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ABSTRACTS BOOK

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LECTURES AND SYMPOSIA

OPENING LECTURE

A1
TIME WAITS FOR NOBODY. BIOLOGICAL RHYTHMS AND CLOCKS.

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All organisms exhibit periodic variations in their physiology and behavior, which are organized around specific frequencies that span from microseconds to years. Among these variations, circadian rhythms (with periods of about 24 h) and interval timing (from seconds to minutes) stand out. These biological rhythms are generated by endogenous clocks, not well characterized for interval timing, but well-known for circadian rhythms. In mammals, the main clock is located in the hypothalamic suprachiasmatic nuclei, which are entrained by environmental cues, mainly the light-dark cycle.
In this presentation we shall describe recent advances in the mechanisms of such circadian clock, its synchronization and its relevance for metabolic variables in animal models

SYMPOSIUM 1: DEVELOPMENT OF VACCINES AND IMMUNITY

A2
LEISH-TEC RECOMBINANT VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a severe vector-borne disease of humans and dogs caused by Leishmania parasites. Approximately 0.2 to 0.4 million new human VL cases occur annually worldwide, while the disease occurrence is spreading to regions where it was not previously reported. These alarming figures are primarily due to the impracticality of current control methods based on vector reduction and dog culling and limited diagnostic tools. Thus, efficient prophylactic vaccines and highly sensitive and specific diagnostic tools appear to be essential for VL control and badly needed. The current efforts and challenges to develop an efficacious vaccine and more accurate diagnostic tests will be presented and discussed. Special emphasis will be given to the translation research that led to the development of the Leish-Tec® canine vaccine. This amastigote-specific protein A2 plus saponin vaccine is on the Brazilian market since 2008, has been used safely on the prophylaxis of VL in millions of dogs and more recently, as an immutherapeutical alternative for CVL treatment. Moreover, A2-based vaccine formulations have been tested extensively in mice and monkeys; in combination with different adjuvants approved for human use and are therefore a solid base for further vaccine improvements, towards a human VL vaccine. The process of antigen discovery for vaccines against VL also led us to the identification of candidates for serological diagnosis. A new chimeric molecule was used to prototype new ELISA and rapid immunocromatographic tests with high performance, which are ready for registration and commercialization.

A3
EVALUATION OF NEW ADJUVANTS TO ENHANCE IMMUNE RESPONSES AND THE PROTECTIVE EFFICACY OF A UNIVERSAL INFLUENZA VACCINE

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Influenza is a respiratory disease caused by influenza virus. This virus provokes annually a high impact in terms of morbidity and mortality worldwide. Most influenza vaccines induce antibodies that recognize surface viral proteins, hemagglutinin and neuraminidase. These antibodies are capable of neutralizing the virus. However, these proteins mutate constantly on antigenic sites which are recognized by antibodies. Therefore, the virus is able to evade the immune system.
Every year the viral strains contained in the vaccine should be updated. The proposed strains are based on the prediction of the strains that will circulate that year. Nevertheless, antigenic mismatches between vaccine strains and circulating virus are frequent. As a result, the protection conferred by vaccines is often not optimal.

New vaccines are under development in order to provide an improved immune response which confer protection against new strains. Our laboratory has been developing experimental influenza vaccines which contain a highly conserved antigen, the nucleoprotein (NP). It has been demonstrated that this antigen is able to induce cellular immune responses which confer protection against different strains of influenza. We formulated vaccines with a recombinant NP and new experimental adjuvants. The adjuvants which have been used, are molecules which are recognized by pattern recognition receptors and cytosolic DNA sensors. These compounds are able to activate innate immune response and lead to the stimulation of adaptive immune response. Our studies suggest that the combination of a recombinant NP and new adjuvants promotes an effective antigen-specific immune response which protects mice infected against influenza. In conclusion, the use of adjuvants in new influenza vaccines provides potential benefits to enhance immune responses which confer protection against influenza.

A4
IMMUNE RESPONSE TO DEVELOP A VACCINE AGAINST LEISHMANIASIS

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Leishmaniasis is an infectious disease caused by flagellated parasites belonging to Leishmania genus and transmitted by phlebotominae sandflies. Leishmaniasis is distributed in 98 countries of the world; in Argentina, the endemic areas are the Northwest and Northeast regions of the country.
Leishmania parasites present two different stages: the intracellular amastigote localized in the mammalian polymorphonuclear cells and the extracellular promastigote, presents in the sanfly vector. This disease manifests different clinical forms: cutaneous, mucocutaneous or visceral leishmaniasis, mainly depending on the species of Leishmania involved.
There is currently no vaccine against human leishmaniasis. In order to develop that, it is important to considerate the immunology of susceptibility and resistance to leishmaniasis, which depends on the genetic background of host and the specie, and even the specie of Leishmania involved. In consequence, it is possible that a vaccine is effective against one Leishmania specie but not against others.
Our research group has been developing first generation vaccine, using Total L. amazonensis Antigens (TLA) which combined with Poly(I:C) and/or Montanide ISA 763 produce a Th1 like immune response and protect against L. amazonensis infection. Using serums of vaccinated mice, immunoproteomic assay was made in order to identify and select the immunodominant antigens, and therefore develop third generation vaccines.

SYMPOSIUM 2: CHRONOBIOLOGY

A5
IT'S WORM TIME. CIRCADIAN RHYTHMS IN *Caenorhabditis elegans*

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Circadian rhythms are driven by endogenous biological clocks and are synchronized to environmental cues. Diverse model systems – including mice, flies, fungi, plants and bacteria – have shed light on the mechanisms of circadian rhythmicity. However, general principles that govern the circadian clock of Caenorhabditis elegans have remained largely elusive. We have demonstrated circadian rhythms in several variables in C. elegans, including locomotor activity, stress resistance, enzymatic activity, pharyngeal pumping, oxygen consumption and defecation. In addition, we have found evidence of photic and thermal synchronization of locomotion through a lite-1/tax-2/4 related pathway. Finally, we have also developed a bioluminescent system to record oscillations in gene expression in C. elegans and demonstrated the main features of its circadian system.

A6
CIRCADIAN DYSFUNCTION AND ALZHEIMER'S DISEASE: A RELATIONSHIP OF DEPENDENCE?

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Circadian rhythms are generated by an internal biological clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus. This clock entrains the peripheral clocks located in most cells and tissues of the body. The circadian system maintains these 24-h rhythms in physiological functions, including the sleep–wake cycle, and synchronizes them to the light–

dark cycle. Disturbances of both sleep and the underlying circadian rhythms have long been associated with many neurological diseases, including Alzheimer Disease (AD). AD and circadian rhythm physiology display a dependency relationship. On the one hand, AD pathology leads to sleep and circadian disturbances, which have a negative impact on the quality of life of patients. On the other hand, sleep and circadian system may in turn have a causal role in the pathophysiology of AD. Thus the circadian clock could be a new therapeutic target for the treatment of this neurodegenerative disease

A7
THINKING WITH RHYTHM ABOUT THE BENEVOLENT SIDE OF THE INNATE IMMUNITY

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The immune system is responsible for surveillance and monitoring, and for reacting against pathogens and insults. The central nervous system (CNS) is a highly protected area that utilizes its own immune resources, due in part to anatomical barriers. These are the microglia, which are the resident phagocytes of the CNS. Microglia are highly dynamic cells in both shape and phenotype. They originate from myeloid precursors in the yolk sac that colonize the developing CNS. Beyond their defensive role, microglia participate in many processes that shape and maintain the CNS, and also implement its plasticity. One question that emerges is whether microglia adjust their functions based on their own innate temporal capacity, or whether they merely react to external temporal cues. Our group has been working with the pineal gland, as a circadian model within the CNS, in order to better understand microglia phenotypes in both ontogeny and daily cycles.

SYMPOSIUM 3: PREBIOTICS AND PROBIOTICS AND THEIR IMPACT IN HUMAN AND ANIMAL HEALTH

A8
PROBIOTICS: A CHALLENGE FOR THE FOOD MARKET

Rigalli A; Ramadán SS; Casabonne C; Chulibert ME.
The Codex Alimentarius and the Argentine Food Code define probiotics as "*livemicroorganisms that, when administered in adequate amounts, confer benefits for theconsumer's health*".

For a strain to be used as a probiotic ingredient for food, it must undergo an evaluation protocol, having to meet minimum requirements in order to guarantee scientific evidence of it. They are products that contain microorganisms defined and viable enough to modify the microflora of a compartment of the host, thus exerting a beneficial effect on the health of the latter. The use of ferments with beneficial properties has promoted the development of products that contain microorganisms that exert a positive effect on human health because they colonized or remain for a long time in the intestine. Among the beneficial effects we can mention the displacement or inhibition of pathogenic microorganisms, the increase of the immune response, the degradation of lactose and the decrease of mutagenic and carcinogenic compounds in the colon. The development of a non-dairy beverage based on kefir, fruit juice and egg shell has been proposed as a source of calcium and accessible to the population and can replace the intake of dairy products, which is limited for several reasons. From this development arises the study of the composition of the kefir present in the drink, whose first results found that it could be a potential probiotic product due to its composition and microbiological quantification.

A9
BREAST MILK MICROBES. FUNCTIONAL ROLE AND POTENTIAL PROBIOTICS.

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The intestinal microbiota is the set of microorganisms, mainly bacteria, that colonize the intestinal tract, with higher abundance in the colon. The gut microbiota exerts numerous vital functions for life, such as the fermentation of fibers for the production of short-chain fatty acids, the synthesis of vitamins, protection against pathogens, the development of the gut-associated immune system and the synthesis of neurotransmitters such as serotonin, with impact beyond the intestine. The establishment and development of the intestinal microbiota is a complex and dynamic process that begins before birth and extends beyond the first two years of life. This period is key to the development of the immune system and the establishment of oral tolerance. Numerous

factors (premature birth, cesarean delivery, reduced breastfeeding, antibiotic abuse, western diet, small family size, limited contact with the environment) have led to a progressive aberrant establishment of the microbiota, which is correlated with an increase in autoimmune and inflammatory diseases during childhood (allergies, dermatitis, intestinal inflammation, diabetes, overweight, obesity). Breast milk is an abundant source of microorganisms that exert a putative probiotic role for the baby, but that can also be isolated and used as probiotic supplements in a context of impoverished microbiota. Numerous studies indicate that early intervention can significantly contribute to a better microbiota establishment and to the development and maturation of the intestinal immune system. In our laboratory we isolated and characterized a breast milk bifidobacteria strain, resistant to spray-drying, with promising anti-inflammatory effects in the gut.

A10
PROBIOTICS AS ADJUVANTS AND VEHICLES OF ANTIGEN IN THE PREVENTION OF RESPIRATORY INFECTIONS

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The immunomodulatory properties of probiotics, especially of the lactic acid bacteria, have been amply demonstrated. The use of lactococci and lactobacilli as adjuvants and vehicles of heterologous antigens for the prevention of infections constitutes an important aspect of the use of these microorganisms in human health. Nisin-controlled gene expression was used to develop a recombinant strain of *Lactococcus lactis* that is able to express the pneumococcal protective protein A (PppA) on its surface, which was termed L. lactis PppA. The production of mucosal and systemically specific antibodies in adult and young mice was evaluated after mice were nasally immunized with L. lactis PppA. Immunoglobulin M (IgM), IgG, and IgA anti-PppA antibodies were detected in the serum and bronchoalveolar lavage fluid (BAL) of adult and young mice, which showed that PppA expressed in *L. lactis* was able to induce a strong mucosal and systemic immune response. Challenge survival experiments demonstrated that immunization with *L. lactis* PppA increased resistance to systemic and respiratory infection with different pneumococcal serotypes, and passive immunization assays of naive young mice demonstrated a direct correlation between anti-PppA antibodies and protection. The potential risk of using lactic acid bacteria based on mucosal vaccines is that antibiotic markers may lead to the horizontal transfer of genes other bacteria. Due to that, we evaluated the nasal administration of L. lactis PppA live, inactivated, and in association with a probiotic (Lc) to young mice. The animals that received Lc by the oral
and nasal route presented the highest levels of IgA and IgG anti-PppA antibodies in BAL and IgG in serum, which contributed to the protection against infection. However, only the groups that received the live and inactivated vaccine associated with the oral administration of the probiotic were able to prevent lung colonization by *S. pneumoniae* serotypes 3 and 14 in a respiratory infection model. This would be related to a preferential stimulation of the T helper type 1 (Th1) cells at local and systemic levels and with a moderate Th2 and Th17 response, shown by the cytokine profile induced in BAL and by the results of the IgG1/IgG2a ratio at local and systemic levels. Nasal immunization with the inactivated recombinant strain associated with oral Lc administration was able to stimulate the specific cellular and humoral immune response and afford protection against the challenge with the two S. pneumoniae serotypes. The results obtained show the probiotic-inactivated vaccine co-administration as safe and effective immunization strategy for their application in human health, especially in at-risk populations such as the immunocompromised patients, where a live recombinant vaccine could be harmful.

A11
PROBIOTICS FOR THE IMPROVEMENT OF ANIMAL NUTRITION AND FORAGES QUALITY

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The world demand of meat for the coming years will be growing and demanding in quality and safety. The performance of the production animals depends on the digestibility of the nutrients present in the diets and the degree to which they can be absorbed and used. On the other hand, the challenge to diseases and the increase in the prices of raw materials are a threat to the profitability and competitiveness of the world livestock industry. Two problems today raise the public health, the sub-therapeutic use of antibiotics as growth promoters in animal feed which is banned in many countries, including the European Union due to the potential development of antibiotic resistance in microbial populations associated with human and animal diseases and second, zoonotic diseases transmitted by food (salmonellosis, campylobacteriosis and infections caused by *Escherichia coli*). It is imperative to develop new technologies that positively impact on the performance of animals and their profitability. Probiotics are considered one of the alternatives to antibiotic growth promoters and the control of zoonotic diseases. Maintaining intestinal health through diet manipulation is crucial to maintain or improve the performance of production animals. Improvements in animal productivity due to probiotics may be associated with an increase in digestion and the absorption of nutrients probably by a greater enzymatic activity in the intestine. Diets containing probiotics could modulate the host's immune response. Probiotics can be effective in reducing diarrhea after weaning in piglets and morbidity and mortality in pigs. Focusing on the relevant

research for the identification of the risk associated with probiotics together with the development of capacities of the competent regulatory authority are important aspects to protect the public health. In animal nutrition, international guidelines for the production, commercialization, and use of probiotics are essential, especially due to the growing globalization. These guidelines would help prevent the use of inappropriate microorganisms such as probiotics and maintain the efficacy of them to achieve the specific benefits; they would help the institutions involved in the production, commercialization, and regulation of probiotics intended for use in animal production and for the protection of public health.

SYMPOSIUM 4. AROMATIC AND MEDICINAL PLANTS

A12
SELECTION OF PLANT SPECIES FOR BIOLOGICAL EVALUATION: IMPLICATIONS OF BIODIVERSITYAND FUNCTIONALITY

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The development in the search for pharmacologically active compounds has enabled the use of many approaches for the selection of the plants for biological evaluation. These can be grouped into five, namely (1) the random approach, which involves the collection of many plants with a common characteristic found in a study area; (2) the chemotaxonomic approach, which entails the collection of all members of a plant family known to be rich in certain class(es) of compounds; (3) the ecological approach, in which the relationship between the plant and its ecosystem is used as a means of selection; (4) the ethnobotanical-directed sampling approach, based on traditional medicinal use(s) of a plant; and (5) the information-driven approach that utilizes a combination of ethnobotanical and chemotaxonomic data or a database that contains all relevant information concerning a particular species. These different methods will be briefly described and discussed in detail in particular cases from the Colombia biodiversity. Colombia is a country rich in biodiversity and endemism, inhabited by different types of population. Just like Central and South American countries, Colombia also has rich and diverse healing cultures, which are poorly known and have not been properly recorded. All this wealth allows that the approaches available for the selection of plants can be applied in our territory, some more than others, or in combination thereof. This work attempts to present these strategies in context looks from the perspectives of the research done in recent years. The exploration of the anti-inflammatory effects from traditional medicinal plants, neuroprotective substances from the Amaryllidaceae family following a chemotaxonomic approach, the enzymatic inhibition activities related to cosmetic functionality from various botanic families and the search for antiprotozoal compounds from Annonaceae species may be displayed. Given that nature has performed a pre-selection of molecules that influence specific metabolic roles in all living things, it is likely that the search for pharmacologically active compounds following integrated studies will play an important role in the future.

A13
THE PATAGONIAN PLANT *Nardophyllum brioydes* (ASTERACEAE)

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Nardophyllum brioydes is a plant native to South America, widely distributed in the Argentine-Chilean Patagonia. From its aerial parts were isolated pentacyclic triterpenes, ent-halimanes and seco-ent-halimanes, among which the secochiliolide acid stands out. This is the major compound present in the plant, it possess unusual structural motives and shows activity against *Trypanosoma* spp. Several derivatives were synthesized mainly by modification of the carboxyl group present in this compound, and to a lesser extent by transformation of the tetrasubstituted alkene. Some of the obtained derivatives were active against *T. brucei*, *T. cruzi* and *Plasmodium falciparum* in both in vitro and in vivo assays.
From the ethanolic extract of the roots, several pentacyclic triterpenes were isolated and identified, together with two novel compounds: nardoquinones A and B. These isomeric compounds have an o-quinone structure, probably originated by means of a radical reaction from a common precursor.
The last results obtained from the chemical research of this promising Patagonian species together with its biological activity data will be presented.

A14
POTENTIAL SYNERGY OF ESSENTIAL OILS WHEN COMBINED WITH COMMERCIAL ANTIBIOTICS

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The high rate of fungal infections, along with the limited efficacy and high toxicity of available antifungal drugs, render imperative the need for the development of new potential therapeutic options against the most important clinically fungi. A possible solution may be to combine existing antibiotics with essential oils (Eos) to enhance the efficacy of then. The antibiotics have several modes of action and the essential oil components may act synergistically, affecting multiple targets; by physicochemical interactions and inhibiting antifungal-resistance mechanisms. Many reported assays show additive or moderate synergism, indicating that EOs may offer possibilities for reducing antibiotic use. Herein, it presents el effect of the essential oils obtained from species of the native flora combined with commercial antifungals; the approach was to develop potential antifungal with a minor toxicity and most effective. The checkerboard test, that evaluates the effect of interactions between two antimicrobial substances, was the method used. The combined effects between EOs of eight species from San Juan province and three commercial antifungals, against yeast and dermatophyte strains of clinical relevance, analyzed with the Fractional Inhibitory Concentration Index (FICI) values. Synergistic effect was observed (FICI =0.31) between A. cryptantha EO (4000 m a.s.l.) in combination with fluconazole against C. albicans, while that against C. neoformans, the A. mendozana, A. cryptantha (2700 m a.s.l.). L. integrifolia EOs showed an additive response (FICI = 0.62, 0.75, 0.75, respectively). Regarding dermatophytes, A. seriphioides and A. cryptantha EOs combined with terbinafine displayed an additive effect against T. rubrum (FICI = 0.56) and M. gypseum (FICI=1.03). All combinations showed doses reductions regard evaluated antifungals (dose reduction index, DRI 16.25 and 32). The EOs of species collected in the province of San Juan in combination with commercial drugs could for the development of a new antifungal agent useful for the treatment of infections associated with C. albicans and T. rubrum, thus minimize the side effects, and prevent the emergence of antifungal resistance. Despite the promising results given by in vitro studies there is still need to find molecular basis of mode of action of these EOs. Acknowledgement: CICITCA, UNSJ and PIO.CONICET SECITI Gob de San Juan 022.

CLOSING LECTURE

A15
HEALTH TECHNOLOGY AND INNOVATION

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Countries like Brazil have a huge deficit in their trade balance in inputs for public health. Industry companies prefer to import diagnostic tests, drugs and vaccines rather than investing in their own technologies, given the risk, high cost of the R & D process and internal production. The generation of knowledge and new technologies and human resources is concentrated at the universities that are dammed or are poorly explored. This scenario contrasts with the concept of an entrepreneurial university, already existing in other countries, which assists in the chain of self-sufficiency of these institutions and generates knowledge that impacts on strategic security and economic activity for their countries, which, in contrast, are surpluses in their trade balance. Aware of this scenario, UFMG has sought to transform its many patents into products, creating and stimulating an entrepreneurial and innovative ecosystem around its environment. In addition, there has been considerable legal and regulatory progress in Brazil in recent years that provides legal certainty to public institutions, researchers and technology-based companies. In this talk, our intention is to share this recent experience, with the creation of technology centers, startups, technology parks, incubators and accelerators of companies, which involves UFMG's innovation policy, with a special focus on new technologies for health.

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POSTERS

MICROBIOLOGY AND IMMUNOLOGY

A16

DEATH OF MOUSE PERITONEAL MACROPHAGES INFECTED WITH *Clostridium chauvoei*

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Clostridium chauvoei is an anaerobic bacillus which is the causative agent of blackleg, a clostridialmyonecrosis that has an important impact on the sheep and cattle industry worldwide. Blackleg is considered a gaseousgangrene which is an acute disease with a poor prognosis and often fatal outcome. Initial trauma in the host results in muscle damage and impaired blood supply. Early symptoms are generalized fever and pain in the infected tissue. One of the most outstanding characteristics of the myonecrosis produced by this microorganism is the absence of phagocytic cells at the site of infection. Both the induction of apoptosis and necrosis are considered mechanisms of evasion of the innate immune system caused by some microorganisms, since they achieve the destruction of key cells of the immune system. The objective of this work was to evaluate the ability of *C. chauvoei* to induce apoptosis and/or necrosis in mouse peritoneal macrophages at different multiplicities of infection (MOI): 1:1, 10:1 and 100:1 at different times (4, 10 and 24 h). *C. chauvoei* ATCC 10092 cells obtained in logarithmic phase of growth were assayed. The mouse phagocytic cells where grown in DMEM medium supplemented with 10% fetal calf serum and incubated in atmosphere enriched with 5% $C0_2$ at 37°C. The production of apoptosis and/or necrosis was determined by preliminary tests based on morphological changes observed by optical microscopy (Giemsa stain). All MOIshowed significant apoptotic index at all times assayed as compared to the control. Apoptosis was observed at low MOI and in the early stages of infection. In contrast, necrotic cell characteristics were observed at high MOI and 24 hincubation. On the other hand, in all the conditions tested, a phenomenon of autophagy characteristic of cells subjected to physical stress and infection was observed, triggering macrophage death by necrosis in the last incubation hours and at a high infectious dose. This work is the first approach to the determination of apoptosis and necrosis of mouse peritoneal macrophages at different infective doses and post-infection times with *C.chauvoei* cells. Our results support the study of cell death mechanisms to elucidate the pathogenic potential of this bacterium and the search of treatments aimed to avoid the large economic losses produced in cattle.

A17

Brucella INTERACTION WITH Rab PROTEINS IN THE ENDOCYTIC PATHWAY

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A large number of infectious diseases involve an intracellular life stage of the etiological agent. Many of them remain inside the cell protected of attack of antibodies and antibiotics, determining human chronic pathologies, which do not have an adequate control therapy. *Brucella* is an intracellular parasite that affecting humans and animals. In our country, the strain of *B. abortus* S19 is being used, which has advantages and disadvantages, among the latter, the high capacity to cause postvaccinal disease. The rugose strain, on the other hand, due to the characteristics derived from its nature, makes it a more attenuated virulence. The comparison of their intracellular behavior is of vital importance for the implementation of one or the other in veterinary medicine.The maturation of phagosomes containing *Brucella* can be studied in reconstitution models of isolated cells and cell homogenates, as well as cultured cells. The alteration of the endocytic pathway determined by *Brucella* may involve the participation of Rab proteins, especially those related to early endosomes and in biosynthetic compartments. In fact, the objective is to complement and complete the results obtained up to now, in particular with specific inhibitors of kinases and Rab 5. We proceeded to calculate the infective dose to always work with a constant concentration of microorganisms. For which it was preceded of culture of virulent and vaccinal *Brucella* strains (2308, RB51 and S19), in Brucella Agar medium. Each strain was harvested and placed in ependorf to measure its OD in a spectrophotometer. Subsequently, Brucella agar dilutions and plate counts were performed. The infective dose selected was $30x10^8$ CFU / ml. Then the calculations were performed to know the necessary milliliters of suspension to be used to infect the macrophages. The Midipreps Wizard® plus DNA purification system was used. The transfection of the Raw macrophages with the Rab 5 plasmid was performed. It was determined that the amount of transfected macrophages was not expected when observed under a confocal microscope, so transfection with the electroporation technique, which is being tested at this time, will be used. In this way, we will try to understand convincingly the disorder in the maturation of the phagosome caused by *Brucella* as an intracellular survival strategy.

A18
PURIFICATION AND IDENTIFICATION OF IgY OBTAINED FROM HEN EGGS

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The eggs of the birds contain all the nutrients and growth factors necessary for the development of the embryo, including antibodies that are transported from the blood of the hen to the egg, thus providing passive immunity to the offspring. When the egg is in the ovary, the hen transfers the immunoglobulin "Y" (IgY) to the yolk. IgY is the main immunoglobulin of the serum involved in the secondary immune response and it is analogous to the IgG present in mammals. Obtaining IgY has numerous advantages, like the preservation of animal welfare, since it is not an invasive method like that of mammals, the high concentration of antibodies that are obtained, the easy collection of the egg, the simple methodology of extracting antibodies from them and the low amount of antigens needed to obtain a lasting immunoglobulin concentration from immunized eggs. In addition, the phylogenetic distance between birds and mammals, makes IgY not cross-react with mammalian IgG, nor non-specific reactions with complement, minimizing false positive reactions. The aim of this work was to set up the protocol for the separation, purification and identification of IgY obtained from chicken eggs to obtain specific antibodies after immunization plans. Field eggs were used, which were disinfected with alcohol. The manual separation of the yolks was carried out. Then proceeded to the delipidation by mixing it with sterile distilled water (1: 9), adjusted to pH 5.0 and the lipids were precipitated at 4°C by centrifugation. The lipid-free supernatant was stored at -20 overnight to eliminate proteins sensitive to freezing. The fractionation was carried out with 45% (NH4)2SO4. It was centrifuged at 15.000 rpm and the pellet was dialyzed in order to eliminate the salt used. The sample was separated by chromatography using ion exchange columns (HiTrapDEAE FF). The proteins collected were subjected to electrophoresis in polyacrylamide gel under native and reducing conditions. The bands were visualized with Coomassie brilliant blue and they were characterized by comparison with known molecular mass patterns. The bands corresponding to both whole molecule-IgY (180 KDa) and their heavy (68 KDa) and light (32 KDa) chains were identified. The methodology implemented in our laboratory was successful since it allowed the extraction of immunoglobulins and their subsequent purification, identification and isolation. This methodology opens the doors to the antigen-specific IgY obtention through simple immunization plans in hens. Its use could be extended to the industry since it is an easy, inexpensive and non-invasive procedure to obtain immunoglobulins in high performance.

A19
ANTICANDIDAL ACTIVITY OF *Annona emarginata* (SCHLTDL) H. RAINER

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The infusions and decoctions of the leaves of *Annona emarginata (Schltdl.) H. Rainer (=Rollinia emarginata Schltdl.)* are used in folk medicine as a gargle against throat pain orfor calming the toothache by absorbing juice produced by chewing the leaves. The anticandidal properties of extracts from *Annona emarginata* flowers, fruits, leaves, stem barks were tested against an extended panel of standardized and clinical isolated of *Candida albicans* and *Candida tropicalis*respectively,according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The fruits global methanolic extract (FGME) showed a very strong antifungal activity against *C. albicans* ATCC 10231 and clinical isolated (MICs 62.5-100 µg/mL).The bioassay-guided fractionation by Sephadex LH 20 and silica gel column of the FGME extracts led to the isolation of compound(R)-2-(4-methylcyclohex-3-en-1-yl)propan-2-yl (E)-3-(4-hydroxyphenyl)acrylate(1), which showed a strong anticandidal activity (MIC =6.25 µg/mL) against *C. albicans* ATCC 10231.The main antifungal isolated compound was chemically elucidated using 1D and 2D NMR, IR, UV, HRMS experiments and previously published date.Authors are grateful to CICITCA-UNSJ, UNSL and UNC.

A20
CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS FROM *Acantophilia deserticola* (RICA RICA) AND *Artemisa copa* (COPA COPA) EXTRACTED BY MICROWAVE-ASSISTED HYDRODISTILLATION (MAHD).

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Acantophilia deserticola (RR) and *Artemisa copa* (CC) are herbs native to the Chilean highlands, used ancestrally for medicinal purposes by the Atacama peoples. The essential oil, extracted by Microwave-Assisted Hydrodistillation (MAHD) was analyzed

by GC-mass to determine its composition. The antimicrobial activity was evaluated against 32 bacteria, by disc diffusion test and the MIC was determined for those that showed a strong antimicrobial activity. The major compounds were tanacetone (45%), ß-linalool (16%), eucaliptol (9%) for RR and cis-3 hexenylisobutyrate (38%), g-terpinen (16%), 2-cyclohexen-1-ol, 1 methyl-4 (1 methylethyl) cis (12%), for CC. The RR-EOs showed a significant inhibitory effect (> 20 mm) on 8 bacteria, including *L. monocytogenes* and *S. aureus* while the CC-EOs affected *E. coli*. The anti-*E.coli* effect of *CC* (MIC = 8µg/mL) was higher than *RR*(MIC = 64µg/mL). The essential oils of RR and CC, extracted by HDMO present compounds and antimicrobial activity of industrial interest, which would allow to project their use in areas such as the clinic, food, cosmetics, sanitizers, detergents, among others.

A21
IMPROVED IMMUNE RESPONSE TO TOTAL *Leishmania* ANTIGEN FORMULATED WITH A TOLL LIKE RECEPTOR AGONIST

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A proper adjuvant has a relevant role in vaccine formulations to generate an effective immune response. Our proposal was to develop a first generation vaccine adjuvanted with a toll like receptor agonist, polyinosinic-polycytidylic acid [Poly (I:C)], able to induce a Th1 response. Total *Leishmania* antigens (TLA) were obtaining by a sonication process. Mice were vaccinated with two doses of TLA-Poly(I:C) administered by subcutaneous route at 3-week interval. Humoral and cellular immune responses induced by the immunization were measured. Mice vaccinated with TLA-Poly (I:C) showed a high anti-*Leishmania* IgG levels, as well as increased IgG1 and IgG2a subclass levels compared with mice vaccinated with the TLA alone. The high IgG2a levels indicated a Th1 bias response induced by the TLA-Poly (I:C) immunization. Accordingly, the cellular immune response elicited by the formulation was characterized by an increased production of IFN-γ, delayed-type hypersensitivity and cell proliferation. Our findings demonstrate a promising vaccine formulation against leishmaniasis that improved the Th1 immune response to TLA and which should be taken into account for preventing leishmaniasis in developing countries.

A22
DETERMINATION OF PATHOGEN-FREE CONDITION IN *Larrea divaricata* CAV. SAMPLES FROM DIFFERENT AREAS OF SAN LUIS, ARGENTINA

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Larrea divaricata Cav. (Jarilla), a shrub widely distributed in South America, is used in folk medicine. Studies in our laboratory have shown that proteins from aqueous crude extracts of "jarilla"(JPCE) exhibit cross-reaction with *Pseudomonas aeruginosa* proteins. The aim of the present study was to characterize the microbiota and to determine protein profiles of "jarilla" specimens collected from different geographical areas of San Luis, Argentina, in order to ensure that the plant is pathogen free. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is an analytical technique used to separate proteins based on their molecular weight that allows reveal the defense response of the plants at the level of protein expression when they are subjected to stress caused by biotic agents such as pathogens. Special care was taken in choosing healthy specimens in terms of color and structure of leaves and stems, absence of spots, etc. For microbiological analysis, homogenates of the different samples were serially diluted in peptone water and plated for counting and characterizing colonies. Appropriate culture media were used for *Pseudomonas aeruginosa,Escherichia coli*, *Staphylococcus aureus*, yeast and filamentous fungi, and mesophilic aerobes identification. They were incubated under the conditions required for each species. Triplicate trials were performed. The microbial colonies were counted and reported as colony forming units per ml (cfu/ml) and they were identified by their biochemical and morphological characteristics. The microbial load analysis was negative for *P. aeruginosa, E. coli and S. aureus*. A few colonies grew in Sabouraud agar (fungi) and Plate Count Agar (mesophilic aerobes). Regarding the JPCE analysis by SDS-PAGE, a high percentage of similarity between the protein profiles of plants from different geographical areas was found.The results of this study provide interesting data referred to the "jarilla" microbiota since Gram negative and Gram positive pathogenic bacteria were not detected. Also, the high degree of similarity in JPCE protein profiles observed among the different "jarilla" specimens would confirm the absence of a potential pathogen agent that could induce a proteomic variation related to a defense response by the plants.

A23

STUDY OF SAFETY OF EXTRACTS OBTAINED FROM *Arachis hypogaea* L (PEANUT)

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The peanut plant (Arachis hypogaea L) is a legume that belongs to Fabaceae family and Arachis genus. It is native to South America and is grown around the world in tropical, subtropical and temperate regions. The plant contains several active components that include flavonoids, phenolic acids, phytosterols. Scientific reports have indicated that these components can cause various biological effects, including cardio-protective, anti-inflammatory, antioxidant, anti-cancer, antimicrobial, anti-diabetic, anti-obesity and sedatives. There are no reports on toxicity. The aim of this work was to evaluate the ethanolic extracts of Arachis hypogaea L in its cytotoxic and genotoxic capacity in vivo. For this, ethanol extracts of seeds (EES) and tegument (EET) of peanuts were obtained by a simple alcohol extraction method. Cytotoxicity studies: Vero cell monolayers were treated with increasing concentrations (0-1400 µg/ml) of EES and EET. Cytotoxicity was determined at 48 h of incubation by: Neutral Red (NR) uptake and MTT reduction. For the genotoxicity studies, the Micronucleus Test was carried out in mouse bone marrow. Healthy Balb/c mice of 2 months age (25 g) were used. They received, by intraperitoneal injection, 0.2 ml of EET at concentrations of 25, 50, 100 and 250 mg/Kg of body weight (b.w.) and of EES at the concentrations of 500, 1000 and 2000 mg/Kg b.w. prepared in physiological solution and DMSO (Dimethylsulfoxide). Negative (NC), positive (PC) and vehicle (VC) controls were included. The animals were sacrificed by cervical dislocation at 24 h post-injection. The marrow samples from the femoral bones were spread on slides, stained with May-Grünwald-Giemsa solution and observed under a microscope. The genotoxicity index was determined: number of MNPCE (micronucleated polychromatic erythrocytes) in a total of 1000 PCE (polychromatic erythrocytes) per spread and the toxicity index (TI), considering the PCE/NCE ratio (normochromatic erythrocytes) every 1000 PCE. The cytotoxicity results indicated CC50 values of 600 µg/ml (MTT) and >1600 µg/ml (NR) for EET and >1400 µg/ml (MTT) and 1600 µg/ml (NR) for EES. EES was less toxic than EET by the MTT technique. The genotoxicity indexes were 6, 8.5, 9, 10.7 and the TI: 1.68, 1.47, 1.43, 0.95 for the concentrations 25, 50, 100 and 250 mg/Kg of EET, respectively. The EES presented as genotoxicity indexes: 7.5 (500 mg/Kg), 7.4 (1000 mg/Kg) and 9.6 (2000 mg/Kg) and as TI: 1.08 (500 mg/Kg), 1.31 (1000 mg/Kg), 1.95 (2000 mg/Kg). The genotoxicity indexes were for NC: 8.5, PC: 27 and VC: 8.25. TI: 1.32 (NC), 1.5 (PC), 1.12 (VC). None of the extracts were found to be genotoxic ($p>0.05$). Only the highest concentrations of EET and EES showed some toxicity, with respect to the negative control ($p <0.05$ and $p<0.001$, respectively). It is concluded that the EET and the EES of Arachis hypogaea L exert toxicity in vitro and in vivo at high concentrations and do not generate genotoxic damage at the evaluated doses. These extracts are safe for human consumption and use in phytomedicine.

A24

EFFECT OF CELL-FREE SUPERNATANT OF *Lactobacillus fermentum SL36* ON THE BIOFILM FORMED BY *Staphylococcus aureus* AND *Candida albicans.*

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In the last decades, there is an increasing interest on Lactic acid bacteria (LAB) due to their antimicrobial activity by production of lactic acid and metabolites. Numerous investigations have shown that natural products with antimicrobial activity can affect biofilm formation. Bacterial biofilms are communities of cells adhered to surfaces, immersed in a matrix of extracellular polymeric substances preventing the action of antibiotics. The purpose of this study was to evaluate the effect of cell-free supernatant of L. fermentum sl36 isolated from raw goat milk, on planktonic and biofilm (sessile cells) formed by S. aureus and C. albicans. The antimicrobial activity of cell-free supernatant (CFS) of LAB strain was evaluated in previous studies. For biofilm formation, Petri dishes containing sterile coverslips (22 x22 mm) and TSC medium for S. aureus or YPD broth medium for C. albicans were used. At 48h of incubation a good production of biofilm was obtained from both pathogens strains. The effect, on biofilm formated, of CFS (pH: 5.5) and of CFS neutralized (CFSN, pH: 7.0) was evaluated by incubation for 24 h. Planktonic and sessile cells treated with CFS and CFSN were analyzed by viability using plate counting and Optical Microscopy (OM). For the analysis of planktonic cells, measurements of DO 580 were made. Quantification of sessile cells was made by absorbance measurement at 570 nm from stain of coverslips using 0.1% violet crystal. For OM, coverslips with biofilm were stained with violet crystal. After both treatments, no significant difference was observed in the biofilm formed by S. aureus compared to controls at 570 nm. Optical microscopy exhibited low decrease in biofilm in both treatments. The decrease in the viability of the biofilm in the presence of acidic CFS was appreciable while in the presence of CFSN there was a slight tendency to increase viability. The planktonic cells were affected in both cases, with a greater effect of the CFS of pH: 5.5, this result agrees with that observed by OM. As regards biofilm formed by C. albicans, no significant difference was observed, compared to controls, after treatment with both CFS .Optical microscopy exhibited pronounced decrease in biofilm in both treatments. Concerning the viability of biofilm, it decreased significantly in both treatments, with a largest reduction in the presence of acidic CFS. The planktonic cells were affected in both cases, with a greater effect of the CFS of pH: 5.5, coinciding with that observed by OM. These results indicate that L. fermentum sl36 produces antimicrobial substances that significantly affect planktonic cells of S. aureus and C albicans. Although these substances do not affect the biofilm formed, viability of sessile cells decrease. Acid production increases the inhibition. These data are important in regard to pathogenesis and resistance provided by the biofilm that difficult treatment of pathologies caused by these microorganisms.

A25
INTERACTION OF COMMERCIAL INSECTICIDES WITH THE ENTOMOPATHOGENIC FUNGUS *Metarhizium* SP. TO CONTROL *Lobesia botrana*

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For many years, pest control has been carried out only using chemical insecticides. However, the indiscriminate use of these products has brought negative consequences to the environment, the health of the workers, the quality of harvest, and the economy of small, medium and big farmers. Currently, the control of *Lobesia botrana*, which is the plague that causes major damagesto the vine crop, depends almost exclusivelyon the use of chemical insecticides. Alternatives to this type of control are a key factor for the sustainability of agricultural ecosystems. On the other hand, entomopathogenic fungi (ETP) are currently considered as important natural control agents that limit insect populations worldwide, making them an interesting biological pest control tool.

The aim of this study was to analyze the integrated effect of a commercial insecticide (Spinosad® 48%) applied together with the three ETP *Metarhizium*sp. strains (CEP413, CEP589, and CEP591.) on L_4larval instar of *L. botrana*.

To carry out these trials, 10 larvae of *L. botrana* were used with three replicates per treatment. The treatments were: 1-Spinosad 48% (0.15 µL/mL), 2-CEP413 ($1x10^8$ c/mL), 3-CEP589 ($1x10^8$ c/mL),4-CEP591 ($1x10^8$ c/mL), 5-Spinosad 48% (0.075 µL/mL) + CEP413 ($1x10^4$ c/mL), 6-Spinosad 48% (0.075 µL/mL) + CEP589 ($1x10^4$ c/mL), 7-Spinosad 48% (0.075 µL/mL) + CEP591 ($1x10^4$ c/mL), and 8-distilled water as control. The treatments were administered with a repeating dispenser (Hamilton PB600-1) placing 1µL per larva according to each treatment. The treated larvae were placed on individual bunches of grapes in sealed plastic trays. The cadavers were counted after 96 h and symptoms of the ETP infection were observed to confirm the cause of death.

The variance analysis detected differences among treatments and control ($F=23.78$; $p<0.0001$). Treatment 1 (only Spinosad) was the less affective with a cumulative mortality of 75%. The greater mortality percentage in L_4 instar (100%) was obtained when insecticide was applied together with each strain at half dose (treatments 5, 6, and 7).

According to this study results, it is more effective to apply Spinosad® 48%mixed with entomopathogenic *Metarhizium* at 50% of their recommended dose. These findings are important because it aims to reduce the excessive use of some insecticides and to implement the use of entomopathogenic on Integrated Pest Management programs to control *L. botrana*.

A26
ISOLATION OF BACTERIOPHAGE AGAINST STRAINS OF TEROHEMORRHAGIC *Escherichia coli* IN MENDOZA

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Strains of Enterohemorrhagic Escherichia coli (EHEC) are human pathogens whose most severe clinical manifestation is Hemolytic-Uremic Syndrome (HUS) and / or Thrombotic Thrombocytopenic Purpura (TTP). It is important to control the spread of this pathogen in our region due to its high prevalence in meat products.HUS is a serious, life-threatening disease that mainly affects children under 5 years of age, but it can occur at any age.

Like any bacterial infection, its growing resistance to antibiotics is of great concern. An alternative is the use of bacteriophage (phages), as a biocontrol tool for these pathogenic bacteria, present in meat foods. Phages are viruses that are naturally selected to infect and lyse specific bacterial cells and do not infect human cells.Objective: To isolate specific EHEC phages. To determine and identify these phages, ground meat, sausages and residual water were used. The technique used was to enrich the culture medium and on the 2nd day, Cl2Ca was added, followed by centrifugation and filtration. The Spot Test was carried out in order to isolate the phages.Results: A total of 6 specific EHEC phages were obtained, isolated from ground beef, sausages and wastewater. The phages were selected to form a therapeutic cocktail and the lytic phages were chosen. Their effectiveness was evaluated with the reduction of viable cells (VC) of EHEC, which were sown on the surface of the meat, and then the phages were planted. They were allowed to act the required time. Subsequently, a new EHEC count was performed and the results were compared, showing a reduction of the VC.

It is necessary to consider the existence of spontaneous phage-resistant mutants. The EHEC strains were provided by outbreaks of HUS from a pediatric hospital.

A27
BIODETOXIFICATION OF AFLATOXIN B1 USING PURE COMPOUNDS FROM *Achyrocline satureioides*

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Aflatoxin B1 (AFB1), produced by strains of Aspergillus flavus and A. parasiticus, is carcinogenic, teratogenic, hepatotoxic and immunotoxic and, at high doses, can be lethal. Also, fungal toxins are important because they generate great economic losses. Natural products have been shown to have detoxifying capacity of mycotoxins. The objective of this work was to evaluate in vivo, by oral administration in Wistar rats, the ability of luteolin (L) and chlorogenic acid (CHL) to attenuate or revert cytotoxic and genotoxic effect of AFB1 by supplying the toxin for 30 days. For this experience Wistar rats (Rattus norvegicus albinas) of 200 g of body weight were used, which were divided into 6 groups of 4 individuals each one. They were orally administered 0.2 ml of the different treatments. AFB1 was dissolved in 0.05% of dimethyl sulfoxide (DMSO) and 0.1 M of sodium bicarbonate (pH 7.4). The compounds were administered at non-cytogenotoxic concentrations by gastric tube. Groups: Control AFB1 (400 ppb); Treatment 1: AFB1 (400 ppb) + L (0.5 mg/kg b.w.); Treatment 2: AFB1 (400 ppb) + CHL (5 mg/kg b.w.); Control drug 1: L (0.5 mg/kg b.w.); Control drug 2: CHL (5 mg/kg b.w.); Negative Control: 0.1 M sodium bicarbonate. In all cases they were administered water and food ad libitum. The treatment was extended for 30 days. The detoxifying effects were evaluated by: 1. Evaluation of genotoxicity and antigenotoxicity by Micronucleus test in rat bone marrow: from the femurs of rats, the bone marrow samples were obtained with fetal calf serum, were homogenized, centrifuged, and plated on slides which were fixed by soft flutter and stained with May-Grünwald and Giemsa. The frequency of micronuclei in 1000 polychromatic erythrocytes (PCE) was determined per slide. Cytotoxic and anticytotoxic effects were evaluated through the determination of the toxicity index (TI): polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE) by 1000 PCE counted. 2. Studies of oxidative stress and antioxidant action: it was performed by determining substances reactive to thiobarbituric acid (TBARs) from liver and kidney samples. The determination of TBARs was carried out using malondialdehyde (MDA) as a reference substance. To the homogenates obtained in phosphate buffer, BHT was added, the samples were centrifuged, and the supernatant was incubated at 90°C for 30 min with thiobarbituric acid (TBA, 0.375%). To calculate MDA concentrations in the tissues, a calibration curve was used. The tests were carried out in duplicate and each sample was read in triplicate. The results are expressed as nmol of MDA/g of tissue. The results obtained indicated a significant increase in the genotoxicity index in rats treated with AFB1 (20 ± 1 MN/1000 PCE) respect to NC (6 ± 0.5). Treatment with L and CHL managed to reverse/protect from damage induced by AFB1, both, L + AFB1 and CHL + AFB1, indicated a normal value of MN/1000 PCE. On the other hand, TI showed no significant difference between any of the treatments. With regard to studies of oxidative stress, it was observed that there was an increase in TBARs in livers of rats treated with AFB1 in relation to NC but without statistical significance. On the other hand, kidneys showed a significant increase in TBARs in rats treated with AFB1 respect to NC. Treatments with L and CHL protected from damage induced by AFB1.

A28
INFLAMMATORY PROPERTIES OF *Streptococcus dentisani*. A NEW ALLY AGAINST DENTAL CAVITIES?

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The manifestation of dental caries is mediated by complex mechanisms that are initiated by genetic, behavioral, environmental and microbial factors. In the case of the latter, the presence of bacteria is essential for the onset and progression of caries lesions. It has been shown that some bacterial species predominate only in the initial stages of the lesion, while others are present exclusively in the advanced stages. Each caries lesion represents a unique ecosystem, where the microbial species form a biofilm. Regarding oral cavity microorganisms with dental caries, the main importance of Streptococcus mutans and related organisms has been established. However, Streptococcus species may constitute more than 50% of the oral microbiota in healthy individuals. In addition, they also have positive effects on human health, and some of them belonging to the Mitis group; have begun to be used as probiotics in disorders of the digestive system. Recently, Streptococcus dentisani has been isolated from caries-free human dental surfaces. This novel bacterium could be involved in the modulation and prevention of dental caries by inhibiting S. mutans by producing inhibitors of peptidic nature such as bacteriocin-like peptides, which makes it an ideal candidate as a probiotic agent for dental caries. However, there is little literature that identifies different mechanisms of interaction of this bacterium with the individual and the protection against various pathogens from the oral cavity. Therefore, we propose to study the profile of interleukins produced by cell lines in response to infection with S. dentisani and S. mutans since they have a fundamental effect on the regulation of the mechanism of inflammation. The hypothesis of this work is that the presence of S. dentisani promotes the production of inflammatory interleukins in a cellular model in vitro. HeLa cells (human epithelial cells) were cultured in DMEM medium, with 10% fetal bovine serum. S. dentisani and S. mutans were inoculated into 50 ml of brain infusion broth and incubated aerobically at 37°C without stirring for 48 h. After growth, the culture was centrifuged at 3200 rpm for 10 min to separate the supernatant from the precipitate containing the bacteria. Culture cells were infected with bacterium or incubated with supernatant for 1 h, and then the cells were incubated at 37°C, 5% CO2 for different

times. The samples were harvested in TRIzol reagent and processes to obtain DNA copy and polymerase chain reaction (PCR) assay. The data were analyzed by non-parametric Kolmogorov-Smirnov tests and parametric ANOVA in combination with the Tuckey and Dunnett test.

A29
DETERMINATION OF PATHOGENIC FACTORS OF *Candida albicans* FROM CLINICAL SAMPLES

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Candida is a microorganism that is always valid in the context of infections in immunocompromised patients. In developed countries, invasive candidosis is the most common mycosis among hospitalized patients and with a high mortality rate. The pathogenicity of *C. albicans* is a complex phenomenon that includes colonization, adhesion, invasion and damage to host cells, cell wall composition of the fungus, as well as the production of toxins and proteolytic enzymes. On the other hand, determining the virulence factors makes it possible to establish, more precisely, how the relationship established with its host is modified once the resistance mechanisms have been deteriorated. The main objective of this work is the study of the pathogenic characteristics of the mycotic agent. We started with 32 strains, isolated from different clinical samples and from both hospitalized and ambulatory patients. Typing was carried out by micro-morphological and biochemical methods: production of pseudohyphae, germ tube, chlamyconidias, micromorphology on corn flour agar, characteristics of the colonies in the Chrom Agar *Candida*® medium, assimilation tests and carbohydrate fermentation. Those corresponding to *C. albicans* (22/32) were determined enzymatic activity: phospholipase Sabouraud agar supplemented with 8% egg yolk and glucose; protease (by the plaque method in the culture medium containing bovine serum albumin as the sole source of nitrogen) and esterase (by the plaque method in the Tween 80 half-opacity). The results were expressed by the Pz and Prz indices, which is a quotient between the diameter of the colony without and with halo, which can vary between 0 and 1, corresponding the greater activity when the value more approaches the lower limit. For the phospholipase activity it oscillated between 0.88 and 1, of which 43% corresponded to a weak activity, 73% to a very weak activity and 4% no activity; for the protease activity between 0.62 and 0.85, 23% to strong activity, 45% to moderate and 32% to weak activity and for esterase activity between 0.85 and 1, being 9% of weak activity, 59% very weak and 32% null. This is a work that is in development, therefore, the conclusions that we can express are preliminary. However, the most obvious activity is that of protease enzymes.

A30
THE SUSCEPTIBILITY OF *Metarhizium* sp. NATIVE STRAINS TO CHEMICAL FUNGICIDES AND INSECTICIDES COMMONLY USED IN VINEYARDS

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The use of entomopathogenic fungi (ETP) is a strategy that is currently increasing and has proven to be highly effective to control insect pest populations. These microorganisms are mainly considered as important natural control agents, however they can have different roles in nature, such as fungal endophytes, phytopathogen antagonists, beneficial associations in the rhizosphere and even as plants growth promoters. Over the years, different research shows that the use of ETP integrated with other control methods can be an alternative to the exclusive use of chemical insecticides. Nevertheless, is also necessary an analysis of some factors that intervene in the management of a crop and their effect on the performance of ETP as biocontrol. The objective of this work was to evaluate the susceptibility of a Metarhiziumsp. native strain of San Juan (CEP591) to fungicides and insecticides commonly used in the vine (Vitisvinifera) crop. Strain CEP591 was selected mainly for its pathogenicity against L. botrana in previous bioassays. The fungicides (Carbendazim, Copper oxychloride 36% WP, Cyprodinile-Fludioxonile, Dicarboximide, Fenhexamide 50%, Iprodione, Miclobutanil 24% p/v) and the insecticides (Spinosad 48%, Metoxifenoxide, 10% p/v Lambda-cihalotrin, Clorpirifos-Cipermetrine) were added individually to separated 500 mL Erlenmeyers with APG Britania® (Agar, Potato, Glucose) after sterilization of the culture mediaaccording to manufacturers recommended dose. Strain CEP591 was inoculated in the center of each Petri dish containing each chemical compound. In addition, a control treatment was carried out inoculating the strain in Petri dishes containing only APG. Three replicates of each treatment were performed. The Petri dishes were preserved at 27 °C and after 48 hours their radial growth was measured in two perpendicular axes using a Vernier's digital caliper. The measurements continued every 48 hours for 12 days. The inhibition percentage generated on CEP591 with each chemical compound was calculates using the Jiang et al., (2014) formula. Regarding to fungicide trials, ANOVA test for inhibition percentages detected significant differences among treatments. CEP591 was completely inhibited (100%)byCarbendazim,and highly inhibited by Miclobutanil (97.8 ± 3.7%),Cyprodinile-Fludioxonile (94.5 ± 5.17%), Dicarboximide (87.5 ± 11.5%), and Copper oxychloride (73.1 ± 13.7%). On the other hand, CEP591 resulted less susceptible to Fenhexamide 50% (41± 12.5%). The susceptibility of CEP591 to insecticides showed mediuminhibition percentage by10% p/v Lambda- cihalotrin,Clorpirifos-Cipermetrine, and Spinosad 48% (67.6, 68.5 and 75.6% respectively). On the other hand, Metoxifenoxide showed a high inhibition percentage (86.3%) regarding to control treatment.

A31

ANTIBODIES OBTAINED WITH JARILLA SPECIFIC PROTEINS RECOGNIZE MEMBRANE PROTEINS OF *Pseudomonas aeruginosa*

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Larrea divaricata Cav., commonly known as Jarilla, is native plant to arid regions of South America that is used in folk medicine for the treatment of different pathologies. *Previous studies have demonstrated* a *cross reaction* between proteins from *L. divaricata* and whole cell-bacterial proteins of gram negative bacteria. *Pseudomonas aeruginosa* is an opportunistic pathogen responsible for both acute and chronic infections associated with a high mortality in vulnerable populations. In this study, we investigated the cross-reactivity of sera obtained from purified proteins fractions of jarilla crude extract (JPCE) by *Fast Protein Liquid Chromatography* (*FPLC*) with *membranes proteins ofP. aeruginosa. T*he aim was evaluating the most immunogenic bands of JPCE proteins. Outer-membrane proteins (OMPs) and inner-membrane proteins (IMPs) were obtained from *P. aeruginosa* culture in exponential growth phase. JPCE were prepared from leaves. The extract was partially purified by using membrane concentrators with a 10kDa cut off. Different proteins fraction were separate by *FPLC*. The protein concentration was determinate by *Bradford* protein *assay* and the different fractions were resolved by SDS-PAGE. Antigenic preparations were obtained according to similar band patterns fractions and protein concentration: J1 (33-103 kDa), J2(22-26kDa), J3 (14-26 kDa) and J4 (14kDa). Mice were immunized with J1, J2, J3 and J4. The cross reactivityof anti-JPCE proteins sera (S1, S2, S3 and S4,respectively) was evaluated against OMPs and IMPsby ELISA test. ELISA Index (EI) was calculated and EI≥1 were considerate positive sera. In the case of IMPs, the reaction obtained with the four sera was slightly significant with respect to the control. When OMPs were used as a sensitizing antigen, S1 showed cross-reactivity greater than four times the control, demonstrating the immunogenicity of jarilla proteins between 30 and 103 kDa for obtaining sera capable of recognizing outer membrane proteins of *P. aeruginosa*. The low reactivity of S2, S3 and S4 against OMP and IMP could correspond to the absence of similar antigenic determinants and/or the immunogenicity of bands of low molecular mass. These findings could be important in the development of newmethods to prevent *P. aeruginosa* infection, based on *plant specific proteins* of cross reaction without the need to expose patient to pathogen, in a safe and effective way.

VETERINARY: ANIMAL ANATOMY, HISTOLOGY AND PHYSIOLOGY

A32

HOURLY BEHAVIOR OF A BIOCLIMATIC INDEX FOR BOVINES IN POCITO, SAN JUAN

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Livestock is one of the most increasing activities in San Juan, having already got long-term goals established by the provincial Government. Livestock production depends on many factors and the meteorological ones are among the most important. Climate comfort and animal welfare are part of the equation to achieve a successful activity. Due to the Province's high temperatures and low relative humidity, it is very important to determine whether they will turn into limiting factors. High temperatures and high relative humidity can cause thermal stress that alters the physiology of the animal, reducing the biological efficiency and the productive capacity. Among the many bioclimatic indexes to monitor environmental conditions for heat stress in livestock, one of the most used is the temperature and humidity index (THI). THI for beef cattle indicates alert with values up to 75, danger from 79 to 83 and emergency over 84. These categories have been used in different regions of Argentina to evaluate the effect of climate on some productive responses in dairy cows. In this analysis it is proposed to characterize the time behavior of this index for the months of higher temperature of the year, December and January, in the Department of Pocito, province of San Juan, during years 2014 and 2015. THI was calculated according to the equation: THI = (1.8 T) + 32 – (0.55 – 0.55 HR/100) * (1.8 T – 26), with T being the hourly temperature and HR the percentage relative humidity both obtained from the meteorological station located in the EEA INTA San Juan, (31° 39´ S; 68° 35´ W and 615 m a.s.l.). The average value of THI, 71 and 72 for December and 70 and 75 in January (for 2014 and 2015 respectively), showed apparently more than acceptable comfort. However, the hours registered for THI alert were 209 and 259 in January and 172 and 192 in December (for 2014 and 2015 respectively). For level of THI ≥75, January had the maximums with 113 hours (2014) and 148 (2015), while December registered 65 and 83 respectively. Regarding to THI danger levels, January as well as December had 6 hours in 2014 and 1 hours in 2015. THI emergency levels, happened from 8 am to 10 pm (with peaks at 2 pm and 5 pm) in December and from 8 am to 11 pm,(with peaks at 5 pm) in January. Livestock were exposed to alert levels, between 32% and 55% of the hours of January and December in 94% and 79% of the days, respectively. A strong dependence of the THI of the temperature was detected and it was also observed that only the first hours of the day have no stress situation (THI > 75).

A33
TEMPERATURE AND HUMIDITY INDEX (THI) ESTIMATION FOR BEEF CATTLE WITH TEMPERATURE AND HUMIDITY VALUES RECORDED AT 4:00 PM

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This work was due to know the optimal environment (climatic comfort and animal welfare) where the cattle production would be developed to get the optimal livestock productivity. The meteorological data are usually restricted to series that only have maximum and minimum values of both relative humidity and temperature in daily time scale, making impossible the hourly parameters calculation. For this purpose, the bioclimatic indicator, temperature and humidity index (THI), was used to monitor whether environmental conditions generate heat stress in livestock. So, it was obtained the THI using the highest temperature and humidity data of the day (it occurs at 4:00 pm (THI4), to establish if it is representative of a daily average. For calculations, the data were obtained from the meteorological station located in the EEA INTA Pocito San Juan, whose coordinates are 31° 39´ 14´´ S and 68° 35´ 15´´ W and 615 m a. s. l. The data used correspond to the warmest months, December and January of the years 2014 and 2015. THI was calculated according to the equation: THI = (1.8 T) + 32 – (0.55 – 0.55 HR/100) * (1.8 T – 26), with T being the temperature of 4:00 pm and HR the relative humidity percentage corresponds to the same time. The hourly THI values were calculated and the daily average obtained (THI24); THI4 was validated by correlation coefficient. The ITH4 obtained were corrected by adding the mean difference between THI4 and THI24. The correlation coefficient obtained between the THI daily calculated with the hourly records, and the corresponding THI4, showed higher than 91% for both months and years analyzed. The adjusting by the algebraic add of the difference THI24-THI4, improved the estimation mainly for December and in less accuracy for January. It is concluded that a good estimation of the THI daily for the months of December and January could be obtained using the temperature and humidity at 4:00 pm.

A34
CIRCADIAN VARIATIONS IN THE EXPRESSION OF ESTROGEN RECEPTOR α IN RETINA OF VISCACHA

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In some species the presence of estrogen receptors α (ERα) has been demonstrated in retina. ERα are involved in neuroprotective functions through their ability to regulate apoptosis, autophagy and antioxidant processes. Morphological variations in the retinal layers of viscacha have been demonstrated in response to changes in ambiental lighting. The objective of our work was to study the expression of ERα in retina according to the morphological variations described previously. Retinas of adult male viscachas (*Lagostomus maximus maximus*) were obtained at 08:00, 16:00, and 24:00 h (n = 4 for each group). They were processed for light microscopy and histological sections were made for the immunohistochemistry with the primary antibody anti-ERα (MC20): sc-542. The visualization of reaction was performed using an amplified biotin-streptavidin system (DAB chromogen). The immunoreactive (-ir) cells were quantified by image analysis and the number cell was expressed by microscopic field (cell/field). The data was statistically analyzed. In retinas from viscachas sacrificed at 08:00 h, intense nuclear immunostaining was observed in cells of the inner nuclear layer (INL) and in retinal ganglion cells (RGC). At 16:00 h, the ERαwere not expressed in the retinal layers. However, in samples obtained at 24:00 h was observed nuclear labeling less intense in the INL and RGC. The morphometrical study revealed that the number ERα-ir cells in the INL was significantly increased at 08:00 h (15.52 ± 2.90 cell/field) compared to the corresponding values at 24:00 h (4.45 ± 0.92 cell/field, $p < 0.05$). In addition, the number of RGC that express ERα were higher at 8:00 h (0.96 ± 0.25 cell/field) compared to those obtained at 24:00 h (0.20 ± 0.05 cell/field, $p < 0.05$). These results show that the expression of ERα is detected at 24:00 h when the photoreceptor layer (PL) thickness was maximal. The expression of ERα reaches a maximum at 8:00 h when the thickness of the PL began to decrease and they are absent when the thickness of the PL was minimal. This suggests that ERα expressed in INL and RGC might be involved in processes that regulate autophagy and the daily photoreceptor renewal cycle through the specific connections established into the retinal layers. However, future studies will benecessary to elucidate the role of ERα in viscacha retina.

A35

CHARACTERIZATION AND EVALUATION OF *IN VITRO* ACTIVITY OF ESSENTIAL OIL *Cryptocarya alba* AND *Laurelia sempervirens* AGAINST *Staphylococcus intermedium*

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Essential oil (EO), represent an attractive therapeutic potential in a significate number of pathologies. In veterinary medicine, the staphylococci most frequently isolated from the skin and external ear of dogs is Staphylococcus intermedius (S. intermedius). This is the most causal agent for pyoderma, a bacterial skin condition that usually results from a primary underlying skin disorder, such as ectoparasitism, hormonal imbalances, or immune-mediated dermatitis. The present study had as objective to characterize and to assess the biological activity of bioactive compounds against S intermedium and cytotoxicity in a primary culture of fibroblasts. The EO from Cryptocarya alba (C. alba EO) and Laurelia sempervirens (L sempervirens EO) was obtained using the technique of stripping steam in a Clevenger team, and was subsequently analyzed by GC-MS. The antibacterial activity of EO against 7 clinical isolates strain of S. intermedius was assayed by disc diffusion and microdilution methods. The cytotoxicity in fibroblasts was evaluated by using the MTT (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide) assay. Antimicrobial screening showed that the antibacterial activity of C. alba EO was higher than current reference antibiotics. The anti- S. intermedius effect of C. alba EO (MIC = 8µg/mL) was higher than L. sempervirens EO (MIC = 64µg/mL). We propose that the antimicrobial activity could be due to the synergism between its different components of EO, since it was observed that the C. alba EO has an activity higher than single components. The cytotoxicity was moderate in both C. alba EO and L sempervirens EO (IC50: 665,2µg/mL and CI50: 592,8 µg/mL respectively). Therefore, C. alba EO can be applied as an alternative agent for treatment of pyoderma infections, but more studies would be required to better clarify its mechanism of action on S. intermedium.

A36

EFFECT OF HEAT STRESS AND ITS MITIGATION BY CAPSAICIN IN HOUSE SPARROWS

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Heat stress due to climate change affects the health of organisms. This stressful event predisposes to physiological disorders at different levels, modifying hematological parameters, as well as the activity of digestive functions or the expression of some proteins (e.g. heat shock proteins). Due to this negative effect of heat stress on the animal health, nutritional research has begun to use additives in the diet to mitigate its effect. On the other hand, these additives, such as capsaicine, are used as growth promoters in poultry.The aim of this work was to evaluate the effect of heat stress on the house sparrow and its mitigation by capsaicin (an active component of chili peppers but not irritant in birds) and its effect on digestive enzymes. To achieve our objective, 26 birds were kept in separate cages, with a light/dark photoperiod 12/12hs. Birds were assigned to 4 independent treatments for 3 days: at 22°C without capsaicin (control), 22°C with capsaicin, 32°C without capsaicin and 32°C with capsaicin (control). Capsaicin was administered by gavage every day after the lights were on, at a concentration of 31.25×10^{-3} mg/gr of body $mass^{-1}$;with water and food *ad libitium*. Body weight measure, blood extraction and subsequent removal of the intestine were performed at the same time (8:00 am) to avoid disturb by circadian variation. We analyzed the data using a one-way and RM-ANOVA with Tukey post-hoc test ($p<0.05$). We observed an increased Heterophil/Lymphocyte ratio (stress index, $P<0.05$) and the levels of uric acid in plasma during heat stress ($P<0.05$). These situations were reverted to their normal values when capsaicin was administered ($P<0.05$). On the other hand, no effects were observed in hematocrit ($P>0.52$), triglycerides in plasma ($P>0.56$) and body mass at the time of exposure ($P>0.75$). Despite heat stress producing a decrease in the intestinal mass ($P<0.05$), no changes were observed in theactivity of digestive enzymes: maltase, sucrase and aminopeptidase ($P>0.1$). Regarding the effect of stress on the digestive physiology, no modifications were detected in the enzymatic activity due to heat stress or the administration of capsaicin ($P>0.1$). Studies in animals agree that a variation in 10°C produces stress and uric acid increase, which act as an antioxidant, on organisms under stress, but we found a lack of effect in enzyme activity. Capsaicin proved to be a stress-relieving compound but we are missing an effect on intestinal enzyme activity in contrast with studies in poultry and mammals. Supported by CyT-UNSL PROICO 2-0516 and FONCYT PICT-201–0595.

A37

DIFFERENCES IN THE HIND LIMB MUSCULATURE BETWEEN THE BLACK-CHESTED BUZZARD EAGLE (*Geranoaetus melanoleucus*) AND THE DOMESTIC CHICKEN (*Gallus gallus domesticus*) IN RELATION WITH THEIR LIFESTYLES

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Different lifestyles in birds might be reflected in differences in the muscular morphology, especially in the limbs. The goal of this work wasthe anatomical study of the muscles of the hind limb of two species of birds to evaluate morphological differences between them. One species is raptorial with prevalent aerial locomotion (*Geranoaetus melanoleucus*) and the other with a predominantly terrestrial locomotion (*Gallus gallus domesticus*). For this purpose, it was dissected the hind limb of two adult specimens of the mentioned species. Among the observed differences stand out the absence of the *m. flexor crurislateralis* in *G. melanoleucus*, as also occurs in other accipitrid birds. By contrast, this is a well-developed muscle in basal Neornites, which are characterized by a predominant or exclusively terrestrial locomotion. This muscle is an important extensor of the hip during the contact phase of the hind limb with the ground, and also prevents the hyper-extension of the knee articulation, so it has a significant functional role in birds with a terrestrial locomotion. Therefore, the loss of this muscle in *G. melanoleucus*is linked with the mainly aerial habit of this species. Moreover, it was observed a greater development of the muscles of the tarsometatarsus in *G. melanoleucus*, which is related with the predatory lifestyle of this bird. The function of these muscles is the flexion of the pedal digits, especially the talons, and thus generating the gripping force necessary to hold the prey. Additionally, it was observed the fusion of the tendons of the *m. gastrocnemius* and the *m. digitorum longus* in *G. melanoleucus*, whereas in *G. gallus domesticus* these two muscles have different insertion points. Thus, this study shows how the muscular anatomy is tightly related to the lifestyle. Furthermore, it was possible to observe, regarding the phylogeny of birds, the derived and specialized hind limb musculature of a raptorial bird and to compare it with the muscles of a more basal bird, i.e. the domestic chicken.

A38

VARIATIONS OF THE PROLIFERATING CELL NUCLEAR ANTIGEN IN THE TESTIS 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 testis by immunohistochemistry, relating the results with the animal sexual maturity. The animals were captured in their habitat near San Luis city between 2012 and 2017 and were classified into adult (5–7 kg; n = 5), pre-pubertal (3–4 kg; n = 5) and immature (1-2 kg; n=5), according to their corporal weight and the light microscopy observations of testes. The blood samples were obtained from anesthetized animals. The testicular 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 ng/dl) than in prepubertal (139.74±30.77 ng/dl) and immature (< 20 ng/dl) animals. The immunostaining was observed only in germinal cells while the Sertoli cells were negative for PCNA-immunodetermination. The percentage of PCNA-positive cells in the seminiferous tubules was significantly higher in adults (29.75±0.68) than in prepubertal(7.36±0.47) and immature (7.27±0.51) animals. The immunostaining was moderate in the germinal and interstitial cells of the studied groups. Our results suggest that the cellular proliferation might be strongly in germinal cells related to the serum testosterone levels while the Sertoli cells did not show proliferative activity after birth. These results could be important to ensure the maintenance of the testicular structure and function during the animal reproductive life.

A39

PREGNANCY RATE VARIATION IN AN ABERDEEN ANGUS COW CALF RODEO FROM ÑACUÑAN, MENDOZA AND ITS RELATION WITH EFFECTIVE RAINFALL

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Eastern Mendoza plain gathers 9 million hectares where cow-calf operations take place. This study aims to demonstrate the relationship between pregnancy and effective rainfall in a cow-calf operation. Pregnancy rate diagnoses were performed for two years, on winter 2017 and 2018, on an Aberdeen Angus (n= 265) cow-calf system from Ñacuñan, Santa Rosa, Mendoza province (-34.131357;-67.998952). Four pregnancy groups were made according to pregnancy status: head, body, tail and not pregnant.

Every group corresponds with a calving date previous to natural breeding season 2017-18. Overall pregnancy rate 2017 was 91 %, and 2018 was 75.8% (over total cows exposed to breeding). Both breeding seasons had the same bulls' proportion (5%). The 2018 pregnancy rate by groups was: head 76.3%; body 84.8%; tail 68.0%. Minor pregnancy rate found between head and body groups may be associated with phase shift among nutritional requirements of cows with forage offer. Body and tail groups increase adjusting to forage offer available. Head group calved early in spring, framing its last pregnant third and calving season on limited forage availability and quality. The calving-breeding season extends from October to April in both years. Rainfall during calving-breeding seasons in 2016-17 was 326 mm and in 2017-18 was 264 mm. Effective rainfall is the water that penetrates soil surface and is available for pasture growth. On arid rangelands, 20 mm is the minimal accumulated effective rainfall necessary to produce pasture growth. One hundred years rainfall record is available for Ñacuñan. 20 mm accumulated effective rainfall happens on December in seven out of ten years. The present study shows that pregnancy distribution depends on effective rain, resulting from that in traditional breeding seasons (December- March) cows getting pregnant in head group end up calving in October or November wherein seven out of ten years will found limited forage offer, resulting in body lost and lower pregnancy probability.

A40
DEVELOPMENT OF A DEVICE TO RECORD AND CLASSIFY BEHAVIOUR ON GRAZING GOATS

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Behavioral studies of grazing animals allow a better understanding of how animals use the resources of grasslands. They also help to develop herd management tools to improve the sustainability and productivity of goat production systems. However, behavioral assessment is difficult and, until recently, animal activity was only quantifiable through direct observation or video monitoring,a very laborious and time consuming method. Recent advances in sensor technology allow predicting behavior from information recorded on devices carried by the own animals. In this work, the objective was to build a device to record and classify the behavior of goats grazing in a desert rangeland. A Pixhawk autopilot was used as a platform to store the measurements of different sensors: 3 accelerometers, 3 gyroscopes and a GPS receiver. GPS and Pixhawk were powered by a 4000 mAh battery. All sensors, Pixhawk, GPS and battery were stored in a plastic box custom built using 3D printing techniques. This box was fixed to the head of a goat with a camera to record the behavior. Then the goat grazed freely in a natural pasture of the Monte desert (Mendoza, Argentina). After grazing, the device was recovered and the information processed. An activity predictor was built using the recorded data. The video record was used to classify the behavior in four states: resting in the pen (RP), resting in the field (RF), walking (W) and grazing (G). Time-series corresponding to each sensor was splitted in a one-minute time window and labeled according to the four types of activities. A procedure known as bag-of-features was then applied to extract the predictor variables used by the model. The bag-of-features is a method that consists of applying a clustering algorithm on the vector composed of the sensors measures and then building a histogram of the number of vectors belonging to each cluster. This is used to train a statistical learning model known as Random Forest (RFO). The evaluation of the RFO performance was conducted on a dataset of 777 one-minute time windows. The 70% of the original dataset was used for building and fine tuning the model. Then, the model was evaluated on the remaining 30% of the dataset. The total time of data collection was 12 hours and 57 minutes, from which goat spent 15.5, 18.3, 34.3, and 32.0 % in the activities of W, RP, RF and G, respectively. The precision achieved with the device to correctly detect and classify the activities was 96.1, 73.0, 89.0, and 75.9 % for W, RP, RF and G, respectively. These results show that it is possible to register and classify, with an acceptable precision, the behavior of each goat grazing in the Monte desert.

A41
MAINTENANCE ENERGY REQUIREMENTS OF CRIOLLO AND SAANEN GOATS.

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In Argentina, goat production is carried out mainly under extensive conditions in natural grasslands. Criollo goats are the most common breed and the main products obtained are suckling kids for human consumption. The Criollo goats have adapted for centuries to the rigorous agro-ecological conditions of arid zones. For several years, indiscriminate crosses have been practiced with exotic breeds, such as Saanen of high milk production, with the aim of increase the milk productivity of Criollo breed. However, these exotic breeds are usually unable to adapt and demonstrate their genetic potential in arid and semi-arid areas. This

could be due, among other factors, to the fact that high production breeds have very high nutritional requirements, as energy needs. Therefore, it is necessary to evaluate different physiological characteristics of exotic breeds and their crosses before their diffusion in traditional Argentinean goat production systems. The objective of this research was to determine the maintenance energy requirements in Criollo and Saanen goats under stabling conditions. Five goats of each breed were individually housed and fed with alfalfa pellets for 8 weeks. To determine energy requirements, adjustments were made in the food supply until achieving dry matter consumption (DMI_m) that maintains constant bodyweight (BW is bodyweight and $BW^{0,75}$ is metabolizable bodyweight). Animals were weighed twice a week and adjustments in the offer were made according to the changes in BW in the immediate previous period. The maintenance energy expenditure (ME_m) was estimated with the DMI_m value and the metabolizable energy (ME) concentration of the ration. The nutritional composition on dry matter (DM) basis of alfalfa pellet was: 16.7 % Crude protein; 36.8 % Neutral detergent fiber, 31.8 % Acid detergent fiber, 9.3 % ash; and 2.1 Mcal kg DM^{-1} of ME. The BW of Saanen goats (49.1 ± 1.2 kg) was higher ($p < 0.05$) than that of Criollo goats (43.4 ± 0.9 kg). No differences were found ($p > 0.05$) in the DMI_m between Criollo (53.9 ± 3.6 g kg $BW^{0.75}$ d^{-1}) and Saanen (45.4 ± 2.8 g kg $BW^{0.75}$ d^{-1}) goats. The ME_m requirements for Criollo goats were 106.7 kcal ME kg $BW^{0.75}$ day^{-1}; while for Saanen goats were 97.0 kcal ME kg $BW^{0.75}$ day^{-1}. No differences were found in maintenance energy requirements between Criollo and Saanen breeds. This could be due to the fact that diet energy availability was not limiting, as in low energy diets, where goats could reduce their metabolic rate and energy requirements differentially between breeds. However, this should be checked under other test conditions, mainly with diets with lower energy availability.

A42
DESCRIPTION OF PRODUCTIVE PARAMETERS IN A FEED-LOT SYSTEM

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A feedlot of cattle for meat is a confined area with adequate comforts for a complete feeding for productive purposes. This definition does not include temporary enclosures to wean calves, enclosures due to sanitary or climatic emergencies, or other transitory enclosures. The facilities for storing, processing and distributing food are considered part of the feedlot structure. Due to the nature of the feeding, afeedlot allows a greater efficiency of conversion (ECMS), daily increase of live weight (ADPV), better degree of animal termination (greater weight of the carcass and better performance), provides greater tenderness than the traditional fattening in the field. The objective of this work was to describe the variables of meat production in a feedlot system in San Luis. 221 closure forms were taken from the Ser Beef SRL livestock establishment in 2016. According to the main nutritional contribution they make, the food supplied were: highly degradable energy (cracked corn and grain of barley: 43.1% of the diet on a wet basis); protein (wet gluten-feed, soybean pellets, wet distillers grains, 32.1% of the diet on a wet basis); fibrous (corn silages and alfalfa roll, 19.6% of the diet on a wet basis); and salts and vitamins (5.2% of the diet on a wet basis). The bovines were classified into 5 categories according to their live weight (100-150, 150-200, 200-250, 250-300, 300-500 kg). The variables that were taken into account were: daily increase in live weight (ADPV: kg / day), dry matter intake (CDMS: kg MS / animal) and green (CDMV: kg MV / animal), conversion of food to produce meat on dry (ECMS: kg DM consumed / kg meat) and green matter basis (ECMV: kg MV consumed / kg meat), relative intake capacity (C% PV: live weight percentage). The data were analyzed using the statistical software InfoStat / P version 2018. The descriptive statistics yielded the following results of mean and standard deviation: ADPV from 1040 ± 0.2 kg (100-150) to 1.2 ± 0.1 kg (250-300); CDMS: from 7.0 ± 1.3kg (100-150) to 12.6 ± 1.5 kg (300-500); CDMV: from 11.0 ± 2.3 kg (100-150) to18.6 ± 1.6 kg (300-500), ECMS: from 6.8 ± 0.8 kg food.kgcarne-1 (100-150) to 11.7 ± 4.5 kg food.kgmeat^{-1} (300-500); ECMV: from 11.1 ± 1.9 kg food.kgmeat^{-1} (150-200) to 18.1 ± 6.6 kg alimen.kgcarne-1 (300-500); C% PV: from 3.2% ± 0.2 (100-150) to 2.9% ± 0.2 (300-500). Although this results don´t define the animal behavior according to categories, indicate a productive tendency of the variables under study.

A43
DESCRIPTION OF SEASONAL VARIATIONS OF DIFFERENT PRODUCTIVE PARAMETERS IN A FEEDLOT SYSTEM

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The objective of this work was to describe variables of meat production in a feedlot system according to their entry season. 221 closure forms were analyzed from the Ser Beef SRL livestock farm in San Luis, 2016. The animal entry seasons were defined as autumn (O: from March 1 to May 31), winter (I: from 1 June to August 31), spring (P: from September 1 to November 30) and summer (V: from December 1 to February 28). In each of these, the number of entry pens was 86, 46; 42; and 45 in O, I, P and V respectively. In the same seasonal order, the average weight (kg) of the animals was 214 ± 67, 222 ± 65, 203 ± 37 and 212 ± 37. At the end of the fattening, depending on the time of entry of the animals, the variables considered were: daily weight gain (ADPV: kg / day), daily dry matter intake (CDMS: kg MS / animal), conversion of feed into meat on a dry matter basis (ECMS: kg DM consumed / kg meat) and wet matter (ECMV: kg WM consumed / kg meat), relative consumption capacity (C% PV: consumption as a percentage of live weight). The variables were analyzed using the statistical software InfoStat / P version 2018. According to the data record, the descriptive statistics showed the following range of results of mean and standard deviation:

ADPV from 1.1 ± 0.2 kg (O) to 1.2 ± 0.2 kg (I) ; CDMS: 8.2 ± 2.0kg (O) at 9.1 ± 1.8 kg (I); ECMS: 7.9 ± 2.9 kg food.kgmeat^{-1} (O) at 7.5 ± 1.3 kg food.kgmeat^{-1} (V); ECMV: 12 ± 3.5 kg food.kg meat^{-1} (I) at 13.0 ± 2.1 kg food.kg meat^{-1} (P); C% PV: 2.9% ± 0.2 (O) to 3.2% ± 0.2 (P). It should be noted that the highest income occurs in O, mainly due to the fact that the producers dump a large part of their weaning calves to this production system. The average weight of income is similar for all seasons, although there is a great variability in the weight of income in O and I, because in addition to entering weaning calves, heavy categories enter for their termination.

A44
GUT HISTOMORPHOMETRY IN BROILERS FED STEVIA (*Stevia rebaudiana* BERTONI).

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In avian nutrition, the international regulations related to the use of Antibiotics Growth Promotor (APG) have banned their use of in-feed and they forced to broiler production to looking for natural additives alternatives to APG, asStevia (*Stevia rebaudiana*Bertoni*).* The objective of this research was to determinate the Stevia effects, added to water drinking (0.5-1 %) to broilers during the first fifteen days old, in productive parameters and intestinal health. The bioactive extract obtained from Stevia by alcohol-water/pressure (SE) was added to water drinking. Sixty broilers Cobb, males (a day of life) were divided into**Control** (**C)**, without additive, **Group 1(G1)**: 0.5 % SE; **Group 2(G2)**: 0.75 % SE y **Group 3(G3)**: 1 % SE. Avian was divided into four pens/treatment, with five broilers each one. It was determined the Weight Gain (WG)(g) into each group and the broilers were sacrificed at fifteen days old, gut extracted for histomorphometric study, stained with hematoxylin/eosin and analyzed with an optical microscope with the digital camera annexed to it. It was measured: Villi Hight (HV)(µ), Crypt Deep (CD)(µ) and VH/CD relation, using AxioVision Release program.The data were analyzed by ANOVA and posteriorly test (Infostat), p≤ 0.05 was considerate significative. WG media was similar between all the broilers.**G2** has registered hight HV (p≤ 0,05) than all the groups [HV(X±SD): **G2**:1088.82±21.44; **C**:958.08±26.11; **G1**:928.06±11.07 y **G3**:902.79±12.36]. CD were similar between **G2** and **G3**; both of them were high than **G1**and**C** (p≤ 0.05) (PC: **G2**:101.36±10.01; **G3**:102.43±6.9; **G1**:91.50±9.03; **C**:81.86±7,01). HV/CD ratio were: **G3**‹**G1**‹**G2**‹**C** (**G3**: 8.81; **G1**:10.72; **G2**: 10.74; **C**: 11.7). Also, it is noticed increased plasmatic cells (IgA producers) and goblet cells number and hight mucus layer in broilers receiving SE. In conclusion, SE (0.5-1 %) had better gut histomorphometric variables, increasing the intestinal health, mainly when SE is administered at 0.75 % in drinking water to broilers during the first fifteen days old.

A45
EXPRESSION OF GONADAL HORMONE RECEPTORS IN PARS DISTALIS AND PARS TUBERALIS OF VISCACHAS IN RELATION TO PREGNANCY

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In mammals, parsdistalis (PD) is the anterior pituitary region mainly involved in the regulation of metabolism, growth and reproduction. Pars tuberalis (PT) has long been thought to be a support for the PD,supplementing endocrine output during times of high demand.Recently it has been shown that PT plays a role in the regulation of reproductive mechanisms. Estrogen and androgen receptors (ERα, AR) are expressed in both pituitary regions and they are sensitive to the variation of gonadal steroids. The aim of this work was to studythe expression of ERα and AR in pituitary PD and PT of non-pregnant and pregnantviscachas, and to relate it to the serum levels of estrogens and androgens. Non-pregnant (NP) viscachas pituitaries of and pregnant from early pregnancy (EP), mid-pregnancy (MP) and late pregnancy (LP) (n=4 per group) were processed for light microscopy. ERα and AR were detected by immunohistochemistryand morphometrically quantified by image analysis. The labeled nuclei and cytoplasm were counted and expressed as a percentage of the total number of cells per microscopic field. The values were expressed as mean ± SEM. The serum levels of estradiol and testosterone were determined by radioimmunoassay. In PD, the immunostaining pattern for ERα and AR was nuclear (n) and cytoplasmic (c). The %ERαn-immunoreactive (-ir) cells in PD increased significantly in LP mainly in the medial and ventral regions. The %ERαc-ir cells did not differ significantly among different groups and were distributed throughout parenchyma. %ARn-ir cells and %ARc-ir cells did not vary significantly in relation to the reproductive status. ARn-ir cells were numerous in the medial region and caudal end. In PT, the immunostaining pattern for ERα and AR was only nuclear.The %ERαn-ir was not statistically significant variationsbetween the groups studied, but %ARn-ir cells increased during LP.The ERαn-ir cells were observed mainly in the upper end of the caudal region in NP and MP animals. The AR-ir cells were observed in all the groups studied, they were located in the caudal zone of PT. Estradiol and testosterone serum levels were higher during MP compared to other groups. These results demonstrated that in PD the increase of ER is after the estradiol serum peak, whereas the expression of AR does not vary with the variations of testosterone serum levels. However, in PT the expression of ER appears to be independent of estradiol levels and AR increases after the maximum levels of

serum testosterone.It is likely that these differences are related to the presence of specific cell populations and the function of both receptors in each adenohypophyseal portion.

A46
SEASONAL MORPHOLOGICAL CHANGES IN OVARIES OF MATURE AND IMMATURE VISCACHAS (*Lagostomus maximums maximus)*

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The timing of the reproductive cycle in female viscacha, a South American rodent of wildlife, is related to the testicular development of males, the photoperiod, concentrations of some hormones and the morphological and morphometric characteristics of the reproductive organs. The aim of the present work was to study the probable morphological variations of the ovarian follicles and luteal bodies of viscacha. Twenty mature and immature female viscachas were captured in the San Luis province, Argentina, during February –March(presumable activity period) and August (possible period of gonadal regression). Animals were anaesthetized and sacrificed then the ovaries were quickly removed and processed for optical microscopy. Cuts of 3-5 µm were stained with Hematoxylin-Eosin and then observed. Follicular structures and luteal bodies were cuantificated. The results showed significative variations in the number of different follicles and luteal bodies between the reproductive periods were analyzed. Primary and secondary follicles were increased in regressed and active immature females, respectively. Atretic follicles were present mainly in mature active animals. Luteal bodies showed a major number in mature regressed females. The ovary showed structural variations in the follicular development and the luteal bodyformation related to seasonal periods. These changes are coincident and appropriate with the reproductive changes experienced by malesalong the annual reproductive cycle. This allows us to postulate that both female and male show synchronization and regulation of their reproductive activities according to their habitat, in order to guarantee the reproduction of the species.

INTERNAL MEDICINE AND ODONTOLOGY

A47
GINGIVAL GRAINS INDUCED BY CALCIUM CHANNEL BLOCKS

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Gingival enlargement is a common side effect of the use of antihypertensive drugs such as dihydropyridines. There is a relationship between periodontal health, development and severity of gingival enlargement induced by nifedipine and amlodipine. Gingival enlargement causes the morphological alteration of the gums and affects nutrition and access to oral hygiene, which results in an increase in the susceptibility of oral infections, caries and periodontal diseases. Objective: to demonstrate the involvement of periodontal disease in the gingival enlargement induced by calcium channel blockers. Material and methods: A cross-sectional study was conducted with patients attending the FOUNLP between 2014 and 2017. The sample consisted of 10 hypertensive patients medicated with calcium blockers and a control sample of 10 patients who received another type of antihypertensive medication. All patients were asked to sign an informed consent form. A medical history, plaque index and periodontal measurement of the pouch were obtained. Results: The bacterial plaque index in the study sample was 7 (70%) severe, with bags larger than 4 mm and bleeding in the sounding and 3 (30%) moderate with bags smaller than 4 mm and without bleeding in the sounding. In the control sample it was 8 (80%), mild without bag and 2 (20%) moderate with bags of less than 4 mm, without bleeding. Four gingival enlargements were found in the study sample, and none in the control group. Conclusions: 40% of patients who used antihypertensive calcium channel blockers had gingival enlargement, which was statistically significant in comparison to the control group.

A48
PERIODONTAL AND CARDIOVASCULAR PATIENTS: ANALYTICAL METHOD FOR MEASUREMENT THE REACTIVE PROTEIN C

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The initial inflammatory process of periodontal disease is characterized by an increase in the levels of C-reactive protein and other inflammatory markers, which are also found in cardiovascular diseases, suggesting an association between periodontitis and episodes where cardiovascular diseases are aggravated. The objectives of this project are: 1 – To study the degradation photochemistry of C-reactive protein. 2- To select a degradation product suitable for follow-up. 3- To determine the values of C-reactive protein in patients with periodontal chronic disease between moderate and advanced and cardiovascular disorders. 4- To assess whether C-reactive protein levels decrease after periodontal treatment to improve the systemic condition. The sample was selected in the first stage of the project. A history was recalled. Serial periapical bacterial plaque index and measurement of the depth of the periodontal characteristics were performed. Saliva was collected for testing the presence of reactive protein C. Plaque index was moderate at 77% and severe in 23%. The record of tooth mobility was grade 1 in 73% with bags of 4 mm and bleeding on probing and grade 2 in 27% with bags of 4 mm and bleeding on probing. In this second phase, the saliva samples, taken prior to periodontal treatment patients, were injected and analyzed with a modular system Agilent 1100 LC-MSD. The configuration was: binary pump, autosampler and detector, temperature-controlled column compartment of matrix of diodes and selective mass detector using API (electrospray) and APCI (atmospheric pressure chemical ionization). An Agilent RP-C18 column of amino acids at 25° C was used. The mobile phase was 1:1 methanol: water. The flow rate was 1 ml/min and the injection volume 5 ml. The MSD parameters were: interfaces API and APCI, positive and negative modes, 50-600 amu mass range (0.5 amu mass resolutions). Results: The sample was run and the products found in the first 10 samples studied levels of C-reactive protein from 1.0 to 3.9 mg/dL were analyzed. Conclusions: It is necessary to complete the sample and to correlate the results with the clinical parameters in order to achieve valid conclusions. Thus far, the findings allow to suggest that the values of C-reactive protein found are between moderate and high risk groups.

A49
CALORIC INTAKE OF SUGAR DRINKS CONSUMED BY CHILDREN OF 8 AND 9 YEARS OF AGE, OF THE NORMAL SCHOOL JUAN PASCUAL PRÍNGLES, OF THE CITY OF SAN LUIS.

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The consumption of beverages with a high sugar content and the excessive addition of sugar to infusions has been increased in the last decade by the school population, a situation that favors the increase in weight and the prevalence of obesity in childhood. Given this situation, we hypothesized that the students of the Normal School Juan Pascual Pringles exceed the limit established by the WHO as a healthy parameter, in relation to the caloric intake by simple sugars, at the expense of sugary drinks and added sugar to infusions, in detriment of water intake. This research was carried out with the purpose of determining the caloric contribution that implies the consumption of sugary drinks and the addition of sugar to infusions in schoolchildren of both sexes of 8 and 9 years old.The material used was a semi-structured interview, with open and closed questions, applied to a sample of 85 children of 8 and 9 years old during 5 consecutive days of the Juan Pascual Pringles School of the City of San Luis. The type of study was Descriptive- Exploratory- Transversal- Quantitative. The objectives of this study were to: 1) determine the type of sugary drinks and caloric intake provided by these in children of both sexes, 8 and 9 years of age who attend the normal school Juan Pascual Pringles. 2) Investigate what type of sugary drinks are preferred by children of 8 and 9 years. 3) Inquire the amount of sugary drinks and amount of sugar added to infusions. 4) Calculate the caloric intake of sugar-sweetened beverages and the addition of sugar to infusions ingested by schoolchildren under study. 5) Determine the prevalence in terms of adequate and inadequate caloric intake of the same.6) Know the consumption of water ingested daily in relation to the corresponding water requirements for this age group. The results obtained in this research show a high intake of sugary drinks that replaces water consumption and that the amount of sugar added to infusions is well above the values compatible with a healthy diet. When unifying the calories contributed by sweetened drinks and added sugar to infusions, it was observed that 64.58% of the children of 8 years and 70.27% of the children of 9 years presented an inadequate caloric intake. The average caloric intake was 744.68 kcal / day and 687.2 Kcal / day for children of 8 years and 9 years respectively. As it was stated in the hypothesis, it was widely demonstrated that there is a statistically significant difference (student test $p < 0.05$) between the population studied and what is healthy acceptable established by the WHO (155 Kcal / day for children of 8 years and 185 Kcal / day for 9 year olds).This allows us to conclude that the students of the Normal School Juan Pascual Pringles exceed the caloric intake by simple sugars, which do not provide nutrients for their growth and development, at the expense of sugary drinks and added sugar to infusions to the detriment of water intake. An increase in weight and obesity has been observed in school-age children, which is accompanied by a high aggregate of sugars in the infusions and consumption of sugar-sweetened beverages.

A50

NUTRITIONAL PATTERN AND PREVALENCE OF INSULIN RESISTANCE IN A SAMPLE OF PATIENTS OF THE CITY OF SAN LUIS CAPITAL, SAN LUIS, ARGENTINA

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The nutritional pattern is linked to changes in the prevalence of overweight (OW) and obesity (OB), which are risk factors for insulin resistance (IR). However, this link varies from population to population. There are no studies on the population of the Capital City of San Luis, Argentina, on the possible link between nutritional pattern, nutritional status and IR. Our central hypothesis is that changes in nutritional habits determines changes in the prevalence of OW / OB and therefore changes in the incidence of IR in this population. To test this hypothesis we conducted a quantitative, observational, transversal and descriptive study in a sample of 78 patients including men (n=40) and women (n=38) between 18 and 80 years living in the central neighborhoods of the city of San Luis between 2015 and 2017. Of these patients nutritional data were obtained through a standardized food-frequency questionnaire, anthropometric measurements (weight, height and waist circumference) and a blood sample for biochemical analysis. These data allowed us to relate the nutritional pattern, nutritional status and IR. The total population was composed of 22% OW and 9% Type 1-OB according their body mass index (Kg / m^2). Analysis of the nutritional pattern showed that the studied population consumes: 14% of the group of grains and legumes, 18% fruits and vegetables, 8% meat and eggs, 7% milk, 1% oil seeds and nuts and 17% sweets and fats. Water consumption was 30% of the average consumption. Waist circumference (cm)—a marker of central obesity was greater in 37% and 17% of women and men, respectively, thus those have an increased metabolic risk. Of all patients surveyed baseline glycemia (mg / dL) was normal in 90%, while 6% was hypoglycemic. 4% of the total population showed cholesterol concentration at the upper limit (*i.e.*, close to 200 mg/dL). The HDL-c was reduced (<30 mg / dL) in 24% and 55% of women and men, respectively. However, the total cholesterol (mg / dL) / HDL-C (mg / dL) ratio—an atherogenic-risk marker was higher in women (8%) than men (5%). 11% of total patients showed elevated (200-499 mg / dL) plasma triglycerides (TG), while 5% showed high TG (≥500mg / dL). The TG (mg / dL) / HDL-c (mg / dL) ratio—an indirect indicator of IR was elevated in 23% of the total population. In this group the CRP—a marker of systemic inflammation was positive in 11% of patients. The data obtained in this sample indicate a close relationship between the consumption of sweets and fats, the prevalence of OW/OB, central OB, dyslipidemia, atherogenic risk, systemic inflammation and IR. This study was supported by PROICO 023418/100218.

A51

RENIN-ANGIOTENSIN SYSTEM POLYMORPHISMS ASSOCIATION WITH OBESITY AND OTHER RISK FACTORS IN HYPERTENSIVE PATIENTS

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Hypertension (HTA) is the clinical expression of genetic, physiological, and biochemical interaction tomaintain cardiovascular homeostasis. The renin-angiotensin system (RAS) has an important role in the regulation of blood pressure. RAS polymorphisms have been studied as candidate risk factors for hypertension. The study of polymorphism is very controversial, and its identification in HTA patients is required. The aim was to investigate a possible involvement of angiotensin converting enzyme insertion/deletion (ACE I/D) and angiotensin II receptor 1 (AT1) A1166C polymorphisms association with obesity and some clinical variables related to HTA. A total of 230 hypertensive patients (48% women) and 167 controls subjects (68% women) from Juana Koslay Hospital (2014-2015) were recruited. Mean age (years): 54.2 ± 9.3 HTA and 39.2 ± 13.7 control. Blood pressure and body measurements were recorded and a blood sample was obtained for glucose, lipid profile and uric acid determination, and also polymorphism identification. Subjects were grouped based on hypertension and excess body weight (overweight and obesity) based to WHO classification determining by body mass index (BMI). ACE I/D polymorphisms were identified by polymerase chain reaction (PCR) and AT1 A1166C was performed using PCR-RFLP (restriction fragment length polymorphism) methods. The BMI>25.0 kg/m2 in hypertensive patients represented 87.4% (52.7% men, 47.3% women) and normotensive 63.9% (37.2% men, 62.8% women). The allele and genotype frequency of A1166C polymorphism showed no significant difference between HTA and controls. The genotypes CC in studied population (BMI>25.0 kg/m2) was different statistically significant compared to AA ($p<0.004$) and AC ($p<0.003$). The CC vs. AA, AC genotype differed significantly in overweight and obesity hypertensive ($p<0.05$) and normotensive ($p<0.009$), whereas no association with ACE gene polymorphisms was obtained. Significant differences in the alleleand genotype frequency of I/D polymorphism were observed between HTA and controls ($p<0.01$, $p<0.008$, respectively).The DD genotypeshownsignificant associationin normotensive females, which suggest its protective role in studied population (OR=2.02, 95%CI=1.12-3.64, $p=0.01$).A positive correlation between overweight and obesity hypertensivevs.systolic ($r=0.23$, $p=0.02$) and diastolic blood pressure ($r=0.25$,$p=0.01$) were found. The correlation between BMI>25 kg/m2and biochemical parametersin normotensive patients: glycaemia ($r=0.28$, $p=0.005$), HDL ($r=-0.21$,$p=0.003$), uric acid($r=0.28$,$p=0.02$) was obtained.The RAS polymorphism is associated with the pathogenesis of HTA in patients with overweight and obesity.The genotyping for the variants of RAS gene could in the future become an important part of the clinical process of risk identification for HTA.

A52
EPIDEMIOLOGIC PROFILE OF CONGENITAL SYPHILIS DIAGNOSED AND NOTIFIED AT A MATERNIDAD PROVINCIAL "DRA TERESITA BAIGORRIA", SAN LUIS

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Congenital syphilis (CS) remains a major public health problem worldwide. Vertical transmission of syphilis (S) can occur during pregnancy, childbirth, postpartum and breastfeeding. In Argentina, the National System of Health Surveillance (SNVS) has observed an increase in reports of congenital infections. The aim of our work was establishing the epidemiologic profile of CS in pregnant women (PW); being necessary to identify factors influencing S prevalence; and to recognize the causes involved in inadequate S prenatal consultations (PC) and/or those that are often not treated properly or even do not receive any S treatment (T). A descriptive and retrospective study was carried out from January to September 2018, in MPDTB. Serum/ Perinatal Information System (PIS) and serum/cerebrospinal fluid of PW and newborn respectively were analyzed. A total of 11 cases were studied by VDRL (Venereal Disease Research Laboratory test), TPPA (Treponema pallidum particle agglutination assay) and socioeconomic factors, risk behaviors and maternal factors were analyzed through the PIS. Of cases analyzed with CS, 4 (36%) PW came to give birth without starting T and 7 (64%) received incomplete T. The average age of the patients (P) was 22 years old, with 10 (90%) of them being attended by public entities. Only 1 (9%) P finished high school, while 3 (27%) and 5 (45%) had an incomplete high school and primary education respectively, while 2 (19%) cases have no data. Respect for PC, 5 (46%), made less than 5 PC. 5 (46%) P reported having stable couple, 4 (36%) being single, 1 (9%) other marital status and 1 (9%) without data. Only 1 (9%) case was stillborn and 5 (50%) were premature. In turn, 5 (50%) cases of neurosyphilis (N) were reported. On the other hand, only 6 (55%) sexual partners (SP) of the PW were tested and 3 (50%) of them did not have S. From the analyzed cases, it can be concluded that the P are young, in reproductive age, with a low education level, and most of them received the T inadequately; most of them had an acceptable number of PC, however, S was diagnosed close to the date of childbirth, resulting in a high percentage of newborn premature or N and the no compliance with the guide recommended by the Ministry of Health. Although these findings are not unexpected, they show associations found indicate the need for the reorganization of actions, prioritizing PW with the risk characteristics identified.

A53
PREVALENCE OF CARDIOVASCULAR RISK FACTORS IN RURAL POPULATION OF THE JUAN M. DE PUERREDÓN DEPARTMENT OF THE PROVINCE OF SAN LUIS

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Chronic no communicable diseases (NCDs) are mainly represented by cardiovascular diseases (CD), diabetes (DBT), cancer and chronic respiratory diseases (CRDs). These pathologies are associated with a series of common risk factors, among which the most important are: inadequate nutrition, physical inactivity, tobacco consumption and alcohol consumption. Worldwide, it is estimated that 6 out of 10 deaths and 70% of adjusted life years due to disability (DALY) are due to NCDs. Also, it is projected that by 2030 more than three quarters of deaths will be for this cause. Morbidity and mortality due to NCDs has increased in low and middle income countries, estimating that 80% of deaths and almost half of the burden of disease is a consequence of this group of pathologies.There is no information about what happens with the rural population, with different lifestyles and the possibility of access to health. Our objective was to know the frequency of cardiovascular risk factors in a population of 18 years of age or older in a rural area of the Juan Martín de Pueyrredón department of the province of San Luis.A cross-sectional descriptive, observational study was conducted in the rural localities of Zanjitas, Cazador, Alto Pelado and Beazley.. The number of inhabitants according to the 2010 National Population and Housing Census is 1377 inhabitants, of which 808 are over 17 years old. Results (%) The population interviewed (N = 375) represented 46.41 of the registered population according to the census source. The distribution according to sex, male (M) 36.00, female (F) 64.00; according to age range, between 18-24 years M: 9.63, F: 24.58, between 25 and 34: M: 18.52, F: 20.00, between 35 and 49 M: 25.19, F: 27.50, between 50 and 64 M: 25.19, F: 12.08, from 65 to more years M: 21.48, F: 15.83.Perform physical activity on 50.40. Add salt in the preparation of the food 95.46. Add salt in cooked food 27.46. Prevalence of arterial hypertension (AH) 43.4. Smoking 24.00 Hypercholesterolemia 22.40. Cardiovascular history 13.33.History of cerebrovascular accident (CVA) 1.6. The prevalent sex was the feminine one. It is a population with a predominant age between 18 to 49 years. Only half of the studied population performs physical activity and a quarter is a smoker. The most prevalent risk factor is AH, with an important use of salt being observed mainly when cooking food. Almost a quarter of the population reported having high cholesterol values. The information obtained can be used to develop actions of promotion, protection and prevention of health in the rural population and in this way reduce the inequities that limit the opportunities to lead a life with better health conditions.

A54
PREVALENCE OF DIABETES MELLITUS TYPE 2 AND RISK FACTORS IN RURAL POPULATION OF THE JUAN M. DE PUERREDÓN DEPARTMENT OF THE PROVINCE OF SAN LUIS

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Diabetes Mellitus is within chronic noncommunicable diseases (CNCD), and is a worldwide public health problem. The World Health Organization (WHO) estimates that there are more than 170 million people with diabetes, a number that could double by 2030. It is the most frequent endocrine disease. In developed countries, the main cause of death in type 2 diabetics is acute myocardial infarction. In our country the National Survey of Risk Factors provides information on the prevalence of NCDs in populations of more than 5000 inhabitants, not knowing the reality of rural populations, of few inhabitants, with different social determinants and lifestyles. Our objective was to know the frequency of diabetes mellitus type 2 and risk factors in a population of 18 years old or more from a rural area of the Juan Martín de Pueyrredón department of the province of San Luis. A descriptive, cross-sectional observational study was carried out by means of a survey (N = 375) and weight and height determination (N = 250) in the rural population of the localities of Zanjitas, Cazador, Alto Pelado and Beazley. The number of inhabitants according to the 2010 National Population and Housing Census, is 1377, of which 808 are over 17 years old.The body mass index (BMI = Weight / Height^2) was determined, considering overweight BMI 25-29.9 kg / m^2 and obesity BMI ≥ 30 kg / m^2. Results (%): Population interviewed (N = 375). The distribution according to sex, male (M) 36.00, female (F) 64.00; according to age range, between 18-24 years M 9.63, F 24.58, between 25-34: M 18.52, F 20.00, between 35-49 M 25.19, F 27.50, between 50-64 M 25.19, F 12.08, from 65 to more years M 21.48, F 15.83. Levels of education of population: no schoolingM 5.19, F 6.72, elementary schoolingM 75.00, F 61.61middle or secundary schooling M 25.00, F 38.39. Health coverage M 69.47, F 73.31. Perform physical activity M 61.65, F 46.72. Low consumption of fruit M 68.63, F 70.00.Low consumption of vegetables M 64.29, F 61.50.Prevalence of diabetes M 25.86, F 20.90.Family history of diabetes M 41.80, F 52.07. Overweight (N = 250) M 34.94, F 28.74. Obesity (N = 250) M 38.55, F 46.10. The prevalent sex was the feminine one. It is a mostly young adult population. Physical activity is greater in males than females. Feeding is scarce in fruits and vegetables. The prevalence of diabetes is higher in men, while women have a greater family history. Overweight prevails in men and obesity in women. The magnitude of morbidity and mortality, loss of quality of life and the individual and social cost of diabetes make preventive actions essential even from an early age, tending to changes in lifestyles. On the other hand, it is essential to establish health plans aimed at monitoring risk factors and the effectiveness of preventive actions.

A55
ASSESSMENT OF THE REACTIVE STRIP TO DETERMINE SIGNIFICANT PROTEINURIA COMPARED TO THE PROTEINURIA / CREATININURIA RELATIONSHIP

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High blood pressure (HBP) is the most frequent complication in pregnancy and the main cause of morbidity - perinatal mortality. In Latin America perinatal morbidity from pre-eclampsia is 8-45% and mortality from 1-33%.Significant proteinuria is an important sign of pre-eclampsia, caused by glomerular damage which produces increased permeability of the basement membrane, with loss of proteins of low selectivity.Renal perfusion and glomerular filtration are diminished. A diagnosis of preeclampsia without significant proteinuria is questionable. The standard gold method for protein determination in urine is the measurement of proteinuria in a 24-hour urine sample. Other alternatives for measurement of proteinuria in pregnant hypertensive patients are considered, including the urine test strip method and the proteinuria / creatininuria index (P / C), in occasional samples with 3 hours of urination retention.
These methods have the advantage of being fast and inexpensive and offering results with good sensitivity. Significant proteinuria is considered when the test strip yields two crosses (++) of proteinuria and the P / C index is> 200 mg / g.We proposed to compare the results obtained by the use of the urine test strips to determine urinary proteins versus the P / C ratio, and to determine the diagnostic value of the test strip method by assessing sensitivity (S), specificity (E), positive predictive value (VPP) and negative predictive value (VPN).We studied 84 pregnant patients from their 20th week of gestation, hypertensive, with clinical preeclampsia and complete laboratory for hypertension. The samples processed were occasional urine at which the P / C index and proteinuria were determined by reactive strips. The determination of proteinuria for the P / C index was carried out by quantitative colorimetric method using pyrogallol-molybdate red as a reagent and reading at 600 nm.Creatininuria was determined by kinetic method. A Wiener CM250 autoanalyzer was used. The measurement of proteins in urine was performed visually by the test strip method (multitix).The interpretation of results is reported as negative (-) or positive by crosses, from traces, (+) <30 mg / dl, (++) 30 to 90 mg / dl, (+++) 100 to 299 mg / dl and> 2000 mg / dl (++++). According to the test strip method, we obtained 49 negative samples, where the P / C ratio was on average 96 mg / g, yielding 4 false negative test strips in this group.The sensitivity was 78.9%, specificity 88.2%, PPV 58.3% and NPV 91.8%. The data was analyzed through the GraphPad Prism 5 program. Therefore, the methodology of the test strips is a very good quick diagnostic option to evaluate the entry of a patient into a guard. The intensity of proteinuria must be evaluated by quantitative methods.

A56
EFFECT OF A FLAVORED WATER ON THE MORPHOLOGICAL AND CHEMICAL COMPOSITION OF MICROSTRUCTURE OF HUMAN DENTAL ENAMEL IN VITRO

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There is a remarkable interest in the dental area about the effect produced by the acidic agents contained in commercial non-alcoholic drinks on dental enamel due to their ability to produce caries or erosive lesions. The objective was to characterize the morphological and the chemical alterations of the adamantine structure exposed to the action of a flavored water. Human dental crowns were sectioned in the buccolingual direction and included in polymer. Flat and highly polished surfaces were obtained by wear with descending granulation sandpaper. Observations and chemical analysis in the radial enamel before and after exposure to the drink were carried out on the ESEM FEI QUANTA 200-EDS (SeMFi-LIMF. FI- UNLP). For the morphological description, the etching patterns of the enamel were considered. The chemical elements sodium, magnesium, chlorine, and the calcium/phosphorus ratio were studied. The samples were immersed in 100 ml of a flavored water for 12 minutes. At the ESEM the prisms presented different patterns of acid etching which may affect the core or the profile. The chemical composition showed variations according to the area, before and after the treatment. Although the studied elements were present in the healthy enamel, both in the radial and in the Hunter Schreger bands, the percentage values were different. In the bands sodium and magnesium increased while chlorine decreased. After the action of the flavored water, sodium and magnesium increased even more and the chlorine dropped markedly. A significant difference was found in Ca/P ratio before and after treatment. The beverage used contains acid agents in its composition that produce loss of minerals from the adamantine tissue. We conclude that the exposure of tooth enamel to flavored water produces demineralization compatible with erosion lesions.

A57
MECHANICAL CHARACTERIZATION OF THE ENAMEL MICROSTRUCTURE TREATED WITH NON ALCOHOLIC DRINKS

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In previous studies, it has been shown that the microstructure of prismatic dental enamel presents differences between the external and internal zone. Radial enamel is found in the outer third of enamel and has higher microhardness values than enamel with Hunter- Schreger Bands (HSB) that occupies the inner 2/3. Our aim was to evaluate the mechanical behavior of radial enamel and HSB due the action of a non-alcoholic beverage *in vitro*. Longitudinal sections of dental crowns were used, wich were included in polymer, worn and polished with sandpapers of decreasing granulation. The samples were immersed in a flavored natural water for 12 minutes. Nanohardness tests (Triboindenter Hysitron) were carried out on the radial and HSB enamel before and after the exposure to the drink. Hardness determinations "H", reduced modulus "Er" and contact depth "hc" were performed. The percentage of reduction of hardness was determined. The values found in healthy radial enamel were H: 5.48±0.23 GPa; Er: 86.97±8.11 GPa; hc: 149.73±4.25 nm and in HSB H: 4.24±0.43 GPa; Er: 75.24±7.09 GPa; hc: 176.36±11.29 nm. After exposure to beverage, it was found in the radial enamel H: 2.22±0.31 GPa; Er: 58.73±10.79 GPa; hc: 270.29±21.22 nm, and in HSB H: 1.54±0.42 GPa; Er: 48.11±6.54 GPa; hc: 350.10±63.33 nm. After the action of the drink the values of hardness of the radial enamel and HSB decreased and the trend observed in healthy enamel was maintained, where the highest values corresponded to the radial enamel. The percentage reduction of H in the radial enamel was 59.48% and in the HSB enamel it was 63.67%. The contact depth increased by about 50%.The decrease in hardness is related to the mineral loss produced by the acids contained in the drink. We conclude that the action of the non-alcoholic beverage produces a decrease in the mechanical properties in both the radial enamel and the HSB.The lower values in the reduced module Er indicate the formation of a superficial softened layer, the enamel withHSB being more vulnerable.

A58
RENAL HYPERFILTRATION AMONG ADULT POPULATION IN PRIMARY HEALTH CARE. INFLUENCE OF BODY MASS INDEX

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The prevalence of glomerular hyperfiltration (GH) and chronic kidney disease is increasing worldwide in parallel with obesity epidemic. In obesity, a compensatory hyperfiltration occurs to meet the heightened metabolic demands of the increased body

weight. Although not a unique cause of chronic kidney function loss, GH is thought to play an important role in the initiation of glomerular damage. We explored the association of body mass index (BMI) with GH in a sample of apparently healthy adult population that attends a medical consultation of a first level of care (A. Luchini Hospital, San Luis city).A total of 171 ambulatory patients aged 30-69 years were studiedfrom July 2017 to May 2018. BMI was calculated as weight (kg) divided by height squared (m2) and categorized in three groups according to WHO recommendations: lean (<25 kg/m^2), overweight (≥25-<30 kg/m^2) and obese (≥30 kg/m^2). A 24h urine collection was performed following standardized oral and written instructions. History of chronic kidney disease, diabetes, cardiovascular disease, diuretic or steroids treatment or pregnant women was not included in the study. All samples were collected after patient consent. Serum creatinine (Screat) and urinary creatinine (Ucreat) were measured by kinetic colorimetric Jaffe method (Wiener Lab, Argentina). Creatinine clearance (CrC) was computed from the creatinine concentration in 24h urine collection and a single measurement of serum creatinine [CrC (mL/min)=Ucreat (g/24h)/Screat (mg/L)*694]. CrC reported as absolute value and indexed to body surface area. GH was defined as a CrC ≥140 ml/min. 24h urine protein excretion was determinated by pyrogallol red colorimetric method.Proteinuria ≥0.15 g/day was considered positive. Median age in years among the groups was, for woman: lean 41.37 (CI: 33.21-49.53), overweight 55.00 (CI: 44.26-61.74), obese 54 (CI: 48.1-59.9) and for men: lean 52.63 (CI: 43.15-62.1), overweight 62 (58-66.7) and obese 59 (52.84-63.16). The overall prevalence of obesity and overweight was: for woman 58.43% (BMI: 37.49±5.46) and 20.22% (BMI: 26.67±1.15), and for men: 56.10% (BMI: 36.63±5.83) and 24.39% (BMI: 27.51±1.71), respectively. GH was present in 20.73% men and 20.22% woman. Among patients with GH, the proteinuria was observed in 64.7% men and 50% woman.The frequency of GH increased across BMI categories in men (6.25%, 15%, and 21.74% for lean, overweight, and obese participants, respectively; p<0.05) but not in woman (15.79%, 16.67% and 19.23% for lean, overweight, and obese patients, respectively; p=ns). SCr levels were normal without difference between all groups. Although, SCr levels tended to increase with BMI raise. GH is frequent in the studied population and is influenced by BMI, particularly in men. Because hyperfiltration may contribute to renal dysfunction, public health initiatives to enhance weight loss will improve renal hemodynamic avoid nephropathy onset or progression.

A59
VAGINAL INFECTIONS IN A POPULATION OF SEXUALLY ACTIVE ADOLESCENTS ATTENDED TO PRIMARY CARE LEVEL OF SAN LUIS CITY

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Vaginal infection is a high-prevalence gynecological affections and vaginal discharge is one of the most common reasons for which women seek medical attention. The origin of vaginal dysfunction (VD) may respond to systemic etiologic factors (mainly from the hormone-immune system), or external factors (sexual behavior, contraception practice used, vaginal showers, among others). In particular, during adolescence stage, reproductive hormones cause physiological and tissue changes which may increase susceptibility to infections and could facilitate the acquisition of sexually transmitted diseases. Balance of the vaginal content (BAVACO) is a very efficient laboratory study developed to allow diagnosis of the most frequent vaginal pathologies with a high predictive value. This study aimed to determine the frequency of vaginal infection in adolescents from lower income neighbourhoods who attending in a Public Primary Health Care Center "West Hospital Dr. Atilio Luchini" of San Luis city, Argentina. The study was conducted among 120 girls aged 13-19 years who consulted gynecology service during the year 2018. Not selected by signs or symptoms of vaginal infection were done. Sociodemographic characteristics, obstetric history and contraceptive and hygiene practices (vaginal bathing) were collected by face-to face interviews using a structured questionnaire. Samples of cervico-vaginal smears were analyzed by wet mount, Gram and Giemsa stains, according BAVACO methodology. Girls who received antimicrobials in the last month were excluded. The mean age of participants was (16 ±1.50) years. In 55 adolescents (45.9%), vaginal infections were diagnosed. The most frequent infections were bacterial vaginosis (21.4%) and vulvovaginal candidiasis (18%). We found 1% of intermediate microbiota cases, 5% of trichomoniasis and 19% of aerobic vaginitis. The others cases corresponded to normal microbiota (19.6%) or normal microbiota associated with vaginal inflammatory reaction (16%). Already 48% of patients with VD were asymptomatic of disease complaints. More frequent clinical manifestation was the leucorrea or gray or yellowish vaginal discharge. From the analysis of the surveys we observed than only 57% of respondents were using temporary methods of contraception. A strong association was observed between vaginal discharge and the usage of toilet (vaginal bathing). The results showed a high frequency of vaginal infections among adolescents in a disadvantaged social environment from San Luis city. The problem of vaginal discharge can be best understood not only in the biomedical context but also with the sociocultural perspective. The early onset of sexual intercourse (13.6 ±1.1) and the intercourse without protection could be related to presence of these vaginal infections.

A60
A SPECIFIC MLH1 GENE MUTATION IN FAMILIES FROM MENDOZA ASSOCIATED WITH LYNCH SYNDROME

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Lynch syndrome (LS) is the most common cause of hereditary colon cancer which predisposen to colorectal, endometrial, and other cancers. LS is caused by germline mutations in the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). The MMR system detects and corrects replication errors, maintaining the stability of the genome. Consequently defects in the MMR increase the mutation rate causes microsatellite instability (MSI) and increased cancer risk. The main objective of our study was to analyze clinical characteristics and diagnostic algorithms of two unrelated families from Mendoza, Argentina, carrying a specific mutation in *MLH1* gene. The clinical importance of these mutations and their significance in the general population were also examined. After carrying out the genealogical study, MMR proteins MLH1, MSH2, MSH6 and PMS2 were evaluated in paraffin-embedded tissue sections from colorectal tumors using Ventana Benchmark automated immunostaining. MSI analysis was performed using STRs markers (NR-21, NR-24, BAT-25, BAT-26 and Mono-27) and Illumina next-generation sequencing (NGS). Family characteristics and evidences are presented below. Family A: a male patient, 36 years old, with right-sided colon cancer and MLH1/PMS2 proteins absent by immunohistochemistry (IHC). Two sisters with colorectal cancer before 40 years of age. The father and the paternal grandmother died from colon cancer. A pathogenic mutation was localized in *MLH1* c.1890dupT(p.Asp631Ter1) by NGS. Family B: a 33-year-old male patient with right-sided colon cancer. IHC staining showed the absence of MLH1 expression. The patient also presented MSI. The mother had endometrial and colon cancer, a maternal uncle had colon cancer and papillary urothelial carcinoma, and the maternal grandfather had colon cancer. Using NGS, a mutation in *MLH1* c.1890dupT(p.Asp631Ter1) was found. Our results demonstrate the important implications of clinical and molecular algorithms to improve the efficiency of LS diagnosis, as well as the detection of asymptomatic carriers. These data allow to established guidelines for the follow-up, risk-reduction management and treatment strategies for patients found to has pathogenic mutations. In addition, our data contribute to determine frequencies of specific mutationsin the general population. The mutation of the *MLH1* gene described above is prevalent among families with LS in South America.

A61
CASE REPORT: HEREDITARY BREAST CANCER AND LARGE REARRANGEMENTS IN PATIENT OF ASHKENAZI ORIGIN

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Hereditary breast cancer represents 5-10% of cases of breast cancer and is mainly associated with mutations in *BRCA1* and *BRCA2* genes. Patients of ashkenazi Jewish origin have an increase in the prevalence of mutations in these genes, with 90-95% carriers of founder pathogenic mutations in BRCA1/2, while 2-4% carry another type of mutation. To date, there are no reports of large rearrangements in patients of this origin. In our experience, the frequency of mutations produced by large rearrangements is 0.0045% (9/2000). We present the case of a patient of Ashkenazi-Sephardi origin with breast cancer, carrying a pathogenic deletion in the *BRCA1* gene. Case: 37 year-old patient with breast cancer and negative hormone receptors. Her mother, of Sephardic origin, ovarian cancer was diagnosed at 60 years of age. From a sample of genomic DNA, the *BRCA1/2* genes were studied by Next Generation Sequencing (NGS) and Multiple Ligation-dependent Probe Amplification assay (MLPA) for the study of large rearrangements. The presence of pathogenic mutation was not detected by sequencing. By MLPA (probemix P002 MRC Holland®) the heterozygous deletion including exons 23 and 24 of the BRCA1 gene (c.5407-?(*1_?) Del) was detected. This mutation is reported in the reference bases as pathogenic. It is considered important to search for a rearrangement in patients of this ethnic group in our country and to study these genes in full form to establish prevention measures, risk reduction and family counseling.

A62
PREVALENCE OF HYPERPROLACTINEMIA IN WOMEN WITH MENSTRUAL DYSFUNCTION AND ITS ASSOCIATION WITH SUBCLINICAL HYPOTHYROIDISM

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Presence of abnormally high values of prolactin, termed as hyperprolactinemia (HPRL), have been linked to menstrual irregularities which may lead to infertility. Although primary hypothyroidism is a well-known cause of HPRL, data on hyperprolactinemia in subclinical hypothyroidism (ScH) is scant. The objective of this study was to know the prevalence of HPRL among women who consulted for menstrual cycle alterations and to find its correlation with subclinical hypothyroidism (ScH). ScH was defined as patients having normal free thyroxine (fT4) levels with thyroid stimulating hormone (TSH) levels above the normal range. Data were collected in 287 women attending private endocrinology laboratory in San Luis city (San Luis, Argentina), from January 2017 to July 2018. History of pituitary adenomas, thyroid disease or thyroid medication was excluded. All patients with regular cycles were tested in the early follicular phase (days 3–5 of the menstrual cycle). Women with infrequent menses or amenorrhea had their blood collected at any time provided the progesterone was less than 0.2 ng/mL. Serum prolactin (PRL; RV: 4.79-23.30 ng/mL), TSH (RV: 0.27-4.2 mUI/mL), total thyroxine (TT4; RV: 5.52-12.6 ug/dL) and fT4 (RV: 0.89-1.71 ng/dL) were measured by electrochemiluminescence assay (Elecsys Roche 411). Triglycerides (Tg; RV: 35-150 mg/dL) and total cholesterol (TC; RV: <200mg/dL) were determinated by enzymatic assay (Wiener Lab, Argentina). HPRL was staged into three levels: mild (26-50 ng/mL), moderate (51-75 ng/mL) and marked (>75 ng/mL). Participants were divided into two groups according to their age: group 1 (20-29 years; 24±2.95; n=111) and group 2 (30-42 years; 36±3.7; n=176). Prevalence of HPRL was 56.75% and 40.34% in group 1 and 2, respectively ($p<0.05$). HPRL staging: group 1, 79.36% (n=50) mild and 20.63% (n=13) moderate HPRL; group 2, 85.92% (n=61) mild, 11.26% (n=8) moderate and 2.81% (n=2) marked HPRL. When the association of raised prolactin levels and thyroid hormones was analyzed, 10% in group 1 (median PRL: 36.94; CI 33.38-40.43; median TSH: 5.39; CI: 4.73-6.19) and 7.95% in group 2 (median PRL 38.30; CI: 34.0-43.44; median TSH: 5.73; CI: 4.76-6.28) were found to have ScH. An increase CT levels were observed in 9% of group 1 and 33.52% of group 2, without changes in Tg levels between groups. The HPRL was common in women with menstruation-related problems and its prevalence varies according to age. Routine prolactin evaluation may be warranted in patients with ScH. Elevated prolactin levels may be one of the indications for treatment of asymptomatic ScH as a part of menstrual dysfunction approach.

A63
STUDY OF SPECIFIC ANTIPHOSPHOLIPID ANTIBODIES IN WOMAN WITH RECURRENT PREGNANCY LOSS

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Recurrent pregnancy loss (RPL) is an important reproductive health issue. The etiology of RPL includes chromosomal, anatomic, endocrine and autoimmune abnormalities. Antiphospholipid syndrome (APS) is a heterogeneous autoimmune disorder characterized by arterial and venous thromboembolic events and obstetric complications, in association with laboratory evidence of anti-phospholipid antibodies (aPL). The affected women have usually no other signs or symptoms referring an autoimmune disease. The aim of this study was to evaluate the presence of aPL [anticardiolipin antibodies (aCL), lupus anticoagulant (LA) factor and anti-β2-glycoprotein I antibodies (aβ2GPI)] and haemostatic parameters related in patients of recurrent unexplained pregnancy loss. In a retrospective study, a total of 83 women who had suffered from two or more consecutive miscarriages were evaluated in a private clinical laboratory in San Luis city, Argentina, from November 2017 to September 2018. Patients with history of bleeding or coagulation disorder or anticoagulant therapy were excluded. To evaluate plasmatic element of hemostatic system, activated partial thromboplastin time (APTT; RV: 28-48 sec), thrombin time (TT; 17-21 sec) and prothrombin time (PT; RV:70-100%) tests were carried out using hemostatic analyzer (Fibrintimer II, Wiener Lab) while hemoglobin (RV:≥12g/dL) and platelet count (RV:150-450x10^9/L) was measurements by automated blood count analyzer (Sysmex Corporation, Japan). LA (IgM, RV: 31-44sec; IgG, RV: 28-38sec), aCL (IgM, RF:≥14; IgG, RV:≥16) and aβ2GPI (IgM, RV:≥10; IgG, RV:≥20) were detected by ELISA assay (Inova Diagnostics). Patient's ages ranged from 20 to 41 years (34.4±5.5 years). The 42.5% of the women were in their peak reproductive age group (25-34 years). The 18% women had abnormally high circulating aPL levels: 14 cases (16.87%) had LA (IgM and/or IgG isotype), 7 case (8.43%) had aCL antibody (IgM and/or IgG isotype) and 3 cases (3.61%) were positive for aβ2GPI antibody (IgM and/or IgG isotype). The 46.7% women had more than one positive aPL. In aPL(+) group, 66.6% course with mild anemia, without changes in platelet count, APTT, TT, and PT in relation to aPL(-) group. Factor V Leiden, prothrombin G20210A and methylenetetrahydrofolate reductase C677T mutations could not be found for any of the positive aPL patients. APS is one of the few treatable causes of pregnancy loss and successful pregnancy can be achieved with appropriate treatment. Our data emphasizes the role of aPLresearch in RPL,in combination with others hematological parameters.

A64
EPIDEMIOLOGY OF DERMATOMYCOSES: MULTICENTRE STUDY IN SAN LUIS CITY, ARGENTINA

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Dermatomycoses are considered an important group of fungal diseases and a public health problem. The most common types of such infections are dermatophytosis, pityriasis versicolor and candidiasis. A multicentre study was carried out to determine the prevalence, etiological agents, and clinical presentation of dermatomycoses. Samples were analysed by two different techniques: direct microscopic examinations (DME) with KOH at 20-40%, and cultures on Sabouraud agar added with cycloheximide at 28°C for 3 weeks. Filamentous fungi were identified by their macroscopic and micromorphological characteristics, while the yeasts were typed by development on chromogenic agar and the production of germ tubes and chlamydoconidia on agar milk. In this work, we present the data collected by three laboratories in San Luis city. A total of 1369 samples obtained between 2013 and 2018 were analysed. The average age of patients was 38.83 ± 16.03 years and the male:female ratio was 1:1.4. Samples were collected from nails (38.86%) –fingernails (9.57 %) and toenails (29.29%)-, scalp injury (9.64%), ear skin (0.29%), skin (34.92%) and chest and abdomen skin (16.29%). The 51.50% of the total of samples were positive by DME and 68.23% were positive by culture. These results were similar to those obtained in studies of the same characteristics in other regions of Argentina. DME produced positive results for 49.63%, (n: 199 samples) of toenails, 54.20 % (n: 71) of fingernails, 38.64% (n: 51) of scalp lesions and 33.68% (n: 161) of skin samples. Cultures produced positive results for 50.12% (n: 201) of toenails, 60.31% (n: 79) of fingernails, 36.36% (n: 48) of scalp lesions and 31.17% (n: 149) of skin samples. Malassezia spp. was the fungus most commonly observed in the direct examination with methylene blue from skin of chest and abdomen with a 96.86% (n: 216) frequency. The etiological agents in descending order of prevalence were identified as Malassezia spp.(31.39%), Trichophyton rubrum (16.28%), Candida species other than albicans yeasts (12.5%), Candida albicans (11.78%), Trichophyton mentagrophytes (11.34%), Microsporum canis (7.70%), Trichophyton spp.(4.65%), Fusarium spp.(1.31%), Trichophyton tonsurans (1.02%), Aspergillus spp.(0.87%), Epidermophyton floccosum (0,43), Alternaria spp.(0.44%) and Microsporum gypseum (0.29%). Currently, tinea pedis occupies the second place in incidence among all clinical forms of dermatophyte infections in skin and skin appendages. The current epidemiologic trends and the knowledge of the causative agents demonstrate the need of new strategies for the prevention and therapy of dermatomycoses.

A65
TOTAL SERUM IgE IN ATOPIC AND NON-ATOPIC INDIVIDUALS IN SAN LUIS: SIGNIFICANCE IN THE DIAGNOSIS

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Total serum immunoglobulin (IgE) test is usually performed to aid in the diagnosis of allergic diseases and atopy. In both atopy and allergies, the symptomatology is superimposed, such as pruritus and lichenification on the face, trunk, and neck.Therefore, it is important to differentiate them in order to reach an accurate diagnosis.The present study aimed to investigate the distribution of total serum immunoglobulin E (IgE) levels in atopic and non-atopic adults individuals to evaluate its utility in the prediction of atopy and allergic diseases. In this work, we present the data collected by one laboratory in San Luis city. Total IgE levels were measured in 200 patients, 104 were male (52%) and 96 females (48%). The mean age of the patients was 32.64±12.13 years (ranged from 16-79 years old). The median total IgE level was 168.19 U/L±185.28 (range: 10.7-1000 U/L). A total of 105 atopic patients and 95 non atopic of both sexes, were classified in two groups to evaluate IgE levels.
The presence of atopy was determined through history, physical examination and reactivity to skin prick tests with allergens. The serum IgE was evaluated by Quimioluminiscencia, Centaur XP, Siemens, USA. It was observed that IgE levels are higher in women. The medium values of total IgE in atopic patients were 277.02±199.52 are significantly higher than those observed among non-atopic individuals 47.91±27.89. This study allowed us to observe that the data found are similar to studies conducted in other countries. In addition, it was possible to determine the usefulness of the IgE value for identify atopic individuals from those who are not.

A66
STRUCTURAL IMAGES IN A SERIES OF ODONTOGENIC KERATOCYSTS

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It is attributed to Mikulicz in 1876 the first description of a lesion of the maxillary bones of what is interpreted as Keratocyst received different names: cholesteatoma, epidermal disembrioplasia, primordial cyst and Keratocyst. The latter adopted in 1976

by Who. The main characteristic of this entity is its histological constitution, formed by Malpighiano epithelium with 6 to 10 cells of thickness, a basal layer of cells and palisade and with the property of producing keratin towards the cystic cavity, with conservation of Nuclei (parakeratin) or without them (ortho keratin). This last condition formed the 2017 classification of Ortho keratinized odontogenic Cyst . The epithelium in its contact with the connective tissue does not form epithelial retes. The connective represented by fibroblasts, Fibrocitos and collagen Fibers. Other structures may be observed. In order to evaluate structural images, the histopathological parameters observed to the optical microscopy in specimens were taken into account considering an n = 35, from the surgical Pathology Laboratory of the Faculty of Dentistry of UNLP included in paraffin and coloured with Hematoxylin-eosin. To specify the nature of the epithelium is used monoclonal antibodies Anticitoqueratinicos (AE1 – AE3). The results were: parakeratosis 97.14%; inflammatory components 31.43%; epithelial islets in cystic wall 11.42%; micro keratocysts 8.57%; proliferations of the wall 5.71%, presence of epithelial papillae 5.71%, cholesterol 5.71%, discontinuity of basal 2.86%, hyalinization 2.86%, dysplasia – cancer 0%; ortho keratosis 2.86%; Gorlin areas 2.86%. In conclusion, keratocyst has peculiarities in clinical, behavioral or histopathological aspects that differentiate them from the rest. In the year 2017, who separates Ortho keratinized odontogenic cyst from Odontogenic keratocyst.

A67
FETAL LUNG INTERSTICIAL TUMOR (FLIT): CASE REPORT

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Fetal lung interstitial tumor (FLIT) is a recently (2010) reported type of congenital lung lesion comprising solid and cystic components. There are approximately ten cases described in the international literature. We report the case of a three-day-old female patient admitted for vomiting. A chest x-ray showed a nodular image in the right lung, and computed tomography revealed a heterogeneous, hypodense nodular lesion with regular contours measuring 4.2 x 3.4 cm. Therefore, surgical resection was decided. Light microscopy showed proliferation of embryonic cells, arranged in numerous papillae, delimited by a dense fibrous capsule, cells with hyperchromic ovoid nuclei and scarce cytoplasm. Immunohistochemical staining for Vimentin was positive while staining's for Desmin, Myf-4 and CD-99 were negative. The epithelial and interstitial cells contained abundant glycogen granules. Although primary pulmonary tumors are rare, this possibility should be considered in all isolated pulmonary masses in childhood, especially in patients under three months of age, born at term, neonates with weight appropriate to gestational age and with a single lung lesion, of solid or mixed characteristics, composed of interstitial immature mesenchyme resembling the fetal lung of 20 to 24 weeks of gestation. In addition to the case report, we expose a brief resume of the state-of-the-art knowledge about the histopathology and the molecular cell biology of this neoplasm.

A68
REFERENCE VALUES OF HEMOGRAM PARAMETERS OF PREGNANT WOMEN TESTED AT THE LABORATORY OF THE MATERNITY CLINIC DR. TERESITA BAIGORRIA.

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Numerous pathologies can affect patients in stages of pregnancy. The quarterly follow-up serves not only to evaluate if not to detect the presence of acute and important infections, and to decrease the frequent incidence of anemia and thrombocytopenia in this period. To reduce possible complications, it is necessary to perform a blood count in each trimester of pregnancy. It must be taken into account that the reference values change gradually throughout it. We encourage staff to determine the reference intervals in the study population in its different stages in order to reduce the error caused by its misinterpretation. We obtained 22000 samples of anticoagulated blood with $EDTAk^3$ from pregnant patients who attend for control in the laboratory of the Maternity Dra. Teresita Baigorria. The gestational period was divided into quarters according to the weeks of gestation. Until week 13, they will be included in the first trimester, the second trimester between weeks 14 and 27, and from the 28th until delivery they conclude the third trimester. In addition, the study included the preconception and the puerperium period. The hemograms were analyzed in an Advia 560 hematology counter. This fully automated system uses the impedance method to mesure, leukocytes, erythrocytes and platelets. The hemoglobin concentration was measured by photometry. The differential of the leukocyte formula of 5 populations was carried out using laser-based optical flow cytometry technology. The data were analyzed through the GraphPadPrism 5 program. In the firstquarter of 586 samples studied the following average values were obtained: leukocytes 8986 / mm3, red blood cells 4.23 $x10^6$ / mm^3, packed-cell volume 37%, hemoglobin 12.1 g / dl and platelets 241 $x10^3$ / mm^3. In the second quarter, 500 samples were analyzed and their values were: leukocytes 10092 / mm^3, red blood cells 3.68 $x10^6$ / mm^3, packed-cell volume 32%, hemoglobin 11.6 g / dl and platelets 238 $x10^3$ / mm^3. In the last quarter, 6835 samples were analyzed obtaining the following results: leukocytes 9640 / mm^3, red blood cells 4.0 $x10^6$ / mm^3, packed-cell volume 35%, hemoglobin 11.7 g / dl and platelets 222 $x10^3$ / mm^3. It was observed that when comparing the different parameters between the quarters a $p < 0.001$ it was obtained. Therefore, it is vital to have reference values for the parameters under study. This helps the discrimination between pathological and normal processes.

A69
AGING AND MEMORY. THE POTENTIAL OF LEARNING AS A PREDICTOR OF PERFORMANCE IN OLDER ADULTS

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Population aging brings challenges and the need to respond to new demands. There is a certain consensus regarding the neurocognitive changes produced by age, being the memory the most studied function as a fundamental element for learning. Currently it is agreed that learning is present throughout life due to neuroplasticity. In this field, compensation and cognitive plasticity come into play, being the Learning Potential (LP) a measure or indicator of this (Baltes and Singer, 2001, Kempermann and cols., 2002, Fernández-Ballesteros and cols., 2007). LP is the difference between the performance or initial capacity of a subject and the level of performance after a training phase or obtaining aid (Baltes and Willis, 1982). The goal of this study was to know if the Learning Potential (LP) is related to the recall measures in a verbal auditory learning test in older adults. The work consisted of a non-experimental study (N = 60, age mean= 73.02, SD 6.17). The measure of LP was made by "Rey Verbal Auditory Learning Test" and with the difference between A1(1° trial) and A5(5°trial). This value reflects the individual gain and benefit of the training). A significant positive correlation was obtained, between the LP and the memory measures (Post-interference r = 0.70 p <0.01, Deferred memory = 0.69, p <0.01 and Recognition r = 0, 72 p <0.01);while the learning potential increases, memory tasks performance increases. We conclude that LP (level of cognitive plasticity in the development of the task) would predict performance in memory and recall tasks in older adults. This finding is an important tool in the Neurocognitive Evaluation and Rehabilitation processes.

A70
LOW PHYSICAL ACTIVITY AND SMOKING EXACERBATE METABOLIC SYNDROME PARAMETERS: A STUDY IN A SAMPLE OF THE ADULT POPULATION OF SAN LUIS, ARGENTINA

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Metabolic syndrome (MS) occurs in most overweight (OW) and obese (OB) patients. There are no studies in our population about how physical activity and smoking affect metabolic syndrome parameters. Our hypothesis is that in OW and OB patients, smoking and a reduced physical activity exacerbate MS parameters. To test our hypothesis we conducted a population-based study in 316 adults (mean age 28.6 year-old) of both genders (50.6% male) between 18 and 80 years-old in the city of San Luis, Argentina (2015-2017). Data of smoking habit and physical activity were obtained by questionnaire. Body-weight (Kg) and height (m) were measured and subsequently the body-mass index (BMI, Kg/m^2) was calculated. In addition, a fasting-blood sample was drawn from 79 patients for the measurement of biochemical parameters. We considered as "active" those patients who perform aerobic exercise of moderate intensity at least 30 min/5 days a week. Patients with OW and OB were those with a BMI≥25 Kg/m^2. Smokers were those who smoke at least one cigarette per day. We categorized as MS patients those having at least 3 of the following: central obesity (waist circumference> 102 cm (men) / 88 cm (women)), arterial hypertension (AHT, systolic blood pressure> 130 and diastolic blood pressure > 85mmHg or those who take prescribed medication for AHT), hyper-triglyceridemia (HTG, TG> 150 mg/dL), and low plasma HDL-c (<40 men or <50 in females, mg/dL). The population sample (n=316) consisted of 69.6% active patients. The BMI in this sample population was 25.2 ±5.21, 38% were OW or OB, 7.6% were smokers and a 3% had SM. In the group of 79 patients, 19% had central obesity, 10% had AHT, 18% had HTG, and 38% had a reduced plasma HDL-c concentration. Patients with central obesity had less physical activity (Spearmen correlation, CS p = 0.031), HTG (CS p = 0.036), reduced HDL-c (p = 0.040 CS) and AHT (CS p = 0.061, a significant difference at 10%). In those patients with BMI≥25 an association was observed with HTG (χ^2 p = 0.0001). We only found an association between smoking and central obesity (χ^2p = 0.020). The MS correlated with gender (CS p = 0.006), and had an average correlation with physical activity (p = 0.446 CS) and BMI (CS, p = 0.026). In OW and OB subjects, smoking is associated to central obesity—a hallmark of metabolic risk. Smoking is a modifiable conduct that may help reducing the metabolic risk in this population. Data from this study would provide appropriate health policies to reduce metabolic risk and improve life-quality in OW and OB patients.
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A71

Streptococcus agalactiae COLONIZATION IN PREGNANT WOMEN IN THE MATERNIDAD PROVINCIAL DRA. TERESITA BAIGORRIA OF SAN LUIS CITY.

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Streptococcus agalactiae maternal colonization is the main risk factor for early onset disease. *S. agalactiae* can be part of the human microbiota mainly the genitourinary and gastrointestinal tracts. About 50 to 75% of the exposed newborns become colonized and 1-2% developed invasive neonatal disease. In pregnant women *S. agalactiae* may cause abortion, urinary tract infection, premature birth, chorioamnionitis and puerperal endometritis. Various studies have shown colonization rate from 3.3 to 22.8%. This different prevalence can be attributed to differences in the studied population or the microbiological methods employed. We analyzed 322 samples of pregnant women (35- 40 gestational weeks) in the Maternidad Provincial Dra. Teresita Baigorria of San Luis city. There were performed rectal and vaginal swab to evaluate *S. agalactiae* colonization. The swabs were inoculated into Todd Hewitt broth incubated at 37°C 24hs and then were cultivated in CHROMagar StrepB. Susceptibility diffusion test were performed according Clinical Laboratory Standard Institute's rules on *S. agalactiae* strains isolated. Data were analyzed by GraphPad Prism 5.0. The *S.agalactiae* total prevalence was 20.18%, lower in the group of patients under 20 years (17.39%) and higher in the group of 31-35 years (23.81%). Clindamycin resistance was 27.69% while Erythromycin resistance was 24.62% and the phenotypic mechanisms observed in the disk diffusion test were the commonly described in other previous studies: Lincosamine nucleotidiltransferase, efflux pump, cMLSb and iMLSb. No resistance to Penicillin or Vancomicin was found in the strains studied. These results were according to a national and international data reviewed but the prevalence found was higher in comparison of our previous data in San Luis city (less than 10%). This study shows an increasing macrolide and lincosamide resistances, both of them are used in clinical patients with anaphylaxis to β lactam antibiotics. The present study contributes to the knowledge of local epidemiology of *S. agalactiae* colonization.

A72

PROGNOSTIC VALUE OF γH2AX IN PRE-CHEMOTHERAPY PERIPHERAL BLOOD LEUKOCYTES (PBL) FROM CANCER PATIENTS TREATED WITH PLATINUM ANALOGS

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Cisplatin (cisPt) is a widely used chemotherapeutic drug for the treatment of solid tumors. Its mechanism of action is based on the formation of DNA-interstrand and -intrastrand crosslinks, which interfere with normal DNA function. Histone H2AX phosphorylation (γH2AX) facilitates the recruitment of DNA damage repair proteins to the chromatin surrounding DNA lesions. The expression of γH2AX has been proposed as a tool to monitor DNA damage and repair in translational cancer research. The aim of our work was to determine the prognostic and predictive value of γH2AX in peripheral blood leukocytes (PBL) from cancer patients treated with cisPt. We isolated PBL from 6 healthy persons and 32 cancer patients (mean follow-up 13 months) before chemotherapy. PBL were exposed *in vitro* to cisPt (200 µM, 1 h). Cells were harvested both immediately after cisPt exposure and after 24 h and 48 h. γH2AX expression was evaluated by immunocytochemistry. At basal conditions, there were no statistically significant differences in the number of γH2AX foci per nucleus and in the percentage of γH2AX-positive cells between healthy subjects and cancer patients. PBL from healthy individuals and patients showed a significant increase ($P < 0.05$) in the number of γH2AX foci per nucleus 24 h after cisPt. However, γH2AX foci per nucleus decreased after 48 h of recovery in PBL from healthy subjects, but they were not modified in PBL from cancer patients. Statistically significant differences between patients with complete clinical response to chemotherapy and those with partial response, stable or progressive disease were not found for the number of γH2AX foci per nucleus. A high number of γH2AX foci per nucleus in cancer patients were associated with better overall survival ($P<0.05$). Our preliminary findings indicate that detection of number of γH2AX foci per nucleus in pre-chemotherapy PBL from cancer patients may be a useful prognostic marker for cancer patients treated with platinum-based chemotherapy.

A73
RELATIONSHIP BETWEEN ATHEROSCLEROSIS, BIOMARKERS IN METABOLIC SYNDROME

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A metabolic syndrome (MS) diagnosis makes it possible to identify individuals exposed to a 2 to 3 time higher risk of suffering a serious cardiovascular event. Inflammation, thrombophilia and endothelial dysfunction represent a pathophysiological link between the MS, its components and the atherosclerotic process. Our objectives were to evaluate the variables of inflammation, of thrombogenesis, of endothelial dysfunction and insulin resistance in patients with MS, with coronary atherosclerosis and without it. We studied women older than 16 years with MS, with evidence of atherosclerosis or without it. Diabetic and hypothyroidpatients, those with other systemic diseases, with heart disease, with peripheral or brain arteriopathy and with a history of smoking were excluded. The patients were divided into two groups: I) without clinical expression of atherosclerosis and II) with evidence of coronary atherosclerosis. The variables studied were: anthropometric, metabolic (diagnosis of MS and insulin resistance), inflammation (ultrasensitive C-reactive protein [CRP]), of thrombophilia (fibrinogen), of endothelial dysfunction (e-selectin) and microalbuminuria. Statistics: Student's *t* test for unpaired data, Fisher's exact test. We studied 40 female patients with MS (20 patients in each group). Average ages were 56 ± 8 years in group I and 59 ± 9 years in group II (ns). No significant differences were found between groups in the anthropometric variables. In the metabolic variables, a significant decrease in HDL-cholesterol was found in group II with respect to I (36 ± 9 vs 54 ± 13 mg/dl, p <0.0001); without significant differences in the other variables studied. CRP levels were 4.8 ± 1.4 vs 4.0 ± 0.8 mg/dl, for group I and II, respectively (ns); fibrinogen concentration was 399 ± 102 vs 426 ± 119 mg / dl (ns); e-selectin was 41.3 ± 26.1 vs 49.1 ± 24.7 ng / ml (ns). Microalbuminuria was present in 9 patients (22%) of group I and in 12 (30%) of group II (ns). In all patients with MS (with atherosclerosis or without it), insulin resistance and biomarker variables (of inflammation, thrombophilia and endothelial dysfunction) were increased. The only variable that differentiated both groups was the level of HDL-cholesterol.

A74
HSP27, γH2AX AND 53BP1 AS PREDICTIVE/PROGNOSTIC FACTORS IN CANCER PATIENTS TREATED WITH PLATINUM COMPOUNDS

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Over the past 30 years, platinum agents have been extensively used for the treatment of various solid tumors. Their main target is the DNA molecule, forming covalent DNA adducts, thereby inhibiting replication and transcription and inducing cell death. Resistance to platinum-based chemotherapy that some patients acquire during treatment limits the scope of its full potential. The biochemical mechanisms related to such resistance are multifactorial, including tolerance to nuclear DNA damage and heat shock proteins (HSPs). HSP27 (HSPB1)is overexpressed in many cancer types and influences cellular processes such as apoptosis and DNA repair and has also been related to drug resistance. One of the first events in the activation of DNA damage repair pathway is phosphorylation of histone H2AX, yielding γH2AX foci, that recruits other factors such as 53BP1, an important mediator of DNA damage checkpoints. Our main objective was to determine the predictive/prognostic value of HSP27, γH2AX, 53BP1 and the DNA repair proteins, ERCC1, MLH1 and MSH2 in tumor biopsies from cancer patients who received platinum-based chemotherapy (cisplatin/carboplatin). Immunohistochemistry was employed to detect the expression of HSP27, γH2AX, 53BP1, ERCC1, MLH1 and MSH2 in paraffin-embedded tumor tissues from 31 cancer patients before chemotherapy (mean follow-up 11.7 months). Patients with complete clinical response to chemotherapy showed lower expression of cytoplasmic and nuclear HSP27 than patients with partial response, stable disease and progressive disease (P<0.05). Low expression of nuclear HSP27 associated with longer disease-free survival, DFS (P<0.05). Patients with low expression of cytoplasmic HSP27 had favorable overall survival, OS (P<0.05). Percentages of γH2AX and 53BP1 greater than the mean values (55%) correlated with better DFS (P<0.05). High percentage of γH2AX positivity was significantly associated with longer OS of the patients (P<0.05). No statistically significant relationship was found between ERCC1, MLH1 and MSH2withclinical response and outcome of the patients. Our preliminary data indicate that HSP27 could be a useful predictive biomarker and along with γH2AX and 53BP1, may be used as prognostic molecular markers in cancer patients who receive chemotherapy based on platinum compounds.

A75

DISTRIBUTION OF MYELINATED AND NON-MYELINATED NERVE FIBERS IN HUMAN FEMALE AND MALE DENTAL PULP TISSUES

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Clinical practice indicates that there is different tolerance to pain in men and women. The cell bodies of the sensory neurons of the dental pulp are found in the trigeminal ganglion.Most nerve bundles reach the coronal dentin where they open to form the Raschkow nerve plexus. There, they anastomose and end as free nerve endings in the odontoblast layer and in the odontoblastic cellular processes, (approximately 100-200 μm deep in the dentinal tubules).Based on these initial observations, we aimed at: determining the distribution of myelinated and unmyelinated nerve fibers in human pulp tissues in relation to sex. Healthy teeth were collected from patients aged between 14 and 28 years old (14 in total, 8 from female patients and 6 from male patients, Protocol BIOETHICS COMMITTEE of the UNCUYO School of Dentistry, No. 000003/2016). For the phenotypic determination by triple immunofluorescencethe following antibodies were used: a mouse monoclonal antibody (RT97) directed against high molecular weight neurofilaments (NF200) that selectively labels myelinated fibers, and a rabbit raised antibody against trkA, the high affinity receptor for nerve growth factor (NGF) that labels selectively peptidergic fibers both myelinated and unmyelinated. These antibodies were combined alternatively with either an antibody against β tubulin 3 (selective neuronal marker) or binding of isolectin B4 which stains non-peptidergic fibers and neurons.Our results showed, both in the samples stained with usual colorations and in the immunostained ones, the predominance of fast-conducting peptidergic myelinated fibers forming bundles that are located both in the central region of the dental pulp and in the proximity of the odontoblast layer.This differential distribution could indicate a production of peptide mediators capable of interrelating as messengers with the odontoblast line.Our observations did not show a different distribution in men or women. This suggests that differential perception of dental pain may be better explained by functional differences rather than by a particular anatomical disposition.

DEVELOPMENTAL BIOLOGY AND REPRODUCTION

A76

CHANGES IN MANNOSE-6-PHOSPHATE RECEPTORS AND GLYCOSIDASES INDUCED BY ACROSOMAL REACTION IN BOVINE SPERMATOZOA.

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Mammalian spermatozoa must undergo biochemical and morphological changes before the oocyte fertilization. Some of these changes are induced by capacitation and acrosomal reaction that occurs in the female genital tract. In previous studies, we have described the presence of mannose-6-phosphate receptors (MPRs) on the bovine sperm (BS) surface and their interaction with glycosidases. The aim of this study was to analyse whether capacitation and/or acrosomal reaction may induce changes on these receptors and glycosidases. Epididymides from adult bulls (Aberdeen Angus) were carefully dissected and spermatozoa from cauda were collected by retro-perfusion. By flow cytometry, using specific antibodies, we observed that both MPRs, the cation-independent (CI-MPR) and the cation-dependent (CD-MPR) were detected in BS. After 2 hours of incubation in capacitation media (SP-TALP) and ulterior 20 min with the ionophore A23187, we observed that CI-MPR detection increases significantly due to capacitation. Meanwhile, the detection of both receptors increased significantly after acrosomal reaction. Some glycosidases, such as β-N-acetyl-glucosaminidase can be partially removed from the sperm due to capacitation and acrosomal reaction, whereas α-fucosidase detection was increased after acrosomal reaction. No changes were observed for other glycosidases, such as β-galactosidase, β-glucosidase and α-mannosidase. These results suggest that MPRs and some glycosidases may be affected by the capacitation and acrosomal reaction in BS. These changes could provide new insights into molecular rearrangement that can take place before the fertilization of the oocyte.

A77
CHANGES IN THE SEMINIFEROUS EPITHELIUM OF THE LIZARD – *Liolaemus cuyanus*- DURING REPRODUCTIVE CYCLE.

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The arid regions have a heterogeneous availability of resources in space and time because of the marked climatic seasonality. As a strategy to guarantee reproductive success, vertebrates are adapted to hostile environments presenting a circannual rhythm. They possessed a single reproductive period, relatively short, followed by a stage of complete sexual inactivity, with resting gonads. The objective of this work is to describe the changes of the seminiferous epithelium of the lizard *Liolaemus cuyanus*, from the Monte Desert, San Juan, throughout its reproductive cycle. Twenty one adult male specimens were collected between October 2017 and March 2018. Both gonads were isolated and then fixed by immersion of small pieces in a fixing solution (phosphate buffered saline, containing 2% paraformaldehyde and 2% glutaraldehyde, depending on whether it is for light (MO) or transmission electron microscopy (MET), respectively). Once the samples were processed by routine histological/ultrastructural techniques, they were observed with an upright microscope - Nikon 80i for histological architecture or with a Zeiss EM 900 for ultrastructural characterization. Pictures were taken and analyzed with Image J, version 1.37v. At the beginning of the reproductive season, seminiferous tubules with developing epithelium and abundant interstitial space were observed. In the following three months, a remarkable increase of the surface of the seminiferous tubules was observed, with luminal spaces decreased, almost filled with released sperm. The interstitial space in this period was scarce due to tubular development. Towards the end of mating season, we appreciated a fall of the seminiferous epithelium, corresponding to cessation of cell differentiation that occurs during spermatogenesis and spermiogenesis. Observing the MET images, we appreciated different stages of chromatin condensation, elongation of the nucleus and formation of complex structures over the months of activity. In conclusion, the seminiferous epithelium presents circannual rhythmicity for a successful reproduction adapted to desert conditions.

A78
PROSAPOSIN IS INVOLVED IN MALE FERTILITY

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Luminal proteins of mammalian epididymis interact with the surface of in-transit spermatozoa as a requirement for gamete maturation and acquisition of fertilizingability. The major sulfoglycolipid of the sperm plasma membrane is a sulfogalactosylglycerolipid (SGG), which is a substrate for Arylsulfatase A (ARSA). ARSA modifies SGG in the presence of a sphingolipid activator protein, termed saposin B (SapB). Both, the precursor of SapB, Prosaposin (PSAP), and ARSA are secreted by the epididymal epithelium, and they interact with the sperm plasma membrane. Moreover, the intracellular processing of PSAP into saposins occurs at acidic pH, and requires the protease cathepsin D (CatD). Given that CatD is secreted by the epididymal epithelium and that the female genital tract has an acidic environment, we tested whether CatD can process PSAP at acidic pH and whether the fertilization rate is affectedunder these conditions. Additionally, we evaluated whether the inhibition of PSAP/SapB results in alteredmotility and/or fertilization. Mouse epididymal fluid and spermatozoa were collected with buffers adjusted to pH 5.5, 6.3 or 7.2, without or with pepstatin A (CatD inhibitor) or with an antibody raised against PSAP/SapB. Luminal epididymal proteins were then subjected to immunoblotting. Sperm motility (by CASA) and *in vitro* fertilization (IVF) were also tested. Although PSAP processing by CatD was enhanced at pH 6.3, with subsequent increasing of fertilization rate, the acidification did not alter sperm motility parameters.Moreover, sperm motility and IVF were significantly decreased by blockade of PSAP/SapB with a specific antibody.These results suggest that PSAP/SapBplay an important role in modifying the sperm plasma membrane during sperm capacitation and fertilization.

A79

EFFECTS OF A HIGH FAT DIET ON MALE NEW ZEALAND RABBITS PRO/PROSTATE

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The addition of fat to the normal diet of adult male rabbits leads to hypercholesterolemia (HC) with multi organ / systemic consequences and it is associated with seminal and spermatic changes. HC was related to decrease seminal volume and morphological and functional sperm disorders. The first could be attributed to changes in sexual glands physiology. The aim of this study was to analyze prostatic histology and quantify the content of prostatic acid phosphatase in seminal plasma of male rabbits - under different diets - and their testosterone levels. New Zealand White rabbits were fed commercial rabbit pellet (normocholesterolemic rabbits: NCR), plus 14% bovine fat (HCR) or 7% bovine fat plus 7% olive oil (OO) (½HCR + ½OO). In HCR, prostate epithelium height and pro prostate villi length significant decreased ($p<0.05$) compared to NCR. Only pro prostate villi length was recovered in ½HCR + ½OO. Therefore, cholesterol intake affects mainly pro prostate villi and prostate epithelium. Prostatic acid phosphatase and testosterone did not show changes in their levels at three months of experimental diets. In conclusion, high fat diet promotes prostatic epithelial modifications at three months of fat diet but this impact at the functional level has not been detected yet as a fall in phosphatase or testosterone levels.

A80

CHARACTERIZATION OF MEPC5 CELLS (MOUSE EPIDIDYMIS PROXIMAL CAPUT 5) AS A MODEL FOR THE STUDY OF EPIDIDYMAL EXPRESSION OF PEDF (PIGMENT EPITHELIUM DERIVED FACTOR)

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Serpine 1F, also known as pigment epithelium-derived factor (PEDF), is a glycoprotein that belongs to the serine proteases family initially found in retina. PEDF is widely spread in different mammalian tissues. It has functions in cell survival and repair, as an antioxidant, antiapoptotic and antiangiogenic factor antagonizing VEGF (Vascular Endothelial Growth Factor). There are many antecedents regarding PEDF role in female reproductive tract as an antioxidant and anti-apoptotic factor protecting the ovarian granulosa cells. Recently, it has begun its study in physiological and pathological situations in male reproductive system. Our laboratory recently described PEDF expression and androgen-dependency in the male tract of Wistar rats. In this species, PEDF is expressed mainly in epididymis, seminal vesicles and prostate, but notably, not in testes. We extended the study to an in vivo model (C57bl/6 wild type mice) and also in a cell culture model (MEPC5 cells obtained and immortalized from C57bl/6 mice). MEPC5 cells showed PEDF, P21 and Estrogen receptor alpha (ER α) expression by immunocytochemistry. Androgen receptor (AR), VEGF (a known PEDF antagonist), were also detected by western blot and indirect immunofluorescence (IFI). In mouse tissues, we observed PEDF expression over testicular peritubular cells as it was described previously in human and primates but no in rats. These results will foster the development of new lines of research to characterize the functional role of PEDF in the reproductive tract of rodents.

A81

DEXAMETHASONE MODIFIES OVARIAN STEROIDOGENESIS RESPONSE IN RATS WITH POLYCYSTIC OVARY

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder of women, characterized by an heterogeneous presentation of hyperandrogenism and ovulatory dysfunction. Several lines of evidence indicate that the systemic low-grade inflammation compromises multiple aspects of fertility in PCOS. In vitro studies suggest that pro-inflammatory stimuli may be capable of directly inducing hyperandrogenism in PCOS. Treatment options include dexamethasone (Dexa). Despite their therapeutic efficacy in modulating pain and inflammation, their influence on reproductive disturbance associated with PCOS is still under discussion. The study was carried out to investigate, in a PCOS-induced rat model, if Dexa affects ovarian progesterone (P), estradiol (E2) and nitric oxide (NO) release, and its relationship with enzymes and cytokines involved in ovarian steroidogenesis. Polycystic ovary condition (PCO) was induced in adult female Holtzman rats via i.m injection of estradiol valerate (2 mg/rat). PCO ovaries were incubated with RPMI medium (basal value), dex (10^{-6}M), androstenediona (A2; 10^{-6}M) or Dexa+A2, for 6h in metabolic bath. The P and E2 release were measured by electrochemiluminescence (Cobas e411), while NO (as nitrites) were

quantified by Griess reaction. The mRNA expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) and P450 aromatase (P450arom) (enzymes of P and E2 synthesis, respectively), interleukin (IL)-1β (pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine) were assessed in ovary by RT-PCR. Compared with basal value, Dexa-treatment decreased E2 release and P450arom mRNA expression ($p<0.05$). In contrast, no elevation in P release was found in PCO ovaries treated with Dexa alone, although 3β-HSD expression was up-regulated in relation to basal value ($p<0.05$). A2-treatment increased ovarian E2 and P release ($p<0.01$), as well as P450arom mRNA expression ($p<0.01$), in comparison with basal medium. This stimulatory effect was reduced when the PCO ovaries were simultaneously incubated with Dexa ($p<0.05$). In addition, Dexa-treated ovaries were associated with a decreased expression of IL-1β mRNA compared to basal, A2 or A2+Dexa condition ($p<0.05$). No difference between treatments was observed in the expression of mRNA for IL-10. The NO release pattern was: A2>basal> A2+dex>Dexa ($p<0.05$). The results reported here provide evidence for a local effect of Dexa in PCO ovary. This synthetic glucocorticoid induced a reduction of local estrogen production by inhibiting aromatase expression. For P release, the Dexa inhibitory effect is only observed in the presence of A2. Glucocorticoid regulated paracrine factors such as IL-1β, NO and local steroids, in concert, could be part of a mechanism to improve reproductive events in PCO condition.

A82
CALCIUM OSCILLATIONS PATTERN IN IN VITRO AND IN VIVO MATURED OOCYTES

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Before fertilization, mammalian eggs are arrested at the metaphase stage of the second meiosis (MII). Sperm entry triggers a series of increases in the intracellular free-Ca^{2+} concentration, termed calcium oscillations, which enable exit from the MII and induce egg activation. *In vitro* maturation has become an important tool in assisted reproductive technology in humans and animals, but it is unknown how it affects oocyte activation and embryo development. One of the first steps in oocyte activation is the cortical reaction, which consists in the exocytosis of small vesicles called 'cortical granules'. This exocytosis is activated by the first arise of intracellular calcium after the first sperm entry and is responsible for blocking polyspermy. We have developed a method that allows the analysis of cortical reaction in live oocytes activated parthenogenetically with $SrCl_2$, which produces calcium oscillations mimicking the sperm entry. Using this method we have shown that *in vitro* maturation alters cortical reaction in mouse MII oocytes. Based on this observation, we hypothesized that the altered cortical reaction is due to altered calcium oscillations in *in vitro* matured oocytes. In consequence, the aims of this work were: 1) to set up the standard method to observe and analyze calcium oscillations in live mouse oocytes and 2) to compare calcium oscillations in *in vivo* and *in vitro* matured oocytes. Ovulated oocytes (*in vivo* matured) were obtained from superovulated CF-1 females (8-12 weeks). *In vitro* matured oocytes were collected 45 h after PMSG injection and *in vitro* matured overnight in G-IVF medium. *In vivo* and *in vitro* oocytes were loaded with Fura2-AM and activated parthenogenetically with $SrCl_2$. The increase of intracellular free-Ca^{2+} concentration in live oocytes was registered every 10 seconds during 1 h in a thermostatic chamber by confocal microscopy. Results showed that *in vivo* matured oocytes had 3-4 calcium oscillations during the first hour; however, *in vitro* matured oocytes showed 0-1 calcium oscillations during the same time. In conclusion, our results showed that *in vitro* maturation affects the calcium oscillations pattern in mouse MII oocytes.

A83
PRENATAL DEVELOPMENT OF THE PERITUBULAR WALL IN THE RAT TESTIS

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The peritubular wall surrounds the seminiferous tubules in mammal testis. In adult rodents, the wall consists of an inner monolayer of myoid cells and an outer monolayer of endothelial cells, separated by spaces with collagen fibers. The aim of our work was to describe the appearance of these elements during the testicular development since 14 pcd (post-conception day), when seminiferous cords are just formed in 12 pcd, until birth. This study was done in male rat embryos by light and electron microscopy. On 14 pcd, the cords are well defined into a very dense interstitium. Inside the cords, there are Sertoli cells and gonocytes. Sertoli cells show a dense net of actin filaments in front of the basement membrane, which is thin and continuous. The wall is not organized on 14 pcd, but the neighbor cells, oval and similar, are already surrounding the cords. As the days proceed, more concentric layers are added, being 2 or 3 on 18 pcd and 5 layers on 20 pcd. The cells have an elongated shape, and on 18 pcd they show a thinner thickness. They can be distinguished more easily around the cords because the interstitium becomes lax. They are clear cells, with few organelles, without basement membrane or caveolae yet. On 20 pcd alpha actin filaments are seen in the inner face of these cells. The inner space, between the cord basement membrane and the surrounding cells, is narrow, with some collagen fibers on 14 pcd. On 16 pcd, some collagen fibers are also seen among the overlapped layers. Large peripheral blood vessels appear first, and afterwards the small capillaries that pull apart the cords. Blood vessels have endothelial cells well defined since 14 pcd and then other cells are recruited to form the muscle layer. There was no evidence of lymphatic vessels during the period studied. We conclude that the cells of the peritubular wall have been recruited in the fetal stage. Close to birth, the first actin filaments are seen in the myoid cells, near the cords. They are covered by other cell layers, which would acquire the final characteristics of endothelial cells after birth.

A84
TRIIODOTHYRONINE INDUCES CHANGES IN THE PROTEOME AND TRANSCRIPTOME OF ECC1 CELL LINE

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During women´s reproductive life, steroid hormones govern the periodic changes of the endometrium. Estradiol (E2) and progesterone (P4) are essential to regenerate and differentiate the endometrium after menses, in order to provide an adequate milieu for embryo implantation. Both implantation and maintenance of pregnancy depend on the correct function and interaction between the corpus luteum, the receptivity and functionality of the uterus, and the action of thyroid hormones (THs). THs dysfunction causes irregularities during the menstrual cycle, failure of implantation and early pregnancy loss. Therefore, we hypothesize that interaction between THs, E2, and P4 in the endometrium are fundamental for development and differentiation, providing the right environment for implantation. The goal of this study was to investigate how endometrial cells respond to HTs-controlled signaling, through the analysis of the genomic and proteomic expression pattern. For this purpose, the human endometrial ECC1 cell line was used to determine the expression of E2 (ERS1 and ERS2), P4 (PGR) and TH (TRα and TRβ) receptors and also how it responds to E2, P4, and triiodothyronine (T3) stimulation. Besides, the pattern of protein and genomic expression in response to the combination of E2 and P4, with or without T3 was analyzed. The protein identification was performed by LC and tandem mass spectrometry. The data were analyzed using Mascot server, followed by PROTEOIQ (Premier Biosoft) and Functional Enrichment analysis tool (FunRich) software. The relative expression of mRNA was analyzed by RT-qPCR. The mRNA analysis confirmed the expression of PGR, ERS1, ERS2 and TRα, TRβ in ECC1 endometrial cells. The mRNA expression of PGR increased significantly ($p < 0.05$) with T3. The proteomic analysis showed that cells treated with E2 and P4 present a greater amount of proteins involved in biological processes such as energy metabolism; protein transport; and cell growth. Whereas the combination with T3 expressed a greater amount of proteins involved in immune response and signal transduction. This work elucidates that ECC1 cells respond differentially to the hormonal treatments with E2, P4, and T3, showing that these cells are good model for the study of the interaction between steroid and THs. On the other hand, the proteomic analysis allowed us to infer that T3 induces changes in the relative expression of proteins when it interacts with maternal hormones. Altogether, the changes observed, demonstrated that the presence of THs is essential to provide an optimal environment for embryo implantation and growth.

A85
NEUROBEHAVIORAL DEVELOPMENT OF RAT OFFSPRINGS EXPOSED TO AT2 RECEPTOR BLOCKAGE.

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Renin-Angiotensin system is known primarily for its effects on blood pressure and electrolyte homeostasis. Several studies suggestthat may play a role in the regulation of growth, maturation and neuronal functions. The brain has two major angiotensin II receptors: type 1 (AT_1) and type 2 (AT_2). A high expression of AT_2receptors has been found on rat embryonic and postnatal phases, and it is rapidly down-regulated in adult tissues. In young rats, the AT_2 receptor expression was determined in areas involved in learning, control of motor activity, sensory areas, and selective limbic system structures. Adult knockout AT_2 receptor mice presented a phenotype with a selective reduction of exploratory behavior but not locomotors activities.The aim of this study was to test the effects of prenatal AT_2 inhibition on pup's neurobehavioral development. Pregnant Wistar rats (200g) on the 13th day of pregnancy wereimplanted mini-osmotic pumps subcutaneously withAT_2 antagonist (PD123319, 1mg/kg/day, n=6) and saline solution (SF, n=6). The effects of prenatal blockage of AT_2 receptor were evaluated on physical landmarks and behavioral indicators at different postnatal ages (n=18/each test). Treated pups didn't show any change on somatic growth or developmental landmarks in comparison with control pups. Neurobehavioral tests shown significant differences in righting reflex (PD: 11.62±1.10 sec vs SF:8.13±1.17 sec, $p<0.05$) and negative geotaxis performances during first postnatal week (PD:15.49±1.70 sec vs SF:11.09±0.72 sec $p<0.05$). Rota-Rod test result in delay latency to fall of PD123319 treated pups at 12 postnatal day (PD:1.38±0.11 sec vs SF:0.84±0.12sec, $p<0.01$). Open field test shown decrease motion time (PD:86.3±4.1% vs SF: 67.7±5.1%, $p<0.05$), latency to start moving (PD:0.5±0.0 sec vs SF: 0.2±0.0sec, $p<0.05$) and exploratory activity (PD: 31.43±3.20 % vs SF:41.15±3.89 %, $p<0.05$) and significant increase on locomotor speed(PD:9.2±0.8 cm/sec vs SF: 7.6±0.7cm/sec, $p<0.05$).Rota-Rod test result in delay latency to fall of PD123319 treated pups on 12 postnatal day (PD:1.38±0.11 secvs SF:0.84±0.12 sec, $p<0.05$). At postnatal day 12, treated pups spend more time on transition areas (intermediates) of the open field (PD:35.6±4.5% vs SF: 15.7±2.0%, $p<0.05$) and control pups remains on the center areas (PD:12.8±2.6% vs SF: 21.9±5.0%, $p<0.01$).These results indicate that AT_2 receptorof the central nervous system could be involved on locomotorsestablishment, spontaneous activity and anxiety behavior during postnatal development.

A86
HYPOTHYROIDISM PRODUCES CHANGES IN THE UTERINE VASCULATURE DURING THE IMPLANTATION PROCESS

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Hypothyroidism is one of the most common endocrine abnormalities implicated in the recurrent loss of pregnancy. Our laboratory, have shown that hypothyroidism in the rat is associated with a lower number of pups per litter due to a lower number of implantation sites and a decrease in the proliferation of the endothelial and decidual cells during the process of implantation of the embryo. On the other hand, is known that angiogenesis is a critical process in the uterine endometrium for embryo implantation, maintenance of early pregnancy, and development of the placenta. During this period, steroid hormones (E2 and P4) stimulate the synthesis of vascular endothelial growth factor (VEGF-A), the main modulator of angiogenesis during peri-implantation period. Therefore we hypothesize that hypothyroidism affects the normal vascularization of endometrium during implantation. The aim of this work was to study the effect of hypothyroidism on the degree of vascularization of the uterine decidua during the implantation process. Hypothyroidism was induced in female Wistar rats by daily administration of 6-propyl-2-thiouracil (PTU) 0,1 g/L in drinking water. In addition, hormone replacement therapy with T3 was administered simultaneously to the treatment with PTU (PTU+T3), in daily physiological doses of 0.6ug/100g. Both groups were compared to rats that only drink tap water (Control),on day five (G5) and seven (G7) of gestation. Uterine vascularization was evaluated by immunofluorescence. Besides,mRNA expression of PECAM (Platelet endothelial cell adhesion molecule, an indicator of the presence of endothelial cells) and VEGF-A were evaluated during the same peri-implantation periods (G5 y G7)by RTqPCR. Our results demonstrate that hypothyroidism decreases vascularization density of the uterine tissue during the process of implantation of the embryo ($p<0.05$). On the other hand, our results demonstrated a significant increase of expression of VEGF mRNA when hypothyroid rats were treated with T3 before implantation, in comparison to the control group and hypothyroid group ($p< 0.05$). However, no changes were found in the levels of PECAM expression among the different groups. In conclusion, the failure of implantation due to hypothyroidism may be directly linked to expression of VEGF-A, and consequently to vascularization of the endometrium before implantation in early gestation. Although are necessary further studies that corroborate the exact mechanism, our results identify molecular targets regulated by thyroid hormones that may link hypothyroidism to implantation failure and recurrent miscarriage.

GENERAL, CELLULAR AND MOLECULAR BIOLOGY

A87
LONG-TERM HIPOTHYROIDISM PRODUCES MAMMARY GENETIC AND EPIGENETIC CHANGES

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The mammary tissue is one of the few ones whose terminal differentiation is completed in adult life through lactation. In this process, the mammary genome is subject to epigenetic modifications that result in the selective expression of differentiation-associated genes and the establishment of the mammary transcriptome. After weaning, massive involution occurs and the tissue returns to a pregestation-like stage. This proliferation, differentiation and apoptosis cycle is regulated by lactogenic, ovarian and thyroid hormones. Thyroid pathologies such as hypothyroidism (hypoT) have an impact on the lactoma (lactation transcriptome), impairing the mammary epithelial cell ability to express differentiation genes. Despite this, the long-term impact of hipoT on mammary gland, and if hypoT alters the mammary epithelial cell epigenome are both unknown. To determine the long-term hypoT impact on mammary transcriptome, we used female virgin Sprague Dawley rats of 55 and 130 days old without treatment, female 130 day old rats that underwent a cycle of pregnancy, lactation and involution without treatment and a PTU treated 130 day old female rats that underwent a cycle of pregnancy, lactation and involution. Using real-time PCR, we evaluated the mRNA expression levels of differentiation-associated genes, such as GATA-3, PINC, NCOA-1, NCOA-2, and STAT6; and by methyl-specific PCR (MSPCR) we analyzed the methylation of two promoter regions of STAT6 gene. The analysis of gene expression showed that the long-term hypoT altered the mammary transcriptome differentially and that it had a special impact on NCOA-2, a gene associated with histones modification. Also, hypoT changed the mRNA expression of two mammary epithelial differentiation-associated genes, STAT6 and GATA3, both related to alveolar development. In addition, the methylation analysis

of the STAT6 gene showed that hypoT increases the CpG island methylation level upstream of the transcription initiation site. Our results show that the hypoT deleterious effect on the mammary gland is not limited to lactation but in long-term alters the mammary epithelial cell ability to express differentiation-associated genes and genes associated to transcriptional modification of histones. Also, changes in the methylation level of STAT6 gene show that the hypoT effect is beyond the function of the transcription factor associated to the receptor and that the pathology itself leads changes in the mammary cell epigenome.

A88
IMPACT OF LONG-TERM HYPOTHYROIDISM ON MAMMARY EPITHELIAL CELL SURVIVAL AND CELL CYCLE CONTROL

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After a reproductive event, at weaning, the mammary tissue involution is characterized by programmed death of epithelial cells and return to a pre-gestational like tissue histoarchitecture. Hypothyroidism (HypoT) alters intracellular events that regulate mammary cell differentiation and involution, and cause both, mammary development in virgin rats and premature involution of tissue in lactating rats. Beyond lactation, the long term impact of hypoT on the mammary gland is unknown. Therefore, we investigated the mammary glands of hypothyroid Sprague Dawley (HypoT) and euthyroid (Ctrl) rats that have undergone a complete cycle of gestation, lactation and involution. On day 28 after weaning we dissected inguinal mammary glands for western blot and for histological analysis. We evaluated the hormonal receptors level, cell cycle regulatory proteins and apoptosis related proteins. HypoT increased the p53 and p21 protein level and decreased the Retinoblastome phosphorylation level. Despite these results indicating cell cycle arrest, we also found an increase in the Ki67, Survivin and Bcl-2 protein level, which suggest an atypical cell survival. The thyroid hormone receptor expression increased with HypoT and although it did not change the protein level of the estrogen beta receptor, progesterone receptor decreased by HypoT. The tissue histological analysis revealed architecture similar to the pre-gestational stage in the Ctrl group. Surprisingly the HypoT group showed mammary tissue architecture altered with atypical alveoli, with epithelium areas stratified and disordered, with hyperplasic characteristics similar to fibroadenomas. Added to this, one of the animals showed alveolar secretory development and cell masses similar to *in situ* carcinoma. These results proved that the impact of hypothyroidism in mammary physiology is not only limited to gestation and lactation but also after the reproductive event, the mammary epithelium remains subject to intracellular and extracellular modifications which promote cell survival and an atypical epithelium, possibly more sensitive to the pathologic development.

A89
CADMIUM EXPOSURE MODIFIES SERUM LIPIDS AND THE EXPRESSION OF INTESTINAL LIPOGENIC GENES

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Cadmium (Cd) is a persistent environmental and occupational contaminant that accumulates in humans and shows adverse effects on health, including alterations in the serum lipid profile with increased cardiovascular risk. The aim of this work was to evaluate the response of Cd on lipid metabolism in the intestine and serum of male rats. Adult male Wistar rats, 180 to 200g body weight, were exposed to $CdCl_2$ (15 ppm) in drinking water, controls drank drinking water alone. Feeding was *ad libitum.* After 15 days (15d), 30 days (30d) and 45 days (45d) of treatment the animals were sacrificed and the intestine was extracted for further study and stored at -80 °C. Serum lipids (triglycerides TG, total cholesterol C, HDL-C) were measured by Wiener kits. The mRNA expressions of diacylglycerol acyltransferase (DGAT), fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and sterol regulatory element-binding protein-2 (SREBP-2) were determined by RT-PCR, using S28 as internal control. Cadmium treatment caused an increase of TG in 15d when compared to control, 30d and 45d groups; C increased in 30d and 45d respect to control group, HDL-C did not show differences and estimated LDL-C increased in all treated groups. HMGCR expression increased in 15d respect to 30d group; FAS increased in 30d and 45d respect to the control and 15d groups; SREBP-2 decreased in 30d vs control, 30d vs 15d and 45d vs 15d, DAGT did not change. In conclusion, Cd affects the content of serum lipids in a variable manner in the different exposure times.The variations observed in HMGCR expression correlate with those presented by SREBP-2, primarily responsible for the activation of genes involved in cholesterol synthesis. All changes vary according to the exposure time. The results in serum would agree with the variations in the expression of lipogenic enzymes. It remains to be determined if Cd affects the transport of exogenous lipids.

A90

STRUCTURAL AND CONFORMATIONAL DIFFERENCES BETWEEN WILD TYPE AND F278C MUTANT VARIANT OF THE RENAL OUTER MEDULLARY POTASSIUM CHANNEL

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Bartter syndrome (BS) is an inherited autosomal-recessive, salt-losing tubulopathies, characterized by hypokalemic metabolic alkalosis. BS is reportedly caused by mutations in genes encoding ion transporters or channels leading directly or indirectly to a loss of function. The gen KCNJ1 encodes the apical renal outer medullary potassium channel (ROMK). The homozygous missense F278C mutant is located in a domain that is involved in ROMK regulation. Mutations within this region may disrupt channel regulation and alter ROMK function. We studied the wild type and F278C mutant to research structural and conformational differences between both of them using *in silico* tools. We have homology modelled the wild type and the mutant variant using the software MODELLER (9v10). Proteins of known structure that share sequence similarity with both proteins were chosen from the protein databank (PDB) using BLASTp. Models were validated using the PROCHECK and ERRAT software. The visualization, analysis and comparative studies were made using Pymol and Chimera program. The comparison of the structural elements obtained from the homology modeling shows significant structural and conformational differences between the wild type and the mutant variant. These differences could be responsible for the functional changes in the F278C mutant variant of the renal outer medullary potassium channel. This work represents the first structural characterization for this outer medullary potassium channel mutant present in Bartter syndrome.

A91

AGING ABOLISHES CIRCADIAN RHYTHMS OF RORα AND ANTIOXIDANT ENZYMES EXPRESSION IN THE TEMPORAL CORTEX OF FREE RUNNING RATS

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The retinoid-related orphan receptor α (RORα) is a transcription factor that binds RORE motifs in the promoter of clock Bmal1 and other target genes. Antioxidant enzymes contribute to the cellular redox state which is crucial for the molecular clock function. Previously, we showed that antioxidant enzymes activity follows a daily variation in the temporal cortex (TC), which was abolished in aged rats. Herein our objectives were: 1) to investigate endogenous rhythms of catalase (Cat), glutathione peroxidase (Gpx) and Nrf2 genes expression as well as RORα protein levels in the rat TC, and 2) to evaluate the aging consequences on those temporal patterns. Three- and 22-month-old male Holtzman rats were maintained under constant darkness for 15 days before the experiment, in order to validate the endogenous nature of circadian rhythms. On the experiment day, TC samples were isolated every 4 h during a 24h period. RORα protein levels were assessed by immunoblotting. Cat, Gpx and Nrf2 mRNA levels were determined by RT-PCR. Specific software was used to analyze circadian rhythms. We observed circadian endogenous rhythms of RORα, Cat and Gpx expression in the TC of free running young rats (Chronos fit, $p<0.05$, $p< 0.001$ and $p<0.05$, respectively). A bioinformatic analysis revealed Ebox, Ebox-like and RORE sites in the Cat, Gpx and Nrf2 genes' regulatory regions. RORα rhythm's acrophase occurs at the second half of the subjective day, preceding Cat and Gpx mRNA peaks. Consistent with our previous results, aging abolishes RORα, Cat, and Gpx circadian rhythms in this brain area. Interestingly, Nrf2 gene expressions become rhythmic in the TC of aged rats. Our observations would contribute to the knowledge of circadian alterations in the TC of the aged brain.

A92

Coxiella burnetii INTERNALIZATION IS REGULATED BY CHOLESTEROL AND DYNAMINS IN EPITHELIAL CELLS

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Cholesterol is an essential component of mammalian cell membranes. The level of cellular cholesterol results from the tight control of cholesterol neosynthesis and uptake. Dynamin proteins are a family of GTPases whose canonical function is the release of nascent endosomes from the plasma membrane; nevertheless, several works have also shown their participation in the metabolism and trafficking of cholesterol in different cell lines such as HeLa cells. A wide variety of bacteria preferentially interact with cholesterol-rich membrane microdomains, called "lipid rafts", to invade host cells. C. burnetii, a causal agent of Q Fiber, is an intracellular pathogen that enters into non-professional phagocytes through a mechanism poorly characterized. The goal of this work was to determine the role of cholesterol and dynamins in the internalization process of C. burnetii into non-professional phagocytic cells. To analyze the role of cholesterol in the internalization process, a cholesterol depleting agent Methyl-β-cliclodextrin (MβCD) and the inhibitor of cholesterol transport U18666A were used. HeLa cells seeded on coverslips

in 24-well plates were infected for 6h with C. burnetii in the presence of MβCD or U18666A. Then, the cells were processed for indirect immunofluorescence and analyzed by confocal microscopy to quantify internalized bacteria. A significant decrease in the uptake of C. burnetii was observed in cells treated with MβCD. Internalization was lower in cells treated with U18666A than those treated with MβCD. To analyze the participation of dynamins in the bacterial entry into host cell, HeLa cells were infected in the presence of the inhibitor of dynamin Dynasore. The C. burnetii internalized by cells treated with the inhibitor decreased by 60% in comparison of untreated cells. To confirm the participation of dynamins in the internalization process, HeLa cells were transfected with pEGFP-dynamin I-WT (Wild Type), pEGFP-dynamin I-K44A (dynamin negative mutant defective in GTP binding and hydrolysis) or pEGFP-vector (control) and then infected with C. burnetii for 6h. While the overexpression of dynamin I WT showed a slight increase in the internalization, the overexpression of the mutant K44A produced a significant decrease. These results suggest that cholesterol and dynamins are involved in the internalization of C. burnetii by non-professional phagocytic cells.

A93
SREBP 2 (STEROL- REGULATORY- BINDING- PROTEIN 2) PATHWAY IN TESTIS AND LIVER OF HYPERCHOLESTEROLEMIC RABBITS PROMOTED BY FAT DIET. PROTECTION BY OLIVE OIL ADITTION

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Fat diet promotes hypercholesterolemia and infertility in adult male rabbits without obesity. This metabolic-reproductive disorder is corrected with a decrease in fat diet accompanied with the intake of extra virgin olive oil (AOVE). The underlying cellular and molecular mechanisms are still under research. The general regulation of cholesterol also relies on intracellular regulation. The cellular / molecular balance of cholesterol levels depends on the SREBP 2 pathway and its dependent molecules, such as LDLr or Methyl-Coenzyme A, which are involved in *de novo* synthesis of this lipid. The high fat diet promoted a decrease in the expression of SREBP 2 (mRNA and protein), in rabbits after 3 months compared to control animals that consumed balanced diet for rabbits (GEPSA ®), in both liver and testicle. This drop in expression is more pronounced at 6 months of diet. Interestingly, the addition of AOVE to the diet promoted an increase in the expression of SREBP 2 (both mRNA and protein) in both tissues, hepatic and seminiferous tubule. In addition, it was possible to observe the subcellular localization of the SREBP-2 protein in testes and hepatocytes by immunofluorescence (IFI). In the testicle, the SREBP 2 protein was located in interstitial cells and, depending on the stage of the seminiferous epithelial cycle, Sertoli and/or germline cells. We can infer that dietary fat / circulating cholesterol affects the hepatic and testicular levels, generating alterations such as fatty liver and germinal disorders with abnormal sperm cells observed in previous studies. These changes are due to an imbalance of the intracellular cholesterol regulatory pathway. The AOVE contributes in the correction of the hepatic and testicular histo-physiology.

A94
EVALUATION OF THE ACTION OF NEW MOLECULES ON THE REDOX SYSTEM OF *Trypanosoma cruzi*

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Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*) and affects to millions of people worldwide, mostly in Latin America. Despite its sanitary importance, there are currently only two drugs available for its treatment: benznidazole and nifurtimox, both exhibiting serious adverse effects on patients. To complete its life cycle, *T. cruzi* undergoes drastic cellular morphological and biochemical changes as it passes from extracellular epimastigote and trypomastigote forms, to intracellular/tissue non-motile stage, as well as it faces extreme fluctuations such as oxidative environment. It is known that antioxidant defense system in the trypanosomatids is different from mammalian cells, since the parasites have exclusive molecules and reducing enzymes. Due this, parasite redox pathway is an attractive target for antiparasitic therapies. Our study is focused on the action mechanisms of the natural sesquiterpene lactones (STLs) dehydroleucodine (DhL), and here we expanded the study to derivatives: DC-X1, DC-X2, DC-X3, DC-X4, DC-X5, DC-X6 and DC-X7 obtained by chemical substitutions. We have previously described to DhL as a leishmanicida drug by oxidative stress generation. In this work, it is shown an antiproliferative effect of DhL and its chemical derivatives, being the most actives DC-X1 and DC-X3 on *T. cruzi* epimastigotes. This effect was blocked by 3 mM reduced glutathione, suggesting that compounds are reactive upon intracellular sulfhydryl groups. Moreover, *T. cruzi* overexpressing reducing enzymes, showed a protective effect against these compounds. Consistent with these results, the active STLs induced

ROS generation in the wild type parasites, and this effect was at lesser extent in *T. cruzi* overexpressing reducing enzymes. These results indicate that the induction of oxidative stress is, at least, one of the mechanisms of STLs antiparasitic action.

A95
A PENTAMERIC THREE-DIMENSIONAL MODEL FOR *T. cruzi* RIBOSOMAL STALK P PROTEIN COMPLEX

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Trypanosomacruzi is the protozoan parasite that causes of the Chagas´ disease in humans. Ribosomal proteins are involved in important metabolic processes and the development of Chagas´ disease pathology. The stalk is formed by the P proteins: TcP0, TcP1α, TcP1β, TcP2α and TcP2β. TcP0 protein has 34 kDa, TcP1 and TcP2 proteins are smaller with a molecular weight of 10 kDa. The three dimensional structure of *T. cruzi* P proteins and the stalk complex TcP0-TcP1α-TcP1β-TcP2α-TcP2β have not been solved to date. Homology models for the five TcP proteins have been obtained in our laboratory using the Modeller program. TcP0 model shows four structural regions: a N-terminal globular domain, an alpha domain, a disordered region and a C-terminal negative tail. The TcP small 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 α–domains for the five P proteins are implied on the protein-protein interactions for the pentameric complex formation. Analysis of the molecular electrostatic potential and the hydrophobic surfaces of this domain on the P proteins suggest a model for the formation of a pentameric complex. Complementarily, observations of the three-dimensional structure of the alpha-domains allowed proposing a structural model for the pentameric complex. Several programs such as Frodock and Z-dock were used to study binding. PIC and PISA servers were used to identify protein-protein interactions that may be involved in conformational complex stability. An association model for the *T. cruzi* stalk complex proteinsis proposed in this work. It represents an important advance for the structural characterization providing clues to understand its functional properties.

A96
CONSEQUENCES OF AGING ON THE CIRCADIAN VARIATION OF LIPID PROFILES

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Aging is associated with declines in multiple physiological systems, resulting in increased risk for the development of disease such as the metabolic syndrome (MetS), among others. MetS is defined as a cluster of metabolic abnormalities including central obesity, hypertension, highserum triglycerides, decreased high density lipoprotein (HDL), cholesterol and glucose intolerance.Epidemiologic studies have demonstrated a marked increase in prevalence of MetS in younger people (20–65 years).Also, aging is associated to alter circadian rhythms. The objective of this work was to investigate the consequences of aging on the circadian variation of lipid profiles. Holtzman rats3-month-oldand 22-month-old(aged) groups were maintained under constant darkness conditions, during 10 daysbefore the experiment. On the experiment day, they were sacrificed every 4hs and blood samples were collected.Serum triglycerides, total cholesterol and HDLc levels were determined by colorimetric assays. Triglycerides and total cholesterol levels display an endogenously-controlled temporal variation. We also found HDLc levels do not vary throughout a 24h periodunder constant darkness conditions, in serum of young rats. Noteworthy, aging abolished circadian rhythms of triglycerides and total cholesterol levels. This could be a biochemical basis of altered temporal patterns of lipid profiles in aged persons.

A97
ESTROGENS MODULATES THE EXPRESSION OF RABS PROTEINS IN ENDOMETRIAL CELLS INFECTED WITH *Chlamydia trachomatis*

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Sexually transmitted diseases (STDs) are highly prevalent pathologies. There are more than 30 bacteria, viruses and parasites that can cause sexually transmitted infections. Among them, Chlamydia trachomatis (CT) is the most frequent causative agent of STD in the world. It is an obligate intracellular gramnegative bacterium that multiplies into a single non-acidic, non-degrading vacuole, called inclusion. This bacterium prevents degradation in the phagolysosome route and, at the same time, sequesters the biosynthetic route to obtain nutrients from the host cell. CT disrupts vesicular transport by hijacking certain host traffic controllers, such as Rab proteins. In addition, chlamydial infections mainly affect women of reproductive age. Therefore, sex hormones may play a role in the course of CT infection. At this time, it is unknown whether estrogen could regulate the expression and / or function of the Rab protein and its effectors in CT-infected cells. We analyzed whether the expression and / or

localization of Rab11, 14 and its interactive FIP2 protein could be affected by sex hormones in CT-infected cells, using advanced confocal microscopy, Western blotting and real-time PCR techniques, we observed that the The presence of estrogen in cells infected with CT decreases the expression of both Rab14 and its effector FIP2. Our results show that the hormonal microenvironment affects the course of Chlamydia infection, Rab proteins and their effectors. These findings could open new scenarios that justify new therapeutic options for the treatment of chlamydial infections.

A98

SECONDARY STRUCTURE OF DOMAINS IV AND V OF THE 16S rRNA GENE OF *Heleobia hatcheri* (Pilsbry, 1911) (Gastropoda: Tateidae)

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Phylogenetic studies aim to recover the accumulated historical signal in the evolution of nucleotide or amino acid sequences. The alignments of these sequences represent hypotheses that allow us to infer genealogies in a set of species. Thus, an adequate alignment is essential to reconstruct evolutionary relationships. Mitochondrial markers commonly used in molluscs to infer such relationships include rRNA genes. The alignment of sequences of this type of genes is usually difficult due to the presence of insertion/deletion events, which increase in number with the inclusion of divergent species, evidenced by multiple ambiguous positions in the final alignment produced. These difficulties can be overcome through the use of complementary information such as the secondary structure of rRNA. Based on the complementarity of bases along the molecule, the ribosomal RNA sequences form complex structures composed of helical stems connected by loops. Consequently, at a structural level, in these molecules it is feasible to recognize sections, subdivided in turn into domains. Here we present a structural model of the fourth and fifth domains of the 16S rRNA gene of *Heleobia hatcheri* (Caenogastropoda, Rissoidea). The partial sequences of the 16S rRNA gene were obtained using universal primers from a specimen of *H. hatcheri* collected in the town of Uspallata (Mendoza, Argentina). The amplified product was sequenced in both directions in the laboratories of the Genomics Unit of the Institute of Biotechnology (INTA, Castelar).Once the consensus sequence was obtained, and after alignment with reference sequences available in GenBank, the molecule was folded manually by direct comparison with reference models for molluscs. The secondary model derived here represents the first one for the *Heleobia* species and it is expected to allow future comparative approaches of structural changes in this genus, as well as to complement the phylogenetic analyzes of the group.

A99

PRELIMINARY STUDY ON PREVALENCE OF CLASS 1 INTEGRONS AND EXTENDED-SPECTRUM β-LACTAMASES (ESBL) IN CLINICAL ISOLATES FROM MENDOZA HOSPITALS, AND TUNE-UP TOOLS FOR SURVEILLANCE OF INTRAHOSPITAL INFECTIONS

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The dissemination of genes for resistance to antibiotics by genetic elements, especially those related to class 1 integrons, is a growing concern worldwide due to the substantial increase of strains resistant to multiple drugs, especially those resistant to carbapenems and/or third and fourth generation cephalosporins. Resistance cassettes dissemination studies is an extremely relevant issue that concerns antibiotic administration policies. In this work, we determined the occurrence of class 1 integrons and extended-spectrum beta-lactamases (ESBL) in clinical isolates from local hospitals, using PCR techniques. On the other hand, nosocomial infections are among the most common complications in intensive care units. Bacteria are capable of surviving the adverse conditions found at the hospital environment, and the use of antibiotic pressure turns them multi-resistant to various antimicrobials. Therefore, we were interested in acquiring adequate and flexible tools to identify possible reservoirs of strains that propagate clonal infections inside the hospital. For this aim, we tune-up the Random Amplification of Polymorphic DNA (RAPDs) in order to be able to quickly identify infection focus, making possible an efficient control outbreak of clonal infection within hospitals.For this aim, we tune-up the Random Amplification of Polymorphic DNA (RAPDs) in order to be able to quickly identify infection focus of P. aeruginosa, making possible an efficient control outbreak of clonal infection within hospitals. We found that only 2 of the 4 primers tested for RAPDs were optimal for reproducibility. Also, our preliminary studies based on the identification of class 1 integron show that only 4 of the 12 strains obtained so far (33%), are positive for this integron. This rate of occurrence of class 1 integron is in accordance with the ones obtained in other countries such as the United States, Brazil and the European Union.

A100
CELLS WITH PHAGOCYTIC ACTIVITY ON THE WALL OF SEMINIFEROUS TUBULES OF MOUSE TESTIS.

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In the wall of the seminiferous tubules (TS), of rodent's testis, the peritubular myoid cells (MP) form a monolayer providing peristaltic action and structural support. Recently macrophages located in the wall of mouse TS, known as peritubular macrophages (MacP) were described as cells capable of releasing colony stimulating factors and enzymes involved in the biosynthesis of retinoic acid for the differentiation of spermatogonia A. However, there are no studies detailing the interaction of MacP with MP cells and whether they have phagocytic activity. Due to this, we set out to locate, characterize and quantify MacPs in the wall of TS mouse testis and determine if they present phagocytic activity of the germinal epithelial cell. Microscopy images showed that MacPs have a cytoplasm with long projections that extend up to 100 μm long. They adhere to the outer face of the MP cells and they are observed introducing their extensions between the spaces of the adjacent MP cells. The cell count revealed that in stage VII-VIII of the TS, there are 6.1 ± 0.62 MacP per 40,000 μm² of the surface. Interestingly, consecutive confocal planes showed that 13.5 ± 2.74% of the MacPs contacted and enveloped undifferentiated spermatogonia A and 7.56 ± 3.58 % of MacP had cytoplasmic granules that were labeled with the antibody that identifies spermatogonia A (anti-E-cadherin). By labeling with an anti-Lamp-1 antibody, it was found that cytoplasmic granules corresponded to the phagocytic compartment pathway. These results indicate that in the TS wall MacP are activated and phagocyte spermatogonia A. Tests are being carried out to determine if other cells of the TS germinal epithelium are also phagocyted by MacP.

A101
ANTIPROLIFERATIVE EFFECT OF CHLOROFORMAL EXTRACTS (MEDIUM POLARITY) OF *Artemisia mendozana* DC *mendozana* ON TUMOR CELLS B16F0.

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The basic pathophysiology of cancer includes aberrations in the different points of the cell cycle. Due to the increasing incidence of cancer worldwide, there is an intense search for new therapeutic strategies to treat this disease. In this field, many investigations have focused on exploring the action of compounds of plant origin. The ajenjo, *Artemisia mendozana DC mendozana*, is a plant belonging to the Asteráceas family, typical of the Mendoza piedmont, used as a medicinal plant with antispasmodic, antifungal properties, among others. By means of column chromatography, 120 fractions (extracts of medium polarity) were collected and then grouped according to the similar chromatographic profile, finally obtaining 9 fractions (F 1-9). Their major compounds were identified, being: terpene compounds for F1-3, sesquiterpene lactones (LS) for F4-6, LS and flavonoids for F7 and phenolic compounds for F8-9. We analyzed the *in vitro* effect of F1-9 on the proliferation of B16F0 cells (obtained from a murine melanoma) for which the cells were cultured with DMSO (vehiclecontrol) or with 0.1 mg/mL of the fractions dissolved in DMSO.The growth index (CI) ± SE was calculated from 5 trials for 0 to 72 h and analyzed statistically. After 72 h of culture, the CI of the control was 9.6 ± 0.49 and those treated for F1 6.18 ± 0.63, F2 5.76 ± 1.53, F3 4.42 ± 1.32, F4 4.74 ± 1.15, F5 5.12 ± 1.65, F6 8 ± 1.57, F7 7.26 ± 0.69, F8 6.88 ± 0.74 and F9 5.32 ± 1 , 53 being significant with $p < 0.001$ for F2, F3, F4, F5 and F9. The results show that fractions obtained from the ajenjo plant significantly inhibit the proliferation of B16 F0 cells at low doses such as 0.1 mg/mL.

A102
TAMOXIFEN ALTERS LYSOSOMES OF BREAST CANCER CELLS BY A MECHANISM INDEPENDENT OF ITS ANTI-ESTROGENIC ACTIVITY

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Breast cancer is one of the most important causes of morbidity and mortality worldwide. It has been shown that the cells of some tumors have an increased lysosomal biogenesis in response to metabolic alterations, which also has an impact on the integrity and/or lysosomal functionality, showing increased levels of lysosomal proteases, such as cathepsin D (CatD). It has been demonstrated that this enzyme induces apoptosis when is released into the cytoplasm. Since the lysosomes could play a role either as initiators or executors of apoptotic processes when the membrane integrity is altered, this organelle could be taken as a potential therapeutic target against tumors. In breast cancer cell lines positive to estrogen receptor RE (REα), CatD is positively regulated by this hormone, while in cell lines negative for REα the enzyme is constitutively overexpressed. Tamoxifen (TAM) is one of the most common anti-estrogenic drugs used in breast cancer therapy. It interacts with RE and inhibits transcriptional

activity in the mammary gland. The aim of this study was to evaluate the effect of estrogens and tamoxifen on lysosomal acidification and CatD processing in breast cancer cells. Mammary cell lines MCF-7 (tumorigenic expressing REα), MDA-MB231 (tumorigenic non-expressing REα) and MCF-10A (non-tumorigenic) were treated in the presence or absence of 17-β-estradiol and/or TAM. For quantification of acidic lysosomes, cells were treated with Lysotracker. Cultures were subjected to immunoblot analysis and fluorescence microscopy. As expected, TAM blocked the effect of estrogen on CatD processing in MCF-7 cells. However, TAM used alone, also altered CatD processing and decreased the number of acidic lysosomes in both cell lines. Neither effect of TAM was observed on MCF-10A cell line. In addition, a decreased level of another lysosomal protein, GM2AP (related to the development of tumors), was observed in the cells due to TAM. All these results suggest that TAM has additional effects independent of its anti-estrogenic activity, possibly due to lysosomotropic action.

A103
AGING MODIFIES TEMPORAL PATTERNS OF OXIDATIVE STRESS AND PROINFLAMMATORY MARKERS IN THE RAT PREFRONTAL CORTEX

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Aging is often accompanied by a decline in cognitive function in conjunction with a variety of neurobiological changes, such as neuroinflammation and oxidative damage. The brain is vulnerable to oxidative damage because of its large amount of polyunsaturated fatty acids and relative deficiency in antioxidative defense mechanisms. Recent research has shown that the expression of proinflammatorycytokines increases with aging in brain. Besides cognitive deficit, older personsshow alterations in their circadian rhythms.The objective of this work was to investigate the consequences of aging on 24h patterns of oxidative stress parameters (TBARS and protein carbonyls)as well as TNFα, BMAL1 and RORα protein levels, in the rat prefrontal cortex (PFC). Holtzman rats from young (3-month-old) and aged (22-month-old) groups were maintained under constant darkness conditions, during 10 days before the experiment. Tissues samples were isolated every 6 h during a 24h period. TBAR‘s levels were measured by a colorimetric assay and protein carbonyls by ELISA. Protein levels of TNFα, BMAL1 and RORα were determined by immunoblotting. As expected, we observed an increase in the protein carbonyls levels in the PFC ofaged rats. We also found that lipoperoxidation as well as protein levels of TNFα, BMAL1 and RORα follow a robust circadian rhythm in this tissue.Interestingly,aging abolishes the oscillation of endogenous circadian patterns oflipoperoxidation, TNFα, BMAL1 and RORα protein levels.These findings may constitute, at least in part, the molecular basis of the relationship between TNF-mediated neuroinflammation and altered circadian rhythms of protein clock in aged individuals.

A104
BENEFICIAL EFFECTS OF AQUEOUS EXTRACT OF *Tessaria absinthioides* AND *Prosopis strombulifera* ON HUMAN ENDOTHELIAL VASCULAR CELLS

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The use of herbals in the treatment of disease is a well-established practice in traditional medicine. *Tessaria absinthioides* (*Ta*) and *Prosopis strombulifera* (*Ps*) are native plants from South-America with reported ethnopharmacological uses. Despite recent scientific reports about plant-derived compounds effects, there is not a well-conducted research about its effects on cardiovascular disease (CAD). The aim of this work was to analyze the potential antioxidant effects of aqueous extracts from leaves of *Ta* and *Ps* on human endothelial cells (HUVEC) isolated from the human umbilical cord. After cell exposure to different concentrations of *Ta* and *Ps*, intracellular reactive oxygen species (ROS) generation was measured by lucigenin-enhanced chemiluminescence and dihydroethidium assays. Total antioxidant status (TAS) of treated cells was measured by colorimetric assay. qRT-PCR was utilized to examine NADPH oxidases (NOX) subunits mRNA expression. Angiotensin II-induced HUVEC intracellular ROS generation was significantly diminished by the addition of 1 and 4 μg/ml of *Ta* or *Ps* extracts. *Ta* and *Ps* extracts (1 and 4 μg/ml) significantly increased TAS in HUVEC (*Ta* in a dose-dependent manner). Ps (4 μg/ml) was able to downregulate NOX2 ($p< 0.05$) and NOX5 ($p<0.001$) expression. Ta (4 μg/ml) could diminish NOX2 ($p<0.01$) and upregulate NOX5 ($p<0.0001$) expression. On the other hand, Ps ($p<0.01$) and Ta ($p< 0.001$) increased NOX4 mRNA expression. In conclusion, these herbal extracts may reduce antioxidant status on HUVEC which are one of the main therapeutic targets in the treatment of CAD.

A105
CIRCADIAN RHYTHMS IN EX VIVO SPLEEN MACROPHAGES

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Accumulating evidence links the immune system and circadian regulation. Spleen macrophages (MΦ) play acritical role in innate immunity, acting as sentinels to fight against circulating pathogens, and orchestrating thedevelopment of the specific acquired immune response. However, the temporal organization of those processesand the significance of circadian regulation in the spleen, have not been completely elucidated yet.Communication between the central clock in the suprachiasmatic nucleus and peripheral tissues occurs throughthe autonomic nervous system. Other authors reported daily oscillation of norepinephrine in spleen. Based on this,our general objective is to study the circadian rhythms of clock and clock-controlled genes in the spleen MΦ. Male Holtzman rats were maintained under 12h-light: 12h-dark conditions, and *ad-libitum* food/water intake,from weaning. Sixteen-week-old animals were euthanized at different times during a 24 h period (ZT2, ZT6,ZT10, ZT14, ZT18 and ZT22) and spleens were aseptically removed for *ex vivo* cultures of MΦ. In order tooptimize these cellular cultures in our lab, we tested different incubation times (2 h and 24 h) and the number ofinitial total spleen cells (2 x 106; 4 x 106 and 8 x 106 splenocytes). Once we defined optimal experimentalconditions of our model, BMAL1 protein levels were analyzed from adhered cells by western blot, and normalized
againstβ-actin. We observed BMAL1 displays a circadian rhythm ($p < 0.001$) in the spleen MΦ of rat. Therhythm's parameters were: mesor 0.92 ± 0.05; acrophase 08:52 ± 01.58; amplitude 0.27 ± 0.05. BMAL1 circadianrhythm in the *ex vivo* MΦ, would impact in the temporal organization of the immune activity of these cells.Probably, BMAL1 peak occurring at middle of the day triggers the expression of clock-controlled genes involvedin the defense of the organism against pathogens, during the diurnal (activity) period.

A106
FOREIGN MITOCHONDRIAL GENES IN A PARASITIC PLANT

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Parasitism is a highly successful life strategy originated independently in 11-13 lineages during the evolution of flowering plants. Parasitic plants are characterized by their ability to feed directly from other plants, invading the roots or stems of their plant hoststhrough a specialized structure called haustorium. This vascular connection allows the passage of water, nutrients, pathogens andnucleic acids. Consequently, parasitic relationships facilitate the exchange of genetic information, a process known as horizontalgene transfer (HGT). HGT refers to the movement of genetic information between unrelated organisms, and most cases of plant
HGT involve the mitochondria, in contrast to nuclear and chloroplast compartments. Mitochondrial sequences of the donor are transferred to the mitochondrial genome of another angiosperm and incorporated through homologous recombination. Parasitic plants are particularly susceptible to this phenomenon and represent an excellent system to examine the role of HGT in the evolution of the mitochondrial genome. In this study, we sequenced and analyzed the mitochondrial genome (mtDNA) of theholoparasite Ombrophytum subterraneum (family Balanophoraceae). Total DNA extraction and massive sequencing with Illuminatechnology were performed. The assembly of the DNA sequences was carried out with Velvet Assembler and Consed v29 based oninformation of paired-end reads from the Illumina dataset. The mtDNA is composed of 56 circular molecules (1-27kb in length)totaling 713,111 bp. This multipartite chromosomal architecture was recently described in a close relative, Lophophytum mirabile.The mtDNA was annotated in Geneious using BLAST. The O. subterraneum mtDNA encodes 2 ribosomal RNA, 14 transfer RNA,and 30 protein genes. The majority of these genes were present in multiple copies, resulting in a total of 65 genes. Only 29chromosomes contain complete mitochondrial genes, while the remaining are devoid of known genes. Maximum Likelihoodphylogenetic analyses were performed for 24 protein genes to assess their evolutionary history. About 37.5% of them wereassociated with species of the Family Asteraceae, indicating that they were transferred from asterid hosts. Most foreign genes coexistwith native homologs in the mitochondrial genome of O. subterraneum. Additional studies are required to understand whichcopy is functional in O. subterraneum. Comparative analyses of the mitochondrial genomes of O. subterraneum and L. mirabile suggest that each holoparasite acquired foreign sequences from their hosts in independent events.

A107

STUDY OF SREBP1 AND SERBP2 IN PERIPHERAL BLOOD MONONUCLEAR CELLS FRON HYPERCHOLESTEROLEMIC RABBITS

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Peripheral blood mononuclear cells are a possible biomarker that could reveal molecular alterations before the development of the disease. Therefore, the objective of the present investigation is to study molecular and genetic changes that indicate metabolic modifications even with normal biochemical values. SERBP1 and SERBP2 are proteins involved in lipid metabolic. These molecules can be expressed in peripheral blood mononuclear cells. This allows study tissue changes without resorting to biopsies. In this study, one control group of New Zealand rabbits was fed with a balanced feed (C) and another group received the same balanced feed supplemented with 17% fat (F). These animals did not receive fructose overload, maintaining constant concentrations of carbohydrates and protein in both groups. In biochemical tests from both groups were observed similar levels of glucose (C group: 140.7 + 28.4 mg/dl / F group: 118.3 +12.0 mg/dl) and triglyceride (C group: 144.1 +15.5 mg/dl / F group: 135.6 +8.3 mg/dl), while F group showed increased levels of cholesterol (42.8 +21.6mg/dl) compared with C group (27.1 +4.5 mg/dl). However, there is variability in the cholesterol values because some animals of the F group do not experience significant increment despite the intake of fat. This interesting finding leads to the hypothesis that changes in lipid metabolism can be examined by the expression of different genes early. As preliminary results, we observed by immunohistochemistry the presence nuclear of SREBP1 and SREBP2 in lymphocytes of the F group, while in C group was not observed immunoreaction or cytoplasmic signal. This result is indicating an activation of the lipid metabolism before being able to observe changes at a biochemical level.

A108

EFFECTS OF HYPOTHYROIDISM ON THE VISCERAL ADIPOSE TISSUE OF RATS

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Abnormal thyroid stimulating hormone (TSH) and thyroid hormones levels are frequently associated with changes in body weight (BW) and in the functioning of adipose tissue. Our aim was to characterize the influence of Hypothyroidism (HypoT) on the rat visceral adipose tissue (VAT). Sprague Dawley female rats (55 days old) were divided into two groups: hypothyroid rats (HypoT, 0.01% 6-N-propyl-2-thiouracil in drinking water, n=10) and untreated control euthyroid rats (EUT, n=10). BW and body length were assessed. On day 70 of the experiment, animals were decapitated. Trunk blood was collected for measurement of serum hormone concentrations. The VAT was removed for histological, western blot and real-time PCR analysis. We evaluated the morphology and differentiation of adipocytes, and the expression of adipokines and their receptors in the VAT. HypoT showed decreased BW and body length. Serum growth hormone, estradiol, and progesterone levels were significantly lower in HypoT rats. The percentage of body fat, the amount of lipids and size of adipocytes were similar to EUT. Nevertheless, leptin and adiponectin levels were altered by HypoT. Finally, the expression of Perilipin A1 and HSL, both indirect biomarkers of adipocyte maturity, and UCP1 and PRDM16, both biomarkers of browning adipocytes, were significantly lower in HypoT. These findings suggested that adipocytes showed a less differentiated state; despite the fact that the expression of PPARγ, a master regulator of adipocyte differentiation, was increased in the VAT of rats with HypoT. In addition, the increase of MCT1 expression indicated that the metabolic demands on the adipocyte tissue were altered in HypoT. We concluded that hypothyroidism in rats induced a decrease in body size without changing the percentage and the morphology of the VAT, but interfering with white adipocytes differentiation.

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A109
DEFICIENCY OF VITAMIN A OF 6 MONTHS: EFFECT ON PROTEIN OXIDATION AND ITS CORRELATION WITH THE LIVER GLUCOSE LEVELS.

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The vitamin A (VA) is an essential nutrient for cell growth and differentiation. Previous work done in our laboratory has shown that vitamin A deficiency modifies oxidative stress parameters and lipid metabolism in heart and aorta. Oxidative stress is the exposure of living tissue to various sources that produce a rupture of the balance between pro-oxidant factors and antioxidant mechanisms. This generates alterations in the structure-function relationship of any organ. The objective of this work was to study of the parameters of the oxidative stress, metabolism of lipids and glucose in liver of ratsvitamin A deficiency (VAD). We determined reactive species to the tribarbituric acid (TBARS), carbonyls groups of proteins by ELISA, quantification of tissue and serum lipids and serum glucose by enzymatic assays using an autoanalyzer CB350i (Wiener Lab). Statistical analysis: One-way Anova, tukey post test, regression and correlation analysis with Graphpad Prism. P <0.05 was considered significant.We worked with Wistar female rats separated at weaning in three groups: one was fed a sufficient diet in VA (8 mg of retinyl palmitate / kg) for 6 months (+A group), another group was fed a VA deficient diet for 6 months (-A group) and the last one was fed with a VA deficient diet for 150 days and then was fed with a VA sufficient diet for 30 days (R group).No differences were observed in TBARS levels. The carbonyls groups increased between the +A group and the -A and R groups ($p < 0.05$). A negative correlation was found between carbonyls and glucose levels ($p = 0.0465$; R^2:0.5103) and between carbonyls and HDLcol levels from liver ($p=0,033$; R^2: 0.59).The results suggest that the absence of vitamin A for a period of 6 months would generate protein oxidation and that this does not revert with the time of refeeding. The analysis of the correlations would suggest that the deficiency would cause a decrease in the levels of HDLcol not only by reduced biosynthesis but also by an increased catabolism in the liver. This could cause a decrease in serum glucose by depuration through this route, as a metabolic alternative at low sensitivity and insulin release. The decrease of glucose would lead to the increase of protein oxidation to be used in gluconeogenesis, as an alternative metabolic compensation.

A110
SHIGA TOXIN 2 FROM EHEC PRODUCE INFLAMMATORY RESPONSE THROUGH MICROGLIAL REACTIVITY AND Gb3-pERK SIGNALLING

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Shiga Toxin 2 (Stx2) is released during enterohemorrhagic *Escherichia coli* (EHEC) infection. Stx2 causes fever, hemorrhagic colitis, hemolytic uremic syndrome and neurological dysfunctions through its cell membrane receptor globotriaosylceramide (Gb3). Previous reports from our group have confirmed that proinflamatory agents play a critical role in the development of the pathology. The aim of this study was to determine the cell signalling mechanisms that occur in an *in vivo* and *in vitro* rodent experimental inflammatory model triggered by Stx2, validated by different methodological approaches. Male Swiss mice were injected intravenously with control (saline solution) or Stx2 (1ηg per mouse).After 2 days, each group was submitted to hole-board test to measure anxiety, stress, neophilia and emotionality. Besides, fixed hippocampi were subjected to immunofluorescence (IF) with anti-GFAP and anti-Iba1 to determine reactive astrocytes and microglial score activation respectively. In addition, another set of hippocampi were homogenized for western blot analysis to determine pERK activation at 2, 6, 12 and 24 hours. On the other hand, primary microglial cell (MG) cultures were incubated with either DMEM or 100ηg/ml of Stx2to test by IF the uptake of Stx2, microglial reactivity and the expression profile of Gb3 receptor at day 2. Hippocampal deterioration was observed after Stx2 treatment, which was correlated with memory failure. GFAP expression levels were increased in Stx2 treated mice in comparison to the control ones (0.19±0.02 control vs 0.52±0.02 Stx2, in IOD, $p<0.05$), while microglial activation score was increased in Stx2 treated mice in comparison to control ones. The expression of pERKwas decreased in Stx2 treated mice in comparison to controls at 24 hours (0.96±0.02 control vs 0.49±0.02 Stx2, $p<0.05$). Finally, MG resulted to be sensitive (12.53±0.89 Stx2, in IOD, $p<0.05$)and responsiveness (1±0.00 control vs52.01±1.02 Stx2, in IOD, $p<0.05$)to the toxin as it was determined by Iba1 expression level, and Stx2 incubation changed the distribution of Gb3 expressionin MG. In conclusion,Stx2 produced an inflammatory response both *in vivo* as *in vitro* through Gb3-pERK signaling pathway.

A111
HYPOGLYCEMIC AND ANTIOXIDANT EFFECTS OF MULINUM SPINOSUM IN MICE WITH METABOLIC SYNDROME INDUCED BY HIGH FAT-FRUCTOSE DIET

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Mulinum spinosum, popularly known as "neneo", has a broad distribution in the Patagonian steppe. Traditionally, an aromatic bitter decoction prepared from the root of this plant is used for different purposes. Due to the widespread utilization of the plant as a traditional medicament, the present work investigated the effects of its consumption in an animal model of Metabolic Syndrome (MS). Neneo roots were dried, crushed and used to prepare an infusion according to the traditional method. To generate the MS model, C57/BL6 adult male mice were fed with a 20% w/v fructose (Fru) in drinking water and a 30% w/w high fat diet (HFD) over a period of 5 weeks. After MS was confirmed by intraperitoneal glucose tolerance test (IPGTT), the neneo infusion was administered (8g/Kg/day) together with Fru-HFD during 12 weeks. At the end of the exposure period, mice were anesthetized and sacrificed by cardiac puncture to obtain blood samples. The Fru-HFD group significantly increased body weight, total serum cholesterol, HDL-col, LDL-col and serum lipid peroxidation (MDA) compared to control group. The treatment with neneo during 12 weeks, decreased glycemia and MDA compared to Fru-HFD group ($p < 0.05$).Our study showed that in our conditions neneo infusion has antioxidant and normoglycemic effects in a MS animal model. The possibility of evaluating the actions of neneo would provide experimental data that, together with the empirical understanding transmitted by popular practices, would increase the knowledge about the medicinal use of this plant.

A112
FOODS WITH A HIGH CONTENT OF ANTIOXIDANTS MINERALS (ZINC, COPPER AND SELENIUM)

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Zinc (Zn), copper (Cu) and selenium (Se) contribute to body antioxidant activity, preventing premature aging and the development of chronic diseases. There are reports in relation to Zn and Cu deficiencies in several foods however the problem is further complicated by the lack of data and the variability of mineral content. Therefore, it is essential to review and complete the existing information referred to the mineral content in food chemical composition, from nutritional software or database tables. This work aims to evaluate the contents of zinc, copper and selenium in Spirulina powder (*Spirulina max*), maca powder (*Lepidium meyenii*), germ of wheat (*Triticum aestivum*), and (*Curcubita maximum*) pumpkin seeds. In order to quantified zinc, copper, and selenium, the samples were treated by acid digestion, and subsequently, the minerals concentrations were quantified by plasma mass spectrometry inductively (ICP-MS). Spirulina showed 11.07 mg of zinc; 2.36 mg of copper and 425.44 µg of selenium, in 100g food. Maca displayed 18.58 mg of zinc; 6.38 mg of copper and 425.44 µg of selenium in 100g. The germ of wheat presented the following results: 25.39 mg of zinc; 3.59 mg of copper and 384.94 µg of selenium in 100 g. Pumpkin seeds had 16.79 mg of zinc; 9.08 mg of copper and 379.02 µg of selenium in 100g. The results show that the incorporation of small amounts of studied food can cover the Dietary Reference Intakes (DRIs) in adults. It is necessary to consider that high mineral content in food not secured its biological utilization or bioavailability. Analyzed food can be used as a dietary supplement to help meet the DRIs concerning some essential minerals, which would help to prevent specific deficiencies of micronutrients and chronic diseases linked to the oxidative stress.

A113
INTERSTITIAL CELLS IN THE PINEAL GLAND OF PREGNANT AND NON-PREGNANT VISCACHAS (*Lagostomus maximus maximus*): A MORPHOMETRIC AND BIOCHEMICAL STUDY

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The pineal gland of mammals undergoes morphological and biochemical changes throughout the gestation period. In viscacha, a seasonal breeding rodent, pregnancy lasts approximately 154 days and three stages can be defined: early, mid, and late pregnancy. The purpose of this study was to analyze morphometric variations in the expression of S-100 protein, glio-fibrillary acidic protein (GFAP) and vimentin in the interstitial cells (IC) of pregnant and non-pregnant viscachas by immunohistochemistry. We also aim to evaluate a probable relation between glandular activity and pregnancy. In this research, 16 females (12 pregnant and 4 non-pregnant) were used. Pregnancy stages were classified according to the number and size of embryos or fetuses (early pregnancy: 2 or more 1- to 3- cm embryos; mid pregnancy two 9 - to -11 cm fetuses; or late pregnancy

2 fetuses > 19 cm). The immunopositive percentage area (%IA) for the studied proteins and the number of immunoreactive cells against the S-100 protein with a visible nucleus (nº IC-S-100) were analyzed. Estradiol (pg/ml) and progesterone (ng/ml) serum levels were also determined by RIA. The results were expressed as means ± SEM. The groups were analyzed using Kruskal-Wallis test. Differences between 2 glandular regions (proximal vs. distal regions) were evaluated by means of the Mann-Whitney test. In the proximal region the %IA-S-100 and %IA-GFAP were significantly higher during middle (19.36 ± 0.54 and 11.09 ± 0.54 %, respectively) and late pregnancy (16.24 ± 1.30 and 8.37 ± 0.69 %, respectively) compared to non-pregnant and early pregnancy viscachas ($p < 0.001$). In the distal region, the expression of these proteins varied significantly according to the pregnancy stage. The non-pregnant ones exhibited the lowest values of %IA-S-100 (1.81 ± 0.38 %) and %IA-GFAP (0.96 ± 0.20 %), whereas in middle pregnancy exhibited the highest values (10.69 ± 1.34 % and 6.49 ± 0.71 %, respectively, $p < 0.001$). Likewise, the nº IC-S-100 varied following the same pattern in the proximal and distal regions. In addition, the comparison between these regions in each pregnancy stage also showed significant differences for the %IA-S-100 ($p < 0.01$ for each pregnancy stage) and %IA-GFAP ($p < 0.01$ for mid-pregnancy and non-pregnant; $p < 0.05$: early and late pregnancy). In the biochemical study, variations in estradiol and progesterone serum levels were reported. For both hormones, the highest values were found in mid-pregnancy (77.50 ± 1.44 pg/ml and 55.05 ± 1.81 ng/ml) compared to previous stages and significantly decreased during late pregnancy (23.75 ± 1.75 pg/ml and 19.03 ± 1.94 ng/ml; $p < 0.05$ and $p< 0.001$). On these grounds, we may conclude that IC undergo changes in relation to ovarian hormone levels and participate in the regulation of glandular activity during this physiological stage. However, further research is necessary to elucidate this relationship.

A114
DIFFERENT ANDROGEN DEPRIVATION PERIODS INFLUENCE ON BLOOD PRESSURE AND HAEMATOLOGICAL PARAMETERS

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Androgen deprivation during elderly has been related with many pulmonary and cardiovascular diseases. We previously demonstrated that the absence of testosterone induces oxidative stress and lipid peroxidation in lung and aorta, and some oxidative stress parameters were significantly increased in castrated rats, returning to the control values after the administration of testosterone (T) and zinc (Zn). There is an increasing interest in the involvement of trace elements such as Zn in the pathogenesis of cardiovasculardiseases.Many enzymes that are involved in the regulation of arterial blood pressure (BP)contain Zn in their structure.Zn has beendocumented to act as an antioxidant, to have membranestabilizingproperties, to block apoptotic cell death, and to beessential for endothelial integrity. We tested the effect of androgen deprivation 30 and 60 days after castration. Wistar male rats (200±20g) were separated in five groups: controls (Co), controls supplemented with zinc (Co+Zn), castrated (Ca), castrated supplemented with testosterone (Ca+T) and castrated supplemented with testosterone and zinc (Ca+T+Zn), for five days. Blood pressure (BP) was measured every 15 days. After 30 and 60 days, rats were euthanizedand hematological parameters were quantified.Concerning to BP, we found significant differences between 0, 15 and 30 days post castration ($p<0.0001$), but after 30 days of androgen deprivation BP were similar in all treatments. Likewise there are significant differences between 0, 15, 30, 45 and 60 days post castration ($p<0.01$). After 45 and 60 days of androgen deprivation, BP did not show changes between treatments. Hematological profile showed a significant decrease in the levels of RBCs 30 days post castration ($p<0.05$) and a significant increase in Co+Zn vs Ca and Ca+T+Zn after 60 days ($p<0.05$). WBCs in Ca+T is significantly increased 30 days post castration vs Co and Ca rats, while a significant decrease in Co+Zn vs all other treatments, occur after 60 days of castration. We can conclude that time impact more in BP than treatments, suggesting an adaptation of this parameter at longer periods. T deficiency impacts on RBC and the changes produced are time dependent, while T and Zn affect WBC in a different way, depending on the treatment and time period.

A115
SHIGA TOXIN 2 CHANGES NEUROTRANSMITTER EXPRESSION OF NEURONS FROM MURINE MOTOR CORTEX AND STRIATUM

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Shiga toxin 2 (Stx2) from enterohemorrhagic *Escherichia coli* (EHEC) causes hemolytic uremic syndrome (HUS) and acute encephalopathy, which may lead to fatal outcomes in patients. Neurological signs of thisdisorder include decerebrate posturing, hemiparesis, ataxia, seizures and changes in the level of consciousness (from lethargy to coma). When neurological symptoms are evidenced mortality rate may rise up to 40%, significantly higher in comparison to that produced by HUS (5%).The motor areas of the brain are frequently affected in patients infected with EHEC. The aim of this study was to determine whether Stx2 changes the expression of neurotransmitters and/or the number of dopaminergic, GABAergic and glutamatergic neurons from the motor cortex and striatum. Swiss male mice (n=4) were treated intravenously with vehicle (control) or1ηg of Stx2(100µl per

mouse). Allanimals wereintracardiallyfixed on the 4th day (day of the injection considered as day 0)and their brains were subjected to immunofluorescence with an anti-tyrosine hydroxylase (TH) antibody to identify dopaminergic axons from substance nigra, anti-GABA and anti-glutamate antibodies to identifymotor cortexGABAergic and glutamatergic neurons. Stx2 increased the striatal expression of TH in comparison to control-treated mice (36.17±5.44 vehicle vs 98.89±2.52 Stx2; in IOD; $p<0.05$).On the other hand, Stx2 reduced the expressions of GABA (2.1±0.24 vehicle vs 1.38±0.09 Stx2; in IOD;$p<0.05$) and glutamate (1.82±0.13 vehicle vs 0.9±0.03 Stx2; in IOD; $p<0.05$) per neuron in motor cortex, while no significant changes were found in the number of GABAergic and glutamatergic positive neurons between control and Stx2 groups. These results may suggest a compensatory mechanism carried out by substancia nigra´s nigrostriatal neurons. The presented results together with previous published ones may indicate that the increased expression of TH compensates a lack of striatal neurons from the extra pyramidal system that suffered from a neurodegenerative process. In addition, the decreased on GABA and glutamate expression in the murine motor cortex could be a consequence of a reduction on neuronal metabolism produced by ribotoxic stress from Stx2 action. We concluded that Stx2 changed the expression of the 3 main neurotransmitters that are responsible for the pyramidal and extra-pyramidal signalling pathways involved with the reported motor alterations in patients and animal models.

A116
EFFECT OF MELATONIN ADMINISTRATION ON THE MALE VISCACHA ADRENAL CORTEX PROLIFERATIVE ACTIVITY

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The adrenal cortex, under control of the hypothalamic-pituitary-adrenal axis, produces specific steroid hormones which are key to ensure physiological adaptation and improvement of survival chances when facing homeostatic challenges. The viscacha (*Lagostomus maximus maximus*) is a hystricomorph rodent that lives in burrows and has nocturnal habits. The adult male viscacha exhibits an annual reproductive cycle synchronized by the environmental photoperiod through the pineal gland and its main hormone, melatonin. The aim of this study was to assess the effect of melatonin administration on the adrenocortical cells proliferative activity in adult male viscachas. For this study, animals were divided in two groups: an experimental group (n=4) that receive two daily injections of melatonin; and a control group (n=4) that receive two daily injections of the diluent. After 9 weeks, animals were sacrificed and adrenal glands processed for light microscopy. Adrenal cortex proliferation activity was assessed trough immunohistochemical detection of the proliferating cell nuclear antigen(PCNA), an S-phase related cell proliferation marker. The number of PCNA-immunoreactive adrenocortical cells (PCNA-ir) was counted per reference area.Differences between the two groupswere evaluated using Mann–Whitney U test. In both experimental and control animals, cell proliferation was observed mainly in the outermost portion of the adrenal cortex, near the zona Glomerulosa and outer zona Fasciculata. In the innermost portion of the cortex PCNA-ir cells were scarce, except in some control animals where proliferation could also be observed in the inner zona Fasciculata. In melatonin administered animals, the number of PCNA-ir was (1.31 ± 0.2) significantly lower than in control animals (2.31 ± 0.16). These results indicate that proliferation in the adrenal cortex of the viscacha occurs mainly in the outermost portion of the cortex as described in other mammal species. Furthermore, melatonin is capable of modulating the adrenal cortex proliferative activity, reducing proliferation under stressful conditions of daily manipulation and subcutaneous injections.

A117
ANANDAMIDE DECREASES BLOOD PRESSURE, NEUROINFLAMMATORY AND OXIDATIVE MARKERS IN A RAT MODEL OF HYPERTENSIVE DISEASE

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Essential hypertension is considered one of the significant risks of cardiovascular diseases; it is frequently related with: upregulation of the renin-angiotensin-aldosterone system (RAAS), over-excitation of brainstem centers, sympathetic hyperactivation, systemic and neural inflammation. Spontaneously hypertensive rats (SHR) are a validated model of hypertension, plus several neurocognitive deficits.To highlight, the endocannabinoid anandamide (AEA) protects neurons from the inflammatory damage, and cannabinoid signaling decreases in brains of hypertensive animals. However, several side effects were described at the central nervous system level by anandamide, while drugs delivery by nano-formulations could reduce it. To assess whether nano-formulated anandamide could produce a decrease in blood pressure, values as well as a reduction of the systemic inflammatory state and the central nervous system. We used adult male rats (n=7) of 250-300 g normotensive (WKY) and hypertensive (SHR), treated or not with nano-formulated AEA in polycaprolactone (AEA/PCL), at a weekly dose of 5 mg/Kg IP, for four weeks. Systolic blood pressure was obtained by the tail-cuff method. Plus Maze Test and Open Field Test

were performed at the end of the treatment. Animals where sacrificed, peripheral blood extracted and frozen, and brain cortex harvested for Western Blot. Inflammatory markers (IL-1, IL-6, TNFα, ultrasensitive CRP and plasma Hsp-70) were dosed in plasma by ELISA. AEA/PCL produced a significant reduction of systolic blood pressure, a decrease of inflammatory markers (IL-1, IL-6, TNFα, ultrasensitive CRP and plasma Hsp-70), and oxidative stress markers (NADPH oxidase and nitrites).Protein expression of WT-1, AT-1, and iNOS was higher in SHR brain cortex, while AEA/PCL decreased it. Conversely, Hsp-70 expression increased after treatment within the cerebral cortex. Abnormal behaviors observed in Plus Maze Test (time of permanence in the open arm) and Open Field Test (time of exploration) also decrease after AEA/PCL treatment. These preliminary results suggest antihypertensive and anti-inflammatory properties of AEA, at the level of both peripheral and central nervous system. This nano-formulated cannabinoid might regulate inflammation through the AT-1-Hsp-70-iNOS pathway.

A118
EFFECT OF MILD HYPERTHYROIDISM ON GLUCOCORTICOID SECRETION INDUCED BY ACUTE STRESS IN PREGNANCY AND LACTATING RATS.

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Disorders in thyroid function are associated with anxiety and depression. Therefore, responses to stress may be altered in patients with thyroid diseases. Stress is associated with functional changes in brain areas such as the hippocampus (HpC) and medial basal hypothalamus (MBH) by activating the hypothalamic-hypophysis-adrenal axis (H-H-A) and the consequent release of glucocorticoids in response to the stressor. In this study we analyzed the effects of mild hyperthyroidism on corticosterone response (measured by RIA) to acute stress (exposure to ether vapors for 2 min.) and on the expression (by qPCR) of the glucocorticoid receptor (GR) and the long isoform of the prolactin receptor (PRLRL) in MBH and HpC in Wistar female rats in different reproductive states (day 19 of gestation (G19), 2 (L2) and 12 (L12) of lactation). Hyperthyroidism (hyperT) was induced with T4 (0.1mg /kg/day, s.c.) at a dose that allows the maintenance of lactation. Control (Co) and hyperT rats were bled from the tail vein during the 2 min ether exposure (s1) and 5 min after ether exposure (s2). In G19 acute stress induced corticosterone release in Co group ($P< 0.001$ and $P < 0.01$). In L12 ether stress increased corticosterone release in the hyperT group ($P< 0.001$), while the Co group showed unchanged levels. In MBH the expression of GR in the Co group increased in L2 compared with G19 ($P< 0.05$) and remained at the same levels in L12. In the hyperT group an increase of GR expression in L12 was observed with respect to G19 and L2 ($P< 0.01$). The expression of PRLRL in the Co group increased in L12 compared with G19 and L2 ($P< 0.01$), while hyperT advanced the increase of PRLRL expression to L2 compared with G19 ($P< 0.05$). On the other hand, hyperT increased PRLRL expression on L2 compared with the Co group ($P< 0.05$). In HpC the expression of the GR increased in L12 with respect to G19 in both groups (Co $P< 0.05$ and hyperT $P < 0.001$). No effects of hyperT nor of reproductive stage were observed in HpC PRLRL expression. Once lactation is established, the H-H-A axis is still reactive in hyperT rats in response to an acute stress evidenced by adrenal corticosterone release, whereas in Co rats the axis showed a physiological adaptative hyporeactivity. These evidences indicate that mild hyperthyroidism induces a differential expression of hippocampal and hypothalamic GR and PRLRL at early lactation that could be involved in the maintenance of the H-H-A axis reactivity.

A119
ROLE OF INFLAMMATION IN THE ENCEPHALOPATHY PRODUCED BY SHIGA TOXIN 2 FROM ENTEROHEMORRHAGIC *Escherichia coli*

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Shiga toxin 2 (Stx2) from enterohemorrhagic *Escherichia coli* (EHEC) causes hemolytic uremic syndrome (HUS) and acute encephalopathy, which may lead to fatal outcomes in patients. The clinical sings of this neurological disorder includes decerebrate posturing, hemiparesis, ataxia, seizures and changes in the level of consciousness (from lethargy to coma). When neurological symptoms are involved the mortality rate may rise up to 40% compared with the death produced by HUS (5%).The motor areas of the brain are frequently affected in patients infected with EHEC. In addition to Stx2, LPS is released by the bacteria and may also contribute to the observed striatal dysfunction. The aim of this study was to determine: i) whether LPS exacerbates the deleterious effect of Stx2, ii) whether Stx2 alters the motor performance, and iii) the existence of a proinflammatory component. Swiss male mice were injected intravenously with Stx2+LPS or Stx2 or LPS or vehicle (control). In addition the same described groups were treated with dexamethasoneintraperitoneal (i.p.). Mice were perfused with fixative solution. Their brains were subjected to immunofluorescence with an anti-NeuN (neuronal nuclei marker) and anti-Gb3 (Stx2 receptor). Open field test were assayed to study motor performance. Also, SD male rats were injected intracerebroventricular with Stx2 (20pg/gr) and Stx2+etanercept (3.10ηM, soluble TNF-α receptor) to immunolocalize Stx2 in striatal neurons by

immuno-gold electron microscopy. Stx2+LPS maximally increased the neuronal damage (34±4%) and increased the expression levels of Gb3 (85±7.57 A.U.). Mice treated with Stx2+LPS showed an altered motor performance (64±11% less) in comparison to controls. Dexamethasone significantly reverted the changes observed on NeuN and Gb3 expression and in motor performace. Etanercept reduced the immuno-gold particles that corresponded to Stx2 into striatal neurons ($p<0.05$ by ANOVA-Bonferroni *post hoc* test). We concluded that Stx2 alters striatal neurons and motor performance; LPS enhances the deleterious action of Stx2; dexamethasone and etanercept treatments determined a proinflammatory participation generated by these toxins. The observed proinflammatory component should be taken into account in order to establish new studies and possible therapies to treat this encephalopathy.

A120
LOSS OF WEIGHT AND MINERALS BY COOKING IN COMMERCIAL NOODLES

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Pasta plays a fundamental role in human nutrition as a source of energy and it is widely consumed in the world. Due to its low-cost, service life and the importance of their nutritional values can be used as a good source for the nutrients incorporation. This work aims to evaluate therelationship between cooking time and the loss of weight and minerals of commercial noodle (spaghetti type). We assessed the loss of weight, zinc (Zn), and copper (Cu) at different cooking times (optimal time indicated by the manufacturer) and an overcooking, without adding salt to cooking water and adding salt (2g and 10g / 200ml). The raw and cooked commercial noodle samples were treated by acid digestion, and subsequently, the minerals concentration were quantified byinductively coupled plasma mass spectrometry (ICP-MS). Solids losses were 15.55%, 13.35%, and 6.16% with optimal cooking time (unsalted, with 2g of salt and 10g of salt, respectively) and 16.9%, 13.86% and 1.83% with overcooking (unsalted, with 2g of salt and 10g of salt, respectively). There were losses of Zn: 24.38%, 7.08% and 2.53% during the optimal cooking time (unsalted, with 2g of salt and 10g of salt respectively), which have not significantly increased with overcooking. The Cu losses were 22.91%, 8.15%, and 7.14% during the optimal cooking time (unsalted, with 2g of salt and 10g of salt % respectively), which have not significantly increased overcooking. The Zn loses (fortification mineral) was similar to Cu (endogenous mineral). The results show that lower loss of copper and zinc occurs when adding salt to the cooking water, which is relevant from the nutritional point of view. On the other hand, at a time of overcooking does not significantly increase the loss of solids and minerals.

A121
OXIDATIVE CHANGES IN THE ERYTHROCITE MEMBRANES INDUCED BY CHRONIC STRESS AND ITS INCIDENCE IN THE BLOOD RHEOLOGY

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Oxidative modification of erythrocyte membrane protein is considered very significant due their important role in transport, enzyme activity and membrane fluidity. In disease, red blood cell (RBC) membrane abnormalities promote thrombosis and could lead to RBC adhesion to the endothelium and microvascular occlusion. The objective of this work was to evaluate the chronic stress effects on plasmatic and erythrocyte oxidative state, RBC aggregability and hemorheological parameters. Control and stressed (movement restriction, 1 h/day, 3 times/week /45 days) adult Wistar male rats were used. After the last stress, blood samples were obtained by decapitation. Corticosterone (CORT), glycemia, blood and plasma viscosity, antioxidant capacity of the plasma (FRAP), osmotic erythrocytes resistance (OER), the erythrocyte aggregation, the hematocrit and the globular sedimentation rate (GSR) were measured. Carbonyls and Malondialdehyde (MDA) levels in erythrocytes membrane fraction and superoxide dismutase (SOD) and catalase (CAT) activity in cytosolic fraction were evaluated. Stress increased corticosterone levels ($p=0.000001$), glycemia ($p= 0.00001$), blood viscosity ($p=0.02$), hematocrit ($p=0.0001$), OER ($p=0.006$), erythrocyte aggregability ($p=0.05$)and globular sedimentation rate (p=0.05). No changes in plasma viscosity were observed. A decrease in FRAP ($p=0.01$) was found in stressed rats. SOD ($p=0.03$) and catalase activity ($p=0.01$), MDA ($p=0.04$) and carbonyls ($p=0.000001$) increased in response to stress. A positive correlation between CORT and MDA levels (p=0.03), and CORT and carbonyls (p=0.002) was found. In addition, a negative correlation between FRAP and MDA (p=0.03) was observed. Moreover, a positive correlation between CORT and OER (p=0.009) and OER and carbonyls (p=0.00006) was found, but no significant correlations between MDA and OER was observed. The increase of corticosterone together with the decrease of the plasma antioxidant capacity would be responsible for the greater oxidation of the erythrocyte membrane. These structural alterations in the erythrocyte membrane induced by chronic stress would lead to greater aggregation and viscosityblood. The oxidation of proteins of the erythrocyte membranes seems to be more related to the changes in aggregation and globular resistance than lipid oxidation. This abnormal RBC membrane architecture could alter blood rheology and microcirculation.

A122

TEMPORAL PATTERNS OF BLOOD PRESSURE AND BMAL1 PROTEIN ARE MODIFIED IN THE HEART IN A RAT MODEL OF AGING AND CALORIC RESTRICTION

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Aging has been extensively associated with a decline in cardiovascular performanceand an increased risk of cardiovascular diseases, such as atherosclerosis, hypertension and myocardial infarction. Additionally, the circadian system coordinates and synchronizes numerous physiological processes, including cardiovascular function. The performance of this system is also disrupted with aging. On the other hand, caloric restriction (CR) has been recognized as a preventive factor for atherosclerosis and vascular aging. In the present study, we aimed to evaluate if the circadian variation of the blood pressure (BP) and BMAL1 protein levels are altered in the heart of aged rats and aged rats under CR. Holtzman 3- and 22-month old male rats and 22-month old male rats under 3 months of 40% of CR were maintained in constant darkness conditions for 15 days, in order to study the endogenous rhythms. Blood pressure was determined under 12h/12h light/dark (LD) conditions, as well as under 12h/12h dark/dark (DD) conditions. Young rats subjected to LD conditions showed a circadian variation in systolic BP (SBP) and diastolic BP (DBP) (rhythm percentage 47.4% and 20.54%, respectively). Under the same conditions, SBP and DBP underwent a loss of circadian oscillationin aged rats. When aged rats were subjected to CR, we observed a restoration of the circadian variation and a phase advancement of SBP and DBP, in comparison to young animals ($p<0.0001$ and $p< 0.01$, respectively). On the other hand, in DD conditions, all three groups of animals showed a circadian oscillation in SBP values (rhythm percentage 27.27% in young, 35.52% in aged and 33% in aged subjected to CR). We also observed that aged rats presented a higher rhythm's amplitude than young animals ($p<0.05$). DBP values in DD, also varied in a circadian way in young and aged rats (rhythm percentage 24.57% and 20.94%, respectively), showing the last group, a decrease in the rhythm's mesor and a phase advancement when compared to young rats ($p<0.01$ and $p<0.05$, respectively). The rhythmicity of DBP was lost in aged rats under CR. In previous studies, we observed that BMAL1 protein levels vary in a 24-hour period in the heart of young and aged rats. In the present work, we observed that BMAL1 protein levels present a circadian oscillation in the heart of aged rats under CR, with a decrease in therhythm's mesor, an increase in the rhythm's amplitude and a phase advancement when compared to de young rats ($p<0.001$, $p<0.001$ and $p<0.05$, respectively). In this work, we observed how the circadian rhythm of BP values and BMAL1 protein levels are altered the in heart during aging. CR modifies these alterations, restoring the circadian oscillation of some parameters in old rats. Further researchneeds to be done in order to elucidate the possible positive impact of the CR on the altered circadian and cardiovascular systems in elderly individuals.

A123

EFFECT OF A 6 MONTHS VITAMIN A DEFICIENCY ON THE EXPRESSION OF CELLULAR RETINOL BINDING PROTEIN-1 AND ITS CORRELATION WITH HORMONAL LEVELS

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Deficiency in vitamin A (DVA) is an evil in underdeveloped countries where the minimum recommended intake is not achieved. This may have long-term implications on the health of the population.. Our objective was to relate the DVA with the expression of cellular retinol binding protein-1 (CRBP-1) and its endocrine effects. We worked with 3 groups of females rats Wistar separated at weaning: one group was fed with a vitamin A (VA) sufficient diet (8 mg of retinyl palmitate/kg de diet) for 6 months (+A), another group with a VA defficient diet for 6 months (-A) and the last one was fed with a VA deffcient diet for 150 days and then was fed with a VA diet sufficient for 30 days (R). The levels of retinoic acid in serum and intermediary of the vitamin A pathway (CRBP-1) in mammary gland were studied by the Neeld and Person technique and RT-PCR, respectively; and the correlation was made with the levels of colesterol (CL) and estrogen (E) in serum and expression of the estrogen receptor α (REα) in mammary gland. It was used one way Anova, Tukey test, regression and correlation analysis with Graphpad Prism, $p<0.05$. A decrease in the expression of CRBP-1 of group + A was observed with respect to -A and R ($p<0,0001$). A positive correlation was observed between: CL and the levels of retinoic acid (R2: 0.80, $p = 0.02$), CL and E (R2: 0.84, $p = 0.001$); Retinoic acid and CRBP-1 (R2: 0.85 and $p = 0.001$); and a negative correlation of E and REα (R2: 0.59 $p = 0.0425$). The DVA would generate a decrease in the levels of retinoic acid that would regulate the expression of CRBP1 and the levels of circulating cholesterol, being able to generate a decrease in the synthesis of E that negatively regulates the excretion of the REα.

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BIOTECHNOLOGY AND GENETICS

A124
EXTRACTS OF *Erythrina crista-galli* L. and SACAROSA ENCAPSULATED IN CALCIUM ALGINATE DISCS TO BE USED AS A NATURAL SCARLET

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The natural products obtained from the vegetables provide a great variety of compounds, among which the flavonoids, products of the secondary metabolism of plants, are untied. Among the multiple properties that flavonoids possess, is also its antifungal and bactericidal effect. The presence of flavonoids in nature and their potential benefits for human health has attracted increasing interest in their study.Our original communities already used the ceibo bark, or seibo, *Erythrina crista-galli L*. (also known as coral tree, bucaré, cachimbo, coral flower, cock, pico de gallo, crest of rooster, Sylvá, zuinandí) for its properties as a sedative, disinfectant, deodorant and anti-inflammatory. The decoction of the bark of the ceibo is used for washing wounds, in seat baths against hemorrhoids, in poultices to heal wounds, and also in gargles and as an infusion to cure ulcers and in enemas in rectitis and blenorrhagic vaginitis of external application. Taking into account the use of ceibo to clean wounds and accelerate the restoration of affected tissues, the objective of this work is to determine the concentration of flavonoids in the bark of the ceibo and to encapsulate the extract thereof, in calcium alginate discs for make its application as a scarring more practical and effective. To prepare the extract, the ceibo bark was harvested, then 30 g of it was weighed and placed in 300 ml of water and boiled for half an hour. Then the sample was filtered and the concentration of total flavonoids was determined by the Folin Cioucalteu method, measuring the absorbance at 760 nm. Gallic acid was used as a standard. A 30% sucrose solution was prepared using the extract as solvent. To encapsulate the solution, 3% sodium alginate was prepared, which was poured into a Petri dish containing 2% calcium chloride and sucrose and ceibo solution, forming a plate containing the solution of ceibo and sucrose inside it. The plates were placed on equal pieces of crude potato which were previously weighed and the osmotic power was measured by gravimetry. They were incubated for 1 h, dried with absorbent paper and their weight recorded again. The osmotic power was taken as the quotient between the difference in the mass of the disc before and after the incubation and the initial mass multiplied by 100. The concentration of flavonoids was 2.6 mg / L. The potato discs presented lower weight after incubation, as did the alginate discs with sucrose and ceibo solution. From the results obtained we can say that the osmotic power of the sucrose solution allows extracting water from the tissue, but the ceibo is permeable to the calcium alginate membrane and by concentration difference flows to the potato, in this way, the solution encapsulated is more effective and practical to use.

A125
Piptochaetium napostaense (SPEG.) HACK. Y *Leptochloa crinita* (LAG.) P. M. PETERSON & N. SNOW: *IN VITRO* ORGANOGENESIS

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In San Luis (Argentina), the different causes of environmental impact (socio-economic, climatic, technological and cultural) led to biodiversity loss and habitat fragmentation and destruction. In this sense, technological systems that allow stability and profitability in the short term compromise the long-term existence of native flora. The present work aims to apply biotechniques as alternatives for an appropriate propagation of this gene pool. The study focused on key species of the natural pasture with forage aptitude: *Piptochaetiumnapostaense* (Speg.), Hackel and *Leptochloacrinita* (Lag.) P.M. Peterson &N.Snow. The *in vitro* organs formation induction study from the stimulation with growth regulators was evaluated in nutritive environments Murashige and Skoogmedia. The explanto suitable for direct and/or indirect morphogenesis was determined.The experimental design was completely random, ten explants were planted per repetition, with three repetitions in total.It was used a nonparametric test (Kruskal Wallis)and chi square test of independence. The difference was determined with a *posthoc* test using the R software (PMCMR library) using the Bonferroni-Dunn test.Seven days after sowing, the following were evaluated: number of contaminated explants and type of contamination. 90 days after sowing, the following was evaluated: rate of callus formation, presence / absence of direct or indirect organogenesis.It was obtained calluses(p-valor: 0.000<0.05) in *L. crinita* by treating it with 6 mg/l of 2.4-dichlorophenoxyacetic (2.4-D). In the case of *P. napostaense*, treated with 2mg/l of 2.4-D and treated with 0.5 mg/l of 2.4-D and 2mg/l of Acetic acid naphthalene were the ones that presented calluses in the embryonic zone of the fruit-seed. Regarding *in vitro* organogenesis in both species, a Chi square test of independence was applied and it was determined that the presence and absence of organogenesies is not independent of the treatment (p-value: 0.000 <0.05). This work was able to determine the effect of different regulators of adequate growth to achieve *in vitro* organogenesis in each evaluated species. The development of this biotechnology allowed us to improve methodologies to initiate *in vitro* conservation processes, analysis of genetic variability and initiation of domestication and genetic breeding programs.

A126
Anti-Cp4-EPSPS SOYBEAN PROTEIN ANTIBODY PRODUCTION FOR THE DEVELOPMENT OF ELECTROCHEMICAL IMMUNOSENSORS FOR GENETICALLY MODIFIED SOYBEAN DETECTION

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Biosensors have aroused great interest in genetically modified organisms (GMO) detection assays. In this regard, immunosensors presents high specificity due to the antigen-antibody interaction. In this work, New Zealand rabbits were injected with a synthetic peptide from the soybean Cp4-EPSPS protein, which provides glyphosate resistance, by injecting a synthetic peptide. The anti-Cp4-EPSPS antibodies production was evaluated by electrophoresis (SDS-PAGE) and an ELISA was developed in order to study their specificity. The ELISA showed that the polyclonal antibodies were specific to Cp4-EPSPS. In addition, the anti-Cp4-EPSPS were immobilized onto a gold disk electrode and the antigen-antibody interaction was evaluated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The CV showed that the current detected by the modified electrode decreased after incubation with Cp4-EPSPS peptide solution, whereas EIS increased after the incubation. These results suggest that the Cp4-EPSPS peptide was immobilized onto the electrode, due to the specific interaction with the anti-Cp4-EPSPS. On the other hand, these results show that this antigen-antibody interaction can be detected by electrochemical techniques, suggesting that the anti-Cp4-EPSPS produced can be used in CP4-EPSPS electrochemical immunosensors development.

A127
EVALUATION OF TOXICITY OF SILVER NANOPARTICLES SINTETIZED BY BIOLOGICAL MEDIATORS

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Nanotechnology is a rapidly developing area that can have substantial impacts on the economy, society and the environment. In recent years, silver nanoparticles (AgNPs) have been studied for their antimicrobial capacity and their possible applications to the food industry. This is why it is necessary to monitor the toxic potential of nanoparticles. Plants have been used as indicators of toxicity because they have a variety of well-defined biological parameters. The objective of this work was to study the possible toxic and genotoxic effects of AgNPs synthesized by biological mediators (biosynthesis) such as *Aspergillus niger*(AgNPs-*A.n*),*Cryptococcus laurentii* (AgNPs-*C.l*)and *Rhodotorula glutinis* (AgNPs-*R.g*) on plants.Formeasure the toxicity effects of the AgNPs, standardized bioassays were performed using *Lactuca sativa* (lettuce)seeds. Ten seeds were placed in a Petri dish with filter paper in the bottom as a support and then, 2.5 mL of each treatment: 1-AgNPs-*A.n*, 2-AgNPs-*C.l*, 3-AgNPs-*R.g*, and 4-AgNPs-chem (AgNPs by chemical synthesis), adjusted at concentration of 3 $mg.mL^{-1}$were added. Distilled water as negative control and Cu^{+2} solutions (5 $mg.mL^{-1}$) as positive control were used. After 5 days of incubation at 25 ± 1°C, the germination percentage (G%) and the root length were determined. To measure the genotoxicity effects of the AgNPs, bioassays using onion (*Allium cepa*) roots were performed.Each onion bulb was placed in aerated cuvettes and four bulbs of uniform size (3 cm diameter) were used per treatment. The previously mentioned treatments 1, 2, 3 and 4 were applied, while dechlorinated water and Griseofulvin solution (250 $mg.mL^{-1}$) were used as negative and positive controls, respectively. The bulbs were partially submerged and after 30 h of exposure, cells were observed to identify the following parameters: mitotic index and chromosomal aberrations. In the toxicity tests, our results showed no significant differences of G% and root length among *L. sativa* seeds treated with different biosynthesized AgNPs or when they were compared to the negative control; whereassignificant differences among AgNPs-chem and the positive control were observed. Ingenotoxicity analysis,taking into account the *Allium cepa*mitotic index, no significant differences of AgNPs-*A.n*, AgNPs-*C.l* and AgNPs-*R.g*treatments when compared to the negative control were observed;meanwhile, AgNPs-chem and Griseofulvin treatments presented significant differences. In contrast, considering chromosomal aberrations, all the treatments presented significant differences compared to the negative control, except for AgNPs-*R.g*treatment.. We can conclude that the biosynthesized AgNPs did not show toxicity. In the case of the *Allium cepa* assays, AgNPs-*R.g* was not genotoxic and although AgNPs-*A.n* and AgNPs-*C.l*showed some genotoxicity degree, it was lower than that of AgNPs-chem and the positive control. We can conclude that AgNPs derived of biological synthesis might be used as antimicrobials applied to the food sector.

A128
THERMAL PRE-TREATMENT WITH WATER ADDITION OF ALPERUJO AND BIOGAS PRODUCTION

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Alperujo (AL) is the waste of the extraction process in olive oil mills. Its composition is suitable for Dry Anaerobic Digestion (DAS), but AL contains high concentrations of phenolic compounds (CF) that should be inhibitors for biogas production. The aim of this work was to perform a thermal treatment with water addition of AL for CF extraction and the posterior DAS of AL for biogas production. Three tests were done, on 10 g AL at pH 5.49, in thermostatic bath at 80°C for 120 min: one test was carried out without addition of water; another portion of AL was treated with 40 ml of tap water; and in the third test 5 ml water were added each 30 min. DAS were performed for 25 days, in 60ml syringes, packed with 40 ml of treated AL inoculated with 10^8 bacteria/ml, incubated at 32°C. The inoculum used was an isolation obtained from an anaerobic digestion of AL and horse manure. The biogas produced was purified with NaOH 3M (for CO_2 absorption). The best reduction of the CF, in solid phase, was obtained in the treatment with total water addition at the initial moment, 57% CF were reduced compared to thermal pre-treatment without addition of water. The pre-treatment with 5 ml water dosage, presented 28% CF reduction. The better production of biogas was obtained in DAS using AL thermal pre-treated with addition of water at the beginning, and this production was 23% higher than that using AL without pre-treatment and 12% higher than that using AL thermal pre-treated without water addition. It can be concluded that thermal pretreatment with water addition in an interesting method for CF extraction from AL and should be optimized.

A129
ADSORPTION AND DESORTION STUDY OF EGG WHITE LYSOZYM USING AFFINITY CHROMATOGRAPY

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Protein purification is an essential process in scientific research. Considering its final application, these biomolecules need to be isolated and purified. Affinity chromatography is the most effective affinity purification technique, which combines conventional column chromatography with affinity interactions. The aim of this work is to study the adsorption and desorption of Lysozyme (Lys) using affinity chromatography with Cell-Cibacron macroligand immobilized in solid gels. Affinity macroligand was prepared from yeast cells chemically modified. Cibacron Blue F3GA ligand molecule was immobilized to the cell wall through a covalent bond. A fixed-bed column system with immobilized agarose-macroligand cubes was prepared. Lys adsorption from hen egg white and Lys desorption from agarose-macroligand were studied. Agarose-macroligand cubes with Lys absorbed were put in elution buffer, 0.05 M Tris/HCl containing 2.0 M NaCl or 1.5M NaSCN. The degree of recovery and purity of Lys was analyzed. The supernatants of adsorption and elution were analyzed by SDS-PAGE. Protein concentration was determined by spectrophotometry at 280 nm. About 94% of Lys was eluted using 1.5 M NaSCN as eluent and 64% Lys was obtained using 2M NaCl as eluent. Lys purity in the gels was analyzed with Gel-Pro Analyzer 4.0 software, obtaining purity values from 96.7 to 99.9% using both elution buffers. . The effect of ionic strength of the Lys desortion on the affinity column was studied by the addition of NaSCN or NaCl. We concluded that the elution using NaSCN is better for the dissociation of Lys from agarose-macroligand adsorbent than NaCl. This is because NaSCN is a chaotropic agent that affects the electrostatic interactions between the positively charged groups of Lys and the negatively charged groups, favoring protein desorption. In addition, the SCN- ions can bind easily the Lys molecules, affecting the conformational structure of the protein. There is therefore a decreasing of the hydrophobic interactions between Lys and Cibacron Blue. However, is convenient to use NaCl as eluent to prevent the loss of enzyme activity.

A130
MICROPROPAGATION DE *Lippia integrifolia* (GRIS.) HIERON

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*Lippia integrifolia (*Gris.) Hierb, "Incayuyo", is a medicinal species that due to its industrial importance has been over-exploited, with risk of genetic erosion and reduction of natural populations. Therefore, for the purpose of promoting the domestication and genetic improvement of this species, biotechniques were used to multiply this species on a large scale and in the shortest possible time. The objective of this work was the micropropagation of chemotypes of this species from Tuclame (Córdoba, Argentina). Thus, the appropriate disinfection method was determined for the explants (nodal segments) that came from the field. Growth was evaluated in Murashige and Skoog medium with different concentrations of naphthaleneacetic acid (ANA) and 6-benzylaminopurine (BAP): without growth regulators (T); 0.1 $mg.l^{-1}$ ANA (T1); 0.1 $mg.l^{-1}$ BAP (T2); 0.5 $mg.l^{-1}$ ANA (T3); 0.5 $mg.l^{-1}$ BAP (T4); 1 $mg.l^{-1}$ ANA (T5); 1 $mg.l^{-1}$ BAP (T6). The neoformation of shoots and roots was measured at six weeks of *in*

vitro culture. The cultures were incubated at 27 ± 1 ° C and 14 hours of photoperiod (116 mmol.m-2.sec-1 PAR radiation). To achieve the acclimatization of the micropropagated plants, the combination of substrates was evaluated: TA = vermiculite; TB = organic soil; TC = vermiculite: organic soil (1: 1); TD = vermiculite: sand: organic soil (1: 1: 2). The data were analyzed using a nonparametric test (Kruskal Wallis) and for the categorical variables the Chi-square tests were used. Regarding disinfection, 100% of aseptic explants were obtained in the treatment with water with detergent (10 '), 70% Alcohol (7'), 20% sodium hypochlorite (7 ') and rinses with sterile water. Significant differences were observed between the treatments for the variable number of shoots per explanto standing out T and T2, with average ranges of 3.88 shoots / explanto and 3 shoots / explanto respectively. No significant differences were obtained for the number of roots per explanto. In the acclimatization stage it was found that there were no significant differences between the substrates (p-value = 0.417> 0.05). However, it was determined that there was influence on the viability of the plants when the bud / root ratio at the beginning of the acclimatization stage was between 1 and 2. Observing 82% of viable plants for said proportion at the time of transplanting to the substrate. These results make a contribution to initiate the development of native germplasm with added value.

A131
EVALUATION OF PEANUT (*Arachis hypogaea* L.) GENOTYPES BASED ON POD YIELD, STABILITY AND REACTION TO SMUT (*Thecaphora frezii*)

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The genetic progress is focused in the obtaining varieties adapted to the production environment due to be a high impact management practice. Aspects such as yield stability and diseases reaction, such as smut (*Thecaphora frezii*), should be considered in genetic breeding programs of peanuts (*Arachis hypogaea* L.) to obtain superior genotypes, since this disease causes losses of up to 35% of production. The present work was carried out to identify peanut genotypes higher pod yieldsand stability of them which present also a good performance with tolerance to *T. frezii*. The 18 genotypes evaluated in 3 environments of the Córdoba province were: 3 varieties (FAVar1 to FAVar3) and 12 advanced lines from the FAV-UNRC (LAx1 to LAx12), 2 INTA varieties (Ascasubi Hispano and Asem Pepe) and a control variety (Granoleico). The experimental design using was randomized complete blocks with three replicates. At harvest, 2 m^2 per plot were sampled, where pod yield (kg/ha) and smut incidence (%) were evaluated. The traits were statistically analyzed in individual environments and across environments by analysis of variance (ANOVA). Later, the sites regression (SREG) model was using for the analysis of multi-environmental data and the GGE biplot graphs was obtained. In the ANOVA by environment, significant differences between genotypes were observed. The combined ANOVA showed significant effect of genotype (G), environment (E) and interaction G × E in both traits. The GGE biplot allowed the differentiation of three varieties (Ascasubi Hispano, FAVar2 and FAVar3) with greater yield and great stability compared to the variety most seeded in Argentina (Granoleico). Meanwhile, the varieties with the lowest incidence the *T. frezzi* were Ascasubi Hispano and FAVar2, coinciding with those that presented highest yield. This is of great significance because there are a high amount of smut teliospores in the province of peanut crop of the Córdoba province. In addition, four advanced lines (LAx8, LAx9, LAx10 and LAx11) showed smut high tolerance and stability, characteristic that gives them great potential for their contribution to this crop´s genetic base expansion

A132
INTERRELATION BETWEEN AMARANTH (*Amaranthus* spp.) GENOTYPES AND ITS AGRONOMIC TRAITS

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Amaranth (*Amaranthus*sp.) is an interesting alternative for semi-arid environments due to its excellent performance under drought conditions. Nutritionally, the amaranth grain is very rich in proteins, vitamins and minerals, which, makes it stand out over classic cereals. The phenotypic expression of many characters of the amaranth plant is determined by genetic and environmental factors (edaphic, climatic, management, etc.) to which the crop is exposed during the growing season. Variability is important when selecting parents to hybridize in breeding programs. Grain yield is a complex quantitative trait therefore direct selection for this trait may not be efficient. Twenty amaranth genotypes, including commercial varieties and advanced lines, were evaluated in three environments (sowing dates) in Río Cuarto-Córdoba, during the 2017/18 summer cycle, using a randomized complete block design with three replications. In each genotype, the following traits were measured: plant height (PH), panicle length (PL), days of emergency to panning (DEP), days of emergency to physiological maturity (DPM), fertility index (FI), thousand seeds weight (TSW), grain yield (GY) and popped index (PI). The study of the interrelation among genotypes and traits was carried out through the cluster analysis and the principal components (PC) analysis. The genotypes were grouped in 6 clusters according to phenotypic similarity and the dendrogram showed that clusters I, II and VI had 6, 9 and 2 genotypes respectively, while the groups III, IV and V had one genotype each.The first three main components accounted 78.1% of the total variation (40.40, 21.50 and 16.20% for CP1, CP2 and CP3, respectively).The highest GY was corresponded to the clusters V and VI, while the group II associated better with the TSW. The greatest divergence was observed in the conglomerates IV and V, and

the lowest distance was found between I and II. A negative correlation is observed between DEP and GY, and between DPM and TSW. The RG and FI traits showed positive association, as did DPM and PL. The highest GY were obtained with the genotypes H22II, AmanG1/3, AhybG1/1 and H17a, standing out this last genotype for being associated with the highest TSW. The AcaG10/3 genotype was shown as the longest cycle genotype. Interpreting the relationships among genotypes, traits and genotypes-traitsis important in amaranth breeding programs.

A133
SOLID STATE FERMENTATION OF AGROINDUSTRIAL SOLID WASTE FOR ENZYME PRODUCTION. GRAPE AND OLIVE MARK EVALUATION AS SUBSTRATES

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In Cuyo region, he industrial processing of vines and olives generates large quantities of by-products or agro industrial solid waste (AISW). The treatment of these solid waste or by-products, for their final disposal, represents not only a significant cost for the processing industries but also a potential negative impact on the Environment. Due to their chemical composition (tannins, poly-phenols, poly-alcohols, proteins, sugars and lipids among others), are highly polluting and their direct disposal is not allowed. Even though, there are microorganism such as filamentous fungi, which can use AISW as nutrient source for growth and product formation. Solid State Fermentation (SSF) would be a suitable technology for agro-industrial solid waste treatment. The aim of this work is to evaluate two different substrates prepared using grape marc (GM), olive mark (OM) and grape stalk (GS) for enzyme production in SSF by filamentous fungi. For substrate evaluation, two culture media were prepared and inoculated with two different filamentous fungi. Substrate were: GM + OM (50/50 % w/w) humidified to 60% (wet base), pH=4.5 and OM+GS (70/30 %w/w), humidified to 65%, (wet base), pH=4.5. Microorganisms were filamentous fungi isolated from Olive pomace named *Aspergillus spp LR1-IBT* and *A.spp M9-IBT*. Taking into account substrate and microorganism combination, four SFF essays were carried out: SSF-A (GM + OM with *A.spp M9-IBT*), SSF-B (GM + OM with *A.spp LR1-IBT*), SSF-C (OM+GS with *A.spp M9-IBT*) and SSF-D (OM+GS with *A.spp LR-IBT*). Culture medium was set in petri dishes, inoculated at 10^7 spore/g and incubated at 28 °C, during 15 days. Samples were taken periodically for analytical determinations. An aqueous extract from SFF was prepare for the enzymatic activities detection which includes, Cellulase (Cel), Exo-Polygalacturonase (Exo-PG), Laccase (Lac) and Lignin Peroxidase (LiP). All SSF developed well, since weight loss was detected along the whole incubation period, showing log phase between days 2 and 5. Major weight loss was found when SFF was carried out on OM (SSF-C and SSF-D). Enzymatic activities were detected in all SSF (A, B, C and D). Maximum activities where shown during first seven days, then enzymatic activity was barely detectable. Cel and exo-PG activities were major in SFF on GM (SSF-A and SSF-B). Ligninolytic activities were major in SFF on OM (SSF-C y SSF-D). In the future, the trials will focus on the study of variables to optimize the production of Lac and LiP enzymes in OM substrates and Cel and Exo-PG enzymes in GM substrates.

A134
ISOLATION OF TOTAL DNA IN *Hedeoma multiflora* Benth.

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Due to the diverse uses of the native aromatic species from the province of San Luis, "Peperina de las Lomas" (*Hedeoma multiflora* Benth.) is suffering from a high extraction pressure and therefore,it is at risk of genetic variability losses. There is no background on molecular markers in this species for the study of the population genetic structure. The aim of the present work was to obtain *Hedeoma multiflora* genomic DNA with quality, optimal purity, and adequate quantity for subsequent analysis by molecular techniques, such as inter simple sequence repeats (ISSR). Leaves and stems were used as plant material applying the CTAB Protocol 7 for total DNAisolation with modifications of the original protocol. It was possible to extract total genomic DNA with high yield and purity from leaves without the use of liquid nitrogen. A rapid technique was standardized to obtain genomic DNA from this species and to initiate genetic characterization protocols.

A135
BACTERIOSIS IN A DIVERSE MAIZE GERMPLASM IN SOUTH CÓRDOBA, ARGENTINA

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The maize is one of the most important crops of the Argentinian pampa region. Plant pathogens, such as fungi, bacteria, and virus can cause serious damage to agriculture and significantly reduce the yield and quality of crops. Diseases caused by bacteria,

however, are the few studied. The objective of this work was to identify bacterial pathogens based on symptoms observed in a diverse maize germplasm. Therefore, a population of 200 maize inbred lines developed and provided by the International Maize and Wheat Improvement Center (CIMMYT) were evaluated at Río Cuarto location during the summer cycle of 2017/2018. Light-colored necrotic streaks or yellow irregular blotches were observed on leaves from symptomatic maize inbred lines. The leaves with symptoms were rinsed with sterile distilled water and cut into small bits. These pieces were immersed in 0.85% (w/v) NaCl (physiological solution) and macerated. The samples were serially diluted and plated onto Luria-Bertani (LB) medium, containing dicloran (to prevent fungal growth). Plates were incubated at 30 °C. A total of twenty isolates were obtained from symptomatic maize inbred lines. Gram staining, pigment production in LB medium and catalase reaction were tested in all isolates. In addition, β-galactosidase production was assessed by X-Gal test. Most of the strains were Gram-negative and five isolates were catalase positive. The isolates were distinguished by their different colonial morphologies. Light yellow, orange, red or white convex colonies were observed on LB medium. Moreover, three strains produced blue color colonies on X-Gal containing plates, indicating the presence of β-galactosidase enzyme in those bacteria. Representative isolates were chosen for further identification through the use of phylogenetic analysis of 16S rRNA gene sequences. A single product of about 1.5 kb was amplified by PCR with the primers fD1 and rD1 from each of strains analyzed. The purified PCR products were sent to Macrogen Inc. (Seoul, South Korea) for the sequencing of the gene encoding 16S rRNA. The identification of bacterial pathogenic strains in maize is relevant. Since the study of these emerging diseases in the maize region of Argentina is yet little explored.

A136
AGROINDUSTRIAL WASTE FROM THE CUYO REGION REVALORIZATION. PRELIMINARY TESTS USING SOLID STATE FERMENTATION

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The agroindustrial activity of the Cuyo region is based mainly on the processing of two crops: the vine and the olive; and to a lesser extent fruits and vegetables. The wine industry and the olive oil industry generate large amounts of solid waste, such as grape marc, grape stalks and olive mark (OM). At present, the final disposal of these residues is difficult due to their high concentrations of phytotoxic substances, causing environmental problems if they accumulate in large quantities. Due to its chemical composition (carbohydrates, fiber, fats, proteins and mineral salts),agro-industrial residues are suitable substrates forbiotechnological proceesing to obtain value-added products. The objective of this work was to carry out a preliminary study of the production of hydrolytic enzymes using *Aspergillusniger*in Solid State Fermentation (SSF) using solid regional wastes. The following residues were used: red grape marc (RGM), olive mark (OM) and mixture RGM-OM (50% w/w of each one). SSF were carried out in Petri´s dishes at 27°C, with solid medium initial moisture content of 60% w/w and an inoculum size of $1x10^7$ spores*g dry^{-1} of *A. niger*. Two samples of culture were taken per day during one week for subsequent analytical determinations. The enzymatic activities that were measured in the aqueous extracts of SSF were: α-L-arabinofuranosidase (α-L-arf), α-L-rhamnosidases (α-L-rha), exopolygalacturonase (exo-PG), laccase (lac) and lignin peroxidase (LiP). The maximum enzymatic activity values were obtained for α-L-arf and exo-PG. The maximum activities for both enzymes were:2.62U/g for α-L-arf, at 58 hours of cultivationand 4.53U/g for exo-PG at 70 hours, in SSF of RGM-OM. As for lac and α-L-rha activities, lower values were obtained. No significant amounts of LiP activity were determined. The results obtained indicate that it is of interest to optimize the SSF tests with the mixture of the two solid substrates to maximize the values of the enzymatic complex, investigating the culture conditions and nutrient additions.

A137
DETECTION OF IMPORTANT GENE POLYMORPHYMS IN SWINE PRODUCTION

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Applied genetics allows increasing the production of pork meat and improving its quality. Some of the most wanted production parameters in pigs are litter size, number of piglets born alive, feed conversion, backfat thickness and meat quality. For litter size and number of born alive, two genes that have the greatest impact are the estrogen receptor (*ESR1*) and the prolactin receptor (*PRLR*). For the pork meat quality one of the important factors is related to a polymorphism of the ryanodine receptor gene (*RYR1*). The objectives of this work are: 1) to establish the frequency of favorable polymorphisms for the estrogen receptor gene and for the prolactin receptor gene in gestating sows and 2) to establish the frequency of the HAL-1843 polymorphism of the *RYR1* gene in juvenile pigs, sows and boars. All specimens come from small and medium-sized establishments in the UNNOBA area of influence. The end is to contribute to the knowledge of breeders to achieve a genetic improvement, increasing productivity. A total of 51 samples were analyzed for *ESR1* and 50 for *PRLR*, from 2 porcine establishments and 154 samples for *RYR1* from 11 establishments. The study of the different genotypes was carried out by PCR followed by RFLP. Hair bulb samples were washed with ethanol, incubated with Proteinase K; DNA was extracted by phenol-chloroform-isoamylalcohol and amplified by PCR.The fragments were digested for the three genes in question, using the restriction enzyme *Pvull* for the *ESR1* gene, *Alul*

for the *PRLR* gene and *BsiHKAI* for the *RYR1* gene. For the estrogen receptor gene, where the B allele is preferred 54.9% was detected with the AB genotype and 3.9% with the BB genotype. For the prolactin receptor gene, which the A allele is favorable, 38% was registered with the AA genotype and 40% with the AB genotype. The genotypes found for the *RYR1* gene were: 88 NN: 6 boars, 52 sows, 4 young males and 26 young females; 64 Nn: 8 boars, 31 sows, 7 young males and 18 young females; nn: 2 young males. We detected 1.3% of recessive homozygotes and a heterozygosity of 41.5%, corresponding the 25.3% to breeding animals. The report to the pig breeders allows them to know the presence of gene polymorphisms in order to maximize better crosses avoiding unfavorable alleles. The HAL-1843 mutation permits also to detect the presence of animals susceptible to Swine Stress Syndrome and to understand the variability observed in the meat quality, so to control the crosses of heterozygotes will contribute to reduce the incidence of the mutation.

A138
DESIGNING AND OPTIMIZATION OF TETRA PRIMER ARMS-PCR PROTOCOL FOR GENOTYPING SINGLE NUCLEOTIDE POLYMORPHISM RS12107982 (C/A) OF RECEPTOR II OF TGF-β

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Type 2 diabetes mellitus (T2D) is one of the most important global public health problems. T2D prevalence is rapidly increasing and it is considered an epidemic disorder worldwide. In the last years, several studies have demonstrated that chronic and low-grade inflammation is closely involved in the T2D development. TGF-β1 is an anti-inflammatory immune mediator which inhibits or reverses the activation of macrophages. High levels of TGF-β1 were associated with T2D. On the other hand, receptor II of TGF-β (TGFBR2) is a key mediator of growth inhibitory signals of TGF-β. The TGFBR2 receptor contributes as a risk factor for the onset and severity of coronary artery disease risk. The most common techniques used to analyze SNPs are time consuming, multi-step process and require expensive instruments. Therefore, in order to overcome these problems, we have developed a new, rapid and cost effective T-ARMS PCR assay to genotype rs12107982 (C/A) in TGFBR2. However, the optimization step can be hardworking and laborious. Hence, we propose to demonstrate and discuss critical steps for its development, in a way to provide useful information. In a first step, we design and validate two specific primer pair for T-ARMS PCR. Later, the amplification conditions were optimized for DNA concentration, $MgCl_2$concentration, annealing temperature, *Taq* DNA polymerase units and primers concentration. The last one was considered the main interference factor for a correct amplification and appropriate band intensity. Finally, the results obtained by T-ARMS PCR were concordant with modified standard PCR-ASO assay. T-ARMS PCR assay developed in our laboratory for genotyping rs12107982 (C/A) in TGFBR2 gene is time saving and cost-effective compared to the available methods used for SNP studies.Our results provide direct evidence that T-ARMS-PCR is a rapid, reliable, and cost-effective method for SNP genotyping of TGFBR2 gene in type 2 Diabetes Mellitus individuals.

A139
GENOMIC REGIONS FOR RESISTANCE TO FUNGI AND VIRUSES IN MAIZE

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Discovering communalities among studies reporting quantitative trait loci (QTL) for maize diseases resistance is crucial. First, we performed a literature systematic review to search published QTL for maize disease resistance by searching multiple databases with the term ("*Zea mays*" or maize or corn), ((resistance or tolerance) and "disease"), (QTL or loci or "Quantitative Trait Loci"). A total of 110 studies containing QTL information related to fungi and virus resistance were found. Second, we performed a meta-analysis aimed at identifying genomic regions carrying major-effect QTL for resistance to fungal and viral diseases. For each chromosome, reported QTL were counted, and a global odd ratio of carrying major effect loci was calculated by averaging findings across studies. Results show that the greatest number of QTL was reported in chromosome 1, but the odds of finding major-effect loci for fungus and virus resistance in chromosome 10 were twice as high as the odds of finding those main loci in the rest of the genome. The major-effect QTL reported for resistance to fungal diseases were not located on the same chromosomes as those with virus resistance, except for chromosome 10. There was no agreement among studies in the occurrence of major-effect loci on chromosome 3 and 8. Bins 1.03, 1.04, 1.05, 1.06, 1.10, 2.04, 2.07, 5.03, 6.02 and 10.06 in chromosome 1, 2, 5, 6 and 10 were recognized as genomic regions where major-effect QTL are located. Our results summarize and confirm published findings about key genomic regions for maize molecular breeding against diseases that can cause significant yield losses.

A140
MAIZE INBRED LINES PERFORMANCE FOR THREE MAIN DISEASES IN SOUTH CÓRDOBA, ARGENTINA

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Germplasm collections are a valuable resource in genetic plant breeding. The CIMMYT (International Maize and Wheat Improvement Center) Maize Lines (CMLs) have been developed over the last 25 years. The CMLs represent one of the most widely distributed sources of publically generated elite lines, which are freely available to both public and private sector breeders, research and growers, worldwide. The objective of this study was to describe the performance of a 200 maize inbred lines collection developed and provided by the CIMMYT against the three main diseases that occur in south Córdoba, Argentina. The collection was evaluated by a partially replicated experiment with a 12% of test lines in three replications. The experiments were located at Río Cuarto and Sampacho during the summer cycle of 2017/2018. In each location, for each genotype, the common smut incidence (INC_{CS}), common rust severity (SEV_{CR}), Mal de Río Cuarto Virus incidence (INC_{MRC}), severity (SEV_{MRC}) and disease severity index (DSI_{MRC}) were estimated. A multivariate approach, principal component analysis (PCA), was used to describe the relationship among genotypes, traits and genotypes-traits. A cluster analysis, to group genotypes, was also used. The first two principal components of the biplot graph explained 80% of the variation. Positive correlations were observed between INC_{CS} and SEV_{CR}, and among INC_{MRC} SEV_{MRC} and DSI_{MRC}. There is not correlation between these two groups. The 35% of the genotypes did not present disease. The cluster analysis indicated eight groups. Such as shown by the PCA, one of the groups included the 35% of the genotypes that did not present disease. These results allow us to identify promising genotypes to obtain maize hybrids with good behavior against common smut, common rust and Mal de Río Cuarto Virus.

A141
ON-LINE CHROMATOGRAPHY PROCESS FOR CHIRAL SEPARATION USING CARBON NANOWIRES AS A STATIONARY PHASE AND LYSOZYME AS CHIRAL SELECTOR ADDED TO MOBILE PHASE

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Many pharmaceutical compounds have one or more chiral centers that are responsible for their optical activity. The importance of chirality in the activity of these compounds has long been recognized due to the fact that those enantiomers exhibit different activities in human and animal bodies. Being able to define the amount of each in a mixture has become of utmost importance for the pharmaceutical industry. Consequently, developments of methods for enantiomer separation are extremely valuable.The applicationsof nanoparticles (NPs) are increasing in separation science; many past papers reported the use of NPs as stationary phases in chromatography to improve selectivity, chemical stability, and separation efficiency of chromatography. In addition, carbon nanotubes (CNT) have been also subjected to an intense research owing to their unique their physical, mechanical and chemical properties.Other nanoscale carbons, such as carbon nanowires (CNWs) which is a new member of one-dimensional (1-D) nanomaterials with solid core, large aspect ratio and highly curved structures, have attracted much less attention.Nanowires have a cross-section in the nanometer scale, and a length-to-width ratio in the range of 1000 or higher, a size regime where quantum effects produce new electrical, physical, and mechanical properties.In this work we proposed a methodology to separate a racemic mixture tryptophan, an essential amino acid which has chiral characteristics and deeply involved in the synthesis of serotonin, a hormone strongly related with stress.Aseparation column was developed using CNWs as support. For the assembly of the column 2.7 to 12 mg of CNWs, were weighedand a mobile phase containing lysozyme, a protein well known as chiral selector was studied. The injected samples were determined using a Hewlett Packard spectrophotometer. The preliminary results showed a resolution (Rs) =1.3 between both chiral analytes.

A142
PHENOTYPIC VARIATIONS IN POPULATIONS OF *Hedeoma multiflora* BENTH.

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The biological and cultural diversity of a country is constituted in the heritage of the society that integrates it; which is why in order to continue exercising your identity you must conserve, protect and improve them. The rational use of these genetic resources is a source of income of genuine resources that allows achieving a sustainable development of the regional economies. In the case of native species with aromatic-medicinal value their extraction and use has determined that the sustainability of these systems presents high fragility and commitment to their productive efficiency. One of these species is *Hedeoma multiflorum*, a

perennial herb with aromatic, digestive and analgesic properties. In the present work, the evaluation of the phenotypic variability of four natural populations of this species is reported. Seven morphological and phenological variables were evaluated: leaf width, leaf length, plant diameter, plant height, number of stems, presence of inflorescences and presence of seed-fruit. The sites evaluated were areas of the Sierra de Comechingones (northeast sector of San Luis, Argentine): Carpintería, La Cordobesa, Boca de Rio and Loma Blanca. The data was analyzed by correlation matrix, principal component analysis and grouping. A high correlation between plant diameters with number of stems and also between leaf width and leaf length was determined. Significant differences were obtained ($P < 0.01$) between the populations for leaf length, height, diameters and number of stems. The first two components were considered sufficient, since they concentrate 80% of the total variation of the characteristics included in the analysis. Based on the first two main components, two different groups were identified, one of them composed of the populations of Boca de Río, La Cordobesa and Loma Blanca and the other group by the population of Carpintería. It is possible that the inherent variation observed in the latter population is restricted to a local adaptation to specific environmental conditions, due to the great ecological plasticity of the species. Through the dendrogram, the results of the principal component analysis are corroborated. The grouping suggests the perfect separation of two groups. This shows that the variables evaluated are satisfactory to phenotypically classify the populations studied. With this preliminary essay, the aim is to initiate the development of native germplasm with aromatic and / or medicinal attributes, and to reduce the impact of genetic erosion on natural populations, constituting a proposal with commercial interest for the region, based on an indigenous natural resource.

PHARMACOLOGY AND TOXICOLOGY

A143
. *Tripodanthus flagellaris*: STUDY OF ACUTE TOXICITY

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Tripodanthus flagellaris (Cham.& Schlecht.)Tiegh.(Loranthaceae), popularly known as “liga blanca”, “liguilla”, is used in folk medicine for therapeutic on circulatory system and as antihypertensive. Infusion of the plant material (10%, Del Vitto LA & EM Petenatti 8553, UNSL) was prepared, separated by filtration and the aqueous extract was concentrated and lyophilized to preserve it.The *Tripodanthus flagellaris* lyophilized extract (TFLE) was studied for acute toxicity, using different routes of administration, as per revised OECD guidelines N°423: Acute Toxic Class Method (a stepwise procedure with the use of a minimum number of animals of a single sex per step).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 and intraperitoneally (i.p.) increasing doses of TFLE (5, 50, 300 and 2000 mg/kg) respectively. 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.The Irwin observation test was used to evaluate the effects of TFLE on behavior and physiological function. Both oral and i.p. doses of 2000 mg/kg of TFLE did not produce any sign of acute toxicity in the animals (male and female). Over the 14 days following the oral and i.p. administration of TFLE, none of the animals died and no significant changes organ weight were observed through the end of this period ($p>0.05$). No gross lesions were noted in any mice on necropsy. There were no signs on symptoms of ataxia, catalepsy, excess curiosity, scratching, restlessness, respiratory distress, urination, diarrhea, convulsions, and coma. Oral and i.p. doses of TFLE 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 and i.p. 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, TFLE had not presented signs of toxicity.

A144
NEW EXPERIMENTAL LINES *Amaranth* SEEDS: ANTINUTRIENTS FACTORS

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In Argentina Amaranth production is very low despite its nutritional attributes and economic potential. Numerous investigations show the bio-physiologic properties of the grain, high protein level and its excellent amino acid profile, vitamins, minerals, absence of gluten make Amaranth seeds a potential food, recommended for celiacs patients, and an alternative nutritional source to human food. Rio Cuarto National University works since 1980 in the optimization of Amaranth agronomic characters to develop a series of advanced lines, such as, Alin-G7 / 8; Alin- G14b / 1; H21 / 8 and H10 / 1, with outstanding characteristics of adaptability in order to promote the use of these grains in alimentary industry. The Amaranth grain is processed mainly to obtain

flour, this by product have many application but have antinutritional substances in their composition that affect the nutritional value, hindering the assimilation of nutrients, causing undesirable physiological effects and even diminishes the flour taste. Therefore the knowledge of these substances leads to a better use of the nutritional potential and give a guideline of the quality and organoleptic properties of the new lines of Amaranth grains used to obtain food products. Determinations were performed for oxalates, obtaining 6.61 – 8.54 mg of oxalic acid/100g of sample; nitrates, which ranged between 206,82 and 209.06 mg%; saponins, which gave a foaming index <100, lectins which presented agglutination in dilutions between 1/64 and 1/128, and phytic acid, 0.0365 to 0,0393 mg of phosphorous/100g of sample. The results obtained of amaranth flour from new experimental lines studies are within the allowed ranges, do not represent a risk for human consumption, and can be used in food industry.

A145
WHEN CADMIUM ATTACKS LUNGS: DIFFERENCES BETWEEN ANIMAL AND VEGETAL PROTEIN SOURCES IN DIET

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Cadmium is a naturally occurring toxic heavy metal with common exposure in industrial workplaces, plant soils and from smoking. Among its effects are the generation of oxidative stress, lipid peroxidation, alteration in gene expression and apoptosis/cáncer. Pulmonary response might lead to the development of diseases such as asthma, COPD, and lung cancers. On the other hand, it is known that consumption of soy protein has beneficial health effects, e.g.: lowering cholesterol, decreased weight in cases of obesity and protection against breast cancer. We studied Cd effects on lung morphology, bronchoalveolar lavages (BAL) and oxidative stress markers under different diets. 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots received soybean (Soy) as protein sources. Within each group: 1 lot received regular water (Co) and the other 15ppm of Cd in the drinking water, for 60 days. BAL was performed and nitrites concentration was measured. Total RNA was isolated with Trizol and cDNA was obtained. SOD, GPx and Nrf-2 were determined by PCR. S28 was the control. TBAR'S and Catalase (CAT) activity were determined. Lungs were examined for evidence of injury and analyzed morphometrically. A lung section was mineralized and Cd, Cu, Zn, Se and Mn concentrations were measured by ICP-MS. Cd was higher in intoxicated groups, but only significant in Cas-Cd ($p<0.01$). TBARS showed a significant increase ($p<0.01$) in So-Cd. SOD-2 decreased in So-Co vs Cas-Co ($p<0.01$), and also showed an increase in So-Cd vs So-Co ($p<0.01$) and Cas-Cd ($p<0.05$). GPx showed an increasing trend in Cd groups. Nrf-2 augmented in So-Cd vs So-Co and Cas-Cd ($p<0.01$). CAT activity increased in Cas-Cd vs Cas-Co ($p<0.01$) and in So-Co vs Cas-Co ($p<0.005$). Nitrites decreased in Cas-Cd and So-Co vs Cas-Co ($p<0.01$). Zn and Cu showed no changes; Se concentration decreased in Cas-Cd vs Cas-Co ($p<0.05$); Mn decreased in So-Co vs Cas-Co ($p<0.01$) and increased in So-Cd vs So-Co ($p<0.001$). Non-functional areas are widely spread in Cas-Cd when compared with Cas-Co ($p<0.005$); there are also larger non-functional areas in Soy-Co vs Cas-Co ($p<0.01$) and no differences were seen among soy groups. Cas-Cd showed the presence of numerous non-functional spaces and advanced pulmonary fibrosis. On the other hand, when we analyzed Soy-Cd group, it also showed evidence of pulmonary fibrosis but this wasn't homogeneus and the non-functional spaces (with presence of connective tissue) were in the periphery of lobes. We report that Cd produces oxidative stress (and not nitrosative stress) on lung and the protein source in the diet is important because the antioxidant response (enzymes, transcription factors and metals involved) showed differences between the casein group (animal protein source) and the soy group (vegetal protein source).

A146
Minthostachys verticillata ESSENTIAL OILS AS POTENTIAL CHEMOPROTECTIVE STRATEGY ON AFLATOXIN B1 CYTOTOXICITY

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Aflatoxins (AFs) are toxic secondary metabolites produced by *Aspergillus* species which grow in a wide variety of animal feeds and foods. Aflatoxin B_1 (AFB_1) is the most prevalent and the most carcinogenic, teratogenic, genotoxic and immunotoxic mycotoxin. Exposure to and the adverse health effects of AFB_1 can be limited by chemopreventive and chemoprotective strategies to reduce the AFB_1-induced toxicity. *Minthostachys verticillata* (Griseb) Epling, also known as peperina, is a well-known South America aromatic plant rich in essential oils widely used in folk medicine for the treatment of a variety of diseases. Antiviral, antibacterial, antifungal and immunoenhancing activities of *Minthostachys verticillata* essential oil (Mv-EO) have been reported; however, little is known about the interaction of Mv-EO with AFs. Thus, the aim of the current study was to investigate the possible protective effects of Mv-EO against toxics effects caused by AFB_1 on Vero cells.Leaves and thin stems from *M. verticillata* were used to obtain Mv-EO. The essential oil was extracted by the hydrodistillation procedure for 2 h in Clevenger's apparatus and dehydrated using anhydrous sodium sulfate. The chemical composition of peperina oil used in the present work was previously determined using GC–MS. Vero cells were exposed to increasing concentrations of AFB_1 (0-30 µg/mL) and Mv-

EO (0-500 µg/mL), alone and in combination for 48 h. The cells treated with Mv-EO did not show cytotoxic effect. However, the treatments with AFB_1 caused cytotoxicity in a dose-dependent manner. The 50% inhibitory concentration (IC_{50}) of AFB_1 was 8.41µg/mL. When cells were treated with AFB_1 and Mv-EO, the essential oil decreased AFB_1-induced cytotoxicity in an inverse doses dependent-manner. Highest concentrations of Mv-EO did not inhibit AFB_1-induced cytotoxicity. The cell viability was 40.72% at 10 µg/mL AFB_1 exposure, and significantly increased to 87.82%, and 75.94%, when 50µg/mL and 150µg/mL Mv-EO were added, respectively.These preliminary results indicate that *M. verticillata* essential oil has potential to counteract the effects of AFB_1-induced cytotoxicity.

A147

HEALING ON ETHANOL-INDUCED GASTRIC LESIONS BY *Lithraea molleoides* IN RATS

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Lithraea molleoides (Vell.) Engl. (Anacardiaceae), known in Argentina as “molle”, “molle de beber”, “molle blanco”, “molle dulce”or “chichita”, is a tree which grows in South America, especially in Argentina, Brasil and Uruguay. Decoctions and infusion of the leaves are used by people of these countries for its medicinal properties which include digestive diseases. In a previous work, we have demonstrated that a pretreatment with *L. molleoides* induced inhibition of gastric ulcers, compared with the control groups, using as necrotizing agents 0.6N HCl, 0.2N NaOH, 200 mg/kg acetilsalicilic acid and absolute ethanol (pretreatment of *L. molleoides*). The aim of the study was to evaluate the in vivo healing activity of *L. molleoides* (post-treatment of *L. molleoides*).The 10% infusion of *L. molleoides* was prepared according to Argentinean Pharmacopoeia. The experiments were carried out taking into account international guiding principles and local regulations concerning to the care and use of laboratory animals for biomedical research. The experiments were approved by the local Committee CICUA (Protocol F-283/17).The experiment was performed according to the method described by Sibilia et al. Before the experiment, Wistar rats (200-220 g) were unfed for 24 h but allowed free access to water until the beginning of treatment. 50% ethanol, as necrotizing agent, induced severe gastric haemorragic damage. The test groups were administered different doses of an infusion of *L. molleoides* lyophilized (250 or 500 mg/kg, orally). At the end of the experiments, the animals were euthanized by inhalation of carbon dioxide (groups: 3 h or 24 h after the treatment). The stomachs were removed and incised along the greater curvature and examined for the severity of mucosal gastric lesions. The macroscopic ulcer lesion area in mm^2 was determined (Image J, NIH). Statistical analysis was performed by a statistical package (Prism, GraphPad Software, USA). All values were expressed as the mean ± SEM. Data were analyzed by analysis of variance (ANOVA) and posterior comparison by Tukey-Kramerand p values less than 0.05 were considered statistically significant. Oral administration of the damaging agent to the control group clearly produced mucosal damage characterized by multiple hemorrhage bands of different sizes. A postreatment with *L. molleoides*induced the healing of gastric ulcers, compared with the control group, at the time 3 and 24 h (*L. molleoides* lyophilized 250 mg/kg: $p<0.001$ vs. control and *L. molleoides* lyophilized 500 mg/kg: $p<0.001$ vs. control).The percentage of healing was between 92 and 98%. In conclusion, these results demonstrate that acute *L. molleoides* infusion treatment induced healing of ethanol-induced gastric lesions in rats. *L. molleoides* exerts protective and curative effects in different models of experimentally induced gastric ulcer in rats.

A148

ETHNOBOTANICAL STUDY ON THE KNOWLEDGE AND USE OF MEDICINAL AND AROMATIC PLANTS BY THE COMMUNITY OF SCHOOL No 3 MADRE TERESA DE CALCUTA (SELF-MANAGED), SAN LUIS

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Medicinal and aromatic plants make an important contribution to the health system of local communities, since they are frequently used by most rural populations. A study was conducted to establish the current state of local knowledge about the use of medicinal plants in School No 3 "Madre Teresa de Calcuta "(self-managed), San Luis, Argentina. Data were collected from popular informants of the educational community by structured and semi-structured surveys, observations and interviews. A total of 257 subjects were asked about the plants used to treat some diseases, the traditional medicinal uses, preparation methods (infusion, poultice, juice, cooking, macerated, crushed), and the form of administration (drink, food, baths, external use. In addition, information was obtained on sociocultural aspects such as age and sex of the subjects. The results obtained were as follows: 80% of the population uses traditional medicine, due to safety (15%), illness (47%) and low cost (18%). Aromatic plants are used to treat gastrointestinal, respiratory, and high blood pressure disorders, and as a diuretic. The 125 medicinal species of the following plant families are included: Asteraceae (39%), Lamiaceae (26%) y Verbenaceae (14%), Euphorbiaceae y Fabaceae (12%), Moraceae (10%), Passifloraceae (9%) Rutaceae, Tiliaceae, Anacardiaceas y Valerianaceae (5%), others(4%). Most of the species were collected in the field. Only the 10% of the subjects have plants in pots and vegetable gardens, the rest, does not cultivate them.

The senior members of the community (parents and grandparents, 55%; uncles, aunts and neighbors, 25%) gave the necessary information to gather an important collection of popular knowledge and traditional use of medicinal plants. This work gives new information about the use of plants for medicinal purposes, and provides a scientific base for further studies on the field.

A149

MEDICINAL PLANTS FROM ARGENTINA WITH ANTILITHIATIC ACTIVITY: A REVIEW

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The use of medicinal plants for healing purposes is a practice that has been used since time immemorial. This made it possible to deepen the knowledge of plant species that possess medicinal properties and expand their experience in the use of the products extracted from them. Lithiasis is the formation of stony concretions (calculi) in the body, most often in the gall bladder or urinary system. These calculi can have a preeminence of calcium and phosphates, which makes them extremely difficult to expel or extract from the body.
The objectives of this work are the following: (a) to search and analyze available bibliography on herbal medicines from Argentina used for urinary lithiasis; (b) to observe the taxonomic contribution of the medicinal flora of Argentina and (c) to identify the species and parts of the plant that are used by lithiasis treatment. The information on flora having antilithiasis activity was collected from various journal, websites and books. The information extracted was included in an Excel-based database and organized by: (a) family / scientific name, (b) vernacular names, (c) parts of the plant used, (d), category for the Argentine flora, (e) use related to the review, (f) bibliographic sources of the Citation, (g) distribution in the Argentine provinces and (h) use in the market.
A total of 65 species of plants used for lithiasis from different regions of Argentina were found. The species used belong to botanical families, mainly Asteraceae (15%) and Fabaceae (11%) the rest the families they are in different percentages. Leaves, fruits, bark, flowers, inflorescences, seeds, mucilage and stems were the most used parts being leaves the most used (10%). The reproductive organs (flowers, fruits, seeds and inflorescences) (5.5%) and the underground organs (7%) were mentioned less frequently. Forty native species (14%) were found in different provinces. Five species used in medicines were detected; the others were herbs and phytomedicines. This data collection can serve as a tool of rapid and easy access to all people interested in natural anthilitiasics.

A150

TOXICITY AND REPELLENCY OF THE ESSENTIAL OIL FROM *Fluorensia oolepis* BLAKE (ASTERACEAE) ON *Triatoma infestans* KLUG

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The misuse of pesticides, especially in undeveloped countries, is one of the causes of pest control failures and a widespread and growing problem of environmental pollution. Chemical pesticides have played an important role since the mid-twentieth century. However, their continued use has a strong environmental impact. *Triatoma infestans* Klug (Reduviidae) is a medically important pest. The control of these triatomines by pyrethroid insecticides represents currently an alternative among the Regional Initiatives. The use of botanical insecticides is an accessible and low-cost control alternative for peasants and communities that inhabit the triatomine-infested zones, due to the fact that several plant species, which possess recognized insecticidal activity, grow easily or are native to these geographical areas. Likewise, insecticides of vegetable origin have the advantage of being biodegradable and immediately available. For this reason, essential oil (EO)from*Flourensia oolepis* Blake were selected and their ovicidal activity, contact toxicity, fumigant and repellent activity were determined. Considering the results obtained in the laboratory, a formulation was prepared with the EO from *F. oolepis* in order to carry out a field experience. The prepared formulation, whose main component was the EO showed very good activity, was similar to the activity of Deltamethrin©. In conclusion, the EO tested here shown interesting biological activities against nymphs of *T. infestans* and could be a potential basis for new preparations for peridomicile treatment in order to minimize the presence of the Chagas disease vector.

A151

BEHAVIORAL MODIFICATIONS INDUCED BY KETAMINE SUBANESTHETIC DOSES INJECTED 5 TO 30 MINUTES IN THE FORCED SWIMMING TEST.

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Ketamine, by antagonizing the NMDA (N-Methyl-D-Aspartic Acid) receptors of glutamate, produces psychotomimetic effects. In previous studies we have verified the action of antagonists of this type injected intraperitoneally in behavioural tests in rats. In the present work the effect of the administration of ketamine at low doses (2.5, 5 mg / kg) is studied in the forced swimming test, at 5 and 30 minutes. Holtzmanderived rats weighing 280 to 310 g were used. This test is based on the principle of learned hopelessness, which establishes that every organism that is subjected to a stressful event that it cannot control and from which it cannot escape develops, initially, anxiety and subsequently, if the event remains in time, depression. There was a significant decrease in swimming with the dose of 2.5 mg/kg ($p < 0.05$), an increase in climbing in the saline groups and 5 mg/kg ($p<0.001$, $p<0.01$) and the consequent decrease in resting only in the saline group ($p<0.05$). We conclude that the administration of ketamine at the doses studied produces behavioural alterations in the times in which the tests were performed.

A152

ACTION OF KETAMINE ON ACQUISITION IN THE NOVEL OBJECT RECOGNITION TEST AND THE METABOLIC ACTIVITY IN HIPPOCAMPUS

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Psychotogenic effects of ketamine, due to antagonism of NMDA (N-methyl-D-aspartic acid) glutamate receptors, are widely known, and they are subject of study in our laboratory. In the present study we searched for the effect of systemic administration of ketamine at low doses in the novel object recognition test. Holtzman derived colony rats weighing 240-290 g were used. Novel object recognition test involves variables related to memory and perception. We used an intraperitoneal administration of ketamine in sub-anesthetic doses (1.25, 2.5 and 5 mg / kg ip) 3 min before training. Test was realized 120 min after training. Additionally, groups of five rats treated with saline or different sub-anesthetic doses were paired for measurement of hippocampus metabolic activity 8 min after injection. It was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) through spectrophotometric wavelength measurement. We observed that treatment produced a significant increase in total scan time in the evaluation session ($p < 0.05$) at all doses. It also led to a significant decrease in the discrimination rate ($p < 0.001$) at doses of 2.5 and 5 mg/kg. These findings allow us to postulate that treatment induced an inhibitory effect on short-term memory. Metabolic activity showed an evident and significant decrease in hippocampus (1.25 mg/kg; 2.5 mg/kg, $p<0.001$). We conclude that systemically administered ketamine in low doses produces inhibitory effect on short-term memory. These behavioral findings are accompanied by a decrease in metabolic activity in the hippocampus.

A153

SAMPLING AND IDENTIFICATION OF POLLEN, ASSOCIATED WITH THE DETERMINATION OF METALS AS AN INDEX OF ENVIRONMENTAL POLLUTION

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People are exposed to a great diversity of environmental pollutants, coming from anthropogenic activities. Metal pollution has been increasing rapidly over the past century, and at the same time, the human population has continued to rise and producing contaminants. Thus, particles such as pollen grains may contain various trace elements, and their amounts vary from one species to another. They can present traces of metals such as lead (Pb), barium (Ba), cobalt (Co) and manganese (Mn), which are among the most common soil contaminants and are considered toxic to living beings. In the present work we propose a simple and fast method for the multielemental determination of traces in aerobiological samples. The sample of aeroparticles was obtained with a volumetric Lanzoni sensor, which is located on the terrace of the National University of San Luis. This device allows the capture

of particles suspended in the air with a week periodicity, and with a suction flow of 10 l of air min^{-1}, similar to the volume of air inhaled by the human lung. The pollen content was analyzed for three months of continuous aerobiological sampling, in the atmosphere of the city of San Luis, Argentina. Pollen samples were readed with an optical microscope at 400 X and they were identified through palynological atlases and with the pollen library belonging to Aerobiology´s laboratory. For multielement determination, a mass spectrometer with inductively coupled plasma (ICP-MS) was used. The aeroparticle samples were subjected to acid digestion at 90 ° C for one hour. The conditions of analytical determination in the sample were optimized. The nebulizer gas flow used was 0.87 l min^{-1} and the RF power was 1100 W. The pollination period extended from August to October. Comparatively with others pollen types, Moraceae, Cupresaceae and *Ulmus* were the most abundant in the atmosphere of San Luis. Linear regression models were used to analyze the metals vs pollen content. Positive associations were found between Mn (y=0.12x + 8.53; r= 0.12, P=0.0012) and Ba (y=0.03x + 4.85; r= 0.03, P=0.73) with Cupresaceae. In the same sense it was observed positive associations between Pb (y=0.12x + 1.59; r= 0.12, P<0.0001), Ba (y=0.09x + 6.90; r= 0.09, P=0.58), Co (y=0.07x + 0.71; r= 0.08, P=0.0001) and Mn (y=0.22x + 12.11; r= 0.21, P=0.11) with *Ulmus*. The maximum concentration values for Cupresaceae (166 pollen grains/m^3 of air) and *Ulmus* (> 50 pollen grains/m^3 of air) were recorded in August, while the maximum concentration values Moraceae (500 pollen grains/m^3 of air) was recorded in September. In this study, during the pollination period an increase in the elemental content was observed. Therefore, the metals transported in the pollen could act as a factor that increases respiratory disorders.

A154
OBTAINING, CHARACTERIZATION AND EVALUATION OF IN VITRO ACTIVITY OF ANANDAMIDE/ε-POLYCAPROLACTONE NANOPARTICLES

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Endogenous cannabinoids, such as anandamide, represent an attractive therapeutic potential in a significate number of pathologies. The present study had as objective to synthesize, to characterize and to assess the biological activity of anandamide/ε-polycaprolactone nanoparticles (Nps-AEA/PCL) in cell culture of epithelial cells of human proximal tubule (HK2). Nps-AEA/PCL were produced by electrospraying method and characterized by Differential Calorimetry Scanning (DSC), Thermogravimetry (TGA), Scanning Electron Microscopy (SEM), Infrared Spectroscopy by Fourier Transform (FTIR), UV-Visible spectroscopy (UV-Vis), and Dynamic Light Scattering (DLS). Physical stability studies were carried out on these nanoparticles. Also, we measured inducible nitric oxide synthase (iNOS) expression by Western blot, nitric oxide (NO) levels (nitrites) by Griess reaction, and Na^+/K^+ ATPase activity by a colorimetric method in HK2 cells. The obtained particles had a size between 100 to 900 nm of diameter with a predominance of 200 to 400 nm, with a Z potential value of -20±1.86 mV, indicating their good stability in solution. We observed the stable encapsulation of AEA in Nps-AEA/PCL, as well as its ability to induce the expression of iNOS and NO levels and to decrease the Na^+/K^+ ATPase activity in HK2 cells, in a dose-dependent manner. We concluded that obtaining Nps-AEA/PCL by electrospraying would be a useful methodology for a new pharmaceutical formulation development with optimal physicochemical properties, physical stability and biological activity on HK2 cells. Future works should increase the know-how about this novel alternative and its feasibility as treatment of cardiovascular diseases, particularly of arterial hypertension.

A155
THE PROTECTIVE ROLE OF A SOYBEAN DIET IN CEREBELLUM OF CADMIUM INTOXICATED RATS

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Cadmium is a toxic agent and is also an environmental contaminant. We studied its effects and the protective role of a vegetarian versus animal protein diet in cerebellum. Histoarchitecture, morphometric parameters, cadmium concentration, antioxidant enzyme activities and oxidative stress markers were determined. Female Wistar rats were used; 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source (n=6). 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 mRNAs were determined by RT-PCR. S28 was used as internal control. Histological studies were performed in H&E stained tissues. Morphometric parameters were measured in ten random visual fields in four sections (X40) and were analyzed with IMAGE J Software. Cadmium concentration was determined with ICP-MS. 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 SoyCd ($p<0.05$). CAT decreased in SoCd ($p<0.05$) and VCAM-1 did not change. iNOS increased in CasCd ($p<0.001$) and decreased in SoyCd ($p<0.001$). CAT and GPx activity decreased in both Cd groups ($p<0.01$). Cadmium concentration was incremented in CasCd ($p<0.05$). Not significant difference was found in Soy Groups. We found that Cd induces changes in the morphology of Purkinje and granular cells. Purkinje cells number/ mm PCL were decreased in CasCd ($p<0.0001$). Not significant difference was seen in

Soy Groups. The distance between Purkinje cells increased in CasCd ($p<0.05$). In Soy groups it was not significant. The Purkinje cells area was decreased in SoCd ($p<0.0001$). Soy diet attenuated the oxidative stress and the morphological changes in cerebellum of cadmium intoxicated rats. Soy could exert a protective role against this heavy metal.

A156
ALLICIN EXERT A PROTECTIVE EFFECT ON INJURED BV2 CELLS THROUGH AT1-Hsp70-iNOS CROSS-TALK MODULATION

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Lately, there are a lot of reports on the use of phytochemicals and their implications for human health as the neuroprotection effect of allicin (Alli) -an organosulfur compound from garlic- linked to a reduction of the oxidative stress, inflammation, and apoptosis. Oxidative stress and mitochondrial dysfunctions are common features leading to neuronal death in cerebral ischemia, traumatic brain injury, Parkinson's disease, Huntington's disease, and Alzheimer's disease; where the nitric oxide (NO) is a predominant effector of neurodegeneration. Parallel, heat shock protein 70 (Hsp70) upregulation may protect depression by downregulation of inducible nitric oxide synthase (iNOS) expression. To highlight, Alli attenuated loss of cell viability and neuronal apoptosis by iNOS decrease expression with an increased in the Hsp70 protein. Besides, Alli exerts protection like to losartan, and in silico analyses suggested that Alli and losartan could have a common mechanism involving AT_1 interaction. AT_1 overexpression is an early deleterious factor in many brain diseases. Aim: To evaluated if Alli may exert a critical anti-apoptotic/anti-inflammatory/anti-oxidative role in a model of neuronal injury, a murine microglial cell line (BV-2 cells), through AT_1-Hsp70-iNOS axis modulation. We also assessed if there is a possible protein's interaction related to BV-2 injury as well as the Alli improvement. Methods: BV-2, were cultured and after 80% confluence, cells were placed in quiescence for 24 hours. Immediately, BV-2 cells were injured with lipopolysaccharides (LPS, 100 ng/mL) and treated or not with Alli (50 μM) for 72h. The culture medium containing the LPS, Alli or both was replaced every 24 hours. All treatments were done in triplicate, and all experiments were replicated five to ten times. Cells were collected to perform the following determinations: Cell viability (MTT), pro-inflammatory cytokine levels, cellular oxidative stress (DHE), mitochondria isolation, NADPH oxidase activity (cells and mitochondrial fractions), iNOS, Hsp70 and AT_1 expression (WB), nitrite levels (Griess), and protein-protein interactions by immunoprecipitation technique. Results: LPS in BV-2 cells provoked morphological changes, reduction in mean cell viability ($p<0.05$), a significant increase in NADPH oxidase activity ($p<0.01$) as well higher levels of $O_2^{\bullet-}$ ($p<0.01$). Also, TNF-α as well IL-1β levels were significantly increased in the culture medium of LPS-treated BV-2 cells ($p<0.01$). Furthermore, LPS induced a higher iNOS/AT_1 and a lower Hsp70 protein expression. To highlight, the co-treatment LPS+Alli significantly reversed all the parameters mentioned previously.Unprecedented, we demonstrate iNOS-Hsp70 and AT_1-Hsp70 interactions, and more especially that Alli can modulate it. Conclusions: Alli prevents neuronal damage through the reduction in the oxidative stress/inflammation/apoptosis linked to an unprecedented modulation in AT_1-Hsp70-iNOS proteins cross-talk.

A157
SELECTION OF PLANT SPECIES FOR BIOLOGICAL EVALUATION: IMPLICATIONS OF BIODIVERSITY AND FUNCTIONALITY

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The developments, in the search for pharmacologically active compounds has enabled the use of many approaches for the selection of the plants for biological evaluation. Between them, (a) the random approach, which involves the collection of many plants with a common characteristic found in a study area; (b) the chemotaxonomic approach, which entails the collection of all members of a plant family known to be rich in certain class(es) of compounds; (c) the ecological approach, in which the relationship between the plant and its ecosystem is used as a means of selection; (d) the ethnobotanical-directed sampling approach, based on traditional medicinal use(s); and (e) the information-driven approach that utilizes a combination of ethnobotanical and chemotaxonomic data or a database that contains all relevant information concerning a particular species. These different methods will be briefly described and discussed in greater detail in particular cases from the Colombia biodiversity. Colombia is a country rich in biodiversity and endemism, inhabited by different types of population. All this wealth allows that the approaches available for the selection of plants can be applied in this territory, some more than others, or in combination thereof. This work attempts to present these strategies in context looks from the perspectives of the research done in recent years (a review). The exploration of the anti-inflammatory effects from traditional medicinal plants (e.g. *Cuphea calophylla***,** *Tibouchina kingii*, and*Pseudelephantopus spiralis*), neuroprotective plants from the Amaryllidaceae family following a chemotaxonomic approach (e.g. *Crinum jagus* and *Zephyrantes carinata*), the enzymatic inhibition related to cosmetic functionality from various species (e.g. *Gaulteria erecta*, *Passiflora tarminiana*, *Ugni myricoides*) and the search for antiprotozoal compounds from Annonaceae species may be displayed. Given that nature has performed a pre-selection of molecules that influence specific metabolic roles in all living things, it is likely that the search for selection of plants for

biological evaluation following a criteria combination strategy, it could increase the finding of bioactive compounds.

A158
INDOLE-3-CARBINOL REDUCES HYPERTENSION-INDUCED CARDIOVASCULAR REMODELING AND ARRHYTHMIAS

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Introduction: Hypertension and arrhythmias stand out as main causes of morbidity and mortality. During hypertension, nitric oxide (NO) and heat shock protein 70 (Hsp70) contribute to arrhythmogeniccardiac remodeling. A diet with low phytochemical content could worsen hypertensive remodeling through inflammation and oxidative stress modulation. Functional foods claim to reduce inflammation and oxidative stress by its high content of active phytochemicals. The indole-3-carbinol, present in cruciferous vegetables (broccoliand cabbages), protectsagainst inflammatory response triggered by ischemia-reperfusion. Aim: To evaluate the effects ofchronic treatment with indole-3-carbinol in hypertensive and normotensive rats summited to acute myocardial ischemia-reperfusion. Methods: Male SHR and WKY rats received indole-3-carbinol, or not (2000 ppm/day, *per os*) from birth to 8 weeks of age (n=10, each group). We assessed blood pressure and collected blood samples to evaluate biochemical/inflammatory markers.Per group, five cardiac samples we preserved for histology and molecular assays andfive hearts underwent the following ischemia-reperfusion protocol: 10 min of pre-ischemia, 10 min regional ischemia and 10 min reperfusion. We evaluated action potentials, electrocardiogram and arrhythmias (regularity of rhythm, presence, and severity). Results: SHR rats exhibited histological, structural and functional changes with increased systolic blood pressure and inflammatory-oxidative markers (IL-6, TNF-α, NADPH activity, NO/iNOS/Hsp70, $p<0.01$). The indole-3-carbinol reduced oxidative-inflammatory markers and blood pressure in SHR rats ($P<0.01$). To highlight indole-3-carbinol prevented the ventricular fibrillation in both WKY and SHR ($P<0.05$). Conclusions: Although the etiology of the hypertensive disease remains unclear, it is recognized that inflammation and oxidative stress are crucial elements. In this sense, our results demonstrate a novel protective mechanism linked to the use of indole-3-carbinol and its impact on cardiovascular arrhythmogenic remodeling associated with the hypertensive inflammatory-oxidative process. Indeed, the use of certain phytochemical -presents in the so-called functional food- and their possible anti-inflammatory effects in CVD open up promising perspectives in the area of clinical research.

A159
. NEW ARGENTINEAN EXPERIMENTAL BUCKWHEAT LINES: NUTRITIONAL COMPOSITION

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The buckwheat belongs to *Poligonaceae* family, is a pseudocereal, whose origin is in China, used in human food since ancient times. There are approximately 19 species, in addition to the wild varieties, although the most commonly used are common buckwheat (*Fagopyrum esculentum Moench*) and tartaric buckwheat (*Fagopyrum tartaricum* (L.) Gaertn). Nowadays, its use has resurfaced because it has proteins of high biological value free of gluten, essential polyunsaturated fatty acids, fibers, and the presence of fagomins (imino sugar with properties to anti-obesity); these characteristics convert at this cereal in a good resource in food industry. In central region of Argentina has been obtainednew experimental lines of buckwheat, called ALMNO13, in order to improve their adaptability, uniformity and performance to the growing conditions and promote their use in food industry. The objective of this work was to determine the chemical compositionand nutritional value (moisture, carbohydrates, caloric value, total fiber, proteins, fat, and ash) of the flour obtained from this new experimental variety (ALMNO13). The nutritional composition obtained was: proteins 12.0%; carbohydrates 69.6 g%; moisture 13.5 g%; caloric value 378 kcal; total fiber 15%; fat 6.1 g% and ashes 1.8 g%. The results obtained show that the studied flour can be used as a promising ingredient in the elaboration ofnovel foods, contributing to expand the diversity and variety of the human diet.

A160

EFFECT OF *Ligaria cuneifolia* ON DIURESIS IN RATS

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Ligaria cuneifolia (Ruiz & Pav) Thiegh. (Loranthaceae) is a widespread hemiparasitic plant in Argentine and it is employed as antihypertensive agentin folk medicine. It is well recognized that the diuretics are used for the management of mild to moderate hypertension. This study was designed to determine the diuretic activity of*L. cuneifolia*. The test was performed as described by Lipschitz et al. The experiments were carried out taking into account international guiding principles and local regulations concerning to the care and use of laboratory animals for biomedical research. The experiments were approved by the local Committee CICUA (Protocol F-300/18). The 10% infusion of *L. cuneifolia* was prepared according to Argentinean Pharmacopoeia. Wistar rats (200-230 g) were employed. The animals, randomly assigned into groups (n=7), were deprived of food for 24 hours prior to starting the experiments and had free access to water. The test groups were administered different doses of an infusion of *L. cuneifolia* lyophilized (250 or 500 mg/kg, orally) or furosemide as standard drug (10 mg/kg by intra-peritoneal route). The control group received only the vehicle (50 ml/kg, orally). At the end of the experiments, the animals were euthanized by inhalation of carbon dioxide. Urinary volumetric excretion (UVE), urine chemical parameters and urinary levels of sodium and potassium were measured in 3 hours diuresis. The urinary concentration of sodium and potassium were determined byinductively coupled plasma-mass spectrometry (ICP-MS).All values were expressed as the mean ± SEM. Graph Pad Prism was used for the statistical analysis and p values less than 0.05 were considered statistically significant. Student's t-test was performed to evaluate the differences between the control and the experimental samples for each time point. The lots treated withinfusion of *L. cuneifolia* (250 and 500 mg/kg)showed diuretic activity (UVE infusion of *L. cuneifolia* 250 mg/kg group: between 45 and 180 min, $p<0.05$ vs. negative control; UVE infusion of *L. cuneifolia*500 mg/kg group: between 45 and 180 min, $p<0.05$ vs. negative control). The urine samples presented normal chemical parameters in all the cases. The treatment with infusion of *L. cuneifolia* caused an increment in the urinary excretion of sodium (infusion of *L. cuneifolia* 250 mg/kg: $p<0.01$ vs. negative control) and potassium (infusion of *L. cuneifolia* 500 mg/kg: $p<0.01$ vs. negative control). The data reported in this work indicate that the infusion of *L. cuneifolia* showed a moderate diuretic activity, compared to furosemide, a loop diuretic potent. This diuretic activity could be due, in part, to the presence of flavonoids in this plant. Further investigations are necessary prior to their recommendation for use as diuretic.

A161

IMMOBILIZED ARTIFICIAL MEMBRANE CHROMATOGRAPHY OF SUBSTITUTED CHALCONES AND CORRELATION WITH BACTERIOSTATIC ACTIVITY

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The high performance liquid chromatography (HPLC) on a stationary phase composed of phospholipids, the so called immobilized artificial membrane (IAM), has been widely recognized as a valuable alternative method to extract and quantify information about the structure and physicochemical properties of organic compounds, particularly hydrophobicity parameters that are extensively used in studies of quantitative structure-activity relationships (QSAR). In the present study, the chromatographic capacity factors (log $k_{(IAM)}$)for 19 natural and synthetic substituted chalcones were determined by IAM-HPLC. In order to evaluate the ability of the IAM phase in assessing lipophilicity of the compounds under study, it was of interest to study to what extent the log $k_{(IAM)}$values were related to the octanol-water partition coefficients (CLOGP) values. Because a moderate correlation was found between log $k_{(IAM)}$andCLOGP values, several quantitative structure-retention relationships (QSRR) were derived to explore fundamental intermolecular interactions that govern the retention of compounds under study on the IAM phases.Thus, the multiple linear regressions (MLR) techniquewas used for modeling the IAM-chromatographic datadetermined for these compounds, and the molecular characterization was carried out by calculating several nonempirical descriptors, which weresubsequently used in the construction of QSRR models. The selected MLR-model for all compounds (n=19),which was highly significant statistically, was as follows: log $k_{(IAM)}$= - 4,34 + 0,298 CLOGP + 0,135 PCWTe + 16,5 RNCG ($r = 0.953$, $r(pred) = 0.943$, $s = 0.05$ and $F = 48.6$). From the analysis of the obtained results, it can be inferred that, as expected, the hydrophobic factors are of prime importance for the IAM-retention of these compounds, but the specific polar interactions such as expressed by charge descriptors (PCWTe, RNCG)are also involved. On the other hand, in the present work we also attempted to evaluate the ability of the IAM phase in assessing the antimicrobial activity of chalcones under study. Thus, the bacteriostatic activities exerted by a subset of 11of these compounds against *Staphylococcus aureus* ATC25923 strains, were investigated by means of QSAR modeling and by using the partial least squares (PLS) approach. The PLS analysis of minimal inhibitory concentrations (MICs) data for the 11 chalcones tested, yielded a two-component PLS model with the following statistics: $R^2 = 0.984$, $R^2(pred) = 0.890$ and $F = 254.5$. From the analysis of the obtained results, it may be concluded that the bacteriostatic activityof the chalcones under study is strongly dependent on hydrophobic factors as expressed by log$k_{(IAM)}$.Especially dependent on the geometric factors mainly accounted for by the directional and three-dimensional WHIM descriptors (G3u, E1m and E3m),

which encode the influence of the different aspects of the molecular shape.In conclusion, the present work provides evidence for the great potential of the IAM phases in the development of QSAR models.

A162
QUINUA BREAD: NUTRITIONAL AND SENSORIAL EVALUATION

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Food and Agriculture Organization of the United Nations (FAO) gave to Quinua the role in attaining food nutritional security and its potential for eradicating poverty. Quinua grains are highly nutritious in comparison than traditional cereal grains. Its protein content is remarkable and the essential amino acids composition is excellent, quinoa also provides complex carbohydrates, essential fatty acids, vitamins, minerals, dietary fiber and has a low glycemic index. Due to all beneficial characteristics of these grains the objective of this work was elaborated wheat flour breads with 30% and 50% of quinoa added-flour with the purpose to obtain a distinctive product with nutritional and sensorial benefits. The proximal composition of breads were determined (Protein, Fat, Humidity, Ashes, Total Fiber and Carbohydrates by difference), according Official Methods of Analysis, Argentinian Alimentary Code. The Caloric Value and Percent Daily Value (%DV) for each macronutrient was determined.
The breads obtained were sensorial evaluated according to a hedonic scale with to untrained panel of 50 members to establish the acceptability in function of the presentation, color, flavor, smell, and texture.
The bread with 50% quinua flour- added presented better nutritional characteristics than 30%. Conversely, 30% bread quinua flour-added obtained best sensorial evaluation, receiving the major acceptance between the judges surveyed.
Our results allowed conclude that both proposed quinoa breads improve nutritional facts and sensorial characteristics, mainly the % of dietary fiber and protein are high than conventional wheat bread and the % DV was high in both breads preparations and also the Caloric Value. These quinua breads can be consumed as potential functional food with a well balance of nutrients and can be recommended in a healthy and well-balanced diet as a replacement for common white bread

BIOCHEMISTRY, PHYSIOLOGY, PATHOLOGY AND PLANT PRODUCTION

A163
PRELIMINARY STUDIES IN VIVO OF THE EFFECT OF *Amaranthus hybridus* ALIN G16/9 ON ANTIOXIDANT ACTIVITY

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To environmental pollutants generates the production of reactive oxygen species and reduction in cellular antioxidant systems. Epidemiological studies have examined the possible interaction between food habits and oxidative stress. The elevation in the production of reactive oxygen species seems to be influenced not only by the amount of energy provided by the diet but also by the composition of macronutrients in the food. Amaranths have relevant nutritional properties and antioxidant capacity that is why in recent years there has been a great scientific and technological development for its integral use. This work evaluated the effect of a diet based on *Amaranthus hybridus* Alin G16/9 on lipid peroxidation and protein oxidation in serum and liver of Wistar rats. Previous studies in vitro on the seed extract showed an adequate content of lipids, proteins and an elevated antioxidant capacity, which motivated the initiation of studies in vivo, using the following model: Two groups of six rats each were studied for 28 days. Rats were fed with an AIN-93 M diet, with casein in group I and amaranth as protein source in group II. In serum and liver, the levels of products of lipidic peroxidation, malondialdehyde, were assessed according to Draper and Hadley. The content of carbonyls groups was determined by the 2,4-dinitrophenylhydrazine by ELISA in liver. Proteins were determined by the Biuret method. In both groups, there were no significant differences in the content of malondialdehyde in serum and live rindicating that there placement of casein by amaranthin our experimental model does not modify lipid oxidation. The presence of carbonyl groups in liver showed significant differences between the groups, with a significant increase in the group fed with amaranth (1.11±0.09mM/mgprot) compared to the group fed with casein (0.27±0.11mM/mgprot)($P<0.001$), possibly because with a protein-based diet of vegetable origin there is greater oxidative damage due to increased content of aminoacids susceptible to oxidation such as arginine, lysine and threonine. There is increasing scientific evidence supporting that Amaranths are a good alternative to prevent pathologies associated with oxidative stress due to its content of phenols and the presence of some minerals such as Zn, Se, Mn, Mg, Cu and other bioactive

compounds that have a protective effect. However in our model the effects of *Amaranthus hybridus* Alin G16/9 are still inconclusive, so it is necessary to continue making determinations.

A164
IDENTIFICATION KEY OF WEED SPECIES FROM THE BRASSICACEAE FAMILY FOUND IN SAN LUIS, ARGENTINA

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An increasing number of species of Brassicaeae are weeds of difficult control, in fact, three of these species were reported to have evolved resistance to glyphosate herbicide and *Brassica napus* has shown to become a feral plant in Buenos Aires province.In the last 30 years, the province of San Luis has been exposed to different changes in land use management, passing from afarming production to a mainly agricultural activity. These changes in the usage of farmland, which were similar to those adapted throughout the country, resulted in new perspectives in weed management. In San Luis there are 31 taxa of *Brassicaceae* family, some of them identified as crop weeds. The *Brassicaceae* family was previously studied in San Luis province. However, these studies were made before the actual agricultural transformation and did not consider the intensive use of agrochemicals. The aim of this work was to build a key in order to identify the species of the family *Brassicaceae* which represent agronomic crop weeds in San Luis.For this purpose, specimens at the adult state were collected in different cultivated fields, photographed in situ, determined and described following the classic botanical methods. Additionally, specimens were drawn, highlighting seeds and fruits, and deposited in the Herbarium of Departamento de Ciencias Agropecuarias, of Universidad Nacional de San Luis, Villa Mercedes (San Luis).A dichotomous key based on vegetative and reproductive characters wasmade, allowing the recognition of the following species: *Descurainia erodiifolia, Lepidium bonariense, Lepidium didymum, Rapistrum rugosum, Diplotaxis tenuifolia, Hirschsfeldia incana, Sisymbrium irio, Eruca vesicaria, Raphanus sativus, Capsella bursa-pastoris, Brassica rapa, Lepidium draba*. In conclusion, this study develops a useful tool to recognize those members of *Brassicaceae* family which representcrop weeds in San Luis.

A165
STUDY OF THE ARACHNOFAUNA AS AN AGROECOSYSTEM SERVICE USING THE RBA TECHNIQUE IN A VINEYARD OF MENDOZA, ARGENTINA

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Spiders are very abundant predatory species. They are key animals due to the impact they have on terrestrial ecosystems, since they make an important contribution in the depredation of pests in different agroecosystems, attenuating considerably the incidence of them in different crops. They are a link in the trophic chain of the system, positioning them as biological control agents constituting a direct benefit for the producer. They can be categorized as an agroecosystem service in this viticulture region.They are also considered good bioindicators for sustainable management of plantations, environmental transformation at agricultural and forestry level and natural environments. In the region of Cuyo, there is only one research on arthropods carried out by Torres Echeverría et al. (2015) in vineyards of Mendoza, using RBA technique (Rapid Biodiversity Assessment), so this work tries to make an important contribution using such technique to the knowledge of the arachnofauna present in vineyards of Mendoza. It was proposed the following aim: Identify and group by morpho-species the arachnofauna of a vineyard, conducted in “parral”, sited in Maipú, Mendoza, in order to establish the population dynamics of the most frequent species. The vineyard had a biological corridor and a nearby olive crop, so it was decided to install three monitoring stations in each site (vineyard, corridor, olive crop).Each station had a pitfall trap and an intercept trap. The monitoring was carried out during a week on September, December and March. In the laboratory, the captured individuals were classified and put in 70% ethyl alcohol. It was used RBA parataxonomic technique for the identification, in order to differentiate the specimens captured by their shape. The results obtained are part of a database used to evaluate biodiversity from the Shannon-Weaver index. The index corresponding to the place of study considering the three sites was low as well as the one corresponding to the analysis of the monitoring date. According to the results obtained, it can be concluded that the diversity of spiders is low and therefore this ecosystem service should be increased, however the fact that there are no pests that exceed the threshold of economic damage could mean that the spiders are fulfilling its function by keeping pest species at low levels.This indicates that studies on the subject should be continued. In addition, this work aims to adopt and adapt the foreign RBA methodology to this region and continue the study over time and in other agroecosystems of interest.

A166
EVALUATION OF CORN YIELD RESPONSE TO THE APPLICATION OF A SLOW-RELEASE NITROGEN FERTILIZER

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Nitrogen (N) is a nutrient of great importance for the cultivation of corn. If nitrogen is properly supplied from the early stages, good root development, rapid growth and high biomass production are enabled. The maximum rate of accumulation of N in plant occurs between V10 (tenth leaf) and R1 (silking), which determines that nutrient provision must be balanced until the beginning of the reproductive period, if good grain yield (GY) is sought. Conventional nitrogenous fertilizers, such as granulated urea, have low efficiency and their application throughout the crop cycle depends on rainfall and available machinery. The objective of this work was to evaluate the response of the GY and its components to the simultaneous application at the time of sowing of the corn (Hybrid *LT621MGRR2*), of a slow-release nitrogen fertilizer (SULFAMMO META 29). This process was carried out in the town of Tilisarao (San Luis) in a design with witnesses at par, by modeling the spatial correlation (Gaussian) during the 2016/17 cycle. At the time of sowing the availability of nitrogen in the soil (0-60 cm) was 65 kg ha^{-1}. The fertilization treatments were: 0, 50 and 100 kg ha^{-1} of SULFAMMO, equivalent to 0, 14.5 and 29 kg ha^{-1} of N, respectively. GY showed differences ($p<0.01$) between treatments with values of 8822, 7131 and 5742 kg ha^{-1} for the respective decreasing levels of fertilization, exceeding in all cases, the control at par (5416 kg ha^{-1}). The kernel number (KN) per surface unit and the grain number per spike showed differences ($p<0.05$), where the treatments fertilized with 50 and 100 kg ha^{-1} exceeded the treatment without SULFAMO and the control at the same time. Although the grain weight showed differences ($p<0.05$) between treatments, it was not consistent with the doses of the added fertilizer. The use of a slow-release nitrogen fertilizer applied to the sowing of corn allows a balanced supply of N during an important part of the crop cycle with a significant response in GY, in dry land, where the fractional application of the fertilizer is difficult.

A167
PHYTOSANITARY AND ENVIRONMENTAL CHARACTERIZATION OF MAIZE CROPS IN THE EAST CENTRAL REGION OF THE PROVINCE OF SAN LUIS, ARGENTINA

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According to global tendencies related to food demand, research efforts must be oriented towards a coexistence of production and conservation in order to achieve sustainable production systems. In the semi-arid central region of Argentina, the cultivation of maize is the second most important in relation to the cultivated area. Agricultural production systems are complex and may be affected by several factors simultaneously, including biotic factors. For this reason, it is appropriate to apply a multidisciplinary approach to characterize and understand how each of these factors affects productivity. The objective of this work was to make a description of different aspects of the phytosanitary status of maize cultivation and environmental variables in the region. For this, censuses were carried out in 25 lots distributed along a climatic gradient between the isohiets of 600 and 700 mm and average annual temperature between 14 and 16 ºC. In each site (1) the severity of corn common rust, (2) weed cover, (3) damage severity caused by the corn earworm, (4) grain production, was recorded. The severity was evaluated in the functional sheets expressing as the percentage of damaged tissue / plant, according to Peterson's scale, the average of 10 plants in three replications per sampling site (Peterson R. F., A. B. Campbell, A. E. Hannah. 1948). The coverage of weeds was registered with the modified Braun-Blanquet method (Mueller-Dumbois and Ellenberg 1974) in an area of 10 x 10 m and the percentage of lost grains was calculated as the number of missing grains / total grains in the spike. Additionally, soil samples were taken for analysis and characterization. Descriptive statistics was performed to all data set. At the regional level, the average severity of damage caused by phytopathogenic agents was almost 5%, the average cover of weeds was 13.8% and the average percentage of grains affected by insects was 2.1%, on an average production of grains of 204 grams / plant. With regard to soil characteristics, the average percentage was 0.4% C, 0.83 mg / g N, 21.26 mg / kg P, with 0.75% MO, a pH (1 / 2.5) of 6.32 and conductivity (1 / 2.5) of 0.04. The weed cover was lower than in other more arid west region in the same province. This sanitary and environmental characterization will allow, based on the parameters obtained to propose trials to determine the effect of each of these factors and their interaction on maize yield and then be able to design integrated management strategies for sustainable agriculture.

A168

IDENTIFICATION OF *Gomphrena* L. SPECIES PRESENT IN THE AGROECOSYTEMS OF GENERAL PEDERNERA DEPARTMENT (SAN LUIS, ARGENTINA)

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Gomphrena belongs to the family of *Amaranthaceae* and comprises almost a hundred species worldwide, mostly of them from warm temperate and tropical regions of America and Australia. Fifty three are accepted botanical entities present in Argentina. The species of this genus are generally perennial or annual herbs or sub-shrubs with striated stems, thickened knots, opposite leaves more or less pubescent, hermafrodite flowers grouped in capituliform inflorescences with free tepals partly joined in the base. Within this genus there are C4 metabolism species and few others C3. Some species behave like weeds and are difficult to control; such is the case of *Gomphrena perennis*, given the presence of a structure called xilopodium. *Gomphrena perennis* particularly, has increased its presence in the agricultural area of eastern central Argentina, due to its tolerance to glyphosate. The aims of this work are to identify the species of *Gomphrena* genus present in Department of General Pedernera, San Luis and to provide a tool for an easy identification of them. Specimens were collected, documented and identified by classical botanical methods and deposited in the Herbarium of "Departamento de Ciencias Agropecuarias, Universidad Nacional de San Luis". An illustrated dichotomous key was built; diagnostic characters were photographed and drawn. The species collected correspond to *Gomphrena perennis* var. *perennis*, *Gomphrena pulchella* and *Gomphrena martiana* and they mainly occur on the edges of lots and shoulders. As result of herbaria revision within the species registered: *Gomphrena martiana* var. *martiana*, *Gomphrena martiana* f. *austrina* and *Gomphrena pulchella* subsp. *rosea* are also reported.This key contributes to a further knowledge of the different species of the genus present in the agroecosystems of the Department Pedernera, included those that are problematic weeds and to provide better assesment to professionals and farmers.

A169

STORAGE OF HARVESTED PRODUCTS IN A CAMPESINO AGRICULTURAL SYSTEM OF THE SANTIAGO DEL ESTERO IRRIGATION AREA

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At present, there is a tendency to carry out productions with diverse agroecological practices due to the problems originated by traditional agriculture. Many of these traditional production systems are carried out by rural families; an example is the "fence" in the irrigation area of Santiago del Estero, within the region of Chaco Semi-Arid. Although the technical productive characteristics at farm level are known, there are no publications in our country referring to the storage of the products of the fence. The purpose of this work is to document the traditional knowledge related to these practices in the irrigation area of Santiago del Estero. A semi-structured survey was elaborated and carried out referring to aspects such as products that are stored, characteristics of the storage structures, main problems during storage and destination of the stored production. There were surveyed 32 rural families that possess fences and that store what they harvest of the same ones. In general, rural families call "troja" or "pirhua" the structure where they store seeds, mainly corn and fruits of various species of Cucurbitaceae. The principal problem that concerns the stored corn, in 90.63 percent is the species *Sitophilus oryzae* L. (Coleoptera: Curculionidae) that they attack to the grains of corn in the field and in the troja; secondly the rodents in 59.38 percent, that attack both the corn and the cucurbits and, finally, the fungi that affect principally the cucurbits producing the rots of the same ones.

A170

SIDEROPHORES PRODUCTION BY SOIL CYANOBACTERIA OF SAN LUIS

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The plant growth promoting rhizobacteria, present different mechanisms that directly or indirectly improve the growth of the plant. The absorption of iron by the plant is of vital importance, since it is one of the micronutrients that are demanded in a greater proportion and in addition, its bioavailability depending on varied edaphic conditions. Siderophores are organic molecules of low molecular weight that facilitate the absorption of this nutrient by forming iron-siderophore complexes. These molecules can be secreted by rhizobacteria, helping the uptake of this nutrient by the plant. The objective of this work is to evaluate the qualitative capacity of the edaphic cyanobacteria of the San Luis province on the production of siderophores. Samples of agricultural soils and natural grassland were extracted in a field located 20km north of Villa Mercedes at a depth of 0 to 10cm and cultivated in a Petri dish with liquid medium specific for cyanobacteria, Rippka BGº11, at 12-hour intervals of light and 12 hours of darkness at 28ºC. After obtaining biomass production, successive peals were made in agar medium at 1% to isolate different potential species for the production of siderophores. The species *Anabaena* oryzae Fritsch, *Nostoc calcicola* Brébisson ex Bron et Flah, *Nostoc muscorum* Ag. Ex Born were isolated. Et Flah, *Nostoc comune* Vaucher ex Born. et Flah., *Calothrix clavata* Wets, G and *Nostoc sp*. These were sown with ansa with equidistant points in Petri dishes with Rippka BGº11 medium

with Chromium Azurol Agar (ACS) with the blue CAS-Fe chromophore, without carbon or nitrogen source. They were cultured for 15 days at 28 °C with 12 hours of light and 12 hours of darkness. The species *Anabaena* oryzae Fritsch, *Nostoc muscorum* Ag. Ex Born. Et Flah and *Nostoc* sp. segregate siderophores showing an orange halo around the colony. In this work it is concluded that San Luis soil cyanobacteria has the capacity to produce siderophores for plants, contributing to a direct mechanism in the promotion of plant growth. This is of great importance for future work in the application of cyanobacteria as biofertilizers or inoculants of agricultural crops.

A171
"IN VITRO" AND "IN VIVO" STUDY OF INHIBITION OF BANANA PEROXIDASE WITH ULTRAVIOLET LIGHT

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Enzymatic browning is a phenomenonthat causes losses in the quality of fruits. This browning is produced by two enzymes, polyphenoloxidases and peroxidases (POD), which must be inactivated. The chemical agents and thermal treatments are effective to inhibit the enzyme activity. However,exposure to ultraviolet light may promote photo-oxidation processes that cause the modification of proteins and consequently the inactivation of the enzymes. In comparison with traditional preservation methods, this process would produce less loss of quality in the fruits, prolonging the shelf life of these products. In the present work, the inhibition of the activity of banana POD by treatment with ultraviolet light (UVL_T) *"in vitro"* and *"in vivo"* was studied. For the *"in vitro"* evaluation, the peroxidase was exposed at UVL_T (254nm and 366nm) during different times. Subsequently, the enzymatic activity was measured by a spectrophotometric method at 490 nm using as substrate 4-methylcatechol and pH 7.5 buffer solution added withH_2O_2 as enzyme activator. For the *"in vivo"* study, color development was measured in slices of banana sprayed with pH 7.5 buffer solution added with H_2O_2 and exposed to UVL_T using a colorimeter-durometer. The color data were obtained in RGB scale and transformed to scale CIELab with an online calculator where the parameter are L (whiteness or brightness), a* (redness or greenness) and b* (yellowness or blueness). At 254nm, the peroxidase activity decreased by 57.60% in relation to the sample without UVL_T after 390min (6.97 Abs min^{-1} ml^{-1} sample without UVL_T and 2.95 Abs min^{-1} ml^{-1} sample with UVL_T). In the fresh sample,the color parameters after ~170min of exposure at UV were: L= 49.55/50.37; a*=-7.86/-7.85; b*=41.69/44.42 (without/with UVL_T). All these parameters indicate that the browning-inhibition was practically nul. After ~480min ofUVL_T there were no changes in relation to the untreated sample. At 366nm, after 390min of exposure the peroxidase activity decreased by 14.38% (1.97 Abs min^{-1} ml^{-1} sample without UVL_T and 1.68 Abs min^{-1} ml^{-1} sample with UVL_T). The color parameters after ~560min of exposure were: L= 39.08/41.46; a*=-6.52/-7.22; b*=26.49/29.48 (without/with UVL_T). The inhibition of browning was minimal, as well as at 254 nm, without significant increases at longer times. The differences between the treatments *"in vitro"* and *"in vivo"* could be due to the little depth reached by ultraviolet light in fresh samples. However, UV exposure could be used as an alternative method of food conservation to traditional methods that are more aggressive at a nutritional level.

A172
THERMODYNAMIC STUDY OF THE STABILITY OF THE PEROXIDASE ENZYME OF BANANA

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Peroxidases (POD) are a group of hemoproteins that catalyze enzymatic reactions in vegetables. They use phenols and simple diphenols as substrates, and electron-withdrawing compounds must exist in the medium, such as superoxide radicals or peroxides. This phenomenon deteriorates the quality of fresh fruit and its industrial products, from the sensory point. The activity of the enzyme is a reason for study because it is inhibited by chemical or physical methods. In the present work,the inhibitory effect of temperature on banana POD was evaluated. This study was carried out to determine the magnitude of the thermodynamic variables associated with this process. The concentrated extract of the enzyme was obtained from banana of Ecuadorian origin (*Musa* spp.).This concentrate was added in buffer pH 7.4 and subjected to defined temperatures for different periods of time. The variation of the activity of the enzyme, defined as ΔA/min (Absorbance variation/minute) was followed by a spectrophotometric method at 490 nm, zone of maximum absorption of the colored product resulting from the oxidation of the substrate 4-methyl-catechol by POD. A thermostatted cell of 1 cm optical path was used to perform absorbance readings during different periods of time. Thermal deactivation follows first-order kinetics so that kd (deactivation constant) has a linear relationship with time at each temperature. From the analysis of the values of kd with temperature, it is possible to obtain the values of activation energy (Ea) and thermodynamic parameters such as variation of enthalpy, entropy and free energy of Gibbs. The values obtained from kd range from 0.0123 h^{-1} at 303 K to 0.5146 h^{-1} at 328 K. The analysis of the data shows that the process is thermodynamically non-spontaneous with a majority contribution of the enthalpy variation (≈ 85%). The activation Ea obtained (125 kJ/mol) is similar to that found by other authors in other fruits, but no banana data are reported at the moment. The Z

value, found for the deactivation of banana POD is 15.3 °C. The data and results obtained are very useful to establish fruit conservation mechanisms.

A173
CADMIUM AND ZINC IN SOYBEAN CROP, GREAT CHALLENGERS IN THE METABOLISM OF OXIDATIVE STRESS

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There are serious problems associated with the contamination of soils by heavy metals, generating competition between polluting metals and essential metals. The Zn is an essential element for both, plants and animals, however in excess causes alterations in important processes for the development of the organism. Cadmium (Cd) is a heavy metal without biological functions; even in low concentrations it causes damage at the cellular and molecular level. By having the same charge as an essential metal, the plant absorbs by the same transporters (IRT1-ZIP) Cd and Zn, interfering in the entry, transport and use of microelement. The similarity between both ions lead to comparable toxicity symptoms, such as the reduction of biomass, elongation decrease and darkening of the main roots, chlorosis and leaf necrosis, and also causing changes in oxidative stress parameters. The objective of this study was to establish the mechanisms of tolerance in the presence of cadmium and zinc, evaluating morphological and biochemical changes in soybean plants. In our study model, leaves and roots of *Glycine max* were used. After 10 days of development and adaptation to hydroponic conditions in Hoagland nutrient solution, they were exposed to contamination with Cd and Zn, for 6 days. The following work concentrations of $ZnCl_2$ (0; 1.2 and 6.1mM) were selected from a concentration curve, while the concentration of $CdCl_2$ was constant (40 μM). Dry weight, root and stem length and leaf area were determined; MDA was measured as oxidative stress parameter and catalase (CAT) as antioxidant enzyme. The results of the morphophysiological parameters showed a significant decrease, both in root and stem length, in all the treatments compared to the control ($p < 0.001$). Regarding the MDA content, a significant increase was observed either in leaf and root, from the treatments with Cd + Zn (6.1mM) ($p < 0.001$). With respect to CAT determinations in root, the activity with the treatment Cd + Zn (6.1mM) decreased with respect to the control ($p < 0.05$), in leaf a significant increase of the enzymatic activity was observed in all the treatments with respect to the control ($p < 0.01$). We can conclude that there are differential responses between both organs. Antioxidant and prooxidant activity have a greater response in leaves than in root, mainly in the highest concentrations of Zn, which could be attributed to the plant reaction to Zinc high toxicities.

A174
PRESENCE OF *Zygina nivea* (HEMIPTERA: CICADELLIDAE) IN SALICACEAE FROM MENDOZA, ARGENTINA

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In recent years it has been registered new species of hemiptera pest in forest trees of Mendoza, such as *Rhytidodus decimusquartus* Schank (Hemiptera: Cicadellidae) on *Populus* spp. (2008); *Thaumastocoris peregrinus* (Carpenter & Dellapé) (Hemiptera: Thaumastocoridae), on *Eucalyptus* spp. (2013); *Monosteira unicostata* Mulsant & Rey (Hemiptera: Tingidae), on *Populus* spp. and *Salix* spp. (2016). Also, it has been observed during two years the presence of a Cicadellidae causing damages in *Populus* spp. This phytophagous is increasing its populations and its dispersion, which represents a potential pest risk with economic damages. The aim was to identify the Cicadellidae and establish its current distribution. In this communication, it is reported the first records of this species for Mendoza. Since 2015, when the phytophagous under study were observed for the first time, it has been visited the different departments of Mendoza collecting leaves with specimens which were extracted with a brush and stored in 70% alcohol. Then the material was sent for identification to the Entomology Division (FCNyM, UNLP). The taxonomic studies reported that the species collected correspond to a Typhlocybinae *Zygina nivea* (Mulsant & Rey, 1855), whose damage is direct and consists of small internerval chlorotic areas on the adaxial side of the leaf and are associated with the injection of its phytotoxic saliva. In severe attacks it produces defoliation. It is distributed in the three oases of Mendoza, where its dispersion could be a threat to the bounding provinces.

A175
OXIDATIVE STRESS BY ARSENIC IN *Glycine max* L. EXPRESSION OF GENES INVOLVED IN ANTIOXIDANT DEFENSE AND CELLULAR DEATH

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Soybean crops in our country are exposed to soils and water contaminated with arsenic (As). The incorporation of As to water results from natural processes, the atmospheric deposition of materials emitted by volcanic activity and anthropogenic contributions. The objective of this work was to evaluate the expression of genes involved in the antioxidant defense reaction generated by oxidative stress due to the presence of As. For this purpose, soybean seeds were germinated and hydroponic cultivation was carried out. The growth period was 10 days, the temperature: 26 -28°C and the light/ dark period: 16/8 h respectively. The treatments were carried out withtwo different concentrations of As: 2.5 ppm (T1) and 5 ppm (T2) and two times (24 and 72 h). Control plants for each time, did not have contact with As. The RNA extraction protocol was carried out on 36 samples: control and treated leaves and roots. The integrity gels were made and RNA was quantified by spectrophotometry and the 260/280 ratio to evaluate the purity. The retrotranscription of the RNAs to cDNA was carried out and the following genes were amplified by PCR: tubulin gene, as a constitutive gene and three genes that participate in the antioxidant response and cell death: isocitrate dehydrogenase (ICDH), Glutathione Reductase (GR) and Mitogen Activate Protein kinase (MAPK). The expression of ICDH gene increased both in leaves T1 and T2, in both periods of time. In the case of the 72h roots, the same behavior was observed, that is, the increase was greater with a higher concentration of As and the greater time of exposure. Only a decrease in the expression of this gene was observed in the samples of the roots T1- 24 h compared to the control. There was an increase in leaf and root samples with T1 24 h when the expression of the GR gene was studied. In the case of the roots T2 24 h, the expression of this gene was lower with respect to T1, but it is greater than the control. If we observe the expression of the MAPK gene, the samples of leaves and roots of 24h and in the roots of 72h increases with T1 and still increases more with T2. However, in the 72h leaves a decrease in the T1 was observed with respect to the control samples and in the T2 this decrease is more marked. From these results we conclude that the expression of the genes involved in the antioxidant response by the action of As in soybean is increased both in the roots and in the leaves of stressed plants.

A176
TAXONOMIC IDENTIFICATION OF SPECIES OF "CUTWORM LARVAE" IN THE CULTURE OF ALFALFA (*Medicago sativa* L.)

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In the Semi-arid and Subhumid Pampean Region, the production systems are, in general, mixed. These systems are based on rotations of annual crops and perennial pastures, acquiring value the cultivation of alfalfa (*Medicago sativa* L.), this species being the main forage in the country and the basis of meat and milk production in the region. In Argentina, more than 80% of the damage caused to the cultivation of alfalfa is caused by four main groups of insects, among them the complex of "cutworm larvae". This group of insects corresponds to the order Lepidoptera, family Noctuidae, subfamily Noctuinae, tribe Noctuini and is considered a pest of economic importance. The correct identification and quantification of the problem species allows to predict the moment of the occurrence of attacks and their intensity, being able to exert more efficient containment measures on the different species, which constitutes the first step in a pest management planning. In the case of the Noctuidae, differences among different authors have been observed regarding the genera that it includes and the determination of some species, being under review.The objective of this work is to identify different species of "cutter caterpillars" -both of young and adult forms- for which 9 sampling stations, of 1 m^2, were made every two weeks in alfalfa crops located in INTA Experimental Station San Luis (Villa Mercedes). The adults were captured using a light trap placed close to the crop. Identifications were made in the Laboratorio de Manejo Integrado de Problemas Fitosanitarios (FICA-UNSL), using the keys of Fichetti and San Blas, 2017 and Triplehorn and Johnson, 2005. The species identified are:*Agrotis robusta* (Blanchard), *Agrotis* sp., *Helicoverpa gelotopoeon* (Dyar), *Dargida albilinea* (Hübner), *Feltia hispidula* (Guenée), *Feltia gypaetina* (Guenée), y *Feltia irritans* (Köhler).

A177
MACROPHYTES SPECIES IN “NUEVO” CREEK OF SUB-BASIN “RÍO NUEVO”, SAN LUIS, ARGENTINA

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The basin of “El Morro” has attracted national attention because factors as deforestation, agricultural expansion, and the increased rainfall,whose have had negative effects on agricultural lands and infrastructure.In this area, the predominant hydrologic characteristic is great erosion present in all channels of the system. In recent years the flow increased in water courses

have developed gullies and this affected water dynamics of the middle and lower portion, due to the transport and deposition of sediments and increase of water salinity in these areas. Macrophytes are considered useful for the detection and monitoring of physicochemical pressures that produce: Reduction of water transparency, variation in conductivity and salinity, and eutrophy. Macrophytes are also sensitive to the hydromorphological pressures they produce variations in flow regime, river continuity and morphological characteristics of riverbed. This study site is part of the "El Morro" basin. The studies were carried out on the "Río Nuevo" sub-basin of 372 km2. The aim of this work was to investigate the taxonomic identity of the macrophytes growing in Nuevo creek. The specimens were collected in winter-spring stations of 2018 and were identified following the classic botanical methods and incorporated to the VMA Herbarium. It was observed that Nuevo creek is characterized by sandy soils of recent formation due to deposition of sediments and these unstable soils were susceptible to erosion. At the sampled areas there were shallow water courses with broad salty alluvial terraces. A little number of macrophytes species were found, mainly belonging to the families: Cyperaceae (*Schoenoplectus pungens, Schoenoplectus americanus*), Poaceae (*Cortaderia selloana*), Ranunculaceae (*Ranunculus bonariensis*), Plantaginaceae (*Veronica anagallis-aquatica*)and Typhaceae (*Typha sp.*). The low richness suggests that channels recent formation and salinity could be influencing the development of other species. For this reason, macrophytes species will continue to be monitored in the coming months/years.

A178

Jodina rhombifolia: NUTRACEUTICAL POTENTIAL OF A CUYO REGION FRUIT

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Jodina rhombifolia is a member of the *Santalaceae* family, originally from San Luis region, has a red fruit, spherical, rough, drip-shaped capsule, almost 1 cm in diameter. The objective of this work was to evaluated *Jodina rhombifolia* fruits, vulgarly called "Peje or Sombra del toro", in terms of its proximal composition, nutritional value and antioxidant activity (moisture, ash, carbohydrates, proteins, fat, total fiber, caloric value, polyphenols, vitamin C and mineral micronutrients, associated with antioxidant defense), for its possible application as a nutraceutical food. The proximal composition obtained was: humidity 51.03%;ashes 2.74%; 26.6% carbohydrates; 0.94% proteins; fat 0.58%; total fiber 18.11%; caloric value 115.38 kcal. The polyphenols and vitamin C was 40 mg AG/100g and 288 mg/100g respectively and microminerals: Se (8.1), Fe (5.1), Mn (7.80), Cu (6.80) and Zn (2.1) mg/100g. According to these results, this fruit can be consider, as a very good source of carbohydrates and fibers, its energy value seems to be influenced mainly by carbohydrates, the main macronutrient. Although the protein and the fat content was very similar to native fruits previously reported and has higher antioxidant metal values than most conventional fruits; showing a potential antioxidant capacity comparable to other fruits considered as powerful antioxidants. In conclusion, the fruit of *Jodina rhombifolia* has nutraceutical potential and antioxidant properties highlighted for the food industry, either as a natural additive to increase the nutritional value of a specific product or preserving its consumption as a traditional fruit, thus valorizing its use allowing the preservation of regional cultural and gastronomic identity.

A179

EFFECT OF THE ACCELERATED AGING IN *Onobrychis viciifolia, Astragalus cicer* var. Lutana AND Monarca

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Onobrychis viciifolia and *Astragalus cicer* are perennial Leguminoseae, originated from eastern Europa, introduced in Canada, adapted to a wide range of soil and climatic conditions, from drylands with no more than 400 mm rainfall annually to irrigated soils. It presents a high productive capacity in the semiarid cold drynesses. The aim of the study was to evaluate the seed quality by its germination with methods of intensive aging (IA),since the experimental sowings in the plots of the EEA San Luis had problems of emergence uniformity. The experiment was realized in a growth chamber with 100 % RH and at 42°C where the seeds remained suspended without touching the liquid water. IA's treatment was realized for 7 days and every 24 h were extracting 4 repetitions of 50 seeds of every species and were placing in tray of germination in stove of incubation at 25°C in a period of 14 days with controls every 3 days. Viability of seeds was analyzed by method of tetrazolium. In *Onobrychis viciifolia*it was observed that in the witness after 24 h of IA the germination had a maximum of 34 %, while during the last 6 days the percentage of germination was 0 %. These results would be due to the aged material. In *Astragalus cicer* var *Lutana* the witness following 24 h of IA reached 10 % of germination, and its maximum (45%) scoped at 3 days. Finally they decreased gradually up to 8 % of germination on the seventh day. In *Astragalus cicer* var *Monarca* both the witness and the first 24 h of IA resulted in 25 % of germination, its maximum value (40 %) was achieved after 5 days, and later it declines. These results in Monarca as much as in Lutana reflect not uniform field sowings presuming that they need a previous priming treatment by IA's method.

A180

EVALUATION OF THE DORMANT RUPTURE IN *Onobrychis viciifolia, Astragalus cicer* var. Lutana AND *Astragalus cicer* var. Monarca

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Onobrychis viciifolia and *Astragalus cicer* (Leguminoseae) are species perennial adapted to a wide range of climatic conditions, amount of rainfall and types of soil, with great adaptability to arid environments. They arenative to Eastern Europe and cultivated in Canada.The aim was to evaluate the quality of the seed by its germination with methods of chemical scarification due to the fact that the experimental sowings in the plots of the EEA San Luis had problems of emergence uniformity. There were realized two types of chemical scarification, one with sulphuric concentrated acid (H) and the other one with10 % sodium hydroxide (OH), with 4 treatments and 4 repetitions of 50 seeds each one. For H the treatments were, T0 (witness), T1: 5 minutes (m), T2: 10 minutes and T3: 15 minutes, in dip. For OH the treatments were, T0 (witness), T1: 10 m, T2: 20 m and T3: 30 m, in dip. After the treatment the seeds were washed with abundant distilled water, were conditioned in trays with germination paper, and placed in growth chamber at 25°C for 14 days with germination controls every 3 days. Viability of seeds was analyzed by method of tetrazolium. For *Onobrychis viciifolia* T0 had a germination of 21 %with H the T1 was 1 % of germination and other treatments were 0 %. With OH the T1 was 15 % of germination and others 0 %. The results for this lot of seeds show that a chemical scarification is not necessary. For *Astragalus cicer* var *Lutana* the T0 had a germination of 46 %, with H had a maximum of 51 % in T3 coinciding with the OH for T3 with 49 %. For this lot the chemical scarification was better asthe time of treatment increased. For *Astragalus cicer* var *Monarca* the T0 had 37 % of germination and with H the major germination was obtained in T2 by 69 % of germination and with OH the maximum was obtained in T1 by 46 %. We can conclude that higher times of dip to T2 with H and higher times to T1 with OHaffect the tegument decreasing germination.These values for *Astragalus cicer* would indicate that to obtain a uniform germination to field they need a chemical scarification before the sowing.

A181

GC-MS ANALYSIS OF THE ALKALOID EXTRACT FROM THE LEAVES OF *Hippeastrum argentinum* (PAX) HUNZ.

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Alzheimer´s disease (AD) is the most common cause of dementia. The main pharmacological treatment comprises the use of acetyl- and butyrylcholinesterase inhibitors. The Amaryllidaceae family is a source of a wide range of cholinesterases inhibitors alkaloids including galantamine, a long-acting, selective, reversible, and competitive acetylcholinesterase inhibitor, approved by FDA in 2001, and currently used for the treatment of mild and moderate AD. As a part of an ongoing study of Argentinean Amaryllidaceae species in search of new bioactive compounds, the alkaloid extract from the leaves of *Hippeastrum argentinum*. The aim was the characterization and identification of bioactive compounds in the alkaloid extract from the *H. argentinum* leaves by GC-MS analysis, and comparing the results with those of the bulbs (previously obtained). The extract was prepared from dried and crushed leaves. Then, was analyzed by GC-MS, and these compounds were characterized by comparing their mass fragmentation patterns with standard reference spectra in homemade databases. Major alkaloids detected were 7-OH-clivonine, montanine, undulatine y pancracine. The alkaloid GC-MS profile was similar to those of the bulbs excepting the proportion. Thus, the relative alkaloid concentration depending on the plant´s parts can vary in this species. The alkaloid 7-OH-clivonine isolated from *H. argentinum*is a cholinesterase inhibitor, more abundant in the leaves than the bulbs. Acknowledgement: CICITCA- UNSJ, ANPCYT PICT 2014-3425. Red CYTED–BIFRENES. Proy 416RT0511

A182

MARKERS OF TOLERANCE TO SALINITY IN *Medicago sativa* VAR. CW 660

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Alfalfa is a species of great plasticity, morphologically and physiologically adapted to tolerate abiotic stresses. The objective of this study was to determine the tolerance of alfalfa var. CW 660 to salinity, through the analysis of growth parameters, physiological and osmocompatible response. For the test, seeds were sown in a pot system with sterilized vermiculite and Hoagland nutrient solution (NS) modified at 25%. All the plants grew in the sowing chamber at 21-22ºC of temperature; with a photoperiod of 16 hs. of light, and 8 hs. of darkness. Seven days after sowing, the saline treatment was started, which consisted of watering the plants with 3 saline solutions (NS + NaCl) of different concentrations, 50mM, 100mM and 200mM NaCl, and a control with nutrient solution. After four weeks of treatment, we measured: aerial length (AL), fresh weight of aerial portion (AFW) and root (RFW) and aerial dry weight (ADW) and radical dry weight (RDW); In addition, the following were determined in the aerial portion: proline and photosynthetic pigments: chlorophyll a and b, carotenoids. The statistical analysis performed

was a unifactorial ANOVA, in which the means between the salinity treatments were compared with respect to a control. Both AL and AFW showed significant differences ($p<0.05$) starting at 50mM salinity, while ADW decreased significantly ($p<0,05$) at 200mM NaCl. In the radicular part, the length and the RFW presented significant differences ($p<0.05$) with respect to the control from the 200mM of NaCl. In the RDW, no significant differences ($p<0.05$) were observed between treatments and control. From a T test for related samples, an increase in Proline was determined in osmocompatible response to salinity and the colorofilian pigments decreased as well as the carotenoids. In addition, a strong negative correlation (0.97) between these parameters was determined from a Pearson linear correlation analysis.From the results it can be concluded that the variety studied tolerates concentrations between 50 and 100 mM NaCl, and in response to stress increases the concentration of proline and decreases its photosynthetic capacity.

A183
GERMINATION UNDER SALT AND CADMIO CONDITIONS: RESPONSE IN NEW VARIETIES OF ALFALFA

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Alfalfa is a species of great plasticity, morphologically and physiologically adapted to tolerate abiotic stresses. The objective of this study was to determine the tolerance of four varieties of alfalfa to salinity and cadmium in germination by measuring energy and germinative power. *Medicago sativa* varieties were var. CW 197, Trinidad 87, CW 660 and Salina PV. For the salinity test, 20 seeds of each were planted in Petri dishes in triplicate and watered with 5 ml of saline solutions: 10mM, 25mM, 50mM, 100mM, 150mM, 200mM NaCl and a control with distilled water; for the Cd assay, 20 seeds of each were seeded, in Petri dishes in triplicate, with support 1% agar in increasing concentrations of Cl2Cd: 100 μM, 200 μM, 300 μM, 400 μM, 500 μM and control without Cd. Germinative Energy (GE) was measured on the 3rd day and Germinative Power (GP) on the 7th day since sowing. The statistical analysis performed was a unifactorial ANOVA, in which the means between the salinity and cadmium treatments were compared with respect to a control. Regarding the salinity test during the GE, in var. Trinidad 87, Salina PV and CW 660 significant differences ($p<0,05$) were observed from 100 mM, while in CW 197 at 200 mM NaCl. In GP, significant decreases ($p<0,05$) in germination are found, starting at 150 mM in varieties CW 197, Trinidad 87 and Salina PV, while CW 660, tolerates up to 150 mM NaCl. The results of the Cd tolerance test indicate that CW 197 and Salina do not showed significant differences ($p<0.05$) between the control and 400 uM of treatment (GE and GP); to wash. Trinidad87 shows tolerance to Cd at 500 uM Cd in the GE and in the GP at 300 uM Cd. Var. CW 660 did not present a significant decrease in GE or GP ($p<0.05$). Therefore, var. CW 660 is the most tolerant to salinity and Cd during germination.

A184
EFFECT OF SALT AND BORO STRESS ON SEED GERMINATION OF *Chenopodium quinoa* WILLD

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Quinoa (*Chenopodium quinoa* Willd) is an ancient Andean crop and could be a productive alternative for irrigated areas in arid and semi -arid regions. High levels of salts and Boron (B), an essential micronutrient but toxic in excess, in soils are commonly observed in the Jáchal valley (NW of San Juan province). One of the most important effects of abiotic stresses on plants is the overproduction of reactive oxygen species (ROS) that cause cell death. The germination is the most critical stages of plant growth and has a direct impact on productivity. The aim was to evaluate the germination percentage, germination velocity (GV), the emergence rate index (ERI) and the activity of ascorbate peroxidase (APX), catalase (CAT) and malondialdehyde (MDA) of *C. quinoa* var. "Amarilla de Marangani" under conditions of stress due to salinity and B. The treatment were C1B1 (150 mM ClNa+0,025% B); C1B2 (150 mM ClNa+0.075% B); C2B1 (200 mM ClNa+0.025% B); C2B2 (200 mM ClNa+0.075% B); C1B0 (150 mM ClNa); C2B0 (200 mM ClNa); C0B1 (0.025% B); C0B2 (0.075 B) and COBO (distilled water Control). The experimental design was in random plots with five replicates of 25 seeds per treatment. The number of germinated seeds was recorded every 24 h for one week. A seed was considered germinated when the emerging radicle was longer than 2 mm. Statistical analysis was performed using ANOVA followed by LSD Fisher and Tukey-Kramer Multiple Comparisons Test. The germination percentage was significantly higher in the non-salts and boron treatment (COBO) compared with C1B2, C2B1, C2B2 and C2B0 treatments. Similar results were observed in the ERI evaluation. The seeds germinated under the high concentration of ClNa and B (C2B2) and C1B2 treatment have a significant lower GV compared with the rest of the treatments. The activity of CAT (μmol H_2O_2/mg protein min) and APX (μmol Ascorbato/mg protein min) in COBO treatment were significantly higher than the C2B2 treatment($p<0.001$). Contrarily, the concentration of MDA was significantly higher in the control treatment (COBO) compared with the higher concentration of ClNa and B treatment (C2B2). The combination of 200 mM ClNa+0.075% B, in the germination media, negatively modified seed performance (low germination and germination velocity) as well the activity of antioxidant enzymes. Acknowledgement: CICITCA, UNSJ, UNSL, INTA.

A185
CONCENTRATION OF PHOTOSINTETIC PIGMENTS AND WATER RELATIONS IN *Borreria spinosa* UNDER CONDITIONS OF SALINITY

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B. spinosa is an important weed of summer crops in the rain fed area of Santiago del Estero, considered tolerant to glyphosate herbicide.There is a lack of information about the influence of different environmental factors on photosynthesis and water relations.The objective was to determine the effect of salt stress on these physiological variables. For this, seeds were incubated in germination chamber at 38/28 °C and 12 hours of light.They were irrigated with NaCl solutions with osmotic potentials of 0; -0.2; -0.4 MPa. The photosynthetic pigments were extracted with ethanol, and quantified spectrophotometrically.The water relations were estimated through the determination of the RWC of the seedlings.The experimental design was completely randomized with four repetitions. The RWC data were transformed (arcsen (√P)) and analyzed by means of ANOVA, the photosynthetic pigment concentrations by the nonparametric test of Kruskal Wallis. The concentration of photosynthetic pigments was significantly reduced with the decrease of the osmotic potential, with significant differences between the control and -0.4 MPa for chlorophyll a (4.16 and 2.42 µg / ml, respectively), chlorophyll b (2, 92 and 0.91 µg / ml, respectively) and carotenoids (1.21 and 0.23 µg/ml, respectively), indicating severe stress.The RWC decreased with the increase of the salt concentration, finding significant differences between the control (98.95%) compared to -0.2 (90.37%) and -0.4 (90.02%) MPa of NaCl. This result indicates that the species was not adjusted osmotically, and that salt stress produced a loss of cell turgor. *B. spinosa*, during the germination stage, behaves like a glycophyte species, with water relations being more sensitive to salinity than the concentration of photosynthetic pigments.

A186
MORPHOLOGY AND CYTOGENETICS CHARACTERIZATION OF LOCAL POPULATIONS OF *Adesmia bicolor* (LEGUMINOSAE)

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Adesmia bicolor (Poir) DC is a perennial, herbaceous species with undefined prostrate growth habit. Because this species is a promising native fodder, in previous works were studied taxonomic aspects, distribution, growth forms, reproductive traits and biological nitrogen fixation. Bibliographic references indicate that is a diploid species 2n=20. To contribute to the knowledge of this native species, the aim of this job was to characterize the morphology and cytology of three populations (P1; P2; P3) collected in the Argentinian central semi-arid region. Five morphological variables were analyzed using a complete random design with an unequal number of repetitions. The number of bivalents in the meiosis of pollen mother cells (II/CMP) was determined and the populations were compared by t tests. Significant differences were recorded between populations ($p<0.0001$) in all the morphological traits. P1 recorded the highest height, P3 the largest width and length of the leaflet, and P2 recorded the highest number of flowers per bunch. Number chromosomes and ploidy level 2n=2x was confirmed. P1 presented 10.08±0.47 II/CMP and P3 9.83±0.39 II/CMP. However, P2 showed 11.58±1.74 II/CMP, irregulars' diakinesis and some univalent that could explain the differences found. To confirm the possible presence of a 2n=24 cytotype, more exhaustive cytological studies should be carried out in new populations with another regional origin. Non-significant differences between P1 and P3 (2n=20) were observed, but both of them registered differences with P2 ($p<0.0001$). Meiotic behavior of P1 and P3 was considered normal. The data indicate that these populations are reproductively stable and phenotypically different, so it would be possible to use them in a breeding program.

A187
EVALUATION OF PLANT EXTRACTS OF AUTOCHTHONOUS SPECIES AS BIOPHARMACEUTICAL PRODUCT AGAINST *Plodia interpunctella* IN LABORATORY

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The use of plant extracts to counteract phytosanitary problems, taking advantage of the biocidal activity granted by the presence of secondary metabolites produced by the plants themselves, could constitute a valid alternative either because they are considered less toxic by the consumer or less negatively affect the environment. The aim of the present work was to evaluate the biological activity of the plant extracts of silverleaf nightshade (*Solanum elaeagnifolium*) when is ingested by larvae of *P. interpunctella* (Lepidoptera: Pyralidae). This species affects nuts and is generally used as a model in laboratory tests for its ease of rearing. These tests will be used as a basis for the study of the biocidal effect against *Lobesia botrana.* It was made a test with 5 treatments at random and 5 repetitions in order to evaluate the activity of the extract. The treatments were: C: (control) which

corresponds to the peanut purchased in herbal shop; A: peanut submerged in distilled water; Q1: peanut submerged in 100% silverleaf nightshade extract; Q2: peanut submerged in silverleaf nightshade extract diluted at 50%; Q3: peanut submerged in silverleaf nightshade extract diluted at 25%. After 10 seconds of immersion, the peanuts of each treatment were dried in air on paper. It was used glass containers and was put 10 g of peanuts according to the corresponding treatment and it was added five *Plodia* larvae. Each container contituted a repetition. The observations were made at 48 hours, 7 days, 30 days and the last was made when the last adult emerged. It was determined the percentage of survival of *Plodia* as adult. It was made variance analysis and Duncan test. The effect of extracts on survival was statistically significant ($p = 0.0022$), considering a level of significance of 5%. The survival average of extract A (peanut submerged in water) has statistical differences with respect to the rest of the extracts and concentrations used. The best dose corresponded to the silverleaf nightshade extract diluted at 50%.

A188
AGRONOMIC OPTIMAL PLANT DENSITY IN "LATE-SOWN CORN", IN VILLA MERCEDES (SAN LUIS)

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Plant density (D) is one of the main management variables that affect the grain yield (GY) of a corn crop. "Late-sown corn" are defined as those that are sown after a long fallow, unlike the "second maize", which is planted after a winter crop. In corn, the GY response to variations in D is of the optimal type. The component of the GY most affected by D is kernel number (KN). The agronomic optimal plant density (AOPD) is a value of D in which the maximum GY is produced for a certain region and hybrid, and varies according to technological aspects (planting date, irrigation, fertilization and others). In order to determine AOPD for different maize hybrids, a research work was carried out in Villa Mercedes (San Luis). In December 2015, three hybrids were planted in five D (3.6, 4.0, 4.4, 4.8 and 5.4 plants m^{-2}, at harvest) using a random block design in split plots, with four replications, measuring GY, KN and the grain weight (GW). In silking stage (R1) the content of chlorophyll (CC) in the spike leaf was measured, to estimate the availability of resources per plant in the critical period. The data were analyzed by ANOVA, Tukey test, multiple regression and principal component analysis (PCA). The GY average was 7056 kg ha^{-1}. GY differences were detected ($p<0.01$) between D, not between hybrids. There was no ***HybridxD*** interaction. The responses of GY and KN to D were of the quadratic type. GW, however, had a linear and decreasing response with the increase in D, presenting a strong association with GY and CC. The main components (CP1 and CP2) of the CPA explained 78.4% of the total variability of the data set, indicating that the variability was mainly determined by the environment, i.e. by D. AOPD values of 3.95, 4.47 and 4.59 plants m^{-2} were obtained for the hybrids *KM3800*, *KM4321* and *KM4200*, respectively. It is concluded that the D must be an agronomic decision based on the hybrid of corn to be sown, since it determines the availability of resources per plant during the critical period.

A189
DETERMINATION OF EDAPHIC CYANOBACTERIA IN AGRICULTURAL SOILS OF THE ESTABLISHMENT LAS MELLIZAS VILLA MERCEDES (SAN LUIS).

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In the edaphic microbiota we find microorganisms such as the cyanobacteria that actively participate in soil quality.They are prokaryotic organisms, resistant to adverse conditions; they are nitrogen fixers in aerobic and anaerobic conditions and soil fertility enhancers.The objective of this work was to determine edaphic cyanobacteria in agricultural soils of the establishment "Las Mellizas", located 20 km south-west of the city of Villa Mercedes (Pedernera Department).The sampling site has been under a direct sowing system for twelve years.It was divided into two treatments (Tt: bare soil, T1: standing corn), with five blocks systematically distributed at random.At each sampling point, a composite soil sampling was extracted at 0-10 cm.Cultivations were carried out in Watanabe's medium under controlled conditions to achieve growth of cyanobacteria.The taxa of agronomic interest were identified and photomicrographs were taken.The results obtained were 41.48% and 42% of *Phormidiumsp*. in the treatments Tt and T1 respectively; 23.63%, 19.31% of *Oscillatoriasp*. in the treatments Tt and T1 respectively; 34.89%, 30.60% of *Nostoc* sp. in the treatment Tt and T1 respectively and 0%, 6.02% of *Nodularia* sp. in the treatments Tt and T1 respectively.According to the results *Phormidium* sp, *Oscillatoria* sp and *Nostoc* sp were found in approximately similar proportions in both treatments, whereas *Nodularia* sp, an atmospheric nitrogen fixer speciesunder aerobic conditions, was only found in T1.In soils with standing corn cultivation, taxa with nitrogen fixing capacity are predominant. These results contribute to the study of edaphic cyanobacteria as possible soil management tools and quality bioindicators.

A190
ENDOPHYTIC FUNGI IN SELECTED TALL FESCUE PLANTS OF CENTRAL REGION OF ARGENTINA

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The perennial grass *Festuca arundinacea* Schreber (Tall fescue) is used as forage in temperate climate environments. It has autumn-winter-spring growth habit and widely geographic distribution. It has favorable agronomic traits as grazing resistance and tolerance to biotic and abiotic stresses. Tall fescue has an asymptomatic relationship of mutualism with the endophytic fungus *Epichloë coenophiala*. Toxic alkaloids produced by this fungus cause lower weight gains and decreases the percentage of pregnancy in grazing livestock. The aim of this study was to determine the presence of *E. coenophiala* in 21 genotypes of tall fescue, selected from naturalized populations of central region of Argentina, and three commercial varieties. A 100 seeds sample from each genotype was put in a 5% NaOH solution during nine hours. After rinsing them, each seed was stained with a Rose Bengal solution on a slide. The presence or absence of fungal hyphae colored in the endosperm's aleurone cells was recorded by microscopic observations with a 40X magnification, and the percentage of seeds with fungal infection in each genotype was determined. To verify if there were significant differences of infection among genotypes, an analysis of variance with Generalized Linear Mixed Models was made. Also, in order to identify groups with different percentages of infection a test DGC was made. All genotypes showed infection by *E. coenophiala*, with an average of 85%. Significant differences between the 25 genotypes were found, and two groups with different percentages of infection were identified. The group composed of commercial cultivars showed average infection values of 2 to 4%, while the rest of genotypes presented average values of 52 to 100%. Among the 25 genotypes tested, NOR-52 (from southwest of Córdoba province) was the one with the lowest infection percentage (52%). This demonstrates the need for an antifungal treatment of the genotypes tested.

ECOLOGY, ETHOLOGY AND BIODIVERSITY

A191
NEW RECORDS OF *Lucilia purpurascens* AND *Lucilia ochricornis* FLY SPECIES OF FORENSIC INTEREST FOR THE PROVINCE OF SALTA (DIPTERA: CALLIPHORIDAE), ASSOCIATED WITH DIFFERENT ENVIRONMENTAL AND PHENOLOGICAL PARAMETERS

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The species of flies of the genus *Lucilia* (Diptera: Calliphoridae) are of great forensic importance because of their affinity for meat. They are the first to find and deposit their eggs in the corpses, initiating the succession of species that invade it. The exact establishment of the geographical distribution ranges is important for the realization of entomological skills. In Argentina, the species of the genus *Lucilia* were assigned alternately to *L. cluvia* or *L. eximia*, but in the last years, a complete revision for the Neotropical region proposed a complex of species: *L. ibis*, *L. purpurascens*, *L. vulgate* and *L. ochricornis*. Our objective was to make catches using diverse environmental and phenological parameters to establish the presence or absence of the species previously classified as *L. cluvia* for the province of Salta, in order to establish precise search criteria for future research. We carried out samplings from urban to rural and native environments, for two consecutive years and once a month during all the months of the year (morning, noon and afternoon). We used decomposing bovine lungs as bait and carried out catches with entomological nets. Subsequently, individuals were placed in individual covered polyethene cups with fresh liver inside to control oviposition. *L. purpurascens* appears strongly associated with all native environments, while *L. ochricornis* it is strongly associated with rural environments. Overlaps of both species occur in the area limits between the rural and native environments, with a preponderance of *L. ochricornis*. In the same way, *L. ochricornis* it appears scarcely in the peripheries of the city, close to rural environments. We could observe a strong phenological effect throughout the year, because during the warmer months these species are abundant in the catches, while in the coldest months they disappear (June and July). We could conclude that both species have different distributional ranges and based on the variables considered; temperature, time of the day and sun exposure, shown a relationship with the abundance. Also the oviposition, for both species, is generally irregular in the number of individuals that achieve the set by capture, except in two summer months, where the lay of individuals by capture is usually maximun.

A192
GENETIC DIFFERENTIATION OF *Rosa rubiginosa* L, AN INTRODUCED SPECIES IN ARGENTINA.

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Studies characterizing genetic diversity can provide new knowledge in relation with the dynamic of invasive taxon´s colonization and propagation. Exotic plants introduced in a new environment, free of the natural controls present in their native ecosystems, experience a rapid and unrestricted growth. Some of these species become quite abundant and could cause serious economic and environmental problems. *Rosa rubiginosa* L. is a widespread shrub species, introduced to Argentina, widely distributed in different environments and climatic conditions such as woodland and steppe. *Rosa rubiginosa* presents a wide distribution in the Andean-Patagonian region, gradually expanding to the central area of the country. Molecular markers are tools that can provide information about the amount of variation introduced. The objective of the present work is to evaluate the genetic differentiation and diversity by means of randomly amplified polymorphic DNA (RAPDs) in *R. rubiginosa* from eight experimental sites located in the provinces of San Luis and Neuquén. DNA samples (115 individuals), obtained from foliar material were amplified using 9 primers from kit A (Operon Technologies). The average percentage of polymorphic *loci* observed for the 8 populations was 26.95 %. AMOVA analyses revealed high genetic variation within populations (71%) and low variation between populations (29%), in agreement with values estimated by the Shannon–Weaver index. Genetic differentiation (Gst) between populations estimated by AMOVA showed the correlation coefficient PhiPT = 0.29 ($P<0.001$). The Gst based in genetic distance of Nei (0.35) was lower for interpopulation variation. The low interpopulation value obtained suggests genetic homogeneity between the populations. Clusters analysis (UPGMA) by Jaccard coefficient and PCoA clearly suggest a geographical differentiation of the provenances of *R. rubiginosa* populations. The present results contribute to the characterization at the molecular level of the genetic structure of populations of *R. rubiginosa*, an invasive species in different environmental ecosystem of Argentina. This information contributes to a better understanding about phenomena such bottleneck and multiple introductions related with the success of the invasion by an introduced species. Besides, these data can be useful to design strategies of management of invasive species.

A193
WINTER EMISSION OF CO2 OF A WETLAND OF SAN LUIS

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Wetlands are flooded environments or with groundwater levels near the surface that can play another aspect of vital importance as sequestering carbon due to the low rates of mineralization of the organic matter. Our aim was to estimate the emission of CO_2 in the winter of the physiognomy types of the Wetland "Bajo La Salada". The sample area is located 5 km from the town of Villa Mercedes (San Luis) to 33º 37' south latitude and 65º 25' west longitude, with a height above the sea level of 505 m. In each physiognomy type determined in previous work, we evaluated the C of CO_2 released by the soil in closed trap of alkaline solution. A control sample, not closed, was placed in the halophyte open prairies (in the middle of the wetland) that correlates positively with the sums of all physiognomy types ($r=0.99$). The results show significant differences ($p <0.05$) in the emissions, with four groups being formed after 10 days of cumulative progression (mg CO_2/100 cm^2): open control sample; halophyte mount; halophyte scrub (*Atriplex spp*.) and prairies halophyte dense (*Distichlis spicata*); and scrubland patches of halophyte crawling(*Sarcocornia neei*), halophytes open prairies(*Distichlis spicata*) and saline beach. Each of the regression curves of these physiognomic types shows R2 greater than 0.92 (for the control y = 62.55 days). The production of CO_2 responds to the organic matter content of the upper horizon ($r=0.91$). The emission of total CO_2 of the wetland (control sample) reached values of 618.85 kg of CO_2/ha for the period of 10 winter days considered. It is concluded that: (a) there is a good efficiency to measure the production of CO_2 with the methodology used, b) the open control sample showed high correlation with the sums of all physiognomy types, (c) the emission of CO_2 correlated with the organic carbon content of the soil, and d) the winter emission was 618.85 kg of CO_2/ha for the wetland and period considered.

A194
PLANTS USED AS COMBUSTIBLES IN PLACES OF THE SILIPICA DEPARTMENT

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A large part of the population of the Silipica Department lives in rural villages; its population performs productive subsistence activities related to agricultural work and livestock. The vegetation of the Silipica Department has been disturbed and even totally eliminated by human intervention. However, marginal patches of forest persist and villagers use their plants for different purposes. The objective was to survey the species used as fuels and the properties attributed to them; For this purpose, semi-

structured interviews were conducted for residents of 10 localities of the department, to recover the local names, the uses given to the plants, the methods of use and the organs that are used.There were 17 species of which the most mentioned are *Prosopis nigra, Prosopis alba* and *Aspidosperma quebracho-blanco*; they are included in 7 families, with Fabaceae being the most represented (10 species). Within the fuel plants, the categories for firewood, for coal and for flame were differentiated; the majority (59%) is used both for firewood and charcoal. *Vallesia glabra* was the most mentioned among the plants that are appreciated because they "light fast and make good flame". The parts of the plants most used are trunks and twigs. The fuels species are the most used, after the medicinal and fodder, and are the only source of energy in most of the homes visited; this is why reforestation with fast-growing species that provide for these purposes would be important.

A195
FLORA OF APICULTURAL INTEREST IN APIARIES OF THE EAST OF SILIPICA DEPARTMENT, SANTIAGO DEL ESTERO

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Towards the east of the Silipica Department, it is common the numerous paleo-capes that the Dulce River leaves in its journey, printing differential characteristics to the predominant landscape and vegetation. Beekeeping is an activity carried out by people from the east of the department and is intimately related and conditioned by the plants available in the area, which is why beekeepers have a deep knowledge of the local flora of interest for the foraging of bees. The objective of the present work was to inventory this melitophylous flora around the apiaries installed in dry environments of the east of the Silipic Department. Semi-structured interviews were carried out with the apicultural producers of the area, consulting about the species visited by the bees in their apiaries, the type of contribution (nectar, pollen) and the time of flowering. Their presence of plants was confirmed and material was collected for herborization and determination. Seventy one species were cited, of which 90% are native, distributed in bushes-sub-shrubs (40.8%), trees (28.2%) herbaceous (22.5%) and Vines-Creepers (8.5%), of which 83% correspond to perennial species and 16% per year. With regard to botanical families, 24 were registered, the most representative being Asteraceae (17%), Fabaceae (17%), and Cactaceae (16%). The most mentioned species were *Atamisquea emarginata* Miers ex Hook. & Arn, *Prosopis alba*Griseb, *Schinopsis lorentzii* (Griseb.) Engl, *Geoffroea decorticans* (Hook.& Arn.)Burkart and *Sarcomphalus mistol* (Griseb) Hauenschild. In relation to the contributions 48% of the mentioned plants provide nectar and pollen, 20% pollen and 8% nectar. The flowering time evidenced two pulses in the offer, the first, the largest, from June to December with a peak in October, and the second from January to March with peak in February. The native and woody species of the dry lands of the department constitute a valuable resource of interest for beekeeping.

A196
DISTRIBUTION AND ECOLOGY OF *Pisidium chiquitanum* (ITUARTE, 2001) (BIVALVIA, SPHAERIIDAE) IN CENTRAL-WEST FROM ARGENTINA.

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The first record of genus *Pisidium* in central-west from Argentina was in 2008 when *P.chiquitanum* was found on a site from northern Mendoza Province (MP), which greatly enlarged the southern range distribution of this species (central Bolivia to NW Argentina). The first data on distribution of this clam and environmental variables associated with the species from the North to the South of MP (Southern portion of Cuyo Malacological Province, CMP) were reported three years later. As part of comprehensive surveys of CMP, we explored the Northern portion of this malacological region, from South of La Rioja Province (LRP) to South of San Juan Province (SJP). The goal is to provide unedited data on presence and environmental variables associated with this clam in North of CMPand to complete the available information about ecology of this clam in all CMP. Twenty-one lentic and moderately lotic sites were sampled between716 and 2731 m a.s.l., from 29º20.72S (Bermejo dam, LRP) to 32º7.04S (Santa Clara, SJP), and from 67º19.478W (Las Tumanas, SJP East) to 69º50.301W (Manantiales, Andean mountains). At each site biological samples and environmental variables were recorded. The ranges (21 sites in all) of conductivity (C), water temperature (WT), pH, Depth (D), dissolved oxygen (DO), turbidity (T), current velocity (CV) and vegetation cover (VC) were: 0.241–22.2 mS cm^{-1}; 7.4–32.5 °C; 7.4–9.1; 1–27 cm; 4–15.8 mg l^{-1}; 0–432.7 NTU; 0–1.7 m sec^{-1} and 0–90%, respectively. *P. chiquitanum* only was recorded at Vega El Leoncito, a shallow and moderately lotic site with high plant cover located in South of SJP at 2184 m.a.s.l. In this site high clam densities were associated to soft and relatively warm waters (C mean: 0.24 mS cm^{-1}; WT spring mean: 15.7 °C) and substratum dominated by very fine sand (mean %: 78.5). On a total of 94 lentic and lotic sites prospected to date in all CMP from 29º20.72S to 35º39S and 402 to 4000 m a.s.l.,*P.chiquitanum* was found in 9 sites between 965 and 2386 m a.s.l.,8 from Mendoza, Tunuyán, Atuel and Llancanelo basins (mostly shallow margins of streams and brookletswith abundant vegetation cover), and the spring of Vega El Leoncito reported hear. In all, ranges of C, WT, pH, D and very fine sand % where the clamoccurred were 0.18-1.21mS.cm^{-1}, 6.2-25.1ºC, 6.84-9.40, 5-50cm

and 9.4-78.5%, respectively. In central-west of Argentina, *P. chiquitanum* inhabits mostly shallow, vegetated and slightly running waters from Andean piedmont, and it has a relatively broad range of tolerance to environmental conditions.

A197
STUDY OF THE COMMUNITIES OF SOIL AND FRESH WATER CYANOBACTERIA IN THE PROVINCE OF CÓRDOBA

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Cyanobacteria have a wide ecological distribution, colonizing numerous ecosystems, not only aquatic and terrestrial, but also extreme environments such as deserts and hot springs. The ability of some cyanobacteria to fix atmospheric nitrogen in aerobic conditions gives them a special interest in agricultural issues, but other species that develop in bodies of fresh water are the cause of serious environmental problems due to the development of toxin-producing strains. This work consists of the evaluation of the specific richness of cyanobacteria in agriculturally used soils, in native forest and in 4 dams located in the province of Córdoba, which are reservoirs of water to supply the population. For the study of soil cyanobacteria, soil samples were taken at a depth of 0 - 10 cm; then, cultivations in chambers with controlled conditions were made. From the aquatic environment, samples were taken with a Van Dorn type sampler and with a phytoplankton net of 20 μm mesh. The taxonomic determination was made with a Leica CME microscope and the assistance of specific dichotomous keys.The study allowed the analysis of the specific richness of the different environments and the taxonomic description of the new citations for Córdoba. Forty-eight (48) species in aquatic environments were identified; 17 of which are reported in the literature as potential toxin producers. From the four dams studied, San Roque is the most compromised in terms of blooms of cyanobacteria producing toxins; the most frequent species are *Microcystis aeruginosa, M. flos-aquae, M. wesenbergii* and *Dolichospermum spiroides*.In the soil environment, a total of 42 species were recognized. Regarding the index of relative diversity of importance per family calculated from the number of species observed, it was found that the most important family is the Oscillatoriaceae, which stands for more than the 53.66% of the identified species, followed by the Nostocaceae family with 21.95%, the Leptolyngbyaceae with less than 9.76%; the remaining families stand for less than the 5% of the species. The heterozygous species (Nostocaceae, Rivulariaceae, Scytonemaceae and Tolypothrichaceae) developed dominantly in the soil with agricultural treatment compared to those developed in the native forest soil. The common species between aquatic and terrestrial environments were: *Oscillatoria subbrevis, O. limosa, Phormidium corium, P. retzii, Geitlerinema tenuis* and *Nostoc commune*. The perspective of our work is, on the one hand, to contribute to the knowledge of the edaphic cyanobacteria as new bio indicators, complementary to those already described for bacteria and fungi. In contrast, the blooms of cyanobacteria in water reservoirs, represent a health risk because they are potentially toxigenic; thus, the recommendation in this case is to intensify the tasks of monitoring and control of water purification processes.

A198
PRELIMINARY STUDIES OF SOIL PHYCOFLORA OF SALINAS DEL BEBEDERO, SAN LUIS, ARGENTINA.

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The presence of plants determines the environment of where they are. To know the soil phycoflora allows us to characterize and establish degrees of interrelationship in an integral way. In this sense, our aim was the identification of the soil phycoflora of saline environments. In this preliminarystudy we show the results of the qualitative analysis. The sampling was realized during 2018 the dry season, in different points of Salinas del Bebedero, from the north to the south following a longitudinal pattern. The soil samples were taken to a 1m depth, considering the physical chemical parameters. In the laboratory the samples were preserved in 4% formaldehyde solution and then were subjected to continuous dilutions with distilled water to obtain a relatively clean solution of sediments for the observation in the optical microscope (40X and 100X). A total of 10 samples were analysed in which we identified 33 taxas, from which 3 correspond to *Cyanophycea* and the other 30 correspond to *Bacillariophyceae*. *Ephitemia* and *Campylodiscus* were dominant genera. Being the *Campylodiscus* genera reported in San Luis Province for the first time. The composition and distribution of the algae was determined by the physical and chemical factors of the soil. To emphasize on this type of studies will allow us to extend the knowledge of these species adapted to extreme environments.

A199

FREE RADICAL SCAVENGING ACTIVITY OF *Annona emarginata* (SCHLTDL) H. RAINER

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Reactive oxygen species (ROS) are derived from many sources, including mitochondria, xanthine oxidase, uncoupled nitric oxide synthases and NADPH oxidase. Oxidative stress mainly caused by ROS damage normal organs, leading to a gradual loss of vital physiological function.Extracts from *Annona emarginata* flowers, fruits, leaves and barks were evaluated regarding their antioxidant activity by free radical scavenging methods (DPPH and TEAC), ferric-reducing antioxidant power (FRAP) and lipoperoxidation inhibition in erythrocytes (ILP). The total phenolics (TP) and flavonoids (F) content were determined by the Folin-Ciocalteu and $AlCl_3$ colorimetric methods. Polar extracts obtained from *A. emarginata*showed strong free radical scavenging activity in the DPPH, FRAP, ABTS and lipid peroxidation in erythrocytes assays.The results showed that fruits, leaves and barks methanolic extracts prevented the hemolysis caused by the rupture of cell membranes induced by lipid peroxidation (83-93%, at 500 µg/mL). This percentage value is similar to reference compound catechin compound (72%,at 100 µg/mL). The same extracts showed a high content of phenolic compounds (96-562 mg GAE/g extract). The bark methanolic extract showed the highest capture capacity of the DPPH free radical (IC_{50} 2 µg/ml). The DPPH assay is widely used for quickly assessing the ability of polyphenols to transfer labile H atoms to radicals, which is a likely mechanism of antioxidant protection.The chemical characterization of the most active extracts by HPLC-MS is in progress. Authors are grateful to CICITCA UNSJ, and UNSL.

A200

GENETIC VARIABILITY OF PARENTALS AND F1 POPULATION OF *Aspidosperma quebracho-blanco* Schltdl.

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A potential in situ preservation strategy for species of economic forest, like *Aspidospermaquebrachoblanco*Schltdl. is to select seed trees, collect seeds from known, reliable sources in order to use them for seedling aiming to maintain the genetic structure of the forest. The aim of this study was to evaluate the genetic variability of parental and F1 population of *Aspidosperma quebracho-blanco*, in order to use genetic variability for the selection of parental that could be used as best seed tree for production of seedlings for economic forest use or reforestation in conservation areas. Parentals from different locations, Bajo de Veliz (**BV**), La Punta (**LP**) and San Luis (**SL**) were selected for this study. Genetic variabilitywas analyzed by using molecular markers RAPDs. Leaf material (young meristematic tissue) and fruits were collected fromparentalsof *A. quebracho-blanco* Schltdl.from natural forests of Dry Chaco (San Luis, Argentina). Fruits were collected from each parental and the seeds germinated in a greenhouse. Young leaf from parentals and F1 were used for DNA extraction and RAPDs evaluation, following previously described methods.We analyzed 137 loci obtained by RAPDS from 30 studied individuals. We observed a number of bands conserved between the parentals and F1, polymorphic bands and bands specific of F1 individuals. The percentage of polymorphic bands was 59.12% for the **LP** group, 63.5% for **BV**and 64.96% for **SL**groups.A percentage of the polymorphic bands (32%),was present and shared for all the three groups BV, SL y LP.Specific bands were 12 for BV, 18 forSL and 11 for LP. AMOVA analyses of the RAPDs pattern evidenced higher intra-population (85%) and lower inter-population (15%)variation. Two different analyses were performed: UPGMA cluster analysis by using DICE coefficient and principal coordinate analysis (PCA). All of them support the presence of two populations genetically different, each of them with subpopulations. The cluster evidenced two main subgroups, **1** and **2**. The first of them comprise two subgroups **1a** and **1b**. Individuals from **BV** (parental and F1) associate in group **1a**. Subgroup **1b** includes parental and F1 from **SL**. Group 2 contains the F1 from **LP**. Surprinsingly,parental **LPp** grouped to **1b** with the lowest similarity coefficient (0.65).After timber harvest or a forest fire, reforestation is essential for a productive working landscape and healthy ecosystem. The high genetic variation obtained between parentals and F1 support the use of these seedlings for reforestation purpose in order to preserve the genetic resources of the specie.

A201

DIET, DIMORPHISM AND REPRODUCTION OF THE VULNERABLE SPECIES *Olivaichthys cuyanus* (SILURIFORM: DIPLOMYSTIDAE), IN THE RIVER CORDILLERANO LOS PATOS, SAN JUAN, ARGENTINA: BASES FOR THEIR CONSERVATION

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The studies dedicated to know the biology of the species are essential, not only from the generation of knowledge but also applied to conservation, generating a theoretical support from which to plan and execute management plans, especially even more so if they are vulnerable species as *Olivaichthys cuyanus*. The Diplomystidae family is the oldest of the siluriform order. Its distribution is limited to South America, precisely in Chile and Argentina, with three species each. In Argentina, *O. cuyanus* shares the genus with two other species, *Olivaichthys viedmensis* from the Río Negro, *Olivaichthys mesembrinus* from the Río Chubut and Senguerr. *Olivaichthys cuyanus* is categorized as a vulnerable species and there is no information about its biology. We studied 26 individuals from the Río Los Patos, province of San Juan, in a span of 4 years (2013-2017) in the spring and summer seasons, not having success in autumn and winter. Global diet studies were carried out, differentiating sex, seasons, and functional grouping in the ecosystem. Sexual dimorphism: a scheme of 31 morphometric measurements was contemplated. Reproduction: In this aspect we proceeded through the macroscopic analysis of the gonads. *Olivaichthys cuyanus* is a generalist predator frequenter of foods of terrestrial origin (Isopoda) less common (Hymenoptera) and aquatic (Trichoptera and Coleoptera). It presents sexual variations in the percentages consumed of each item males present the order Trichoptera as a fundamental item, females Isopoda. There was also seasonal variation, in the spring season the diet was more diverse and in summer more influenced by dominant components. The species presents sexual dimorphism in some corporal variables (LAa) length of the anal fin; (PPC) depth of caudal peduncle; (PCAP) depth of the body in the pectoral fin and (LbAD) length of the base of the dorsal fin. In the first three variables (LAa), (PPC) and (PCAP) the difference observed was towards males, which are more robust than females, possibly due to territoriality and reproductive function. The females presented higher measures in the variable (LbAD) measure presumably associated with the weight of these and their livelihood in the environment. The highest reproductive activity was observed in the spring season, it is a species with characteristics of vulnerability due to its low fecundity, depending on its sexual proportion is not currently in a period of population expansion. (EB)

A202

COMMUNITIES OF TARDIGRADES (ECDYSOZOA, TARDIGRADA) IN URBAN HABITATS OF THE CITY OF SALTA

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The urban growth in recent decades has increased markedly in the city of Salta, so it was proposed with this work to evaluate the biodiversity of tardigrades in three urban habitats of the city of Salta with high (A), medium (M) and low (B) vehicular traffic to detect the changes that occur in their communities. A total of 48 lichen and moss samples were extracted, and they were treated following the usual methodology and specimens and eggs were mounted in polyvinyl-lactophenol. Data analysis was performed using PAST, PC-Ord7, iNEXT, SPADE and R programs. Three thousand forty-nine specimens (3049) were collected belonging to sixteen species probably some of them are new to science. Habitat A showed the highest abundance (N = 1339), however habitat B turned out to be 1.61 and 1.16 times more diverse than communities M and A respectively. The assemblages of the three urban communities showed to be quite similar, with a strong dominance of a few species: *Macrobiotus hufelandi*, *Milnesium* sp3 and*Echiniscus rufoviridis*, standing out *Paramacrobiotus areolatus* in Habitat B. The Principal Components Analysis (PCA) explained 62% of the total variation in two axes ordering the communities in a nested pattern. In this study, we conclude that there is a process of biotic homogenization in urban habitats, which implies an important loss of species. This process included the tendency to increase the number of individuals in urban communities, similar community structures and nested pattern among habitats.

A203
URBAN TREES GROWTH AND WATER STRESS. RELATIONSHIP BETWEEN ANNUAL TREE-RINGS AND CALCIUM SIGNAL: RESULTS USING LASER-INDUCED BREAKDOWN SPECTROSCOPY (LIBS).

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The water availability incidence in the urban trees growth can be quantified by dendrochronological analyzes. The tree-rings time serie constructions allow evidence the differential thickness of the annual rings and their chemical composition. *Platanus hispanica* Tend, a frequent and representative tree-specie in the urban landscape of Mendoza-Argentina,was studied. We analyzed at anatomical and dendrochemical level, in a comparative way, the tree-rings growth of different thickness: wide -that indicates an adequate water availability- and narrow -resulting by growth under water restriction-. Methodologically, the analytical technique called Laser-Induced Breakdown Spectroscopy-LIBS- is applied, and also tree-ring measurements. This method allows us to evaluate the chemical content present in wood, particularly those related to drought periods, such as Calcium. The first results for studied cases -Urban and Periurban- indicate as tendency that the average Calcium tenor is proportional to the thickness of the tree-rings. The periurban trees have a lower content than those located in an urban condition, where the soil content of this element is higher given the greater urbanization of the area. In future studies and with a larger number of cases, we expect to develop a water stress indicator based on the qualitative and semi-quantitative content of Calcium in tree-rings, which will allow us to know the response and adaptation mechanisms of this tree-specie, to drought and water deficit.

A204
ATTRIBUTES OF BIOLOGICAL CRUST FOR USE IN THE RESTORATION OF ECOSYSTEMS, MENDOZA

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In Argentina, more and more human activities result in a significant deterioration of the biological surface of the soil. Biological soil crusts (CB) provide significant ecosystem services to drylands. With the intention of knowing CB attributes that allow evaluating their role in restoration, our objective was to determine the stability, compaction, moisture retention, content of organic matter, nitrogen, phosphorus, temperature and radiation in soils covered with biological crusts. The study area was located in the Dept. Las Heras, Mendoza. Thirty blocks of 2 m^2 were built at random in the extension of the interparche. Each block was made up of two zones, one with a biological crust (CCB) and another without a biological crust (SCB), separated by 50 cm. In each block soil stability (Herrick's test), compaction (dual penetrometer), moisture retention (saturation and weighing), content of organic matter (calcination), nitrogen (Kjedahl), phosphorus (photometry of flame), temperature (digital thermometer) and radiation (point radiometer) were measured. Significant differences were found ($p < 0.001$) in the compaction, stability, content of organic matter, nitrogen, temperature and humidity between CCB zones and SCB zones. The stability, organic matter, nitrogen, phosphorus and moisture retention turned out to be higher in CCB zones, but not the radiation, temperature and compaction that showed a decrease. The biological crusts seem to resist high levels of radiation, improve the fertility and physical properties of the soil and act as thermal shock absorbers, becoming a plausible alternative for the recovery of degraded ecosystems.

A205
SUSTAINABLE EXTRACTION AND ISOLATION OF ALKALOIDS FROM THE LEAVES OF *Hippeastrum argentinum* (PAX) HUNZ.

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The Amaryllidaceae alkaloids have shown a broad range of pharmacological and biological activities, including acetyl cholinesterase (AChE) inhibition, and antitumoral, antibacterial, antifungal, antiviral and antimalarial activities. The extensive structural diversity of this group of alkaloids promotes the research for the development of new drugs. Until now, only galanthamine is being for the palliative treatment of mild to moderate Alzheimer's disease (AD). It is a long-acting, selective, reversible and competitive inhibitor of AChE and an allosteric modulator of the neuronal nicotinic receptor for acetylcholine. The extracts obtained from Amaryllidaceae bulb usually show complex alkaloid profiles. To isolate these alkaloids bulbs are used, this implying the loss of the plant. Sometimes is requiring more biomass, but due to ecological and conservation aspects cannot be performed. The alkaloid 7-OH-clivonine is a cholinesterase inhibitor previously identified in the leaves of *Hippeastrum*

argentinum. The aim was in a sustainable way to isolate the 7-OH-clivonine using the leaves of *H. argentinum*, to deepen the studies of bioactivity. The fresh and totally developed leaves were collected (between November 2017 and March 2018), and then dried and crushed. Then, the alkaloid extract was prepared. The extract yield related to the dried plant material was >1% w/w, showing a higher yield than that of the bulbs. Chromatographic methods (LC, Sephadex LH20, PTLC) and crystallization were applied to isolate the alkaloid 7-OH-clivonine. The studies based on *H. argentinum* leaves represent an ecological, sustainable and effective alternative for Amaryllidaceae alkaloids research. Acknowledgement: CICITCA- UNSJ, ANPCYT PICT 2014-3425. Red CYTED–BIFRENES. Proy 416RTO511

A206
CORN FERTILIZED WITH NITROGEN: STUDY OF THE DYNAMICS OF NITRATES IN SOIL

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The soils of this region presented as main limitation the low content of organic matter and nitrogen (N). For this reason fertilizers are an essential tool to maintain high levels of N in soils and therefore of production. The purpose of this paper is to study the dynamics of nitrogen from nitrates (N-NO3) in the soil under cultivation of maize as a function of different doses and applied nitrogen source. The study was developed in the agricultural establishment "Santa Monica", which is located 12 km from the city of Villa Mercedes at coordinates 33º 40' 2.62" S and 65º 35' 23.95" or to 660 meters above sea level. The management of the test was similar to the one used in production batches of the region with a density of 50,000 plants ha-1 and a distance between rows of 0.52 m and with normal weed control carried out by the producers in the area. Implemented an experimental design of plots divided into blocks at random, with 7 treatments (T; U20-urea, 20 kg N2 ha-1; U50; U100; S20-Sulfammo MeTA, 20 kg N2 ha-1, S50 and S100) and 3 repetitions. In phenological stage with 6 leaf (V6), maturation and milky grain (R2-3) and physiological maturity (PM) of maize conducted a soil sampling in strata of 0-20 cm, 20-40 cm, 40-60 cm and 220-240 cm (this stratum only for the phenological stage V6). The ANOVA results shows that there are significant differences in the concentration of N-NO3 in the soil profile for all sources of variation (time, depth and treatments). Statistically significant differences were also observed for time-treatment interaction. For the time variable specifically in PM is found greater concentration of N-NO3 (13.45 ppm) with respect to V6 and R 2-3 (3.11 and 2.81 ppm) for a significance level of $p<0.01$. In treatments highly significant differences were observed between the doses nitrogenous bases: U100 (7.29 ppm); S100 (7.62 ppm) and U50 (6.69 ppm); S20 (6.61 ppm), S50 (6.54 ppm) and Urea 20 (5.37) and T (4.13 ppm). In depth found highly significant differences when analyzing the concentration of N-NO3 in the 3 sampled strata. Being for the first layer (0-20) 8.15 ppm; for the second (20-40) and 6.11 ppm for the third (40-60) 4.71 ppm. It is concluded that: (a) there are significant differences in the concentration of N-NO3 in the soil profile for all sources of variation (time, depth and treatments); (b) on PM are the largest concentrations of N-NO3 in soil; (c) the treatment U100 is the most concentration of N-NO3 presented; (d) higher values were found in the stratum 0-20 cm depth.

A207
RELATIONSHIP BETWEEN DIVERSITY, BIOMASS AND PRIMARY NET PRODUCTION IN GRASSLAND OF SOUTH OF CÓRDOBA (ARGENTINA) UNDER TWO DIFFERENT CONDITIONS

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The study of functionality of natural grassland ecosystems with different levels of intervention requires the analysis of indicators that allow us to infer their operation and provide tools for their conservation and sustainable use. The relationships between diversity (H), aerial biomass (Ba) and net primary productivity (PPN) would be good indicators of this operation and management. The present work proposes to establish correlations between H with Ba and PPN; determined to field. Two areas of natural grassland with different levels of disturbance were sampled, at the end of the Chucul stream basin: "La Felipa" Natural Reserve (R) and an adjacent lot with livestock use from August to September with intermediate animal load (P). In each site 15 random samples of 0.25 m^2 were taken seasonally, from June 2016 to June 2018, relieving floristic list, coverage and cutting Ba, which was separated into green, dry and mulch compartments and taken to a 60 °C stove until constant weight. The H indices were calculated with coverage data (H (C)) and green biomass (H (Bv)) and PPN. Linear correlations were established for the following parameters: Ba vs H (C); Bv vs H (C); Bv vs H (Bv), PPN vs H (C) and PPN vs H (Ba). The richness of R oscillated between 5 and 11 species, and between 9 and 16 in P. In R it was observed that at higher values of Bv and Ba corresponds an increase of H (C), while P did not have a similar behavior. The highest correlation values were obtained by contrasting H (C) vs PPN ($R = 0.34$ and $P = 0.71$). While the lowest correlation was obtained with H (Ba) vs PPN ($R = 0.08$ and $P = 0.16$). This would indicate that the diversity calculated with coverage would be an indicator of dynamics and functioning of these systems, adjusting better in this particular case to the situation of use with low level of disturbance.

A208

NATURAL FUNGICIDES – MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION AND THE FUNGISTATIC/FUNGICIDAL EFFECT ON *Lasidioploidea crassispora* ISOLATED FROM SAN JUAN'S PROVINCE VINES WITH THE DECAY OF THE VINE SYMPTOMS.

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Vine diseases, grouped under the term Decay of the vine, caused by ligninolytic fungi are internationally considered to be of great importance since they cause the death of the plant in the long term. Viticulture production is the most important in San Juan's regional economy, hence every kind of pathology that diminishes the grape's output or quality has strong implications for the economic development of the province. These pathologies are produced by a pool of ligninolytic fungi, responsible for Eutypiosis, Yesca, Petri's Disease, BAD (Black Dead Arm) and Hoja de Malvón, being *Lasiodiploidea crassispora* one of the latest fungus globally reported as responsible of this disease. In investigations carried out in San Juan province, over plantations affected by the disease, *Lasidioploidea crassispora* was isolated and identified by the culture's characteristics, morphology and sequencing of the ITS and LSU of ribosomal DNA. Aiming to control this fungus development, the minimum inhibitory concentration (MIC) for phenolic compounds of natural origin: Carvacrol, Timol, Eugenol e Isoeugenol, was determined. This was accomplished by measuring the growth halo in agar/drug plate in final concentrations of 4 mM, 3 mM, 2.5 mM, 2 mM, 1.5 mM, 1 mM, 0.75 mM and 0.5 mM and in triplicate. It was also determined the fungistatic/fungicidal effect. Resulting that the MIC for Timol, Eugenol, Carvacrol and Isoeugenol is 0.75 mM, 4 mM, 1.5 mM and over 4 mM respectively. Also, concluding in the fungicidal effect of Timol and Carvacrol at a 3 mM concentration.

A209

REPRODUCTIVE BIOLOGY OF *Hatcheria macraei* (TORRENT CATFISH) IN THE PROTECTED NATURAL AREA LA CIÉNAGA, SAN JUAN.

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Throughout their evolutionary history, species have developed different reproductive strategies to optimize the viability of their offspring. In fish, the strategies are closely linked to the environment, especially the seasonal changes in light and temperature, which vary considerably depending on the species. *Hatcheria macraei* belongs to the family Trichomycteridae and is endemic to South America. It is widely distributed in Argentina and to a lesser extent in Chile and is the southernmost species of the family. The La Ciénaga Protected Natural Area preserves an important natural, biological and geological legacy. However, there are no records of the fish populations present. Therefore, the objective of this work is to know the reproductive biology of *H. macraei* with the purpose of generating base information as a tool for the management of these populations. There were 5 samplings (March, April, July, August and December). 200 individuals were collected, each one was recorded the total length (LT), standard length (LE), long snout cloak (LHC), total weight, eviscerated weight, liver weight and gonads. The latter were classified in a macroscopic way taking into account three phases: I) Immature, II) In-maturation and III) Mature. The LE of females (n = 105) and males (n = 91) showed significant differences (H = 4.31, P = 0.0378) and, the female-male ratio was balanced 1: 1. Juvenile females were captured in all months and adults only in July and August. While the males, both adults and juveniles, were observed every month. It was determined that the minimum reproductive size for females was 55.85mm and for males of 62.19mm. The reproductive time was determined according to changes of the gonado-somatic index (IGS) and absolute fecundity (FA). The IGS (gonado-somatic index) in females showed significant differences (H = 31.67, P = <0.0001) being minimum in December (mean = 1.01), increasing in March (mean = 3.05), low in April (mean = 2.14) and increases progressively in July (mean = 2.61) and the maximum is recorded in August (average = 5.54). In males the IGS also showed significant differences (H = 11.58, P = 0.0207) being minimum in April (mean = 0.37) and maximum in August (mean = 0.49). It was estimated that FA varied between 206 ± 442 oocytes with a mean of 342 oocytes. The species has an extensive reproductive period, extended from March to July and the highest reproductive activity was recorded in the month of August. (EB).

A210

LEAF CONSUMPTION OF *Diaphania hyalinata* (L) (LEPIDOPTERA, PYRALIDAE) IN AN ANQUITO (*Cucurbita moschata* DUCH)

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Diaphania hyalinata (L.), known as caterpillar of the cucurbits, is the main causal agent of losses in the yield and commercial quality of crops of Anquito *Cucurbitamoschata* (Duch.), developed in the irrigation area of the province of Santiago del Estero,

Argentina.The objective of this work was to determine the foliar consumption (mm^2) of the different larval stages and the total consumption by *D. hyalinata*.Newborn larvae were placed separately on previously-scanned *C. moschata* leaves (CanonScan LIDE 100) to determine the initial leaf area; each leaf was placed in a plastic cup with the corresponding identification, in total 33 larvae were bred.The sheets were reviewed daily; when it was detected the presence of exuvia or pelt (skin that is eliminated in each moult), the larva was separated and the consumed leaf was scanned (final leaf area); later a healthy scanned sheet was placed in its original passenger compartment.The leaf area consumed by the larvae of each stage of *D. hyalinata*, was determined by the difference between the initial area of the leaf placed for food and the final area not consumed.The average leaf area consumption was 5672 mm^2, with the highest consumption value corresponding to the IV stage with 2315 mm^2.

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