showed that villi from control prostate were longer than the ones from FD, where villi were almost absent depending on the time of diet. PD prostates were richer in prostatic concretions and their villi were shorter than the ones from the control, but thicker and more abundant.These preliminary measurements suggest that the function of prostate glands is affected and this could explain the reduction in semen volume. Thus, olive oil could reverse this negative effect.

A70
EXTRINSIC CHOLINERGIC INNERVATION OF RAT OVARY REGULATES THE MOLECULAR MECHANISMS OF APOPTOSIS UNDER THE INFLUENCE OF NITRIC OXIDE

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Using the *ex vivo* coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) system of Holtzman rats in the first proestrous, we previously reported that Acetylcholine (Ach) in CG increases the levels of nitric oxide (NO) and causes oxidative stress in the O. The aim of this work was to determine whether the effects caused by the ganglionic cholinergic stimulus promote apoptosis in the O. The CG-SON-O system was incubated at 37°C for 180 min in Krebs-Ringer bicarbonate buffer with 2% albumin. The CG and the O were placed in separate compartments, connected by the SON. Cholinergic stimulation of the CG was achieved by 10^{-6}M Ach [$(Ach)_{CG}$]. To analyze the influence of NO in altered ovarian function by the extrinsic cholinergic input, 400µM Aminoguanidine (AG), a selective inhibitor of inducible NO synthase, was added in both compartments: ovarian [$(AG)_O$] and ganglionic [$(AG)_{CG}$], separately, with and without ganglionic cholinergic stimulus $(Ach)_{CG}$. At the end of the experiments, ovaries were cleaned and flash-frozen in liquid nitrogen. The gene expression of Bax, Bcl-2, Fas and Fas ligand (FasL) was evaluated by RT-PCR and correlated with the apoptotic index. Statistical comparisons were performed by one way ANOVA followed by the Tukey's test. A probability of $p<0.05$ was used to denote statistical significance. $(Ach)_{CG}$ increased the expression of Bax, Fas and FasL (pro-apoptotic genes), and decreased the expression of Bcl-2 (anti-apoptotic gene), thus shifting the Bax/Bcl-2 ratio in favor of apoptosis ($p<0.05$). The opposite effect was obtained with $(AG)_O$ ($p<0.05$). In addition, $(Ach+AG)_{CG}$ decreased Fas expression ($p<0.05$). In conclusion, the results suggest that cholinergic stimulation of CG promotes the extrinsic and intrinsic pathways of apoptosis in the O as a result of oxidative stress mediated by NO.

A71
METFORMIN MODULES OVARIAN STEROIDOGENESIS IN RATS WITH POLYCYSTIC OVARY

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Polycystic ovary (PCO) is a complex endocrine disorder associated with anovulation, hyperandrogenism and ovarian hyperinnervations. Although metformin (N,N′-dimethylbiguanide) is employed widely in the treatment of patients with PCO, the mechanism involved remains unknown. The aim of this study was investigated the effect of metformin on steroidogenesis of PCO ovary and its relation with kisspeptin (Kiss), a neuropeptide with direct action on gonadal tissues. PCO condition was induced in adult rats by a single i.m. injection of estradiol valerate (2 mg/rat) and the animals were sacrificed, on estrus, two months later. Ovaries from PCO and no-PCO (control) rat were incubate for 3 h in metabolic bath with and without metformin (10^{-3} M) to measure the release of progesterone (P) and estradiol (E2) by RIA, and nitric oxide (NO) as nitrites, by Griess reaction. The Kiss mRNA expression was assessed in ovary by RT-PCR. Metformin-treatment increased P and decrease E2 release from PCO ovary compared with respective basal value ($p<0.01$), without changes in control ovary. In basal conditions, both, the NO release ($p<0.01$) and Kiss mRNA expression ($p<0.05$) increased from PCO ovary in relation to control ovary. Metformin-treatment increased NO release and Kiss mRNA expression compared with PCO ovary in basal conditions ($p<0.05$). Results suggest a direct effect of metformin to module the release of P and E from PCO ovary, in which are involved molecules as NO and Kiss. This mechanism could offset the low production of P that characterizes the PCO ovary, in an attempt to improve ovarian function.

A72

MACROPHAGE SECRETIONS OF SPLEEN INDUCE APOPTOSIS IN ANTERIOR PITUITARY OF RAT IN A POLYCYSTIC OVARY MODEL

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Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder in reproductive-age women. The Hypothalamic-Pituitary-Ovary axis plays a central role in its pathogenesis. Little is known about the interaction of immune cells with anterior pituitary (AP) in PCOS. We have shown that the androgenic environment of rats with polycystic ovary (PCO), induces a higher release and mRNA expression of tumor necrosis factor alpha (TNFα) from cultured macrophages (Mø). In this work, the effect of PCO Mø secretions on AP apoptosis is studied. PCO was induced in adult rats by 2mg/rat (single im. injection) of estradiol valerate, and the rats were sacrificed after 2 months. Spleen Mø, from PCO rats were cultured ($1x10^6$ cells) for 24 hs in RPMI medium. Their secretions were used to stimulate Control (C) and PCO AP for 3 h in a metabolic bath. Gene expression of Bax, Bcl2 and estrogen receptor alpha (REα) was determined by RT-PCR. Apoptosis was detected by TUNEL assay. The Bax/Bcl-2 ratio in PCO-AP was higher than in C rat ($p< 0,01$). When C-AP were incubated with PCO Mø-secretions the Bax/Bcl2 ratio increased in relation to basal incubation ($p< 0,001$), but this effect was not observed in PCO-AP. Apoptotic nuclei stanning increased in PCO-AP (22,8% *vs* 10,1%) and it was not modified after PCO-Mø stimulation (17,1% and 18,2%) compared to C-AP. In PCO-AP stimulated with SOP-Mø secretions, the expression of REα was lower than C-AP ($p< 0,01$). These results show an interesting relationship between immune and endocrine modulators, suggesting that PCO-Mø secretions may be involved in the regulation of AP apoptosis and it could be due to the high levels of TNFα synthesized by Mø, and increased levels of estradiol observed in this PCO model.

A73

INFLUENCE OF GONADOTROPIN-RELEASING HORMONE ANTAGONIST, CETRORELIX, FROM COELIAC GANGLION ON THE OVARIAN ESTRADIOL AND NITRIC OXIDE RELEASE

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There are precedents that demonstrate the extrahypothalamic origin of gonadotropin-releasing hormone (GnRH) as well as its inhibitory effect on ovarian steroidogenesis. Taking into account this information, the aims of this work were to study the effect of a GnRH antagonist on: 1) the ovarian release of estradiol (E_2) and nitric oxide (NO), 2) the ovarian gene expression of P450 aromatase (P450arom, E_2 synthesis enzyme), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) on day 21 of pregnancy in the rat. We used the *ex vivo* coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) system. The system was incubated in Krebs Ringer at 37ºC, keeping CG and O connected by the SON, in separate compartments. The GnRH antagonist: cetrorelix 10^{-6} M (Ctx) was added in the ganglion compartment. E_2 was determined by RIA and NO by the Griess technique in the ovarian compartment at 30 and 180 min of incubation. Ovarian gene expressions of P450arom, eNOS and iNOS were assessed by RT-PCR at 180min. One-way ANOVA and Tukey test were used ($p<0.05$). Ctx added in CG increased the E_2 and NO release significantly at 180 min ($p<0.01$ and $p<0.05$, respectively) without modify the enzymes expression analyzed in O. In conclusion, Ctx from CG stimulate the ovarian E_2 release being the NO the possible messenger molecule involved.

A74

ANDROSTENEDIONE IN SUPERIOR MESENTERIC GANGLION STIMULATES THE RELEASE OF PROGESTERONE IN OVARY ON DIOESTRUS II DAY. IMPORTANCE OF NITRIC OXIDE

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Androstenedione (A_2) is the principal androgen in the rat. The Superior Mesenteric Ganglion (SMG) has numerous steroid hormone receptors. Previous studies have shown that stimulation of these receptors is capable of modifying ovarian steroidogenesis.The objectives were to investigate whether A_2 in SMG modifies: 1) the ovarian release of progesterone (P_4); 2) the activity and gene expression of 3 β-HSD (P_4 synthesis enzyme); and to analyze whether such modifications are related with changes in the Nitric Oxide (NO) levels and gene expression of endothelial and inducible nitric oxide synthase (eNOS/ iNOS) on DII day. The *ex vivo* SMG-Ovarian Nervous Plexus (ONP)-Ovary system was incubated with A_2 added in ganglion. For this, we used a cuvette with two compartments with Krebs Ringer solution, pH 7.4, in a metabolic bath at 37 °C. P_4 (RIA) and nitrites (Griess method) were determined at 15, 30, 60 and 120 min. The gene expression was analyzed by RT-PCR at 120 min. Student's t Test and ANOVA-1 followed by Tukey test ($p <0.05$) were used for statistical analyses. A_2 in ganglion increased the ovarian P_4 release at 30, 60 and 120 min ($p<0.001$) and NO release ($p<0.001$) in all times of incubation ($p <0.001$). No changes were observed in the gene expression of 3β-HSD, but eNOS increased

($p<0.005$) and iNOS decreased ($p< 0.05$) with respect to the control group. In this work we demonstrated that A_2, through of ONP, is able to increase the ovarian P_4 release due to an increase in NO levels in ovary by an eNOS overexpression. These results may help to elucidate the role of A_2 in hormone-dependent women pathologies as polycystic ovary.

A75
Leishmania (Leishmania) amazonensis INFECTION IMPAIRS REPRODUCTIVE PARAMETERS OF FEMALE MICE

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Leishmaniasis is a group of parasitic zoonotic diseases caused by intracellular protozoa of the genus *Leishmania*. In Argentina, the transmission of cutaneous leishmaniasis has increased in intensity and frequency of cases since 1980. Although information exists about the great diversity of its clinical manifestations, little is known about the effects of this parasitosis on reproductive parameters and pregnancy of infected humans and pets. It is believed that there is an increase in the severity of the disease during pregnancy due to a change from a resistant immune response (Th1 profile) to a susceptible profile (Th2 response). The objective of this study is therefore to evaluate the influence of leishmaniasis on reproductive parameters and to describe the clinical signs it produces during pregnancy using a murine model. A control group of female BALB/c mice (n=8) and a group infected with *L. (L.) amazonensis* (n=16) were mated with healthy males. Body mass of both groups was registered and a clinical monitoring of injuries was made during the pre-gestation and gestation periods. Females were euthanized before delivery. Five embryonic resorptions and a fetal death were observed in the infected group. Furthermore, a decrease in the fertility rate was registered in the infected group (37.5%) compared to the uninfected control group (87.5%). The body mass of offspring from infected mothers was significantly lower than that of the healthy group ($p=0.004$). In conclusion, this study suggests that cutaneous leishmaniasis causes a decrease in fertility rates and body mass of fetuses, as well as fetal death and embryonic resorptions. The next step of the project will be to evaluate transplacental transmission of cutaneous leishmaniasis.

A76
OVERNUTRITION INDUCES NEURO-ONTOGENICAL CHANGES IN OFFSPRING BEHAVIOR PATTERNS.

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Altered nutritional experience during the lactancy period can impact on offspring health. Several studies have investigated the effects of artificial litter size adjustment on development. Electrophysiological studies performed on coronal slices obtained from brains of small litter (SL) and age-matched normal litter (NL) rats revealed significant changes in the response to various stimuli/inhibitors. The aim of this study was to investigate if neurobehavioral development in the pup was affected by the size of the litter. Wistar rat litters were divided into two groups: NL (11±1 pups) and SL (were adjusted gradually, 2-3 pups were sacrificed in 0, 3, 5, 7, 15 postnatal days). The pups were carried to the testing laboratory, identified and weighed. The spontaneous neurobehavioral patterns were determined in an open field. SL offspring exhibited significant increased weight at the end of first postnatal week (SL: 15.8 ±0.3 g vs NL: 13.3±0.3 g, $p<0.01$) and second postnatal week (SL: 32.3 ±2.4 g vs NL: 23.0±1.0 g, $p<0.001$). The open field test at postnatal day 15 shows significant increased time exploratory behavior in SL vs NL (59.4 ±7.5% vs 33.34 ±4.1 %, $p<0.01$), square crossing (40.0 ±4.8 sq vs 18.0±3.1 sq, $p<0.02$) and significant decrease grooming (3.2 ±1.0% vs 15.5 ±4.8%, $p<0.01$). Our results indicated advance maturation landmarks in SL litter due to a change in the neuro-ontogeny of the rat behavior patterns which could be direct influence of overnutrition.

Animal Anatomy and Histology

A77

EXPRESSION OF PROLIFERATING CELL NUCLEAR ANTIGEN AND ANDROGEN RECEPTOR IN BULBOURETHRAL GLANDS OF VISCACHA (*Lagostomus maximus maximus*).

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The viscacha is a caviomorph wild rodent and in their habitat the adult male exhibit a seasonal reproduction with a period of maximal reproductive activity in summer (activity) and minimal in winter (regression). The objective of the present work was to study the immunohistochemical variations of proliferating cell nuclear antigen (PCNA) and androgen receptor (AR) in the bulbourethral glands (BG) of immature animals and adult male viscacha. The adult animals (5-6 Kg) were captured between the months of february and march (n=5) and between july and august (n=5) and the immature animals (2 Kg, n=5) were captured during all the year. The BG were removed and processed for optical microscopy. The PCNA and AR immunodetermination was performed by the antibody AM 252-5M Biogenex and (N-20): SC-816 Santa Cruz Biotechnology, respectively. The BG parenchyma is divided in lobules constituted by glandular acini. In adult animals, the percentage of PCNA-positive cells of the glandular acini was significantly higher during the regression (70,41±2,7%) than during the activity period (9.90±1,27%). In immature animals was observed an intense and abundant immunostaining (90,19±0,98%) in the glandular acini cells. The AR distribution was scarce in the glandular acini cells but a moderate immunostaining in the stromal cells was observed in the three studied groups. In relation to our results, we observed variations in the PCNA-expression in relation to the reproductive cycle and the sexual maturity. The scarce AR-expression observed in the studied groups might suggest that the cellular proliferation, in the glandular acini cells of the bulbourethral glands, is not directly regulated by the androgens.

A78

VARIATIONS OF THE PROLIFERATING CELL NUCLEAR ANTIGEN IN THE EPIDIDYMAL INITIAL SEGMENT OF VISCACHA (*Lagostomus maximus maximus*) IN RELATION TO SEXUAL MATURITY.

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The proliferating cell nuclear antigen (PCNA) plays an essential role in nucleic acid metabolism as a component of the mechanism of replication and repair in mammals. The viscacha (*Lagostomus maximus maximus*) is a wild South American rodent with nocturnal habits and seasonal reproduction. The objective of the present work was to study the PCNA expression in the epididymal initial segment by immunohistochemistry, relating the results with the animal sexual maturity. The animals were captured in their habitat near San Luis city between 2012 and 2015 and were classified into adult (5–7 kg; n = 5) and pre-pubertal (3–4 kg; n = 5), according to their corporal weight and the light microscopy observations of testes. The blood samples were obtained from anesthetized animals. The epididymal samples were surgically removed and processed for optical microscopy. The serum testosterone levels were determined by a solid phase competitive chemiluminescent enzyme immunoasay and the PCNA immunodetermination by the antibody AM 252-5M Biogenex. The serum testosterone levels were significantly higher in adult (490,66±40,76) than in prepubertal (139,74±30,77). The percentage of PCNA-positive cells in the epithelium was significantly higher in adults (87.91±1.07) than in prepubertal animals (15.29±1.23). The immunostaining was moderate in the epithelial and stromal cells of adult and prepubertal animals. Our results suggest that the cellular proliferation might be strongly related to the serum testosterone levels, ensuring the maintenance of the epididymal structure and function during the animal reproductive life.

A79

CHYMOTRYPSIN AND TRYPSIN ACTIVITIES IN A FRESHWATER MOLLUSK

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Mollusks are the second largest and diverse animal phylum in nature. In general these animals present a well developed digestive tube with associated exocrine glands. Although there are few studies of enzymatic digestion in mollusks, chymotrypsin and trypsin have been reported in some species of the phylum. *Pomacea canaliculata* is a freshwater snail with important serine-protease activity in the intestine; however, the specific substrate activity has never been assayed. Here, we studied the chymotrypsin and trypsin activities in the digestive tract of this animal. Snails cultured in our laboratory were acclimated to a diet rich in protein content (~40%). Contents of crop, style sac and coiled gut, as well as samples of salivary and digestive glands were

obtained. Trypsin specific activity (mU/mg) was assayed using Bz-Arg-p-nitroanilide (BAPNA) as a substrate (A_{410}, 5min, 25°C). Chymotrypsin specific activity (mU/mg) was assayed using Bz-Tyr- Ethyl ester (BTEE) as a substrate (A_{256}, 5min, 25°C). Higher trypsin specific activity was found in coiled gut. The activities of all gut contents were higher than glands. Chymotrypsin specific activities of crop and style sac contents were higher than coiled gut content. In general, chymotrypsin activity was higher than trypsin activity along the digestive tract of this animal. The enzymatic activities were dependent of pH; higher enzymatic activities were recorded between 8.5 and 9.5. Our observations are similar to those found in some marine gastropods and bivalves.

A80
MORPHOLOGICAL DESCRIPTION OF VISCACHA (*Lagostomus maximus maximus*) HARDERIAN GLANDS.

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The Harderian glands (HG) are accessory lacrimal glands located in the posterior ridge of the ocular globe. The objective of the present work was to perform the anatomical and histological description of the HG of viscacha, a wild South American rodent with nocturnal habits and seasonal reproduction. For the histological study, the samples were fixed in Bouin's solution, dehydrated in an increasing ethanol series, embedded in paraffin and sectioned at 3–4 μm thickness. The sections were stained with Haematoxylin and Eosin and Masson's trichrome and the histochemical technique of PAS was performed. The HG is in the lacrimal fossa, located in the upper and external ridge of the orbit, outside the conjunctival sac. The HG is serous glands formed by tubulo-alveolar adenomeres constituted by cubical/cilindrical and myoepithelial cells, which flow into the lacrimal ducts. The glands are surrounded by a thin connective capsule and scarce connective tissue divides the glandular parenchyma. The histochemistry was negative in most of their cells, only the secretion was poorly positive. The literature related to these glands is scarce and our results constitute the first description of the HG morphological characteristics in a rodent with nocturnal habits. These results might be important to the development of further morphophysiological studies, contributing to elucidate the HG function and their relationship with the pineal gland activity.

A81
EFFECTS OF DIET ON FATTY ACIDS PROFILES IN MEAT FROM CREOLE KIDS

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Meat fatty acid composition is influenced by feeding regime. The objective of this study was to analyze the effect of two diets on meat from Creole kids. Maternal milk was supplemented with alfalfa pasture (diet A) or with concentrate (diet B); five males from each diet were slaughtered at 10 kg live body weight average. In muscles *Longissimus dorsi* (LD) and *Semimenbranosus* (SM) were determined: intramuscular fat % (IMF), cholesterol content and fatty acid composition. Lipids were extracted using the Folch et al (1957) technique. Fatty acids were determined using capillary gas chromatography with a column coated with CPSil 88 and a temperature program. Saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), conjugated of linoleic acid (CLA), omega 3 (n-3) and omega 6 (n-6) fatty acids were identified. Data were analyzed by ANOVA: IMF, Cholesterol and SFA were not affected by diet. IMF varies from 1.01 to 1.42%; Cholesterol was between 56.73 to 66.61 mg/100g and SFA differs from 35.63 to 36.81%. MUFA, PUFA, n-3, and CLA showed significant differences between diets in both muscles. MUFA had lower values in diet A (LD = 29.46% and SM = 33.74%) than diet B (LD = 33.74% and SM = 35.92%). PUFA were higher in diet A (LD = 20.12% and SM = 20.54%) than B (LD = 15.51% and SM = 15.07%). Kids from diet A showed higher proportion of n-3 in both muscles (LD = 6.18% and SM = 6.51%) than kids from diet B (LD = 3.61% and SM = 3.38%). Proportions of n-6 only showed superior values for LD in diet A = 14.35% and in diet B = 11.90%. CLA was higher in diet A for both muscles (LD = 1.74% and SM = 1.79%) than diet B (LD = 0.98% and SM = 1%). Although proportions of IMF in LD and SM were similar between diets, kids supplemented with alfalfa pasture had better fatty acids profiles, appropriate for human diets and health.

A82
APPARENT DIGESTIBILITY OF GARLIC WASTE IN ADULT CRIOLLO BIOTYPE GOATS

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In ruminants, normal rumen functioning requires 25-45 % of fiber in diet. Main source of fiber used on ruminants feeding on intensive systems is alfalfa. The inclusion of other sources of fiber requires information about nutrients balance and digestive behavior. The objective of this work was studying apparent digestibility of garlic herbaceous waste in adult goats. Ten adult goats

- criollo type - with similar age and weight were housed in individual pens located in the Experimental Ranch of Facultad de Ciencias Agrarias, UNCuyo. Three diets were formulated based in alfalfa, as fiber source, corn as energy source and with 0; 15 and 35% garlic herbaceous waste as alfalfa replacement. After 14 days of treatment, dry matter consumption and total feces production was quantified for another 7 days. Aparently digestibility was 54.0; 55.4 and 52.8 % respectively, with no significant differences between treatments (α=0.05). It concludes that inclusion up to 35% of garlic herbaceous waste in replacement of alfalfa does not modifies diet digestibility. Every nutritive fraction digestibility must be evaluated in order to determinate behavior as effective fiber of garlic herbaceous waste.

A83
GRAZING BEHAVIOR OF BREEDING COWS IN THE ARID ZONE

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Extensive arid rangelands used to breeding system (fewer than 350 mm annual precipitation and 15-20 ha.ev-1 receptivity) force a long walk to cows for collect their daily ration. Objectives of this work were to determinate: grazing travel and time of arid rangelands breeding cows according to distance from the source of water, quantifying grazing times and schedule and frequency between water intakes. Collars carrying GPS system were put in 22 adult Aberdeen Angus breeding cows in two different breeding rangelands of General Alvear, Mendoza, in intermittent periods for 2 years, during winter and summer. Data from GPS was obtained using Holux TM software allowing visualizing cows travel and overlapping over Google Earth images. Using math modeling it was verified that animals grazed 8.77 h rest or ruminate 14.31 h and walk 2.5 km per 0.92 h. daily (mean values). In winter season animals dedicate 2.33 more hours to rest or ruminate than in summer season. Grazing and walking activities were done 60% in daytime and 40% nighttime. Mean distance from grazing zones to water source was 2133 m, observing intense grazing activity until 3500 m. Mean water intake frequency was 1.6 days (mode value 2 days). It concludes that grazing activity in arid rangelands takes place further than the distances recommended for humid or sub humid grazing areas.

Pharmacology and Toxicology

A84
ANALYSIS OF DIAGNOSES AND PRESCRIPTIONS IN A HOSPITAL OF SAN LUIS, ARGENTINA

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The purpose of this research was to analyze the drugs prescriptions in a hospital of primary attention of San Luis (H). An observational, descriptive, transversal study, and of indication-prescription was designed. Data of the Pharmacy Service corresponding to 2551 registers from all Services, were collected during June 2012: socio-demographic characteristics, prevalent diagnoses (Di), prescribed drugs (D), polypharmacy (P). D and Di were classified according to ATC and ICD-10 classifications, respectively. Existence of P was considered when 3 or more D were prescribed simultaneously. Statistic method: Chi square. Results as % were: Gender: M (41), F (58) unknown (1). Age: ≤15 (22), 16-65 (27), 61-93 years (1), unknown (50). Services: Medical Clinic (MC, consultation: 55, guard: 30), Odontology 13, Gynecology 2. Di: caries (12), cold (8), pharyngitis (4.6), laryngitis (3.5), syndrome bronchial (3), hypertension (2.5), gastroenteritis (2.4), obstructive bronchitis and urinary infection (2). P: 6.9. D (ATC group): M (27.4), R (16.3), A (13), J(11), H(8.4). D: Ibuprofen (17.4), diclofenac (9.7), diphenydramine (8.4), dexamethasone (7.6), amoxicillin (7). The female sex, adults and child were prevalent. The service most consulted was MC. The prevalent pathologies were acute type, might be because the H is of primary attention, where child and young adults are the majority of patients, and because the respiratory diseases are frequent in the season of data recollection. Drugs most used were los NSAIDs, following by antiallergics, corticoids and antibiotics. The prevalent Di were of the respiratory, digestive and musculoskeletal systems. Although the P detected was low, equally this favors the emergence of interactions that can endanger health or life of the patient. It is worrying the elevated prescriptions of drugs with a low benefit / risk ratio, unnecessary use of antibiotics. It is necessary periodically report these results, and train to health professionals respect to the appropriate use of drugs.

A85

DIFFERENTIAL BIOCONCENTRATION OF CADMIUM IN TISSUES AND ENDOSYMBIONTS OF THE SENTINEL ORGANISM *Pomacea canaliculata*

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Two morphs of an endosymbiont (identified as C and K corpuscles) are found within cells of the digestive gland in *P. canaliculata*. Since previous evidence suggested that cadmium produced a decrease in the frequency of copulation and the number of egg masses, and an increase of non-embryonated eggs in this snail, experiments were launched to measure cadmium concentrations in both morphs of the symbiont, as well as in samples of digestive gland, kidney, uterus and testis. Snails were cultured in reconstituted water (prepared with American Society for Testing and Materials type I water) and then exposed to different concentrations of cadmium in the aquarium water (50, 250, and 500 ug/L) for 28 days. Graphite furnace atomic absorption spectroscopy (instrumental detection limit = 0.03 ug/L) was used to determine cadmium. This element was detected in all tissues and endosymbionts of all exposed snails. High levels of cadmium were found in the digestive gland (50 µg/L) and kidney (250 and 500 µg/L), while they were lower in testis and uterus. The bioaccumulation in the digestive gland was at the expense of both morphs of the endosymbiont. These observations indicate that, after the ingress in the snail, cadmium is distributed preferentially in the digestive gland (which includes the endocytobiont) and kidney of *P. canaliculata*. Bioaccumulation of cadmium in testis and uterus (which was much lower) could explain the deleterious effects in reproductive functions. Together, these results suggest that this freshwater snail may be used as a bioindicator of cadmium pollution in limnic environments.

A86

TREATMENT DURING LATE PREGNANCY WITH ACE INHIBITORS MIMICS BRONCHOPULMONARY DYSPLASIA IN PRETERM BORN ANIMALS

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Preterm birth affects 8-10% of human pregnancies. Individuals born preterm, especially if they develop bronchopulmonary dysplasia (BPD), have an increased risk of impaired lung function in infancy, childhood and adulthood. The etiology of BPD is multifactorial. Genetic susceptibility and environmental factors have all been implicated in the etiology of BPD. The renin-angiotensin system (RAS) during fetal or neonatal stages has been involved in lung growth and differentiation process. The present study tests the hypothesis that exposure during critical stages of lung development to ACE inhibitors could be associated with alterations in pulmonary structure that mimics human BPD. Pregnant Wistar rats were administered subcutaneously (osmotic mini-pumps, Alzet) during late gestation (E13-E21) with captopril or enalapril (2,85 mg/kg/day) and lungs from their offspring were analyzed at P0, P8 and P15 postnatal ages. We performed an histological and morphometric analyses, as well as immunolocalization of proliferating cell nuclear antigen (PCNA). At the different ages, animals showed impaired alveolarization, hyperplasia of airway smooth muscle and increased muscularization of pulmonary vessels. These anatomical changes were present as early as P0 with both treatments. Differences between treatments were observed with regard to the level of PCA. This model could be useful in understanding the pathogenesis of BPD and to define therapies to ameliorate human BPD.

A87

ANGIOTENSIN RECEPTOR NEPRILYSIN INHIBITOR LCZ696 ATTENUATES CARDIAC REMODELING IN EXPERIMENTAL MODEL OF METABOLIC SYNDROME

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Hypertension and heart failure (HF) are major causes of death and morbidity in the Western world, and their prevalence is projected to increase. Increasing recognition that sustained overdrive of neurohormonal systems such as the renin–angiotensin–aldosterone system (RAAS) is involved in hypertension pathophysiology has led to the introduction of drugs inhibiting key components of the RAAS into clinical practice. The aim of this study was demonstrate the participation of LCZ969 (sacubitril valsartan; L) in cardiovascular remodeling. Methods: Male WKY and SHR were separated into five groups: Control, FFR: WKY rats receiving a 10% (w/v) fructose solution during all 12 weeks, SHR, FFHR: SHR receiving a 10% (w/v) fructose solution during all 12 weeks and FFHR+S: (68 mg/kg per day for 6 weeks) (n=8 each group). Metabolic variables and systolic blood pressure were measured. Cardiac remodelling was also evaluated. Results: L reverted an increase in systolic blood pressure but did not modify metabolic laboratory variables. Thus, chronic administration of L was able to revert cardiovascular hypertrophy. Conclusion: All these data suggest the involvement of RAAS and neprilysin system on the expression of different inflammatory

and growth factors proteins in myocardial area, allowing the origination, perpetuation, amplification and destabilization of cardiovascular injury.

A88

DIURETIC EFFECT OF *Lithraea molleoides* (ANACARDIACEAE) FRUITS IN RATS

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In the present study evaluated the diuretic effect of *Lithraea molleoides* fruits (Vell.) Engl. (Anacardiaceae), known as "molle de beber", "molle dulce" or "chichita". It is a tree which grows in South America, especially in Argentina, Brazil and Uruguay. The aerial plant parts are used in folk medicine as diuretic and digestive, and the fruit infusion is used as natural sweetener. The aim of this study was determine the diuretic activity of the fruit in Wistar rats, using the Lipschitz method. Infusion (10%) was prepared and subsequently lyophilized. Rats were treated with the lyophilized of *L. molleoides* fruits (*Lmf*) using as standard 10 mg/kg of furosemide. Acontrol (saline solution) was established. Urinary volume was measured at 15 min for 3h to determine urinary volumetric excretion (UVE). A procedure determination of minerals by ICP-MS in urine was optimized using deionized water. The statistical difference was performed by Student´s test. Rats treated with *Lmf* showed a significant diuretic effect between 30 (UVE: 68.85 ± 5.99; $p<0.001$) and 105 min (UVE: 97.03 ± 4.62; $p<0.001$). Saline solution (UVE: 40.86 ± 6.60) vs furosemide (UVE: 104.27 ± 5.72) showed significant difference starting at 15 min ($p<0.001$). Urinary density and pH were similar to controls. Analysis of the data obtained by ICP-MS (by ANOVA) showed a significant increase in excretion of Na and K ($p<0.001$) in the batches treated with the lyophilized. In conclusion, infusions of the fruits of *L. molleoides* could act like natriuretic and kaliuretic, and new studies are being carried out to obtain evidence of this effect.

A89

PRELIMINARY STUDY OF DRUG USE IN ELDERLY PATIENTS OF A HOSPITAL OF MENDOZA

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The drug use studies in elderly patient (E) are very important because of their vulnerability. The purpose was to conduct a preliminary assessment of drug use in E in a Mendoza hospital in order to determine the feasibility of doing in a larger population. An observational, descriptive, transversal, indication-prescription study was designed. Data of 122 sheets of Pharmacy Service, corresponding to 52 E of Clinic Medical (CM) Traumatology (T), Gynecology (G), and Surgery (S) Services, were collected: socio-demographic characteristics, prevalent diagnoses (Di), prescribed drugs (D), polypharmacy (P), and interactions (I) during Sept-Dec 2014. D and Di were classified according to ATC and ICD-10 classifications, respectively. I were assessed by Horn's algorithm. Existence of P was considered when 3 or more D were prescribed simultaneously. Statistical method: Chi square. The results as % were: Gender: M 59, F 37. Age: 61-93 years. Services: CM 72, T 15, S 3, G 2. Di: fracture 12, chronic obstructive pulmonar disease 8, heart failure 8. P: 67, less than 3D: 33, 3D 18, 4D 12, 7D 8, 8D 7, and 5D 6. D (506): Ranitidine 13, heparin 7, enalapril (En) 6, diclofenac 4, ampicilina-sulbactam 3, metoclopramide 3. I: En-antacids 19, aspirin-antacids 8, dexamethasone-antacids, aspirin-En, diclofenac-antihypertensive, digoxin-spironolactone and digoxin-furosemide 5. Prescriptions/patient: 1(40), 2(26), 3(15), 4(10), 5(5), 6(2), and 7(2). I: 39 in 126 recipes. The male sex was prevalent. Antacids, Antimicrobials, antithrombotics and NSAIDs were drugs most used. Musculoskeletal, respiratory and metabolic disorders were prevalent. The detected P was high, more than 4D/prescription and more than 10D/patient. I are favored by this P, decreasing or increasing the effectiveness of one of the D, such as an antihypertensive, or a D with a narrow therapeutic margin as digoxin, respectively. Situations that can endanger health or life of the patient. These results are considered satisfactory and a stimulus to continue in a larger population.

A90

PHARMACOLOGICAL STUDY OF CB_1 RECEPTOR MODULATION IN HYPERTENSION AND POSSIBLE INTRARENAL NANOIMPLANTS APPLICATIONS

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Arterial hypertension is a medical condition considered to be one of the major public health problems in developed countries. An innovative therapeutic option would involve the use of cannabinoids as activators of type 1 receptors (CB_1) in order to cause vasodilation and inhibition of ion absorption at the renal medulla by a nitric oxide (NO) mechanism, resulting in decreased blood pressure. However, cannabinoids have adverse effects in the central nervous system. In this sense, nanotechnology would allow the controlled release of drugs at specific sites, directing its therapeutic action, and also limiting the occurrence of undesirable

effects. Therefore, our objective is to design an intrarenal scaffold of cannabinoid controlled release, comprising polycaprolactone nanofibers obtained by the *electrospinning* technique, in order to increase NO bioavailability. It will work with six groups (SHR/WKY control, SHR/WKY sham, and SHR/WKY with nanofibers). Hemodynamic parameters, NO levels, oxygen consumption by the loop of Henle cells and sodium excretion, will be monitored. We hope to demonstrate that activation of CB_1 receptors in the renal medulla increases diuresis in hypertensive rats as consequence of increased excretion of NaCl by NO-mediated mechanism. In addition, we aspire equally to use nanotechnology to demonstrate its potential to enhance the activity of therapeutic agents while minimizing toxicity and adverse side effects. Thus, intrarenal implants could be a promising therapeutic alternative for the treatment of hypertension.

A91
EFFECT OF A SOY BASED DIET ON RAT CEREBELLUM EXPOSED TO CADMIUM

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Cadmium (Cd) is an important environmental contaminant. We studied its effects and the protective effect of a soy-based diet in cerebellum. Histoarchitecture, antioxidant enzymes activities, oxidative stress markers, myelination proteins and metallothioneins were determined. Female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (Co) and the other, 15 ppm of Cd for 60 days. NF-E2 related nuclear factor (Nrf-2), SOD 2, CAT, GPx, iNOS, VCAM-1, metallothioneins (MT1 , MT2, MT3), neuregulin (NRG 1), myelin-associated glycoprotein (MAG) and proteolipid protein (PLP) mRNAs were determined by PCR. S28 was used as internal control. Histological studies were performed in H-E stained tissues. CAT and GPx activities were measured in cerebellum homogenates. Nrf-2 decreased in both Cd groups and in Soy Co ($p<0.05$). GPx decreased in both Cd groups ($p<0,001$). SOD 2 increased in Soy Cd. CAT increased in Soy Co ($p<0.001$) and VCAM-1 did not change. iNOS increased in Cas Cd ($p<0,001$) and decreased in Soy Cd ($p<0,001$). CAT and GPx activity decreased in both Cd groups ($p< 0.01$). MT 1 and MT 2 did not change while MT 3 decreased in Soy Co vs Cas Co ($p<0.001$), with a decrease in Soy Cd vs Cas Cd ($p< 0.05$). Myelination proteins showed a trend to increase in Cd groups. Some disorganization was observed in the Cd groups, especially in the Purkinje cell layer. We found that Cd induces oxidative stress and changes in the morphology of Purkinje and granular cells. This was mildly attenuated in the Soy fed group, suggesting that this could be a potential therapy for those individuals exposed to this heavy metal.

A92
Jungia polita: STUDY OF ORAL ACUTE TOXICITY

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Jungia polita Griseb. (Asteraceae-Multisieae), popularly known as “zarzaparrilla”, “viña”, is used in folk medicine as depurative and antisclerotic. Infusion (10%) of the aerial parts (Del Vitto LA & EM Petenatti s.n., III-2007 (UNSL # 835) was prepared, separated by filtration and the aqueous extract was concentrated and lyophilized to preserve it. The *Jungia polita* lyophilized extract (JPLE) was studied for acute oral toxicity as per revised OECD guidelines No. 423. Thirty albino mice (20 - 25 g) of both sexes were randomly divided into five groups of six animals each (3 male and 3 female). Mice were fasted for 4 hours and given oral increasing doses of JPLE (5, 50, 300 and 2000 mg/kg). The fifth group, served as control, was treated only the vehicle (distilled water). Animals were observed daily, for 14 days. The parameters studied were weight and macroscopic analysis of the vital organs: heart, lungs, liver, spleen and kidneys. Acute toxicological studies have showed that an oral administration of 2000 mg/Kg of JPLE did not produce any sign of acute toxicity in the animals (male and female). Over the 14 days following the oral administration of JPLE, none of the animals died and no significant changes organ weight were observed through the end of this period ($p>0.05$). There were no signs on symptoms of restlessness, respiratory distress, diarrhea, convulsions, and coma. Orally administration of JPLE up to 2g/kg produced no mortality and visible signs of delayed toxicity 14 days post-treatment. These results ensured the continuance of pharmacological studies on this species using the oral route and motivated us to proceed with the biological assays. The highest dose did not induce noticeable signs of toxicity. In conclusion, under the present experimental conditions, JPLE had not presented signs of toxicity.

A93

5,5-DIMETHYL-1-PYRROLINE *N*-OXIDE PREVENTS ENDOTOXIN-INDUCED M1-PHENOTYPE IN MACROPHAGES

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Macrophages are cells from the innate-immune system where they play a number of homeostatic and defense functions. Inside the tissues and under tissue-specific microenvironmental pressures monocytes are recruited and differentiated to specific phenotypes. This phenotype is a consequence of the expression of specific genes that are under the control of one or more transcription factors. In this context, inflammatory phenotype of adipose tissue (AT) macrophages (ATM-M1) is responsible for adipose tissue oxidative stress and inflammation mediators that reduce whole-body insulin sensitivity and cause a number of metabolic abnormalities-associated to obesity. Intratracheal instillation of the nitrone spin trap 5,5-dimethyl-1-pirroline *N*-oxide (DMPO) to diet-induced obese-mice reduced markers of AT oxidative stress and inflammation, reduced serum concentration of inflammatory cytokines and improved insulin sensitivity. Thus we hypothesized that DMPO may produce transcripcional effects in macrophages at the AT and maybe other tissues. To approach this hypothesis we determined the transcripcional effects of DMPO in RAW264.7 cells after 6h incubation and with or without lipopolysaccharide (LPS) to model transcriptional profile of ATM. Microarray data showed that LPS caused an M1-transcriptional pattern, whereas DMPO reduced these changes. Remarkable effects were observed in the expression of IRF-7 and PPAR-d, two master regulators of genes that determine M1 and M2 macrophage phenotype, respectively. LPS induced IRF-7, but reduced PPARd expression; whereas DMPO reduced IRF-7, but induced PPAR-d expression. Taking together our data suggest that DMPO may serve as a structural platform for the design of novel compounds to reduce AT inflammation and, thus other inflammatory abnormalities-associated to obesity, such as insulin resistance and metabolic syndrome.

A94

PREVALENT CHRONIC DISEASES AND IMPLEMENTED PHARMACOLOGICAL TREATMENTS IN A HEALTH CENTER OF MENDOZA

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The purpose was to determine the prevalence of chronic diseases, mainly hypertension (H) and analyze the drugs prescribed at the Health Center of Mendoza (HC). A descriptive, cross-sectional and retrospective study was conducted in June-2015. Data of medical consultations and pharmacy records were collected. Formulary of Sanitary Area, Health Ministry biostatistics, and ICD-10 and ATC classifications were used. DDD/patient/day were calculated. Inclusion criteria: outpatient, >15 years with chronic conditions, treated at HC. Results as %: 700 patients were examined: women (F) 59, men (M) 41. Main age group: 50-69 years (60%). Specialists: generalists 48, diabetologist 28, psychiatrist 16, cardiologists 7. Pathologies: anxiety 35, H 32, DM1 29, DM2 18, hyperlipidemia 11. H: Prevalence: for the total 13, in chronic patients 32. Sex: F 52, M 48. Main age range: 50-69 years 71. Comorbidities: DM1 23, anxiety 22, DM2 12 hyperlipidemia 8. Drug number (D): 1D (31), 2D (39), 3D (15), 4D (11); average: 2.5D/patient. D: enalapril (E) and losartan (L) 28, insulin (I) 23, metformin (M) 20, amlodipine (Am) and atorvastatin (At) 19; clonazepam (C) and spironolactone (S) 11, ranitidine (R) 8; D en combination: E+M (9); E+At, L+At and L+S (5), E+M+I and E+R (4). DDD/1000 patient/day: L: 480, Am: 479, E: 469, M 157 and At 111. The largest proportion of H was found in males and in the age group increased cardiovascular risk. H associated with DM1 and anxiety were prevalent, and the most prescribed drugs were specific for them. The found profiles might be consequence of that HC is the unique of the Sanitary Area that have psychiatric and diabetologic ambulatory attention. It is necessary to reduce the prevalence of these diseases that increase morbidity and mortality, strengthen the acquisition of healthy habits and promote appropriate use of medicines.

A95

Aristolochia argentina GASTROPROTECTION: A HISTOLOGICAL STUDY. ROLE OF PROSTAGLANDINS, SULFHYDRYL GROUPS, AND NITRIC OXIDE

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Aristolochia argentina (family Aristolochiaceae) is popularly known as “charrúa”, “charruga”, “patito”, “buche de pavo”. The roots of this plant are used in folk medicine. The aim of the study was to evaluate the effects of *A. argentina* lyophilized extract (AALE), 500 mg/kg, in ethanol-induced gastric ulcer in Wistar rats (200-250 g) by histological study. Moreover, AALE mechanism of action and the role of sulfhydryl groups, nitric oxide (NO) and prostaglandins were studied. Histological study: samples for optical analysis were blocked in paraffin and stained by haematoxylin-eosin. Ethanol provoked evident damage. The stomachs of rats pretreated with AALE (before ethanol) showed a significant reduction in the severity of the lesions ($p<0.001$). The microscopic observations revealed that the surface damage gastric mucosa of the stomachs after oral ethanol. The epithelium

of stomachs pretreated with AALE had similar appearance to control rats. Peptic and parietal cells with preserved morphology were observed. The activity of AALE on ethanol-induced lesions continued even after the inhibition of endogenous sulfhydryls following pretreatment with NEM (N-ethymaleimide). Both indomethacin, a prostaglandins synthesis inhibitor, and L-NNA (Nω-nitro-L-arginine), a nitric oxide synthase inhibitor, antagonized AALE gastroprotective activity (p<0.001). Histological evidence shows that AALE prevents formation of gastric lesions induced by ethanol. Present findings suggest the possible involvement of prostaglandins and nitric oxide in the antiulcer effect of AALE.

A96
SELF MEDICATION IN STUDENTS OF THE FACULTY OF CHEMISTRY, BIOCHEMISTRY AND PHARMACY (UNSL)

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Self-medication is the use of medications on their own initiative, or by the advice of others persons, without consulting a doctor. It is widely known that this usual practice, brings with it serious consequences as the masking a disease, the occurrence of adverse reactions and increased antimicrobial resistance. The aim of this study was to analyze the behavior of self-medication in students of the Faculty of Chemistry, Biochemistry and Pharmacy of the National University of San Luis (FQBF-UNSL) during July-2016. A survey to 290 students of a total population of 1,400 was performed. An anonymous and structured questionnaire with closed questions, and multiple choice was used. Sex: F 73% and M 26%. Age range: 18-34 years. There was self-medication in 95% of the total, and in the 99% of the students in the upper cycle. Reasons: speed: 75%, does not like going to physician 10%, lower cost 2%, others reasons 13%. The most consumed drugs were: analgesics 38%, antibiotics 14%, both simultaneously 30.2%, and anxiolytics 2.5%. Duration of treatment with antibiotics: 1 day 28%, 3 days 38.5%, 7 days 20%, and 15 days 8%; with analgesics: 1 day 64%. Person who recommended consumption: family member: 39%, pharmacist: 33%, by self-choice: 26% and by prior medical advice: 2%. Beliefs about self-medication: 76% believe it is inappropriate, mainly due to lack of knowledge about the disease and/or the medicines, and because antimicrobials could generate resistance. The self-medication in these group of students is elevated, even with ethical drugs, despite they know the downside of this practice. It occurs even in senior pupils of FQBF who already have Pharmacology knowledge. Perhaps, this knowledge give them a false sense of security respect to medicines, unknowing in many cases the potential risks that imply.

A97
VACCINATION AND EVENTS SUPPOSEDLY ATTRIBUTABLE TO VACCINATION OR IMMUNIZATION IN UNIVERSITY STUDENTS, 2016

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Vaccination is the intervention that has had greater impact on health after water purification. The vaccines are not exempt of adverse effects. The events supposedly attributable to vaccination or immunization (ESAVI), are clinical pictures that appear after the administration of a vaccine that may eventually be attributed to it. At the National University of San Luis (UNSL), Operatives of Vaccination with Influenza, Hepatitis B, and Double Bacterial were conducted in May and August 2016. The purpose was to determine the socio-demographic data, the type of vaccine administered, presented ESAVI, and knowledge on existence of notification system in students of the University community during the above operatives. From a total of 639 vaccinated students, 147 were randomly selected for a survey. The included data in it were the following: age, sex, vaccines, number, type and symptoms of ESAVI, treatment received and reasons for not reporting. Symptoms were classified as: general and local; lethal, severe, moderate, mild; and according the need of treatment or not. The results obtained were: age range: 18-52 years; Sex: Female 128, Male 19; vaccines: influenza (83.7%, n: 123), hepatitis B (6.1%, n: 9); double bacterial of adults (14.3%, n: 21) and double viral (2%, n: 3). Events supposedly attributable to immunization (40.1%, n: 59); ESAVI type: mild (96.6%, n: 57), moderate (3.4%, n: 2). Symptoms: Pain, swelling, induration, malaise and fever. Reasons not to notify: ignorance of the existence of the report (n: 2) and where and how to do it (n: 2), believe the expected ESAVI should not be notified (n: 133). There are high percentages of ESAVI and an elevated ignorance about of the notification system. It is important to know and monitor ESAVI of each vaccine and promote its notification to ensure the safety of vaccines and patients.

A98
NEW METHODOLOGY FOR DETERMINATION OF ALUMINIUM IN URINE SAMPLES BY SOLID SURFACE FLUORESCENCE

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Aluminum (Al) is a toxic metal, capable of interfering with a variety of cellular and metabolic processes of the nervous system and tissues of the organism. Al has been considered as a possible cause of renal osteodystrophy, Parkinson's and Alzheimer's disease. In this work, the separation/preconcentration of Al (III) on nylon membrane (0.45 µm pore size) is carried out employing quinizarina $1x10^{-5}$ mol L^{-1} and SDS micellar solution $2x10^{-2}$ mol L^{-1} (acetic/acetate buffer pH 5) for later quantification by solid surface fluorescence (λexc = 490 nm; λem = 570 nm; slits = 1.5/3). Experimental parameters that influence efficiency of separative and determinative step were studied and optimized (nature of membrane, nature and concentration of fluorophore, pH and buffer concentration). Working at optimal conditions, quantitative recovery > 99.9% of Al (III) was reached with detection limits of µg L^{-1}. Calibration of the new methodology presents a linearity of three-orders of magnitude. The developed methodology was successfully applied to determination of Al (III) in urine samples. The proposed methodology represents an alternative to the routine metal analysis methods, with the advantage of using a simple inexpensive instrumental as a spectrofluorometer.

A99
SEQUENTIAL DETERMINATION OF NICKEL AND CADMIUM IN TOBACCO AND MOLASSES SAMPLES BY MOLECULAR FLUORESCENCE

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Smoking cigarettes is harmful for health. In last time, alternatives like electronics cigarettes and narguille or water pipe (WP) have appeared. However, security is no proven. In present research, a new methodology for sequential determination of Ni(II) and Cd(II) is proposed and applied to tobacco and snuff used in WP (molasses). In a first step, a chemofiltration on nylon membrane is carried out employing eosine (Eo) and carbon nanotubes dispersed in SDS solution (phosphate buffer pH 7). In this conditions, Ni(II) was selectively retained on the solid support. Filtrate liquid was re- aconditioned with acetic acid /acetate (pH 5) to Cd (II) determination. Experimental parameters that influence efficiency of separative and determinative step were studied and optimized, like nature of membrane, nature and concentration of fluorophore, pH and buffer concentration. Working at optimal conditions, quantitative recoveries > 99.9% were reached for both analytes with detection limits of ug L^{-1}. Calibration of the new methodology presents a linearity of three-orders magnitude. The developed methodology was successfully applied to determination of Ni(II) and Cd(II) in molasses and conventional tobacco samples. The found concentration of both metals enables us to ensure that the use of water pipe is as harmful to health as smoking cigarettes.

A100
CHANGES IN REPOLARIZATION AND VENTRICULAR FUNCTION IN METABOLIC SYNDROME

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Metabolic syndrome (MS), as a pre-diabetic state, could affect repolarization and ventricular function, regardless of coronary atherosclerosis (ATS), anthropometric variables and markers of inflammation. A transversal clinical trial was performed in two groups of patients with MS: Group I with, and Group II without clinical expression of ATS. The study variables were: anthropometric, metabolic; inflammation and cardiovascular involvement: QT electrocardiogram and diastolic function sisto-dimensional and Doppler echocardiography. Statistics: Student's t test for unpaired data and Chi square test. Fifty female patients with MS were studied (25 in each group). Ages in years were 53.76 ± 10.26 in Group I and 58.96 ± 6.29 in group II (p = NS). There were no significant differences in anthropometric variables or inflammation between the two groups, although the C-reactive protein was in high risk levels in both groups (5.06 ± 6.74 vs 3.6 ± 4.8 mg/dl, p = NS). HDL-C was lower in Group II than in Group I (39.84 ± 8.06 vs 51.32 ± 15.19 mg/dl, p <0.002). No differences were found in the levels of glucose, uric acid, triglycerides or LDL-C. All patients had abnormal diastolic function with preserved systolic function, with abnormal QT dispersion (Group I, 70 ± 9 vs Group II, 80 ± 10 ms; p = NS). MS could be more than a pre-diabetic state; with cardiac disease like diabetes, independent of coronary atherosclerosis ischemic heart disease and cardiovascular risk factors.

A101

ORAL REPEATED DOSES STUDY OF *Jodina rhombifolia* IN RATS

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Jodina rhombifolia (Hook.&Arn.)Reissek (SANTALACEAE) is popularly named "Peje", "Quebrachillo", "Quebracho flojo", "Sombra de toro". Traditionally, has been utilized as anti-alcoholic, digestive, antidiarrheic, anti-inflammatory, hypocholesteremic, hypouricemic, antidysenteric, among other popular applications. The acute toxicity test after oral administration of 2000 mg/kg of *Jodina rhombifolia* lyophilized aqueous extract (JRLE) revealed non-toxicity at this dose. The aim of this study was to evaluate the toxicity of JRLE at 14 days in male and female Wistar rats. Infusion was prepared at 10% from the dried powdered plant material, separated by filtration and the aqueous extract was concentrated and lyophilized to preserve it. JRLE was administered, *p.o*., at concentrations: 0 (control group), 125 mg/kg (low-dose group), 250 mg/kg (middle-dose group) and 500 mg/kg (high-dose group), respectively. Routine clinical observations and body weight were measured. Peripheral blood was collected, hematology (red and white blood cells) and clinical chemical (aspartate aminotransferase [AST], alanine aminotransferase [ALT], glucose, total protein, albumin) values were evaluated. The organs of each rat were observed grossly (lungs, heart, liver, kidneys, spleen, testes and ovaries). Parametric ANOVA method was used. No abnormal symptoms and clinical signs or deaths had been found in rats in each group during the test. The general conditions of all rats were normal. There were no signs on symptoms of restlessness, respiratory distress, salivation, pilomotor erection, lacrimation, diarrhea, convulsions, coma. In addition, no significant differences were found in each hematology value, clinical chemistry value and body weight ($p>0.05$). The highest dose did not induce noticeable signs of toxicity. In conclusion, the JRLE possesses no obvious significant toxic effects.

A102

PRELIMINARY STUDY ON PHARMACOLOGICAL AND NON-PHARMACOLOGICAL TREATMENTS IMPLEMENTED IN SMOKING CESSATION GROUPS OF SAN LUIS

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The purpose was to analyse the pharmacological and non-pharmacological treatments (FT, NFT) in Smoking Cessation Groups (SCG) of San Luis, and identify potential contributions from Nursing, in order to determine the feasibility of implementing it in all SCG of the province. A descriptive, retrospective and cross-sectional study (2012-2014) was performed. Data of 69 medical records of 2 SCG were collected. The notions of self-care (A) and individuation-affiliation (I-A), belonging to the theory of Modelling and Modelling of Roles (MMR), nicotinic replacement therapy (NRT)+V 13, NRT+B 9, NRT 9. NFT: Group without FT (59.4). Self-care: Internal resources (IR): activity in free time: no (38), sedentary (27), moving (29), sedentary and movement (6). Reasons to smoking cessation (SC): do physical activity (38), disease prevention (44), improve the life quality (44), improve the image (16), live more time (26), and be an example (38). Motivation evaluation: highly motivated (F 13, F 16), quite motivated (F 23, F 25), unmotivated (F1). External resources: coexistence: single (F 17, F 28), in couple (F 17, F 28); presence of a significant social network (10; friends or family 46.4). Previous methods of SC (16); types: RNT (3), B (6), V (1). Self-care actions: previous SC attempts (85). SC (45). I-A to SCG (58). Variables of MMR were operationalized with own tools of a Nursing theory. Eleven reasons to quit smoking were found as IR. B was the FT most prescribed. Affiliation to SCG and FT might contribute to the SC. These results are satisfactory and stimulate to implement this study in the three SCG of San Luis.

Ecology, Ethology and Biodiversity

A103

FORAGE AVAILABILITY, GROWING AND GRAZING EFFICIENCY RELATED TO WATERING POINT, ON CENTRAL SANDY GRASSLAND OF SAN LUIS, ARGENTINA.

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Grazing gradient is a concern in the management of natural resources in the semiarid and is widely studied. On 730 has paddock grassland with only one watering point, grazing by cattle for 150 days, the initial forage availability (FA) and leaf remaining (LR) was evaluated at the end of the grazing period (PP), by cutting and drying of the aerial forage biomass. The native species are mainly spring-summer, so nine enclosure cages were installed during the days that animals grazed to evaluate forage species growth (Gr), at a constant stocking rate (0.41 livestock units per hectare). 27 determinations for FA, distributed at 300 (N), 1000 (M) and 2200m (F) from watering point were conducted, and variables relationships allows the estimation of harvest efficiency or dry matter disappearance rate of period (HE= (FA+Gr-LR)/(FA+Gr)*100). Growth of 200, 450 and 840 kgDM.ha^{-1} were recorded at increasing distances from the watering point, with statistical significant differences ($p<0.10$) as well as the initial forage

availability (FA) and leaf remaining (LR). Taking as reference (100%) the forage availability of F sector, C sector has a biomass of about 26% of F whereas M 54%. ANOVA and Tukey mean differences test was performed for the variables (DF, Gr) in each sector, resulting in significant differences between N, M and F ($p<0.10$). Estimated HE was about 73% in N, 48.0% in M and 30% for F. The cattle grazing gradient, increasing to watering point, generates a deterioration of vegetation in the same direction.

A104
PRESENCE OF FLOWERING STEMS AND HEIGHT OF BOTANICAL COMPONENTS IN THE ACUMULATED GROWTH OF *Digitaria eriantha*, AT DIFERENT GRAZING INTENSITIES

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The leaf height of pastures is closely related to forage productivity, and reproductive structures can foster future reseeding. Distance to water point determine the intensity of grazing to which forage sources are subjected, and allow evaluate the vegetation response to a decreasing gradient far away from it. The number of flowering stems (FS), leaf height (LH) and flowering stems height (FH) were determined, on accumulated and deferred *Digitaria eriantha* implanted pasture growth. The study was located in the sandy grassland area of San Luis (Argentina), at the south of Villa Mercedes city. In a paddock of 400 hectares with only a water point, 90 sampling sites were established, distributed at three increasing water point distances (400, 1200 and >2300 m). These distances was near, middle and far away sectors, respectively. LH was determined to the top of the leaves accumulation (canopy) and evaluated with non-parametric statistics, while FH was analyzed by ANOVA. The number of FS in far sector was twice that in near. FH reached 94-108 cm on average between sectors, LH being 7 cm in near sector and 20 cm in middle and far sectors. The heights were significantly lower in near ($p<0.01$) for middle and far sectors, which have an increasing trend. The three analyzed variables were linear and positively correlated with distance from water point ($r>0.70$). Grazing intensity alters the growth of implanted Digitaria eriantha, negatively affecting the productivity and persistence of the pasture.

A105
EVOLUTION OF THE SALINITY AND PHREATIC LEVEL OF "BAJO LA SALADA"

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The wetlands of San Luis are frequently flooded environments and with waters of saline characteristic near to the surface. Our aim was characterized the contents of salinity and the groundwater level of a saline wetland of the center East of San Luis province. The area locates to 33 º 37 ' S and 65 º 25 ' W, with a height of 505 meters above sea level. The groundwater level measured up every month for 14 months and the chemical characteristics of his waters every 2 months of agreement to the physiognomic types established in a previous work. The results of the phreatic level showed an increase understood between 39 to 154 % being observed three groups that differ statistically in the majority of the months: the saline beach and the prairie halophyte dense, the prairie halophyte open and the scrubland patches of halophyte crawling and the halophyte scrub. All the chemical parameters certain show statistically significant differences for the dates of sampling, equally for different physiognomic types except the pH. The electrical conductivity ($dS.m^{-1}$), the dry residue ($g.L^{-1}$), sulphates ($mEq.L^{-1}$), sodium ($mEq.L^{-1}$) and the sodium absorption ratio (SAR) show significant differences for the scrubland patches of halophyte crawling, the saline beach, prairie halophyte open and dense and halophyte scrub in descending order. It was found a model of regression to sulphates with $p<0.01$. It is concluded that the parameters studied presented higher values in winter than in summer, even with increases in groundwater levels.

A106
FIRST APPOINTMENT OF *Monosteira unicostata* (Mulsant & Rey, 1852) (HEMIPTERA: TINGIDAE) FOR ARGENTINA, FOUND IN MENDOZA PROVINCE, ON SALICACEAE

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As part of an emerging globalized world in which trade in agricultural products it has increased both among countries in the region and between continents, has increased the occurrence of insects including some considered a pest for agricultural production. In order to identify arthropod species present in regional crops, the Salicaceae found in plantations and walks in the province of Mendoza, are periodically monitored, authors made field observations. Samples were extracted and analyzed under stereomicroscope. In this context, the authors cite to *Monosteira unicostata* for the first time for the Neotropical region in the province of Mendoza, Argentina. This species is known in the Old World as the "almond's tiger " or "false tiger." It is a common

pest in Southern Palaearctic region till the Turkestan and Iran, which attacks there fruit trees as *Amygdalus*, *Cydonia*, *Malus*, *Prunus* and *Pyrus*. It also attacks *Juglans*, *Populus* and *Salix*. It is considered as one of the two species of Tingidae of major economic importance for the western Palearctic region, along with *Stephanitis pyri* (F.). Feeding bites produce a weakening of the tree, leaf loss and reduced harvest. If the attack is intense at the end of summer can be reached complete defoliation of the tree. This defoliation affects the development and maturation of the fruit. In the case of poplars, they can be completely defoliated. In February 2014 this species was found in leaves of *Populus* sp. in Tunuyan Department, and in January 2016 in *Salix* sp., in Las Heras Department, both in Mendoza. We conclude then that, being the fruit production an important resource for the Mendoza province, the discovery of this pest species must be taken into account to prevent future declines in yields of fruits produced in this province.

A107
ISOLATION AND PURIFICATION OF EDAPHIC CYANOBACTERIA IN THE PROVINCE OF SAN LUIS (ARGENTINA)

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Cyanobacteria applied as bio-fertilizers improve soil properties and promote plant growth. To assess these skills individually and by species, it is necessary to isolate and purify them. The aim of this work was to obtain unialgal crops of native soil cyanobacteria for subsequent agronomic testing. Sampling was carried out in agricultural-livestock lots of corn, soybeans and alfalfa, 25 km north of Villa Mercedes, on July 2014. Soil samples were taken from 0 to 10 cm deep and placed in Petri dish with Ripkka liquid culture medium and were put into a growth chamber at 25-30 ° C with a photoperiod of 12 hours light – 12 hours darkness. After a month, algal biomass was extracted and microscopically identified. The species of agronomic interest were chimed in Petri dishes with Rippka agarized medium at 1%. Depending on the mobility of the species in the agarized medium two forms of chimes were made in order to collect their edges with greater purity - one in the form of rifling for species with low mobility and the other in the form of islands for species with greater mobility. Each chime delayed its growth between 10 to 15 days. 12 to 15 chimes were performed and purity was microscopically tested to confirm each unialgal crop. Then, chime in test tube with Rippka liquid medium with Cycloheximide 100 mg / l was made for 15 days in order to eliminate contaminating fungi. Subsequently, chime was made in Erlenmeyer flasks with Ripkka liquid medium and the samples were cultivated with periodic agitation for biomass growth. Isolated and multiplied species of agronomic interest. were Calothrix sp, Nostoc muscorum, Nostoc calcicola, Nostoc sp, Anabaena oryzae, Anabaena sp. Genera and some species of cyanobacteria obtained are consistent with those isolated in South Africa, Italy, India, Pakitan, Czech Republic and Egypt, where they had good results as biofertilizer. Therefore, they isolate obtained in this study could have future applications such as bio-fertilizers. Isolation unialgal through island shaped chimes on petri dish is a useful method to cyanobacteria of fast growth.

A108
DESCRIPTION OF POPULATION GROWTH CURVE OF *Varroa destructor,* AND EVALUATION OF APLICATION OF OXALIC ACID FOR ITS CONTROL

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Varroosis is a parasitic disease of *Apis mellifera* L. caused by *Varroa destructor,* that cause negative effects for hemolymph extraction and indirect inoculation of virus that triggers a reduction in adult longevity, malformations, bees smaller, poor development of the glandular system, etc. Knowledge of the growth curves of mites, allows to know the time when the hives reach prevalence values that bind the colony. To reduce varroa populations, should apply Integrated Pest Management, this implies knowledge of parasitic levels and the use of synthesis and organic acaricides. The use of organic oxalic acid, has limitations as a liquid solution because this low residual effect. The aim was to describe the population growth curve of varroa throughout the season growth of bees in Villa Mercedes, San Luis and evaluate the effectiveness of a new acaricide formulated with oxalic acid called AluenCAP ®. To study the population evolution an indirect count was monitored from the daily natural mortality of mites which was adjusted by modeling the total population of mites by using technical hive floors and effectiveness was measured from the contrast of acaricide synthesis (Flumetrin) and control treatment. The curve showed a slow initial increase with an exponential growth from February 17, where in just 15 days went from 500 to 2500 mites per hive. Natural mortality was highly correlated with the percentage of prevalence measured by sampling in phoresy (R2: 0.92). The effectiveness of AluenCAP reached 80% as acaricide control. This low value was explained by the constant re-infestation and showed significant difference from the control ($p > 0.05$). Population curve shows that from February 17 mite population should be controlled. AluenCAP ® had no effect on the brood rearing. This organic acaricide allows the start of treatment earlier.

A109
INTAKE AND DIET DIGESTIBILITY IN GOATS FED SIMPLE AND MIXED DIETS OF NATIVE WOODY PLANTS IN NE MENDOZA, ARGENTINA

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Grazing Creole goats in NE Mendoza incorporate over fifteen forage species into their diet. The shrubs *Tricomaria usillo* (TU) and *Mimosa ephedroides* (ME) stand out for their contribution to the bulk of the diet and high content of tannins. Diet diversity allows goats to reduce negative effects of tannins and to improve food intake and nutrient utilization. This study investigates whether offering multiple choices among tanniniferous (T) and non-tanniniferous (NT) plants has an effect on intake and diet digestibility. Fifteen female Creole goats were placed in individual pens, and were assigned to three groups (N=5) with two T (TU, ME) and three NT species (*Prosopis flexuosa*, *Capparis atamisquea*, *Atriplex lampa*). Two groups were offered single tannin-containing shrubs (SDTU; SDME) and the third group received a combination of all five forages (Mixed diet, MD). Dry matter intake (DMI), *in vivo* apparent digestibility of diet (ADD) and nitrogen (ADN) were determined. Goats offered the SDME reached greater ADN ($P<0.05$; 81%) than goats fed MD (75%) and SDTU (73%). Goat offered MD had greater ADD ($P<0.05$; 58%) and trended to increase DMI ($P<0.10$; 60 g/kg $BW^{0.75}$). The forage species most preferred was *M. ephedroides*. Mixed diet led nutritional benefits which contribute to explain the intake of diverse array of food items when the goats graze in the NE Mendoza.

A110
USE AND EVALUATION OF NATIVE SPECIES OF WILDLIFE AREA MORRO (PROVINCE OF SAN LUIS).

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The aim of this study was to analyze the use and assessment of the residents of the surroundings of San José del Morro to native species of flora and fauna. The study area is located in the semiarid and is a small historic town, which rises around its old church. San José del Morro was post and fort of the royal road between Buenos Aires and Cuyo. To analyze the knowledge that the population has of flora, interviews were set up with knowledgeable people (called "yuyeras" by locals) who used especially native plants with medicinal value. Once completed, surveys were conducted to the population, with questions of open and / or closed type. Surveys were left in the Municipality and in the Town School. In the case of flora surveys resulted in extensive knowledge of plant abundance in the area, but not of its use. Regarding fauna, unaware of their use in traditional medicine, although they identified species. Another activity was the preparation of posters with photographs of the most representative plants and animals; they were left for a time in the school and in the municipality and asked the population to vote for the ones which most attracted them. There were significant differences in opinion between adults and young men and women, regarding the most voted animal species. While the selection of the plant, males (youth and adults) selected the same species and the same happened with people of female gender.

A111
IMPACT ON THE CALDENAL WOODLANDS OF THE INTRODUCTION OF EXOGENOUS *Prosopis* SPECIES

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The aim of this study was to analyze the proposals emerged from the executive of the province of San Luis to ease the situation of producers, due to the emergence of new streams in the catchment area of El Morro of this province. The affected area covers an area of 20,000 km^2. Through studies analyzed, changes in the water table, determined the main cause of this process is a change in the water balance resulting from an increase in precipitation and a decrease in evapotranspiration losses, changes in vegetation cover in the catchment area. A law was passed that stated owners of agricultural establishments in the catchment area must reforest 5% of their holdings. Among the selected species indicated the use of exotic species, some with clear invasive dynamics in these environments as the elm (*Ulmus pumila* L.). The most problematic would be the introduction of species of Prosopis genus from the province of Chaco. The species of the genus *Prosopis* have a tendency to cross between them, producing interspecific hybrids. According with geneticists, at present, is almost impossible to find pure natural populations of a particular species of the genus *Prosopis*, so it is concluded the introduction of specimens of this genus that naturally do not grow in the area and whose impact has not previously analyzed, especially threaten the calden (*Prosopis caldenia* Burkart.) endemic to Argentina that would entail the disappearance of pure genomes.

A112

MORPHOLOGICAL CHARACTERIZATION OF *Ephedra* L. (GNETALES) POLLEN GRAINS FROM SAN LUIS PROVINCE

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There is great variation in morphological characteristics such as size, number and shape of ridges and outline of pollen grains in different species of the genus *Ephedra* L. Three species of *Ephedra* are present in San Luis province; *E. ochreata* Miers, *E. americana* H. and *E. triandra* Tul. In this preliminary study, we provide the morphological characteristics of their pollen grains with intent to contribute to the palynotaxonomy of these species and provide a basis for further fossils pollen studies. The primary objective of this work is to differentiate between taxa with these results. One for specie male strobili was removed of specimens deposited in a herbarium. The pollen grains were stained with basic fuchsin. A minimum of 20 pollen grains per samples were studied and measured using a light binocular microscopy (1000 x). The pollen grains are monad, medium sized, ellipsoidal, inaperturate, characterized by a series of psilate longitudinal ridges (or plicae). The wide gently domed ridges are straight and psilate, separated by a psilate region (furrows).There are difference between pollen grains of *E. ochreata* and *E. triandra* in the number and length of plicae. *E. ochreata* presents less plicae (7) than *E. triandra* (9). The long equatorial axis of *E. ochreata* (28 to 37 µm) is bigger than *E. triandra* (28 to 33 µm) and *E. americana* (33 to 34 µm). *E triandra* presents bifurcated plicae at the polar axis; this morphological characteristic is not found in pollen grains of the other two species. Our results show that these morphological differences might contribute to taxonomic, aerobiology and others studies.

A113

COMPARISON OF TARDIGRADE DIVERSITY IN ENVIRONMENTS OF SALTA (ARGENTINA)

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Relatively little is known of the Tardigrada fauna of Argentina, and especially for areas such as Salta province. environments of Salta, from urban ones (with a high, medium and low vehicular traffic) to natural areas, passing through rural areas. Selected sites (1100-1400 masl) belong to phytogeographic province of the Yungas were sampled. Each sample was taken from lichens and mosses growing on bark of trees during rainy season of 2014 (autumn). They were treated following the usual methodology and specimens and eggs were mounted in polyvinyl-lactophenol. Data analysis was performed using PAST, PC-Ord and R programs. Three thousand four hundred and three specimens were collected belonging to heterotardigrades (three species of echiniscid) and eutardigrades (six species belonging to: Milnesidae, Macrobiotidae, Hypsibidae). Probably, some species are new to science. Inventories completeness was complete for the high and medium urban (H and M) communities, of 92% for low urban ones (L); 89% for native (N), and of the 90% for the rural communities (R). The later community (R) was 1.66 times more diverse than H, meanwhile the least difference in the diversity of the communities was reported between M and L, with value of 0.02 times higher for the first community. Non-metric multidimentional scaling (nMDS) explained in two axis the 86,10% (Axis 1: 58.5%, Axis 2: 27.6%) of the total variation, separating native community from those of R and urban ones (stress=6.67). In this work, we can conclude that regional biota shows a nested pattern between different studied environments with an homogenization process in urban areas. This pattern implies an important species loss from the rural to the urban environments.

A114

DEMOGRAPHY AND POPULATION VIABILITY OF *Phymaturus williamsi* (IGUANIA: LIOLAEMIDAE): AN ENDEMIC AND VULNERABLE SPECIES OF CENTRAL ANDES IN SAN JUAN

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There are a great number of ecological and genetic factors that may threaten population stability and persistence. Particularly, saurian populations constitute a central issue in ecology as their reproduction rates are not quite high and are also subject to risk processes at a global level. Consequently, due to an insufficient thermophysiological adjustment and a high vulnerability to climate change, they are prone to a demographic collapse. *Phymaturus williamsi* is a viviparous species, with a biennial reproductive system. It is endemic of Central Andes in San Juan and has been categorized as “vulnerable”. Therefore, the aim of this study was to estimate vital and demographic parameters of a *P. williamsi* population. In addition, we carried out a population viability analysis (PVA) in order to model its demographic destiny. Data were collected in the locality “Quebrada Vallecito”, Calingasta. Individuals were captured using the Triple Capture method and they were marked with colorful beads, numeration with paintings, and amputation of phalanges. We built life tables with fecundity, mortality, and survival rates for different age

groups and genders. The PVA was performed using VORTEX software, based on demographic parameters and natural history data of *P. williamsi*. Results showed fecundity in female adults was higher than in juveniles. It was also found that newborns reached the lowest survival rate, while adults and juveniles presented similar values. PVA results showed a low growth rate, and a high local extinction probability. Our data suggest a low persistence probability of the local studied population over time. Future studies are aimed at providing information to take decisions on their management and conservation.

A115
SPATIAL ANALYSIS OF A CHACO SERRANO FOREST IN SANTIAGO DEL ESTERO

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We analyze Chaco Serrano forest located to the north of Santiago del Estero province. The aim is to analyze the horizontal structure of the tree layer to determine their conservation status. We apply a systematic sampling design with a sample of 16 plots of 10 m x 100 m, distributed in two altitudinal (400/500 m s n m). We consider the population of individuals with dbh (diameter at breast height) greater than 10 cm, which were measured: dbh and total height. We have analyzed density, frequency, basal area (m^2/ha), diameter distribution and aggregation indices: Mac Guiness and Fracker & Brischle. We estimate that 45% of individuals per hectare belong to Libidibia paraguarensis and Schinopsis marginata. The most common species are *Libidibia paraguarensis* Guinness and Fracker & Brischle reveal that 80% of the species have aggregation tendency or grouped. In both altitudinal distribution curves of individuals by diameter class at intervals of 5 cm recorded high frequency in classes of 10 to 20 cm with a preponderance of *Libidibia paraguarensis* (27%), *Phyllostylon rhamnoides* (16%), *Ziziphus mistol* (15 %) and *Schinopsis marginata* (13%). In classes 20 to 30 cm recorded 39%, and the remaining individuals in classes between 30 and 60 cm. The basal area is 7.8 m^2/ha in lower sector and 7.9 m^2/ha at the highest; 62% is concentrated in three species, *Schinopsis marginata* (24.8%), *Libidibia paraguarensis* (19.6%) and *Ceiba chodatii* (18%); 32% is concentrated in the following five species: *Aspidosperma quebracho-blanco, Schinopsis lorentzii, Phyllostylon rhamnoides, Ziziphus mistol* and *Sideroxylon obtusifolium*. According to the Kruskal Wallis, no significant differences (p=0.3920) for variable basal area. It is inferred that the Chaco Serrano characteristics species are sufficiently represented to keep the forest structure, although it should analyze natural regeneration for greater support in the statement.

A116
EXPLORATORY STUDY ABOUT THE DEVELOPMENT OF NATIVE SOIL CYANOBACTERIA IN THE PROVINCE OF CORDOBA

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Cyanobacteria are one of the most important components of the soil and the role of this group of microorganisms it is clear in the maintenance of fertility, through the process of biological nitrogen fixation. Besides, they are related to the process of infiltration and retention of water, erosion and resistance of disturbances when they are part of the biological crusts. In this paper the species richness of Cyanobacteria of native soil in the northern center of the Province of Córdoba was evaluated. Soil samples were taken from native soils at a depth of 0-10 cm. In vitro cultivation using Watanabe's medium and chamber at a temperature of 28 ° +/- 2 ° C, with photoperiod of 12 hours of light and 12 hours of darkness was made. The lighting was provided at an intensity of 4.500 to 5.000 lux. The taxonomic determination was made with a Leyca DMS 100 microscope and the assistance of specific dichotomous keys. For the quantitative evaluation, the counting technique in Sedgewick Rafter Chamber by direct microscopy was used. The study allowed to recognize in this soil seven species from the Nostocales Order: *Anabaena oryzae, A sphaerica, A. Anabaena sp, Calothrix sp, Cylyndrospermum muscicola, Nostoc commune* y *N. muscorum*. Regarding the Oscillatorial Order, veintitrés especies developed: *Geitlerinema tenuius, Lyngbya lutea, L. majuscula, L. putealis, L. truncicola, Oscillatoria subbrevis, O. rupícola, O. limosa, O. proteus, Oscillatoria sp, Phormidium crouanii, P. tenue, P. corium, P. clorinum, P. amoenum, P. subuliforme, P. ambiguum, P. aerugineo-caeruleum, P. fragile, P. puteale, P. retzii, Phormidium sp.* y *Microcoleus vaginatus,* and four species of the Synechococcales Order: *Leptolyngbya edaphica, L. tenuis, L. foveolara.* y *L. maius.* These results constitute a contribution to the knowledge of diversity and richness of the soil microbiota as a baseline study for the assessment of agricultural practices that are applied in this resource.

A117

WATER QUALITY OF AN URBAN RIVER FROM THE PROVINCE OF SAN LUIS: ESTIMATION THROUGH PHYSICOCHEMICAL VARIABLES AND BIOTIC INDICES

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Urban rivers are between the ecosystems most affected by contamination. Aquatic macroinvertebrates are bioindicators of widespread use in biotic indices, which complement physicochemical data in assessments of ecologic health of water bodies. The objective of this study was to evaluate the degree of pollution of Chorrillos River, San Luis, integrating biotic indices, a diversity index and physicochemical variables. In four sites along the course of the river, during a high flow period, samples (n=8) of macroinvertebrates, using a D net, and water for physicochemical analysis were taken. Physicochemical variables measured were pH, temperature (ºC), turbidity (NTU), conductivity (μS cm^{-1}), total dissolved and suspended solids (mg L^{-1}), color (PtCo), dissolved oxygen (mg L^{-1} O_2), organic matter (mg L^{-1} O_2), and nitrates, sulfates, fluoride, iron and phosphorus concentrations (mg L^{-1}). A simplified index of water quality (SIWQ) was calculated. Organisms were identified to the required taxonomic level for the application of the Biotic Index of San Luis Sierras (BISLS), the Biological Monitoring Working Party index (BMWP) and the Shannon diversity index (H). Differences between sites in physicochemical variables, evaluated through ANOVA, were not significant, except for nitrates. The SIWQ evidenced sites moderately contaminated to contaminated, and a quality decrease that correspond to the urban gradient. Water quality judgments based on biotic and diversity indices showed a gradient from slightly to heavily contaminated environments. The application of biotic and diversity indices, complementing physicochemical data, allowed to perform a preliminary evaluation of the environmental condition of the river, to assess impact of human activities and to generate basic information that may be used in remediation and restoration actions.

A118

BURIAL DEPTH OF GROWING POINTS OF TWO NATIVE SPECIES IN A MEGATHERMIC PASTURE OF A SANDY GRASSLAND AREA OF SAN LUIS, ARGENTINA

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The burial depth of growing point of forage grass seems to be an adaptive strategy to persist to an herbivory history. Preliminary results obtained in the sandy grassland area of San Luis show differences in the burial of phytomers, comparing native species with different animal preference. The objective was to compare burial depth of meristematic apex of the perennial native undesirable specie *Elyonurus muticus* and desirable specie *Sorghastrum pellitum*, on *Digitaria eriantha* pasture, in different grassland intensity sectors related to watering point distance. On 400 ha paddock, 75 km away from Villa Mercedes, 20 plants for each specie were selected at random on August, which according to the distance of the watering point were in the sectors: near (400 m), middle (1200 m) and farthest (> 2300 m). The depth in cm, between emergency site of roots and soil surface was measured at three species, and associated with sector. Kruskal Wallis analysis was applied, because data series did not show a normality distribution. Whatever was the distance to the watering points, *E. muticus* presented deeper fitomers (4) than *S. pellitum* (2) and *D. eriantha* (1.6). The species did not show statistics differences of medians between sectors ($p > 0.05$). As a response to bovine grazing, *S. pellitum* and *D. eriantha* compromises their adaptation with high and sustained grazing intensities, considering the shallow location of meristems in the paddock.

A119

FLORISTIC DIVERSITY OF CALDENAL FOREST CHANGES AFTER YEARS OF STRIP CROPPING

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In San Luis, Argentina, there was an advance of agriculture into semi-arid areas and deforestation caused fragmentation of the forest and impacted on biodiversity. Organic farmers use practices such as crop rotations, intercropping, strip cropping, establishing wildlife cover and providing habitat for beneficial organisms.The aim of this study was to evaluate in two areas of Caldenal Forest, the effect of deforestation on the floristic diversity measured by the number and coverage species. Zone 1: field distant 15 km north and zone 2 other field distant from 20 km south of Villa Mercedes, where there was deforestation10 (T1) and 30 (T2) years ago. The strip cropping has performed defining surface area of native forest (FB) and surface area of deforested (FD) of carried out in 5 sites in each zone. .Diversity indices as Shannon-Wiener (ISW), Simpson (IS) and floristic richness (S) were calculated. Multiple regression analysis relating the different indexes with variables "time" (T) and surface area of FB and FD was performed.In zone 1 with shorter deforestation and increased surface area of FB more species and floristic diversity were found, while in zone 2 with longer deforestation and lower surface area of FB dominance of species, mainly shrubs were detected. Strip cropping with FB promotes greater floristic biodiversity and the development of larger trees

A120

SURVEY ON CYANOBACTERIA PRESENCE ON THE AGRICULTURAL LAND OF LAS MELLIZAS ESTABLISHMENT (SAN LUIS)

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Soils with permanent agricultural activity may be exposed to a steady deterioration of their structural stability and fertility levels. When cyanobacteria develop in environments with low nitrogen concentration, they present taxonomic groups which develop specialized cells called heterocyst, thus presenting the consequent morphological changes. The aim of this study was to identify nitrogen fixing cyanobacteria under aerobic conditions (with heterocystand without heterocyst) and non-fixing cyanobacteria under aerobic conditions in a lot of "Las Mellizas" establishment located 20 km south-west of the city of Villa Mercedes (Pedernera Department). The sampling site has been treated with a direct seeding system for ten years. The site was divided into three treatments (Tt bare soil, T1 stover of chopped corn with cover crop (rye) fertilizing the sowing with 50kg /ha of urea and T2 standing corn without cover crop) with five blocks systematically distributed at random. At each sampling point, a composite soil sample was extracted at 0-10 cm. deep. Cultivation was made using Watanabe's medium under controlled conditions to achieve the growth of cyanobacteria. Species of agronomic interest were identified and photomicrographs of the taxa present were taken. The results were 38.86%, 23.71% and 46.24% of aerobic nitrogen-fixing cyanobacteria in the Tt, T1 and T2 treatments respectively. The remaining percentages are aerobic non fixing cyanobacteria. According to the results obtained in T1, the community of cyanobacteria was modified by the contribution of inorganic nitrogen to the soil, reducing the percentage of heterocyst fixing.In the three treatments, *Phormidium fragile* (aerobic non fixing), and *Nostoc sp.* (aerobic fixing) were found. Also, in Tt and T2, *Nodulariasp*, and *Calothrix* (both aerobic fixing cyanobacteria with heterocyst) were found respectively. These results contribute to the study of soil cyanobacteria as possible tools of soil management and quality bioindicators them.

A121

NEST STRUCTURE AND COMPOSITION OF *Troglodytes aedon* (Passeriformes) IN THE MONTE OF SAN LUIS, ARGENTINA

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The nest structure of each species is relatively uniform. One function of the nests is the protection against predators and unfavorable climatic factors, maximizing reproductive efficiency. The analysis of the composition of the nests is a good indicator of habitat use and requirements related to the functions of thermal insulationagainst the wind and cold, which favor the eggs incubation. The *Troglodytes aedon* nests are built by analyzed. The area has characteristics of the Monte phytogeographical province. The nests were placed in individual bags, labeled and taken to the laboratory, where the length and weight was measured and its composition was analyzed. Thin rods labeled with letters (A, B, C, D and E) and numbers (1, 2, 3, 4, 5 and 6) were placed vertically and horizontally at specific points of the nest in order to optimize the study. Thelengthand weight of those materials (stems, branches, sticks) that were touching the union of two rods was measured. The results showed that there wasno significant difference between the measured weights and lengths of materials between the points of intersection of all nests ($F = 1.45$, $p = 0.19$; $F = 0.62$, $p = 0.77$ respectively). Comparing the total material examined between nests, a significant difference is observed with respect to lengths ($F = 13.44$, $p < 0.0001$) and weights ($F = 7.51$, $p < 0.0001$). These results are showing that thisspecie use elements with the same characteristics to buildthe different partsof the nest, but the nests differ each other. This may be explained by the amount and type of material used. Supported by UNSL-grant PROICO 2-0516 to Cid FD

Biochemistry, Physiology and Neurochemistry

A122
CADMIUM INDUCES HYPERTENSION TRHOUGH INCREASED EXPRESSION OF ROCK I, OXIDATIVE STRESS AND MORPHOLOGYCAL ALTERATIONS IN THE AORTA

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. It is one of the main contaminants in cigarette smoke. We studied its effects on blood pressure, vascular smooth muscle proteins, histoarchitecture and oxidative stress markers of rat aorta. Male Wistar rats were used: 1 group received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water for 60 days (Cd). During the treatment, blood pressure was measured with a CODA system. Total RNA was isolated with Trizol and cDNA was obtained. Nrf2 factor, NOX2, GPx, SOD, Heme oxygenase 1 (HO-1), COX-2, Myosin Light Chain Kinase (MLCK), RhoA/ROCK I and II were determined by PCR. S28 was used as control. Aortas were fixed, sectioned, stained, and examined for evidence of injury. Cd induced significant increase in systolic and diastolic blood pressure ($p<0.05$). NOX2 increased ($p<0.001$) in the Cd group even though p47 did not show differences. Nrf2 decreased in Cd ($p<0.05$) while GPx did not change and SOD significantly increased in Cd ($p<0.05$). HO-1 and COX-2 showed a significant increase in Cd. MLCK and ROCK II did not show changes while ROCK I showed a significant increase in Cd ($p<0.05$). Regarding the morphology, irregular luminal layers of endothelial cell linings were observed in aortas of Cd-treated animal. Structural changes in tunica intima and tunica media cells were found, exhibiting clearer and bigger cytoplasms than cells from Co aortas. This shows that Cd induces hypertension probably through an increase of ROCK1 expression, therefore inhibiting Myosin Light Chain Phosphatase (MLCP); together with oxidative stress and architectural changes in the aorta.

A123
A STUDY OF EARLY MOTOR BEHAVIOUR AND POSTNATAL CEREBELLUM DEVELOPMENT IN SPONTANEOUSLY HYPERTENSIVE RAT (SHR) AS A MODEL OF INTRAUTERINE GROWTH RETARDATION

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Infants suffering intrauterine growth retardation (IUGR) experience increased incidence of neurological and intellectual impairment. The offspring of SHR have been proposed as an animal model of IUGR. The effects of maternal hypertension in the SHR on brain development and cerebellar function are not fully understood. In this study, we evaluated the neurologic development and early motor behaviour of SHR rat pups and we examined morphological aspects of the postnatal cerebellum. Physical indicators of growth were evaluated throughout early postnatal life (P0-P30) in SHR (n=20) and Wistar Kyoto (WKY, normotensive strain) (n=20) rat pups. Body weight, sensory (eye opening, incisor eruption, ear unfolding) and neurological reflexes (righting reflex, negative geotaxis, gait) tests were performed under controlled environmental conditions. Weight at birth was significantly lower in the SHRs compared to WKYs ($p<0.01$). SHR pups present a delay in weight gain, being significantly different from P16 to P30 ($p<0.001$). The emergence of sensory and neurological reflexes was restrained in SHR compared to WKY; however, once reflexes were acquired, SHR performed better in motor coordination tests than WKYs. Cresyl violet stained mid-sagittal sections of cerebellum were processed to measure whole cerebellar area and to quantify thickness of cortical layers (external -EGL-, molecular -ML- and internal granule cell -IGL- layer) in preculminate, primary and secondary fissures. SHR rat pups at P7 and P14 showed a significantly thinner ML and thicker EGL, compared to WKY, indicating a possible delay in granule cell migration. Cerebellar area was diminished in SHR at P7 and P9 compared to WKY. By P30 the measured parameters are normalized in SHRs. The delay in maturation of central nervous system structures sustains behavioural and neurological impairment in the pups of hypertensive progenitors and may also indicate possible alterations at cellular and molecular levels in these areas.

A124
CUTANEOUS INFLAMMATION REGULATES THIK1 EXPRESSION IN DORSAL ROOT GANGLION NEURONS

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THIK1 is a two-pore-domain potassium channel present in a variety of tissues including arterial endothelium, kidney and in neurons of the retrotrapezoid nucleus and the trigeminal ganglia. We previously demonstrated that THIK1 mRNA is present in dorsal root ganglia (DRG) and that its levels dropped ipsilaterally 1 day after CFA-induced cutaneous inflammation (CFA1). However, the identity of the DRG subpopulations expressing THIK1 and the specifics of any relationship to inflammatory pain, remain unknown. Using a combination of immunohistochemistry, western blotting and behaviour in normal and CFA-treated rats we elucidated the cellular localization and inferred physiological properties of THIK1. In normal rats, we found that all small neurons and subpopulations of medium and large DRG neurons express THIK1. THIK1 staining is weakly positively correlated with IB4-binding but strongly positively correlated with trkA suggesting that THIK1 is expressed by nociceptors. At CFA1, cytoplasmic THIK1 staining was significantly reduced only in small neurons ipsilaterally compared to normal. At 4 days after inflammation (CFA4), THIK1 staining increased significantly ipsilaterally in medium and large neurons compared to normal. THIK1 levels in small neurons increased ipsilaterally compared to contralateral but did not differ significantly from normal. Finally, ipsilateral (but not contralateral) mean %intensities of THIK1 in small neurons at CFA1 correlated strongly negatively with spontaneous foot lifting duration (a marker of spontaneous pain). Our results demonstrate THIK1 expression in DRG neuron subpopulations that are likely to include nociceptors. Interestingly, THIK1 is regulated by cutaneous inflammation and it might be implicated in the pathogenesis of spontaneous pain.

A125
NOCICEPTOR-LIKE DORSAL ROOT GANGLION NEURONS EXPRESS THE ANGIOTENSIN-II AT2 RECEPTOR THROUGHOUT DEVELOPMENT

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Pharmacological and histochemical evidence suggests that the Angiotensin-II type 2 receptor (AT2R) is present in the dorsal root ganglia (DRG) and that this receptor plays a role in neuropathic pain in humans. However, the expression pattern of AT2R during development and the identity of the subpopulation expressing it remain unknown. Using a combination of semi-quantitative PCR, western blotting and immunohistochemistry we examined the expression of AT2R at mRNA and protein levels in rat DRGs from embryonic day 15 (E15) until postnatal day 30 (PN30). We found that AT2R mRNA is present at constant levels from E15 to PN30. A similar different ages. Detailed quantitative analysis of ABC/DAB AT2R staining showed a) that this receptor was present in most neurons at E15 and E18 and b) that postnatally it was predominantly expressed by small DRG neurons. Given that small neurons are putative C-nociceptors and the proposed role of AT2R in neuropathic pain, we next examined whether these AT2R+ve neurons co-localized with Ret and trkA embryonically and with IB4-binding postnatally. We observed that most AT2R+ve neurons were trkA+ve embryonically and IB4-binding postnatally. We also found strong positive statistically highly significant correlations between cytoplasmic AT2R and trkA at E15/E18 and with Ret only at E18. Cytoplasmic AT2R was also strongly and positively correlated with IB4-binding at PN3, 15 and 30. Our evidence demonstrates that AT2R is continuously expressed by a subpopulation of C-nociceptor-like neurons during development.

A126
ANDROGEN DEPRIVATION AND LUNG DISEASE RISK-THE ROLE OF TGFβ AND HO-1

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Previously, we demonstrated that inflammatory parameters changed in lung parenchyma after castration. The antioxidant response elements (ARE) are involved in gene activation coding for a number of antioxidant proteins. Androgen deprivation triggers of oxidative stress and exacerbates lung injury. We studied if castration produced changes in oxidative markers and inflammation parameters.

Male Wistar rats (200 ± 20 g) were separated in 3 lots: controls (Co), castrated (Ca), and castrated supplemented with testosterone (Ca+T) for five days. 60 days after castration rats were killed and lungs were obtained. Before that, bronchoalveolar lavages (BAL) were collected and cell count was performed. RNA was extracted by using TRIzol. mRNA levels of TGF-β, HO-1, Neuregulin (NRG-1), VCAM, COX2 and FOXO3a were quantified by RT-PCR. ANOVA was used for statistical analysis. After 60 days of castration, COX_2 significantly increased its expression in Ca group ($p<0.05$). The expression of TGF-β, and HO-1 decreased in Ca ($p<0.05$), with no changes in Ca+T. Other markers as: NRG-1 and VCAM decreased in Ca + T ($p<0.05$) while FOXO3 did not change. Considerating the HO-1 gene expression in response to TGF-β, this relation is crucial in designing

interventional strategies in TGF-β-mediated diseases. Generated oxidative stress and inflammation orchestrate further up-regulation of COX-2 and the particular importance of TGF-β, which may have a key role in mediating inflammation and fibrosis. We conclude that castration significantly affected the antioxidant status, and modified the inflammatory balance in pulmonary parenchyma.

A127
ANGIOTENSIN II AT2 RECEPTORS EXPRESSION IN *SUBSTANTIA NIGRA*

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The renin-angiotensin system (RAS) has been recognized for its critical role in physiological regulation of arterial pressure as well as sodium and fluid homeostasis. Angiotensin II (Ang II) is the major effector component of RAS and recognizes two receptor subtypes: AT1 (AT1R) and AT2 receptors (AT2R). There is evidence about a role of the central RAS in the establishment of Parkinson disease and other neurological disorders. AT2R has been implicated in processes occurring during brain development and tissue regeneration. AT2R is widely distributed in fetal tissue, but their expression is dramatically decreased after birth. In adult animals, AT2R expression is restricted to a few organs and limited brain areas. The mesencephalic nucleus Substantia nigra (SN) plays a pivotal role in the control of movement, acting as a major input and output center of the basal ganglia. Conflicting results have been reported concerning the distribution of AT2R in the striatum and the SN of mammals. Data about the distribution of AT2R in animal models of Parkinson disease are limited to a few studies.The aim of this work was to study the localization of AT2R in SN of adult rats. We investigated the expression of AT2R gene in SN of Wistar adult rats, detected and semiquantified it by multiplex RT-PCR. We observed the presence of AT2R mRNA in SN by RT-PCR. Indirect immunofluorescence staining with anti-AT2 receptor primary antibody revealed scattered immunoreactive cells in SN. AT2R might play an important role in neuroregeneration of damaged cerebral areas. Additional research is required in order to further unravel the implications of the RAS in Parkinson disease.

A128
GENDER AND AGE DIFFERENCES IN DIETARY ZINC AND SELENIUM, AND IT ASSOCIATION WITH OVERWEIGHT, OBESITY AND HYPERTENSION: A STUDY IN SAN LUIS CITY, ARGENTINA

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Arterial hypertension (AHT) and altered intake and loss of zinc (Zn) and selenium (Se) have been reported in obesity (Ob) and overweight (OW). Herein we report preliminary data showing an association between consumption of dietary Zn and Se with OW, Ob, and AHT; and how it relates to gender and age in a population between 18 and 80 years-old from a population-base sample performed in San Luis City. Patients with chronic diseases, mental disabilities or those that do not sign the informed consent were excluded. A validated food-frequency questionnaire (Aballay, 2009) was used to assess socio-demographic data, dietary intake of Zn and Se, nutritional status (body mass index, BMI, Kg/m^2); and systolic (SBP)/diastolic (DBP) blood pressure (AHT: SBP>120/DBP>80). Average consumption of dietary Se (ug/day) was categorized either as sufficient (SU, ≥55) or insufficient (INS,<55); and Zn (mg/day) in men either as SU(≥11) or INS(<11); whereas in women either as SU(≥8) or INS(<8). T test and Chi-square were used to assess differences by age and gender. Adjusted models of multiple-logistic regression included as response the presence/absence of AHT; and as co-variables consumption of dietary Se and Zn, Ob, gender and age. AHT prevalence was of 41.2% without gender differences (p=0.927). In woman prevailed OW (32%) whereas in men prevailed Ob (32%). The average consumption of dietary Zn was higher in men than women (12.16±5.22 *vs* 9.11±5.11). 52% of the population showed INS consumption of dietary Zn, among these 43%, 34.0% and 18.9% has AHT, OW and Ob, respectively. The average consumption of dietary Se was 71.38±49.02. 48% of the population had INS consumption of Se, among them 38.8%, 28.6%, 16.3% had AHT, OW and Ob, respectively. INS consumption of Se or Zn did not associate to AHT or BMI. AHT was associated with the age (p=0.011) and with the Ob (p=0.002). This preliminary study shows that INS consumption of dietary Zn and Se associates with AHT in aging OW and Ob.

A129
ROLE OF ANGIOTENSIN II AT_2 RECEPTORS IN PURKINJE CELL MIGRATION AND APOPTOSIS IN DEVELOPING CEREBELLUM

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Apoptosis plays an important role in neonatal cerebellar development. Angiotensin II (Ang II) exerts its physiological effects through binding to two receptor subtypes: AT_1 and AT_2 receptors. Recently, it was shown that Ang II participates in cerebellar organogenesis. Previous data suggest a potential role of Ang II AT_2 receptors in Purkinje cell (PCs) migration during cerebellum development. The aim of the present study was to evaluate apoptosis in neonatal cerebellum of animals treated with AT_2 receptor antagonist. Treatment was performed in Wistar rats during the last week of pregnancy with vehicle (control) or PD123319 (AT_2 antagonist, 1.0 mg/kg/day) and offspring were analyzed at different ages: P5, P8 and P15. Apoptosis was evaluated by using acridine orange staining and fluorescence microscopy. In control animals, higher number of Purkinje cells was observed in comparison with treated animals at the different ages. Sagittal sections showed the expected pattern of PCs in a caudal-to-rostral sequence in control animals. At all ages, control animals showed a high number of apoptotic PCs as compared to treated animals. The apoptosis of Purkinje cells in control animals showed a caudal-to-rostral sequence in agreement with their time-of-origin. Animals born from mothers treated with PD123319 showed a delay in the apoptosis process encompassing the lower number of PCs present at the different areas at all studied ages. The detailed analysis of apoptosis pattern revealed regional and cellular differences with respect to postnatal ages studied. These results suggest that there is a developmental stage-specific mechanism of apoptosis in cerebellum and that Ang II AT_2 receptor plays an important role in proper maturation of cerebellum. Besides, these results confirm our previous hypothesis related to a role of AT_2 receptors in PCs migration.

A130
NEUTROPHILIC INFLAMMATION IN THE LUNG IMPROVES INSULIN SENSITIVITY: A MECHANISTIC STUDY IN A MOUSE MODEL OF METABOLIC SYNDROME

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Neutrophilic inflammation (NI) is a poorly known process occurring in the lung of obese subjects exposed to indoor airborne pollutants that may worsen obesity-associated metabolic abnormalities, including insulin resistance (IR). Indeed, obese patients exposed to indoor-airborne pollutants show worse IR than those unexposed, however the mechanisms for this disparity are partially known. Bacterial lipopolysaccharide (LPS) is a stressor that when administrated by intratracheal instillation (ITI) causes NI in the lung. At sites of NI, myeloperoxidase (MPO) oxidizes chloride anions to hypochloroses acid/hypochlorite ($HOCl/OCl^-$), which can damage the lung, increase systemic oxidative stress/inflammation and thus worsen IR. Hydrazide 4-aminobenzoic acid (ABAH) is a fairly-specific inhibitor of MPO. Taurine is a non-cell-permeable scavenger of HOCl. Herein we hypothesized that inhibition of oxidative processes mediated by MPO in the lung can improve insulin sensitivity in an animal model of metabolic syndrome (MS). To test this hypothesis we used male B6 mice which were fed for 16 weeks a high-chicken-fat diet and fructose (MS) and a low-fat diet and tap-water (control). During the last week of diet and on a daily basis both groups of mice were ITI with either PBS (vehicle), ABAH (10 µmol/mouse) or taurine (5nmol/mouse). The last day of treatment animals were ITI with 2.5 µg LPS/mouse or PBS alone; and 6 h later an intraperitoneal glucose tolerance test was performed. A total of 6 groups, for both control and obese mice, were compared (PBS, LPS, ABAH, ABAH+LPS, Tau and Tau+LPS). Compared to control, LPS treatment to obese mice increased lung MPO activity, chlorotyrosine, nitrotyrosine and TNF-α; but reduced insulin sensitivity. These differences were abrogated when animals were pre-treated with either ABAH or taurine. We conclude that MPO-driven oxidative modifications in the lung of obese animals responsible for worsening IR and thus NI may provide a therapeutic target to reduce IR in obese subjects exposed to airborne pollutants.

A131
SEDATIVE EFFECT OF GAMMA-AMINO-BUTYRIC ACID IN THE OPEN FIELD TEST IN RATS

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Gamma-Amino-Butyric Acid (GABA) is a natural compound that mediates an important inhibitory effect within the Central Nervous System (CNS). It is peripherally administered in clinical medicine, but it has been proposed that it may not pass the Blood Brain Barrier (BBB). The aim of the present study is to elucidate the possibility of a central effect when administered peripherally. Male rats weighing 240-270 g from a Holtzman derived colony were used, divided in four groups. These rats were injected with saline or GABA (12.5 and 25 mg/kg) 5 min before testing. The test was the Open Field (Columbus Instruments) in

the conditions of our laboratory. Classical parameters were considered: vertical movements, ambulatory movements, non ambulatory movements, and total GABA ($p<0.05$) when compared to saline controls. This parameter is classically linked to sedation. Present findings may be considered as an evidence of central effect of GABA. Further experiments will elucidate the achievements or scope of present findings. We may conclude that GABA has a sedative like effect in the whole board test, and that it is compatible with an effect within the CNS, suggesting a passage through the BBB, or the action on a zone devoid of BBB.

A132

KETAMINE DISRUPTS WORKING MEMORY IN A HOLE BOARD TEST AND THE METABOLIC ACTIVITY IN PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS

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Ketamine, a N-methyl-D-Aspartic-acid (NMDA) receptor antagonist, is an anesthetic that has psychotogenic effects, and it led to use it inducing animal models of schizophrenia. In the present study the effects of systemic administration ketamine (1; 2,5; 2,5; 5; 10; 15 y 20 mg/Kg ip) were evaluated in a hole board test (HBT). Besides the metabolic activity of the Pre-Frontal Cortex (PFC) and the Nucleus Accumbens Septi (NAS) were simultaneously evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) through wavelength measurement using a spectrophotometer. Male rats derived from a Holtzman colony weighing 240-290 g were used. Hole exploration strategies and locomotor activity were significantly modified in the HBT. Non ambulatory movements were significantly decreased at the dose of 10 mg/kg ($p<0.05$). Ambulatory movements were significantly decreased at the doses of 15 mg/kg ($p<0.01$) and 20 mg/kg ($p<0.05$). Horizontal movements were significantly decreased with 15 mg/kg ($p<0.01$) and 20 mg/kg ($p <0.05$). Metabolic activity showed an evident and significant decrease in the PFC ($p<0.001$) at the doses of 1,25 mg/kg, 2,5 mg/kg and 20 mg/kg. It was also observed a significant decrease in the metabolic activity of the NAS at the doses of 1,25 mg/kg ($p<0.05$) and 2,5 mg/kg ($p<0.01$). We conclude that ketamine at the doses administered interferes exploration and strategies, as well as spontaneous activity in the HBT. It may be interpreted as interference in working memory. These behavioral findings are accompanied by a decrease in metabolic activity in the studied brain areas (PFC and NAS), classically related to this function.

A133

EFFECT OF D-PHENYL-ALANINE ON ANXIETY IN THE PLUS MAZE TEST IN RATS

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D-Phenyl-Alanine (DPA) has a recognized antidepressant action, and it has been shown in several clinical studies. Some patients refer a mild anxiogenic effect receiving it. Due to this reason we studied in the present experiment the possible anxiogenic effect of this drug. Male rats weighing 280-300 g from 15 and 30 mg/kg) 3 min. before testing. The test was the Plus-Maze test, in the conditions of our laboratory, with the classical parameters. Clear results were obtained with all doses. Time spent in the open arm was significantly decreased by all doses ($p<0.001$), and time spent in the closed arm increased ($p<0.001$). Time per entry was also reduced in a very significant manner ($p<0.001$ for 7.5 and 30 mg/kg, $p<0.01$ for 15 mg). Open arm entries were reduced by all doses ($p<0.001$), but closed arm entries were decreased only by the higher one ($p<0.05$). The extreme arrivals were decreased by all doses in the open arm ($p<0.001$) but they were not affected in the closed arm. Grooming did not show significant modifications. *Rearing* was increased by the middle dose ($p<0.05$) and decreased by the maximal dose ($p<0.05$). Expelled fecal boli were increased by the two higher doses ($p<0.05$). We may conclude that DPA has an effect compatible with an anxiogenic property.

A134

TUMOR NECROSIS FACTOR ALFA (TNFα) EXPRESSION IN THE HIPPOCAMPUS FROM THE PROGENY OF EXPERIMENTAL DIABETES RATS. CHANGES BY EARLY INTAKE OF VEGETABLE OILS.

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Uncontrolled maternal hyperglycemia during pregnancy produces alterations in the developing nervous system of the offspring. The aim of this study was to characterize the expression of TNFα in the hippocampus of adult males (SD, 8 months old) born from control mothers (CO) or mothers with experimental diabetes (streptozotocin, 30 mg /Kg iv; DO) that were submitted to an early dietary supplementation with water (C), corn oil (Mz), extra virgin olive oil (OL) or pistachio oil (PS). Treatment were

orally administered from day 2 to 62 (8µl /15g). TNFα expression was analyzed by western blot. Inmumoreactive bands of 51 kDa (inactive, I) and 17 kDa (active, A) were quantified. Two way ANOVA showed significant changes ($p<0.001$) for I, in the condition (CO vs DO), treatment (C vs Oils) and interaction. For A only significant changes by treatment was observed ($p<0.001$). TNFα I showed DO<CO in C and Mz ($p<0.05$), DO>CO for OL and no changes for PS ($p<0.05$). The level of TNFαA was elevated for DO in C and OL ($p<0.05$), decreased in PS ($p<0.05$) and unchanged in Mz. These results indicate that an early supplementation with oils (rich in ω3) modulate the hippocampal TNFα expression 6 months after the treatment. The PS was the most effective decreasing the expression of the TNFα active protein. (CONICET-PIP0243, PICTO/UCCuyo 2009-0158-CICITCA UNSJ-IDeA1400.0107/2012).

A135
SYNERGISTIC EFFECT OF KETAMINE AND FLUOXETINE IN THE FORCED SWIMMING TEST

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Major depression is a prevalent psychiatric disorder. Different pharmacological treatments have their limitations as regards their efficacy and time of latency, requiring even weeks before showing clinical effects. Recent research has shown that NMDA (N-Methyl-D-Aspartate) receptors might be involved in Major depression's pathophysiology and therefore they are possible therapeutic targets. The present study investigates the possible synergistic interaction between NMDA antagonist Ketamine and SSRI (selective serotonin reuptake inhibitor) Fluoxetine. Holtzmann derived rats where treated with ketamine (2.5, 5 and 10 mg/kg) and fluoxetine (5, 10 and 20 mg/kg) 30 minutes before subjected to the forced swimming test. Higher doses of Fluoxetine (20 mg/kg, $p<0,01$) and Ketamine (10 mg/kg, $p<0,05$) induced a significant immobility time reduction. The simultaneous administration of ineffective doses induced a synergistic effect. When combined at lower doses (Fluoxetine 5 mg/kg and Ketamine 2,5 mg/kg) immobility time was reduced ($p<0,01$) and climbing time was increased ($p<0,01$). Fluoxetine (10 mg/kg) combined with Ketamine (5 mg/kg) induced a reduction of immobility time ($p<0,0001$) and increased climbing time ($p<0,0001$). We conclude that simultaneous treatment with fluoxetine and ketamine induce a stronger antidepressant effect when combined than when they are used alone, evidencing a synergistic effect of combination.

A136
NITRIC OXIDE EFFECT ON STRETCHED TUBULAR EPITHELIAL CELLS LINKED TO WT-1 CYTOPROTECTION DURING NEONATAL OBSTRUCTIVE NEPHROPATHY

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A major feature of the injury sustained by the kidney during an obstruction of the urinary tract is a profound induction of apoptosis in the tubular epithelium. In this sense, mechanical stretch is related to renal tubular apoptosis and nitric oxide plays a key role. Furthermore, a decreased nitric oxide (NO) and heat shock protein 70 (Hsp70) expression associated with Wilms tumor (WT-1) gen low expression were shown in obstructed kidneys from neonatal rats. Therefore, we performed the present study to evaluate if NO cytoprotective effects during mechanical stretch injury may be mediated via increases in WT-1 expression linked to Hsp70 dependent pathways. Wistar-Kyoto rats were submitted to complete unilateral ureteral obstruction within 48 h after birth and treated with L-NAME or L-arginine. Also, NRK-52E cells were subjected to mechanical stress. The expressions of Hsp70 and WT-1 were evaluated in order to measure apoptosis induction and to evaluate NO effect. Nephrogenic and cytoprotective gene expression were decreased in obstruction and with the use of L-NAME but remained as controls with L-arginine treatment. The cells with L-arginine showed a reduction in apoptosis even in control cells without stress. L-NAME increased the apoptosis in the groups exposed to mechanical strain but not in control group. We found a cytoprotective role of NO associated to WT-1 expression as well to Hsp70 dependent pathways during conditions involving both mechanical and oxidative stress as well as in neonatal obstructive nephropathy.

A137

DIFFERENTIAL HYPOTHALAMUS BETA CATENIN EXPRESSION IN RATS WITH DEFICIENT LACTATION

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Cadherins and catenins and their activation by Wnt proteins participate in reproductive processes and interact with PRL signaling. We examined whether changes in the expression of Wnt/catenin proteins are involved in lactation deficiency of OFA *hr/hr* rats by focusing on the regulation of PRL secretion at the hypothalamic level. Our first objective was to characterize the pattern of expression of β-catenin during the transition between gestation and early lactation in OFA rats compared with Sprague Dawley (SD, their strain of origin). OFA and SD rats were decapitated at 12.00 h on days 19 and 21 of gestation (G19, G21) or day 2 of lactation (L2). Serum PRL levels were measured by RIA, and β-catenin expression in the medial basal hypothalamus was determined by Western blot. Comparison of β-catenin expression through days under study, revealed that SD rats decreased β-catenin levels at G21 and re-established their values at L2, while OFA rats decreased protein expression only at L2 ($p < 0.05$). Comparison of β-catenin expression on the same reproductive day revealed that SD rats had significantly lower values than OFA rats at G21 ($p< 0.05$) and higher values at L2 ($p < 0.05$). In conclusion, SD rats diminished β-catenin protein expression at G21 while OFA reached the lowest expression at L2. These results suggest that OFA rats show altered β-catenin expression linked with an increased dopaminergic tone and impaired PRL release during lactation.

A138

EFFECT OF CORTICOSTERONE ON IMMUNOLOGICAL STATUS IN *Passer domesticus.*

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Birds are continuously confronted by a large number of stressors (e.g., predation, climate change, pathogens) that affect homeostasis. In response, allostatic processes activate different systems, one of them is the HPA axis increasing secretions of corticosterone (CORT) in blood. The aim of this study was to determine the effects of CORT in some nutritional and immunological parameters in non-migrant birds (house sparrows). To achieve our goal we analysed variations in heterophil to lymphocyte ratio (H/L), hematocrits, serum protein profile and body weigh in *Passer domesticus* exposed to different CORT concentrations (0, 20, 40 and 80 mg/l) in drinking water for 72hs emulating a long-term stress exposure. The H/L ratio was determined with the microscopic differential count on a blood film. Native PAGE was performed to obtain the albumin, α, β and γ globulins fractions corresponding to 0 and 72hs for each organism. The relative abundance of each fraction was determined by densitometric analyses (ImageJ software).We analyzed the data using an RM-ANOVA with Tukey post-hoc test ($p<0.05$). We found a proportional increased in H/L index and a significant decreased in the hematocrit to all the concentrations treatments. A significant increase in the β fraction of the birds treated with 40 and 80mg/l of CORT was observed while a decreased in the albumin fraction was significant only under the 80mg/l of CORT treatment. Finally, the 80mg/l treatment of CORT shows a significant decline in body weight. In conclusion, high levels of CORT affect the nutritional and immunological parameters in sparrows. Supported by UNSL 2-0516 to FDC and UNSL 2-0814 to ECV.

A139

THE BLOCKADE OF OPIOID SYSTEM INCREASES OXYTOCIN SECRETION IN SUCKLING RATS WITH DEFICIENT LACTATION

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Oxytocin (OT) and prolactin (PRL) are essential hormones for a successful lactation and OT acting at pituitary level was proposed as a putative prolactin-releasing factor (PRF). An opioid restraint of OT secretion is developed during late gestation but declines in the postpartum. Our aim was to evaluate if blockade of opioid action may influence OT regulation in response to suckling in hypoprolactinemic OFA *hr/hr* rats characterized by a failure in lactation compared with Sprague-Dawley rats (SD) their strain of origin. Mid-lactating OFA and SD rats separated from their pups for 12 h and subsequently subject to suckling during 2 and 4 h were treated with the opioid antagonist naltrexone (NTX; 5 mg/kg, ip; 8.00 h) or saline and decapitated at 12.00 h. Serum OT levels were measured by RIA, weight gain of the litters was determined and the expression of OT receptor (OTR) in the anterior pituitary was measured by western blot. After suckling OT release and litter weight gain were higher in SD rats compared with OFA rats. NTX treatment increased OT secretion only in OFA rats and after 2 h of suckling an increase in weight gain was observed. After suckling during 4 h an increase of OTR expression was observed in OFA rats. The results obtained show that the opioid restrain of OT secretion is still operating during lactation in OFA rats suggesting another mechanism involved in the failure of the lactation observed in these rats. The higher OTR expression in anterior pituitary in suckling rat supports the proposal that OT may act as a PRF.

A140

EATING FIRED FOODS TIES TO INCREASED PREVALENCE OF ARTERIAL HYPERTENSION AND CARDIOVASCULAR DISEASES

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Arterial hypertension (AHT) has been associated with overweight and obesity; and is a risk factor for cardiovascular diseases any association with eating fried foods in a population of patients in San Luis City, Argentina. In this study 102 patients (18.6% men and 81.4% women), between 18-80 years-old, were included and grouped in tertiles (% of patients): 18-35 (55.9), 36-59 (21.6) and 60-80 (22.5). Patients with chronic diseases, mental disabilities, or those that do not sign the informed consent were excluded. They were interviewed in their homes using a validated-food frequency questionnaire, and anthropometric data were collected. Patients were classified according to their body mass index (BMI, Kg/m^2) in underweight (UW, < 18.5, 3.9%), normal weight (NW, 18.5-14.9, 44.1%), overweight (OW, 25-29.9, 31.4%); and type 1 (T1O, 30-34.9, 10.8%), type 2 (T2O, 35-39.9, 4.9%) and type 3 (T3O, ≥40, 4.9%) obese. The prevalence of AHT in the population studied was of 20.6% and increased with age (by tertiles, 2.0%, 4.9% and 13.7%) and was higher in women than men (1% vs 19.6%). The prevalence of AHT (%) according to BMI and according to sex (male/female) showed: UW (0/3.9), NW (7.8/36.3), OW(4.9/26.5), T1O(2.0/8.8), T2O(2.0/2.9), T3O(2.0/2.9). The higher prevalence of AHT was observed in patients with OW (6.9%) and T1O (5.9%). As for the culinary habits of food preparation was observed that there was a positive correlation between patients whose use frying their food ≥3 times a week with CVD (Pearson = 0 260 rho, p = 0.008). This preliminary study suggests that eating fried foods is positively associated with AHT and CVD, although prospective studies to establish causality between these associations are required.

A141

INHIBITORY EFFECT ON SHORT TERM MEMORY INDUCED BY SUBANESTHETIC DOSES OF KETAMINE ON NOVEL OBJECT RECOGNITION TEST IN RATS

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Ketamine is a dissociative anesthetic. It was derived from phencyclidine. Pharmacologically, it is a noncompetitive NMDA glutamatergic receptor blocker. It binds intracanalicularly the phencyclidine site. Given its pharmacological action, it has been used to induce animal models of schizophrenia. Within these models novel object recognition test involves variables related to memory and perception. Both variables have been previously studied by us in different models. In this study the effect of Ketamine is experimented. We used an intraperitoneal administration of ketamine in sub-anesthetic doses (1.25, 2.5 and 5 mg / kg ip) 10 min before training. Test was carried out 120 min after training. The effect on novel object recognition test parameters was studied. Rats of a Holtzman derived colony weighing 240-290 g were used. We observed that the treatment produced a significant increase in total scan time in the evaluation session ($p < 0.05$) at all doses. It also showed a significant decrease in the rate of discrimination ($p < 0.001$) at doses of 2.5 and 5 mg / kg. This allows us to postulate that treatment had an inhibitory effect on short-term memory. We conclude that this effect could be attributed to the antagonistic action of ketamine on the NMDA type glutamate receptor.

A142

INHIBITORY EFFECT ON LONG TERM MEMORY INDUCED BY SUBANESTHETIC DOSES OF KETAMINE ON NOVEL OBJECT RECOGNITION TEST IN RATS

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Ketamine is a dissociative anesthetic. It was derived from phencyclidine, an abuse drug used since 1965. Pharmacologically, Ketamine is a noncompetitive NMDA glutamatergic receptor blocker. It binds intracanalicularly the phencyclidine site. Given its pharmacological action, it has been used to induce animal models of schizophrenia. Within these models novel object recognition test involves variables related to memory and perception. Both variables have been previously studied by us in different models. In this study the effect of Ketamine is experimented. Rats of a Holtzman derived colony weighing 240-290 g were used. It was realized an intraperitoneal administration of ketamine in sub-anesthetic doses (1.25, 2.5 and 5 mg / kg ip) 10 min before training. Test was realized 24 hours after training. The effects on novel object recognition test parameters were studied. We observed that the treatment produced a significant increase in total scan time in the evaluation session at 2.5 ($p<0.01$) and 5 mg/kg ($p<0.05$) doses. A highly significant decrease was observed in the rate of discrimination at all doses ($p < 0.001$). This allows us to postulate that treatment had an inhibitory effect on long-term memory. We conclude that this effect could be attributed to the antagonistic action of ketamine on the NMDA type glutamate receptor

A143

HIGH FAT DIETS INCREASE METHYLGLYOXAL BLOOD LEVELS WHICH ARE REDUCED BY OLIVE OIL INTAKE

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MethylGlyoxal (MG) is a glycation end product found in patients with diabetes, a Chronic Adult Disease (CAD). Another CAD is HyperCholesterolemia (HC). A classic animal model to study effects of HC is rabbit fed with high fat diets (HCR). Also HC can be reverse by the addition of Olive Oil (OO) to diets. The purpose of this study was to determinate MG variations under high fat and OO intake. To generate HCR, rabbits were fed with balanced diet supplemented with grease. After 6 months, fat was reduced and replaced by OO (1:1, fat:OO). Blood samples were analyzed by several biochemical parameters. In HCR, cholesterolemia increased three fold (87 ± 12 mg/dl) over control (25 ± 4 mg/dl, $p < 0,001$). The addition of OO to diet promoted a cholesterol lowering (48 ± 5 mg/dl). Curiously, triglyceridemia and glycemia showed a non-significant increment. MG blood levels increased in HCR (Δ=385 µg/dl compared to NCR Δ=96 µg/dl; Δ = differences between 6 months of treatment and 0 month) and decreased in OO rabbits (Δ=-485 µg/dl). MG normally linked to glycemia could be also related to cholesterolemia. OO may benefit the HCR group by preventing the formation of MG.

A144

HYPOXIA-ISCHEMIA INDUCES ENDOSOMAL-LYSOSOMAL ALTERATION IN RAT BRAIN

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The endosomal-lysosomal system plays an important role in the modulation of the cell integrity. The protease Cathepsin D (CatD) and the protein prosaposin (Psap) participate in neuronal homeostasis. Previous studies have shown that endosomal-lysosomal dysfunction participates in neuronal death as a response to excitotoxicity. In this work, we proposed to study the effect of neonatal hypoxia-ischemia (H/I) on compartimentalization of CatD and Psap. Seven-days old Wistar-Kyoto rat pups were subjected to H/I treatment by ligature of the left carotid artery followed by a short exposure to 100% N_2. After 96h, the brain was dissected and both hemispheres from cerebral cortex (Cx) and hippocampus (HIP). CatD, Psap and Lamp-1 (lysosome marker) proteins were evaluated in membranes and cytosols from Cx and HIP by immunoblotting. We observed a significant increase to controls. Moreover, by using anti Lamp-1 we observed a protein of ~50 kDa in the cytosolic fraction of the injured tissue, which could correspond to a cleavage fragment of LAMP-1. We concluded that H/I induce an increase of cytosolic CatD and Psap due to Lamp-1 cleavage and higher lysosomal-endosomal permeabilization. This phenomenon could be responsible of the excitotoxic damage.

A145

HOCL SCAVENGING STRUCTURES IN LIBRARIES OF NITRONES AND DITERPENES

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Neutrophilic inflammation results from activation of neutrophils at sites of chronic and acute inflammation. Activated neutrophils release myeloperoxidase (MPO), the unique enzyme that uses H_2O_2 to oxidize chloride anions to the powerful oxidant HOCl. HOCl damaged proteins are seen and involved in a number of inflammatory diseases. Thus the search for inhibitors of MPO and/or scavengers of HOCl is of vital importance for the treatment of neutrophilic inflammation. The aim of this work is the search for highly characterized synthetic or natural structures that react with HClO/ClO^-. We searched HOCl scavenging activity in 55 synthetic and natural compounds: 47 nitrones and 8 natural diterpenes. For this purpose, we used a screening assay to evaluate the ability to reduce luminescence caused by reaction of HClO with luminol. To avoid luminescent-probe-associated unspecific reactions, a chlorotyrosine assay was performed. Results showed that from the 8 diterpenes compounds, only one lessened the signal below 1 µM concentration. In the case of nitrones, 13 probed to have inhibitory activity in the range of 5-10 µM, 28 compounds in 1-5 µM, 4 compounds between 0.1 and 1 µM, and finally two nitrones showed an interesting activity below 100 nM. Scavenging HOCl at sites of neutrophilic inflammation may be an interesting bioactivity for finding new leader structures in drug discovery. Further studies aimed at determining toxicity, cell permeability, mechanism of action and in vivo activity are 0414 (To SEGM) and PIP2015-2017-112215-0100603CO (To DCR, SEGM & SEGM).

Biochemistry, Physiology, Pathology, Genetics and Plant Production

A146
BEHAVIOR OF FOUR SPECIES IN THE MODERATION OF SUMMER TEMPERATURE ON TILED FLOORING

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The major thermal amplitudes that occur in summertime in the city of Villa Mercedes (San Luis) Argentina, cause the concrete pavement to collapse revealing cracks, breaks and upheavals that result in the loss of the evenness of the levels on the tiled flooring. In order to know the species that can best act in the moderation of temperatures affecting the flooring, Sophora *japonica*, *Ligustrum lucidum*, *Platanus* x *acerifolia* and *Fraxinus americana* were studied. Temperatures were measured with an infrared thermometer on sidewalks and streets, under the shade of the four species mentioned before and without their protection in January, February and March. To analyse the data, the Analysis of Main Components (MCA) was used and they were examined with Biplot. On examination it is evident that in general lower temperature, is due to the presence of Platanus x acerifolia and *Fraxinus americana* while *Sophora japonica* and *Ligustrum lucidum* reduce the impact of temperature to a lesser extent. The MCA reduces the variables and interprets the correlations making subsequently necessary to continue with the analysis of species in relation to the pairs formed.

A147
RESPONSES AND DEVELOPMENT OF MUTANT WHEAT (*Triticum aestivum*) TO WATER STRESS CONDITIONS DURING GERMINATION

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The water deficit is considered as the most important factor related to growth and decreasing yield on many crops. The aim of this study was identify drought resistant wheat mutant genotypes. Two trials were conducted, the first was performed in the laboratory and the second was assessed in the field. The plant material was obtained by mutagenic induction from the genotypes BIOINTA 1005 and ProElite. To stimulate water stress conditions during germination stage, we used PEG-6000. Three osmotic solutions (-0.5, -1 and -1.50 MPa) were used for treatments. Controls were performed with deionized water. Percentage and speed germination were measured. In the field, trials were conducted in a split plot design with four replicates. The plants were supplied with 100% of the ETc (control) and 25% of ETc (stress). Phenology and yield components were evaluated numerically and physiologically in all treatments. Complete data and stress index were performed using ANOVA. In the first trial were observed significant differences among treatments, BioInta1005 mutants was the most susceptible to severe stress conditions. In the second experiment, significant differences were detected among treatments for growth and yield trials, both lower in stress conditions. The results of germination test contrast with those of the field. In the first BIONTA 1005 mutant`s resulted more sensitive, but in the second they were more tolerant, presenting higher yields.

A148
DEVELOPMENT AND EVALUATION OF SSR MARKERS FOR GARLIC AND ONION

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The genus *Allium* includes economically important vegetables, consumed worldwide due to their characteristic flavor. Globally, garlic and onion are the most important *Allium*. INTA La Consulta is home of breeding programs for both vegetables. Despite its importance, the molecular tools available for *Allium* are very limited. In the present study we developed and evaluated 261 microsatellite markers (or SSRs) from genetic and genomic level of polymorphism in four genetically different cultivars of garlic and onions. Analysis of the data revealed that 31 (12%) of these markers were polymorphic in onion cultivars and 44 (17%) were in garlic. Also a significant number of garlic and onion SSRs were successfully transferable across Alliaceae. 75 (EST and genomic SSRs) and 51 (EST-SSRs) primer pairs that produced fragments in garlic and onion respectively, also produced putative SSR-containing products in one or more Alliaceae species. The SSRs markers developed in this study will impact positively the garlic and onion national breeding programs.

A149
GENETIC DIVERSITY AMONG ARGENTINE GARLIC CULTIVARS (*Allium sativum* L.) REVEALED BY SSR MARKER ANALYSIS

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Garlic is an asexually propagated crop, presenting abundant morphological variation. This species represents one of the main exported vegetable products of Argentina. Purple, red and white type garlics are grown and consumed in the country.In this study, the genetic diversity of 21 Argentine garlic cultivars was evaluated using 14 polymorphic SSR markers. In total, 36 alleles were detected with an average of 2.6 alleles per locus. The allele number per locus ranged from 2 to 4. Observed (Ho) and expected heterozygosity (He) ranged from 0 to 1 and 0.3 to 0.7, respectively. The Polymorphic Index Content (PIC) varied from 0.2 to 0.6, with a mean of 0.4 for the 14 loci tested. Our study revealed considerable genetic variation among the garlic cultivars tested, with a range of 38-100%. The UPGMA dendrogram constructed with all 14 polymorphic SSRs revealed the existence of six arbitrary groups at 0.59 similarity coefficient, and showed clustering of the genotypes according to their physiological groups (at 0.59 similarity) and the flowering behavior (softneck *versus* hardneck types) (at 0.51 similarity). This study represents the first genotyping and genetic diversity analysis of Argentine garlic cultivars using microsatellite markers.

A150
SOYBEAN GERMOPLASM DEVELOPMENT WITH NUTRITIONAL AND INDUSTRIAL QUALITY

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The UNSL and INTA within the framework of a technology-linked agreement have developed soybean germoplasm with nutritional and industrial quality obtaining genotypes with absence of anti-nutritional factors (Kunitz), reduced lipoxigenase activity and high protein content. In order to evaluate selected germoplasm we sowed, in Villa Mercedes (San Luis), during 2014/15 and 2015/16 crop seasons 12 soybean advanced lines (triple null for lipoxigenases and null for antinutritional Kunitz factor) and a commercial control variety in a randomized block design with three replications and analyzed the following traits: days to flowering (DF), days Variance analysis (ANOVA) and principal component analysis (PCA) were performed for each year. Very significant differences (p-value<0.01) were found for DF, DM, Y, SW and PC for both crop seasons and PH and OC for 2015/16. PCA explain 66% of the explored variability and showed that, during 2014/15, Y, PH, DF and DM were highly positively correlated and associated with genotypes 35.1, 79.2, 54.2 and the control. While PC, with values higher than 43 %, was negatively correlated with Y and OC and associated with genotypes 34.2 and 42.2 during 2014/15 and with 27.1 and 12.1 in 2015/16. The characterization of the germoplasm allowed us to select soybean genotypes with absence of anti-nutritional Kunitz factor, lipoxigenase low activity and high protein content.

A151
EVALUATION OF TOLERANCE TO SALINITY AND BORO IN 14 GENOTYPES OF THE GENUS *Vitis*

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In the irrigated valleys where viticulture is developed, the problems of salinity and toxicity of some ions in the soil limit productivity and quality of the obtained products. The tolerance to toxic ions (Cl^-, Na^+, B $(OH)^{4-}$) of the commercial grape cultivars is rather low, but it is feasible to use rootstocks through different mechanisms withstand high levels of ions in the soil. The purpose of the study was to determine the tolerance of 14 genotypes of *Vitis* subjected to high concentrations of sodium chloride (NaCl), boron (B) and the combination of both, analyzing seven hybrids and 7 American *Vitis vinifera* varieties. One year old plants were maintained under semi-controlled conditions, watered with a NaCl solution (10dS / m), other boron (2ppm) and NaCl + B (10dS / m + 2ppm) combination. Growth and survival parameters were evaluated for 20 days. Monitoring of the plants the growth parameters obtained growth rates and percentage of leaf damage were determined. The results showed that the Muscat and Syrah, genotypes showed higher tolerance to saline conditions while Poulsen, Malbec and 101-14 were the most sensitive genotypes, with a high mortality rate. With regard to the toxic effect of boron Sultanina was the most tolerant genotype while Paulsen and Ruggeri were the most sensitive to this ion. Respect to boron salt-tolerant genotype combination was Torrontés, and sensitive genotypes were 101-14, Paulsen and Malbec.

A152

DAMAGE EVALUATION OF *Dichelops furcatus* IN LATE CORN IN VILLA MERCEDES (SAN LUIS)

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One of the pillars on which the corn crop rests and which directly influences the yields achieved, is the control of injurious species. Among the arthropods able to determine the yield potential, *Dichelops furcatus* is harmful in early and late maize planting since the beginning of the crop cycle. This species injects toxins into the stem of the seedlings during its feeding process causing reduction of the stand or harming the vigor. Their populations have caused damage of up to 30% of young corn plants in the center of Córdoba (Argentina). In order to determine the presence and degree of damage an experiment of hybrid corn in the experimental field of the Department of Cs. Agricultural FICA-UNSL, sown on December 10, 2015, at a distance of 0.52 cm between rows, was established. The leaf stages 4, 540 plants were analyzed. The severity scale was calculated using the five degrees of damage established by Flores (2012), marking the plants according to their degree, evaluating -in physiologically maturity for each of them: stem diameter 8 cm above the crown secondary roots, plant height from the crown to the insertion of the male flower, number of ears per plant and number of leaves. The data obtained are analyzed by analysis of variance and Tukey's test (alfa = 0.05) using the statistical software InfoStat. The results showed significant differences between grade 4 and grades 0 and 1 for the variables height and number of ears per plant. The diameter of plant showed significant differences between grades 3 and 4 with respect to 0 and 1. No significant difference for the variable number of sheets between degrees of severity was observed. No plants with the presence of grade 2 were observed. The incidence was 3% and the mortality of plants was 50% corresponding only to the grade 4. The variables: plant height, number of spikes and basal diameter were affected by the degree of severity. The incidence occurred in a small percentage compared to the values reported for the center of the country.

A153

EVOLUTION OF WEEDS AND HARMFUL ARTHROPODS POPULATIONS IN THE SECOND YEAR OF IMPLEMENTATION OF A CULTURE OF ALFALFA, IN J. JORBA (S.L.)

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The establishment, crop productivity and persistence depends on, among other factors, the health. In order to know the species and population levels biweekly random samplings were carried out during two productive years in a lot of 40 ha, with three marked physiognomic characteristics: hill (L), medium hill (ML) and low (B). weed species and the percentage of coverage within a framework of a m2 were determined. Arthropods were sampled with insect net. Both the condition B and L decreased 25% fall-winter weeds the first year compared to the second is observed, whereas in ML condition was an increase of 86%. Regarding the spring-summer weeds was observed a decrease in the number there of being for each condition: B, 50%; ML, L 64.7% and 85.7%. The diversity of phytophagous arthropods remained in both years of cultivation, the three conditions (between 9 to 12 species). The important species for its impact on defoliation were: *Colias lesbia*, *Rachiplusia nu* and *Helicoverpa gelotopoeon*, whose relative abundance was 56.4%, 30% and 13.6% respectively. The total number of individuals was 30% lower in the second year compared to the first. Species "cutworms" observed corresponded genus *Agriotis*, the number of individuals showed an increase of 30% over the first year. This will be explained by the increase in size of the crown of the plant.

A154

Na_2SO_4 AND NaCL DIFFERENTIALLY MODULATE THE ENZYMATIC ANTIOXIDANT SYSTEM IN *Prosopis strombulifera* (Fabaceae)

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Prosopis strombulifera (Lam) Benth, is a spiny shrub especially abundant in the salinized areas of central Argentina with soils characterized by similar proportions of NaCl and Na_2SO_4. This species has shown a halophytic response to NaCl, but in contrast, a strong growth inhibition at lower Na_2SO_4 concentrations. In the present study we evaluated the oxidative damage in tissues and the antioxidant response caused by NaCl, Na_2SO_4 and their iso-osmotic mixture in this species. We found that Na_2SO_4 induced an important oxidative damage with significant increase in malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). Also, an intense NaCl-treated plants presented a slight staining. In Na_2SO_4-treated plants, a gradual decrease in catalase (CAT) activity was observed in roots, while in leaves showed a marked increase at high salinity. In contrast, a strong superoxide dismutase (SOD) activity was observed, with increases in both leaves and roots, with activity levels much higher than in NaCl-treated plants. It seems that CAT plays an important role in early detoxification of H_2O_2 in roots, while SOD would have a predominant role in leaves, reflecting its important role in the anion superoxide detoxification. It seems that at higher salt concentrations the antioxidant enzymatic activity allows H_2O_2 to act as a stress signal to trigger adaptive physiological responses such as early

suberification and lignification in roots. An intense activity principally of SOD in Na_2SO_4-treated plants indicates an effort of plants to counteract the severe oxidative stress caused by this salt.

A155

DRY MATTER PRODUCTION OF *Panicum coloratum* (KLEINGRASS) DURING THE GROWING PERIOD.

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Beef cattle husbandry is a widespread activity in the Province of San Luis. Megathermic perennial pastures, such as *Panicum coloratum* L. cv. Green, are adequate alternatives for forage programs in the central semiarid region, reducing risk of erosion and production costs without sacrificing productivity. However, productivity of the pasture is affected by management. The objective of this study is to determine the effect the timing of the first cut during spring growth that has on the productivity of *P. coloratum*. The study was carried in 2014-2015 in field plots in the experimental station of INTA San Luis, near the city of Villa Mercedes (Lat 33 ° 40'Long 65 ° 28'; 512 m a.s.l). Two treatments were applied: T1 = defoliation at the preflowering state (flag leaf / partial onset 1st inflorescences) and T2 = defoliation at the state of 50% heading. Subsequent cuts were performed at fixed time, every 28 days, 4 cuts in the cycle. 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), after the 1st defoliation. 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 leaf (L). The samples were dried at 65°C to constant weight). Yields in both treatments were compared. Dry matter production differed between treatments ($p < 0.05$), with values of 10,290 and 12,143 kg DM .ha^{-1}.$year^{-1}$ T1 and T2, respectively. It is concluded that the time of the first cut in spring affect forage production.

A156

COMBINATION OF EXTRACTS FROM ALGAE *Macrocystis pyrifera* WITH BACTERIA PGPR STIMULATE GERMINATION OF HORTICULTURAL SPECIES

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The evolution of horticulture in recent years has recorded a reduction in acreage but an increase in intensive production based on the incorporation of technological innovations, mainly associated with biofertilization. The aim of this study was to analyze the germination response of tomato, lettuce, radish and chicory to the application of extracts from the alga *Macrocystis Pyrifera* individually, or together with *Azospirillum brasilense* inoculation, to assess whether both types of promoters exert a synergistic action on the germination energy (GE) and the germination power (GP). Seeds from the four horticultural species were inoculated and placed on germination trays (50 seeds per tray and 4 trays per treatment) in growth chamber (Conviron PR48) with controlled conditions : 80% relative humidity, 16 h light / 8 h dark photoperiod and of 24 and 20° C respectively, in triplicate. GE and GP were determined according to ISTA rules. Germination kinetics was also determined. The results showed a positive effect of the combined use of both biofertilizers on the germination parameters in some species, mainly on lettuce, in which co-inoculation produced significant differences. In other species germination was mainly stimulated by the algal extract alone. These results suggest that the use of algal extract individually or in combination with PGPR may be used as bio-fertilizers in horticultural species but taking into account that each species responds to each fertilizer in a particular way. The use of biofertilizers is of great importance in horticulture, to replace chemical agents for a more sustainable food production.

A157

GOOD PRACTICE FOR THE CONTROL OF *Puccinia graminis* IN CROPS OF *Avena sativa*

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Know the epidemiological characteristics of rust allow propound appropriate and sustainable management strategies, such as the application of fungicides and the use of appropriate cultivar. The Agro-ecological conditions of the semiarid region, they make it necessary. In order to guarantee the correct use of active ingredients Azoxystrobin +, Cyproconazole (T1) and Penconazole (T2), to control the disease, its impact on field of FICA -UNSL between July and December 2015. In a completely randomized factorial experimental design, 2x3x3 (Cultivars: CALEN y MANÁ, treatments: T1, T2 y control T, with three repetitions), incidence and severity of rust were evaluated weekly, between stem elongation to senescence, in 10 tillers random. Areas under the curve disease progress (AUCDP) were calculated. ANOVA from the same were made and were correlated with yield data, in R statistical software. AUCDP higher values corresponded to oats MANÁ (*P*: $1.91e^{-05}$) and T2 (*P*: $4.43e^{-06}$). A negative correlation (-0.56) between yield and AUCDP was determined, especially when considering the cultivar MANÁ (-0.92), the most

affected by the disease. It can be concluded that the use in recommended doses of Azoxystrobin + Cyproconazole and cultivar CALEN are appropriate strategies for this disease management in Villa Mercedes, San Luis province.

A158

GOOD PRACTICES IN THE IMPLEMENTATION OF PLANT PROTECTION FOR CONTROL OF RUST (*Puccinia* spp.) IN CROP OF RYE (*Secale cereale*)

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Rye, prominent cereal for seed production in San Luis, its affected by rusts (Puccinia spp.) that influence yield. It is essential the knowledge and application of good plant protection practice guarantee not affect "non target" organisms or the environment. In order to assure the pertinent use and effectiveness of active ingredients Azoxystrobin + Cyproconazole (T1) and Penconazole (T2), to control the disease and its impact on yield in Villa Mercedes, studies were conducted in the experimental field of FICA - UNSL between July and December 2015. In a completely randomized experimental design, (treatments: T1, T2 y control T, with three repetitions), incidence and severity of rust were evaluated weekly, between stem elongation to senescence, in 10 tillers random. Area under the curve disease progress (AUCDP) was calculated. ANOVA from the same were made and were correlated with yield data, in R statistical software. The results show that ABCPE indicated no significant difference between treatments and other yield, T1 being the highest value (P: 0.00107) attributable to the positive effect of strobilurins on the crop and not the effect of fungicides on the fungus. Given the economic point of view and the sustainability of the system, its concluded that should not apply fungicides and consider other alternatives to replace the effect of strobilurin.

A159

CHARACTERIZATION AND ANTIOXIDANT POTENTIAL OF CAROTENOIDS PRODUCED BY *Bacillus licheniformis* Rt4M10

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Carotenoids are the most widespread naturally occurring yellow, orange and red pigments. These can be grouped into two major classes: carotenes (hydrocarbons that can be cyclized at one or both ends of the molecule), and their oxygenated derivatives called xanthophylls. Several species of bacteria accumulate high concentrations of carotenoids. They are of interest due to theirantioxidant, UV protecting and natural food colorant properties. In a previous study *B. licheniformis* Rt4M10 (Rt4M10) was isolated from roots of *Vitis vinifera* L. (cv. Malbec). The aim of this study was to identify and quantify carotenoids produced by Rt4M10 and its biological role *in vivo*. Rt4M10 was cultivated aerobically in orbital shaker. Cells were centrifuged and the upper layer was discarded and residual cells were extracted with methanol. Suspensions were sonicated and centrifuged. After that, the clear coloured organics phases were transferred into a clear tube. Finally, the carotenoids levels in the supernatant were quantified through HPLC. To evaluate sensitivity to hydrogen peroxide (H_2O_2), the cells were cultivated until the stationary phase. Accurate quantities of H_2O_2 solution were added to the cell suspension, it was incubated with H_2O_2 in the dark and it was plated. Colonies were counted after 4-5 days to evaluate antioxidant capacity. Lycopene, β-carotene, g-carotene, violaxanthin, criptoxantine and zeaxanthin were identified and quantified by HPLC. These carotenoids demonstrated antioxidant activity *in vivo*

A160

EVALUATION OF *Eragrostis curvula* GERMINATION SIMULATING DROUGHT TO DIFFERENT DEPTHS OF SOWING AND OSMOTIC POTENTIAL

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Eragrostis curvula is a perennial forage grass originated from South Africa and widely spread in Argentina in the last 70 years. The producer of the arid and semiarid region raises the problems of dry soils, high temperature and different depths of sowing simulated before in a situation of drought, availability and water quality during the germination. The object was to evaluate the germination and survival to different depths of sowing and different osmotic potentials with polyethylen glycol (PEG). Experiment I (EI): the seeds of *Eragrostis curvula* were placed droughts (10 days to 65°C) in trays with land to 25°C and field capacity, to five depths of sowing (0cm; - 0.5cm; -1.0cm; -1.5cm and -2.0 cm) with four repetitions with 50mg of seed for repetition. Experiment II (EII): the witness was distilled water and with polietilen glicol (PEG) to 0.5; 1.0 and 1.5 MPa in a growth chamber to 25°C on germination's paper. In the EI, the witness (without stove to 65°C) germinated with an average of 2000 seedlings, meanwhile in the treatments of 65°C for 10 days: to 0cm 240 seedlings were germinated and to -2cm 100. In the

EII, the witness was germinated in a 98%, in 0.5MPa 50 % and in the rest 0 %. The conclusion is that in EI and EII the survival in the worst conditions was extremely low to coincide with the results of failure of seed to field.

A161
ELATERIDOS (COLEOPTERA: ELATERIDAE), PRESENT IN DIFFERENT AGROECOLOGICAL CONDITIONS AND COMPARISON OF TWO METHODS OF SAMPLING IN SAN LUIS

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Among insects living in the soil, there is a group of larvae of the Elateridae family, "wireworms", which can sometimes become a pest damaging roots and seeds of cultivated plants and their presence is associated with the degree of habitat modification. In the "Area of dunes with grasslands and islets of chañar" (San Luis), the population level of edaphic wireworms was assessed in three situations: natural pasture, weeping lovegrass and soybean and capture bait traps was compared to the efficiency the conventional sampling. To do this fortnightly sampling, simple random type, between the months of November to March were made. The data obtained were results show differences between sampling methods, being significant in the number of individuals collected in traps baited with respect to the traditional sampling in soils with weeping lovegrass crops and soybeans, in contrast to that observed in natural grassland. The low population level wireworm present in soil without disturbance (natural grassland), could explain the lack of difference observed between sampling methods evaluated.

A162
EVALUATION OF THE ANTIDOTE EFFECT OF FLUXOFENIN ON *Digitaria eriantha* AND *Panicum coloratum* cv. Klein Green TO APPLY PRE EMERGENT HERBICIDES

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Digitaria eriantha (De) and *Panicum coloratum* cv. Klein Green (Pc) are C4 megathermal Poaceae originated from South Africa and adapted to the semiarid temperate central region of Argentina with frosts of -17°C. The difficulties of implantation due to competition that the undergrowths produce it is a relevant reason of failures in the implantation. Up to the moment selective herbicides do not exist neither tolerant growing to graminicides. The object was to evaluate the effect of the protector - antidote (safener-fluxofenin), to herbicides like S-metolaclor and Acetoclor used in *Sorghum* spp. Phytotoxicity of the fluxofenin-safaner and the protection that produces the herbicides S-metolaclor and Acetoclor in survival of seedlings of De and Pc was evaluated. The trial was done in the laboratory of seeds of the EEA-INTA San Luis. Twelve treatments were designed by four repetitions to evaluate the effect of four doses of antidote (0; 20; 40 and 60 cc every 100 kg of seed) without the application of herbicides, and the combination of every dose of antidote with two herbicides: SM (S-metolaclor) and A (Acetoclor) with the commercial dose. The trays were incubated in a growth chamber to 30°C with 8 hs of light and 20 °C with 16 hs of darkness. The conclusion is that the treatment with antidote - fluxofenin in low doses (20cc) is not phytotoxic and it didn't affect neither the development nor the number of plants of De and Pc. The best combination of antidote - fluxofenin and herbicide SM was 20 cc for De and 60 cc for Pc. The herbicide Acetoclor was phytotoxic in both species. It is suggested to verify these results in new tests in field conditions.

A163
GROWTH RESPONSES OF *Lactuca sativa* SEEDLINGS UPON BIOFERTILIZATION WITH THE ALGAE *Macrocystis pyrifera* AND THE PGPR *Azospirillum brasilense*, INDIVIDUALLY OR IN COMBINATION

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Horticultural production in Rio Cuarto, Córdoba, occupies approximately 700ha being leafy vegetables such as lettuce the main crops in this area. Due to high demand as food complement for being an excellent source of minerals, vitamins and dietary fiber, lettuce production needs to be increased. Cultivation is carried on mainly under field conditions, or in greenhouse with hydroponic systems. Increased production of leaf biomass could be achieved by the use of environmentally sustainable alternatives such as biofertilizers. The objective of this work was to analyze the physiological responses of lettuce to the application of extracts of the alga *Macrocystis pyrifera* individually or in mixed inoculation with *Azospirillum brasilense*, and to assess whether both types of promoters exert a synergistic action on root growth and crop establishment. The parameters analyzed were root length, dry root biomass and area and aboveground fresh weight and dry biomass, total chlorophyll content and stomatal conductance in response to the different treatments. The results showed a statistically significant increase in all these physiological parameters in seedlings inoculated with the algal extract individually, and in combination with PGPR bacteria with no differences between these two treatments. Chlorophyll content and stomatal conductance control were also favored by biofertilization, suggesting that its implementation could allow optimization of lettuce production by using a natural alternative

A164
MORPHOGENETIC CHARACTERISTICS of *Tetrachne dregei*

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Knowledge of morphogenesis helps to develop management guidelines for forage species. It was established as objective to describe the morphogenetic variables at tillering level for *Tetrachne dregei* (mega thermal grass) for three growing cycles in Villa Mercedes (SL). Morphogenesis is defined by its variables: phyllochron (Fc:°C day), mean leaf lifespan (MLL:°C day), leaf appearance rate (LAR: leaves/°C day) and leaf elongation rate (LER: mm/day). After a cleaning cut (late August) and the beginning of regrowth (September), tillers were identified in different plants (20-15/cycle). Once a week, appearance and number of green leaves/tiller (LN°), leaf senescence and green leaf length (GLL) was recorded. Total LN°/tiller, Fc, MLL (Fc * LN°), LAR (1/Fc) and LER (average daily increase in GLL) was determined. Fc and MLL were calculated between the 3°-4° sheet up to 6°-8° sheet, with based growth temperature = 9°C. On average inter-cycles, variables reached the following values: MLL = 795 ± 224 °C day, Fc = 307 ± 83 °C day, LAR = 0.004 ± 0.002 sheets/°C day, LER = 5.5 ± 2 mm/day. Total LN° was 8 ± 1, and in each weekly observation, were detected 2 to3 leaves/tiller. These variables indicate that *T. dregei* has a slow growth and a long MLL, so it stays green for a long period of their growth cycle. The species requires low frequency defoliation, with rest periods that consider the MLL and the rate of appearance of leaves (Fc).

A165
MORPHOGENESIS OF THE MEGATHERMICAL FORAGE SPECIES *Panicum coloratum*

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The dynamics of generation and leaf expansion and subsequent senescence defines the morphogenesis of plant species. Production and loss of fodder simultaneously occurs as a result of growth and senescence processes. It was proposed to describe the morphogenesis at tillering level of the grass *Panicum coloratum* in Villa Mercedes (San Luis) for two cycles of growth. The phyllochron (Fc) expressed in thermal and chronological time (°Cday and days), mean leaf lifespan (MLL: °Cday and days), leaf appearance rate (LAR: sheets/°Cday or sheets/day) and leaf elongation rate (LER: mm/day) conform the morphogenetic characteristics. After a cleaning cut at the end of August, and the beginning of regrowth in September, tillers were identified in different plants (20-30/cycle). Once a week, appearance and number of green leaves/tiller (LN°), leaf senescence and green leaf length (GLL) was recorded. Total LN°/tiller, Fc, MLL (Fc * LN°), LAR (1/Fc) and LER (daily increase in GLL) was determined. Fc and MLL was calculated between 3°-6° sheet to 7°-10° sheet, with based growth temperature of 10°C. On inter-cycles average, variables reached the following values: Fc = 166°Cday (17 days), MLL= 615°Cday (68 days), LAR= 0.006 sheets/ºCday (0.07 sheets/day), LER= 4.5 mm/day. Total LN° was 10 and in each weekly observation, 4 ± 1 sheets/tiller were detected, being determined 2.5 leaf generations at the end of the cycle in March. These variables indicate that *P. coloratum* requires low frequency defoliation, with rest periods adjusted at the beginning of leaf senescence (MLL) and the rate of appearance of leaves (Fc and LAR).

A166
EFFECT OF NITROGEN ON *Eragrostis curvula* MORPHOGENESIS IN SAN LUIS, ARGENTINA

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Morphogenesis has genetic and environmental control (mainly light, water and nutrient availability). It raised comparing morphogenetic variables in *Eragrostis curvula* (cv. Tanganyika) under nitrogen fertilization. The phyllochron (Fc) expressed in thermal time (°Cday), mean leaf lifespan (LLS: °Cday), leaf appearance rate (LAR: leaves/°Cday) and leaf elongation rate (LER: mm/day) make morphogenetic variables. The plants established in pots, underwent two fertilization treatments (with N and control) under a completely randomized design. After a cleaning cut (August) and the beginning of regrowth (September), 15 tillers/treatment were identified in various plants and fertilized with the equivalent of 200 kg N/ha (NH_4NO_3). Once a week, appearance and number of green leaves/tiller (LN), leaf senescence and green leaf length (GLL) was recorded. Total LN/tiller, Fc, LLS (Fc* LN), LAR (1/Fc) and LER (average daily increase in GLL) was determined. Fc and LLS were calculated between the 3° and 7° sheet, with based growth temperature = 7 °C. There were significant statistics differences between morphogenetic variables (Test T, $p < 0.01$), except for LER and LN. For tillers with N and control, respectively, resulted: LLS: 359 vs. 502 °Cday; Fc: 102 vs. 178 °Cday, LAR: 0.01±0.002 vs 0.01±0.001L/°Cday, LER: 9 mm/day and total LN: 11. Foliar extension responded to the synergic action of N and water received. *E. curvula* fertilized with high doses of N shows higher rate of appearance of leaves but less LLS. This indicates changes in the management of species subsidized with N, adapting to more defoliation frequency and shorter periods of rest and use.

A167

SYNOPSIS OF THE *Amaranthus* L. SPECIES GROWING IN DIFFERENT HABITATS OF SAN LUIS, ARGENTINA

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Amaranthus L. is a large and widely distributed genus of flowering plants that comprises approximately 70 species. The genus includes edible plants and weeds. In Argentina 29 species belonging to this genus are cited. Remarkably, 9 of them are endemic. There is an increasing interest in the species considered weeds of *Amaranthus* between farmers and agronomists worldwide. The aim of this work was to investigate the systematic identity and distribution of the species of *Amaranthus* L. growing in the province of San Luis and to produce reliable information about them. The specimens collected were identified following the classic botanical methods and incorporated to the Herbarium or the "Facultad de Ingeniería y Ciencias Agropecuarias (UNSL)". As a result of an extensive field work and herbaria study 8 species were documented: *Amaranthus albus, A. crispus, A. deflexus, A. muricatus, A. hybridus ssp. hybridus, A. palmeri, A. standleyanus* and *A. vulgatissimus*. Only 3 are important weeds in maize and soybean crops in San Luis: *A. hybridus ssp. hybridus*, *A. palmeri* and *A. standleyanus*. In addition, an easy identification key is given, and synonymy, descriptions, geographical distribution and habitat information are provided for each species.

A168

GENOTYPE-ENVIRONMENT INTERACTION STUDY OF SOYBEAN ADVANCED LINES WITH ABSENCE OF LIPOXIGENASES AND ANTI-NUTRITIONAL FACTORS

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Soybean (*Glycine max* (L.) Merril) based foods are an efficient alternative for feeding the growing population. But it is necessary to develop varieties with specific characteristics that make it suitable for human consumption as the absence of lipoxigenases and anti-nutritional factors. During breeding programs, decision making is hampered by the presence of genotype-environment interaction (GE) and the way to detect and explore it is via multi-environment trials. It has been defined as the difference between phenotypic value and the sum of the corresponding values of the genotype and environment. The objectives were to study GE, its nature and contribution to the variability and the analysis of genotypes and environments interactions. We conducted performance trials of 12 soybean advanced lines and a commercial control variety in two sites of San Luis, during 2013/2014 and 2014/2015 crop seasons .Combined ANOVAs were performed using mixed models to detect the presence of GE, variance components were determinate using restricted maximum likehood estimator (REML) through mixed models and interaction analysis was done using additive main effects and multiplicative interactions model (AMMI). The ANOVA detected the presence of GE in all environments combination (p-value<0.05) and the quantification of its components justified the inclusion in the analysis. Genotype 13 yield was higher in all environments and it also was stable. Meanwhile, genotype 34 present high yield in one of the evaluated sites but also high interaction and therefore low stability. The analysis of the GE allowed us to identified genotypes with differential behaviors across environments.

A169

METABOLOMICS STUDIES IN DROUGHT RESISTANT *Arabidopsis thaliana*

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One of the most common crop productivity limiting is drought. Plants need adapt to environmental changes, which produce modifications in the metabolome. Metabolomics allows characterizing a biochemical response of an organism to a disturbance. Abscisic acid (ABA) is an important regulator of plant growth and development, generally involved in plant responses to stress. Factor transcription HAHB4 operate in the signaling cascade that controls a set of drought responses mediated by ABA, in sunflower. Transgenic Hahb4 *Arabidopsis thaliana* is tolerant to water stress. The objective was to study the role of drought and ABA in the primary metabolism of *A. thaliana*. Transgenic HAHB4 and wild type (wt) ecotype Columbia plants under well watered (ww) and drought conditions, and wt well watered sprayed whit ABA, were assayed. Plants were grown in trays with Canadian Sphagnum Peat Moss as substrate, with periodic irrigation or drought, and photoperiod of 12 h. At 50 days, the aerial part was collected, extracted and derivatized. Then, the extracts were analyzed by GC-EIMS. In the ww transgenic plants decreased fatty acids (tetra, hexa-, octa- and decanoic), and increased sucrose, fructose, galactose and glutamine, respect to wt. Drought increased the most amino acids and sugars in wt plants. However, Hahb4 did not showed the same behavior under the same conditions, instead showed similar results to those of transgenic ww. Fatty acids were diminished by drought treatment in wt and HAHB4 plants. ABA as stress signal, increased glucose, fructose, sucrose, lactic and acetic acid, threonine, and most fatty acids founded. These results show the role of HAHB4 on primary metabolism in drought conditions.

A170

INOCULATION WITH *Pseudomonas fluorescens* RT6M10 AND *Azopirillum brasilense* Az39 INCREASE GROWTH AND PRODUCTIVITY AND MODIFY SUGARS LEVELS IN *Arabidopsis thaliana*

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Plant growth promoting rhizobacteria (PGPR) are bacteria that can actively colonize plant roots and enhance yield. The use of beneficial PGPR as bioinoculants may enhance crop productivity in a sustainable and environmentally friendly manner. *Pseudomonas* sp. are a well-studied group of bacteria that promote plant growth. In a previous work, our group isolated *Pseudomonas fluorescens* Rt6M10 (Rt6M10) from roots and rhizosphere of grapevines from a commercial vineyard. Moreover, *Azospirillum* sp. is one of the best-studied PGPR. *A. brasilense* Az39 (Az39) is used as inoculant in Argentina. The aim of this study was to evaluate the effect of Rt6M10 and Az39 in plants of *A. thaliana* Col-0. Seeds of Col-0 disinfected were placed in plastic pots containing peat moss and perlite. Then, pots were put into each tray and located in a growth chamber. The plants were watered to keep soil water status close to field capacity. Plants with two fully expanded leaves were inoculated with (1) Rt6M10, (2) Az39, (3) Rt6M10+Az39 and (4) Control. After 60 days, leaf area, photosynthetic and photoprotective pigments, relative leaf water content and the dry weight of rosettes were measured. Also, the percentage of plant survival and the total seeds production per plant (yield) were evaluated. The sugars were measured by capillary electrophoresis. Inoculation of *Arabidopsis* with Rt6M10 and/or Az39 improved the vegetative and reproductive plants growth. The inoculation increased maltose levels while sucrose was only detected in inoculated plants.

A171

EFFECTS OF DIFFERENTS MACRONUTRIENTS ON THE GERMINATION OF *Digitaria eriantha* STEUDEL CV. IRENE

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Digitaria eriantha Steudel subsp. *eriantha* is a perennial forage grass with good quality for the ranching, originated from South Africa which was introduced in 1990 in San Luis-Argentine. It has implantation's problems for its slow initial growth coinciding with soils with low level of nitrogen. The seed must be conditioned and seed coating or pelleting for its sowing, being possible the incorporation of macronutrients. The object was to study the germination and phytotoxicity with NPK's different doses (nitrogen, phosphorus and potassium). The trial was done in the INTA-EEA San Luis- Villa Mercedes. The treatments were: 1T (witness), 2NO3K (5gr/l), 3NPK (TRIPLE15 5gr/l), 4UREA 46%N (5gr/l), 5NO3K (10gr/l), 6NPK (10gr/l) and 7UREA (10gr/l), in a randomized block design with four repetitions, which were put in a growth chamber to 25°C. There were two dates of count, seven and fifteen days after the sowing. In a tray, 0.25 gr of anthecium were sowed and the number of germinated plants (NP) and the height (AP) was determinated. In the first date both in the NP and in the AP significant differences were not detected over 1T. In the second date of evaluation it was outlined, with significant differences, 5NO3K (10gr/litro), both for NP and for AP, with 61.4 % of survival or 38.6 % of phytotoxicity. The conclusion is that the best macronutrient was potassium nitrate with a dose high.

A172

EFFECT OF WATER STRESS IN COMMERCIAL WHEAT GENOTYPES: ANALYSIS OF THE RELATIONSHIP SOURCE-SINK

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Water stress in plants can cause a wide range of effects. The plants have developed different strategies to tolerate drought stress. The aim of this study was to growth different wheat genotypes in water stress conditions and to analyze their morphological, physiological and yield responses. The experiment was conducted in a split plot design with four replicates. The plants were supplied with 100% of the ETc (control) and with 25% of ETc (stress treatments). Stress conditions were applied from flowering to harvest. Within each treatment, a thinning of spike was applied and considered as a sub-treatment. Yield, growth and physiological trials were analyzed using ANOVA. The results showed significant differences among treatments. Only in the stress treatment the differences in grain weight between thinned and complete spikes were significant. The grain of the thinned spikes was in average 4 g per 1000 grains heavier than non thinned spikes. When the sink demand is higher than source production, the grain filling is negatively affected. The observed differences in stress conditions among thinned and complete spikes are consistent with a minor biomass production (tillers and stem) in stress treatment.

A173

ARBUSCULAR MYCORRHIZAL SYMBIOSIS IN ALLEVIATION OF DIFFERENTIAL STRESS AT *Medicago sativa*

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The association of agronomic plants with mycorrhizae provides abiotic stress tolerance. This study evaluates physiological responses in *Medicago sativa* plants inoculated and non-inoculated (NM) with the arbuscular mycorrhizal (AM) fungus *Rhizophagus intrarradices* and subjected to drought, cold or salinity. Biomass (B), stomatal conductance (SC), photosynthetic efficiency (PE), proline (P), lipid peroxidation (LP), antioxidant enzyme activities and endogenous jasmonates (JAs) were determined. B increased in all AM plants with statistical difference. Under drought and salinity stress, B, SC and P were increased in AM plants. The PE in NM plants decreases, while in AM plants it remained similar to control. LP in NM plant increased under drought and salinity conditions, while in AM plants it remained low similar to control plants, in both shoots and roots. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were implicated in protecting mechanism. The levels of JAs varied under drought and salinity. *M. sativa* is sensitive to drought and salinity and the protection mechanisms were associated with mycorrhizal symbiosis and include reduced LP, enhanced antioxidants enzymes and regulation of JAs levels

A174

OXIDATIVE STRESS IN THE REGULATION OF SEED DORMANCY AND GERMINATION IN TWO SUNFLOWER INBRED LINES

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Reactive oxygen species (ROS) play important roles in the regulation of seed dormancy and germination. Our aim was to evaluate the content of hydrogen peroxide (H_2O_2) and the activity of two antioxidant enzymes: catalase (CAT) and ascorbate peroxidase (APX) in embryonic axis and cotyledons of dry and imbibed seeds of two sunflower inbred lines (B123: with dormancy; B91 without dormancy) at harvest (day 0) and after storage at 25°C during 33days.Higher levels of H_2O_2 were detected in dry embryonic axis than in cotyledons. At day 0, H_2O_2 was higher in B91 embryonic axis of dry seed than B123. After 33 days of storage, B123 increased H_2O_2 concentration, while B91 decreased. During the imbibition, H_2O_2did not change in B91 embryonic axis at 6 h on day 0; whereas in B123 embryonic axis a decrease was observed at 33 days under storage at room temperature at 6 h. In cotyledons, H_2O_2 decreased at 12 h of imbibition in both lines. CAT activity showed a high level incotyledons respect to the embryonic axis. A decrease in CAT activity was observed in embryonic axis and cotyledons of B91and B123 dry seeds under storage. APX activity decreasedin both lines during the imbibitions. The pattern of H_2O_2 accumulation in B123 embryo axis during dry storage might be a key signal in the dormancy release allowing the germination of embryo. It is possible that H_2O_2 is acting as a signal molecule and could be a response to the changes involucrate in physiological mechanisms as dormancy and germination.

A175

PREDICTION OF THE BASE TEMPERATURE OF LEAF GROWTH OF MEGATHERMAL GRASSES

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The determination of the base growth temperature (Tb) adjusts the calculation of growth accumulated degree days. In order to find number of sheets generated (LN) and the average tillering growth temperature (Tm) was evaluated. In plots of *Tetrachne dreguei* and *Panicum coloratum*, located in Villa Mercedes (San Luis), 15-30 tillers for species with their first leaves were identified. The first specie was evaluated during two cycles of growth and the second for three. In *Eragrostis curvula* plants established in plots and subjected to two fertilization treatments (withase N and control), 15 tillers per treatment were identified and foliar appearance was evaluated during a cycle. In late winter the equivalent of 200 kg N/ha (ammonium nitrate) was supplied. In each species a completely randomized design was applied. From the beginning of the sprout, once a week the appearance and number of green leaf for tiller was registered. It was obtained simple linear models from a range of 3 to 13 sheets and 10 to 30 °C Tm. considering the intersection on the axis "x" the Tb of leaf growth was estimated. For the inter-cycles average value, *P. coloratum* showed a Tb = 9.5 °C and *T. dreguei* = 8.6 °C. In *E. curvula*, Tb = 7.5 °C was the average between control and plants with N (10 and 5 °C). The results indicate a decreasing Tm requirement for foliar generation, without supply of N, in the order *P. coloratum*, *T. dreguei* and *E. curvula*. Such Tb indicated the minimum threshold temperature foliar accumulation for the three megathermal grasses.

A176

CHARACTERIZATION OF PHENOLIC COMPOUNDS IN *Prosopis strombulifera* (FABACEAE) BY HPLC ESI- MS AND THEIR CONTRIBUTION TO TOTAL ANTIOXIDANT CAPACITY

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Halophytes are known for their ability to withstand unfavourable conditions by quenching toxic reactive oxygen species (ROS), since they are equipped with a powerful antioxidant system including enzymatic and non-enzymatic components as polyphenols. *Prosopis strombulifera* (Lam.) Benth. is a woody shrub found in high-salinity areas in central Argentina, with an interesting and contrasting growth response under NaCl and Na_2SO_4. NaCl caused growth stimulation (up to 500 mM) while Na_2SO_4 caused a significant growth reduction and toxicity symptoms. In the present study, 17 phenolic compounds were identified by HPLC-ESI-MS and their antioxidant capacity and contribution to overall antioxidant activity were calculated. Different profiles of polyphenols were found in *P. strombulifera* plants subjected to different salt treatments. NaCl-treated plants presented the major variety of compounds (16), with an increase in the antioxidant capacity which was similar in NaCl+Na_2SO_4-treated plants. In Na_2SO_4-treated plants, only 4 compounds were identified with a decrease in antioxidant capacity. Accumulation of flavonoids in tissues and their powerful antioxidant activity under NaCl indicates a significant role for these compounds in counteracting the oxidative damage induced by high salt concentrations, allowing optimum growth. Whereas compounds found in Na_2SO_4-treated plants may play a more structural role, strengthening the cell walls and increasing tissue lignification as shown in previous work.

A177

GENOTYPE-ENVIRONMENT INTERACTION ANALYSIS FOR PROTEIN CONTENT IN SOYBEAN GRAINS

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Increasing the protein content is one of the main objectives of genetic soybean improvement (Glycine max (L). Merril).In advanced stages of improvement programs, multi-environment trials are conducted to evaluate the performance and stability of the experimental lines. If there is interaction genotype-environment (GE) its study provides valuable information to optimize the genotypes selection.The aim of this study was to analyze the GE for the protein content in soybean grains. To achieve this, they were sowing during the 2013-14 crop season in Marcos Juarez, Sampacho, La Carolina and Villa Mercedes, three experimental lines of soybean breeding program of INTA and elite commercial control, in a randomized block design. The percentage of protein in the grain (PR) was determined in the seeds laboratory of the experimental station INTA Marcos Juárez. To detect the interaction presence and their magnitude, as well as to study the performance of genotypes and environments in their interaction an analysis of variance components and GGE-biplot analysis, were performed. The GE contribution was greater than the effects of environment and smaller than the genotype effects. The FICA42.2 line, was the best performing in Sampacho, La Carolina and Villa Mercedes locations, and ALIM5.09 and FICA58.2 were higher in Marcos Juárez, while the witness was the poorest performance in all environments tested. Sampacho, La Carolina and Villa Mercedes had a similar behavior at GE, being Villa Mercedes the largest discrimination capacity, therefore has ideal conditions to select genotypes with high protein content in soybeans.The identification and analysis of GE for the character protein content in soybeans allowed us to characterize the environments and determine differential behavior of genotypes.

A178

YIELD COMPONENTS EVALUATION OF SOYBEAN CULTIVARS IN TILISARAO (SAN LUIS)

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Soybean (Glycine max (L). Merril) is one of the most important crops in our country, being third among the highest production, after Brazil and US. Crop yield is determined by its components: number of pods, number of grains and weight of grains. The aim of this study was to determine whether there is variation for yield components among different soybean cultivars sowed in Tilisarao (San Luis) and provide possible causal explanations of observed correlations, between yield and its components. DM4913, FN4.50, FN4.97, FN5, FN5.75, N4,57, NA4990 and NA5009 soybean cultivars of maturity groups IV and V and one control were sowed in the town of Tilisarao (San Luis) during the 2014-2015 crop season in an paired witnesses experimental design. The traits yield per plant (RTO), number of pods per plant (NV), number of grains per pod (NG) and weight of 100 grains (PG) were determined The traits yield per plant (RTO), number of pods per plant (NV), number of grains per pod (NG) and weight of 100 grains (PG,) were determined and descriptive statistics and path analysis were performed. All genotypes presented highly significant positive correlations (r= +0,83-0,94) between RTO and NG and RTO and NV (r= + 0,63-0,83) determined in the first case almost entirely by direct effects and through the NG indirect effects in the second. There was no correlation in neither of the genotypes between PG and RTO except for FN 5.55. The analysis enabled us to determine that for DM4913,

FN4.50, FN4.97, FN5, FN5.75, N4,57, NA4990 and NA5009 soybean cultivars, NG was the most important yield determination component, because it was the biggest contributor to the correlation.

A179
CHEMICAL TREATMENT AT DEGRADED SANDY GRASSLAND OF SAN LUIS, ARGENTINA: EFFECTS ON VEGETATION

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The sandy area of the province of San Luis, Argentina, presents a vast area with degraded rangelands, with significant presence of undesirable species grazing and land without vegetation cover. The impact that chemical action generates on *Elyonurus muticus* (Paja amarga) and other sandy grassland species was evaluated by analysis of the vegetation canopy cover. Four treatments resulting from the combination of two doses of glyphosate (1.25 $kg.ha^{-1}$ y 1.75 $kg.ha^{-1}$) applied at two different moments (November and December) were conducted, and a control. The design was in randomized blocks, with 3 replications. On each plot, one transect was set to determinate coverage and Nº of individuals/species, mulch and % coverage of bare soil (SD) at the end of summer season (March). A non-parametric analysis by Test median differences of Kruskal Wallis test was applied, grouped species into: desirable, intermediate and undesirable. Regardless of doses and moment of glyphosate application, did not cause significant changes ($p>0.05$) in coverage of different species groups. However the value of SD tends to increase (principally 1.75 $kg.ha^{-1}$) and the number of desirable species tends to decrease with applications ($p<0.05$). Also, changes in botanical composition were detected. *Elyonurus muticus* persist with glyphosate application at the doses utilized in this experience.

A180
TILLERS DENSITY OF *Digitaria eriantha* STEUD UNDER DIFFERENT CUTTING FREQUENCIES

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The use of high grazing frequencies can modify the pasture structure and affect their productivity over time. The density of tillers of forage grass, is one of the structural variables that define the pasture growth. It was proposed as objective to establish the density of tillers against different cutoff frequencies. It was measured tillers density (tillers/m^2) at cycle end, using a ring of 7.5 cm diameter on plots of *Digitaria eriantha* subjected to different cutoff frequencies. Treatments according to cutting time, were fixed time (FTC: monthly), in mean leaf lifespan (MLLC: at the beginning of first leaf senescence) and the end of the growth cycle (ECC). A completely randomized design with 3 replications was used. Five tillers were identified by plot to determine the beginning of first leaf senescence. The results of the ANOVA and LSD test showed differences between treatments ($p <0.05$): FTC showed the highest values of density (3772 ± 332 tillers/m^2) and was statistically superior to other treatments. There was not significant differences ($p>0.05$) between MLLC and ECC (2263 ± 331 and 2490 ± 331 tillers/m^2, respectively). High grazing frequencies in *Digitaria eriantha* increase the density of tillers and modify the structure of the pasture. Grazing frequencies that respect the morphogenesis of the species, do not alter the density of the pasture during a grazing cycle.

A181
BIOCHEMICAL DETERMINATION OF RESERVE SUBSTANCES IN *Jatropha curcas* L. *AND J. macrocarpa* GRISEB. SEEDS

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J. curcas and *J. macrocarpa* are useful for restoring degraded areas and their seeds contain oils for biodiesel production. The aim of the work was to determine the reserve substances of *J. curcas* and *J. macrocarpa* seeds. Seeds were imbibed in distilled water for 24h, to facilitate removal of seed coat with the aim to separate the embryo and nutritive tissues. 1-The anatomical studies were done by longitudinal cuts of endosperm and were stained with Sudan IV for lipids, iodine-potassium iodide for starch and ethyl alcohol and then iodine-potassium iodide for protein. 2-Quantification of reserve substances in seeds were carried by different methods: lipids were extracted with chloroform / methanol / water solution. Methyl esters of fatty acids were prepared from lipid extracts and analyzed in a gas chromatograph coupled to a flame ionization detector. The sugars are determined by the phenol sulfuric acid method and protein by the Bradford method. In both species, the endosperm contained aleurone grains consisting of a crystalloid and globoid, lipids of red colour and the starch wasn't observe. Four major fatty acids were determined in *J. curcas* seed: oleic, palmitic, stearic, palmitoleic and oleic fatty acid represent about 70% oil content. Oleic acid was the most abundant in *J. macrocarpa* seeds, while, there wasn't palmitoleic acid. Seed with predominantly unsaturated fatty acids is ideal for biodiesel

industry. Sugar and protein contents of *Jatropha* seeds were significantly higher in embryo in both species, which suggests early mobilization towards the embryo during imbibition period.

A182
PURIFICATION OF PPO FROM *Prunus persica* (L.) var. *nectarine*

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Polyphenoloxidases (PPO) are widely distributed in fruits and vegetables, is important to characterize their activity in order to facilitate their control during processing. The enzyme extraction was conducted following the procedure of Gauillard and Richard-Forget with some modification. The enzyme extract obtained was purified by a chemical and a physical methods and enzyme activity was evaluated by measuring the variation of absorbance (A) at 400 nm, which is the length of the maximum absorbance of the reaction product (using 50 μL of 2.737 M of 4-Methylcatechol as substrate and 100 μL of enzyme in 3mL of solution buffer 5.5). The first purification method, consisted of precipitating the enzyme with $(NH_4)_2SO_4$ at two concentrations, 35% w/v and 70% w/v. After purification with 35% $(NH_4)_2SO_4$ activity of the extract was 2.14E-01 ΔA/min mL E, whereas using 70% $(NH_4)_2SO_4$, the activity was 8.00E-03 ΔA/mL min E. The enzyme purified by the second method exhibits lower activity values respect to the first, due to a possible inhibition of the enzyme. The physical method consisted of ultrafiltration, centrifuging the extract in Centriplus tubes with a 30 kDa membrane cut-off. Activities of 1.72 ΔA/ min mL E for purified enzyme and 2.27E-01 ΔA/min mL E for corresponding residue were obtained, indicating that a large part of sugars and amino acids present in the extract with molecular weights less than 30 kDa, pass through the membrane, while the enzyme is retained in the supernatant. This result is expected, according to the molecular weights reported for different isozymes PPO, ranging from 50 kDa to 120 kDa. Considering the extract without any purification showed an activity of 1.42E-03 ΔA/mL min E, we conclude that the most effective method to purify the extract was the physical method.

A183
SEASONAL RAINFALLS MODIFY POLYPHENOLS PROFILE IN A NATIVE GRASS SPECIES FROM SEMIARID REGIONS

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Native species from semiarid regions have developed a powerful antioxidant system to survive during drought seasons. Polyphenols have antioxidant capacity and can help the plant to survive and develop. The aim of this work was to identify the different phenolic compounds produced by *Pappostipa speciosa* (Poaceae) during two seasons with different water availability. We identified 14 molecules determined by HPLC-ESI-MS and the antioxidant capacity of these compounds was calculated by free radical assay techniques. Their contribution to overall antioxidant activity was also estimated based on their chemical structure. The molecules identified were phenolic compounds, as flavonoids and non-flavonoids. Most of the flavonoids conjugated with sugar were identified in the rainy season while no-flavonoids and free flavonoids were identified in the dry season. Despite the fact that the polyphenols profile was different between the two seasons analyzed, the antioxidant capacity was similar. These results suggest that in rainy season the main role of phenolic compounds is acting as intermediaries in metabolic pathways, while in dry seasons their main roles are as antioxidant molecules which reactive groups (HO^-) in their chemical structure are not bond to sugars or any other compound, being available to scavenge reactive oxygen species (ROSs). Also, they can form polymers useful to strengthen the cell walls to reinforce plant tissues such as xylem vessels under stressful conditions.

A184
PARTICLE SIZE DISTRIBUTION AND DRY MATTER IN FREEZING *Sorghum bicolor* SILAGE

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The particle size and tenor of effective fiber of chopped and silage forage are indicators of the nutritional quality of silage and feed efficiency in the animal consuming. Particle size distribution was evaluated in sorghum silage maked with the same material at two moments (5 and 12 days) before a lethal critical frost (-2/-3ºC) and 1, 2, 3, 5, 7, 12 days after the same. Plants were chopped with a precise chopping static machine and were ensiled placed into 4.3 liters PVC pipes, with a compaction pressure of 500 $kg.m^{-3}$. Eight microsilages for each days were prepared for each day, totaling 64 samples. After 60 days, microsilages were opened and the particles size distribution (TP) was evaluated by the Penn State Particle methodology. The relative proportions of different particle sizes were: >19 mm 22.10%, 8-19 mm 43.98%, >8 mm 32.21% and <1,18mm 1.69%, and were consistent with the published standards for silage fractionation with Pen State, recommended for cattle feed. An analysis of Pearson correlation ($p<0.01$) between moments, particle sizes and dry matter was also performed. The correlation matrix showed positive correlations for extreme sizes (r: 0.6 for <1.18 and 0.7 for >19 mm size) and dry matter (r: 0.7), negative for particles between 8 to 9 mm (r: -

0.8) and there was no correlation for <8 mm size (r: 0.15). By performing a multivariate cluster analysis two clusters were identified (euclidean distance^2: 11.14), which indicates homogeneous groups characterized by particle sizes and MS (pre and post frost). The occurrence of the first frost generated differences in particle size and dry matter, tending the effective fiber (>19 mm fraction) to increase after the frost, coincident with dry matter increase.

A185
GERMINATIVE BEHAVIOR IN SEEDS OF *Cercidium praecox* (RUIZ & PAV.) HARMS (FABACEAE), "BREA"

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"Brea" is a species that grows naturally in the Province of San Luis, Argentina. According to studies on plant species from arid and semiarid areas they present a condition called latency, and it has been observed that both, simulated water stress and salt stress, produce decrease in the percentage of seed germination The aim of this study was to evaluate pre-germination tests for breaking dormancy in seeds of "brea" and analyze the effects of salinity and drought simulated on germination. The seeds were subjected to different treatments of scarification: T1) Control (without scarifying); T2) Mechanical, with sandpaper; T3) Immersion in water at boiling point for 24 hours; Immersion in H_2SO_4 T4) for 15 minutes, T5) for 30 minutes and T6) for 45 minutes. On the other hand, previously scarified seeds were placed under the following treatments: I-Control, washed down with 20ml of distilled water; II- Stress, washed down with 20ml of PEG 6000 solution and 20ml of NaCl solution until reaching potential of -0.5; -1 -1.5MPa. In all tests the seeds are placed in Petri dishes according to Standard Method. The number of germinated seeds was recorded in three days (Germination Energy, GE) and seven days (Germinating Power, GP). Results are expressed as Percent of Germination. For the germinative treatments it was observed that T2 and T6 showed the highest values of GE (99% and 98% respectively) and GP (99% for both treatments).Finally, in seeds treated with PEG and NaCl solutions, GP and GE decrease when increases the concentration of both solutions, becoming equal to 0 in -1,5MPa potential.

General, Cellular and Molecular Biology

A186
DIFFERENTIAL METHYLATION OF KLF14 PROMOTER IN PATIENTS WITH TYPE 2 DIABETES

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The results of GWAS (genome-wide association studies) indicated that *Krüppel Like Factor 14* (*KLF14*) may play a role in the pathogenesis of Type 2 Diabetes (T2D). In addition, epigenetic mechanisms, such as changes in DNA methylation patterns, might have a role in the pathophysiology of T2D. This study aimed to investigate the differences in DNA methylation profile of *KLF14* promoter gene between T2D and controls (Co). We included 10 T2D and 10 Co. Genomic DNA was isolated using conventional protocol by Qiagen kits. Epigenetic analysis was performed by bisulfite method. PCR amplification was carried out using BSP primers (*GeneBank* sequence: NG_016152) of *KLF14*. PCR products were sequenced. The sequencing results were analyzed by the Chromas Lite 2.1.1software. Statistical analyses were carried out in both groups. A $p < 0.05$ was considered to be statically significant. Methylation levels in DNA were obtained for 37 sites covering the region between -125 bp and -370 bp according to the ATG position for the *KLF14* gene. Altered levels of DNA methylation was detected in the 37 analyzed CpG sites in T2D compared with Co [RR=1.9 (1.236-2.963),p=0.014]. Further, 18 out of the 37 CpGs (49%) showed a significant difference between T2D and Co (p<0.0001). Our results showed significant differences in the frequency of methylation at overall and individual CpG sites between T2D and Co. Despite the small sample size, this is the first study to report a possibility that the methylation frequency at individual CpG sites in *KLF14* promoter may serve as markers used to distinguish T2D patients. Further research should unveil the potential role of these data in the physiopathology of T2D.

A187

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

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*Trypanosoma cruzi (T. cruzi) i*s the etiologic agent of the Chagas´ disease. The objective of our work is to study the three-dimensional structure of *T. cruzi* ribosomal complex stalk in the large subunit of the ribosomes. The stalk is formed by the P proteins: TcP0, TcP1α, TcP1β, TcP2α and TcP2β. The 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. Previously, an homology model for TcP0 have been obtained in our laboratory. In this work, we have made three dimensional homology molecular models for these four 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. The unstructured A-rich domain was characterized using several disorder predictors. The molecular surface electrostatic potential and the hydrophobic surface were calculated. The surface properties are important for the C-terminal's antigenic properties. We explored and identified protein interactions that may be involved in conformational stability. This work represents the first three dimensional characterization for these *T. cruzi* proteins and provide clues for understand its functional properties.

A188

STUDY OF PROTEIN EXPRESSION IN *Streptomyces* sp. MC1 UNDER Cr(VI) STRESS BY TWO-DIMENSIONAL ELECTROPHORESIS

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In previous works, Streptomyces sp. MC1 has shown the capability to remove 98% of Cr(VI) (20mg*L-1 of Cr(VI) as K2Cr2O7) in presence of 7.5mM of sulfate ions (as Na2SO4) in the culture media (MMm) during 48h of incubation, at 180 rpm and 30°C. The purpose of this study was to analyze the intracellular protein profile expressed by Streptomyces sp. MC1 in the presence or absence of the heavy metal using two-dimensional (2D) electrophoresis to identify proteins induced by Cr(VI) presence. Cells obtained at 48h in MMm in presence and absence of Cr(VI) were chilled in N2(l) and physically broken using mortar. The supernatants of cell lysate were used as protein samples. Intracellular proteins were concentrated by ultrafiltration using Vivaspin® 500 Centrifugal device. After 2D electrophoresis, differential spots (18) were identified, separated from gels and digested with trypsin. Tryptic peptides obtained were analyzed using nano-Ultra Performance Liquid Chromatography, coupled to tandem mass spectrometry. Bioinformatics analysis for protein identification was performed by searching against Swiss Protein database, using Mascot server and ProteoIQ v2.8. Proteins expressed only in the metal presence include proteins involved in protein biosynthesis (5), in oxide-reduction processes (1), proteins of response against stress (1), and one protein of resistance to the metalloid tellurium. Five spots appeared in both conditions and showed differences in spots intensity. These results indicate that nonspecific mechanisms of defense based on the overexpression of proteins capable to cope the metal presence were activated.

A189

ANALYSIS OF VESICULAR DYNAMICS IN *Chlamydia trachomatis*-INFECTED CELLS

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Chlamydia trachomatis (CT) is the major causative agent of bacterial sexually transmitted diseases worldwide. CT resides in a vacuole, called "inclusion", where it acquires sphingolipids from host cell by intercepting multivesicular bodies (MVBs). CT interferes vesicular trafficking by promoting or avoiding the recruitment to the inclusion membrane of certain host Rabs, the master controllers of intracellular transport. CT actively intercepts vesicles, previously characterized by our laboratory as MVBs by subverting Rab39a function. For the analysis of vesicular movement and fusion-fission events between small vesicles (MVBs) and the chlamydial inclusion, we have developed an ImageJ application. This application measures: number of fusion events, number of fission events, average event duration and the areas of greatest activity. *In vivo* experiments were performed in Rab39a-overexpressing HeLa cells infected with CT for 24 h. Our results showed that fissions and fusions seem to take place in different zones of the inclusion, and that there more fusion events than fission ones. Participation of cytoskeleton was analyzed in Rab39a-infected cells treated with Nocodazole (to disrupt microtubules) and Latrunculin (to depolymerize actin). Our observations indicated that Rab39a-vesicle movement and the interaction with the chlamydial inclusion highly depend on microtubule and microfilament integrity and dynamics.

A190
PROTOCOLS COMBINED WITH LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY TECHNIQUES

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The combination of gel "free" and gel-LC protocols, are a useful approach to perform protein identification and relative quantification analysis of complex samples mixtures, such as biological fluids, cell lysate, and extracellular proteins or secretome. Also, both techniques can help to resolve technical limitations related with the sample dynamic range, potential molecules affecting protein digestion, and separate peptides present before the protease (digestion) addition. Both protocols are mass spectrometry-based and employ a bottom-up strategy that involves protein digestion with trypsin (in solution and in gel digestion), followed by the separation and analysis of the tryptic peptides through two-dimensional liquid chromatography coupled with tandem mass spectrometry analysis. The gel "free"+ gel-LC approach was applied to analyze samples from a bacteria to improve the number of proteins to identify, and to resolve certain problems associated with the extraction process. In this particular case, streptavidin affected the peptide mass measurement because of increased "ion suppression" during the ionization and data acquisition at the mass spectrometer.The gel "free" protocols provided preliminary data showing a decrease in the number of proteins identified as a consequence of the presence of streptavidin, suggesting that it was necessary to use another strategy "upstream" of the analysis to decrease or eliminates the interference. Gel-LC as a complementary approach successfully resolved the problems related with streptavidin and improved the dynamic range of the sample, increasing the number of proteins identified.

A191
IDENTIFICATION OF SOLUBLE COMPONENTS IN HUMAN ADIPOSE TISSUE EXPLANTS FROM RENAL CELL CARCINOMA

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We have recently demonstrated that conditioned media (CMs) from human adipose tissue explants from renal cell carcinoma near the tumor (hRATnT) regulate adhesion and migration of tumor (786-O, ACHN) and non tumor (NK-2) renal epithelial cells, contrary to CMs from human adipose tissue explants from renal cell carcinoma farther away from the tumor (hRATfT). Now, we have begun to identify soluble components present: 1) in hRATnT- and hRATfT-CMs by WB; 2) in tumor (786-O, ACHN) and non tumor (NK-2) renal epithelial cells incubated with different CMs. We employed the CMs after 24 h of incubation of hRATnT and hRATfT. We evaluated the expression of proteoglycan versican, metalloproteinase ADAMTS1, leptin and adiponectin in the CMs. We incubated786-O, ACHN and NK-2 cells with theCMsfor 24 h, lisated the cells and evaluated the expression ofAdipoR1, AdipoR2, ObRand leptin.We observed a higher expression of versican, leptin and adiponectin, as well as a lower expression of ADAMTS1 in hRATnT-CMs compared to the expression in hRATfT-CMs ($p<0,05$). In addition, we observed an increase of leptin an decrease of AdipoR2 ($p<0,05$) in786-O, ACHN and NK-2 incubated with hRATnT-CMs compared to the expression observed with hRATfT-CMs and control-CMs ($p<0,05$).These factors could be involved in the biological effects previously observed.

A192
HYPOTHYROIDISM DECREASES JAK/STAT SIGNALING PATHWAY IN LACTATING RAT MAMMARY GLAND THROUGH LACTATION

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Thyroid hormones (TH) are essential for prolactin actions in the mammary gland. Exposure to excessive or insufficient quantities of THs during lactation diminish milk production and quality, and advances mammary involution. To continue investigating the mechanism through which hypothyroidism (hypoT) alters mammary function, we studied its effect on day 2, 7 and 14 of lactation (L2, L7 and L14) on expression and activation of main members of prolactin signaling pathway in the rat mammary gland. We analyzed by western blot, protein expression of prolactin receptor (PRLR), STAT5a/b, phospho-STAT5a/b (P-STAT5), SOCS3, and CIS protein. The patterns of PRLR and STAT5a/b proteins expression decreased gradually through lactation with a significant difference between L2 and L14. HypoT significantly decreased the RPRL protein level in the three days and STAT5a/b protein

level in L2 and L7, abolishing the gradual decrease of control group. The P-STAT5a/b protein level increased through lactation in controls but hypoT decreased them significantly in L7 and L14.SOCS3 and CIS protein levels remained constant between L2 and L14 in the controls, but hypoT significantly diminished the protein levels of both in L2 and of CIS in L7.The present results demonstrate that hypoT has a negative effect on the PRL signaling pathway, evidenced by a reduction of the mammary contents of PRLR and STAT5a/b and particularly of p-STAT5thatis the main transcription factor of lactating mammary gland. However, the decrease in the inhibitor proteins levels could be a compensatory cell response to the PRL signaling deficit. According to the evolutive importance of lactation it is probable that mammary cells are able to deploy compensatory responses to hormonal deficits in an attempt to keep milk production, although it is important to note that in any case, this mechanism could not be maintained up to L14. These results can explain the lactation deficit and advanced mammary involution previously described by us, as well as provide a mechanistic explanation of clinical observations, which show that hypothyroid mothers with inadequate TH supplementation have deficient lactation.

A193
EXPRESSION, PURIFICATION AND PRELIMINARY STRUCTURAL STUDIES OF CRUZAIN PROTEIN

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Chagas' disease is a chronic systemic parasitic infection caused by the protozoan parasite Trypanosoma cruzi. Available drugs are highly toxic and often ineffective, particularly those used to treat the chronic stage of the disease. The aim of our work is the structural and functional study of proteins which are vital for Trypanosoma cruzi as a first step in rational drug design. Cruzain protein is a member of the papain/cathepsin-L family of cysteine proteases, and the major cysteine protease of the protozoan T. Cruzi. The protein is present in all life stages of the trypanosome and is implicated in cellular entry and digestion of immunoglobins. . *E. coli* BL21 (DE3) Star were transformed with pET21a-cruzain vector and single colonies were selected in LB agar containing specific antibiotic. A starter culture of BL21(DE3) Star- TcCruzain was grown in 5 ml LB medium containing 50 mg/ ml ampicillin al 37 °C overnight. This initial culture was increased to 1 l, induced with IPTG at a concentration of 1 mM and grown for an additional 72h at 20°C. The bacteria were recovered by centrifugation. His-TcCruzain was purified using Ni-Sepharose affinity column. Autoproteolysis of the pro-region (~ 14 kDa) from the zymogen (~ 37 kDa) was initiated by adding DTT (1 mM final concentration) to the solution and incubating in a 37 °C water bath. Transformation of the inactive zymogen into the catalytically active domain was monitored by removing 50 uL aliquots of the solution at selected time points. All stages have been analyzed by SDS-PAGE and Western Blot. The preliminary results show a high concentration of active enzyme with a high degree of purity which would allow structural studies as the next step.

A194
LOSARTAN INDUCES CYTOSKELETON REORGANIZATION ON RENAL PROXIMAL TUBULE CELLSs (PTCs) FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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Angiotensin II (AII) binds to AT_1R, the peptide being a potent mediator of oxidative stress. The role of reactive oxygen species (ROS) as signaling molecules that contribute to migration and cytoskeleton remodeling differentiation and remodeling of the cytoskeleton. Nox4, is expressed in PTCs. Previously, we identified Hsp70 and CHIP as Nox4-interacting proteins, mediating the ubiquitination and proteasome degradation of Nox4 included within the Losartan antioxidant effect on SHR PTCs. Here, we evaluate the Losartan (L) effect on the cytoskeletal organization of both actin and junctional-related protein in SHR PTCs. Primary culture of PTCs from SHR and WKY were stimulated with AII, treated with L or left untreated (C). Immunofluorescence confocal microscopy reveals reorganization and stabilization of actin cytoskeleton, without Nox4 colocalization in L–treated SHR PTCs. However, C and AII treated SHR PTCs led to disorganized actin cytoskeleton with increased Nox4 colocalization. AII and C SHR PTCs showed decreased vinculin staining on the cell periphery and lower Nox4 colocalization. In contrast after Losartan, increased vinculin colocalize with reduced Nox4 on the cell periphery leading to focal adhesion stabilization on SHR PTCs. Through Live Cell Time-lapse Microscopy, L induces decreased cell displacement and slowed down cellular rate movement. Also, the cells remained attached and did not change their morphology when compared to C and AII SHR PTCs. By Western Blot, Losartan increased vinculin and E-cadherin levels and decreased Nox4, phospho-ERK and phospho-p38 expression related to AII and C SHR PTCs. Losartan AT_1R blockage induces actin cytoskeleton stabilization and cell migration reduction due to decreased Nox4 and ROS production. These results in the reduced activity of signaling pathways mediated by MAPKs: phospho-ERK and phospho-p38. A protective role of Losartan could be suggested that avoids cell tubular detachment and stabilizes cellular junctions on PTCs from hypertensive patients.

A195

A MOLECULAR AND MORPHOLOGICAL STUDY OF A TREMATODE WORM FOUND IN THE DIGESTIVE GLAND OF *Asolene pulchella* (CAENOGASTROPODA, AMPULLARIIDAE)

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Asolene pulchella (Anton 1839) is a freshwater snail that inhabits the Plata River basin. The population of Regatas Lake (Buenos Aires, Argentina) shows a high prevalence of unidentified cercariae (Digenea, Platyhelminthes). This larval stage lives in hemocoelic spaces and connective tissues from digestive gland of this ampullariid snail, which is an intermediate host of other trematodes. An internal transcribed region (ITS 1) of 900 base pair, codified between two ribosomal genes (that codified for both 18S and 5.8S subunits) was amplified by polymerase chain reaction (PCR) using a set of specific primers for digenean worms (S20T2, 5´-GGTAAGTGCAAGTCATAAGC-3´, 5.8S1, 5´-GCTGCGCTCTTCATCGACA-3´). The phylogenetic analysis (BLAST analysis, Maximum Likelihood phylogenetic analysis) showed that this organism belongs to the order Plagiorchiida and it is associated with other species of the Xiphidiata suborder, which display a stylet associated to oral sucker. In addition, we identified the main morphological characters of cercariae from digestive gland. They have a spined body (98 µm long and 57 µm wide), a well-developed spined tail (81 µm long and 12 µm wide) and penetration glands. Ducts of these glands converge in the oral sucker, which holds a conspicuous stylet. A ventral sucker and a reniform excretory vesicle in the base of tail are also observed. These morphological, morphometric and molecular observations suggest that *A. pulchella* hosts a novel digenean worm which belongs to Xiphidiata suborder.

A196

MORPHOFUNCTIONAL HETEROGENEITY WITHIN THE RAT PINEAL GLAND: A LESSON FROM CREB/PCREB

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The circadian modulation of physiology and behavior facilitates adaptation to environmental changes. The pineal gland (PG) is one of the principal effectors and regulators of the mammalian circadian timing system by producing nocturnal melatonin. The PG is under sympathetic regulation *via* local norepinephrine (NE) release at night. In rat, phosphorylation of the transcription factor CREB (cAMP-responsive element (CRE)-binding protein) at serine 133 is essential to initiate the expression of the *aanat* gene. This gene encodes AANAT, one of the pivotal enzymes in the melatonin synthesis. To challenge the concept of pineal homogeneity, we analyzed CREB and pCREB at different ZTs (*Zeitgeber* time; L:D 12:12). We performed immunolabeling (IHC) and confocal microscopy in rat PG sections and Western blot (WB) analysis in whole PG protein extracts. The immunoblottings mostly confirmed previously published data for both protein forms. On the other hand, IHC revealed heterogeneity within the PG. While total CREB was found in pinealocyte nuclei at ZT6, 14 and 18, diversity in immunoreactive granule sizes and nuclear distribution patterns was seen throughout the L:D cycle. CREB present in the nuclei of interstitial cells showed a finer and more homogeneous distribution. For nocturnal pCREB, the signal varied from pinealocyte to pinealocyte with higher expression at ZT18. The phosphorylated form was negative in pinealocytes at ZT6. The presence of pCREB in interstitial cells at different ZTs infers that its phosphorylation may be independent of the nocturnal NE. These findings are of special interest because they suggest that melatonin synthesis varies significantly among individual pinealocytes. This cellular heterogeneity further implies that the pinealocyte population may have a greater need to synchronize its nocturnal melatonin release.

A197

Chlamydia trachomatis INFECTION OUTCOME IN A MODEL OF IMMUNOSUPPRESSION

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Chlamydia trachomatis (CT) causes long-term subclinical infections, with potentially devastating reproductive consequences, such as pelvic inflammatory disease, ectopic pregnancy, preterm birth and tubal infertility. This is in part due to the strategies that CT has developed to survive within epithelial cells and to evade the host immune response. Current treatment is based on eliminating the infectious agent with antibiotics, without dealing with the associated inflammatory response. Our work analyzes CT genital infection outcome in a murine model, set by this research, immunosuppressed by dexamethasone (Dex) treatment. At the macroscopic level, Dex-treated genital tracts showed less sequelae scarring lesions. Western Blot assays revealed a decreased activation of the Akt pathway and lower levels of interleukin-6 in Dex-treated mice, indicating both, a disturbance in signaling and a depressed inflammatory response. These findings suggest that forthcoming pharmacological proposals should deal with Chlamydia trachomatis infection not only at the bacterial level but also with the inflammatory response to prevent genital tissue damage.

A198

TUMOR EFFECT ON ITS ADIPOSE MICROENVIRONMENT IN HUMAN BREAST CANCER – AN IMMUNOHISTOCHEMICAL STUDY

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Tumor stroma is one of the emerging topics of interest in cancer research. In breast cancer, tumor epithelial cells interact dynamically with different stromal cells. This interaction has been shown to be crucial for tumor progression and dissemination. Adipose tissue is the main component of the breast stroma. Therefore, in this study we aim to identify the effect that breast tumors have on their adipose microenvironment. For this we collected samples of human breast adipose tissue from tumor breasts (hATT) of patients with cancer and from normal breasts (hATN) of healthy women. The explants were fixed with formaldehyde 4%, embedded in paraffin and sliced for immunohistochemical analysis. We found that adiponectin (one of the major adipokines and with anti-tumorigenic effects) expression was significantly increased in hATN vs hATT ($p<0.05$). Interestingly, adiponectin receptor Adipo R1 showed a significantly increased expression in hATT vs hATN ($p<0.05$). We didn´t find a difference in the expression of leptin (the other major adipokine) and of its receptor ObR. Furthermore, caveolin 1 (principal component of caveolae membranes in adipocytes and involved in signal transduction) was significantly increased in hATT compared to hATN ($p<0.01$). When we studied the differentiation state of adipocytes we found that expression of perilipin A (marker for mature differentiated adipocyte) was decreased ($p=0,055$) and of CD44 (marker for adipose stem cells) was significantly increased in hATT vs hATN ($p<0.05$). In conclusion, the 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.

A199

LIPIDS FROM DIET AFFECT CHOLESTEROL METABOLISM IN RABBIT TESTICLES

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The overweigh and the obesity are known causes of infertility in men, but the mechanism involved remains unknown. We have previously found that rabbits fed with a fat diet have a poor semen/sperm quality, and Olive Oil (OO) supplementation improved semen parameters affected by high fat diet. We investigate the intracellular pathway of cholesterol in rabbit testis under different diets (control; 0.05% cholesterol and protected by 7% OO). We found that after 3 months of diet the expression of mRNA encoding a protein related to the cholesterol synthesis and metabolism (Sterol- Regulatory-Binding-Protein 2; SREBP-2) did not change between hypercholesterolemic (HC) and control animals, but it reduced 50% expression after 6 months of fat diet. Moreover, 3 months of protection with OO in HC animals triggered the recovery in the mRNA expression of SREBP. These data showed that the testicle is sensitive to the intake of saturated or unsaturated fats, modulating the expression of SREBP-2 RNAm. In addition, this mRNA also changed expression depending on the time consumption of diets: after 3 months, the testis seemed not to sense chol sterol blood increment, while after 6 months a significant reduction of the mRNA expression is observed. On the other hand, mRNAexpression does not correspond to protein. These data suggest that the poor semen quality found in hypercholesterolemic rabbits may be related to changes in metabolic status and the molecular machinery of testis. So, we are facing a possible target in the protection mechanism by OO already studied organs.

A200

Rab7b PARTICIPATION ON THE TLR4 (TOLK LIKE RECEPTOR) ENDOCYTIC PATHWAY IN HEMOLYTIC UREMIC SYNDROME

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The inflammatory response of host endothelial cells to Shiga toxin and/or lipopolysaccharides (LPS) of E coli is included in typical HUS. LPS stimulation of TLR4 activates signal transduction pathways leading to proinflammatory cytokine secretion. The TLR4-LPS complex is rapidly internalized and TLR4-induced inflammatory signaling is stopped by targeting the complex for degradation. Rab7b, a small GTPase expressed in monocytes, regulates the later stages of the endocytic pathway. We studied the Rab7b participation on the TLR4 endocytic pathway and its effect on monocyte intracellular cytokine production along the acute course of HUS. The studies were performed in monocytes from HUS patients by flow cytometry and immunofuorescence confocal microscopy. Surface TLR4 expression determined by flow cytometry in CD14(+) monocytes from 16 HUS patients remains unchanged at onset and significantly increased by day 4 compared to 10 healthy children monocytes. Significant higher citoplasmatic TLR4 protein expression was accompanied by increased proinflammatory intracellular cytokines, tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) in HUS monocytes at days 1 and 4 vs controls. On the contrary, monocytes display decreased surface TLR4 expression and significant reduction of intracellular TNF-α and IL-6 levels released in a time-dependent

manner after a disease follow up of 6 to 10 days. Furthermore, immunofluorescence confocal microscopy proved colocalization of increased intracellular TLR4/Rab7b determined by Pearson's coefficient in monocytes from HUS patients on day 1. The highest colocalization of both proteins in monocytes was shown by day 4, then decreased TLR4/Rab7b colocalization was shown 10 days after HUS onset. The colocalization of TLR4 and Rab7b allows us to suggest that Rab7b participates in the control of the TLR4 endocytic pathways in HUS patient monocytes. A consequential fall in cytokine production throughout the early follow up of HUS is demonstrated.

A201
TP73 METHYLATION AND UPREGULATION OF ΔNP73 CORRELATE WITH BREAST CARCINOMA AGGRESSIVENESS

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The incidence of breast cancer is high in Argentina. The optimal treatment is based on proteomic and histological prognostic factors. We previously reported the correlation between aberrant methylation of *TP73* gene and high histological grade, one of the key poor prognostic factors in invasive ductal breast carcinomas (IDC). The TP73 protein has numerous isoforms with different biological and antagonistic functions. The goals of our study were to determine whether the TP73 gene methylation produces the silencing of TP73 and ΔN-p73 isoforms and to correlate TP73 methylation, silencing and expression with tumor aggressiveness and disease progression. For this, we evaluated the expression of TP73 isoforms in 42 IDC by immunohistochemistry and western blot. Previously, we have also studied in these tumors the TP73 gene methylation at two promotor regions by MLPA. Results showed a high expression of ΔN-p73 isoform in 69,7% of the samples. Inmunohistochemistry analysis revealed that all of the TP73 isoforms were localized in the nuclear compartment. No association was found between TP73 isoforms protein expression and the methylation status in the promotor region of TP73 gene (p= 0.08). However, Fisher test and Sperarman's p confirmed the association between ΔN-p73 expression with high histological grade (p= 0.042), (p= 0,005). All together our data suggest that TP73 and ΔN-p73 are useful factors to assess tumor aggressiveness in IDC.

A202
OPTIMIZATION OF THE EXPRESSION AND PURIFICATION OF *Yersinia enterocolitica* OMPC PORIN

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Yersinia enterocolitica (Ye) are Gram-negative bacteria that cause food borne acute or chronic gastrointestinal diseases. Porins are abundant proteins found in the outer membrane of Gram-negative bacteria. Porins form pores that allow the passive transport of nutrients. Moreover, porins have been described as activators of the innate and adaptive immune responses. The objective of this study was to optimize the conditions of high expression and purification of recombinant OmpC. To OmpC expression, *Escherichia coli* BL21 (DE3) Star were transformed with pET-OmpC vector and single colonies were selected in LB agar containing specific antibiotic. To optimize the conditions of expression, we analyzed various conditions including the IPTG concentration, induction temperature, glucose addition, induction time and bacterial density. We observed that the highest expression of OmpC was obtained at cellular density (OD600 nm) of 0.7-1.0. Moreover, we demonstrated that OmpC is not toxicity to the host cell. Although the addition of 0.1% glucose improved the bacterial growth, this glucose concentration suppressed the basal expression of OmpC. Moreover, the optimum expression of OmpC started 3 h post-induction with 1 mM IPTG at 25°C. However, OmpC was predominantly expressed as insoluble inclusion bodies. The recombinant OmpC was purified by affinity chromatography and we observed that soluble protein bound to nickel ion-affinity column and was eluted as pure protein with 200 mM imidazole. In conclusion, we have optimized an efficient protocol to produce recombinant OmpC from Ye in *E. coli.*

A203
HOME SWEET HOME: GALECTIN-1 PROMOTES *Chlamydia trachomatis* BINDING TO CELLS

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Chlamydia trachomatis (CT) is an obligate intracellular pathogen that causes a broad range of acute and chronic genital pathologies in both, men and women. Since its entire developmental lifecycle only takes place within human cells, the attachment and entry into them are crucial steps for establishing infection. CT uses several receptors to invade host cells, among them Toll-like receptors (TLR) 2, TLR-4, high-mannose receptor, CFTR. In this work, we investigate if Galectin-1 (Gal-1), a soluble

carbohydrate-binding protein, participates in the recognition and attachment of CT to a human cervical epithelial cell line (HeLa). By western blot, we showed that Gal-1 binds to CT in vitro. In addition, Gal-1 increased CT attachment to HeLa cells in a doses dependent manner assessed by flow cytometry and confocal microscopy. Likely, Gal-1 promotes chlamydial infection by bridging bacterial N-glycans to eukaryotic membrane glycoproteins. Furthermore, Gal-1 could tie CT together, helping bacteria to enter into the eukaryotic cell in groups, rising even more chlamydial infection. Unveiling the mechanisms used by CT to invade host cells could help in finding new therapeutic targets for controlling this highly frequent sexually transmitted disease.

A204

THREE-DIMENSION SHAPE RECONSTRUCTION OF PERITUBULAR MYOID CELLS.

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Peritubular myoid cells (MP cells), are part of seminiferous tubule (TS) wall. These cells are similar to smooth muscle cells but myophilaments are organized in two perpendicular layers. Were constructed the three dimension (TD) shape of individual MP cells using the density of actin filaments (AF). TS were isolated from adult Wistar rat testes, fixed with 4% paraformaldehyde and AF stained with anti-alpha actin antibody conjugated with Cy3. AF distribution were analyzed by confocal microscopy in 40 transverse sections of 0.4 µm. Using Image J program, the TD shape of individual MP cells were reconstructed. MP cells looked like a hexagon of 6 µm high containing a groove of 16 µm depth in the center of the body. We interpreted that the shape of PM is directly related to the way of TS contraction.

A205

IN VIVO AND IN VITRO ANTIPROLIFERATIVE EFFECT OF 4-HYDROXY-3-(3-METHYL-2-BUTENYL)-ACETOPHENONE (4-HMBA) IN B16F0 TUMOR CELLS MODEL

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The basic physiopathology of vancer includes aberrations in different parts of the molecular mechanisms that control the cell cycle. Due to the increasing incidence of cancer worldwide, there is an intensive search for new therapeutic strategies to treat this disease. In this area, the research has focused on exploring the action of compounds of plant origin. 4-HMBA is the main secondary metabolite from *Senecio nutans*, commonly known as chachacoma, a medicinal plant widely used in Andean traditional medicine; this compound exhibits antifungal and tripanocidal activities. We analyzed, *in vitro and in vivo*, the effect of 4-HMBA. B16F0 cells were cultured in presence of ethanol (vehicle) or 5.0-17.5 µg/mL of 4-HMBA dissolved in ethanol. The growing index (GI) ± SE in 3 independent experiments was assayed from 0 to 72 h. At 72 h of culture, GI of vehicle treatment, was 6.3 ± 0.7 and of 4-HMBA treatments were: 4.9 ± 0.7 (A);3.2 ± 0.8 (B) and 1.0 ± 0.3 (C) for 7.5; 15 and 17.5 µg/mL respectively. Both (B) and (C) GIwere $p \leq 0.001$ vs. vehicle.In*i*n *vivo* assays we inoculated B16F0 cells in C57 mice to generate a melanoma. When the tumor reached a volume of 200mm^2, the animals were treated with 25mg4-HMBA/day/Kg in ethanol or vehicle (ethanol, as a control). After 13 days we observedthatcontrol animal melanomas reached a volume of 2198 mm^2± 970, while treated animal melanomas reached a volume of 460 mm^2± 229. These results show that 4-HMBA generated a significant inhibition of proliferation of B16 F0 cell and an important reduction of tumor volume by therapeutic treatment.

A206

DESMOGLEIN-4 DEFICIENCY INCREASES CD45$^+$ LEUKOCYTES POPULATION IN SKIN

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Desmogleins are involved in cell to cell adhesion mechanisms and are crucial in keeping structural integrity of different tissues including skin and heart. These family of molecules, e.g. desmoglein-3, modulate keratinocyte activation and may control key molecules such as actin and p38 MAPK. However, whether desmoglein-4 may drive inhibitory or activating signals to skin-resident immune cells is unknown. The aim of our work was to assess the impact of desmoglein-4 deficiency in the amount of skin leukocyte populations. To this end, OFA $^{hr/hr}$ rats (n=3) which are mutant for the desmoglein-4 gene and their strain of origin, Sprague-Dawley (SD) (n=3) rats were used in this study. Skin biopsies from OFA and SD rats were weighed, minced to obtain cell suspensions and stained with monoclonal antibodies against CD45 (pan-leukocyte marker) and CD3 (T cell marker), conjugated with APC and FITC respectively. Beads were added to stained cell suspensions from skin biopsies derived from OFA and SD rats and acquired by FACS to measure total cell counts per milligram of tissue. OFA rats showed an expansion of CD45$^+$ cells compared to SD control rats (SD 1,2±0,1 vs OFA 3,9±0,5; cells/mg) (*t* test; $p<0,05$). In addition, we found that desmoglein-4 deficiency increased the percentage of CD3$^+$ T cells compared to SD control rats (SD 17,4±9,3 vs OFA 41,6±14,3) but without reaching statistical significance by the *t* test. In conclusion, these results suggest that desmoglein-4 deficiency promotes an

expansion of leukocytes in the skin. Additional work is necessary to effectively evaluate whether desmoglein-4 deficiency in keratinocytes is directly involved in immune regulation. To our knowledge, this is the first report that evaluates the effect of desmoglein-4 deficiency in skin leukocyte population which may have a potential impact in the design of clinical therapies targeting desmoglein-4 mainly in skin tumor progression, dermatitis and psoriasis.

A207
ALLOPREGNANOLONE INDUCES PROLIFERATION AND MIGRATION OF HUMAN OVARIAN CELL LINE IGROV-1 AND SKOV-3

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Ovarian carcinoma is one of the most common causes of cancer death from gynecologic tumors in the world. Allopregnanolone (ALLO) is an active metabolite of progesterone (P4) involved in physiological and reproductive parameters of female rat. Previously we showed that ALLO affects on the hypothalamic-pituitary-gonadal axis in response to stress inducing mood disturbances and generating morphophysiological alterations in ovarian, being able to inhibit apoptosis and promote angiogenesis of corpora lutea. Furthermore, ALLO is able to inhibit ovulation and the formation of cystic structures. Epidemiological studies as well as in vitro assays have shown that P4 has anti-tumor effects. However, the molecular mechanism of the anticancer effect of progestogen had not yet fully understood. In addition, the effect of ALLO in ovarian cancer is unknown. Our hypothesis is that ALLO affects tumor progression. In this work, we analyzed the proliferation and migration of human ovarian cancer cells IGROV-1 and SKOV-3. To this end, both cell lines were exposed to different concentrations of ALLO (10^{-11}-10^{-7} M) for 72 h and proliferation and migration were analyzed by MTS assay and wound assay respectively. We found that ALLO increased proliferation in a concentration dependent manner, reaching a maximum effect of 80% ($p<0.001$ vs. control: untreated cells). In SKOV-3 had an inhibitory effect on proliferation of 20% at 10^{-10} M ALLO ($p <0.01$ vs. control). Interestingly, ALLO increased the migration of cancer cells IGROV-1 in 60 % at 10^{-8} M ($p<0.001$ vs. control: untreated cells) and had the opposite effect in SKOV-3 cells, inhibiting by 25% the migration at 10^{-8} M ($p <0.01$ vs. control). We conclude that ALLO could modify proliferation and migration of ovarian cancer cells, affecting tumor progression. For this reason, we consider very important to continue elucidating the effect of this steroid in the biology of ovarian cancer.

A208
GENE EXPRESSION OF PINC, STAT6, GATA3 AND TBX3 IS MODIFIED ACCORDING TO DIFFERENTIAL BREAST MILK INTAKE IN RATS

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The development of the mammary gland occurs after birth. Breast milk consumption can cause changes in the mammary epithelium of the offspring that result in the expression of molecules involved in the induction of differentiation, reducing the risk of developing breast cancer in later life. STAT6 stimulates alveolar differentiation and proliferation potentially due to the transcriptional induction of GATA3. Together, STAT6 and GATA3, induce proliferation and change of the luminal cell to an alveolar lineage. PINC expression has been associated with inhibition of terminal differentiation of mammary alveolar cells. The proposed objective was to analyze whether adequate breastfeeding generates modifications in the expression of genes involved in mamary differentiation. We use Sprague Dawley female rats divided into litters of 3 (C3), 8 (C8) and 12 (C12) pups per mother. At 55 days of age, half of the animals was sacrificed to study the mammary differentiation by whole mount and real-time PCR. The number and size of terminal end buds were significantly lower in C3 versus C8 and C12 demonstrating its greater differentiation state. Using real time PCR, we analyzed the expression of mammary PINC, Tbx3, STAT6, GATA3 and PTEN genes. mRNA of PINC was increased in C12 with respect to C3 and C8. The expression of Tbx3, a protein necessary for the normal development of murine mammary epithelial, and GATA3 and STAT6, were significantly diminished in C12 compared to C3. On the other hand, the rest of the animals were treated with a single dose of dimethylbenzanthracene (15mg / rat) to study latency, incidence and tumor progression. A lower incidence and greater latency were observed in C3 respect to the other groups. These results demonstrate a direct link between milk consumption and the ability of the mammary gland to activate gene programs associated with cell differentiation, which may decrease mammary carcinogenesis.

A209

REDUCED NRF2 EXPRESSION INCREASES THE METABOLIC RISK: A STUDY IN OVERWEIGHT BOYS AND RATS FED A HYPERCALORIC DIET

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Excess of energy as free fatty acids that should be stored as triglycerides (TG) otherwise; they cause inflammation, and thus a high risk for obesity-associated abnormalities. Nrf2 controls the expression of phase II/III, antioxidant and adipogenic genes. Low Nrf2 expression may determine inflammation and a high metabolic risk in overweight/obesity. To test this hypothesis we performed a study in overweight children and in an experimental model of rats fed a hypercaloric diet (HCD). In overweight boys (OW, n=22) and normal weight boys (NW, n=27) from San Luis City we measured clinical and biochemical parameters of metabolic syndrome, including hypertension, insulin resistance (IR), lipid metabolism, oxidative stress (OS) and inflammation. Compared to NW, OW boys had IR, higher atherogenic index, altered plasma lipid profile, increased markers of OS and an inflammatory lipid profile. Interestingly, GPx activity and GSH/GSSG ratio and leukocyte's Nrf2 expression were lower in those OW children at high metabolic risk. Nrf2 expression negatively correlated with metabolic risk in OW boys. Experimentally we fed male SD rats (n=19) for 16 weeks with a normocaloric (n=7) and HCD (n=12) and found that some rats fed the HCD were obesity sensitive (OS, n=7) whereas the others were obesity resistant (OR, n=5). Compared to OS and in perirenal adipose tissue, OR rats showed a pattern of oxidative stress (increased NOX-2, reduced antioxidant enzymes and increased OS markers), and inflammation (increased VCAM-1, TNF-a, and lipid profile); but reduced lipogenesis (low Nrf2, PPAR-γ, lipogenic enzyme gene expression, total lipids and TG). Low Nrf2 expression determines reduced adipogenesis, but increased metabolic syndrome's risk due to increased OS and inflammation. Supported by PICT-2014-3369 (to DCR).

A210

EMBELIN AND ITS METHYLATED DERIVATIVE ARE ACTIVE AGAINST *Trypanosoma cruzi*

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Trypanosoma cruzi is a parasite responsible of Chagas disease, an endemic pathology that affects millions people in Latin America. On the other hand, the benzoquinone embelin, a natural compound from *Oxalis erythrorhiza*, was studied due to its biological properties (antiinflamatory affects, antioxidant and antitumour activity, among other). The aim was evaluate the effect of embelin (**1**) and its methylated derivative (**2**) on different stages of the parasite. The compound (**1**) and (**2**) were tested on growth and viability of Dm28c strain epimastigotes. Viability was determined by the eosin exclusion method and proliferation by counting the parasites in a Neubauer hemocytometer. The effects on epimastigote ultrastructure were observed by TEM. To evaluate the effect of the compounds on intracellular amastigotes, infected and non infected Vero cells were cultured in DMEM. The number of intracellular parasites was counting after staining the cells with Giemsa. Compounds (**1**) and (**2**) showed to be active on proliferation of epimastigotes without affecting the viability. The IC_{50} value of (**2**) on epimastigotes was similar to benznidazole (control) whereas, the IC_{50} value for (**1**) was higher. Methylated embelin (**2**) induced ultrastructural alterations, such as mitochondrial swelling and cytoplasmic vacuolization. Citotoxicity on Vero cells was similar to that of benznidazole. Proliferating amastigotes were also affected by the methylated embelin, as well as the release of parasites. The results indicate that embelin (**1**) and its derivative (**2**) exhibit cytostatic activity on epimastigotes of *Trypanosoma cruzi*. The effects on mitochondria could be related to embelin inducing ROS generation. The results in amastigotes suggest that methylated embelin could affect the proliferation of amastigotes and/or the release from cells. Further studies will be needed to identify the molecular targets on this parasite. (CICITCA UNSJ PIO-SECITI150 2015-0100022)).

A211

BIOLOGICAL ACTIVITY OF COMPOUNDS FROM PAROTID GLANDS OF *Rhinella arenarum* (ANURA: BUFONIDAE)

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Nature is the main source of active substances for therapeutic treatments. The skin secretions of amphibians stand out as being an important source of compounds of varying structure, with great therapeutic potential, exhibiting antiviral, antibacterial and cytotoxic properties. Several of these compounds isolated are being used as guidance molecules for drug development. The aim of this study was to evaluate the biological activity of compounds present in parotid glands of *Rhinella arenarum*, using the toxicity test over *Artemia salina*. The organisms were cultured for 24 hours in sea water. We performed the toxicity test with 3 replicates (n = 150) which were subjected to concentrations of 100, 75, 50, 25 and 10 µg of venom dissolved in 2 ml of solvents mixture. As positive control, was employed caffeine as active compound at the same concentration (n = 150) and 3 replicates as negative control with artificial seawater (n = 30). After 24 hours we counted the number of dead larvae. The venom toxicity was observed

at 100, 75 and 50 ppm, causing the total mortality of larvae and coinciding with the positive control. At concentrations of 25 ppm of poison the number of dead larvae decreased, being greater than the positive at the same concentration control. Preliminary results show the toxic action of venom parotid glands of *Rhinella arenarum*, since it is the effective defense system in varying degrees against predators and pathogens of the species.

A212
ADIPOSE TISSUE OF HYPOTHYROID RATS REDUCES PROLIFERATION AND MIGRATION OF MAMMARY TUMOR CELLS

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In order to study the involvement of the adipose tissue as possible intermediary between thyroid states and breast cancer, we evaluate changes in the viability, adhesion and migration in ex vivo cultures of mammary tumors and in tumor and non-tumor mammary epithelial cell lines incubated with conditioned media (CMs) from mammary adipose tissue explants (TAM) of hypothyroid (HypoT) and euthyroid rats (EUT). Female Sprague‑Dawley rats were treated with a dose of DMBA dimethylbenzathracene (15 mg/rat) at 55 days of age and were then divided into two groups: HypoT (0.01% 6-N-propyl-2-thiouracil in drinking water, 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. Also, ex vivo cultures of mammary tumors from EUT were performed. Ex vivo cultures, MCF-10A, MCF-7 and MDA-MB-231 cells were grown and incubated with TAM-CMs of HypoT and 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. An aliquot from CMs was lyophilized and the identification of mass spectrometry (MS/MS). Finally, data was analyzed with ProteoIQ (Premier Biosoft) software. The CMs-TAM-HypoT contained fewer proteins that CMs-TAM-EUT ($p<0.05$). Ex vivo cultures and tumor and non-tumor cell lines incubated with MCs-TAM-Hypot survived and migrated significantly less than those incubated with MCs-TAM-EUT ($p <0.05$). MCs-TAM-EUT had a greater diversity of proteins (at equal total protein) and a higher protein quantity (at equal final volume) ($p<0.05$) than MCs-TAM-HypoT. Interestingly, the expression of adiponectin was augmented in MCs-TAM-HypoT ($p<0.05$). In conclusion, hypothyroidism produces changes in the ability of adipose tissue to secrete soluble factors that regulate the survival and migration of normal and tumor mammary cells.

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