



Abstracts from the

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de Biología de la República Argentina)**

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XX Annual Scientific Meeting of the Córdoba Biology Society  
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*The abstracts have been revised and evaluated by a Scientific Committee prior to publication*

## LECTURES

### Opening Lecture

#### A1

### THE LONG GOODBYE: THE RISE AND FALL OF THE ELECTRON SHUTTLE FLAVODOXIN DURING PLANT EVOLUTION

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Ferredoxins are electron shuttles which connect multiple oxido-reductive pathways in organisms displaying very different lifestyles. They employ iron-sulfur clusters as cofactors for electron transfer. Some prokaryotes and algae contain an isofunctional electron carrier, flavodoxin, which contains flavin mononucleotide as prosthetic group. Both electron shuttles are very ancient proteins that evolved in the anaerobic environment preceding the appearance of oxygenic photosynthesis. The advent of an oxygen-rich atmosphere proved detrimental to ferredoxin due to iron limitation and oxidative damage to the iron-sulfur cluster, and many microorganisms induced flavodoxin expression to replace ferredoxin under stress conditions. Paradoxically, ferredoxin was maintained throughout the tree of life, whereas flavodoxin is absent from plants and animals. Interestingly, expression of a chloroplast-targeted flavodoxin in transgenic plants results in increased tolerance to multiple stresses and iron deficit through mechanisms similar to those operating in microorganisms. Then, the question remains open as to why a genetic trait which still confers plants such obvious adaptive benefits was not retained in the plant genome. We review herein the properties of ferredoxin and flavodoxin as electron carriers, and their contrasting modes of expression in response to different environmental stimuli. Distribution of flavodoxin among photosynthetic eukaryotes indicates that the gene disappeared from the plant lineage somewhere between the split of the streptophytes from the chlorophytes and the appearance of land plants. The most plausible hypothesis to explain this loss, despite flavodoxin adaptive value, is a founder effect. This proposal assumes that the coastal/freshwater algal species from which land plants evolved already lacked flavodoxin because they thrived in an iron-rich habitat with no need to back up ferredoxin functions and therefore no selective pressure to keep the flavodoxin gene. Several lines of evidence supporting this contention are presented and discussed.

### "Bernardo Houssay" Lecture (Argentine Biology Society)

#### A2

### COMPARATIVE GENOMICS, PHYLOGENY AND EVOLUTION OF FISHES

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Recent advances in genomic technologies involving next-generation sequencing platforms and improved bioinformatics pipelines increasingly enable compilation of massive comparative databases for phylogenetic and evolutionary analyses. A brief overview of recent advances on fish phylogenomic studies will be presented to highlight new discoveries and possibilities afforded by comparison of whole genomes or transcriptomic sequences. The relatively low number of available genomes of high quality, however, still hampers rapid discovery for non-model organisms. New methods to obtain subgenomic fractions (reduced-representation libraries) targeting loci of interest for phylogenetic analysis have been developed using target capture approaches with probes designed to enrich libraries for hundreds or thousands of genetic loci. These methods and the latest phylogenomic results will be discussed in terms of phylogenetic resolution, information content, and bioinformatics challenges to scale-up comparisons to thousands of species. As a conclusion, a prospect for establishing a complete evolutionary tree for all fishes on the basis of genome-scale data sets will be presented. The challenge is to accelerate integrative evolutionary analysis of multiple trait layers within the context of a complete, species-rich phylogeny. The resolution of phylogenetic relationships among all fishes will empower fundamentally new ways of exploring evolution among basal vertebrates through novel comparative analyses based on the rich data layers of ecological, geographical, and paleontological information available for this group of animals.

**"Jorge W. Ábalos" Lecture (Córdoba Biology Society)**

**A3**

**NEW MITOCHONDRIAL AND NUCLEAR DEFENSES OF *Arabidopsis* AGAINST *Pseudomonas***

*Alvarez ME*

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Plants use many different strategies to cope with the attack of microbial pathogens. For example, they can reprogram their functions to mount inducible defenses upon perception of invaders. The accumulation of reactive oxygen species (ROS) at the apoplast of infected cells reveals the activation of the plant immune cascade. This alteration affects several cellular functions at both the local and systemic levels, providing resistance against biotrophic pathogens. The NADPH oxidase from plasma membrane was early recognized as responsible for ROS accumulation. Later, several laboratories showed that this response also required the functioning of chloroplast and mitochondria, without actually identifying the specific contributions of these organelles. Our work has been focused on evaluating the ability of other metabolic pathways to alter the oxidative balance and induce defenses against biotrophic pathogens in a model system including *Arabidopsis* and *Pseudomonas syringae* pv. *tomato* (*Pst*). The finding that the mitochondrial proline dehydrogenase (ProDH) enzyme is essential for ROS accumulation and cell death activation in infected tissues led us to study how this enzyme affects plant defenses and to evaluate its coordination with NADPH oxidase, reporting new functions for mitochondria in the generation of the oxidative burst. On the other hand, we studied genomic alterations occurring in *Pst*-treated cells. Notably, these cells relax the centromeric heterochromatin, change epigenetic marks and alter the expression of these genomic regions. At present, we have identified a plant protein involved in the chromatin decondensation process, which is part of a base repair system, and affects tolerance to oxidative stress. Thus, we have revealed new responses of *Arabidopsis* plants that may modulate resistance against *Pst*.

**"Miguel Lillo" Lecture (Tucumán Biology Association)**

**A4**

**DEVELOPMENT OF A PROPHYLACTIC VACCINE AND THERAPEUTIC ANTIBODIES AGAINST THE HEMOLYTIC UREMIC SYNDROME USING PROTEIN AND ANTIBODY ENGINEERING**

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The striking feature of Enterohemorrhagic *Escherichia coli* (EHEC) infection is the production of potent Shiga toxins (Stx) implicated in the development of the life-threatening hemolytic-uremic syndrome (HUS). Despite the magnitude of the social and economic problems caused by EHEC infections, no licensed vaccine or effective therapy is presently available for human use. One of the biggest challenges is the development of an effective and safe immunogen to ensure non toxicity but also a strong input to the immune system to induce long-lasting, high affinity antibodies with anti-Stx neutralizing capacity. The enzyme lumazine synthase from *Brucella* spp. (BLS) is a highly stable dimer of pentamers and a scaffold with enormous plasticity for the display of foreign antigens on its structure. Taking into account BLS advantages and the potential capacity of the B subunit of Stx (StxB) to induce antibodies that prevent Stx2 toxicity by blocking its entrance to the host cells, we engineered a new immunogen by inserting Stx2B at the amino termini of the BLS gene. The resulting chimera demonstrated a strong capacity to induce long-lasting humoral immune responses. Llama single domain antibodies (nanobodies) with high neutralizing capacity for Stx are being developed. These antibodies, engineered for avidity, pharmacodynamics and immunogenicity, have been tested in preclinical studies in animals and have shown protective effects at picomolar concentrations. Phase I clinical trials in adult healthy humans and phase II clinical trials in children suffering from EHEC infections are being planned.

**Plenary Lecture 1**

**A5**

**VECTORS ENGINEERED FROM SEMLIKI FOREST VIRUS FOR THE GENE THERAPY OF CANCER**

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Gene therapy is a relatively new paradigm in medicine with enormous therapeutic potential and almost 2000 clinical trials (most of them for cancer applications) registered worldwide. It involves the introduction of genetic material into cells of the organism in order to trigger a therapeutic effect. Although several gene delivery systems have been developed to date, viral vectors are by far the most versatile, popular and effective approach, and are being used in approximately two-thirds of these clinical trials. A viral vector is a virus modified in such a way that it retains its native infectivity and ability to transfer its genome to the infected cell, but does not

cause disease since its original genes have been replaced by the gene to be transported. Our work is focused on vectors engineered from Semliki Forest virus (SFV). These vectors are very promising for cancer gene therapy since they have a broad tropism for tumor cells, allow high levels of heterologous gene expression in infected cells and can be re-administered without being greatly affected by the host immune response. We found that a gene therapy strategy based on the intratumoral administration of an SFV-based vector expressing interleukin-12 (SFV-IL-12) inhibits tumor growth and extends survival in mice models of liver cancer. SFV-IL-12 showed better antitumor potential compared to other viral and non-viral-based IL-12 expression systems due to the strong SFV-mediated induction of apoptosis and activation of type-I interferon responses specifically in the tumor. We have also shown a clear reduction in tumor angiogenesis, a lower number of metastases and a better survival rate when SFV-IL12 was evaluated in an aggressive model of metastatic breast cancer. These antitumor effects were associated with the early and sufficient IL-12 and interferon-gamma expression levels induced by SFV-IL-12 inoculation linked to the strong immunostimulatory and anti-angiogenic properties of both cytokines. Overall, our results demonstrate that SFV-based vectors are efficient biological tools for the delivery of therapeutic genes to tumor cells *in vivo* and SFV-IL-12 can induce highly effective antitumor responses in clinically relevant animal models of cancer.

## Plenary Lecture 2

### A6

#### TRANSGENICS vs. BIODIVERSITY. A REAL DICHOTOMY?

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It has already been 31 years since a bacterial gene expression was obtained in a vegetable by the scientific group of Van Montangu in Belgium, using recombinant DNA techniques and genetic engineering. This combination of genes, which seems so unnatural, exists in nature in many examples. In turn, farmers have used the mixture of genes for breeding in domesticated species since they began to improve crops. This genetic manipulation has allowed the feeding of a population that has grown exponentially during the last 200 years. Like all human activities, innovative technologies have risks and genetic engineering is no exception. However, neither the public nor the scientific community is willing to run these risks. That is why organizations that evaluate, monitor, and authorize the release of genetically modified organisms into the environment and consumption have been created. During the almost 20 years since transgenic plants started to be marketed and consumed in agroproducer countries outside Europe, no evidence has been found indicating that transgenic plants impart additional environmental risks than those caused by other crops in which genes are randomly mixed using traditional breeding techniques.

This dissertation aims at discussing current knowledge on the use of transgenic materials and their impact on the environment, especially whether or not they affect biodiversity. GM and environmental biodiversity are not contradictory and may even be complementary. This mainly is because GM crops in many cases lead a reduction in indiscriminate deforestation, abuse of agrochemicals, particularly insecticides, tillage of the soil and erosion involving the excessive fertilization and excessive use of groundwater for irrigation.

## SYMPOSIA

### Symposium 1: ASSISTED FERTILIZATION

#### A7

#### WHEN DOES LIFE BEGIN?

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The beginning of life has generated controversy in the public opinion, but most arguments do not come from biological events that are clearly defined. In biology, when the sperm penetrates the oocyte, fertilization occurs. Certainly when both pronuclei (male and female) (syngamy) are combined, a new genome, different but typical of the species, is formed. This genome present in a cytoplasm - with the basic information for further development- ensures the development of the embryo. After fertilization there is no cutoff point, since it is an ongoing development plan. It is divided into stages simply to better study and understand it. Life is a dynamic process and, from a strictly scientific point of view, it cannot be said for certain when we start to be a person or a human being. A being is an abstract entity for philosophical purposes; a person implies a legal and religious definition. It is a human being because gametes come from humans. The issue of whether it is alive or not is pointless since there is always life during the process (*vita vivificat*). Gametes are alive – they cannot be dead or damaged - and the first zygote is also alive; if a fault exists and the zygote is not alive there cannot be continuous development. Life passes from one to another even at the cell level. There is no stop, no stage separated from another. It is a human embryo, it is alive, it requires a womb. Eventually, after normal development, it will be a

human newborn. Knowledge gives us better tools, but not always better answers. It is very important to consider that life begins before conception. How? It actually depends on healthy gametes that must come from healthy parents to produce optimal newborns. Reproductive health should be provided for everybody even when inside the womb. True reproductive health is much more than a contraceptive plan. So we must protect the health of children and adolescents against health risks such as exposure to x-rays and adequate food and an unpolluted environment should be part of a reproductive plan. The answer to the question of when life begins is: from a practical viewpoint, life begins before conception.

## A8

### ASSISTED FERTILIZATION: ACHIEVEMENTS AND QUESTIONS

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A timeline of advances in assisted reproductive technologies applied to human beings during the last decades, since the birth of the first human being engendered by *in vitro* fertilization in 1978, is briefly discussed. Then, current alternatives made possible by technical advances in assisted reproduction are analyzed regarding their present application in the light of generally accepted principles of bioethics (non-maleficence, beneficence, autonomy, justice and respect for human life). Because of their cultural and social importance, the foundations of a Christian worldview and its corollaries regarding the application of assisted fertilization techniques are discussed. Finally, the positions of other religious confessions regarding these issues are briefly stated. It is concluded that no technical advance should be applied without a previous thorough analysis of its ethical implications by both the health professionals and their patients.

## Symposium 2: APPLIED BIOTECHNOLOGY IN REGIONAL CROPS

## A9

### TWO ENVIRONMENTALLY SAFE STRATEGIES FOR THE CONTROL OF DISEASES IN STRAWBERRY

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The control of plant diseases requires the use chemical compounds that usually bring about unwanted effects on health and the environment. Here we propose two strategies with very low environmental impact to control fungal diseases as possible alternatives to the use of agrochemicals. The proposal consists in using two natural products, one obtained from strawberry leaves and the other from the fungus *Acremonium strictum*. In our laboratory we detected that strawberry leaf extracts induced the defence response in strawberry plants. Further studies allowed us to determine that the active agent was a 959 Da ellagitannin (HeT). It was demonstrated that strawberry plants treated with HeT acquired resistance to the fungal pathogen *Colletotrichum acutatum*, the casual agent of the anthracnose disease. On the other side, it was observed that strawberry plants treated with the supernatant of *A. strictum* culture also showed resistance against anthracnose. Further studies enabled us to determine that the elicitor of the defence response observed was a 34 kDa (282 aa) protease (AsES) that belongs to the subtilisin family. It was demonstrated that HeT and AsES could activate the plant innate immunity and that this activation is preceded by an oxidative burst (e.g. H<sub>2</sub>O<sub>2</sub> y O<sub>2</sub><sup>-</sup>), and the accumulation of free salicylic acid and callose. It was also observed that plants treated with HeT or AsES underwent a change in the expression level of many genes associated with the defence response (i.e. PR1, ACO, ASO, ETR2, CH1-2, OG5) and that whereas HeT activated the salicylic acid signalling pathway, AsES activated the three major defence signalling pathways i.e. salicylic and jasmonic acids, and ethylene. It was further reported that HeT and AsES can induce a systemic resistance (SAR) that is not restricted to strawberry plants but includes other species and can confer resistance against other pathogens.

## A10

### BIOTECHNOLOGICAL CONTRIBUTIONS TO SOYBEAN CROP IN NOA

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Considering the worldwide level of production and consumption, soybean (*Glycine max* (L) Merr) is the largest oilseed crop. Therefore, it plays a main role in the economy of Argentina and the MERCOSUR. In the last decade, advances in soybean yields came from new technologies such as direct sowing, obtainment of transgenic varieties, development of new genotypes with genetic marker-assisted breeding, and integrated management of pest and crop diseases. However, soybean production is constantly threatened by

biotic stress (diseases and pests) and abiotic stress (drought, heat, salinity). Depending on the predisposing conditions, these stresses can cause significant yield losses and serious economic damage. The aim of this study was to identify DNA fragments linked to resistance to both kind of stresses (biotic and abiotic) through the evaluation and characterization of soybean and associated pathogens genotypes. The genetic diversity of the causal agents of Asian rust and charcoal rot (*Phakopsora pachirizhy* and *Macrophomina phaseolina*, respectively) was evaluated and soybean genotypes resistant to these diseases were identified. Genomic regions associated with resistance to soybean rust and stem canker (*Diaporthe phaseolorum*) were mapped and markers that could be useful in assisting the breeding program of the EEAOC were validated. Potential markers associated with resistance to other diseases such as frog eye spot (*Cercospora sojina*) and sudden death (*Fusarium spp*) were evaluated. A procedure to assess drought tolerance was developed and transcriptomic analyses were conducted among contrasting genotypes.

#### A11

### GENOMIC AND BIOTECHNOLOGICAL TOOLS APPLIED TO SUNFLOWER BREEDING TO IMPROVE DISEASE RESISTANCE TO FUNGAL DISEASES AND MANAGEMENT OF THE SENESCENCE PROCESS

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The integration of genomics, transcriptomics, metabolomics, bioinformatics and phenomics applied to the study of complex traits allows the development of useful tools for assisted plant breeding. Fungal diseases such as sunflower head rot (SHR), caused by *Sclerotinia sclerotiorum*, water deficit and early leaf senescence constitute the main limitations to sunflower production. The objective of this work was the identification and characterization of genomic regions and candidate genes associated with these characters and the development of biomarkers for assisted breeding. Different genomic strategies including the development of medium to high throughput genotyping tools, QTL mapping on biparental populations, association mapping of genetically diverse populations, the characterization of transcriptomic and metabolomic profiles on contrasting genotypes and/or in different growing conditions or different developmental stages as well as the functional characterization of candidate genes associated with leaf senescence and fungal response were assessed. The results obtained include identification of new QTL and the validation of previously detected QTL involved in resistance to SHR in different genomic regions, identification of genes/alleles of candidate genes associated with the character detected by mapping of functional markers and also by integrated analysis of transcriptomic and metabolomic profiles. The integration of these post genomic profiles using different bioinformatics tools allowed us not only to understand the mechanisms involved in these complex traits through the identification and validation of key transcripts and metabolites at different developmental stages but also to generate useful biomarkers for applications in sunflower breeding programs.

### Symposium 3: ARGENTINE BIOLOGY SOCIETY YOUNG RESEARCHERS

#### A12

### CHAGAS: OLD CHALLENGES, NEW TECHNOLOGIES

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More than 8 million people worldwide are infected with *Trypanosoma cruzi*, the etiologic agent of Chagas disease. In Argentina it is estimated 1500 births with congenital Chagas disease annually although only ~ 300 cases are reported due to the limitations of neonatal diagnosis.

**OBJECTIVE:** To develop a simplified test for congenital Chagas diagnosis using isothermal molecular amplification (AMI) that can be useful in all neonatal care conditions.

**MATERIALS AND METHODS:** Samples of control blood and blood artificially inoculated with *T. cruzi* in a serial dilution (liquid or dried blood) were used for AMI reaction. Optimal reaction conditions were determined as well as sensitivity and specificity - comparing with *Real time PCR* (RT-PCR)- revealed by electrophoresis (analytical method) and lateral flow dipstick (LFD) chromatography (field method), and certified clinical samples were tested.

**RESULTS:** AMI reaction was optimal at 65°C-60min. The sensitivity detection was ~1parasite/sample (similar to RT-PCR), revealed both by electrophoresis and LFD in simplified tests dispensed as reaction mixes. All assays with heterologous DNA (human, yeast, *T. brucei* and *Leishmania mexicana*, etc.) were negative with AMI and RT-PCR. The first clinical samples tested showed results in agreement with certificate results.

**CONCLUSIONS:** The adaptation of AMI to simplified conditions, both in artificially inoculated samples and in clinical ones, presented extreme specificity and sensitivity. This encourages us to advance in the development of a suitable kit to be used in all neonatal care conditions.

**A13**

**WHY DO WE GENERATE NEW NEURONS IN ADULT BRAINS?**

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The addition of new neurons into developed brains is the most complex grade of neural plasticity known. In the dentate gyrus of the hippocampus, new-born granule cells (GCs) are constantly added by a process known as adult neurogenesis. After a developmental period of 6 to 8 weeks, adult-born GCs become mature and are functionally integrated into the hippocampal network. During development, GCs switch from an immature stage with elevated responsiveness and integration capability towards a mature stage with sparse activity and increased specificity. How this dual function impacts the circuit remains unclear. To study the way in which adult neurogenesis contributes to neuronal processing, we investigated the output networks activated by new neurons at different maturational stages. For this purpose, we combined in vivo retroviral labeling with optogenetics, chemogenetics, the use of transgenic mice and slice electrophysiology.

We successfully activated specific groups of adult-born GCs at different maturational stages and elucidated the synaptic targets of these neurons, their strength, and the way in which they modulate network activity. Immature (4 week-old) GCs exhibit a mature-like synaptic profile with their CA3 distal targets, whereas their synaptic contacts with dentate gyrus interneurons (proximal targets) is still immature. In this way, immature GCs seem to be banished from the dentate gyrus inhibitory feedback loops. As new-born GCs mature, synapses with local interneurons become stronger and the evoked inhibitory feedback impinges on the dentate gyrus by restricting its activity. This delayed coupling of new GCs to feedback inhibition could be crucial to achieve a fine-grain representation of novel inputs in the dentate gyrus.

**A14**

**SHARK REPRODUCTIVE ENDOCRINOLOGY: PAST, PRESENT AND FUTURE**

Awruch CA

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Sharks are an evolutionarily conserved group that has survived for over 400 million years. Their permanence has largely depended on their reproductive strategies, as the primary requirement for successful propagation of any species is the ability to reproduce. As in other vertebrates, reproduction in sharks is regulated via the hypothalamus-pituitary-gonadal (HPG) axis, where androgens and estrogens are the main steroids during gonadal growth while progestins have maturational activity. In the last years, the decline in shark populations has called for the development of demographic models that address their vulnerability. These models include the size at which an animal sexually matures and the temporal and spatial patterns in reproduction. To obtain these parameters examination of the gonads after sacrificing the shark is required. However, for protected species and bycatch species to be returned back into the water, destructive sampling is not allowed or is ethically inappropriate and should be minimised, yet there remains the need for scientific investigation. Consequently, in recent years different studies have shown that reproductive hormones have the potential to be used as a non-lethal technique to address reproduction. However, although the use of reproductive endocrinology as a non-lethal tool is becoming crucial for the future of shark species, there are still major gaps in understanding the HPG axis, and without a clear understanding on how this axis works mistakes could still be made in researchers' assumptions of the reproductive process. The aim of this review is to assess the use of shark reproductive endocrinology as a basic-applied science for sustainable management programs.

**Symposium 4: EFFECTS OF PLANT SECONDARY METABOLITES ON BIOLOGICAL SYSTEMS**

**A15**

**SESQUITERPENE LACTONES: STRUCTURES – ADVERSE AND BENEFICIAL EFFECTS**

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Among natural products, sesquiterpene lactones (LS) constitute a large group of secondary metabolites, with over 7000 compounds reported so far. They are found mainly in the Asteraceae family, and to a lesser extent in Apiaceae, Lauraceae and Magnoliaceae. They are occasionally found in fungi and liverworts. LS are believed to serve as a defense mechanism of plants against predation.

LS major groups are classified on the basis of their carbon-skeletons as germacranolides, guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides and xantanolides among others.

Interest in LS is justified by their multiple biological activities such as their antifungal, antibacterial, anti-inflammatory, phytotoxic, germination and seedling developmental effects, inhibition of insect larval development with toxicity by contact, inhibition of oviposition and, more recently, antineoplastic and cytotoxic activities, both linked to the function of the  $\alpha$ -methylene- $\gamma$ -lactone group. In addition to the lactone function, LS usually have additional functional groups such as aldehydes, ketones and epoxides, which gives

them a great alkylating reactivity on nucleophiles such as thiol groups of enzymes. This results in the inhibition of various cellular functions and in the study of the relationship between chemical structure and bioactivity, which has been evaluated in several biological systems.

However, the toxicological profile of these compounds must be thoroughly characterized since the properties that make LS useful medicines can also cause severe toxicity. LS containing plants have long been known to induce contact dermatitis in exposed farm workers as well as several toxic syndromes in farm animals

#### A16

### EFFECT OF DEHYDROLEUCODINE ON THE GROWTH OF MICE MELANOMAS

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Malignant melanoma represents the worldwide fastest growing public health risk of all cancer types. Several strategies and anti-cancer drugs have been used in an effort to improve treatment, but because of that melanoma cells have become resistant to anti-neoplastic drugs and chemotherapy failures are frequent.

In our laboratory we showed that the sesquiterpene lactone dehydroleucodine (DhL) promotes the accumulation of DNA damage markers such as  $\gamma$ H2AX and 53BP1 in human tumor cells *in vitro*. This triggers cell senescence or apoptosis in a manner that is dependent on the DhL concentration used. In this study, the effect of DhL on the proliferation of mice melanoma B16 F0 cells was assayed *in vitro* and in an experimental tumor in mice. In *in vitro* experiments, DhL arrested the proliferation of B16 FO cells by inducing senescence or apoptosis according to the DhL concentration used. Also, DhL reduced the expression of proteins that promote cell proliferation such as cyclins D1 and B1 and the protein that inhibits apoptosis, survivin 1. In a skin melanoma generated by the subcutaneous injection of isogenic B16 F0 cell into C57/BL6 mice, treatment with 20 mg/Kg DhL in preventive, neoadjuvant and therapeutic protocols reduced tumor volume by 70%, 60% and 50% respectively. DhL treatments generate a reduction in the number of tumor cells in division, and an increase in the number of cells in senescence and apoptosis. In order to estimate the effect of DhL in a longer treatment, a mathematical model was adjusted to fit experimental data. We observed that preventive and therapeutic DhL treatments were more efficient than neoadjuvant ones.

## Symposium 5: BIOLOGY AND BIOTECHNOLOGY OF ANIMAL REPRODUCTION

#### A17

### GAMETE COMMUNICATION IN MAMMALS AND ITS APPLICATION IN ASSISTED REPRODUCTION

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Capacitated human spermatozoa are chemically oriented by a picomolar concentration gradient of progesterone, a steroid secreted by the cumulus cells at the time of ovulation. This sperm guiding process is called chemotaxis. We developed an assay (SSA) to select spermatozoa at the best functional state by means of chemotaxis towards progesterone. The device consists of two wells connected by a tube, whereas capacitated spermatozoa are placed in one well and 10pM progesterone in the other. The steroid diffuses between both wells, generating a concentration gradient along the connecting tube. The efficiency of SSA was tested for normal and subfertile semen samples. Thus, the selected sperm population is significantly enriched with capacitated spermatozoa, with intact DNA and low levels of oxidative stress. The sperm capacitation state, motility and viability last for at least two hours after sperm selection. In addition, SSA stimulates capacitation in those spermatozoa that could not do it by themselves. In conclusion, SSA selects spermatozoa at the best functional state and prepares some of them for fertilization. Similar results were achieved with subfertile semen samples. The use of SSA might improve the assisted reproduction technology in humans and animals.

#### A18

### IMPROVING METHODS FOR THE PRODUCTION OF TRANSGENIC RUMINANTS

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Animal transgenesis is mainly directed to the production of animal models of human diseases or bioreactors of therapeutic proteins and, to a lesser extent, to the production of animals with improved productivity. Traditional transgenesis techniques are very inefficient and this is exacerbated in ruminants due to domestic species peculiarities. For example, the transgenesis technique most commonly used in mice, blastocysts injection with transgenic ES cells, is not available for livestock due to inefficient maintenance of embryonic stem cells in these species. For ruminants, somatic cell nuclear transfer (SCNT) is one of the most commonly used

techniques. However, in addition to the low SCNT efficiency, only few transgenic animals show the desired expression patterns. This is mainly due to the random integration of the transgene as concatemers that can lead to epigenetic silencing of the transgene. Since 2006, the animal biotechnology laboratory has been working to simplify and universalize transgenesis techniques. Making use of the *gfp* gene under an early expression promoter, we initially approached sperm-mediated gene transfer. Although it was not possible to obtain transgenic embryos by in vitro fertilization or artificial insemination, intracytoplasmic sperm injection (ICSI) resulted in transgenic embryos. However, this transgenesis was transient, without stable transgene incorporation into the genome. In recent years, we began to explore active transgenesis techniques, which are based on the use of enzymes that actively promote the integration of the transgene. The homing meganuclease I-SceI and transposon systems were used with very promising results.

#### A19

##### PRODUCTION OF TRANSGENIC CATTLE EMBRYOS

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Transposon-mediated transgenesis is a well-established tool for genome modification in small animal models. However, translation of this active transgenic method to large animals warrants further investigation. Here, the piggyBac (PB) and Sleeping Beauty (SB) transposon systems were assessed for stable gene transfer into the cattle genome. Bovine fibroblasts were transfected either with a helper-independent PB system or a binary SB system. Both transposons were highly active in bovine cells, increasing the efficiency of DNA integration up to 88 times over basal non-facilitated integrations in a colony formation assay. SB transposase catalyzed multiplex transgene integrations in fibroblast cells transfected with the helper vector and two donor vectors carrying different transgenes (fluorophore and neomycin resistance). Stably transfected fibroblasts were employed for somatic cell nuclear transfer (SCNT) and upon in vitro embryo culture, morphologically normal blastocysts that expressed the fluorophore were obtained with both transposon systems. The data suggest that transposition is a feasible approach for genetic engineering in the cattle genome.

#### A20

##### COMPARATIVE PLACENTAE: SIMILARITIES AND DIFFERENCES IN A UNIQUE ORGAN

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The placenta is the most variable organ of animals. It is a transitory and mixed organ related to nutrition, respiration, excretion, hormone production and immune regulation during pregnancy. This organ is essential for viviparity and represents an important portion of the mamotrophic nutritional condition. Among chordates, the placenta is present in some fish and reptiles and in all therian mammals. In therians, placental variations are found in the macroscopic and microscopic structure as in physiological and biochemical aspects. The placenta of eutherians has been classified following diverse criteria: villi origin, villi distribution, villi morphology, obstetrics and structure of placental barrier. The former classification is related to the lack of maternal layers. Generally the placentae with minor layers have a reduced villi distribution. Most authors think that these features were present in the former eutherian placenta and the placentae with more layers and more distribution of villi are adaptations to longer pregnancies. Carnivores have an endotheliocorial and zonal placenta with an intermediate structure that ranges from the more invasive of rodents and humans to the less invasive of the horse and pig. In dog and cat placenta we found the expression of molecular systems such as galectins, insulin like growth factors (IGF) and metalloproteases associated with the invasiveness and the immunological regulation. Other important aspects of placental biology are related to the carbohydrate expression pattern of the epithelial cells. Saccharides participate in the mechanism of adhesion and implantation. In our laboratory we found differences in the expression of carbohydrates under physiological and pathological conditions.

### ORAL COMMUNICATIONS

#### A21

##### VARIABILITY IN YIELD AND FRUIT CHARACTERS IN TOMATO “PLATENSE” ADVANCED LINES.

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The tomato “Platense”, usually identified by its flatter shape and the presence of “ribes” and for its distinctive and tasty flavor, was introduced by European immigrants in the first half of the XIX century. It became well adapted to the environment of La Plata and its surroundings with a low input management. By selection in old populations it would be possible to improve commercial traits and

thus the possibility of this tomato of being a viable option for production. The objective of this work was to evaluate variability in yield and fruit characters in tomato "Platense" advanced lines. The trial was carried out at the Chacra Experimental Gorina MAA-BA in a greenhouse and began on October 10, 2014. Seven advanced lines of tomato "Platense" (LT) and one commercial hybrid (HC) were evaluated. The experimental design was complete randomized block ( $r=4$ ). In each of 5 fruit per line they were evaluated the following characters: diameter (D), height (A), ratio D/A, weight per fruit, and n° of "ribs". We also calculated yield per plant at the end of the harvest period (April 15, 2015). Differences among treatments were analyzed by ANOVA and Tukey's test. The results showed that: LT had greater ( $p<0.01$ ) diameter, D/A, weight/fruit and number of "ribs" than HC. There were not significant differences ( $p>0.05$ ) in diameter, height, D/A, and number of "ribs" among the LT. One of them had lower ( $p<0.01$ ) weight/fruit than the others. With respect to yield per plant, there were not significant differences ( $p>0.05$ ) among the materials. After 5 years of selection, the advanced lines resulted homogenous for most of the analyzed characters. The yield per plant was similar in LT than in HC, at least in suboptimal conditions of management for commercial hybrids.

## A22

### **BURIAL DEPTH OF *Elyonurus muticus* AND *Sorghastrum pellitum* IN SANDY GRASSLAND PASTURES.**

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The poor condition of sandy grassland pasture is common in San Luis, with considerable presence of *Elyonurus muticus* (a non forage species) to the detriment of *Sorghastrum pellitum* (a summer forage species). The deep planting of forage grass tillers seems to be an adaptive strategy against herbivores. The objective of this work was to compare the burial depth of the meristematic apex in both species at sites with different grazing intensities. Plants were randomly extracted (November) from a pastureland (200 ha) located 40 km. from Villa Mercedes (SL). According to the distance of the watering point, these plants were in the near (500 m), medium (1000 m) and farthest area ( $> 1000$  m). Depth (in cm) between root emergence site and soil surface was measured. The Kruskal Wallis test was applied ( $p<0.05$ ). Whatever the distance to the watering points was, *E. muticus* presented deeper fitomers than *S. pellitum* ( $3 \pm 0.7$  vs.  $0.7 \pm 1.5-2.2$ ). In the middle sector (topographically plain) *S. pellitum* buried them deeper ( $2.2 \pm 1$ ) than *E. muticus* ( $2.9 \pm 0.8$ ). *S. pellitum* was located mainly in the hills (near and far sectors) and presented lower burial depth of meristems, regardless of the grazing intensity to which it is subjected, a condition that, coupled with animal preference, impairs its persistence.

## A23

### **CLARIFICATION OF APPLE AND ORANGE JUICES USING LACCASE**

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The trend towards sediment fruit juices during storage is a persistent problem in the industry of these products. The phenolic compounds present in these drinks are primarily responsible for this deterioration. Laccase is an enzyme widely studied due to its potential application in various areas such as the textile industry, the manufacture of paper pulp, the bioremediation of effluents and the food industry. Laccase could increase productivity, efficiency and quality of food products inexpensively and, up to now, it has the advantage of being a soft technology. In this paper we intend to evaluate the use of laccase, an enzyme that catalyzes the oxidation of phenols, to increase the stability of fruit juices. Orange and apple juices were treated with laccase at various concentrations (0-12 U/ml), with temperatures between 30 and 50 °C. and reaction times up to 6 hours. The effect of this enzyme treatment on the content of total phenols, changes in the color and clarity of the juices was evaluated using a central composite design. The determination of total phenols by the Folin-Ciocalteu method showed a decrease of 25% average for both juices. Data for color showed a slight decrease (8%) for orange juice but a slight increase (9%) in apple juice. The clarity parameter showed no significant changes for both juices. The optimal treatment was one whose conditions were: enzyme concentration: 6 U/ml (100 ul of enzyme extract), incubation time: 2 hours and temperature: 40°C. The laccase treatment is useful for processing orange and apple juices, particularly in the dephenolization process.

## A24

### **EXTRACTION OF POLYPHENOLS FROM STRAWBERRIES GROWN IN TUCUMAN**

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Polyphenols are widely distributed in plants with diverse functions such as protectors against UV radiation, antimicrobial agents, and as an element to attract pollinators. It has been proved that polyphenols have antioxidant, antimicrobial and anticancer properties. Current nutrition in developed countries is very poor in fruits and vegetables, and therefore requires the contribution of dietary supplements, including polyphenols, to supplement the diet. Therefore there is a growing demand for these products by the pharmaceutical and food industries. Tucuman province is an important producer of strawberries, and the fruit selection for packaging

results in a large amount of waste products, whose recovery could generate significant resources for farmers. The aim of this work was to evaluate the extraction of polyphenols in strawberry varieties with an ethanol/water mixture, with different ethanol concentrations and two different temperatures, in order to determine optimum processing conditions and evaluate the antioxidant activity of the extracts obtained to determine if higher temperatures could affect the quality of the extracts. Samples of different strawberry varieties were obtained from the varieties collection of the Experimental Station INTA Famaillá. Total polyphenol content and antioxidant activity were determined by ultra violet-visible spectrophotometry. Statistical analysis of variance was performed on two factors to find significant differences between extraction methods. The best extraction conditions for all varieties of strawberries were a mixture of ethanol/water at 50% and 50 ° C, reaching an average extraction efficiency of 88%.

#### A25

##### IMPROVEMENT OF SANDY GRASSLAND PASTURES IN SAN LUIS BY MECHANICAL ACTION

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The impact of mechanical action on *Elyonurus muticus* and other “sandy” grassland species was evaluated using an analysis of plant coverage and density and soil cover. Five mechanical treatments were applied: Disc harrow (DH) and chisel plows in October, RDA and Chisel plows in April and a control. The design was randomized blocks with 3 replications. One transect in each plot was set and coverage and N° of individuals / species, mulch and % coverage of bare soil (SD) was determined by the canopy coverage method of vegetation analysis of Daubenmire. To assess the delayed action of the tillages, transects were evaluated in March and November, depending on the treatment. For analysis, species were grouped into desirable, intermediate and undesirable. Kruskal Wallis mean difference test and Tukey’s or Duncan’s multiple comparison test were applied. October tillage, evaluated in March, did not cause significant changes in coverage or plant density, SD or mulch ( $p > 0.05$ ). However, changes were observed in the botanical composition (three desirable winter species disappeared, most undesirable species remained and two summer and winter intermediate species appeared). *E. muticus* coverage and number of plants were affected by DH but not with chisel plows. April tillage, evaluated in November, increased SD ( $p < 0.05$ ) and altered the botanical composition of the pasture, increasing the amount of intermediate species and the appearance of desirable ones. Although this tillage affected the coverage and plant number of *E. muticus*, it did not disappear. Mechanical action in October or April failed to improve range condition, but they caused changes in botanical composition.

#### A26

##### STUDIES OF THERMOPHILIC MICROALGAE IN THE HOT SPRINGS OF SANTA TERESITA (PROVINCE OF LA RIOJA) AND THEIR POTENTIAL APPLICATIONS IN BIOTECHNOLOGY

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Microalgae include organisms like *cyanobacteria* prokaryotes and eukaryotes such as algae, which are primary producers in aquatic environments. Some genera are used as biomass to remove pollutants in wastewater. They are producers of energy and compounds of interest in the food, pharmaceutical, and cosmetics industry. Today, the microalgae biotechnology industry has become a matter of research. The objective of this study was to identify thermophilic microalgae present in the Santa Teresita hot springs for further biotechnological study. The springs are located 200 km away from the city of Chilecito, Route 40, Arauco Department. Samples of algae were collected from ponds and temperature, pH and chemical properties were determined. They were placed in sterile flasks with formaldehyde at 40% for microscope observation and identification. They were documented in the High Complexity Lab with microphotographs using a Leica DM2500 microscope. The identification of the specimens was carried out together with the Fundación Miguel Lillo (Tucumán). Water temperature records were: 47° C, pH 6.5. *Calothrix castelli* and *Formidium tenue* were identified and other specimens are still being examined. These are the first records of microalgae in extremophile environments in the province of La Rioja and their identification will be used in future biotechnological studies.

#### A27

##### PRODUCTION OF MEMBRANE VESICLES IN *Pseudomonas putida* IN RESPONSE TO STRESS CAUSED BY CATIONIC SURFACTANTS

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*Pseudomonas putida* A (ATCC 12633) releases outer membrane vesicles (MVs) when is is grown with tetradecyl trimethyl ammonium (TTAB). Using the model of red blood cells we demonstrated that TTAB induced the crenation of the cell membrane (cells with outward membrane protrusions) by the asymmetric expansion of the outer leaflet with respect to the inner one. This effect could be antagonized by compounds such as chlorpromazine, which expands the inner leaflet of the membrane. The high

phospholipids content detected in the cells of *P. putida* grown with TTAB as sole carbon, nitrogen and energy source (30nmol per milligram proteins) led to the loss of contact between the outer membrane and the peptidoglycan. This effect, plus the results that showed that TTAB induces the curvature of the membrane, contributes to MVs biogenesis. MVs exhibit a bilayer structure, and were found to be composed of lipopolysaccharides, proteins and phospholipids, with a marked increase in phosphatidylglycerol content (53% higher than the amount registered in the cellular membrane). Taking into account that TTAB was detected inside the vesicles (710 mol of TTAB per mol of phospholipids), we conclude that this system of TTAB elimination seems to be a part of the tolerance of *P. putida* to cationic surfactants.

### A28

#### EFFECT OF THE CONSUMPTION OF FLOUR FROM FIBER-RICH REGIONAL FRUITS ON THE GASTROINTESTINAL TRACT

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Currently, the study of novel dietary fibers (DF) has acquired relevance due to their proven positive physiological effects on human intestinal health. The flour from *Ziziphus mistol* (HM) and *Geoffroea decorticans* (HCh) contain 13 and 25% of total dietary fiber, respectively. In this work we studied the effect of the DF of these flours on the intestinal tract using Wistar rats as an experimental model. We used 3 batches of 8 animals each with 166±9.6 g initial weight; they were fed *ad libitum* for 63 days, with diets containing 8% DF from different sources: HM, HCh and Cellulose (C, control diet). The animals were slaughtered at the end of the study period with an overdose of anesthesia. Cecum wall (CW) and small (SI) and large (LI) intestine were removed and weighed; SI and LI length were also measured. Cecal content (CC) was weighed and its pH measured. Rats fed with HM presented severe diarrhea and showed greater weight of CW, greater length of LI and pH significantly ( $P \leq 0.05$ ) smaller compared with HCh and C. The increase in LI surface in animals fed with HM was probably due to the nature of the DF and to the lower bowel transit time, which is why a larger surface is required to absorb the nutrients degraded in the cecum. The decrease in pH of the CC was possibly influenced by the type of fermentation involved in the degradation of non-cellulosic polysaccharides. The potential incorporation of HM and HCh in formulated foods requires a deeper study of the nature of their DF.

### A29

#### OFFSPRING QUALITY IN CONSECUTIVE SPAWNS OF THE “RED CHERRY” SHRIMP *Neocaridina davidi*

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*Neocaridina davidi* is a crustacean of ornamental importance; however, several aspects of its reproductive biology remain unknown. The objective of this study was to evaluate the effect of maternal senescence on offspring quality. Nineteen reproductive stocks were inspected once a day for the presence of ovigerous females. Immediately after hatching, juveniles were counted, a sample of 10 was taken for the determination of the initial weight and another sample of 18 was taken for a nutritional stress test. This test consisted of 6 experimental groups: CF (continuous feeding), CS (continuous starvation), S 4 (starvation during the first 4 days), S8 (starvation during the first 8 days), S12 (starvation during the first 12 days) and S16 (starvation during the first 16 days). After the corresponding starvation days, juveniles were fed until day 32. They were weighed on that day for the calculation of mass increment (%MI). This procedure was followed for the 6 consecutive spawns of each female. Juveniles from the first spawns showed the highest initial weight but the lower final weight, as reflected by %MI. This may indicate a higher vulnerability of those juveniles to starvation. Juveniles from the sixth spawns had an intermediate initial weight but reached a higher final weight, indicating a higher resistance to food deprivation. It is worth mentioning that the most extreme starvation treatments (I12, I16 and IC) were analyzed statistically only for the third to sixth spawns, because an adequate number of surviving juveniles were obtained only for those spawns. This suggests that older females produce juveniles with higher starvation resistance.

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### A30

#### EVALUATION OF RECOVERY FROM GENOTOXIC AND CYTOTOXIC DAMAGE INDUCED BY THE INGESTION OF AFLATOXIN B1 (AFB1) IN PIGS

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Mycotoxicosis represents a common problem of environmental pollution in pig farms. In previous studies we demonstrated that Aflatoxin B1 (AFB1) induced *in vivo* the formation of micronuclei (MN) and chromosomal damage in lymphocyte cells of post weaning pigs. The recovery from genotoxic and cytotoxic damage caused by the ingestion of AFB1 was evaluated. The study included 10 pigs (6 females and 4 males) divided into two groups, A: diet free AFB1 and B: fed for 48 days with 48 ppb of AFB1 and then

with an AFB1-free diet for 20 days. Both groups were evaluated cytogenetically and clinically in 5 intervals: 0, 13, 27, 48 and 67 days post ingestion. Genotoxicity and toxicity were evaluated by the cytokinesis block micronucleus (CBMN) assay. The nuclear division index (NDI) and cytokinesis block proliferation index (CBPI) were measured by scoring 500 cells per individual. Frequency of binucleated cells with micronuclei (BNMN) and Micronucleus number in cells BN were also analyzed by scoring a total of 1000 BN per individual. The values of the two indices in group A were stable throughout all intervals evaluated and showed significant differences ( $p < 0.05$ ) in the treated group, corroborating that AFB1 intake at the dose used has a marked cytostatic effect. The comparison between stages IV (48 ppb dpi) and V (20 days after removal of AFB1 from the diet) revealed a significant reduction in the average frequency of BNMN cells. Also, an increase in the two cytotoxicity indices measured was observed. In conclusion, there is a spontaneous recovery from genotoxic and cytotoxic damage once AFB1 is removed from the diet.

### A31

#### ICHTHYC BIODIVERSITY OF THE ALBIGASTA RIVER (SANTIAGO DEL ESTERO) AND POTENTIAL THREATS TO ITS CONSERVATION

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The province of Santiago del Estero has little information about fish biodiversity in its five river systems. Some researches were carried out only in the two main systems, the Dulce River and the Salado River, practically ignoring the others. The objective of the present work is to reveal the fish species diversity in the Albigasta River and map and identify potential threats to their conservation. Two sampling campaigns were conducted in February and May 2015, with studies performed at six sites along the river course. At each site, five hauls were made (2 in each margin 50m apart from each other, and one in the middle of the channel) using a manual drag net of 10m x 0.60m with a flake of 10mm of pore diameter, and a net hand with a pore diameter of 3mm. The mapping of the watershed was made with QGIS software (v. 2.6.1). The land cover was classified up to a kilometer on either side of the river and potential threats were plotted on a point chart. The fish diversity obtained was 9 genera and 10 species, of which 2 had not been previously cited for the province (*Rineloricaria catamarcensis* and *Gambusia affinis*). The material collected in this research is in the Department of Ecology, Forestry Faculty-UNSE. The construction of the dike El Bolson in the province of Catamarca, the discharge waste into the river and the cloacal discharge from the city of Frias, were identified as anthropogenic threats to the conservation of the Albigasta River's fish biodiversity. This paper presents relevant information for the knowledge and conservation of fish biodiversity in the province of Santiago del Estero.

### A32

#### EXPRESSION AND ACTIVITY OF METALLOPROTEASES MMP-2 AND MMP-9 IN EARLY CANINE PLACENTA

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Placentation requires metalloproteases (MMPs) for extracellular matrix (ECM) remodeling. While the expression of these proteases has been studied in different types of placentas, the expression and activity of MMP-2 and -9 was not investigated in early canine placentation. Placentas of each uterine horn from female dogs with 18-30 days of pregnancy were fixed in formalin or frozen for histochemistry PAS, immunohistochemistry (IHC), Western Blot (WB, average UA / actin  $\pm$  SD) and zymography (Zg). The MMP-2 was expressed in the labyrinth (L) and hematoma (H), with strong reaction in trophoblast cells. The expression level of MMP-2 (WB) of L was slightly higher than the H ( $p < 0.05$ ), whereas the expression of MMP-2 in implantative endometrium (IE) was lower ( $p < 0.05$ ). By Zg, only active MMP-2 (54 kDa) was detected in L and H, MMP-2 activity in H being greater than that of L ( $p < 0.05$ ). MMP-9 was strongly expressed in the chorioallantoic membrane and, to a lesser extent, in trophoblast and maternal tissues. By WB, the expression of MMP-9 in L and H was similar but higher than in the non-implantative endometrium ( $p < 0.001$ ). However, no MMP-9 activity (Zg) was detected in any of the areas studied. These data show differential expression and activation of MMP-2 and MMP-9 in early canine placenta. While MMP-2 is strongly expressed in trophoblast cells of the labyrinth, where it is also activated, MMP-9 is only expressed, suggesting that MMP-2 plays a key role in the process of early placentation in dog whereas MMP-9 might become activated under certain circumstances.

**A33**

**EVALUATION OF SEMEN QUALITY IN DOGS SUPPLEMENTED WITH FISH OIL AND VITAMIN E**

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Mammalian sperm contains high proportion of polyunsaturated fatty acids which are sensitive to lipid peroxidation. Dietary fish oil can manipulate fatty acid profiles of reproductive tissues. On the other hand, vitamin E (VE) protects against oxidative damage. Fish oils have omega 3 and are a major source of eicosapentaenoic acid and docosahexaenoic acid. Many studies have evaluated the effect of fish oil supplementation on reproductive parameters in domestic animals. However, there are no studies specifically evaluating semen quality in dogs supplemented with fish oil. The objective of this experiment was to evaluate the effects of dietary supplementation with fish oil and VE on semen quality in dogs. In a 3x2 Latin square, five male dogs were randomly allocated into three groups and fed a control diet (C), a control diet supplemented with 54 mg fish oil/Kg metabolic body weight (FO) or same diet and supplement plus 400 mg VE for 60 days (FOE). Semen samples were collected on days 0, 30 and 60 and their characteristics evaluated by standard methods. Data were analysed using a mixed model with repeated measures (SAS 9.0). In the FO and FOE groups the percentage of sperm motility (P=0.02), total sperm count (P<0.01), total sperm viability (P<0.01) and total sperm normal morphology (P<0.01) were higher than in the C group. The percentage of sperm viability was lower in dogs fed a FO diet than in those given a FOE or C diet (P<0.02). No changes were observed in volume or vigour as a result of diets. These results suggest that long-term fish oil supplementation plus VE may improve semen quality in dogs. However, further studies with a larger number of specimens are required.

**A34**

**2-AMINOETHOXYDIPHENYL BORATE (2-APB) TRIGGERS CORTICAL REACTION IN MOUSE OOCYTES**

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Cortical reaction is a calcium dependent exocytosis that plays a key role in blocking polyspermy. It consists in the fusion of cortical granules with plasma membrane during oocyte activation, which is triggered by fertilization or parthenogenetic activation. 2-APB is an inhibitor of IP<sub>3</sub> receptors that prevents secretion in some exocytotic models; nevertheless, it induces a calcium pulse and activates oocytes parthenogenetically. Based on this, we hypothesized that 2-APB is a new parthenogenetic inducer of cortical reaction. To evaluate the role of 2-APB in this secretory process, we used a method developed in our laboratory that allows us to analyze cortical reaction in real time. Matured oocytes obtained from hormonally stimulated CF-1 female mice were collected from ampulla and incubated with or without 2-APB in MEM/HEPES medium in the presence of *Lens culinaris* agglutinin conjugated with FITC (LCA-FITC). LCA-FITC has affinity for the content of cortical granules and fluorescently labels its secretion. Images were taken every 30 s for 20 min using an inverted microscope with LED illumination and a high resolution camera. Then, cells were fixed and non secreted cortical granules were labeled with LCA-Rhodamine for quantification. The results showed that cortical reaction occurred during the first 5-8min only in those oocytes incubated in the presence of 200 μM 2-APB. Cortical granules quantification using LCA-Rhodamine showed a significant decrease in the density of the remaining cortical granules. In conclusion, 2-APB triggers cortical granules exocytosis during cortical reaction in mouse oocytes.

**A35**

**PARTICIPATION OF UROKINASE TYPE PLASMINOGEN ACTIVATOR (uPA) DURING *IN VITRO* MATURATION OF BOVINE OOCYTES**

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The study of molecules involved in oocyte maturation and early embryo development is crucial to improve the conditions of *in vitro* embryo production. The aim of this study was to analyze if the addition of uPA or an inhibitor of the proteolytic activity of uPA (amiloride) could influence *in vitro* (IVM) bovine oocyte maturation and its subsequent developmental competence. Immature oocytes were obtained by aspiration of ovarian follicles of recently slaughtered heifers. Selected oocytes were *in vitro* matured in 4 different groups: control, 10 nM uPA, DMSO control, and 100 μg/ml amiloride. Nuclear and cytoplasmic maturation of a representative number of oocytes from each group was evaluated. The remaining oocytes were fertilized (D0) and *in vitro* cultured to evaluate cleavage rate (D1) and blastocyst yield (D7-9). In the presence of amiloride, a significant reduction in oocyte maturation was observed at both levels. However, the uPA group was similar to control. Moreover, the amiloride group showed cleavage rate and blastocysts yield significantly lower compared to controls and uPA groups. In conclusion, although the addition of exogenous uPA does not alter oocyte maturation and subsequent embryo development, amiloride, as an inhibitor of the proteolytic activity of uPA present in cumulus-oocyte complexes, reduces IVM of bovine oocytes and therefore fertilization and early embryo development.

**A36**

**STEROID PRODUCTION AND MATURATION IN *Rhinella arenarum* OOCYTE**

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In amphibians it has been suggested that progesterone ( $P_4$ ) synthesized by the follicle in response to gonadotropins is responsible for oocyte maturation. But testosterone (T) also appears to play a leading role in this process. The aim of this work is to study the seasonal variation of two steroids ( $P_4$  and T) released *in vitro* by *R.arenarum* follicles in basal and gonadotropic stimulation conditions. Adult female *R.arenarum* were collected during the non-reproductive period or PNR (March to August) and reproductive period or PR (February to September). Randomized samples of 20 ovarian follicles were incubated for 2 to 12h in 1 mL of Ringer (RA) or RA + hCG.  $P_4$  and T levels were determined by ECLIA in the incubation medium. Meiosis resumption (RVG) was controlled at 20h. The results indicate that the basal and annual secretion of T (PNR =  $0.95 \pm 0.71$ nM; PR =  $1.07 \pm 0.74$  nM) was significantly greater than that of  $P_4$  (PNR =  $0.38 \pm 0.62$ nM; PR =  $0.17 \pm 0.13$  nM). Under stimulation, in PNR T secretion ( $8.05 \pm 6.45$ nM) was 5 times greater than  $P_4$  ( $1.44 \pm 1.36$  nM). In the PR no difference between  $P_4$  levels ( $4.35 \pm 2.19$  nM) and T ( $4.96 \pm 2.38$  nM) was found, with a remarkable increase in  $P_4$  (25 times) and T (5 times) compared to basal secretion. Regarding the dynamics of basal secretion,  $P_4$  levels were unchanged during 12 h of incubation in both periods. T levels tripled between 6 and 12h in the PR (RVG 0%). HCG induced a secretion peak of  $P_4$  in the PR (RVG 100%) between 6 and 9h and of T in the PNR (RVG 73%). We show for the first time the seasonal variation of the follicular secretion *in vitro* of  $P_4$  and T and its effect on *R.arenarum* oocyte maturation.

**A37**

**EFFECTS OF ESTROGEN ON MITOCHONDRIAL FUNCTIONALITY IN AN EXPERIMENTAL PITUITARY TUMOR**

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Pituitary cell senescence process signs during tumor development have been described in our laboratory. Considering that both oxidative stress and mitochondrial dysfunction favor senescence and that mitochondria are a target of estrogen effects, our aim was to evaluate the action of this hormone on mitochondrial function during the evolution of experimentally induced pituitary tumors. Adult male Wistar rats were estrogenized with subcutaneous estradiol benzoate capsules for 10, 20, 40 and 60 days (empty capsules were used in controls). We analyzed mitochondrial number and morphology at the electron microscopy level, formation of reactive oxygen species (ROS) by DCFH-DA probe and the mitochondrial membrane potential (JC-1 probe). Statistical analyses: ANOVA and MLGM ( $p < 0.05$ ). After 10 days of estrogenization an increase in the size and number of mitochondria was detected, which displayed a rounded and less elongated morphology, with evidence of rupture of their internal structure, characteristic of dysfunctional mitochondria. In addition, at 20 days of treatment an increment in ROS levels compared to control cells was exhibited. In contrast, at 40 and 60 days of estrogenization a decrease in membrane polarity was observed. Taking together, these results suggest that during pituitary tumor development, estrogen affects mitochondrial function, triggering cellular senescence in order to protect the gland from uncontrolled growth.

**A38**

**SLEEPING PROBLEMS IN DIABETIC PATIENTS**

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Sleeping problems correlate with a poor quality of life due to physiological and metabolic adverse consequences. Diabetic patients are susceptible to sleeping problems due to risk factors such as advanced age, obesity and specific complications such as neuropathy and nocturia. Furthermore, the biological clock (through chronotypes) affects sleep cycles. Currently there are no reports on the prevalence of these factors, so that their evaluation would help determine whether they pose a public health problem. The aim of this research work is to describe the prevalence of sleeping problems from the perspective of the diabetic patient. A total of 506 type 2 diabetic patients, 154 men (33-90 years) and 352 women (30-93 years) were surveyed to determine age, chronotype (Horne-Östberg questionnaire), amount of daily rest and sleeping problems. 38.34% of the patients reported sleeping problems, mentioning as their origin insomnia, emotional problems and, to a lesser degree, diabetic complications. Sleeping problems were more prevalent among women (41.47%) than men (31.17%). The Evening chronotype reported having sleeping problems in 65.38% of cases. The predominant chronotype for patients with sleeping problems was the Intermediate one for women (61.64%) and the Morning Type for men (58.33%). The results suggest that a high proportion of patients suffer from sleeping problems and that there could be a sex-chronotype association. Potentially, diabetes and its complications would affect sleep; which could favor the metabolic deterioration of patients. Preventively, it is recommended to consider sleep disorders to improve their quality of life.

A39

**RETROSPECTIVE STUDY OF PATHOLOGICAL LESIONS IN PATIENTS ABOVE 14 YEARS OF AGE. EMERGENCY SERVICES. ANGEL C. PADILLA HOSPITAL. SAN MIGUEL DE TUCUMAN. SIPROSA. 2013-2015**

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The epidemiology of diseases in the oral cavity has failed to receive proper attention in the planning of oral health services. There are few references about the types and incidence of disease in oral mucosa in the population. The aim of this paper was to determine and describe the various oral pathologies that affect people over 14 who attended the Emergency Service of the Angel C. Padilla Hospital in the province of Tucumán in 2013-2015, MATERIALS AND METHODS. Information was collected from clinical records, medical exams, and histopathology of the cases where necessary, determining most common injuries, age group, gender and location in oral cavity. Out of a total of 350 patients over a period of two years, the most common injury was traumatic fibroma (20%), followed by mucocele, (17%) and malignant neoplasia (15%) Women were more numerous (54.66%) than men (45.34%). The most affected age group was between 47-57 years (19%) and the most common locations: lower lip (37%), gum (17%) and tongue (17%). These results are valuable because they register oral manifestations in patients attending the hospital emergency services that will allow the implementation of better measures and more effective treatments to improve the quality of life of the population. KEYWORDS: Injury; Stomatology.

A40

***Smallanthus sonchifolius*: BENEFICIAL EFFECTS OF GINGIVITIS ON RODENTS**

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Gingivitis is an inflammatory disease caused by a microbe-immune response that affects tooth-supporting tissues. The purpose of the study was to evaluate the beneficial effects of *Smallanthus sonchifolius* (yacón) roots, rich in fructooligosaccharides, in the periodontal tissues in high-fructose-diet-fed (HFD) rats. Female Wistar rats were separated into two different groups and received either a standard diet or the HFD for 12 weeks. After that, HFD rats were assigned according to the treatment to standard, HFD and HFD+yacón flour (340mgFOS/kg) groups for 8 weeks. Clinical, biochemical and histopathological parameters were evaluated. The results were statistically analyzed (T-test and ANOVA) with a value of  $p < 0.05$ . The histological investigation of the marginal gingiva HFD-fed animals showed diffuse and perivascular focal inflammation of the lamina propria, neo-angiogenesis, and hyperplasia of the squamous epithelium by way of network plugs. A significant increase was also observed in the number of intraepithelial lymphocytes. In the interdental papilla, edema, neo-angiogenesis surrounded by a chronic inflammatory infiltrate was evident. The HFD+yacón flour supplemented group exhibited significantly reduced inflammation indicators. Yacón supplementation improved metabolic parameters and significantly reduced the histopathological signs of marginal gingivitis. In conclusion, yacón flour consumption prevents the development of chronic marginal gingivitis and improves the inflammatory condition of periodontal tissues.

A41

**Entamoeba gingivalis AND Trichomonas tenax IN PERIODONTITIS PATIENTS AND THEIR RELATIONSHIP WITH SUBGINGIVAL MICROBIOTA**

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*Entamoeba gingivalis* and *Trichomonas tenax* can be found in suppurative gingival tissues due to their preference for anaerobic environments. Taking into account that oral infections are the result of complex interactions among microbiota components, we thought it was important to study the relationship between protozoa presence and periodontal pathogens counts in subgingival plaque samples. Fifty-three periodontal patients were clinically examined and subgingival plaque samples were taken with paper points. Gram stain and dark field microscopic examination of the samples were done before inoculating selective culture media to isolate and count periodontal microorganism colonies. Data were analyzed with chi square and Kruskal-Wallis tests. There were no significant differences among protozoa presence and periodontal diagnosis, sex and age. There was significant difference related to diabetes patients. There were also significant differences, with higher counts of *A. actinomycetemcomitans*, *P. intermedia*, *F. nucleatum* and spirochetes ( $p < 0.05$ ). Also, protozoa presence was associated with lower counts of *P. gingivalis* ( $p < 0.05$ ) and *T. forsythia*, but in the latter case without significant differences. *C. albicans* and enterobacteriae counts fell drastically with the presence of protozoa ( $p < 0.05$ ). We also observed significant differences with leucocytes and erythrocytes counts in periodontal samples ( $p < 0.05$ ). We can conclude that these protozoa have a significant influence on the microbial composition of periodontal pockets, so that they would play a role in periodontitis pathogeny when they are present in periodontal pockets.

POSTER PRESENTATIONS

A42

**CADMIUM INDUCES CHANGES IN BUTYRYLCHOLINESTERASE ACTIVITY ASSOCIATED WITH LIPID METABOLISM IN RAT LIVER**

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Butyrylcholinesterase (BChE) is a serine hydrolase synthesized in the liver. There are reports that indicate its participation in both detoxification and metabolism of lipids. Studies in our laboratory showed steatosis in rats treated with cadmium ( $\text{Cd}^{2+}$ ). Until now, a possible role of BChE in response to xenobiotic intoxication had not been investigated. The aim of this work is to show that  $\text{Cd}^{2+}$  administered to rats induces changes in the activity of BChE. Male Wistar rats were treated with 10 mg  $\text{CdCl}_2/\text{kg}$  administered orally for 3 months while distilled water was used in the controls. Each month the liver was dissected and sectioned to quantify  $\text{Cd}^{2+}$  by atomic absorption spectrophotometry and prepare a homogenate. BChE activity was determined by the kinetic method. The results showed that the content of  $\text{Cd}^{2+}$  in liver of animals treated was significantly higher than in the control. The liver homogenate showed a decrease in BChE activities in all periods studied. Structural analysis showed this protein at the C-terminal domain PRAD (Proline Rich Attachment Domain) interacting with a proline-rich peptide and an anchor region to plasmatic membrane. These results show the relationship between lipid metabolism and liver BChE activity in response to treatment. Low levels of BChE reduce the hydrolysis of acetylcholine, which has trophic effects on liver precursor cells, restoring biomass. Therefore, BChE activity could be used as a marker of  $\text{Cd}^{2+}$  intoxication.

A43

**CADMIUM-CALCIUM INTERACTION IN *Rhinella arenarum* OVIDUCTS**

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Cadmium ( $\text{Cd}^{2+}$ ) is used to manufacture mass consumption products that with a finite useful life that, if they are not recycled in a safe manner, will contribute to the global problem of toxic waste. In organisms,  $\text{Cd}^{2+}$  is dangerous because of its bioaccumulation. Previous studies showed that calcium ( $\text{Ca}^{2+}$ ) contained in the jelly envelopes surrounding the *Rhinella arenarum* oocyte induces the acrosome reaction of homologous sperm. The epithelial (ESC) and glandular secretory cells (GSC) of the oviductal pars convoluta (PC) are involved in the secretion of this cation. Taking into account that the toxicity of  $\text{Cd}^{2+}$  could be due to its interference with  $\text{Ca}^{2+}$  homeostasis, the aim of this work is to study the effect of  $\text{Cd}^{2+}$  on the distribution of  $\text{Ca}^{2+}$  in the oviductal PC. Female specimens were injected into the dorsal lymph sac for 15 days with  $\text{CdCl}_2$  2.5 mg/kg and Ringer solution for the controls. After treatment, oviducts were sectorized in pars preconvoluta (PPC) and pars convoluta (pc). For  $\text{Ca}^{2+}$  analysis we used the  $\text{K}^+$  pyroantimoniate technique. In control animals  $\text{Ca}^{2+}$  deposits were found in greater proportion in the secretory granules (SG) but not in the cytoplasm of ESC or GSC. It was also noted as much in the PPC on the PC. In intoxicated animals it showed a marked decrease in deposits of  $\text{Ca}^{2+}$  in the GS and a marked increase in the cytoplasm of CSE and CSG in the areas analyzed. The results show that  $\text{Cd}^{2+}$  causes an interference with  $\text{Ca}^{2+}$  sequestered in the GS of the ESC and GSC, increasing cytoplasmic  $\text{Ca}^{2+}$ , which will lead to alterations in the metabolism.

A44

**STUDY OF THE ACROSOME REACTION IN AMPHIBIAN SPERM**

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The aim of the present work is to analyze the optimal environmental conditions for the acrosome reaction (AR) in *Leptodactylus chaquensis* sperm. Suspensions of  $1 \cdot 10^6$  sperm / mL were incubated for 120 min at 25 °C and samples, for scoring gametes at different stages of AR (F1 - F5), were taken every 20 min. The following experimental parameters were considered in the incubation medium: 1- osmolarity (mother Ringer (100 mM) or 10% Ringer (10%R)) 2- different calcium concentrations (1 . 10 mM  $\text{CaCl}_2$ ) 3- 2 mM  $\text{CaCl}_2$  + 4 mM EDTA (calcium chelator). Our results showed that in mother Ringer (hypertonic medium), 100% of the gametes remained unreacted (F1) throughout the incubation time. In 10% R (hypotonic medium) and at 40 min incubation, all gametes exhibited different stages of AR, completing this reaction (F5) 29% of sperm at 120 min of incubation. At this time, 59% of the gametes incubated with 1 mM  $\text{CaCl}_2$ , reached F5, while at mM 2 and 4 concentrations this percentage was 70% and 66% respectively. 100% of the sperm incubated with the three doses mentioned above showed reaction (F2 – F5) at 40 min, while with doses of 6 and 10 mM  $\text{CaCl}_2$ , this percentage was reached at 60 min. In the presence of EDTA in the incubation medium sperm were arrested at early stages of AR after 20 min of incubation. These results suggest that: 1- hyposmolarity of the incubation medium has a direct effect,

inducing spontaneous RA; 2- calcium has an inductive effect, 2 mM CaCl<sub>2</sub> being the most effective concentration; 3- EDTA inhibits the inducer effect of calcium.

#### A45

### EFFECT OF NERVE STIMULATION ON AMPHIBIAN OOCYTE MATURATION

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Previous work from our research group demonstrated for the first time that the amphibian reproductive system is innervated by the autonomic nervous system. The aim of this work was to determine the effect of nerve stimulation on *Rhinella arenarum* oocyte maturation. Ovaries of the same animal were used as control and experimental material. Electrical stimulation of 7 mA current, 10 Hz frequency and duration of 30 seconds were applied at the mesentery level, the site where the nerves that innervate the organ are located.

In animals captured during the breeding period, the results showed that nerve stimulation induces an increase of 82% in the circulating levels of progesterone (P) determined by the ECLIA method, while values remain unchanged and are practically undetectable in animals of the post-reproductive period. The effect of P in vitro as a physiological nuclear maturation inducer was analyzed in follicles isolated before and after ovarian nerve stimulation. The results show that P at subliminal doses produced a significant increase in the percentage of germinal vesicle breakdown as indicator of nuclear maturation only in the ovaries of females captured in the breeding period after nerve stimulation. Oocyte maturation was also observed in follicle controls incubated without P after electrical stimulation.

The presence of tyrosine hydroxylase and neuropeptide Y revealed by immuno-histochemical techniques indicate that the gonad is innervated by sympathetic fibers of the autonomic nervous system. The results suggest that nerve stimulation induced oocyte nuclear maturation through an increase in P secretion and only during the reproductive period.

#### A46

### MORPHOLOGICAL ALTERATIONS IN *Chinchilla lanigera* SPERMATOZOA WITH ZERANOL TREATMENT

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Mature spermatozoa are divided into four different zones: head, middle piece, main piece and distal piece. The morphology of these areas is relevant to study factors that influence fertility, given the fact that alterations in these segments and their proportions have an impact on successful reproduction. *Chinchilla lanigera* is a South American histricomorph rodent, commercially bred because of its fur. In the last few years, the use of zeranol in chinchilla farms became popular to synchronize fur maturation. Zeranol is a semi-synthetic product with estrogen action, its composition corresponding to a resorcylic acid lactone. Since this is an anabolic compound, we decided to determine if its administration produces morphological alterations in sperm and of which type. The sperm for analysis were obtained by puncture of the epididymis tail of zeranol treated and non treated animals. Sperm samples were processed and colored with Coomassie Blue. Treated animals showed 25% of morphological alterations, while animals without treatment showed only 6%. 70% of the anomalies are located in the middle piece, a loop shape torsion being typical. The distal piece represents 28% of the abnormalities, with rolling of this segment, and 2% in head. These results show that zeranol administration produces important morphological anomalies at the flagellar level, which suggests an effect mainly at male gamete motility, which results in a negative impact on the fertility of the specimens.

#### A47

### EPIDIDYMAL SPERM CAPACITATION OF *Chinchilla lanigera*: EFFECT OF OVIDUCTAL MEDIUM OBTAINED UNDER DIFFERENT CONDITIONS.

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Capacitation, which occurs in the female reproductive tract by interaction with female fluids, comprises a sequence of processes that allow sperm to fertilize the egg. Capacitation and acrosome exocytosis are key events that determine the fertilizing capacity of sperm. Knowledge of the mechanisms involved in the process is still partial and in the case of *C. lanigera* it is nonexistent. The aim of this study is to evaluate sperm capacitation obtained after sperm incubation in oviductal media obtained at different temperatures and in different culture media in a time dependent way. Sperm were obtained by puncturing the caudal region of the epididymis. Sperm and oviducts were isolated from recently slaughtered adult animals. Oviductal media were obtained by incubation of the oviducts in sterile PBS and DMEM / F12 medium (Ham) Gibco® supplemented with L-glutamine and 15 mM HEPES for 24 h in gassed stove (37 °C, 5% CO<sub>2</sub> and 100% Humidity) and at 4 °C. Assessment of capacitation was taken into account capacity to make acrosome reaction

(AR) by progesterone induction. Absence of the acrosome was considered a positive response in preparations stained with Coomassie Blue while its presence was considered negative. Highest AR percentage was observed in sperm with oviductal medium both in sterile PBS and DMEM / F12 at 4 °C. Proteins released by the oviduct are able to induce capacitation in *C. lanigera* epididymal sperm, culture temperature being a determining factor.

#### A48

### OBESITY, GHRELIN AND REPRODUCTIVE SUCCESS IN WOMEN UNDER ASSISTED REPRODUCTIVE TECHNOLOGIES.

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Female obesity has been associated with subfertility and, in some cases, with modifications in serum ghrelin (Ghr) concentrations. A recent clinical study reported a negative correlation between Ghr and embryo viability or cleavage.

In this study, we aimed at investigating the possible association between Ghr, body mass index (BMI), other reproductive hormones and treatment success in women with indication of ICSI (intracytoplasmic sperm injection).

We evaluated 75 women (from July 2014 to March 2015) that attended our fertility clinic (CIGOR). They were younger than 40, with no polycystic ovarian syndrome, and their partners did not show evidences of severe male factor infertility. Thirty patients were normal, 29 were overweight (OW) and 16 were obese (OB). Patients' age or years of sterility did not vary between groups, and were  $34.5 \pm 0.4$  and  $3.6 \pm 0.3$  respectively. Serum Ghr was significantly higher in OB than in OW ( $2559.7 \pm 297.9$  vs  $1695.3 \pm 182.4$  pg/ml,  $p < 0.05$ ). With linear regression analysis, we found a negative association between BMI and basal concentrations of FSH (Coeff.: -0.11), LH (Coeff.: -0.12) or estradiol (Coeff.: -1.78) and a positive association between BMI and insulin levels (Coeff.: 0.68) or days of hormonal stimulation necessary to obtain an adequate ovarian response (Coeff.: 0.09). We did not find differences between groups in the number of oocytes retrieved, their maturity, fertilization index, embryo quality and cleavage or pregnancy percentages. In women under ICSI, BMI seems to be associated with reproductive hormonal alterations, but not with subfertility.

#### A49

### OVIDUCTAL MUCOSA HISTOCHEMISTRY OF *Leptodactylus latinasus* (ANURA, AMPHIBIA).

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*Leptodactylus latinasus* lays its eggs on the ground in mud chambers built by the male, where the first larval stages develop and complete development with the first rains. The objective of this work is to study histochemically the contents synthesized by mucosal cells in different areas of the oviduct. The samples fixed in buffered formalin at pH 7.0 were stained with specific techniques for glycoconjugates (GAG): HE; AB at pH 2.5 and 0.5, combining with PAS and TB at pH 3.2, for identification of different biomolecules. In the mucous cells of the Pars Recta and Intermediate Proximal Zone there is predominance of GAG with acid residues. They are significant products synthesized in the Pars Preconvoluta (PPC) and Pars Convoluta (PC). In the PPC glands synthesize highly polymerized mucoproteins, triggering intensive glandular protrusion into the lumen with the thickening of the epithelial lining, relaxed interfolds and apoptotic bodies. The apical sector glands are released by holocrinia. In the last sections of the PPC and PC, the intraglandular biomolecules arrange themselves into fibrils by the participation of the cell cytoskeleton. In the PC, the mucous epithelial lining cells are metachromatic, contributing sulfate groups to the proteoglycans. The oviduct of this species has not been studied and we postulate that the information provided would be related to the ovoposition mode. In conclusion, each area of the oviduct with its peculiarities supports the hypothesis of the species-specific character of the oviductal content of *Leptodactylus latinasus*.

#### A50

### WHITE BLOOD CELLS AND HEMATOCRIT IN CANINES TREATED AT THE VETERINARY SCHOOL HOSPITAL OF FAZ

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Blood tests are an essential and complementary part of diagnostic evaluation, nutritional assistance and drug administration and even of the development of a large number of other studies. The determination of hematology reference ranges is necessary to correctly interpret the results and obtain a valid conclusion. This study aims at determining hematocrit and white blood cells in adult dogs in the city of San Miguel de Tucumán. This descriptive cross-sectional study was made in 114 adult dogs of 18 different breeds, all patients treated at the Veterinary School Hospital of FAZ.

The proportion of red blood cells was made by microhematocrit determination and white blood cell count was estimated with a Neubauer chamber. The results showed: hematocrit values between 35-48% for apparently normal canines; in animal with clinical symptoms of dehydration between 49-62%; in clinical manifestations of anemia, different values between 7-34%; in apparently normal canines, white blood cell values between 6000-15000/mm<sup>3</sup>, leukocytosis between 15000-27000/mm<sup>3</sup>, and leukopenia between 3000-6000/mm<sup>3</sup>. While the results indicate different values from the ones reported in the literature, we think that the number of animals should be increased to establish reference values, a project to be developed in the future.

### A51

#### EFFECT OF FISH OIL SUPPLEMENTATION ON HEMATOLOGICAL PARAMETERES IN DOGS UNDERGOING TRAINING

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Regular aerobic training and fish oil intake induce changes in blood circulation, improving oxygen transport to tissues. There are no reports on this topic regarding canines. The aim of this work was to assess the effect of fish oil supplementation on hematological parameters in dogs undergoing training. Twelve male dogs from 2 to 6 years old and from 21 to 35 kg were randomly assigned to control (n=5) or fish oil groups (FO, n=7), where they received 54 mg fish oil/kg of body weight<sup>0.75</sup> per day. Dogs were trained on a treadmill twice a week for 12 weeks. Each session was 30 minutes long, at 8 km/h speed and a 7.5% slope. Blood samples for assessing red blood cells (RBC), white blood cells (WBC), platelets (PLT), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were collected at rest at weeks -1 and 13. The data were analyzed using SAS 9.0. Both groups showed a non-significant increase in total RBC (P=0.08). In FO, total PLT and MCV decreased (P≤0.05), with a non-significant increase in Hb (P=0.06). There was no impact on WBC, Ht, MCH or MCHC. The increase in total RBC could show a trend toward increased erythropoiesis as an adaptive response to training. It would be interesting to increase the number of animals in order to assess whether the changes identified in PLT, MCV and Hb could be associated with the effect of fish oil on blood cells, favoring circulation and oxygen transport in dogs undergoing training.

### A52

#### TESTOSTERONE INCREASES CELL PROLIFERATION IN PITUITARY PARS DISTALIS OF VISCACHA

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The proliferative activity of the adenohypophysis has been related to circadian variations and to the effects of sex steroids. The aim of this work was to study cell proliferation in pituitary pars distalis (PD) of adult male viscachas during the reproductive period and gonadal regression period. Four viscachas were captured during the reproductive period (summer and early autumn) and four during the gonadal regression period (winter). The proliferating cell nuclear antigen (PCNA) and androgen receptor (AR) were located and quantified by immunohistochemistry and image analysis. Testosterone serum levels were determined by chemiluminescence enzyme immunoassay. The percentages of PCNA-ir (immunoreactive) cells and AR-ir cells were significantly higher during the reproductive period (PCNA-ir: 8.68 ± 1.18% and AR-ir: 8.05 ± 0.82%) than those found during the gonadal regression period (PCNA-ir: 2.42 ± 0.26% and AR-ir: 2.34 ± 0.34%). Testosterone levels were significantly higher during the reproductive period (4.24 ± 0.47 ng/mL) than the ones during the gonadal regression period (1.27 ± 0.10 ng/mL). These results demonstrate that in PD of adult male viscachas cell proliferation is directly related to testosterone serum levels and to the expression of their receptors during the periods of maximal and minimal gonadal activity. Testosterone serum levels through their specific receptors might stimulate cell proliferation during the reproductive period, which is when glandular activity is increased in the pituitary PD of viscacha.

### A53

#### SEASONAL VARIATIONS OF IMMUNOHISTOCHEMICAL EXPRESSION OF S-100 PROTEIN IN INTERSTITIAL CELLS OF VISCACHA PINEAL GLAND

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The presence of S-100 protein, a glial marker, was reported in the pineal gland of numerous mammals. Viscacha is a native rodent which exhibits seasonal adaptations and a reproductive cycle synchronized by the environmental photoperiod. The aim of this work was to study the seasonal expression of S-100 protein in the pineal gland of adult male viscachas by immunohistochemistry. The glands (n=4 for season) were processed for light microscopy, the polyclonal anti S-100 was used and positive (rat cerebellum) and negative (omission of primary antibody) controls were performed. The percentage of immunopositive area (% IA) was quantified by a computer- image assisted analysis system. The interstitial cells were irregular, oval or stellate-like in shape, with long cytoplasmic processes that form networks extending towards pinealocytes or blood vessels. Immunostaining for the S-100 protein was both nuclear

and cytoplasmic, only nuclear or only cytoplasmic and exclusive for the interstitial cells. The values of % IA in the autumn were  $2.40 \pm 0.53$ , in the winter  $3.40 \pm 0.49$ , in the spring  $2.32 \pm 0.76$  and in the summer  $1.47 \pm 0.03$ . In the winter, % IA was significantly higher than in the summer ( $p < 0.05$ ). Seasonal variations in the expression of S-100 protein suggest biochemical changes in the interstitial cells probably related to the photoperiod dependent activity of pinealocytes. In the pineal gland of viscacha, interstitial cells are involved in glandular histophysiology through paracrine regulation, besides being sustentacular cells as described for other species.

#### A54

### INVOLVEMENT OF SUPERIOR OVARIAN NERVE IN SPLEEN MACROPHAGES FUNCTIONALITY IN RATS WITH POLYCYSTIC OVARY.

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It has been established that ovarian function is regulated by neural and immune influences in both normal and pathological conditions. An increase in the activity of the sympathetic nervous system is linked to the etiology of the polycystic ovary syndrome (PCO). We have shown that the ovarian steroidogenic response is regulated by macrophage secretions ( $M\Phi$ ) from rat spleen through a neuroimmunoendocrine connection at the peripheral level. The aim of this work was to study the effect of the superior ovarian nerve (SON) section on PCO  $M\Phi$  gene expression of sympathetic activation marker (nerve growth factor-NGF) and immune mediators (nitric oxide-NO and tumor necrosis factor alpha-TNF $\alpha$ ) and to determine if this is related to the steroidogenic ability of  $M\Phi$  secretions on the ovary. PCO condition was induced at 60 days of age by *i.m* injection of estradiol valerate, 2 mg/rat (PCO group). In a lot of PCO rats, the SON section was performed 7 days before sacrifice (PCO-SON group).  $M\Phi$  ( $1 \times 10^6$  cells) were cultured for 24 h in RPMI medium, and TNF $\alpha$  (by ELISA) and NO (by Griess reaction) were quantified on cell culture supernatants. Those  $M\Phi$  culture media were used to stimulate PCO and PCO-SON ovaries for 3 h, and androstenedione (A2) release was measured by RIA. TNF $\alpha$  and NGF gene expression in  $M\Phi$  cells were analyzed by RT-PCR. PCO  $M\Phi$  secretions: in PCO-SON  $M\Phi$  secretions, NO and TNF $\alpha$  levels decreased ( $p < 0.05$ ), and these on the PCO ovary reduced the release of A2 ( $p < 0.01$ ). The PCO-SON  $M\Phi$  showed a decrease in NGF and TNF $\alpha$  expression in relation to PCO  $M\Phi$  ( $p < 0.01$ ). The results indicate that the section of the SON, through its effect on  $M\Phi$ , could exert a protective effect against the deleterious consequences of PCO.

#### A55

### PROLACTIN RESPONSE IS MODIFIED BY MACROPHAGE SECRETIONS OF SPLEEN IN RATS WITH POLYCYSTIC OVARY.

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Polycystic ovary syndrome (PCO) is one of the most common female endocrine disorders and a leading cause of infertility. Prolactin (PRL) is a versatile hormone/cytokine secreted in the anterior pituitary gland (AP). Besides the known actions of PRL on reproduction, it participates in the immune system regulation. We have reported a functional relation between the ovary and immune cells in PCO rats, where macrophage secretions ( $M\phi$ -S) regulate ovarian steroidogenesis. Now, we studied whether the  $M\phi$ -S from rat spleen affected PRL release and mRNA expression of PRL receptor (PRL-R) in AP of PCO and Control (C) rats. PCO condition was induced by a single injection of estradiol valerate, 2 mg/rat. After 2 months, rats were sacrificed. Spleen  $M\phi$  from PCO rats were cultured ( $1 \times 10^6$  cells) for 24 h in RPMI medium. Their secretions were used to stimulate PCO and C AP for 3 h in a metabolic bath. PRL release was measured by RIA and gene expression of PRL-R by RT-PCR. AP PRL was detected by Immunohistochemistry. AP from PCO rats showed alterations in acidophil cells (small nuclei and condensed chromatin) and fewer PRL immunoreactive cells in relation to AP from C rats. In basal conditions, lower PRL release ( $p < 0.01$ ) and PRL-R mRNA levels ( $p < 0.05$ ) in the PCO than C AH were observed. PCO  $M\phi$ -S increased PRL release ( $p < 0.01$ ), PRL-R mRNA levels ( $p < 0.05$ ) and R-PRL immunoreactive cells in PCO AH in relation to basal values. The results suggest that PCO  $M\phi$ -S regulate AP PRL release and PRL receptor. A complex interplay among PRL and other mediators of inflammation could be involved in the pathology of SOP.

#### A56

### REGULATION OF STEROID SYNTHESIS IN THE OVARY OF *Rhinella arenarum*

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Ovarian steroid production is essential for many biological processes, including reproduction. In amphibians, ovarian steroids play an essential role in mediating gonadotropin stimulation of oocyte maturation.

This paper analyzes the role of ovarian steroidogenesis during *in vitro* maturation of *Rhinella arenarum* oocytes. *R. arenarum* mature females were used. Time-response curves (6-12 h) were performed with full follicles in the presence of steroidogenesis inducing drugs (hCG, cAMP and Forskolin = Forsk), inhibitors of the ovarian steroidogenic P450s (Ketoconazole and Anastrozole) or steroidogenic precursors (Progesterone= P<sub>4</sub>, Cholesterol= Col). In experiments with ketoconazole and Anastrozole, follicles were incubated with hCG. Meiosis resumption was scored by germinal vesicle breakdown (GVBD). The results indicate that P<sub>4</sub>, hCG and Col induce follicles GVBD in a time-dependent manner. P<sub>4</sub> and hCG were the most efficient inducers (P<sub>4</sub>= 99 ± 1% and hCG= 94 ± 6% at 12h, GVBD respectively). Forsk and cAMP stimulated steroidogenesis (results not shown) but GVBD was completely inhibited. Inhibition of P450s with Ketoconazole blocked maturation of follicles, whereas inactivation of CYP19 with Anastrozole did not affect GVBD produced by hCG, respectively. During *R.arenarum* ovarian steroidogenesis, conversion of Col to Pregnenolone and P<sub>4</sub> to Androstenedione would be the steps of steroidogenesis necessary for meiosis resumption since inhibition of the CYP11 and CYP17 enzymes blocked oocyte maturation. The inhibition of E2 synthesis by Anastrozole did not affect GVBD in the follicles.

### A57

#### ACETYLCHOLINE IN COELIAC GANGLION INCREASES OXIDATIVE STRESS IN OVARY: AN EFFECT MODULATED BY NITRIC OXIDE.

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Reactive oxygen species are necessary to lead to ovulation; however, with the reactive nitrogen species (RNS) they can cause cellular damage by reacting with macromolecules. Using the *ex vivo* coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) system of Holtzman rats in the first proestrus (PE), we demonstrated that stimulating the CG with acetylcholine (Ach) increases nitric oxide (NO) release, an RNS with implication in steroidogenesis and follicular development. The aim of this study was to evaluate if Ach 10<sup>-6</sup> M in CG modifies the oxidative state in O, in relation to changes in levels of NO. The system was incubated in Krebs-Ringer buffer at 37°C for 180 minutes. Aminoguanidine 400 μM (AG), a selective inhibitor of inducible NO synthase, was added both to O and to CG, separately, with and without addition of Ach 10<sup>-6</sup> M in CG. Total antioxidant capacity (TAC), the activity of antioxidant enzymes catalase (CAT) and glutathione peroxidase (GSH-Px), and lipid peroxidation (TBARS) and protein oxidation (carbonyls) markers were determined in O homogenates. For statistical analysis of data ANOVA and Tukey's test (p<0.05) were used. (Ach)<sub>CG</sub> vs control decreased TAC (p<0.05) and increased GSH-Px activity and carbonyls (p<0.001). (AG)<sub>O</sub> vs control increased TAC (p<0.05). (AG)<sub>O</sub>+(Ach)<sub>CG</sub> vs (Ach)<sub>CG</sub> increased TAC (p<0.01) and decreased carbonyl formation (p<0.001). (AG + Ach)<sub>CG</sub> vs (Ach)<sub>CG</sub> increased TAC (p<0.01) and CAT activity (p<0.001); however, it decreased GSH-Px activity and carbonyls (p<0.001). In the first PE, Ach sensitizes the system by increasing oxidative stress in O, an effect that is enhanced by AG.

### A58

#### ACETYLCHOLINE IN COELIAC GANGLION INCREASES NITRIC OXIDE RELEASE WITH IMPLICATIONS IN OVARIAN ESTRADIOL SECRETION.

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Using the *ex vivo* coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) system of Holtzman rats in the first proestrus (PE), we demonstrated that Acetylcholine 10<sup>-6</sup>M (Ach) in CG increased in ovary the release of estradiol (E<sub>2</sub>) and nitric oxide (NO), a gaseous molecule with implications in steroidogenesis. The aim of this study was to determine whether the described effect of Ach on E<sub>2</sub> is *per se* or through the increase in NO. The system was incubated in Krebs-Ringer buffer at 37°C. Aminoguanidine 400 μM (AG) and L-nitroarginine methyl ester 100 μM (L-NAME), inhibitors of NO synthase, and sodium nitroprusside 100 μM (SNP), an exogenous NO donor, were added separately in O, with and without Ach 10<sup>-6</sup>M in CG. E<sub>2</sub> release was determined by RIA at 30, 120 and 180 minutes of incubation, and P450 aromatase gene expression (P450arom, E<sub>2</sub> synthesis enzyme) was determined by RT-PCR at 180 minutes. For statistical analysis of data ANOVA of repeated measures and Student's t test (p<0.05) were used. (Ach)<sub>CG</sub> vs control increased E<sub>2</sub> release at all times (p<0.001) as well as P450arom gene expression (p<0.05). Both groups stimulated with AG showed no change in the parameters studied. (L-NAME)<sub>O</sub> vs control decreased E<sub>2</sub> release at 180 minutes (p<0.05). (L-NAME)<sub>O</sub>+(Ach)<sub>CG</sub> vs (Ach)<sub>CG</sub> decreased P450arom expression (p<0.05). (SNP)<sub>O</sub> vs control increased E<sub>2</sub> release at all times (p<0.01) and P450arom gene expression (p<0.05). (SNP)<sub>O</sub>+(Ach)<sub>CG</sub> vs (Ach)<sub>CG</sub> increased E<sub>2</sub> release at 30 minutes (p<0.01), showing the opposite effect at 180 minutes (p<0.05). The increase in NO caused by Ach in CG is partly responsible for the results obtained on the E<sub>2</sub> release and P450arom gene expression in O.

**A59**

**MORPHOMETRIC STUDY OF POSTNATAL CEREBELLUM TREATED WITH ANGIOTENSIN II AT<sub>2</sub> RECEPTOR ANTAGONIST.**

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The cerebellum has a striking morphology consisting of folia separated by fissures of different lengths. Since folia in mammals are likely to serve as a broad platform on which the anterior-posterior organization is built, it is important to understand how such complex morphology arises.

Angiotensin II receptor type 2 in fetal and postnatal development has been related to neuronal differentiation. In cerebellum, AT<sub>2</sub> receptors are located only in the Purkinje cells, which orchestrate the process of postnatal cerebellar corticogenesis and consequently cerebellar development. Previous studies have shown changes in cerebellum morphology in animals treated with PD123319. The aim of this work was to analyze the morphometric characteristics of postnatal cerebellum treated with the antagonist. Rats during late pregnancy (G13-G21) were subcutaneously administered PD 123319 (1.0 mg/kg/day) and vehicle (control). The offspring were sacrificed at postnatal 3, 5 and 8 days (P3-P5-P8). Slices from cerebellum (14µm) were stained with H&E. The cerebellum perimeter and area showed significant differences between treated and control groups at P5 ( $P \leq 0.01$ ). The cardinal lobes area: anterobasal ( $P \leq 0.05$ ), central ( $P \leq 0.01$ ), posterior ( $P \leq 0.001$ ) and flocculonodular ( $P \leq 0.001$ ) present significant differences at P5, and the anterodorsal lobe at P8 ( $P \leq 0.05$ ). Fissures lengths showed significant differences in secondary and posterolateral fissure at P5 ( $P \leq 0.01$ ), preculminar fissure at P8 ( $P \leq 0.001$ ). The AT<sub>2</sub> receptor blockage affects cerebellum growth at P5, which coincides with the maximum expression of the granular cells mitogenic protein (Shh) secreted by Purkinje cells. The data suggest that the AT<sub>2</sub> receptor could be involved in the cellular proliferation mechanisms in the developing cerebellum.

**A60**

**OBESITY, WEIGHT GAIN AND METABOLIC RISK FACTORS OF KIDNEY STONE FORMERS**

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Significant weight gain has become an epidemic condition around the world together with increased risk of developing nephrolithiasis, suggesting a causal relationship between body weight and urinary stone formers. The aim of this work was to study the metabolic conditions accounting for kidney stone formation in male and female patients with obesity and overweight. A retrospective study included 420 patients, 43% men (M) and 57% women (W). Body mass index (BMI) was classified: obese/overweight patients represented 25/42% M and 9/31% W. Linear regression models were used to analyze metabolic serum and urinary risk factors vs body weight. Serum chemistries: creatinine vs BMI men ( $P < 0.05$ ), significant difference with W ( $P < 0.0001$ ). Uric acid: significant increase classified by BMI in W ( $P < 0.001$ ), significant difference with M ( $P < 0.001$ ) and positive associations with body weight ( $r = 0.32$ ,  $P < 0.0001$ ). Urea vs obesity between M and W ( $P < 0.05$ ). Inverse relation between magnesium vs BMI ( $r = -0.06$ ,  $P < 0.03$ ) in men. PTH obese vs nonobese W ( $P < 0.001$ ), positive associations with BMI ( $r = 0.29$ ,  $P < 0.0002$  W,  $r = 0.30$ ,  $P < 0.0008$  M). Sodium and potassium vs BMI positive relation ( $r = 0.15$ ,  $P < 0.01$  W,  $r = 0.16$ ,  $P < 0.02$  M). Urine 24h: uric acid vs body weight ( $r = 0.40$ ,  $P < 0.001$  W,  $r = 0.63$ ,  $P < 0.0001$  M), phosphate ( $r = 0.20$ ,  $P < 0.001$  W), oxalate ( $r = -0.18$ ,  $P < 0.01$  M), citrate ( $r = 0.01$ ,  $P < 0.05$  W,  $r = 0.11$ ,  $P < 0.05$  M). Correlation BMI vs urine volume ( $r = 0.13$ ,  $P < 0.03$  W,  $r = 0.14$ ,  $P < 0.04$  M), no correlation between BMI and pH. Positive associations between body size on metabolic stone composition were found. There are two distinct metabolic risk factors accounting for kidney stone formation in male and female patients.

**A61**

**MORPHOLOGICAL ANALYSIS OF LARVAL DEVELOPMENT OF *Phyllocnistis citrella* (Lepidoptera, Gracillariidae) IN CITRUS CROPS IN TUCUMAN**

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*Phyllocnistis citrella*, “the citrus leafminer”, is a serious pest in citrus growing areas in northwestern Argentina (NOA). During larval development, *P. citrella* feeds on young leaves, producing galleries and constraining tree growth, which increases susceptibility to bacterial diseases and affects production. Due to the economic importance of the citrus industry in Tucumán and the damage caused by this pest, in the present work we performed a histological study on the larval development of *P. citrella* and its effect on the crop, providing information for integrated pest management programs. The larvae were collected in a lemon plantation free from pesticides to control leafminers. The specimens were fixed in Bouin’s solution, preserved in N-butylic alcohol and stained with hematoxylin-eosin and Mallory’s trichrome. Based on the feeding behavior, the cephalic morphology and body length of the larvae, three stages were established. The morphogenesis processes and anatomical-histological changes in organs and systems were analyzed. In the LII stage the cellular-tissue features and the presence of symbiotic bacteria in the intestinal lumen indicate full system activity that, together with development of abdominal skeletal muscles, would allow the pest to voraciously eat the leaves, causing reduction in

photosynthesis. During larval development, increase in the fat body and active lipids synthesis were observed. In the LIII stage the silk glands produced abundant acidophil secretion. Testes with single follicle and genitalia in LII stage and ovaries with growing ovarioles in LIII stage were differentiated. These results will assess the effect of control methods on the normal development of this species.

#### A62

### IDENTIFICATION of *Chaetanaphothrips orchidii* MOULTON (THYSANOPTERA: THIRIPIDAE) ON THE BASIS OF EXTERNAL MORPHOLOGICAL CHARACTERISTICS OF LARVAE AND FEMALE SPECIMENS

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Lesions caused by *Chaetanaphothrips orchidii* Moulton on citrus fruits, product feeding and oviposition affect marketing in the NOA. The objective of the study was to describe females and larvae to achieve the correct identification and differentiation from other thrips species common in citrus. The study was conducted on specimens obtained from samples of grapefruit and associated weeds in Libertador General San Martín (Ledesma, Jujuy) and Colonia Santa Rosa (Orán, Salta) plantations. Individuals obtained by breeding in the laboratory were also used. Microscopic preparations were deposited in the Agricultural Zoology Laboratory Collection of Yuto INTA. The female of *Ch.orchidii* is characterized by staining of the antennal segments and forewing, absence of the pair mushrooms I ocelares, mushrooms internal posteroangulars of the pronotum longer than the outer, dotted or glandular areas around the spiracles of the tergo VIII, tergos and abdominal cosmetic with *craspedum* posteromarginal in the form of a broadband. The larvae are distinguished by general coloration of the body, distribution and development of the sclerotic areas, number of teeth or spines located between the IX tergo mushrooms 1 and by the presence of mushrooms with apices acute and fringed. Neither eggs nor quiescent stages showed characters of diagnostic value. We were able to perform the diagnosis of this species. Funded by: A/0161 SECTER - U.N.Ju. -INTA SALJU-1232306

#### A63

### OVARY HISTOMORPHOLOGY OF *Odontophrynus americanus* AND *Scinax fuscovarius*

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Anuran amphibians are vertebrates characterized by different reproductive strategies reflected in the morpho-physiological adaptations of the ovary.

In this work we analyze the ovary histomorphological aspects of sexually mature females in the reproductive period of two species of frogs, *Scinax fuscovarius* and *Odontophrynus americanus*.

Ovaries of *S. fuscovarius* and *O. americanus* were processed and fixed in buffered formalin at pH 7 and included in Histowax. 5 µm cuts were stained with hematoxylin-eosin (HE), Mallory's trichrome and Toluidine Blue (TB) at pH 7.

In the ovaries of both species, predominantly vitellogenic and fully grown oocytes were found, with few previtellogenic oocytes. Oogonia were not observed. In vitellogenic oocytes, migration of the germinal vesicle into the animal pole could be seen, with progressive retraction of the nuclear envelope, numerous nucleoli and compact chromosomes at the nuclear periphery. The cytoplasm has large yolk platelets in the vegetative pole and smaller ones at the animal pole. On the periphery of the oocyte, forming the ovarian follicles, follicular cells and theca cells were identified. Follicular layers include fibroblasts, collagen fibers, conspicuous vascularization, follicular cells forming a squamous epithelium and a vitelline envelope. The prominent number of vitellogenic follicles and fully grown follicles show that in both species the ovaries are in the immediate ovulation period.

#### A64

### BIOLOGY OF *Daphnia menucoensis* (PAGGI, 1996) AND *Moina eugeniae* (OLIVIER, 1954), TWO AUTOCHTHONOUS CLADOCERANS, WITH TWO DIFFERENT SALINITIES

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*Daphnia menucoensis* and *Moina eugeniae* are the most common planktonic cladocerans in saline lakes up to 40 g.L<sup>-1</sup> in central Argentina. Field studies indicate that the latter had the greatest tolerance to salinity, so the aim of this study was to compare aspects of their biology in the laboratory. Chronic bioassays were performed using parthenogenetic females of both species, with two salinities (7 and 17 g.L<sup>-1</sup>). The temperature was 22 °C (±1 °C) with a photoperiod of 16/8 hours (light-dark). Every 48 hours the specimens were revised, the molts were measured and the offspring were removed and counted. Survival with 7 g.L<sup>-1</sup> differed (H=9.16; p=0.0026): *D. menucoensis* exceeded 32 days and *M. eugeniae* only reached 18 days. However, with 17 g.L<sup>-1</sup> (H=6.3; p=0.0128) the higher survival was that of *M. eugeniae* (29 days) compared to the 20.6 days of *D. menucoensis*. While with 7 g.L<sup>-1</sup> the number of litters was similar (close to 3), with 17 g.L<sup>-1</sup> it differed (H=12.71; p=0.0005); *M. eugeniae* produced 8 litters compared to the 1.18 of *D. menucoensis*.

The number of neonates was different: With 7 g.L<sup>-1</sup> (H=12.53; p=0.0004) *D. menucoensis* produced a mean of 22.9 and *M. eugeniae* 46.3. With 17 g.L<sup>-1</sup> (H=12.31; p=0.0005) *D. menucoensis* produced 6.5 neonates against the 135 of *M. eugeniae*. The maximal length followed opposite patterns: While *D. menucoensis* reached 3.01 mm at 7 g.L<sup>-1</sup> they only measured 2.44 mm with 17 g.L<sup>-1</sup>. Conversely, *M. eugeniae* were smaller (1.57 mm) with 7 g.L<sup>-1</sup> and they reached 1.97 mm with 17 g.L<sup>-1</sup>. The differences observed show that *M. eugeniae* are adapted to higher salinity, which contributes to explain their presence in lakes over 30 g.L<sup>-1</sup>, where *D. menucoensis* is absent.

#### A65

### MATH AS A TOOL IN BIOLOGY: *Rhinella arenarum* OOCYTE MATURATION

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The use of mathematical models in the biological sciences has increased in the last decades, representing an integration tool in the process of experimental research and promoting interdisciplinarity. The mathematical theory of nonlinear dynamics enables the investigation of the evolution of biological phenomena in different experimental conditions and the prediction of their stability at the end of the process. From this perspective, this paper shows preliminary results of modelling and simulation of the dynamics of spontaneous and progesterone-induced maturation in *Rhinella arenarum* oocytes according to the season.

From the exploratory analysis of the available experimental data it can be deduced that the Gompertz model of nonlinear population dynamics accounts for these data.

Simulation by MATLAB enables the estimation of the parameters related to the speed of maturation and their percentage in the steady state. In turn, we can determine that the dynamics of progesterone-induced maturation is significantly greater than that of spontaneous maturation and that the time required to reach biological effect equilibrium varies seasonally. The solution of the model simulates the temporal behaviour of maturation and predicts the moment when metaphase II is experimentally reached, thus validating it. Finally, modelling allows the estimation of the maturation percentage in less than the 5 hs of the process, a data not always obtainable by experimental means. This work is the result of the integration of mathematics and biology.

#### A66

### CADMIUM ALTERS APOPTOSIS MARKERS AND MORPHOLOGY IN RAT LUNG. EFFECTS OF DIFFERENT PROTEIN SOURCES

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on apoptosis markers and the histoarchitecture of rat lung under different diets. 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein sources. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water for 60 days. Total RNA was isolated with Trizol and cDNA was obtained. Transforming growth factor beta (TGF-β), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), p53, BAX and Bcl-2 were determined by PCR. S28 was used as the control. The lungs were fixed, sectioned, stained, and examined for evidence of lung injury.

TGF-β expression decreased in Soy-Co and Soy-Cd groups vs their respective Caseine groups (p<0.001). NF-κB showed a significant decrease in Soy groups vs Caseine groups (p<0.001) and also a significant decrease in Soy-Cd vs its control (p<0.01). p53 mRNA levels showed a significant decrease in Soy-Cd group vs Cas-Cd (p<0.001). Bax/Bcl-2 ratio increased in Cas-Cd vs Cas-Co (p<0.05), showing no differences among Soy groups. Significant morphological changes in lung parenchyma were observed in intoxicated rats when compared to the control group after 2 months of treatment. Morphological changes were less severe in the Soy-Cd group. This shows that lung histoarchitecture is altered by Cd, which is consistent with the change in apoptotic markers, and Soy might confer protection in lung against the metal.

#### A67

### SIZE VARIATION OF THE POST-EMBRYONIC STAGES OF *Boeckella poopoensis* (COPEPODA, CALANOIDA) AT DIFFERENT SALINITIES

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*Boeckella poopoensis* is a frequent microcrustacean species in neotropical saline environments. This halophilic copepod has a wide geographic distribution that extends from northern Patagonia to southern Peru. Given that salinity directly influences copepod development and information on its effect on *B. poopoensis* in laboratory conditions is lacking, the aim of this study was to determine

the influence of this parameter on the size of its different stages. Five treatments were conducted in 2.8 L aquaria with salinities of 5, 10, 20, 30 and 35 g.L<sup>-1</sup>. Thirty nauplius 1 specimens were placed in each aquarium and incubated at 22 ± 1 °C with a photoperiod of 8:16 hours darkness: light for three months. After that time, a sample of 100 mL was collected every five days for two months in each treatment. From each sample, the total body length of the specimens was measured using an ocular micrometer. No differences in size of the naupliar stages 2 and 4 were found, and in all treatments an approximate length of 252 and 354 µm, respectively, were registered. The other post-embryonic stages were significantly different. A positive relationship between length and salinity was observed, since the larger specimens were registered in treatments with 20 and 30 g.L<sup>-1</sup>.

#### A68

##### KIDNEY MODIFICATIONS IN TWO HYPERTENSIVE MODELS. ROLE OF NITRIC OXIDE

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The kidney is involved in the regulation of arterial hypertension (HT). In rats, the inhibition of nitric oxide (NO) by N<sup>ω</sup>-Nitro-L-arginine methyl ester (LR) and the hydrosaline retention by deoxycorticosterone acetate (DR) produce HT of different etiology. However, the protective role of renal NO in these models has not been studied at all. **Objective:** To evaluate renal histological changes in LR and DR and the protective role of NO in each model. **Methods:** Creatinine (CrC), Na<sup>+</sup> (Na<sup>+</sup>C) clearance and mean arterial pressure were determined in LR and DR. In one kidney, corpuscular, tubular (TA) and vascular (VA) areas, and Bowman's space (BS) were measured by histological techniques. In the other kidney, the bioavailability of NO was measured in cortex and medulla by Griess reaction. Results were compared to control rats (CR).

**Results:** LR and DR developed HT without macroscopic renal changes or CrC alteration. Plasma Na<sup>+</sup> increased in LR and DR. Na<sup>+</sup>C also increased but only in DR. A reduction in corpuscular area and BS was found in LR and DR. TA decreased more in DR than RL, and VA decreased only in RL. Nitrites were lower in renal cortex (CR: 2.7±0.4 vs LR: 0.6±0.1 pmol/mg of tissue; p<0.001) and medulla (CR: 5.9±0.9 vs RL: 2.7±0.3 pmol/mg of tissue; p<0.001) in LR. On the other hand, nitrites in cortex and medulla increased more in DR than in CR.

**Conclusions:** HT may cause microscopic injuries such as progressive atrophy of tubules and glomerules, regardless of the HT model. In DR, Na<sup>+</sup> clearance would increase and remodel tubular area because of a compensatory mechanism to the excess of the plasmatic volume. The decrease in vascular area in LR would indicate a protective role of vascular NO.

#### A69

##### ULTRASTRUCTURE OF TRACHEAL EPITHELIUM IN PLAINS VISCACHA (*Lagostomus maximus*)

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Plains viscacha (*Lagostomus maximus*) is a rodent belonging to the suborder Hystricognathi. Studies on this species include the description of several systems, with limited knowledge regarding the respiratory system. In previous studies we detailed histological and histochemical characteristics of the epithelium and glands of the trachea. The aim of this work is to describe the cell types of the tracheal epithelium using transmission electron microscopy and to establish similarities and differences with patterns observed in other mammals. Three adult animals taken from the ECAS (Bs. As.) were used. The 1mm thick samples were fixed and processed following the protocol of the UNLP FCV electron microscopy service. Basal, ciliated, secretory (goblet and serous) and brush cell types were found. Basal cells are pyramidal and have few organelles. Goblet cells contain round granules with a heterogeneous electron-dense internal frame. Serous cells have abundant rough endoplasmic reticulum, mitochondria, apical electron-dense granules and an indented nucleus. Brush cells have microvilli and abundant apical mitochondria, but lack granules. Viscacha presents common cell types with other mammals, with the peculiarity of the presence of serous cells similar to those described in rat and human.

#### A70

##### ACCUMULATION AND DETOXIFICATION OF HEAVY METALS IN THE SENTINEL ORGANISM *Pomacea canaliculata*

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Model organisms with the ability to produce a specific and measurable response to exposure to xenobiotics can be used as sentinels of water contamination. These organisms can help to overcome the limitations of traditional methods, which are incapable of detecting emissions of xenobiotics released in irregular form that are quickly diluted in large bodies of water. *Pomacea canaliculata*, a freshwater mollusc widely distributed around the world, lodges a prokaryotic organism within its digestive gland cells. This endosymbiont has two morphological types (identified as C and K) that are released regularly in huge quantities from the digestive

gland to the intestine. We have shown that the kidney and digestive gland (and the endosymbionts contained therein) of this apple snail are the main places of incorporation of mercury, arsenic and uranium from the water. Here, we studied the retention time of each metal (at four times; 7, 14, 28 and 56 days post exposure) in tissues, endosymbionts, and faeces of *P. canaliculata* after a chronic exposure period (60 days) to sub-lethal doses (mercury = 2 µg/L; arsenic = 10 µg/L; uranium = 30 µg/L). The elements of these samples were measured by neutron activation. After exposure to the three metals, the 'memory' was retained in the kidney, digestive gland and endosymbionts of *P. canaliculata*. Only the morphotype K of the endosymbiont exhibited a significant decrease in mercury concentration at day 56 post-exposure. Also, elemental concentrations in faeces decreased gradually after exposure, indicating that the metals had been eliminated through the endosymbiont. We suggest that this symbiotic association might be useful for the biomonitoring of freshwater ecosystems.

#### A71

### CADMIUM INDUCES HYPERTENSION, ALTERATIONS IN OXIDATIVE STRESS AND APOPTOSIS MARKERS, AND MORPHOLOGICAL CHANGES IN AORTA.

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on blood pressure, histoarchitecture, oxidative stress and apoptosis markers of rat aorta. Male Wistar rats were used: 1 group received regular water (control-Co) and the other 15 ppm of Cd in the drinking water for 60 days (Cd). During treatment, blood pressure was measured with a CODA system. Total RNA was isolated with Trizol and cDNA was obtained. Nrf2 factor, NOX2, GPx, SOD, p53, Transforming growth factor beta (TGF-β), BAX and Bcl-2 were determined by PCR. BAX/Bcl-2 ratio was calculated. S28 was used as control. Aortas were fixed, sectioned, stained, and examined for evidence of injury.

Cd induced a significant increase in systolic and diastolic blood pressure ( $p < 0.05$ ). NOX2 showed a significant increase ( $p < 0.001$ ) in the Cd group even though p47 did not show differences. Nrf2 showed a significant decrease in Cd ( $p < 0.05$ ) while GPx did not show differences and SOD significantly increased in the Cd group ( $p < 0.05$ ). TGF-β expression did not change while p53 and BAX/Bcl-2 ratio showed a significant increase in the Cd group ( $p < 0.05$  in both). Regarding the morphology, irregular luminal layers of endothelial cell linings were observed in aortas of Cd-treated animal. In this group, light microscopy revealed structural changes in tunica intima cells, exhibiting clearer and bigger cytoplasm than cells from Co aortas. Cells of the tunica media in close contact with the intima also showed these alterations.

This shows that Cd induces hypertension, oxidative stress and changes in the expression of apoptosis markers together with architectural changes in the aorta.

#### A72

### ISOLATION OF OPPORTUNISTIC AGENTS *Cryptococcus neoformans* / *C. gattii* FROM THE ENVIRONMENT.

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Opportunistic fungal infections can affect both immunocompromised and immunocompetent patients. The complex capped yeast fungi *Cryptococcus neoformans* / *C. gattii* are opportunistic infection agents. *C. neoformans* can be found in nature in bird droppings. The disease known as cryptococcosis has a worldwide distribution. The objective of this study was to determine the frequency of isolation of *Cryptococcus* spp in Mendoza city. Two hundred and thirty samples of pigeon stool were collected at different times of the year from sites with a large influx of people, where the presence of these birds is permanent. The isolation and identification of the yeast was conducted with conventional phenotypic techniques. 20 mL of aqueous solution of chloramphenicol 0.5 g/mL was added to 5 g of each sample and allowed to stand for 24 h at 4 °C. From the supernatant 0.1 mL were taken, which were seeded by exhaustion in Sabouraud and Sunflower Seed Agar. They were incubated for one week at 28 °C. Both microscopy (Chinese ink) and biochemical tests were performed in all smooth colonies mucoid, white or cream colored colonies that developed between 24-48 h. Positive isolates for the genus *Cryptococcus* were obtained in 27.8% (64/230) of all samples studied in different seasons. The area of highest incidence was that close to hospitals (30/40%). Other areas showed values ranging from 20 to 50%. We intend to extend the study area to produce a map of the regional distribution of this potential pathogen in different immunological states and pest control campaigns.

#### A73

### SURVIVAL OF *Brucella abortus* IN EUKARYOTE CELLS AFFECTED BY AKT1 KINASE INHIBITION

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*Brucella abortus* can survive and even multiply within macrophages. In order to elucidate the mechanism by which this phenomenon occurs, we determined the involvement of Akt1 kinase of the infected cell in the intracellular replication and survival of different

*Brucella abortus* strains. Raw macrophages were infected with the virulent strain of *Brucella abortus* 2308 and virulence attenuated vaccine strains S19 and RB51. Intracellular bacterial multiplication and survival of the bacteria in control cells and in cells treated with Akt1 kinase inhibitor (Akti) were studied. Survival and bacterial replication capacity were quantified by counting events in flow cytometer. Treatment with a specific inhibitor of eukaryotic kinase Akt1 significantly reduced ( $p < 0.05$ ) the progeny of the virulent bacterial strain 2308 and, to a lesser extent, the vaccine strains, as shown by flow cytometry, using a DNA marker such as propidium iodide (PI) and comparison with an unchecked control. Count viability of *Brucella abortus* was performed with and without Akti outside macrophages to confirm the action of this inhibitor to the level of Rab and discard direct action on the bacteria. These results confirm that one of the intracellular mechanisms used by virulent *B. abortus* strain 2308 for survival involves the Akt1 kinase pathway. The behavior in vaccine strains of attenuated virulence is different. Inactivation of the kinase eukaryotic favors the active state of certain Rab proteins of the host cell, preventing fusion with lysosomes and bacterial degradation.

#### A74

##### ANTIBIOFILM EFFECT OF 2-ISOPROPYL-5-METHYLPHENOL ON *Listeria monocytogenes*.

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*Listeria monocytogenes* is capable of attaching to and forming biofilm on a variety of food processing equipment. 2-isopropyl-5-methylphenol (2-IP-5-MP), an important component of the *Thymus vulgaris* (Thyme) and *Origanum vulgare* (Oregano) essential oils, presents a variety of pharmacological properties including antimicrobial effects. The aim of this work was to compare biofilm production by *L. monocytogenes* with different concentrations of 2-IP-5-MP.

Strain: *L. monocytogenes* CLIP 74902. Culture medium: trypticase soy broth (TSB) with glucose 10 g/l and enriched medium (EM) (g/l): proteose peptone 30, yeast extract 5, trypticase 5, glucose 2, pH 7.6; supplemented with 2-IP-5-MP ( $\mu\text{g/ml}$ ): 0 (control), 250, 750. The experiments were performed in sextuplicate, adding 150  $\mu\text{l}$  of each medium to different 96 well microplates. Aliquots of 10  $\mu\text{l}$  of an overnight culture in brain heart infusion broth were added to each well and incubated at 37°C under aerobic conditions for 24, 48, 72 and 96 h. The negative control wells contained broth only. To estimate the biofilm formed, the content of the each plate was washed with sterile PBS. The remaining bacteria attached were fixed with methanol and stained with crystal violet 1% (w/v). The dye was removed with PBS. The dye bound to the adherent cells was resolubilized with 33% (v/v) glacial acetic acid, and OD<sub>550nm</sub> was measured in a plate reader. At 72 h, sessile biomass (OD<sub>550nm</sub>) obtained in TSB under exposure to 0, 250 and 750  $\mu\text{g/ml}$  of 2-IP-5-MP were: 1.150, 0.710, 0; while in ME: 0.801, 0.504, 0 respectively. Results suggest that 2-IP-5-MP could be used to control *L. monocytogenes* in food processing environments, although further studies in commercial settings are necessary.

#### A75

##### MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) IN OBESE CHILDREN

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Macrophage accumulation in adipose tissue (AT) of obese patients plays a role in chronic low grade inflammatory response through the secretion of inflammatory cytokine molecules such as MCP-1, uPCR and fibrinogen (Fg), MCP-1 being important in exacerbating insulin resistance (IR). The aim of this study was to determine the plasma levels of MCP-1, uPCR and Fg in obese children and the correlation of these molecules with IR and anthropometric indexes.

Forty-five obese children (24 M/21 F) between 7 and 14 years old and 20 normal-weight children of similar age and sex were studied. All patients underwent clinical history consigning age, weight, height, BMI, waist circumference (CC) and Tanner state. Obesity was defined as BMI > 97<sup>th</sup> percentile for age and sex, according to the WHO. Plasma levels of MCP-1 (Met. ELISA, R&D Systems), uPCR (chemiluminescence, Immunolite 2000, Siemens), Fg (Met. Clauss, Diagnostic Stago), fasting blood glucose (Enzyme met, Wiener Lab) and plasma insulin were determined (Met. ECLIA, Roche) and the HOMA index was calculated. Data were expressed as median and interquartile range. Spearman coefficient was used to investigate correlations.

Obese children had higher values of MCP-1 [175(120-200) vs. 95(77-110) pg/ml,  $p = 0.001$ ], uPCR [1.9(0.84-3.1) vs. 0.34(0.20-0.80) mg/l,  $p = 0.001$ ] and Fg [350(315-390) vs. 244(235-260) mg/dl,  $p = 0.0001$ ] than controls. MCP-1 was significantly correlated with insulin, HOMA, BMI and CC. High levels of MCP-1, uPCR and Fg indicate subclinical inflammation associated with RI and obesity in the infant-juvenile population studied.

A76

**RUMINAL BACTERIAL COMMUNITY IN GOATS FED ON FRESH ALFALFA.**

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Ruminants fed with fresh alfalfa develop an alteration in the rumen called bloat. This condition begins when animals ingest fresh forage with a high concentration of soluble proteins. The aim of this study was to analyze the structure of rumen bacterial community, by quantitative PCR, from fistulated goats fed with fresh alfalfa. The animals were monitored for clinical signs of bloat after the change in feeding. The samples were collected 4 days before the dietary change and subsequently according to the intensity of the signs. DNA extraction was performed by a method that combines mechanical cell lysis with the column filtration steps of DNA. The quantification of selected bacteria was performed with the MX3005P qPCR System using PCR primers targeting 16S rDNA gene fragments. The serially diluted DNA isolated from known numbers of cells of the reference strains was used as a standard for the construction of a calibration curve, which allowed calculation of the number of copies of the gene. Mean total copies number of 16S rDNA gene was compared with ANOVA followed by Tukey's HSD procedure. Bacteroidetes and *Bacteroides-Prevotella* group decreased ( $p < 0.05$ ) immediately after the change in the diet, even when the animals did not show signs of bloat. This community did not return to the values observed before the start of the experiment, indicating that bloat generates long-term changes on the structure of this bacterial community.

A77

**BORODIPYRRROMETHENES AS INACTIVATING AGENTS OF MICROORGANISMS**

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Photodynamic inactivation (PDI) is presented as an alternative to conventional treatments of infectious diseases. PDI requires the presence of a photosensitizer and visible light to induce localized formation of reactive oxygen species (ROS).

The objective of this work was to study two new cationic borodipyrrromethenes (BODIPYs) as photosensitizing agents for the PDI of microorganisms. These compounds absorb strongly ( $\epsilon \sim 1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ ) at  $\sim 500 \text{ nm}$ , with high fluorescence quantum yields ( $\Phi_F \sim 0.7$ ). Furthermore, they photosensitize the formation of singlet molecular oxygen with yields of  $\Phi_\Delta \sim 0.1$ . The effectiveness of these agents was studied in *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. BODIPYs bind to cells rapidly in 5 min. Fluorescence microscopy was used to observe the distribution of the agents in the cell suspensions. Photodynamic treatment was performed using 1  $\mu\text{M}$  photosensitizer for *S. aureus*, and 5  $\mu\text{M}$  for *E. coli* and *C. albicans*. Inactivation of *S. aureus* was effective after short periods of irradiations, while the treatment of *E. coli* and *C. albicans* required irradiation longer than 15 min. Also, an increase in the photoinactivation mediated by these BODIPYs was found in the presence of increasing concentrations (0-50 mM) of potassium iodide (KI). Therefore, the results show that the photodynamic effect induced by these BODIPYs in the presence of KI has potential application in the photoinactivation of microorganisms.

A78

**BIOFILM IN STREPTOCOCCUS UBERIS FROM CLINICAL AND SUBCLINICAL MASTITIS**

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*Streptococcus uberis* is the main streptococcal environmental pathogen responsible for a high proportion of clinical and subclinical mastitis. Biofilms are responsible for many persistent infections, including bovine mastitis. Within the biofilm (BF), bacteria are protected from host immune response and antibiotics, making BF-related infections difficult to treat. Limited information is available on BF production in *S. uberis*. The aim of this study was to evaluate the effect of different supplements on *in vitro* BF production in 30 *S. uberis* from 30 cows with clinical and subclinical mastitis from 15 herds of the central dairy area of Argentina. In order to examine the effect of different supplements on BF production, isolates were cultured in broth containing 0.5% glucose, 1% sucrose, 1% lactose or 0.5% milk onto the microtiter plate. The isolates were classified as strong, moderate or weak BF producers. The results showed that most *S. uberis* exhibited a moderate or weak BF production in each test condition. It was also observed that BF production capacity depended on each isolate and each tested supplement. Direct attachment of bacteria to the epithelial tissue of the host mammary gland is the most important step in the pathogenesis of *S. uberis* and is the first step in BF formation. Recently, it was reported that *S. uberis* BF producers are able to colonize the epithelium of the gland and establish persistent infections. An increase in our understanding of the possible role of BF in the pathogenesis of mastitis might contribute to the design of novel immunogens aimed at improving the health status of dairy herds in which *S. uberis* predominates.

A79

**GENETIC AND PARTIAL CHARACTERIZATION OF NONRIBOSOMAL PEPTIDES IN *Bacillus amyloliquefaciens***

SL-6

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Bacterial and fungal synthesis of nonribosomal peptides (NRPs) is a flexible and rapidly adaptable system, which gives selective advantages to compounds with novel properties. Several *Bacillus* species produce nonribosomal lipopeptides with potential agricultural, pharmaceutical and environmental applications. The aim of this study was to look for candidate genes involved in the synthesis of lipopeptides in a native strain of *B. amyloliquefaciens* SL-6 from San Luis, with antagonistic activity against the phytopathogen agents *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alternata*. The presence of NRPs synthetase genes for fengycin (*fenD*), bacillomycin (*bmyB*), surfactin (*sfA*), together with the *sfp* gene (4-phosphopantethein transferase), which is essential for such biosynthesis, were detected by Polymerase Chain Reaction (PCR) using *B. amyloliquefaciens* FZB42 as a control strain. The expression of these genes was analyzed by RT-PCR using purified mRNA from bacterial cultures grown in Synthetic Mineral Broth. Buthanolic extracts of spore and cell-free supernatants were performed on thin layer chromatography (TLC) and bands were visualized with iodine, water, ninhydrin, or UV light. The genes and their respective mRNA transcripts from both strains were confirmed by the presence of expected molecular weight bands on 1% agarose gels. TLC visualization methods allowed the identification of lipopeptides by their retention factors (fengycin: 0.11, bacillomycin: 0.3 and surfactin: 0.7). The SL-6 strain contains genes for the synthesis and release of these metabolites into the culture medium under the assayed conditions.

A80

**PHOSPHATE SOLUBILIZATION AND BIOCONTROL OF PHYTOPATHOGENS BY ISOLATES OF *Bacillus***

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Phosphate (P) solubilization in soils and secretion of bioactive compounds against phytopathogens by the microbiota of the rhizosphere have been described among beneficial prospects of these organisms for enhanced crop productivity. The present report aims at studying five *Bacillus* isolates from San Luis for both capabilities. To detect the phosphate solubilizing activity, native strains SL3, SL4, SL5, SL6, SL7 and *B. amyloliquefaciens* FZB42 (control strain) were streaked onto Pikovskaya's (PVK) and National Botanical Research Institute (NBRIP) agar media. The halo zone formation around the growing colony was considered as positive and was measured after 96h of incubation at 30°C. The Pikovskaya medium was amended with bromophenol blue to detect pH variations. "In vitro" antagonistic activity of the isolates against *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alternata* was studied in potatoe agar after 5-14 days of incubation at 26°C.

The FZB42 strain only showed P solubilización in PVK medium, similarly to all isolates, the strain SL7 reaching similar values to the control strain. A change in color from bromophenol blue to yellow was observed due to acid excretion as a probable solubilizing mechanism. The five isolates showed antagonistic activity against phytopathogens. The maximal inhibition values were 88% for *B. cinerea* by *B. SL3*, while *B. SL7* inhibited 68% and 61% to *A. alternata* and *P. expansum* respectively. All isolates showed P solubilization capability and antagonistic activity against phytopathogens, both capacities with beneficial effects on vegetal growth and development.

A81

**RHINOVIRUS INFECTION IN HOSPITALIZED CHILDREN. FIRST DETECTION IN TUCUMÁN, PRELIMINARY RESULTS**

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Human rhinoviruses (HRV) are the major cause of common colds. Currently the molecular diagnosis of viral infections allows a significant appreciation of their role in lower respiratory tract infections (LRTI) including bronchiolitis and pneumonia in children, acute exacerbations of chronic diseases such as asthma, chronic obstructive pulmonary disease and cystic fibrosis. In Tucuman there are no data available of the impact of HRV infections.

The aim of this study is HRV detection in children under two years hospitalized with acute respiratory infection (ARI) to determine its prevalence. Between March and July 2015, 1335 nasopharyngeal aspirates were processed for immunofluorescence for antigen detection of influenza A and B, respiratory syncytial virus (RSV), adenovirus, metapneumovirus and parainfluenza 1, 2 and 3. 57% were positive for respiratory viruses and 177 of the negative samples were selected and processed for RVH detection using real-time RT-PCR assays amplifying the 207 base pair of the 5' non-coding region (a protocol adapted by Dr. Mancone et al. 2012). 49.7% of

samples were positive, bronchiolitis being the most common clinical diagnosis (70%). Conclusion: HRV is an important cause of LRTI in children, ranking second to RSV. A better understanding of the virological, epidemiological and clinical features, including mechanisms of role in co-infections and in chronic respiratory diseases, is needed to guide future efforts aimed at HRV prevention and treatment.

#### A82

### CHARACTERIZATION OF THE EFFECT OF DOSE AND EXPOSURE TIME OF *Minthostachys verticillata* (Griseb.)

#### Epling ESSENTIAL OIL ON CELLS OF BOVINE AND MURINE MAMMARY GLAND.

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Previous studies demonstrated that the essential oil (AE) obtained from *Minthostachys verticillata* has immunostimulatory activity on human T and B cells. The aim of this study was to characterize the cytotoxic effect of AE on mammary gland cells (GM), both *in vitro* and *in vivo*, to evaluate their possible application as an immunomodulator in intramammary infections. For this, the effect of dose and exposure time of AE was assessed in a line of bovine mammary epithelial cells (MAC-T) and GM of Balb/c female mice in the breastfeeding period. The MAC-T cells were exposed to different concentrations of AE (0, 10, 25, 50, 100, 250 and 500 µg/mL) for 6, 12, 24 and 48 h and viability was determined by MTT assay. The female mice were divided into 4 groups (6 mice per group) and inoculated into the right (R4) and left (L4) abdominal GM. Group 1 (control) received 100 µL of PBS/DMSO (0.03%) while groups 2, 3 and 4 received 100 µL of AE (25, 50 and 100 µg/ml). Animals were sacrificed at 24, 48 and 72 h post-inoculation and the GM were removed and processed according to routine histological methods. AE did not alter the viability of the MAC-T cells independently of the tested concentration and exposure time. The histopathology of the treated GM with AE revealed no injuries or signs of inflammation at any of the tested concentrations or times. The results encourage the study of the immunomodulatory effects of AE in mouse GM and provide the basis for future studies in a bovine model.

#### A83

### ANALYSIS OF THE ANTI-*Listeria monocytogenes* ACTIVITY OF ENTEROCINS AND A COMMERCIAL CURING SALT

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Hurdle technology involves the application of multiple conservation methods designed to prevent bacterial contamination in food in order to improve product safety and quality. The aim of this work was to compare the anti-*Listeria monocytogenes* effect of enterocins present in the cell-free supernatant (CFS) of *Enterococcus avium* DSMZ17511 and a commercial curing salt (Embutir, Salta-Argentina). An adaptation of the chessboard technique was used. Aliquots (2700 µl) of the antimicrobial pair dilutions tested were placed on the ordinate (SLC: 1280, 256 and 128 AU/mL) and the abscissa (salt: 0.03, 0.015 and 0.003% w/v) of a 12-well microplate. Then, an aliquot (300 µl) of a suspension of *L. monocytogenes* 01/155 was added to each well and the survival of the pathogen in each system was monitored at 37°C for 24 h (t0, 4 and 24 h) by plate counting. A control growth of the strain in peptone water was also included. When the pathogen grew in contact with the three salt concentrations tested, its viability remained similar to the control growth throughout the experiment. No growth of the *Listeria* strain was detected in systems containing CFS, except for the well where the lowest concentration of enterocins (128 AU/mL) and curing salt (0.003% w/v) was combined. These results confirm the anti-*L. monocytogenes* activity of the enterocins tested, even at low concentrations; while the curing salt analyzed was not effective against the pathogen tested, even when concentrations over the upper limit set for this food additive by the Argentine Food Codex were assayed.

#### A84

### DIAGNOSIS AND SEROPREVALENCE OF *Tritrichomonas foetus* AND *Campylobacter fetus* IN A BOVINE RODEO FROM SANTIAGO DEL ESTERO.

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Bovine Trichomonosis (BT, *Tritrichomonas foetus*) and Bovine Genital Campylobacteriosis (BGC, *Campylobacter fetus* ssp. *fetus* and *veneralis*) are sexually transmitted diseases that cause important economic losses. Improper or no application of the diagnostic protocol would be responsible for the low efficiency in the health control. The objective of this work was to compare the standard techniques versus seroprevalence in a bovine roundup of Santiago del Estero. Preputial scrapes and extraction of serum of 145 bulls were made. BGC was detected by direct immunofluorescence and BT by culture and observation under the microscope.

Seroprevalence was conducted by "spot-blotting", using as antigens homogenates from each pathogen and the obtained sera. According to the standard technique, 20.7% BGC and 5.5% BT were estimated. Seroprevalence values were 94.5% (*Cf veneralis*); 98.6% (*Cf fetus*) and 80% (*T. foetus*). The latter indicated a strong presence of disease in the herd coinciding with high reproductive losses reported in the rodeo (~ 40%). However, the standard diagnostic yielded much lower values. The lack of standardization of technology or its misapplication partly account for the under-diagnosis of these pathologies. Seroprevalence provides important information that allows the determination of the presence of pathogens, its use being significant in rodeos with no previous health checks for adequate planning and increased productivity.

#### A85

##### POULTRY FARM DETECTION OF *Salmonella* SPP. IN THE PROVINCE OF TUCUMAN

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*Salmonella* infections are responsible for a variety of acute and chronic diseases in poultry. This is manifested in significant economic losses for producers and represents a risk to public health through the food chain.

The aim of this study was to evaluate the presence of *Salmonella* spp. in different types of samples from commercial farms for poultry meat or egg production in the province of Tucuman, Argentina.

Eight layer and 3 broiler farms were sampled in the counties of Trancas, Burruyacu, Leales, Rio Chico and Cruz Alta. Sampling of feed, boot swab, litter, feces, eggs, and dead birds were done in one or two poultry houses in farms with 4 or more houses, respectively. From dead birds from the day of sampling, organs and tarsometatarsal bone marrow were processed. In general, isolation consisted of a pre-enrichment followed by selective enrichment and by the use of selective-differential plating media. Then, at least two isolated colonies were taken and biochemical tests were performed to confirm the genus *Salmonella*.

From the farms studied, only 3 layer farms were positive for *Salmonella* spp. isolation in at least one type of sample, which corresponded to feces or organs of dead animals. *Salmonella* sp. monitoring using different types of samples, both environmentally and in animals, increases the chances of detecting this bacterium in poultry farms.

#### A86

##### EVALUATION OF PATHOGENIC FACTORS FROM STRAINS OF *Candida* ISOLATED FROM RURAL RESIDENTS IN NORTHEAST MENDOZA

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*Candida* is an endogenous opportunist fungus that lives in a fragile balance with the host and can develop disease in the presence of predisposing causes. It has virulence attributes to colonize and cause damage directly, by activating, resisting or deflecting the host immune response. Our objective was to study pathogenic factors of *Candida* strains isolated from rural inhabitants of northwestern Mendoza living in deficient sanitary conditions. Twenty strains were isolated from 72 samples of the oral cavity (average age 22.5 years). Biochemical, morphological, antifungal susceptibility and pathogenicity factor studies in addition to proteolytic and phospholipase activity and biofilm production determinations were performed. The results were: 35% of the samples had a count higher than 100 CFU (7/20), *Candida albicans* was isolated from 75% (15/20) and *Candida non-albicans* was isolated from 25% (5/20). Phospholipase activity occurred in all strains (100%) and in most of them (60-70%) proteolytic activity and biofilm production was evident. All strains had at least one of the virulence factors studied and high antifungal susceptibility. These characteristics were also evaluated in non-rural populations and expressed different behaviors. The strains with greater pathogenic potential should be considered since they may constitute a major challenge to therapeutic management.

#### A87

##### EFFECT OF THE ADDITION OF REGIONAL ADJUNCT AUTOCHTHONOUS CULTURES ON THE SENSORY QUALITY OF GOAT CHEESES

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In the northwest of Argentina most of the goat milk is used in cheese manufacture. In cheeses, starter cultures determine fermentation, while adjunct cultures contribute to flavor and aroma, which are of fundamental importance in the final product. The objective of this work was to evaluate the effect of a mixed adjunct culture, made up of autochthonous strains *Enterococcus faecium* BM6 and BM18, on the sensory characteristics of goat cheese. For this purpose, semi-hard cheeses were manufactured using only the starter culture *Lactobacillus bulgaricus* ETC2 in control cheeses and starter and adjunct culture in experimental cheeses. MRS agar was used for

lactobacillus (Lb) counts, M17 for lactococci (Lc), KF for enterococci (E), fungi and yeasts and VRBA for coliforms. Sensory analysis (24 panelists) of the final product included Triangle Test and Preference Test. During maturation, Lb, Lc and E had a significantly greater development in experimental cheeses ( $p < 0.05$ ), inhibiting coliforms and fungi to undetectable levels. With respect to sensory analysis, 75% of the panelists found significant differences ( $p < 0.01$ ) between experimental and control cheeses, while expressing a preference for the cheese with the adjunct culture. In conclusion, the application of the adjunct culture, *E. faecium* BM6 and BM18, constitutes a valid strategy to improve the sensory quality of goat cheese and can contribute to the development of the local production of cheeses.

#### A88

##### **BIOACTIVITY OF *Tibouchina alpestris* COGN. (MELASTOMATACEAE)**

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The aim of this work was to continue the fractionation of ethyl acetate subextract *Tibouchina alpestris* aerial parts (SAcOEt) and to evaluate antibacterial activity of these fractions. SAcOEt fractionation was performed with DIAION HP 20 resin. Elution solvents were: water/methanol (H<sub>2</sub>O:CH<sub>3</sub>OH) at increasing concentrations of CH<sub>3</sub>OH (100: 0; 0: 100).

Antibacterial tests were performed by the diffusion method in Mueller-Hinton agar (MH). Microorganism strains used were *Staphylococcus aureus* (*St.a.*) ATCC 25922, 27835 and 43300 and *St.a.* isolated from clinical samples, *Escherichia coli* (*E. coli*) ATCC 35218 and 14213 and *Klebsiella pneumoniae* (*Kpn*) ATCC 700603.

Six higher weight fractions were selected for microbiological test (D1 to D6). All fractions showed antimicrobial activity against tested strains. The most active were D1, D2 and D 3 against *St.a.* ATCC 27835 and 43300.

#### A89

##### ***Fusarium graminearum* SPECIES COMPLEX: IMPACT OF NUTRITIONAL CONDITIONS CULTURE MEDIA ON ITS GROWTH**

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*Fusarium graminearum* morphological species generate ear rot of corn, reducing grain yield and contaminating it with toxic trichothecenes for humans and animals. At present these species are considered as a complex of phylogenetic species, the phenotypic significance of this genetic diversity being unknown. The objective of this study was to assess the growth rate of phylogenetic species of the Fg complex in culture media prepared from natural substrates and YES medium recommended for growing *Fusarium*. The effect of the media was studied using YES medium, extract of whole corn and ground corn 3% with and without glucose supplementation on *Fusarium graminearum sensu stricto* (Fgss), *Fusarium boothii* (Fb) and *Fusarium meridionale* (Fm) 25°C by testing microdilution. Assessment of growth was made every day for 20 days through microplate reader by reading absorbance at 630 nm. Growth rates were observed until day 7 in all species. Growth was higher in YES medium followed by extract of ground corn and then by extract of whole corn. In the YES medium, growth rate was higher for Fgss than for other species. The addition of glucose to corn extracts did not cause variations in the response. While a higher growth rate was observed in the YES medium, in the ground corn extract it showed a reproducible response. The advantage of the ground corn extract is that it allowed the detection of reproducible growth rates, easily measurable, in not very long periods and within the dynamic range of detection of the absorbance reader.

#### A90

##### **VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN THE EQUINE FOOT INCUBATED WITH *Bothrops alternatus* VENOM**

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Snake venom metalloproteinase degrades the components of the vascular basement membrane and causes alterations in the expression of vascular endothelial growth factor (VEGF). Snakebites usually cause a direct action of venom on the dermal-epidermal laminae of the equine foot. The aim of this work was to describe the expression of VEGF in the dermo - epidermal horse tissue caused by the action of *Bothrops alternatus* snake venom. Tissue samples of dermal-epidermal foot drop were used. They were cultured for 48 h with culture medium (D-MEM) for controls and with medium supplemented with 10 mg/ml of whole snake venom. VEGF expression was assessed by immunohistochemistry. The histological sections belonging to the control group expressed VEGF immunostaining in some epidermal basal cells with nuclear pattern. Control samples failed to reveal immunostaining in the dermal portion of the dermal-epidermal laminae.

Horse tissues that were incubated with the snake venom exhibited nuclear and cytoplasmic expression of VEGF. They also revealed immunoreactivity in blood vessels and in the extracellular matrix. The semiquantitative analysis revealed 20% of tissue

immunolabeling in the control samples and 65% in samples incubated with the venom. We conclude that *B. alternatus* venom alters the level of VEGF expression as well as its immunoreactivity pattern in dermo-epidermal junction of the equine foot.

### A91

#### GRAPEFRUIT ESSENTIAL OIL INHIBITS THE VIRULENCE OF *Pseudomonas aeruginosa*

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*Pseudomonas aeruginosa* produces several virulence factors such as the toxic pigment pyocyanin and the swarming and swimming motilities to grow and spread on different substrates. Currently, inhibition of virulence factors is a target of research rather than antipathogenic products. For this reason, the aim of this work was to study the effects of essential oil of grapefruit (*Citrus paradisi*) and its main component (Limonene) in pyocyanin production and mobility of *P. aeruginosa* ATCC 27853. The strain was grown under different conditions: in the absence and presence of (4, 2, 1, 0.5 and 0.1 mg/ml) grapefruit essential oil obtained by cold-pressing (APF) and cold-pressing followed by steam distillation, and Limonene. The extraction and quantification of pyocyanin was performed in different growing conditions and the determination of bacterial motility (swimming and swarming) was carried out under the same experimental conditions on solid medium.

The inhibitory effects were similar for all samples tested but higher with APF. The results were correlated with the concentration assayed. The inhibition of pyocyanin was 97% at the highest concentration APF. The inhibition of bacterial motility decreased in a dose dependent manner. At 4 mg/ml APF inhibited swarming by 80% and swimming motility by 70%, respectively. Grapefruit essential oil, a natural product used in the food and pharmaceutical industry, has a potential application as an antipathogenic against *P. aeruginosa*.

### A92

#### RESPONSE OF TROPHOBLAST CELLS (BEWO CELL LINE) IN THE PRESENCE OF *Trypanosoma cruzi* INFECTION

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Chagas is an endemic disease caused by the obligated intracellular protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Congenital transmission is responsible for one-third of new cases each year. During congenital transmission, the parasite breaks down the placental barrier formed by the trophoblast, basal laminae and villous stroma. The observation that only 5% of infected mothers transmit the parasite to the fetus implies that the placenta may impair parasite transmission. This study aimed at evaluating the role of trophoblast cells (BeWo cell line) in the presence of *T. cruzi* infection. We analyzed syncytialisation capacity of BeWo cells by immunofluorescence assays and staining of nuclei. The effect of repetitive reinfections by trypomastigotes of *T. cruzi* (Tulahuen strain) on both types of trophoblast cells, cytotrophoblasts and syncytiotrophoblasts, was studied as well as nitrite production in the supernatant. BeWo cells forming syncytiotrophoblasts are more resistant to infection with *T. cruzi* (21% lesser infection,  $p < 0.05$ ), and produced 1.3 times more nitrites, nitrosative stress being a cause of decreased viability of the parasite affecting its propagation. There were no significant differences between reinfected and control groups with respect to the multiplication of the parasite capacity after 96 h of culture. These results suggest that trophoblast cells are able to modulate *T. cruzi* infection, independently of their reinfected, by forming syncytiotrophoblasts and producing harmful metabolites for the parasite. Grants: PICT2012-1061, SECyT-UNC, SECyT-UNLaR, MINCyT-Cba.

### A93

#### HUMAN PAPILLOMAVIRUS INFECTION IN YOUNG WOMEN LIVING IN TAFI DEL VALLE, TUCUMÁN

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Cervical cancer is the second most common cancer in women worldwide. The aim of this work was to establish the DNA of HPV in women less than 30 years of age living in Tafidel Valle. Cervical cell specimens obtained from 44 women aged 14-29 years attending routine gynaecological control in the public hospital "Médici" located in Tafi del Valle were included in the study and written informed consents were obtained from all study participants. (Helsinki Declaration) Detection and typing of the viral DNA genome was performed by polymerase chain reaction with primers My 09/11 specific for the L1 region of the HPV genome, combined with a restriction fragment length polymorphism assay (PCR-RFLP) or PCR and reverse line blot hybridization (PCR-RLB) for 36 genotypes of HPV. The performance of this assay was validated by the WHO. All women were born and living in Tafidel Valle. HPV DNA was

detected in 54% of the clinical samples, with 25% high risk types. The results showed a variable HPV infection: single infection (38.63%) and multiple infections with 2 to 6 genotypes (15.90%). 16 different HPV genotypes were detected. The most common viral types in the infected population were HPV-16 (11.36%), HPV-31 (9.09%) and HPV-58 (6.8%). This system of PCR-RLB, recommended by the WHO, has the advantage of allowing the identification of all HPV types that infect the anogenital region with analytical sensitivity. The information obtained will be useful as a regional baseline for future epidemiological vigilance within the context of the national HPV vaccination program in Argentina.

#### A94

### REMOVAL OF ANTINUTRITIONAL FACTORS IN LEGUME FLOURS BY BACTERIAL FERMENTATION

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Legume flours are important protein sources, but the presence of anti-nutritional factors (ANFs) may cause detrimental effects on the consumer. Fermentation with bacteria is a technological alternative to improve the nutritional quality of these products. Therefore, the aim of this study was to assess the ability of two strains to decrease/eliminate the concentration of trypsin inhibitors (TIA) and  $\alpha$ -amylase inhibitors (AIU), present in bean and chickpea flours. Doughs prepared with legume flours were sterilized at 121°C for 20min, inoculated with *Lactobacillus* GS30 or *Propionibacterium acidipropionici* Q4 ( $1 \times 10^7$  CFU/g) and then incubated at 37°C for 36 h. ANFs concentration (by colorimetric methods), pH and viability of the strains were determined at regular intervals. Flours sterilization decreased TIA (73-88%) and AIU (11-73%). Fermentation with *Lactobacillus* GS30 increased TIA removal up to 93% in bean flour and 84% in chickpea flour; whereas AIU were decreased by 92% in bean flour and 83.5% in chickpea. Fermentation with *P. acidipropionici* Q4 made it possible to reach 86% of TIA removal in both flours and 60-65% of AIU in chickpea flour and 100% in beans. Both microorganisms were able to grow and acidify these substrates, reaching counts of  $4.0 \times 10^8$  CFU/g and pH  $4.75 \pm 0.17$  for strain GS30 and  $6.5 \times 10^8$  CFU/g, pH  $4.76 \pm 0.32$  for strain Q4. Results show that both strains have the ability to ferment legume flours and may contribute to ANFs removal, which represents an attractive alternative to improve the nutritional quality of these foods.

#### A95

### WORKSHOP: TOWARDS A BETTER UNDERSTANDING OF PROBLEMATIC CONTENTS IN PHYSIOLOGY

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Physiology is an important subject in the training of Pharmacists and Biochemists. Its promotion requires that students acquire the ability to integrate, organize and transmit knowledge. On this basis, in 2009, 2012 and 2015, our chair offered regular workshops as a teaching strategy for students who had failed their final exam. Selected problem contents were dealt with in 3 weekly meetings of 3 hours each, in which teachers played the role of moderators and facilitators while students were the actors responsible for the teaching-learning process. This pedagogical strategy was well received as reflected in the number of students enrolled, the percentage of students present at the meetings, the great interest in discussing the issues raised and the outstanding participation of students who achieved the desired goals. Workshop achievement was evaluated by testing the students' ability to pass the final exam. It is important to note that 80% of the participants took the exam after the workshop ended and 75% of them passed it with high grades (7-10). Some of the students' comments on the workshop were: "The activity was very interesting", "It helped me clarify many doubts that sometimes cannot be properly dealt with in consultation classes", "It was a great opportunity to establish a better contact with the professors", "It helped me to be more positive, to express and release tension before the examination", "I would suggest that this activity be continued for the benefit of other students".

#### A96

### MEANING AND EVALUATION OF HEALTHY EATING IN SECONDARY STUDENTS OF CATAMARCA CAPITAL CITY.

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Previous knowledge of students' opinions can improve the teaching-learning process since it allows the educator to design appropriate and innovative activities. Objectives: to know the meaning, evaluation and foundation of healthy eating in secondary students of Catamarca capital city. Methodology: A mixed transversal descriptive study with randomly chosen 423 2<sup>nd</sup>- year students between 14 and 16 years of age. They were asked by means of structured semi surveys what they thought was the meaning of healthy eating, what were the main components of a healthy diet and why. Results: With respect to meaning: important for health (27.1%); to have a varied and balanced diet (23.4%); in order to provide energy (16.8%); to satisfy hunger (2.1%), undifferentiated answers such as "to be able to live" (4.7%); "to be nurtured" (12.5%), to satisfy bodily needs (1.4%). Components of a healthy diet: 43.23% fruits and

vegetables; 13.48% meat; 4.02 %milk and milk products; 2.84% pasta. The reasons for their choice: 25% because they are healthy, 21% because they provide vitamins and minerals, 14% because it is varied; 9%because it is nutritious, 8% because it contributes energy and strength, 4% do not eat them because they cannot afford them; 6% because they like such foods. Conclusions: Both the meaning and the evaluation that the students assigned to healthy eating were based on biological and sociocultural aspects such as the care and protection of health, hunger, preferences, etc., with coherence between the meaning, the evaluation of the foods and the reasons for their answers.

#### A97

### EFFICIENCY OF THE NTICS AS A DIDACTIC TOOL IN CELULAR BIOLOGY CLASSES

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Kokori is a videogame in which players must control robots that travel around the animal cell; those robots help to overcome different problems. This videogame consists of 7 missions focused on cellular biology subjects. The videogame is an excellent opportunity to show the appearance of the cells in 3D as well as their components, the relative sizes of different structures and also several biological processes. The aims of our work was to teach Cellular Biology using the pedagogical software Kokori as a didactic tool in Biology classes for second year students in Formosa city. A mixed educational research was conducted including a descriptive, explanatory and comparative longitudinal and observational study, with a quasi-experimental design, pre-test and post- test. The population was made up of 2<sup>nd</sup> year students. A Class Observation Guide was used as a measuring device as well as grades records and semi-structured surveys.

In the pre- test, 25% of the students passed with grades between 6 and 10, while 75% of them failed. In the post-test, 96% of the students passed with grades between 6 and 10 and 4% of them failed. There were no students with low grades (1, 2 or 3) at this stage. In general, academic performance improved significantly after the application of the Kokori software. We conclude that the pedagogical and didactic importance of the application of the Kokori software in Cellular Biology classes was proven in our study.

#### A98

### PREVALENCE OF INTERNAL PARASITES IN DOGS AT THE HOSPITAL SCHOOL OF VETERINARY OF THE F.A.Z. FOR THE YEAR 2014

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Internal parasites are one of the most common disorders in dogs of all ages. Some of these parasites are important in public health because there are genres such as Toxocara and Ancylostoma that can affect man -zoonosis-, especially children. This work was developed in the Hospital School of the FAZ. Its aim was to determine the genus of the parasites prevalent in the area in order to establish measures to prevent, control and treat diseases in animals and people. The 406 stool samples of patients who attended the hospital were collected from April to December 2014. These samples were subjected to different diagnostic techniques: 1-Direct Simple Diagnosis, 2- Willis Flotation and 3- Flotation with Zinc Sulfate. In all cases the samples were observed under the microscope. The results indicate that several genera can parasitize dogs and some of them can affect humans and especially infants. The genera found were Ancylostoma (17.3%), Toxocara (7.6%), Trichuris (2.2%), Giardia (0.4%), Isospora (5.4%) and Dipylidium (4.0%).

We conclude that this study gives us an idea of the prevalence of parasites in the environment, which represent not only a danger to animal health but also for public health and gives us tools to take measures to sensitize the community about the risks involved in being in contact with animal feces and the importance of prevention.

#### A99

### PARTICIPANT OBSERVATION OF TEACHING RESOURCES FOR THE UNDERSTANDING OF HISTOLOGY CONTENTS

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It is frequently asserted that students are not formed in a subjective university, that they evidence lack of interest and difficulty in reading comprehension tasks and oral-written skills. In times of information and communication technology, we believe that the development of a pedagogic-didactic model might contribute to solve these problems. The objective of this work was to observe by participating and to analyze the mediation of resources, considering the consequences they have on the learning of histology contents. Qualitative-quantitative surveys were carried out to access the individuals' discourse. Registers were kept concerning the participant observation of resources to teach histology, and analysis categories were established for their study. In the observation process, this taxonomy was applied in its formal (attendance, observance of instructions, acceptance of conditions); content (comprehension of contents, structuring of concepts, systematization, synthesis capacity) and pragmatic (attitude, involvement, emotion, solidarity)

aspects. The mediation of resources in context and the integration of syllabus contents, focused on teachers' task, facilitated the actors' learning and communication process. A reflection involving ourselves will allow the understanding of contemporary situations that require new methods of knowledge acquisition and the establishing of the possibility of constructing ourselves as teachers and students in the development of the teaching-learning process as a way of understanding the world.

#### A100

##### **COMMUNICATION MEDIATION OF RESOURCES FOR THE TEACHING OF HISTOLOGY CONTENTS.**

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The current paradigm of education is based on communication. From this point of view, learning resources comprise objects and individuals of the work process and the word itself, the teachers, the students, the environment, methods of diagnosis and the like. The present work was developed within the framework of the Project "The teaching of histology in Dental education at the university: An epistemological and pedagogical analysis of resources, didactic material and textbooks used in the training practice". The objective was to develop an innovative methodology for the mediation of teaching resources by promoting the construction of integrated contents. A survey about the use of textbooks was carried out among students of the subject Histology II who participated in a pilot program. Taxonomy was developed for the study of resources and their application in formal, content and pragmatic aspects. Group animation techniques were applied at the time of addressing, framing, problematization, production exchange, systematization, application and revising of what was performed. The survey evidenced that students used textbooks. We observed they possessed non systematized knowledge and constructed integrated histology contents. We conclude that the mediation of resources facilitated the construction of pragmatic contents and that students experienced the integration of basic knowledge in clinical textbooks.

#### A101

##### **STRATEGIES FOR STRENGTHENING THE TRAINING OF BIOLOGY TEACHING STUDENTS IN THE BOTANICAL GARDEN OF FCF-UNSE**

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The Botanical Garden "Ing. Ftal. Lucas D. Roic" is one of the most important sites of *ex-situ* conservation of native and exotic species of Santiago del Estero, having among its priorities the training of students on issues related to biodiversity and environmental education. In order to contribute to the training of biology students, strategies were designed aimed at strengthening the learning of contents related to plant systematics, with emphasis on regional groups of plants of provincial interest. Workshops for advanced students were held for recognition of plant species (gymnosperms, legumes and native woody trees belonging to the Santiago flora. They addressed topics related to plant diversity, characteristics of "taxa" (class, order, family) and morphological characters useful to recognize the species on field were analyzed in the Botanical Sectors; skills in the management of botanical literature and optical instruments were strengthened; teaching devices were designed such as guides, data sheets, photographic catalogs and interactive CDs.

A total of 68 senior students of the career of biology teacher of various local institutions participated in these activities in addition to the teachers in charge of related subjects. The activities developed demonstrated the role of botanical gardens as contexts conducive to the promotion of an active construction of knowledge related to biology education. These strategies are expected to have an impact on the formation of students as well as in their future teaching practice.

#### A102

##### **APPLIED ANATOMY INTERNSHIPS AS EDUCATIONAL SUPPORT IN VETERINARY MEDICINE**

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Anatomy is one of the basic pillars in the teaching of veterinary medicine. The number of students since its inception in 2003 has grown yearly so it is necessary to expand and diversify its study, including for this purpose species not normally studied in class. The objective of this internship is to give students experience with non-traditional animal species in dissection practices and thus strengthen their previous knowledge, to contrast with the typical distinctive characteristics of each species and to work respecting biosafety standards. We worked at the Museum that the Chair of Anatomy has in El Manantial with 15 students for six months, 4 hours a week. The species used were porcine, equine, caprine and turkey (*Meleagris gallopavo*). The students worked with surgical dissection instruments and were also provided with overalls, respiratory masks, protection masks, etc. All dissections began with skin, subcutaneous tissue and, muscles of the surface and deep layers in order to expose internal organs. The results were highly positive, as students participated actively and the evaluation standards showed that prior knowledge had been assimilated and that skills in

dissection techniques and in the use of specific material for the job had been acquired. We can conclude that the internship helped articulate theoretical with practical knowledge, strengthened dissection skills and instructed and trained students in group work while implementing biosecurity standards.

### A103

#### EVALUATION OF A UNIVERSITY VOLUNTEER PROJECT FOR STUDENTS AT THE FACULTY OF AGRONOMICS AND ANIMAL HUSBANDRY- UNT

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The impact on the overall education of the students participating in the Strengthening Agricultural University Volunteer Project for the Strengthening of Agricultural and Animal Husbandry (2013-2014 cycle) was evaluated. The project included 10 students and 4 teachers of Agronomics and Animal Husbandry, 20 families from cooperatives in El Timbo and Taco Ralo, Tucumán, whose diversified production is destined to local consumption and to the local market of pigs and beekeeping products, and technical advisers from AER- INTA-Graneros. The methodology consisted of briefings and training of students with later addition of local producers and technicians. Participatory diagnostic workshops were also developed where the needs of producers were detected and a work plan was designed. After each excursion, students had to submit technical reports on each activity performed for further evaluation. Surveys revealed that 56% of the students expressed interest in developing activities in the productive sector and 33% in integrating a multidisciplinary team. 67% were sure of the knowledge acquired during their training. During the course of the project, 30% +/- 10% felt supported by the teaching team and included in the production environment. Regarding achievements: 22% said they developed the ability to talk with the other participants, 21% learnt to work in multidisciplinary teams and 16% to interact with producers. This innovative way of working, had a highly positive impact on the overall education of students and we suggest the continuance of this methodology.

### A104

#### THE MUSEUM AS AN EDUCATIONAL RESOURCE FOR THE TEACHING OF NATURAL SCIENCES

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One of the current challenges in teaching is to abandon the traditional model and place the student as a leading actor in the teaching-learning processes, with new educational alternatives. The Museum is an institution that focuses on the acquisition, preservation, research, and transmission of information for study, education and recreation. The aim is to diagnose the utilization of the Museum as a pedagogical tool at the high school level. The sample consisted of 1038 students of a cycle oriented toward natural sciences from 8 private and state institutions located in the center of San Miguel de Tucumán and its nearby municipalities. Questionnaires, later quantified statistically, were used. 100% (f = 1038) acknowledged the importance of the Museum in teaching. 70.13% (f = 728) had never been in a museum. Out of the 29.87% (f = 310) that had visited a Museum, 120 belonged to public and 190 to private institutions. Out of the 120, 89 students attend institutions located in the downtown and 31 to institution of nearby municipalities. Out of the 190 students from private institutions, 111 attended institutions in the downtown and 70 in nearby areas. The reasons for this situation are: 1 - most teachers do not think that visits to a museum are necessary. 2-lack of means. 3 - some teachers believe that students not living in the downtown area do not require visiting a museum. In conclusion, we can say that there are informal sources that complement, enrich and promote scientific education and improve learning. Museums respond to new approaches in teaching and put emphasis on scientific literacy. The need exists to establish criteria aimed at achieving equal opportunities for all students.

### A105

#### INCORPORATION OF ICTs AS AN INNOVATIVE TEACHING STRATEGY TO THE FARMACOLOGY AND THERAPEUTICS DEPARTMENT.

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ICTs (information and communications technology) and innovative strategies were implemented for the integration of knowledge and the ability to apply it with adequate clinical criteria. Objectives: - To guide students in the use of new tools to analyze learning; to analyze passing rates in the learning of the subject Local Anesthetics (AL); to evaluate the students' development of AL through ICTs. Materials and Methods: ICTs used by students and teachers were Web Quest (wq), Spycinodes (spy) and multimedia materials (mm) (interactive video) in the learning of AL. Students were divided into Group 1: Control (c), Group 2 (spy), Group 3 (wq) and Group 4 (mm). They were evaluated through a written exam and an individual work after training by the teachers on the use of ICTs. The

students were subjected to an opinion poll to determine difficulties in learning the subject using ICTs. The INFOSTAT and SPSS programs for the statistical treatment of the data were used. Results: for 60% of students who used ICTs, teachers' instruction facilitated learning. In the written test and individual work to assess the issue to no significant differences were found between the ICTs employed in the different groups compared to the control group ( $p > 0.05$ ). Conclusions: training students in the use of ICTs stimulates their interest in investigating to learn, to understand biological phenomena and clinical procedures. These tools favor passing rates in the learning of subjects and no difficulties were found in their handling.

#### A106

### TRAINING OF VULNERABLE WOMEN IN THE PRODUCTION OF THE EDIBLE MUSHROOM *Pleurotus ostreatus* – GÍRGOLA, IN CHILECITO, PROVINCE OF LA RIOJA, ARGENTINA.

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The production of the edible mushroom *Pleurotus ostreatus* - Gírgola and its consumption in the province of La Rioja is limited by its cost and by the lack of knowledge of the community since it has not been <sup>incorporated</sup> into the diet. It has a high nutritional value as it provides vitamins of the group of tiamins (B1), riboflavins(B2), pantothenic acid (B3), ascorbic acid and biotin (H). It prevents diseases such as diabetes and cholesterol. In addition to conducting research on its antitumor properties, teachers and students of the UNDeC trained 30 women in gírgola production in the Women's Council. The objectives proposed were: to promote an independent working activity for women in conditions of vulnerability; to train these women in the production, consumption and commercialization of the mushroom Gírgola to incorporate this "vegetable" into the diet. Practical and theoretical classes were taught on the various stages of production and marketing of Gírgola, as well as tasting of the product. Spreading its consumption would provide the possibility of encouraging the production of this nutritive resource in the province of La Rioja as a new communal agricultural activity.

#### A107

### HABITS IN THE CONSUMPTION OF FRUITS AND VEGETABLES: CONCERNS ABOUT THE USE OF PESTICIDES

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Not much is known about the safety of fruits and vegetables for consumption. It is assumed that consumers demand low levels of agrochemicals in food production. The objective of this study was to analyze the habits of consumers from Rio Cuarto city with respect to fruits and vegetables in relation to contaminants, especially pesticides, in order to promote and enhance the fruit and vegetable production chain. The project was based on 580 surveys distributed to both families of preschool students from 8 institutions and participants in the Adults Education Program, UNRC. The results show that 51% claimed that they were aware of the risk of contamination by pesticide residues, 39% by microorganisms and 14% by physical contaminants. 62% of the people did not answer the survey or did not know whether fruits and vegetables underwent inspection. 38% of the people knew that these types of products undergo some controls. As to the use of pesticides, 28.1% answered that they generated profits, 47.1% expressed their rejection to their use and 24.8% did not answer. The issue of the excessive use of pesticides and their potential impact on health and the environment is already installed in society. It is important to highlight that, despite this fact, only 0.17% of those polled stated that they would choose a product if it were organic and 1% would buy a fruit or vegetable with the presence of larvae, a fact indicating the non-use of pesticides. This project provides valuable information about the habits of consumers from Rio Cuarto city with the objective of improving the family diet. This information would allow the regulation of the production management, related to Good Agricultural Practices, and encourage consumption, mainly in school-age children, who are the ones most influenced by junk food.

#### A108

### LIFE CYCLE OF *Plodia interpunctella* IN STORED WALNUTS UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

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*Plodia interpunctella* (Lep.: Pyralidae), known as Indian mealmoth, is a pest of stored products of global significance and one of the main factors involved in the decrease in the production of stored nuts in La Rioja. Considering that its life cycle depends on environmental conditions and on the host, the aim of this study was to describe the life cycle of *Plodia interpunctella* in nuts under environmental conditions similar to those in local nut storehouses.

The study of the life cycle of *P. interpunctella* was performed in a breeding chamber at temperatures of  $24 \pm 1^\circ\text{C}$  and relative humidity of  $55 \pm 3\%$ . Eggs were collected and incubated until larval emergence ( $n=160$ ); larvae were placed in individual containers with nuts

as food. The number and duration of the larval stages were determined by the appearance of a new cephalic cap and each instar was characterized on the basis of morphological and behavioral criteria.

Pupae were measured and sexed and time of adult emergence was monitored. Adult couples were grouped to control oviposition and hatching. The life cycle of *P.interpunctella* lasted an average of 71.42 days from egg hatching to adult death. The egg stage lasted 4-5 days with a hatching rate of 73.5%. Six instars with a total development time of  $52 \pm 2$  days were determined. The duration of the pupa stage was  $10 \pm 1$  days and the percentage of eggs laid per female was  $132 \pm 10$ . The data obtained provide valuable information for pest management strategies during nut storage.

#### A109

### RESPONSE OF EXPERIMENTAL MAIZE HYBRIDS TO HIGH AND LOW DENSITIES

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There is variability in tolerance to high densities in maize crops (Andrade et al. 1992). The objective of this work was to evaluate 20 experimental hybrids (H1-H20) and 3 commercial hybrids (Pioneer P31T17=H21, Monsanto DK390RR2=H22 and Syngenta NK120TDMMaxRR2=H23) under two plant densities ( $4.97 \text{ plants.m}^{-2}$ =D1 and  $8.4 \text{ plants.m}^{-2}$ =D2). For that purpose, an experiment was carried out in Leales, Tucumán, Argentina ( $27^{\circ}03' \text{LS}$ ;  $64^{\circ}15' \text{LW}$ ; 330 masl). Hybrids were sown on 12/30/2011 in  $7 \text{ m}^{-2}$  plots, under a split-plot design (main plot: density; sub-plot: hybrid) with 3 replications. Yield (R), prolificacy (PR) and days to flowering (DF) were determined. Regarding R (CV=9.96), ANOVA was only significant for H ( $p < 0.0001$ ), which indicates that R was not affected by the increase in D. Eight experimental hybrids (17, 8, 5, 1, 12, 16, 10 and 6) showed higher R than H22. Regarding PR (CV=12.19), ANOVA was significant for H ( $p = 0.008$ ) and D ( $p = 0.0002$ ), while as regards DF (CV=2.37), ANOVA was significant for H and D ( $p < 0.0001$ ) and for their interaction HxD ( $p = 0.0001$ ). LSD test ( $\alpha = 0.05$ ) for PR and DF showed significant differences when comparing D main effects. Hybrids had higher PR under D1 (1.06 vs 0.98), while under D2, hybrids had higher DF (49 vs 48.25). Increased density stress did not affect yield in any of the evaluated hybrids, but it did affect PR and DF. The fact that yield was not affected by density indicates stability for this character under the conditions of this experiment.

#### A110

### CHEMICAL CONTROL OF OVERSEEDED *Lolium perenne* IN *Cynodon dactylon* TURFGRASS DURING THE SPRING TRANSITION

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Turfgrasses of *Cynodon dactylon* (Bermuda) of the south center of Cordoba province are overseeded by *Lolium perenne* (Ryegrass perenne) to support a green cover all year round and reduce Bermuda wear in dormancy. New Ryegrass perenne varieties are heat tolerant and persist during the spring, which delays Bermuda regrowth, causing a poor spring transition. A shorter spring transition can be achieved with the use of herbicides to gradually control the overseeded species. Temperatures at the time of application might influence herbicide efficiency and affect the duration of spring transition. The aim of this study was to evaluate the effect of Foramsulfuron, Trifloxysulfuron, Propizamida and Metsulfuron on overall quality, ground cover and Ryegrass perenne injury, in four application dates, recording mean soil temperature (TMS). The lack of Ryegrass perenne control during the spring transition allows high turfgrass quality and cover, but causes less Bermuda cover. This management would cause a weakening of Bermuda if this practice is repeated in successive years. TMS explains better herbicide efficiency so that it would be a good indicator to decide application time. With lower TMS applications, major overall quality and cover can be maintained as a consequence of decreased Ryegrass perenne injury. Propizamida can maintain high overall quality and ground cover without causing a decrease in Bermuda regrowth. Foramsulfuron, Metsulfuron and Trifloxysulfuron produce a great degree of Ryegrass perenne injury, which causes a loss in overall quality and ground cover at 4 SDA, but this behavior does not cause an increase in Bermuda cover towards the end of spring and the beginning of summer.

#### A111

### PEANUT GENOTYPES GERMINATION ACCORDING TO ORIGIN AND SIZE OF SEEDS

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The low quality of seeds can affect plant establishment in early sowing of groundnut (*Arachis hypogaea* L.). The objective of this work was to evaluate the behavior of seeds according to their origin and size at different germination temperatures. The origin of seeds were different sowing dates (FS) in Río Cuarto-Córdoba (FS1: 08/10; FS2: 10/11; FS3: 10/12) and the size of the seeds (sieves: 9, 8, 7 5; 7; 6.5 and 6 mm) of two peanut genotypes (Granoleico: alternating structure and long cycle; Utre: sequential structure and short

cycle). Germination was evaluated by: (1) pattern test and (2) different temperatures (10, 14, 18, 22 and 28 °C) (one month), normal seedlings and dead and fresh seeds being recorded. Pattern test germination (TPG), mean germination time (TMG) for each temperature and the correlation between TPG and dead seeds (DS) and fresh seeds (FrS) at 10 and 14 °C were estimated. At 10 °C there was no germination. Granoleico presented variations in TMG; this increased with delay of FS, and diminished with increasing seed size. There was a positive correlation between TPG and the size of the seeds for Granoleico ( $p < 0.05$ ). At 10 °C, the correlation between DS and TPG was significantly negative in Granoleico (FS1 and FS2) while correlation between TPG and FrS was positive for Granoleico (FS1 and FS2) and Utre (FS1 and FS3). At 14 °C, the correlations between DS and FrS, and TPG were not significant for any of the cultivars. In conclusion, the seeds of these genotypes have different behaviors when exposed to different temperatures during the growth and development of parent plants as well as during germination.

#### A112

### QUANTIFICATION OF INFESTATION POTENTIAL (IP) COMPONENTS OF *Panicum maximum* Jacq. (PANMA) WEED IN SUGARCANE CROPS FROM RIO COLORADO (LEALES, TUCUMÁN).

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*P. maximum* is an important weed of sugarcane crops in the North of Argentina. The objective of this work was to determine the infestation potential (IP) of PANMA and the quantification of the components of that potential. Work was made at cv LCP 85-384, 4-year ratoon, from Río Colorado (Leales, Tucumán) (27°09'S-65°21'22"W) in 80m<sup>2</sup> plots with 5 randomly replications during 2013. Inside each plot, number of stumps, number of stems, seed production per plant and surface were determined. Viability and germinative power of 1000 seeds per plot were studied in the laboratory. For entering seeds, 33% of seeds produced was considered and IRC (individuals with Reproductive Capacity), RRC (Real Reproductive Capacity and IP (Infestation Potential) were calculated. Parametric statistical analysis using ANOVA with Tukey's Test,  $\alpha = 0.05$ , was used. The statistical model was obtained using multiple regression analysis proceeding with software R. Results were: Stem average density of the weed was 45.60 stems.m<sup>-2</sup>; 978 seeds.stem<sup>-1</sup>; 44.708 seeds.m<sup>-2</sup>; seeds entering the bank 14,753.6 (33%); 35% viability; 42% germinative power; IRC=5163.7; RRC=2168.7. Average IP was 47.44m<sup>2</sup>.pl<sup>-1</sup>, which establishes that this surface will be occupied by the descendants of one plant during the next reproductive cycle. A low infestation potential was obtained, indicating that the weed species is slowly invading new sugarcane crops and new natural areas.

#### A113

### SEEDS OF *Sicyos polyacanthus* Cogn. ENTERING THE SOIL BANK IN THE SUGARCANE CROP OF MANANTIAL (TUCUMÁN, ARGENTINA).

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*S. polyacanthus* is a Cucurbitaceae weed of great importance in Tucumán sugarcane crops. The incorporation of conservative techniques for the sustainable management of sugarcane crops favored the new invasions of this weed. The objective of this work was to analyze the seed production of *S. Polyacanthus* and its entrance to the soil-bank. Samplings were made at the Experimental Field of El Manantial (Faculty of Agronomy and Zootechny-FAZ-UNT) on cv LCP 85-384, 4-year ratoon cane, with intermediate to high infestation (0.85-3.65pl.m<sup>-2</sup>) of *S. polyacanthus*, in 2014. Experimental plots were of 32 m<sup>2</sup> with 5 replications. In each plot a 6 m<sup>2</sup>-surface was covered with a plastic cover (200µ) for the weekly counting of the fruit-seeds from May to the end of July. Three controls were kept without covering and the same counting was made. Parametric statistical analysis by means of ANOVA with Tukey's Test for  $\alpha = 0.05$  was used in production analyses and loss of seeds and plantlets. The results were: Weed density: 0.85 pl.m<sup>-2</sup>; Seed production: 1,280 seeds.pl<sup>-1</sup>; Emerged Plantlets: 305 plantlets; Seeds entering the soil bank: 23.82%, Loss of seeds from the plant to the soil: 76.17% due to biotic, abiotic and anthropic causes. Total number of seeds entering the soil bank is 2,025 seeds, from which 482.35 plantlets will germinate in December and 0.25% will survive as a formed plant next year in March.

#### A114

### EVALUATION OF WATER ABSORPTION AND WEIGHT OF QUINOA SEEDS WITH DIFFERENT LEVELS OF NPK FERTILIZATION.

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Water absorption and weight of quinoa grain seeds, cv. CICA, obtained with different levels of NPK fertilization were evaluated. The grains were obtained from Agrotechnical School EMETA Tafi del Valle, Tucumán, at 2000 masl between 2014/2015, from fertilization treatments: T1- Control; T2- 90 kg/ha of N, 75 kg/ha of P2O5 and 60 kg/ha of K2O; T3- 75 kg/ha of N, 50 kg/ha of P2O5

and 80 kg/ha of K<sub>2</sub>O; T4- 60 kg/ha of N, 50 kg/ha of P<sub>2</sub>O<sub>5</sub> and 40 kg/ha of K<sub>2</sub>O; T5- 90 kg/ha of N, 50 kg/ha of P<sub>2</sub>O<sub>5</sub> and 30 kg/ha K<sub>2</sub>O. The water absorption studies were carried out in the CEPA Laboratory of INTA Catamarca; incubation was at 30 °C for 24 h, with the formula:  $Abs. (\%) = (PF_f - PFI/PFI) \times 100$ ; seed weight was performed with Mettler H10 balance in the Laboratory of Phytopathology of the FAZ, UNT. The experimental design was RCB with five treatments and four replicates with 100 seeds per treatment and replicate. The parameters evaluated were water absorption and weight of 1000 seeds of different fertilization treatments. ANOVA, Tukey's test ( $p=0.05$ ) for the comparison of means and Pearson's correlation coefficient between the variables under study were performed. In water absorption by seeds, no differences were observed between treatments; differences in weight were found between 1000 seed treatment T5 (2.949g) compared to control (2.639g). No positive correlation between the parameters evaluated was observed. Fertilization levels of 90 kg/ha of N 50 kg/ha of P<sub>2</sub>O<sub>5</sub> and 30 kg/ha of K<sub>2</sub>O increased grain weight, but did not affect water imbibition by the quinoa seeds, cv. CICA.

#### A115

### RESULT OF DIFFERENT RATIOS OF ORGANIC SUBSTRATES IN LETTUCE SEEDLINGS TREATED WITH ORGANIC FERTILIZER IN SEEDBEDS IN TUCUMÁN

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A substrate provides support to plants, promotes rooting, nutrition, strengthening of the stem and cation exchange. *Azospirillum* sp. colonizes plant roots and fixes nitrogen, solubilizes phosphate, and produces plant hormones and siderophores. The objective of this trial was to evaluate the response of different proportions of organic substrates in lettuce seeds inoculated with *Azospirillum* sp. on the germination and growth of seedlings in nursery. We worked with lettuce cultivar Grand Rapids in the greenhouse of the Facultad de Agronomía y Zootecnia, UNT. The treatments were T1: organic soil + seed without inoculation; T2: organic soil + seed inoculated with *Azospirillum*; T3: soil + seed inoculated with *Azospirillum*. T4: organic soil + sand (80:20) with seed inoculated with *Azospirillum*; T5: organic soil + sand (60:40) + seed inoculated with *Azospirillum*. T6: Soil + sand (40:60) with *Azospirillum* and inoculated seed. T7: Soil + sand (20:80) with *Azospirillum* and inoculated seed. CRB design was with 7 treatments and 3 replicates. At 10 days we evaluated: % of sprouts; at 35 days we determined plant height (cm) (PH); dry weight (g.) of aerial parts (DWAP) and roots (DWR) The results were analyzed by ANOVA, Tukey's test ( $p=0.05$ ) and Pearson's correlation coefficient. The results showed that in (PH) T5, T4, T6, T3 differed from T1, T2 and T6. In (DWAP) T6 differed from T1, T4 and T5; in (DWR) T2e differed from T3, T4 and T5. The correlation was significant between DWR and DWAP. Organic soil nutrients participated with nutrients and the contribution of *Azospirillum* sp., in substrate and/or seeds, offset the decrease in the proportion of soil in lettuce seedlings.

#### A116

### STUDY OF THE HEIGHT OF NURSERY OLIVE TREES TREATED WITH OVINE MANURE, CRUDE OVINE MANURE AND NURSERY SUBSTRATE.

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One way to increase soil fertility and edaphic conditions is by incorporating biocomposted organic residue, biocompost being the product obtained by the inoculation of specific microorganisms into the native bacterial population present in the residue to be composted. The objective of this study was to compare the effects of the biocompost of ovine manure with the crude ovine manure added to the nursery substrate on the height of young olive plants, establishing the nursery substrate as a control. 960 flowerpots with young olive plants, arbequina variety, were formed, distributed in 5 treatments, working with biocompost and crude non composted manure at 12.5% and 25% with substrate and 100% nursery substrate. Each treatment had 192 young trees (seedlings) distributed in 3 replications with 64 individuals each. 20 individuals were taken at random from each replication and their height was measured for 8 months. The work was done with a completely randomized design with measurements repeated over time. The measurements were analyzed with ANOVA determining the source of variability between the different treatments. The result was that the young trees with 12.5% biocompost and substrate showed a growth with a very significant difference with respect to the other treatments. The treatments: 25% biocompost, 12.5 and 25% fresh manure and control did not show significant differences between them. In conclusion, the biocompost at 12.5% had the largest increase in height with respect to the rest of the treatments, significantly surpassing the substrate used in the local nursery.

A117

**VARIATION IN ADULT POPULATIONS OF *Ceratitis capitata* WIED. IN CITRUS GROVES IN SANTIAGO DEL ESTERO**

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The geographic areas in northeastern and northwestern Argentina have a suitable climate for the development of *Ceratitis capitata*, although the extreme conditions prevalent in these regions may impose restrictions on population growth. Seasonal variation of *C. capitata* has been studied in two geographic areas of Santiago del Estero, under irrigation. During the years 2013 – 2014, Jackson traps with sexual attractant for males of *C. capitata* were placed in plantations in the Departments Robles and Banda. The traps were located at a height of 1.5 to 2.0 m from the ground. The host species were: sweet orange and tangerine type orange (*C.sinensis*), grapefruit (*C.paradisi*) and common mandarin (*C.reticulata*).

The traps were monitored every two weeks. Adults captured were identified in the laboratory and expressed as average number of flies per trap per day (FTD) for each department. The data of temperature, relative humidity and rainfall were taken from the meteorological station of INTA La María. In plantations located in the Department Robles (2013), density values ranging from 0.1 to 0.5 FTD were observed, and in 2014 the population showed values between 0-0.3 FTD. Significantly, during the last year the amount of fruit decreased due to hail. In both periods evaluated a similar behavior was observed for the two populations; the apparent differences may be due to the temperatures for the year 2013 which were more favorable for the species.

A118

**STUDY OF MANAGEMENT AND HEALTH OF CITRUS FRUIT FARMS OF SANTIAGO DEL ESTERO- ARGENTINA**

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Citrus production in the study area, characterized by excellent fruit quality and extra early production, reappears after a long period of decline. Plantations were described in relation to phytosanitary, edaphoclimatic and cultural management variables that influence quality and production. Multiple correspondence analyses were used to evaluate 63 variables in 51 groves with 0.5 to 250 ha. Some of the variables studied were: total surface area and for each species, soil and climate factors, machinery and installations, management and technology, varieties and presence or absence of disease and pests. From the analysis, according to the level of similarity, 4 distinct groups emerged on the 1-2 factorial plane, which shows differences related to cultural management, presence or absence of pests and diseases, machinery and facilities used and production destination. We conclude that growers in Group 1 need technical assistance about the most appropriate "foot-crown" type combination and the efficient use of agrochemicals. The citrus groves in Group 2 were found to be the most desirable for small-scale management. Those belonging to Group 3 showed recommended handling characteristics, especially with respect to the "foot-crown" combination, although deficiencies in periodic fertilization and in the implementation of sufficient windbreaks were observed. Citrus groves for Group 4 require the gradual renovation of old and/or sick plants together with an increase in the frequency of thinning, cleaning and pruning, and a more careful handling of machinery.

A119

**BIOMASS YIELD AND ESSENTIAL OILS OF *Mentha arvensis* L. IN SANTIAGO DEL ESTERO**

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The main product of the genus *Mentha* is the essential oil that is produced and stored in glandular trichomes, especially those present in flowers, leaves and stems. The main component of *M. arvensis* is menthol, which has wide applicability in the pharmaceutical, food and cosmetic industries. This study aimed at determining the yield of fresh weight, dry weight and content of essential oils of *M. arvensis*. In the experimental field of FAyA, cultivation was implanted on 04/04/13, under irrigation. Plots of 36 m<sup>2</sup>, consisting of 20 boards with a space of 0.60 m between them were used. An experimental design of randomized blocks with five replications was used. When the culture reached about 70% flowering, harvesting was done by hand, leaving a remainder of 10 cm in height. Two cuts were made on 17/12/13 and 04/03/14. The samples were weighed and dried to constant weight in an oven. Other samples were dried at room temperature for the extraction of essential oils by steam stripping and subsequent chromatographic analysis to determine their chemical composition. Yields of 7 and 9 tn fresh weight, 3 and 2 tn dry weight, and 1.6% and 1.2% essential oils were obtained for the first and second cut respectively. The percentage of menthol content ranged from 65.3 to 59.1% in the first and second cut respectively. These results make the cultivation of *M. arvensis* in Santiago del Estero a promising activity.

**A120**

**CULTIVATION of *Pleurotus ostreatus*-GIRGOLA BY RECYCLING WALNUT SHELLS IN CHILECITO - LA RIOJA, ARGENTINA.**

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Argentina is the country with the smallest fungi consumption in Latin America, although at present it is increasing to their nutritive and medicinal value. *Pleurotus ostreatus* production is performed as a micro-family business or PYMES due to the simplicity of production and adaptability to residual substrata. In Chilecito the main industries are based on walnut, olive and grape production, generating large volumes of organic residues. In this work we investigated the proper substrate for the growth of *Girgola* by recycling agro-industrial residues in the region. We used the technique of cultivation of fungi in plastic bags. Tested substrata: a) poplar sawdust + oak chip, b) sawdust + grape residues, and c) nutshells. Substrata were brought to pH 6 with lime or gypsum. The bags with the moistened substrate (1 and 1/2 kg) were sterilized in an autoclave (1 atm pressure for 2 hs). Later, commercial seeds of *P. ostreatus* were sown. The bags were placed in shelves at 25 °C for micelial development. After 30 days the colonized bags were perforated and taken to a room for 10 more days with a relative moisture of 85-90%, T 15 °C, light and ventilation. Out of the 3 substrates tested, c) was the one with the best micelial development and rapid growth of the mushrooms. Therefore the best adaptability of *Girgola* was in nutshell, this readily available substrate being selected for production purposes.

**A121**

**CONTENT OF PYRROLIZIDINE ALKALOIDS IN HONEY FROM THE ARGENTINE NORTHWEST**

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Pyrrrolizidine alkaloids (PA) and their N-oxides are probably the most widely distributed natural toxins that affect wildlife, livestock and humans. These toxins are a large group of metabolites produced by plants and are found in a wide variety of species in nature. PA are not toxic in themselves, but through their metabolic products they are transformed by liver enzymes into pyrrols, which cause damage at the chromosomal level. In the case of honey, the amounts and varieties of PA observed are very diverse, the same as the variability of the flowers visited by bees. An HPLC MS/MS analytical method was validated for the determination of pyrrrolizidine alkaloids and N-oxides in honey, using solid phase extraction for the cleaning up of samples, with a detection limit of 0.03 µg.Kg<sup>-1</sup> and a quantification limit of 0.10 µg.Kg<sup>-1</sup>. 44 samples of honey from the provinces of Tucumán, Salta and Santiago del Estero were analyzed, with different flower origin. Retrorsine content varied between 30.7 and 80.1 µg.Kg<sup>-1</sup>, and N-oxides of retrorsine varied between undetected and 105.6 µg.Kg<sup>-1</sup>. Crotaline content varied between 28.97 and 409.5 µg.Kg<sup>-1</sup>, crotaline N-oxides not being detected. Senecionine content varied between 1.67 and 13.20 µg.Kg<sup>-1</sup> while N-oxides were not detected. In half of the analyzed samples the presence of echimidine and lycopsamine was also detected. We will continue analyzing samples of honey from different regions of Argentina. At present, there are no maximum limits established for these contaminants in honey.

**A122**

**BOTANICAL PRODUCTS FOR INSECT PEST CONTROL IN STORED FRUIT**

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Walnut production in La Rioja, Argentina, is an activity of great importance in the economy of smallholders. No insecticides are applied in storage sheds and stored walnuts suffer insect damages. Insecticides to control pests in warehouses are too costly, so producers sell their walnuts quickly to prevent infestations. *Oryzaephilus surinamensis* (Coleoptera: Cucujinae) is a common pest in stored walnuts in La Rioja, causing losses in quality and product price. On the other hand the possibilities of insecticides of botanical origin (extracts or essential oils) for pest control is currently observed. These products can have preventive rather than curative action. The aim of this study was evaluate the effects of ethanol extracts of the aerial parts of *Ipomea cairica* (Convolvulaceae) applied to the natural diet of the above beetle. We carried out specific repellency and toxicity bioassays to test the effect of ethanolic extracts of flowers and leaves of *I. cairica* separately, at single doses (250 µg / g natural diet). The bioassays determined that the flower extract resulted attractant and toxic while leaf extract showed repellent and toxic effects on *O. surinamensis*. Both extracts are easy to prepare by smallholders and can be lyophilized for convenient use. *I. cairica* is a common weed in the region. Both products can be used at very low cost as *O. surinamensis* controllers in sheds and warehouses.

A123

**NATURAL PRODUCTS FOR THE CONTROL OF A *Verticillium dahliae* BIOTYPE**

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Verticillium wilt caused by *Verticillium dahliae* is the main problem affecting olive plants in Argentina. The aim of this work was to evaluate the antifungal activity of essential oil (AE), 2R - (-) - 6- hydroxytremetone metabolite of *Xenophyllum poposum* (Asteraceae) and pyrrolizidine alkaloids extract of *S. rubdeckiaefolius* (Sr) on a biotype of *Verticillium dahliae* isolated from olives trees in La Rioja province. Colonies of 5 mm diameter of biotype fungus were placed in the middle of Petri dishes containing APG poisoning with: 2R - (-) - 6- hydroxytremetone (T); essential oil (AE) of *X. poposum*; and pyrrolizidine alkaloids extract of *S. rubdeckiaefolius* (Sr) at two doses: 50 and 100 ppm. The controls were a commercial fungicide at doses suggested on the label (Cf = Carbendazim + thiuram) and untreated medium. They were incubated at 25 ± 2 ° C in the dark. Micellar growth at 72 and 120 hours was evaluated. At 72 hours, the results were: AE (50 ppm) 64.10%, AE (100 ppm) 69.50%; T (50 ppm) 49.51%, T (100 ppm) 77.56%; Sr (50 ppm) 55.69%; Sr (100 ppm) 67.33%; Cf 92.98%. At 120 hours: AE (50 ppm) 76.90, AE (100 ppm) 80.81; T (50 ppm) 90.79%, T (100 ppm) 99.17%; Sr (50 ppm) 95.31%; Sr (100 ppm) 96.15%; Cf: 93.35%. At 120 h, pyrrolizidine alkaloids extract of *S. rubdeckiaefolius* and 2R - (-) - 6- hydroxytremetone were highly effective to control the *Verticillium* biotype isolated in La Rioja, Argentina.

A124

**PRELIMINARY GENETIC STUDIES AIMED AT THE CONSERVATION OF GERMLASM OF AROMATIC PLANTS FROM TAFÍ DEL VALLE, TUCUMÁN**

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In Tucumán, there are native aromatic plants with therapeutic properties such as *Aloysia gratissima* (Gill. et Hook) Tronc, *Chenopodium graveolens* Willd. and *Clinopodium gilliesii* (Benth.) Kuntze. The last two share an ecological niche and grow between 2200 and 4500 masl. *A. gratissima* and *C. graveolens* are required for their digestive properties. *C. gilliesii* stands out because of its antifungal and antioxidant potential. The objective of this work is the genetic and reproductive characterization of *A. gratissima*, *C. graveolens* and *C. gilliesii*. The material came from Tafi del Valle (Tucumán). Traditional cytogenetic techniques of mitosis, meiosis and pollen grain viability were used to evaluate reproductive potential. In *C. gilliesii* clonal multiplication of fifty stakes in clinkstone plus ANA solution 1:1 was made. Results were: for *A. gratissima* 2n=54 with irregularity in meiosis and 7-9% of pollen viability. These values were similar to those of *C. gilliesii*, but the chromosomal number was not confirmed in this case because of its size (0.2µ approx). Chromosomes at diakinesis indicate that it could be a polyploid with x=5, unfertile. The results of the stake clonation of *C. gilliesii* were negative, without root induction. In *C. graveolens* meiosis was regular, with 2n=54 and 93.4% of pollen viability. The situation found in *C. graveolens* reveals an evolution process of diploidization that accounts for the regularity in meiosis and the good seed germinative power, a useful condition for the conservation of its germplasm. In the other two species the clonation tasks should continue for the preservation of their genetic characteristics.

A125

**CYTOGENETIC CHARACTERIZATION OF *Jacaranda mimosifolia* (BIGNONIACEAE)**

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The Bignoniaceae family has a cosmopolitan distribution and grows in neotropical regions. In Argentina, there are 22 genera and near 50 species belonging to 4 tribes. *Jacaranda mimosifolia* D. Don. (Tecomeae) is a deciduous tree, native to northwestern Argentina. Its seed oil has a high content of conjugated linolenic acid with anticarcinogenic properties, and could be used as a functional ingredient for foods. Cytological studies about it are scarce, the aim of this study being the evaluation of the cytogenetics characteristics of this species. Conventional cytogenetical techniques and DAPI fluorescence staining methods were used for root tips and flower buds, to determine chromosome counting, ploidy, karyotype formulae, asymmetry index and meiotic behaviour. A karyotype 2n=36 was determined, with small metacentric chromosomes, length from 1.4 to 2.62 µm, and the presence of terminal satellites in the short arm of the second chromosome pair. Asymmetry indexes were A1 0.28 and A2 0.17 corresponding to a symmetrical karyotype. Meiotic behavior was regular, with 18 bivalents in diakinesis and metaphase I. The family has n=20, the Tecomeae tribe being one of the basal groups, cytologically heterogeneous, with 2n=36 and 40. The present work confirms 2n=36 for this species, which agrees with the data reported in the bibliography; meiosis was normal with regular chromosomal pairing. The presence of satellites was informed for the first time for the Bignoniaceae family, using DAPI as a novel technique for studies on *Jacaranda mimosifolia*.

A126

**ROLE OF PERICARP IN THE DORMANCY OF SUNFLOWER LINE IMI-3**

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Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated oilseed crops in the world, Argentina being one of the world's major exporters of its oil. Sunflowers can display an irregular seed germination due to intrinsic causes which is one of the most important constraints to its commercial production. Such a process, called dormancy, may be caused by the embryo, the seed coat and/or the pericarp. The pericarp has a complex effect on germination and dormancy as a result of both physical and chemical factors. Sunflower dormancy can be reduced during after-ripening or by removing seed coats. Our objectives were to investigate the role of pericarp in dormancy of sunflower cypselas of the IMI-3 line, and to evaluate the dormancy maintenance during dry storage conditions. The germination test was conducted on dry cypselas with and without pericarp at 42 and 70 days after-harvest. Germination percentage was recorded at 10 days. For the histological analysis, permanent slides of pericarp cross-sections were obtained. In our work, the final germination percentage showed significant differences between cypselas with intact pericarp (42 days after-harvest: 26%; 70 days after-harvest: 77%) and cypselas without pericarp (42 days after-harvest: 65%; 70 days after harvest: 96%). This indicates that dormancy release is related to the duration of the storage period. In addition, pericarp anatomy suggests that the establishment of physical dormancy may be related to the sclerification of the middle layer and the increase in a substance similar to phytomelanin in the rays.

A127

**EFFECT OF SEED AGE ON THE GERMINATION OF *Chloris berroi***

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In the halophytic steppe of the Flooding Pampas, a limited presence of new *Chloris berroi* plants can be observed. A possible cause may be failures in seed viability and/or in their preservation in natural conditions. The objective of this work was to assess the effect of seed age on germination behaviour. Seeds were collected by hand in 2010, 2012 and 2014 in the NE of the Flooding Pampas (57° 07'W, 35° 01'S) from a Typic Natraqualf soil (2.9% SOM, pH 7.5 to 8 and Ce 1.4 dS m<sup>-1</sup>) and stored in paper bags at 20 ± 5 °C. In June 2015, only those spikelets (caryopses with their glumes) with normal morphological characters were sown in Petri dishes. The experimental design was completely randomized ( $r = 6$ ); the factor was storage time (1.5, 2.5 and 4.5 years). The experimental unit was spikelets/dish. It was placed in a chamber with alternating 12 : 12 h, 20 : 30 °C, dark : light periods, respectively. They were monitored daily until no germination (radicle ≥ 2 mm) was registered for five consecutive days (22 days). Data were expressed as cumulative germination percentage (CGP %), vigour (velocity) and T50. Percentages were arcsin transformed and analyzed using ANOVA and Tukey's test. A drop in viability ( $p < 0.01$ ) associated with the increased age was registered based on CGP (2014: 84.2%, 2012: 57.3% and 2010: 23.81%), also in vigour (17.64, 4.66 and 1.31) and T50 (2.16, 5.33 and 0 days). Viability preservation in natural storage conditions was high in the first year, but short-lasting in subsequent years. However, the occurrence of new individuals in the grassland studied was lower than the results indicated; therefore, other causes must be explored.

A128

**PRESERVATION OF VIABILITY OF *Lotus tenuis* SEEDS IN NATURAL CONDITIONS ASSOCIATED WITH THE PRESENCE OF IMPERMEABLE SEED COATS**

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*L. Tenuis* seeds, a forage plant naturalized in the Salado Basin, show impermeable coats when they are harvested by hand, without the abrasive effect of mechanical threshing. The objective of this work was to assess if impermeable seed coats contribute to the maintenance of viability for long periods. The germination of scarified (S) and non-scarified (NS) seeds of 12 different genotypes (G), hand harvested in 1993 and preserved in paper bags in natural conditions until June 2015 was assessed. Scarification was performed between sheets of fine sandpaper (P120). The design was completely randomized ( $r=4$ ) and the experimental unit was 50 seeds/Petri dish. The temperature regime was ( $X \pm SE$ ): Tmax = 21.02 ± 0.40 °C; Tmin = 17.06 ± 0.51. For 11 days, the number of germinated seeds (radicle ≥ 2 mm) was recorded daily and, at the end of the test, the number of impermeable seeds (not imbibed) and of imbibed ungerminated seeds was also recorded. Cumulative germination was calculated (CGP %). Two-way ANOVA (factors: G, S and GxS, one-way ANOVA (significant interaction ( $p < 0.01$ ); (GxS) were used as treatment factors), Tukey's test and correlation were applied. A positive effect ( $p < 0.01$ ) of scarification on CGP and variability among genotypes (range: 9–76%) were found. A positive association ( $p < 0.01$ ) between response to scarification and % of impermeable seeds in NS samples was observed. The % of CGP of imbibed seeds in NS samples was minimal (0–2.174%). The preservation of seed viability for 22 years in natural conditions is attributed to the impermeability of seed coats. Variability among genotypes may imply a potential response to selection.

A129

**EFFECT OF CADMIUM STRESS ON TRANSGENIC AND NON-TRANSGENIC SOYBEAN PLANTS (*Glycine max* (L) Merr)**

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In Argentina, 90% of soybeans are resistant to glyphosate, RR (Roundup Ready) transgenic varieties. It is an interesting challenge to find advantageous properties of non-transgenic species (NT) on transgenic plants (T RR) against various stressors, in an attempt to use native species instead of the genetically transformed ones. Cadmium (Cd) is a contaminant that interferes with the uptake, transport and use of essential elements (Ca, Mg, P and K) and water, causing oxidative stress. The objective of this study is to determine the different biochemical behavior of T and NT soybean, under Cd oxidative stress. Soybean leaves NT and T with Cd 40  $\mu$ M contamination for 24 hours was the experimental model. Total RNA extraction was performed by the method phenolic trizol and RT-PCR and the genes expression of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were measured. The results showed that CAT expression is significantly higher in NTCd with respect to TCd. APX showed no significant differences between T vs. NT treated with Cd, nor within each group. APX had lower expression in leaves when comparing TCo vs. NTCo. GR expression increased in TCo with respect to TCd. For NT plants the same pattern was observed although it was less significant. The TCd expressed a greater amount of GR mRNA than in NTCd. Cd exerted an effect on the expression levels of GR Co values that increased both T and NT. NT variety transcription baseline (Co0 h) is markedly higher compared to the T ones, which may be advantageous in relation to the antioxidant defense. There are differences in the mechanisms of response to oxidative stress and antioxidant defense by molecular changes between transgenic soybeans DM 4800 (RR) and non-transgenic DM 48, when they are under stress caused by Cd.

A130

**EFFECT OF EXOGENOUS CALCIUM ON Cr (VI) REMOVAL BY *Salvinia biloba***

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Calcium (Ca) alleviates the effects of heavy metal stress either by ion antagonism or by activating the detoxifying pathway of reactive oxygen species. Since most studies have focused on the Ca-Cd pair, the aim of this study was to analyze Ca-Cr interaction during the removal of Cr(VI) by *Salvinia biloba*. Plants were grown in the presence of both CaCl<sub>2</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, either separately or in combination, under controlled conditions for 5 days. Samples of growth solution were taken daily to quantify remaining Cr(VI) by using 1,5-diphenylcarbazide reagent. On the 5th day, tissue damage (electrolyte leakage) was determined by conductimetry. Results showed that both Cr and Ca individually induce tissue damage; in combined form this increases over 300% compared with control plants (without Cr) and over 100% in relation to Cr-exposed ones. With respect to Cr(VI) removal, the presence of Ca increases metal removal from the treatment solution, but removal pattern becomes dependent on added Ca. Thus, Ca-induced higher accumulation of Cr(VI), could explain the increased damage that occurs in the presence of both metals. Since Cr(VI) interferes with Ca absorption even causing a deficiency of this mineral in extreme conditions, it can be inferred that increased damage observed in plants growing in presence of Ca and Cr(VI) is produced by a synergism between the deleterious effects of increased Cr(VI) accumulation and reduced Ca absorption.

A131

**CHARACTERIZATION OF THE PHYTOTOXIC ACTIVITY OF *Flourensia campestris* AND *F. riparia***

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In endemic species of *Flourensia* (Asteraceae) such as *F. campestris* (FC) and *F. riparia* (FR), the aqueous extracts (AqE) of their leaves, as well as the sesquiterpene (-)-hamanasic acid A ((-)-HAA) -isolated from FC-, have shown elevated phytotoxicity against lettuce. In the present work we aimed at characterizing the phytotoxic activity and selectivity of FC AqE and (-)-HAA through bioassays against weeds and cultivated species. In addition, the phytotoxicity of volatile compounds of FR essential oils was also tested against *Lactuca sativa*. AqE of FC (%) and (-)-HAA ( $\mu$ g/ml) inhibited *Brassica campestris* seed germination at EC<sub>50</sub><0.75% and EC<sub>50</sub><125  $\mu$ g/ml, and *Sorghum halepense* at EC<sub>50</sub>=3% and EC<sub>50</sub>=500  $\mu$ g/ml. Both showed an interesting selectivity against the leguminous alfalfa (*Medicago sativa*) and soybean (*Glycine max*). The EC<sub>50</sub> for (-)-HAA was only achieved at 1500  $\mu$ g/ml in both species. Even at the highest concentration tested, 6%, AqE did not reach EC<sub>50</sub>, and germination was only inhibited up to 30% in alfalfa and 20% in soybean. Essential oils from FR (hydrodistillation, ca. 0.4% v/w), did not show phytotoxic effects at 500-1500 ppm, discarding their involvement in the phytotoxic effects present in AqE. Through GC-MS, from 32 volatiles detected, only 10 were identified, which represented 70% of the total oil: 6 hydrocarbon (50%) and 2 oxygenated (10%) sesquiterpenes, plus 2 non terpene

compounds (10%). No monoterpenes were found. Overall results, especially the selectivity shown against leguminous species, signal the potential of (-)HAA and other compounds present in the AqE of *Flourensia* for weed control in crop systems.

### A132

#### MORPHOANATOMICAL CHANGES IN LEMON FRUIT INFECTED WITH *Phytophthora citrophthora*, THE CAUSAL AGENT OF BROWN ROT

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Brown rot caused by *Phytophthora* spp. (Stramenopila: Oomycota) is a major citrus postharvest diseases. The aim of this study was to determine the structural changes that occur in the peel of infected lemons. Tissues from healthy and infected fruit, artificially inoculated in the styler area with zoospores of *P. citrophthora*, were compared. For the morphoanatomical study, peel sections (flavedo and albedo) of equatorial and styler zones were used. Transverse and longitudinal sections were analyzed in 4 samples: M1- Styler infected, M2 Equatorial infected, M3- Styler healthy, M4-Equatorial healthy. Observations and measurements were made with optical microscopes. Tissues M1 and M2 differ from M3 and M4 controls. This difference is evidenced by inter and intracellular hyphae development that reach the albedo and the formation of lenticular subepidermal structures with tearing of the epidermis; thinner cuticle; cell walls of collenchyma and parenchyma thinner; reduction of calcium oxalate crystals and increase in phenolic compounds deposits. The anatomical changes showed no significant differences between the styler and equatorial areas. These structural changes may be used to characterize the initial stage of infection of *P. citrophthora*, when symptoms of the disease are not yet visible.

### A133

#### FIRST CATALOGUE OF THE PHYCOLOGICAL COLLECTION, CRYPTOGAMIC HERBARIUM OF MIGUEL LILLO FOUNDATION.

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The Phycological Collection has preserved more than 1000 algal species with historical relevance donated by museums and collectors of international prestige. The cataloguing of algal specimens donated by the Herbarium of The New York Botanical Garden (NYBG) was performed under the computerization project of the Phycological Collection. The methodology applied consisted in the review of algal materials, the control of their condition and the simultaneous review of historical inventories. In addition, photographs were taken and the information contained in labels was loaded on the database DATA-LIL. The taxonomy of the species was completed according to AlgaeBase and a search of information regarding the history of the collections, biography of collectors and preservation techniques was carried out. 576 species of 190 genera were cataloged, distributed among: Charophyta (13), Chlorophyta (46) Cyanobacteria (26), Ochrophyta (32) and Rhodophyta (73). These species mainly represent the algal Flora of North America and the Caribbean and belong to collections of The NYBG such as Phycoteca Boreali-Americana, Characeae Americana Exsiccatae of TF Allen and North American Marine Algae, among others. There are eighty collectors represented, the most relevant being Collins (115), Durant (66) Hervey (56), Allen and Gardner (26). Finally we highlight the presence of two duplicates of types *Caulerpa lentillifera subsessilis* and *Symploca howei*.

### A134

#### CYANOBACTERIA OF HIGH WETLANDS FROM NORTHWESTERN ARGENTINA

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The latitudinal and altitudinal variability of our country allows the existence of a diversity of wetlands that, in the broadest sense, includes marsh, fen, peatland or flowing or static water-covered surfaces, permanent or temporary, natural or artificial, brackish or salt, as well as areas of marine water with a depth no greater than six meters at low tide. The objective of this paper was to contribute to the knowledge of Cyanobacteria in the High Wetlands of northwestern Argentina. An interdisciplinary sampling was realized in the summer, from January 21st to February 1st, and twenty-six lakes were studied corresponding to Jujuy, Salta and Catamarca provinces. The qualitative samples were collected with a 20 µm plankton net, filtering 25 litres of water. These samples were fixed in situ with formaldehyde 4% and observed with binocular microscopy and drawing camera in the laboratory. According to the results obtained, the taxocenoses was formed by 23 taxa, most of them cosmopolites and from brackish environments, belonging to Chroococcales (5),

Nostocales (3) and Oscillatoriales (15). We described for the first time *Phormidium inundatum* in the Northwest of Argentina and, apart from this species, *Merismopedia glauca*, *M. punctata*, *Synechococcus elongatus*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc linckia*, *Leptolyngbya fragilis*, *Spirulina major*, *Phormidium autumnale*, *P. breve* and *P. Molle* for the Catamarca Province.

### A135

#### COMMON NAMES OF THE FLORA OF SANTIAGO DEL ESTERO: AN ETYMOLOGICAL APPROACH

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The common names of plants are part of the traditional knowledge of the rural population. Thus, they serve as a practical reference for action and communicate scientific knowledge about plants. The increasing loss of this knowledge leads to the need to safeguard its connotation and origins. The aim of this study is to collect names assigned to local species of our native flora, their meaning and etymology. The information was obtained through literature review and field work consisting of semi-structured interviews with key informants and rural residents of the provincial interior. Plant materials were collected for further identification by following conventional methods. A catalog with 396 local names and popular meanings assigned to 220 plant species belonging to 69 botanical families was created. Popular botanical names registered are associated with the quichua (tacko yuraj, paaj puca), diaguita (sina-sina, huacla), native languages of other regions (aguaribay -guaraní-, tala -aymará-), Spanish (algarrobo, sauce, meloncillo) or conjunctions of different languages (sacha sandía, tala pishpita). The contribution of native languages is highlighted in the construction of vernacular names of plants; they serve as a tool for recognition. Many of these names are assigned in relation to popular uses, properties of the species, morphological characteristics and local legends, and this information may contribute to the rescue of traditional knowledge of the flora of Santiago del Estero.

### A136

#### SALINITY OF PHREATIC WATER IN WETLANDS OF SAN LUIS (ARGENTINA).

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Wetlands are environments frequently flooded and with groundwater levels close to the surface. Particularly in our region, these groundwaters are saline, which determines the development of hydromorphic soils and halophyte vegetation. Our objective was to confirm the salinity contents and the groundwater levels and compare them with those obtained in 2011 in a saline wetland in the center-east of the province of San Luis. The study area is located at 33 ° 37' S and 65° 25' W, with a height of 505 meters above sea level. We determined the water level and chemical characteristics of their waters according to the types through previously established physiognomic studies. The sampling was compared to one previously made in the same place and at the same time of the year. The results indicate that in both samplings each physiognomic type presents different groundwater levels such as salinity, from the halophytic forest (higher sector) down to the beach saline (lower area). The phreatic water rose between 48 and 103% for different physiognomic types in May, while in June the increase was between 36 and 86%. General chemical parameters of the water show a general increase: pH (4 to 19 %), EC (Electrical conductivity, 2 to 25%) and SAR (Sodium Adsorption Relation, 64 to 91%). In regard to the anions, CO<sub>3</sub><sup>-</sup> and CO<sub>3</sub>H decreased their values between 83 and 87%, while Cl<sup>-</sup> and SO<sub>4</sub><sup>-</sup> increased between 4 and 50% and between 145 and 158% respectively. The cations Na<sup>+</sup> and Ca<sup>2+</sup> increased between 82 and 124% and between 49 and 106%, while K<sup>+</sup> and Mg<sup>2+</sup> were dissimilar. We concluded that the increase in phreatic water and the parameters studied show a higher salinity for each physiognomic type in the sector.

### A137

#### MAMMALIAN DIVERSITY IN TWO SALINE WETLANDS OF SANTIAGO DEL ESTERO, ARGENTINA.

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Lagunas Saladas and Huyamampa constitute two of the largest saline wetlands of the Prov. of Sgo. del Estero, covering an area of 400,000 ha. Saladas is a protected area of the provincial system. Huyamampa is a site of high biodiversity conservation value as reported by TNC and WWF. These wetlands belong to the Dry Chaco, and are affected by the same impacts, such as land use change from forest to intensive farms. They are located in the center-east and west of Santiago and share many physiognomic and functional characteristics, the dynamics of the saltwater being the most relevant. During 2011 and 2014 field surveys were conducted to determine the richness and abundance of mammalian species in the wetlands. The methodology employed: 1000 m linear transects (N = 40), recording direct observation and indirect evidence. 16 species were registered, out of which we highlight maned wolf, anteater and quimilero pig due to their declining populations. From the data collected  $\alpha$  diversity indexes were obtained. Using Estimates

software, the Shannon index was calculated,  $H' = 2.13$  for Saladas and  $H' = 2.1$  for Huyamampa, by testing null hypothesis of equal diversity with a confidence level of 95%, which yielded a p value of 0.34 using the R program. From species richness (S), rarefaction curves were developed on the model of Bernoulli. It is concluded that there are no significant differences in the mammals species in these lagoons, a fact that can be closely linked to the great similarity in both environments.

### A138

#### IS THE INVASIVE *Ligustrum lucidum* DISPERSED BY BIRDS IN THE MOUNTAIN FOREST ECOSYSTEM OF CÓRDOBA, ARGENTINA?

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*Ligustrum lucidum* or glossy privet is a fleshy-fruited shrub, native to China. In Córdoba mountains this shrub invades the open forest and scrublands, sharing dominance with other native fleshy-fruited shrubs. Urban areas may function as propagule sources for glossy privet because it has been used as a landscaping tree for over a century. The objective of this work was to know if birds dispersing viable seeds of this species in a mountain forest ecosystem of Córdoba, Argentina. During July, August, September and December of 2012 we randomly collected fresh bird pellets. Since birds can defecate several pellets in the same place, one sample represents a point in a space with a variable number of individual pellets. We collected 11, 24, 15 and 21 samples respectively, in two transects (500m long by 5m wide). In the laboratory, pellets were weighed, crumbled and observed under stereomicroscope. When *L. lucidum* seeds were observed, they were separated, analyzed to determine whether they were intact or physically damaged, and placed to germinate. We counted 279, 232 and 5 seed from bird pellets in July, August and September, respectively. In December no *L. lucidum* seeds were observed. 83.3%, 93.2% and 13.9% of the samples of July, August and September exhibited *L. lucidum* seeds. 99% of the seeds were healthy but none of them germinated after 215 days. According to our results, birds disperse *L. lucidum* seeds but they would not be viable.

### A139

#### CHARACTERIZATION OF A NATURAL GRASSLAND IN THE HEADWATERS OF THE CHUCUL STREAM (CÓRDOBA, ARGENTINA).

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Biodiversity is one of the challenges in the management and conservation of nature. The remnants of natural grassland are a significant source of plant diversity. The need to characterize the study area arises from the limited information available. The objective of this investigation was to study the monthly change in the structure and diversity in a natural pasture of 21 ha located 11.5 km NW of Chucul, with little human intervention. From March 2012 to April 2013, monthly samples were taken randomly with 10 replications of 0.25 m<sup>2</sup>. Presence, height and coverage were recorded. Wealth totalled 42 species distributed in 13 families. Monocots coverage was 54.8%, the most important species being *Cynodon dactylon* and *Muhlenbergia asperifolia*, and Dicotyledons 45.2%, represented by *Bacharis* and *Bidens* sp. 38.2% are annual, 59.5% perennial and 2.3% biennial. 67.4% of the species are spring-summer and the rest autumn-winter. According to the classification of Raunkier (1934), therophytes: 38.2%, hemicryptophytes: 42.8% geophytes: 7.1% and chamaephytes 11.9%. The average height of the herbaceous layer was 17-25 cm, *Elymus scabrifolius* and *Stipa* spp being the highest and *C. dactylon* the lowest. The diversity was higher than 0.8 except in August (0.76). The prevalence (0.18) and evenness (0.81) did not differ significantly. The variation in diversity is due to changes in wealth by the end of winter and early spring and the impact of agriculture.

### A140

#### STOMACH CONTENT ANALYSIS OF *Camelobaetidius* sp. (Ephemeroptera) FROM THE GRANDE RIVER, SAN LUIS, ARGENTINA.

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Knowledge of the alimentary habits of the organisms from lotic ecosystems contributes to a better understanding of organic matter processing. Cummins (1973) assigned aquatic insects to different functional groups (AFG). The aim of this work was to confirm the AFG of the *Camelobaetidius* specimens in the Grande River. Collections were made with Surber net of 300 µm of mesh aperture and 0.09 m<sup>2</sup> µm of area, in periods of high waters (March) and low waters (August) in 2012 in a Grande River station. Specimen were fixed in formalin at 4% and preserved in alcohol at 70%. Stomach content analysis was realized according to the methodology of Palmer & O'Keefe (1992) and Palmer et al. (1993a, 1993b), modified by Albariño (2000). Nymphs were measured and classified according to their total length with and without the tail, length and width of the cephalic capsule, which allowed the classification of these specimens into large nymphs (cephalic capsule of 0.3 µm and abdomen > 2mm) and small nymphs (cephalic capsule < 0.3 µm and abdomen < 2mm). Dissection was practised in the large nymphs while the small ones were made transparent with KOH at 10%. An

analysis of 15 nymphs was made, corresponding to the high waters period, out of which two presented empty stomachs while no specimens were found during the low waters season. The dietary composition was determined under a magnification of 625X. By means of graduated ocular nine random fields were selected and 5 food items were identified which would allow us to assign the specimen of *Camelobaetidius sp.* to the AFG scrapers, considering also the structure of the mouthparts (Nieto, 2003).

#### A141

##### SEED BANK IN A “CALDENAL” DEGRADED BY GRAZING

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The recovery of desirable species in very disturbed grasslands (annual) in the “caldenal” occurs if a sufficient number of growing seasons rest, with adequate rainfall, allowing germination and establishment of “flechillal” species present in the soil. The aim of this work was to evaluate the presence (species and density) of seeds in the seed bank of a disturbed grassland, closed, in “caldenal” in San Luis. Ten sites were sampled. In March (already drop seeds), 4 sub-samples of soil were taken up to 5 cm deep with bore. The species were identified. The soil was sieved and the seeds were extracted and identified and N°/species was counted. The average number of seeds/m<sup>2</sup> /species was calculated. The similarity between sites was analyzed by cluster analysis. The Sorensen similarity index was used to compare the bank with vegetation. In the samples the number of species varied between 2 and 5. The mean number of seeds per m<sup>2</sup> was: 125,634.92 ± 81,892.96. Six species were identified, with 2 dominant species: *Chenopodium sp* (annual) and *Cynodon hirsutus* (naturalized perennial) while no seeds of high forage value were found. Cluster analysis defined two groups, differentiated by the number of *Chenopodium sp* seeds. 18 herbaceous vegetation species were identified. The similarity index bank-vegetation varied between 0 and 0.36, the common species being *Chenopodium sp* and *C. hirsutus*. The possibility of recovering the range condition from the seed bank is negligible. This means that to preserve the forest, intercropping of native or cultivated seeds is required and, if necessary, the forest conditions (cleaning, thinning) should be modified.

#### A142

##### POLYBROMINATED FLAME RETARDANTS (PBDES) TOXICOLOGICAL TESTS ON THE APPLE SNAIL

(*Pomacea canaliculata*, Caenogastropoda, Ampullariidae)

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Polybrominated diphenyl ethers (PBDEs) are a group of industrial chemicals belonging to the family of brominated flame retardants that comprises 209 congeners depending on the number and position of bromine partners. PBDEs are added to the polymers present in the electronic boards, computers, televisions, automobiles and aircraft, among others, to inhibit or delay the onset and spread of fire. Congener BDE-209 is the most widely used and its production is regulated by the Stockholm Convention. The apple snail *Pomacea canaliculata* has been proposed as a biomarker of PBDEs. The aim of this work was to explore the toxic effects of BDE-209 on the species above. *P. canaliculata* individuals, placed in aquaria, were exposed for 42 days to diets enriched with congener BDE-209 at three different concentrations (400 (C<sub>1</sub>), 4700 (C<sub>2</sub>) and 8300 (C<sub>3</sub>) g-1 mg lipid). All animals survived the treatment. A non statistically significant increase in the frequency of copulation in C<sub>3</sub> treatment was recorded. The percentage of embryonated eggs showed no significant differences between treatments for any of the tested concentrations. However, the spawning register showed a significant decrease in the concentration C<sub>2</sub> group, with alteration in the eggs' color, registered using a spectroradiometer. No differences between treatments were observed in the histology of the gonads. The results encourage the exploration of possible detoxification mechanisms to protect *P. canaliculata* from BDE-209.

#### A143

##### CHANGES IN THE LIMNOLOGICAL PARAMETERS AND ZOOPLANKTON OF A SHALLOW SALINE LAKE OF LA PAMPA (ARGENTINA) AFTER FILLING

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Saline lakes are abundant in La Pampa. Since the alternation of rainfall cycles above or below the historical mean is common in the region, their salinity is very variable. The aim of this study was to determine the environmental and biological parameters of Chadilauquen, a large saline lake, after a dry period when it almost became dry. In late 2012, rainfall was abundant and the water level rose. Samples were collected from January to December 2013. In January, the lowest salinity (14.3 g.L<sup>-1</sup>) was recorded but this increased up to 40.38 g.L<sup>-1</sup> in December. Water transparency was low (0.17 ± 0.05 m) due to high concentrations of inorganic (72.61 ± 69.6 mg.L<sup>-1</sup>) and organic (37.46 ± 17 mg.L<sup>-1</sup>) suspended solids. The phytoplankton chlorophyll-*a* concentration showed a winter peak of 147.06 mg.m<sup>-3</sup> (July). The richness was reduced (one cladoceran, three copepods and three rotifers). Among the crustaceans the native species typical of saline lakes of the center of the country were recorded. Among them, *Moina eugeniae*, abundant in the

warmer months, and *Boeckella poopoensis*, present when salinity exceeded  $22\text{g.L}^{-1}$  and the species that contributed the highest density and biomass, were the most important. Rotifers are also halophilic species but with a cosmopolitan distribution. *Brachionus dimidiatus* and *B. plicatilis* were the most important species, and showed a peak in late summer/early autumn ( $1702.2$  and  $2062.2$  ind. $\text{L}^{-1}$  in March and April, respectively), which is different from other lakes in the province, where their peaks were always recorded during the summer.

#### A144

### REFERENCE COLLECTION OF POLLEN FROM PLANT SPECIES OF THE QUEBRACHOS DEPARTMENT, SANTIAGO DEL ESTERO

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For the identification of the botanical origin of honey it is necessary to have a pollen collection of plants in the area where hives are located. The Quebrachos department is located in the Chaco Serrano of Santiago del Estero; the vegetation has peculiar characteristics with respect to the rest of the province. Our aim was to make a collection of pollen from species of flora of the Department that were mentioned by beekeepers as a source of nectar and pollen for bee colonies. Samples were prepared according to the acetolysis method of Erdtman, with material collected in different localities. The flowers were collected at anthesis; they were preserved in alcohol  $70^\circ$ . Pollen grains were photographed and described with an optical microscope according to their morphological characteristics such as length (polar and equatorial), circumference (diameter and area) and type of openings. Pollen types are described for 23 species of the flora of Santiago del Estero, belonging to 14 botanical families. The information obtained will integrate the database available to producers and centers of analysis for the identification of the botanical origin of the honey produced in the region under study.

#### A145

### BENTHIC MACROINVERTEBRATES FROM THE LOWER-MIDDLE BASIN OF THE VALLE RIVER

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Benthic macroinvertebrates are one of the best represented communities in lotic environments. The basin of the Valle River (Catamarca, Argentina) has a main collector, with a length of 140km and an area of more than  $5,910\text{Km}^2$ . This work aims at presenting a preliminary faunal lists of taxa reported along the middle-lower reaches of the Valle River. Five sampling points were established along the river: Isla Larga, Las Pirquitas, Pomancillo Oeste, Collagasta and Banda de Varela. Specimens were collected along a stretch of 10m, at every point, combining manual sampling with "D" and "Surber" nets. Samples were fixed in situ with 90% ethanol. In the lab, with the available keys, we determined up to the lowest discernible taxon. The following were recorded: Nematoda (Mermithidae: two genera); Nematomorpha (Gordiida: three species); Annelida; Mollusca (Gastropoda: two genera; Bivalvia: one genus) and Arthropoda (Acari; Crustacea: Aeglidae; Ostracoda, and Insecta: nine orders and 30 families). The faunal list includes 40 taxa out of which 82.5% are arthropods and 75% are families of aquatic insects. While for many taxa reported here for Catamarca previous records exist in the literature, they do not specify either the watercourse or, in some cases, the place where they were collected. From the above, the importance arises of this first report listing taxa list and precise location along the extended course of the Valle River, which also includes reports of the rich fauna, as a simple biological metric measure of biological diversity.

#### A146

### DIVERSITY OF GORDIIDA (NEMATOMORPHA) AND THEIR PARATENIC HOSTS IN THE BASIN OF THE VALLE RIVER (CATAMARCA, ARGENTINA)

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Gordiida (Nematomorpha) are worms from freshwater environments. During the parasitic phase in their life we can distinguish definitive (terrestrial or aquatic arthropods) and paratenic hosts. The objectives of this research were to know the diversity of Gordiida in the basin of the Valle River and to identify possible paratenic hosts among macroinvertebrates. Four sampling points were established: Las Trancas (LT), La Puerta (LP), Valle River: Banda de Varela height (BV) and Tres Puentes height (TP) height. At each point along a stretch of 200m, gordiidos were collected manually and with wire strainers. Taxonomic determinations were performed by analyzing the rear ends of males and females (binocular magnifying lens) and their cuticles (microscopy preparation). Among the macroinvertebrates in each stream we selected: chironomid larvae ( $n = 20$ ) and *Physa* sp. snails ( $n = 20$ ), collected manually and with "D" nets. Prospection of larvae and cysts was performed with an optical microscope. Out of a total of 30 worms, the following species were determined: *Chordodes brasiliensis* (LT, LP, BV), *Noteochordodes cymatium* (LT), *N. talensis* (LP),

*Paragordius varius* (TP) and *Pseudochordodes dugesi* (LT). In 16 specimens of *Physa* sp. cysts were found, and in 12 chironomid larvae, larvae and cysts were found with morphology compatible with those described for genus *Chordodes*. Out of the 13 species of Gordiida reported for Catamarca, five are present in the Valley River and two taxa of paratenic macroinvertebrates are hosts to at least one species, thus ensuring the life cycle of these organisms. .

#### A147

### CACTI PRESENT IN SAN JOSE DEL MORRO (SAN LUIS)

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Cacti are a group of very interesting plants because of their ability to adapt to arid and semi-arid environments. The goal of this work was to determine and describe the cactus species found in Loma Blanca, part of the Comune of San José del Morro, province of San Luis. The cacti present in the area are usually collected for ornamental purposes; this fact, together with fires, overgrazing and deforestation, has led to a decrease in their density and diversity. Pictures of each plant were taken, along with their location and terrain height and properties. The observations and exomorphological descriptions of the species found match the specific literature. Regarding the natural vegetation, the area belongs to the highlands, with high diversity in its floristic composition. In the survey, we found two native species, *Cereus aethiops* Haw. and *Opuntia sulphurea* Gillies ex Salm-Dyck var. *sulphurea*; an adventitious species, *Opuntia ficus-indica* (L.) Mill. f. *ficus-indica* and four endemic ones, *Echinopsis aurea* Britton & Rose var. *aurea*; *Gymnocalycium monvillei* (Lem.) Britton & Rose; *Gymnocalycium monvillei* (Lem.) Britton & Rose ssp. *achirasense* (H. Till & Schatzl ex H. Till) H. Till and *Trichocereus candicans* (Gillies ex Salm-Dyck) Britton & Rose. The surveyed specimens of *Cereus* are located at 1020 meters above sea level, the rest of the species at 986 meters above sea level. The area shows rocky soils of light sand consistency and moderate soil structuration. The study shows a direct relationship between the habitat of the species and the morphological features they develop.

#### A148

### PHOTOSYNTHETIC CHARACTERIZATION OF *Alnus acuminta* (“ALDER”) OF DIFFERENT AGE IN THE YUNGAS (TUCUMÁN, ARGENTINA)

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In Tucumán, alders form continuous forests on humid slopes covering an area of approximately 1500 km<sup>2</sup>, between 800 and 2000 m asl. It is an important CO<sub>2</sub> sink but there are no data regarding its maximum photosynthesis ( $A_{max}$ ), stomatal conductance ( $g_s$ ), leaf transpiration ( $T_r$ ) and internal CO<sub>2</sub> concentration ( $C_i$ ). Measurements were taken on both young and adult from Provincial Reserve La Florida, Monteros (1,058 m asl), using a gas exchange analyzer, IRGA (Li-Cor, LI-6400XT, USA) under light and CO<sub>2</sub> saturating conditions (1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , 400  $\mu\text{mol CO}_2$ ) and constant leaf temperature (25±0,5°C).  $A_{max}$  was different ( $p \leq 0.01$ ) between young and adult plants (14 y 11.2  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ).  $g_s$  as well as  $C_i$  and  $T_r$  were higher in young plants (57.2%, 37% and 63.1%), while carboxylation capacity ( $A_{max}/C_i$ ) and intrinsic water usage efficiency ( $EUA_i$ ) were higher in adults ones (29.6% and 84.5%). Higher  $A_{max}$ ,  $g_s$ ,  $C_i$  y  $T_r$  values in young plants would be explained by their higher stomatal density (217 against 165 stomata/mm<sup>2</sup>) and highest chlorophyll and carotenoids concentration. While  $A_{max}/C_i$  highest value registered in adults plants might be related to their lower stomatal density (lower  $C_i$ ) and higher  $EUA_i$ , it could also be due to affinity change and/or regeneration speed of RubisCo enzyme. This would indicate that in these plants photosynthesis may be limited by both stomatic and mesophyll components. In this way, the coordinated operation of both mechanisms would allow plants to reach a higher CO<sub>2</sub> accumulating capacity.

#### A149

### CHROMATOGRAPHIC COMPARISON OF PLANT EXTRACTS FROM DIFFERENT SPECIES OF THE GENUS *Flourensia*

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The genus *Flourensia* DC. is constituted by 32 species, among which 12 grow in Argentina. Several authors have classified *Flourensia* into different subtribes according to morphological and chemical studies. In 2005, Panero placed the genus in the new subtribe *Enceliinae*, based on comparative studies of DNA sequences. The aim of this study was to compare the chemical composition of extracts of different polarity from *F. campestris*, *F. fiebrigii*, *F. hirta*, *F. macroligulata*, *F. riparia* and *F. tortuosa*, in order to establish if the species studied are chemically related. The extracts comparison was performed using thin layer chromatography, applying CHCl<sub>3</sub> and CHCl<sub>3</sub>-MeOH (1: 0.1) as solvent system and UV light ( $\lambda$  254 nm), vanillin solution in 3% ethanol and H<sub>2</sub>SO<sub>4</sub>-EtOH (1:4) as developing agent. The presence of compounds with similar  $R_f$  between *F. tortuosa* and *F. macroligulata* species and

among *F. riparia*, *F. campestris*, *F. hirta* and *F. fiebrigii* species was observed. These results agree with the proposal of Dillon, who in a scheme of interspecific relationships based on genus exomorphological characters, recognizes the proximity of *F. tortuosus* and *F. macroligulata* by the presence of large capitula with broad phyllaries and 13-21 ray florets. He also suggests the closeness of *F. riparia*, *F. campestris*, *F. hirta* and *F. fiebrigii* by the trend toward fewer ray florets (5 to 8). Therefore, it is necessary to extend the phytochemical research to confirm the interspecific relationships proposed by Dillon.

#### A150

### PHOTOPERIOD VARIATIONS AND LIGHTING IN THE PROVINCE OF JUJUY AND ITS INFLUENCE ON HUMAN WELLBEING

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The province of Jujuy has a population distributed into four geographical regions: Yungas, valleys, quebrada and Puna (300 to over 4,000 meters above sea level) with a variety of ecosystems and environmental factors that, together with the seasonal variation in photoperiods, could affect the rhythmic production of melatonin, a hormone regulated by light that has not yet been studied in the province. The visual effects of lighting determine our performance, while biological and emotional effects are more important in determining our health and wellbeing. Objective: To study the interaction of environmental factors with melatonin production from differences in photoperiod according to the altitude and latitude of each region. Methodology: representative localities of the regions of the province were selected. Historical weather information from Servicio Meteorológico Nacional and Servicio Meteorológico Andino was collected in order to analyze climate elements and index calculation to determine the influence of environmental factors. With a light meter monthly light curves were plotted, starting measurement when the value was zero, before dawn, and ending when it was zero again. Hourly measurements were collected. The photoperiod was calculated for each day.

Results and conclusions: Differences were found between measured values and calculated values. Although there is no latitudinal variation in astronomical heliophany in the province, we observed that the variations found in relative heliophany resulted from increased atmospheric moisture and cloudiness in the east of the territory, which is due to differences in effective heliophany.

#### A151

### BRYOPHYTA ON LAPACHO ROSADO (*Handroanthus impetiginosus* (Mart. ex DC.) Mattos) FROM SAN MIGUEL DE TUCUMAN.

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#### A152

### RICHNESS, ABUNDANCE AND ORIENTATION OF *Tillandsia* IN SHRUBS OF THE MONTE REGION, IN LA RIOJA, ARGENTINA

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*Tillandsia* (Bromeliaceae) is the most diverse genus of vascular plants, in the Monte region of La Rioja, Argentina. This study analyzes the richness, abundance and orientation preference of *Tillandsia* in six shrub species in a sector of the Central Valley of La Rioja. The study area is located north of the Capayán River (29° 02' 52.2"S; 67° 27' 23.3"W; 1053 masl) in the Famatina Department. Sampling was conducted during September 2014 when the *Tillandsia* species are easily distinguishable. Six species of host plants

were studied: *Acacia gilliesii*, *Bulnesia retama*, *Larrea cuneifolia*, *Prosopis torquata*, *Tricomaria usillo* and *Zuccagnia punctata*. Plants with the largest canopy size in each species (n = 20) were selected within an area of 1km<sup>2</sup>. Height and crown diameter for each host plant and the number of *Tillandsia* plants according to the cardinal direction (north / south) were recorded. A total of 656 *Tillandsia* plants belonging to 10 species were counted in 120 host plants. In order of abundance they were: *T. duratii* (70%), *T. aizoides* (11%), *T. pedicellata* (9%), *T. gilliesii* (6%), *T. myosura* (2%), *T. minutiflora*, *T. angulosa*, *T. capillaris*, and *T. castellanii* (2% overall). The host plants with greatest richness and abundance were *P. torquata* (8 spp; 165 indiv.), *A. gilliesii* (7 spp; 216 indiv.), and *B. retama* (6 spp; 154 indiv.). The richness of *Tillandsia* was relatively similar between orientations, but the abundance was greater in the northern orientation. *Tillandsia duratii* was similarly abundant in the main hosts. The greater abundance and richness of *Tillandsia* was concentrated in host plants with heights of 2.5-4 m and a crown diameter of 2-7 m.

### A153

#### ENTOMOLOGICAL ECOSYSTEM WEALTH IN A MODIFIED ECOSYSTEM IN LULES, TUCUMÁN

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Because the structure of food webs of which insects are a part is complex, they are used to assess disturbance and planning of conservation areas. Since the data is poor regarding the species, the plan is to record their entomological wealth in a disturbed ecosystem. In order to do that, weekly collections were made using two Malaise traps from January to June 2015 in Finca el Manantial FAZ-UNT. The samples were analyzed using stereoscopic microscopy in the Chair of Zoología Agrícola, where orders they were sorted out into orders and, within these, into phytophagous organisms and controllers (predators and parasitoids). The results show that out of all the collected samples (percent values) 46.9 corresponded to the order Lepidoptera followed by Diptera with 18.8, Hemiptera with 12.2, Hymenoptera with 11.9, Coleoptera with 8.2, Orthoptera with 1, Dermaptera with 0.5, Neuroptera with 0.4 and Blattaria with 0.3. Discriminating between Phytophagous and controllers, we found that 66.9 corresponded to Hymenoptera, followed by 17.1 Diptera, 9.2 Coleoptera, 1.9 Hemiptera for controllers and 98.1 Hemiptera, Coleoptera 90.8, 33.1 Hymenoptera for phytophagous. All the specimens of Neuroptera and Dermaptera were controllers and all the specimens of Lepidoptera and Orthoptera were phytophagous. A predominance of phytophagous species was obtained. Predatory and parasitoid species correspond to 13.1. They play an important role in controlling the population density of phytophagous, gaining greater importance when it comes to agricultural ecosystems.

### A154

#### VARIATION OF ATMOSPHERIC TRANSPARENCY TO UV RADIATION AT DIFFERENT ALTITUDES. QUANTIFICATION AND STATISTICAL ANALYSIS.

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The burning of biomass and uncontrolled or deficiently controlled emissions of polluting agents in the atmosphere have certainly become a common practice in the province of Tucumán. This paper presents the results of a statistics study for three stations of the radiometry network in the UV solar radiation band which operates in the province of Tucumán.

Our purpose was to study the atmospheric transparency to UV radiation at different altitudes. Data was chosen according to two criteria: to determine the behavior of this radiation at the ground level in various altitudes above sea level and to use data during the sugar harvest (winter) when fire clearance is frequent. There are three network sites for this study Ampimpa Observatory (26 ° 36 '44.89 "S, 65 ° 50' 30.65" O, elevation 2458 ms / nm), INTA Famaillá Weather Station (27 ° 01 '08.85 "S, 65 ° 22 '49.99 "O, elevation 373 ms / nm) and field Channel 10 Television transmitter antennas National University of Tucumán in Villa Nougues (26 ° 50' 38.79" S, 65 ° 22 '51.83 "O, elevation 1467 masl). The data were collected in August 2013 and comprised a span of about 8 hours of sunlight. Daily averages and variation coefficient were calculated, plotted and processed statistically with ANOVA. There were statistically significant differences (p <0.0001) between the three sites at different altitudes and therefore different tenors of contamination. This was expected and what we seek is to quantify it to develop a statistical model.

A155

**DIVERSITY OF VASCULAR PLANTS ALONG THE NORTH-SOUTH AXIS OF THE ANTINACO-LOS COLORADOS VALLEY, LA RIOJA, ARGENTINA**

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A typical feature of biodiversity is that its spatial distribution is heterogeneous. In arid regions, variations in the relief can generate significant changes in the composition of the vegetation. The Antinaco-Los Colorados Valley (Province of La Rioja, Argentina) is a vast plain ( $\approx 3.000 \text{ km}^2$ ) with a gradual and continuing north-south slope, with a maximum altitude of 1,100 m a.s.l. in the north and a minimum of 660 m a.s.l. in the south. The aim of this study is to examine the variation in species richness of vascular plants and the species turnover along the north-south axis of the valley. The proportion of life forms, the pollination syndromes and seed dispersal were further analyzed. Sampling was conducted during March/2015 at 8 sites characterized by vegetation of Monte. In plots of 10 x 100m (10/site) all species of vascular plants were recorded. A total of 137 species belonging to 94 genera and 34 families (135 angiosperms, 1 gymnosperm and 1 fern) in 80 plots (= 8ha) were recorded. This inventory represented 83 to 94% of the expected richness in the valley according to diversity estimators. The number of species/ha ranged from 56 to 96 (mean = 77). Richness was significantly higher in the centre of the valley and lower in Catinzaco (south, 24 sp). Species composition also varied significantly between sites. The largest species turnover was recorded in the south (Vichigasta-Catinzaco) and north (Antinaco-Capayán) of the Valley. The dominant life forms were Phanerophytes (33%), Therophytes (25%), and Hemicyptophytes (23%). Most species show biotic pollination syndromes, and abiotic dispersion, mainly anemophilous.

A156

**EFFECTS OF SUBLETHAL CADMIUM AND CYCLOPHOSPHAMIDE ON *Cnesterodon decemmaculatus*.**

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Adverse behavioral, morphological and biochemical effects were studied in adult specimens of *Cnesterodon decemmaculatus* exposed to sublethal Cd and cyclophosphamide. Fish were acclimated for 7 days in moderately hard reconstituted water (MHW). Assays were semi-static (96 h); experimental groups were: [1] MHW + 2 ppm Cd; [2] MHW +5 ppm cyclophosphamide (CP); [3] MHW (control). N=10 / group. Aquaria were filmed for 15 min/day, evaluating aggressive and sexual behavior and changes in the swimming pattern. After exposure (no mortality was recorded in either group), fish were anesthetized, weighed and measured. A blood sample was taken for erythrocyte micronuclei (MN) count. Condition Factor (CF) was calculated. Then, individuals were sectioned into two parts (head and trunk). In the trunk, catalase (CAT) and glutathione-S-transferase (GST) activities, glutathione (GSH) and tissue protein (PR) contents were measured; PR head and GSH were also measured in the head. Statistical evaluation was performed by ANOVA or Kruskal Wallis.

Results: A) Behavior: a) aggressiveness increased in CP and reduced in Cd; b) intercourse attempts were reduced; c) short-term hyperactivity episodes were recorded; B) Genotoxicity: erythrocyte MN rate was increased; C) Enzymology: CAT increased and GST reduced in Cd; GSH content decreased in trunk both after Cd and after CP exposure. In no case were differences found in CF or PR. These preliminary results suggest the usefulness of adult *Cnesterodon decemmaculatus* as a sentinel species in short-term toxicity bioassays, providing sensitive and early bioindicators of freshwater environmental stress.

A157

**EFFECT OF GUAIANOLIDES ON MEIOSIS RESUMPTION IN AMPHIBIAN OOCYTES**

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Sesquiterpene lactones (STL's) are a diverse group of plant secondary metabolites with diverse biological activity, such as antitumor, gastric cytoprotective, blocking cell cycle in meristem cells and even meiosis in amphibian oocytes. This activity is due to interference with cellular macromolecules function by forming covalent bonds between STL's electrophilic structures and nucleophilic centers of biological targets. STL's present different groups with potentially reactive sites (PRS) such as  $\alpha$ -methylene- $\beta$ -lactone (MGL),  $\alpha,\beta$ -unsaturated carbonyl, epoxide, etc., which make them more versatile with regard to their biological targets. The aim of this work was to compare the inhibitory effect of guaianolides with different PRS on meiosis resumption in amphibian oocytes.

Fully grown oocytes of *Rhinella arenarum* were incubated with different guaianolides: Dehydroleucodine and its hydrogenated derivative 2H-DhL, Achillin, desacetoxymatricarin and Estafietin. Resumption of meiosis was induced by the addition of progesterone and the oocytes were fixed and sliced in order to observe the presence of the germinal vesicle. Dehydroleucodine inhibited meiosis resumption in a dose-dependent manner while the other guaianolides showed similar inhibitory effects between them

although lower than those obtained with DhL. The inhibitory activity of meiosis in amphibian oocytes of these lactones is related to the combination of different PRS rather than to the reactive potential of a single group.

### A158

#### NITRIC OXIDE EFFECT ON OBSTRUCTIVE NEPHROPATHY

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A major feature of the injury sustained by the kidney during obstruction is a profound induction of apoptosis in the tubular epithelium. After 14 days of obstruction, endogenous nitric oxide (NO) decreases considerably. It has been reported that increased NO availability could be beneficial for unilateral ureteral obstruction (UUO) relief. The goal of this study was to examine the effect of nitric oxide on: 1) apoptosis and fibrosis in UUO of neonatal rat kidneys; 2) apoptosis in response to mechanical stretch in NRK52E cells. Methodologies: Neonatal rats subjected to UUO and controls were treated daily with NG-nitro-L-arginine methyl ester (L-NAME, 50mg/kg/day), L-arginine (L-Arg, 100mg/kg/day) or vehicle for 14 days. They were nephrectomized. Gene expression related to apoptosis and fibrosis was evaluated by RT-PCR and Western Blot. NRK52E cells were exposed to 48 hours of graded mechanical strain using the Flexcell system in the presence or absence of L-NAME (1mmol), L-Arg (1mmol) and sodium nitroprusside (NPS, 2mmol) or their combinations and then apoptosis was assessed with Annexin V/Propidium Iodide based flow cytometry. Nephrogenic and cytoprotective gene expression decreased in obstruction and with the use of L-NAME but remained with respect to controls with L-Arg treatment. Cells with L-Arg or NPS treatments and their combination showed a reduction in apoptosis even in the control cells with no stress. L-NAME and L-NAME/L-Arg treatments increased apoptosis in the groups exposed to mechanical strain but not in the control group. We concluded that NO availability protects against apoptosis during obstructive injury.

### A159

#### NEW ASTERISCANE, LIPPIFOLIANE AND AFRICANANE DERIVATIVES FROM THE MEDICINAL HERB

##### *Lippia integrifolia*

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*L. integrifolia* (Griseb.) Hieron., commonly known as “incayuyo” or “té del inca”, is a herb native to central and northwestern Argentina. Infusions of the aerial parts are traditionally used against dyspepsia, stomachaches, indigestions, as a soft diuretic and emmenagogue, for cough treatment and as a sedative. *L. integrifolia* has been included in the Argentine Food Code as a seasoning since it is used as an ingredient in some well known commercial non-alcoholic beverages, appetizers and teas. Recently we demonstrated that the aqueous extracts of *L. integrifolia* possess anti-inflammatory effects on stomach cells and antiadhesive properties against the main bacterial inducer of gastritis, *Helicobacter pylori* (Marcial *et al.*, 2014. *J. Ethnopharmacol.* **155**:1225-1233). Previously, we reported that the chemotype “lippifolienone” of *L. integrifolia* is a rich source of sesquiterpenoids with the rare sesquiterpene skeletons called integrifoliane, lippifoliane, africanane and asteriscane. Continuing with our investigations on this plant, we report here the isolation of a new asteriscane derivative, 6 $\alpha$ -hydroxy-3 $\alpha$ ,7 $\alpha$ -epoxy-1-asteriscene, a new lippifoliane derivative, 2 $\alpha$ -hydroxy-lippifoli-1(6)-en-5-one and two epimeric hydroxyketones with africanane skeleton, *i.e.*, 2 $\alpha$ -hydroxy-african-1(5)-en-6-one and 2 $\alpha$ -hydroxy-african-1(5)-en-6-one from the more polar fractions of the essential oil. The structure of the new derivatives was elucidated by spectroscopic methods, mainly NMR with 1D and 2D experiments. The possible biogenetic pathway for the asteriscane, lippifoliane and africanane derivatives from an humulene precursor will be discussed.

### A160

#### DECOCTION OF *Smallanthus macroscyphus* LEAVES ATTENUATES OXIDATIVE STRESS IN DIABETIC RATS

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Increasing evidence has suggested that oxidative stress plays a major role in the pathogenesis of diabetes mellitus complications. In a recent work we demonstrated that decoction of *Smallanthus macroscyphus* (Baker ex Martius) A. Grau leaves was effective to reduce postprandial glucose and useful in the treatment of diabetic animals.

In the present study we evaluated the antioxidant effect of 10% *S. macroscyphus* leaves decoction (DEC) on antioxidant enzymes and lipid peroxidation in liver and kidney of diabetic rats. Thirty-day treatment with DEC by the oral route significantly decreased the levels of glucose, HbA1c, Cholesterol and Triglycerides in blood and increased body weight gain. While the untreated diabetic rats

showed high activities of SOD and CAT, low activity of GPx, reduced concentration of GSH and increased levels of lipid peroxidation in liver and kidney homogenates, these parameters were restored to near normal levels by the treatment. In addition, treated diabetic rats showed a restored histoarchitecture of liver and kidney. These results provide a scientific basis that supports the efficacy of *S. macroscyphus* leaves treatment to revert oxidative stress and improve glucose metabolism in diabetic animals.

#### A161

### BENZNIDAZOLE AND CLOMIPRAMINE COMBINATION AGAINST *Trypanosoma cruzi* NATURAL ISOLATES IN A MURINE MODEL

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Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*. Treatment is controversial due to the partial effectiveness and the side effects of the available drugs, Benznidazole (BZ) and Nifurtimox. Clomipramine (CLO) is an inhibitor of *T. cruzi* trypanothione reductase and can be used as an alternative therapy. The aim of this work was to evaluate the effect of the association of BZ with CL at lower doses for the treatment of Chagas disease in the acute stage, in Swiss albino mice infected with natural isolates of *T. cruzi* from an endemic area in Argentina (CASIBLA and SGOZ12). Two groups of mice, n=36, were infected with 50 parasites of each isolate. The two groups of mice were divided into: infected non-treated (INT), infected and treated with CLO 1.25mg/kg/day (CLO1.25), CLO 5mg/kg/day (CLO5), BZ 100mg/kg/day (BZ100), BZ 6.25mg/kg/day (BZ6.25) and BZ6.25+CLO1.25. Treatment effectiveness was evaluated through survival, parasitemia measured through qPCR and liver, skeletal and cardiac muscle histopathological studies were performed on day 35 post-infection. The combined effect of the drugs was evaluated using Principal Component Analysis. All treatment schedules significantly ( $P < 0.05$ ) decreased parasitemia. Groups BZ100, BZ6.25+CLO1.25 and BZ6.25 showed high survival percentages but exhibited different levels of liver injury. Groups CLO1.25 and CLO5 showed low survival percentages and high levels of inflammatory cells in skeletal muscle. Finally, the therapeutic results from the combination of BZ and CLO enhanced the effect of the treatment with respect to BZ6.25 and CL1.25 in mice infected with either isolate.

#### A162

### GENOTOXIC EVALUATION OF HOT AQUEOUS EXTRACT OF *Baccharis articulata* BY MOUSE BONE MARROW MICRONUCLEUS ASSAY

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*Baccharis articulata* (Lam.) Pers., distributed globally, is particularly abundant in the hills of Córdoba (Argentina) and it is often used in regional folk medicine, especially in the treatment of digestive ailments due to its hepatoprotective, coleretic and colagogue action. The species is found in many herbal products of great human consumption. Several studies have demonstrated that different extracts of *B. articulata* present antiviral, antifungal, antioxidant and anti-inflammatory activities. However, few studies have evaluated the potentially cytogenotoxic effect of herbal products that ensure their safe use. The aim of this study was to determine the cytogenotoxic ability of hot aqueous extract of *Baccharis articulata* (HAE) using a mouse bone marrow micronucleus test. No toxicity was detected in any extract-treated groups (polychromatic erythrocytes/ normochromatic erythrocytes). HAE, at doses of 500, 1000 and 2000 mg/Kg, did not produce an increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow with respect to the negative control ( $p < 0.001$ , ANOVA Tukey test). In conclusion, the present work demonstrates the absence of a cytogenotoxic effect in the hot aqueous extract of *Baccharis articulata* on mouse bone marrow.

#### A163

### BIOPROTECTION OF ANTIOXIDANT COMPOUNDS AGAINST THE TOXIC EFFECT OF AFLATOXIN B1 IN VERO CELLS

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Fungal toxins negatively affect production parameters and generate severe economic losses. Aflatoxin B1 (AFB1), produced by strains of *A. flavus* and *A. parasiticus*, is carcinogenic, teratogenic, hepatotoxic, immunotoxic and at high doses it could be lethal. Natural products have been shown to possess mycotoxin detoxifying capacity. *Achyrocline satureioides*, "Marcela del campo", has many bioactivities and, is a promising option to detoxify AFB1. The objective of this work was to evaluate the ability of the main antioxidant compounds, luteolin (L), quercetin (Q), chlorogenic acid (CLA) and caffeic acid (CA), of *A. satureioides* to protect cells from toxic damage exerted by AFB1. AFB1 was obtained by extraction and purification from a culture of *A. parasiticus* NRRL 299. Cytotoxicity studies: monolayers of Vero cells were treated with different concentrations of AFB1 (0.01-10  $\mu\text{g/mL}$ ) and viability was evaluated using the MTT reduction technique. Protection assays: monolayers of Vero cells were treated simultaneously with AFB1

(0.01 and 0.1  $\mu\text{g/mL}$ ) and with non-cytotoxic concentrations of: L (50  $\mu\text{g/mL}$ ), Q (50 and 100  $\mu\text{g/mL}$ ) and CLA and CA (100 and 200  $\mu\text{g/mL}$ ), in independent assays. The results indicated high toxic power of AFB1 ( $\text{CC}_{50}$ = 0.03  $\mu\text{g/mL}$ ). The bioprotection studies revealed that the cells were protected from the toxic effect of AFB1 by CLA, L and Q. When cells were treated with AFB1 at 0.01  $\mu\text{g/mL}$  and CLA or L, a viability percentage similar to that of the cell control was achieved. CA did not protect the cellular system. The results are promising and encourage the continuation of this research.

#### A164

### PREDICTION OF REACTIVITIES AND BEHAVIORS OF 2-AMINO-1-PHENYLPROPANONE IN GAS AND IN ETHANOL SOLUTION

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The compound 2-amino-1-phenylpropanone, known as cathinone, has stimulant properties, because it acts at the level of the central nervous system, with less efficiency than amphetamines and where the presence of the C=O group in its structure favors the lipophilicity at the cellular level. The aim of this work was to predict theoretically their reactivities and behavior in ethanol and gas phases by using frontier orbitals and some descriptors. The S and R structures were optimized in both phases using the method B3LYP/6-31G\* with the Gaussian 09 program. The solvent effects were studied using the self consistent field reaction method and the polarized continuum model. The frontier orbital HOMO-LUMO energies and the chemical potential ( $\mu$ ), electronegativity ( $\chi$ ), hardness ( $\eta$ ), softness (S) and electrophilicity index ( $\omega$ ) descriptors were also calculated. The energy gap showed a greater decrease for conformer S than for R. The descriptors are influenced by the medium giving higher gap values for the S form in ethanol solution than for the R form. The volume from the phase gas to the solution show higher variation for the S form than the R form. It is concluded that the S form is more reactive in both media. The observed volume contraction and the solvation energy values suggest H bonds formation between the  $\text{NH}_2$  and C=O groups with the solvent molecules. The  $\omega$  index in solution is greater for the S form than for the R form. These observations for the S form can be justified by its higher stability and greater tendency to electrophilic substitution. Both conformers in ethanol solution have low  $\eta$  values in gas phase in agreement with their reactivity values.

#### A165

### STUDY OF ANTIFUNGAL MODE OF ACTION OF 4-HYDROXY-3-(3-METHYL-2-BUTENYL) ACETOPHENONE AGAINST *Candida albicans*

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*C. albicans* is an opportunistic fungal pathogen whose proliferation is favored in immunocompromised patients, producing multiple infections. 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (4HMBA) is the main secondary metabolite isolated from *Senecio nutans* Sch. Bip (Asteraceae) aerial parts, which was already described as an antifungal against *C. albicans* (ATCC10231). *C. albicans* strains with calcineurin pathway mutations, iron chelating activity, time-lapse microscopy observation of germ tubes development and electron microscopy observations were used to assess the antifungal mode of action of 4HMBA. Calcineurin activity, calcineurin controlled transcriptional factor and iron homeostasis were not affected by 4HMBA. Microscopy observations indicated that 4HMBA inhibits cell transition between yeast-form (non virulent) to filamentous-form (virulent) and reduced cell wall thickness. Our findings suggest that 4HMBA is a compound of interest to support further studies dealing with chemical modifications to increase the activity and diminish the fungal resistance to the commercial antifungal drugs. Further assays should clarify the cell target for 4HMBA and the action mechanism, though this study sets the first step for those researches.

#### A166

### EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF VARIOUS PLANT EXTRACTS

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There is great concern in the scientific community about the gradual reduction of effective antimicrobial drugs and the toxic effects of their residues in foodstuffs of animal origin. In this context, there has been increasing interest in the study and search for new compounds of plant origin that present antimicrobial activity. In this paper, we evaluate the potential antimicrobial activity of various plant extracts against *S. aureus* (ATCC 26922) and *P. aeruginosa* (ATCC 27853) using the agar diffusion method. Minimal Inhibitory Concentration (in 96-well plate) and Minimal Bactericidal Concentration were also determined for the extract that showed antimicrobial activity against both tested microorganisms. Extracts were obtained from tinctures dried by rotary evaporator and then resuspended in 7.5% DMSO. The following excerpts were evaluated: Lemon verbena, cloves, pitanga, mistol, dandelion, mouse ear, mallow, carob, sage, cow hoof and molle from Cordoba. The only extract evaluated that exhibited antimicrobial activity against both microorganisms was Molle from Cordoba (*Lithraea molleoides* (Vell.) Eng.), showing inhibition halos of 13.5 mm average in both cases. It was determined that the MIC of Molle from Cordoba for *S. aureus* was 14  $\mu\text{g/mL}$  and the MBC of 28  $\mu\text{g/mL}$ ; while for *P.*

*aeruginosa*, MIC was 460 ug / ml and CBM of 920 ug / ml. The results obtained indicate that the ethanolic extract of Molle might be a suitable candidate to carry out further in vitro tests, including a greater number of species and bacterial strains in order to determine their potential therapeutic usefulness in various pathological processes of infectious origin.

#### A167

### COMPARATIVE STUDY OF THREE DERIVATIVES OF THE TROPANE NUCLEUS IN AQUEOUS SOLUTION BASED ON ELECTRONIC DESCRIPTORS

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Tropane alkaloids, derived from the tropane N-methyl-8-azabicyclo [3.2.1] octane nucleus, belong to the *Solanaceae* family. They have pharmacological properties due to their anti-cholinergic or stimulant activities. Their overdose causes convulsions, heart arrhythmia, respiratory inadequacy and even death. Here, scopolamine, called commonly burundanga, (C<sub>17</sub>N<sub>2</sub>NO<sub>4</sub>), atropine (C<sub>17</sub>N<sub>2</sub>NO<sub>3</sub>) and anisodamine (C<sub>17</sub>N<sub>2</sub>NO<sub>4</sub>) were the three derivatives selected in this study. The objective of this work was to predict the reactivities and behavior in gas phase and in aqueous solution of those three tropane alkaloids using calculations based on the density functional theory (DFT) with the B3LYP/6-31G\* method. The three structures were optimized in gas and aqueous solution phases with the Gaussian 09 program and the B3LYP/6-31G\* method. The corresponding HOMO and LUMO energy values and the chemical potential ( $\mu$ ), electronegativity ( $\chi$ ), hardness ( $\eta$ ), softness (S) and electrophilicity index ( $\omega$ ) were calculated. Analysis of the results showed that the reactivity order in the gas phase is: atropine > atrosine > anisodamine, while in the aqueous solution it changes to: atrosine > anisodamine > atropine. On the other hand, atropine presents a higher electrophilicity index ( $\omega$ ) in the gas phase while in aqueous solution the highest value was observed in anisodamine. The behavior observed in both phases is clearly correlated with the functional groups present in scopolamine and anisodamine.

#### A168

### CHLOROGENIC ACID PROTECTS AGAINST TOXIC DAMAGE INDUCED BY OCHRATOXIN A IN A 28-DAY ORAL TREATMENT IN RATS

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Ochratoxin A (OTA) is a mycotoxin with nephrotoxic, genotoxic and immunosuppressive properties. Recent studies showed that treatment for 24 h with polyphenols (chlorogenic acid, caffeic acid and luteolin) reduced the toxic effects of OTA *in vitro* and *in vivo*, chlorogenic acid (ChlA) being the compound with better effects. The aim of this study was to characterize *in vivo* ChlA capacity to reverse the toxic effects induced by OTA by a subacute toxicity test. Wistar rats were fed orally for 28 days with OTA (0.4 mg/kg), ChlA (5 mg/kg), or combinations OTA (0.4 mg/kg) + ChlA (5 mg/kg). PBS/methanol (0.03%) was used as vehicle control. No deaths or decrease in food intake or body weight were recorded in any experimental groups. The control group and the animals treated with ChlA alone showed no changes in any of the parameters evaluated. In animals treated with OTA significant changes were observed such as decrease in urine volume, decrease in urine creatinine values, proteinuria, occult blood, and decrease in absolute and relative kidney weight and histopathological lesions characteristic of kidney damage. The group treated with the combination OTA + ChlA was similar to the negative control group in the evaluation of certain parameters of toxicity, which would indicate a protective effect of ChlA on renal damage induced by OTA. These results provide the basis for further studies related to the protective effects of polyphenols and the mechanism of action exerted on the toxicity of OTA, considering that similar effects could occur by administration of these compounds in the diet of animal species susceptible to OTA.

#### A169

### EFFECTS OF *Flourensia* EXTRACTS AND (-)-HAMANASIC ACID A ON BIOFILM FORMATION AND PLANKTONIC GROWTH OF *Staphylococcus aureus*

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Pathogenic biofilms are associated with persistent infection due to their high resistances to various antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial diseases. In this line, *Flourensia fiebrigii* (Asteraceae) proved to have antipathogenic metabolites. In the present study, the anti-biofilm abilities of *Flourensia campestris* (FC) and *F. oolepis* (FO) dry leaf extracts, and of the sesquiterpene (-)-hamanasic acid A ((-)-HAA) against MRSA (F7, locally isolated), and an international collection (ATCC 6538 P) of *S. aureus* strain, were examined. EtOAc subextracts were obtained from partition of 24 h-22°C-6% aqueous

extracts, and MeOH and CHCl<sub>3</sub> extracts from ground tissue. In order to propose anti-pathogenic metabolites of low toxicity for freshwater species, their activity against the snail *Biomphalaria peregriana* were determined.

All assayed samples at concentrations of 25-50 µg/ml inhibited *S. aureus* strains biofilm formation, FO being more active than FC, without affecting planktonic cell growth. MRSA biofilm was inhibited by 11-66%, the most antipathogenic (66%) being (-)HAA at 25 µg/ml, while ATCC 6538 P biofilm was inhibited by 9-42%. In this case, highest inhibition was obtained with both (-)HAA and CHCl<sub>3</sub> extracts at 25 µg/ml. The inhibitory effects were observed for 24 h (27-48%). The active (-) HAA and EtOAc (FC) extract was non toxic for *B. peregriana* at 100 µg/ml; the other extracts showed toxicity at ≥ 50 µg/ml. Although FO extracts are known to possess antimicrobial activity, this is the first study that reports that both *Flourensia* species studied and, interestingly, the bisabolonoid (-) HAA, have promissory anti-biofilm activity against *S. aureus*.

#### A170

### GASTROPROTECTIVE EFFECT OF THE AQUEOUS EXTRACT OF *Smallanthus macroscyphus* LEAVES ON ULCERATION MODELS

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The “wild yacon”, *Smallanthus macroscyphus* (Baker ex Martius) A. Grau, is an autochthonous perennial herb distributed from southern Bolivia to northwestern Argentina. Peptic ulcer is an increasing disorder resulting of the imbalance between offensive and defensive factors in the gastric mucosa and the search for new treatments is a current challenge. The aim of the present study was to evaluate the *in vivo* gastroprotective and antisecretory effects of the aqueous extract of *S. macroscyphus* leaves. Two models of induced gastric ulcers in male Wistar rats, ethanol and pylorus ligation, were used. The experimental groups (n=6 animals) were: 1-Control, 2-Positive controls received Sucralfate or Ranitidin (100mg/Kg, orally), 3-Treated with aqueous extract of *S. macroscyphus* leaves (140mg/kg, orally). Mucus content and ulceration parameters (number of ulcers, severity and ulcerated area percentage) were determined. Chemical and microscopic study of gastric contents and histological studies were performed. *S. macroscyphus* extract caused a significant ( $p < 0.05$ ) decrease in the ulcerated area, the severity and number of ulcers, conserving the mucus content. There was also a decrease in the volume, free acidity and presence of cellular elements in gastric content with respect to the control group; total acidity was not affected. These preliminary results suggest that wild yacon could have beneficial gastroprotective and antisecretory effects. Additional studies are needed to determine the active compounds and mechanisms involved.

#### A171

### ANTIOXIDANT COMPOUNDS OF *Achyrocline satureioides* INHIBIT WESTERN EQUINE ENCEPHALITIS VIRUS

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Alphavirus (Togaviridae family) are highly virulent pathogens that cause encephalitis in humans and horses, affecting different regions of America. One of its members is the *Western Equine Encephalitis* (WEEV) virus. There are no effective antivirals to deal with its infection. *Achyrocline satureioides* (Asteraceae family) has many medicinal properties. Phytochemical analysis of extract of *A. satureioides* revealed the presence of luteolin (L), quercetin (Q), chlorogenic acid (CLA) and caffeic acid (CA). The aim of this study was to evaluate the *in vitro* antiviral action of the compounds (C) of *A. satureioides* against WEEV. The aqueous extract was obtained from the aerial parts of the plant and analyzed by HPLC-mass. Cytotoxicity was assessed by Neutral Red Uptake (RN) and MTT reduction in Vero cells. Then, the antiviral activity of C during the whole viral replication cycle was evaluated by plaque reduction assay. Further, the action stage in the replication cycle was determined. Finally, the selectivity index (SI) was calculated. Cytotoxicity studies revealed low toxicity of all C. The studies of antiviral ability indicated that WEEV was inhibited 100% by L (15 µg/mL) and 55% by CA (10 µg/mL), Q and CLA were not active against WEEV. Both compounds exert their action in post-viral adsorption and penetration stage. Selectivity indices of L were: 146 (RN) and 99 (MTT). L and CA showed strong inhibitory action against WEEV and may be useful for the treatment of infections caused by alphaviruses.

A172

**LARVICIDAL EFFECT OF EXTRACTS OF *Tagetes minuta* L. (ASTERACEAE) AGAINST *Aedes (Stegomyia) aegypti* Linnaeus, 1762 (DIPTERA: CULICIDAE).**

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Mosquitoes are considered urban pests because they are disease vectors. *Aedes (St.) aegypti* is known to carry dengue, yellow fever and chikungunya fever. An alternative to chemical control of these vectors is the use of natural insecticides. *Tagetes minuta* L., an aromatic herb, has bioactivities and that its essential oil has insecticidal activity, we evaluated the larvicidal effect of extracts of *T. minuta* in larvae of *St. aegypti*. Ethanolic extracts were prepared (P1, P2 and P3), from dried plant material obtained from populations selected for their chemical profile and tested at 0.003, 0.005, 0.007, 0.01, 0.02 and 0.05 mg/ml. Each treatment was performed in triplicate, placing 30 third stage larvae in trays with distilled water (100ml). Chlorpyrifos were used as positive control and water and DMSO (extracts diluents) were used as internal control. Mortality was recorded at 24 hours. Probit Regression was used to determine LD<sub>50</sub>. Using ANAVA, we detected that the profiles presented significant differences between 0.003-0.007mg/ml: P1 (H=19.25; p>0.05), P2 (H=19.56; p>0.05) and P3 (H=19.10; p>0.05). Mortality rates above 95% were obtained in the three occasions. In P1 and P3, LD<sub>50</sub> was 0.008mg/ml, whereas for P2 higher concentrations were needed to achieve 50% mortality (0.1mg/ml). LD<sub>50</sub> estimated for chlorpyrifos was 6x10<sup>-6</sup> mg/ml. The results suggest that the profiles P1 and P3 of *T. minuta* have great larvicide potential and can be used in the control of *St. aegypti*.

A173

**LARVICIDAL ACTION OF *Heterophyllaea pustulata* HOOK F. (RUBIACEAE) AGAINST *Culex quinquefasciatus* SAY (DIPTERA: CULICIDAE).**

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*Culex quinquefasciatus* is a mosquito of great interest in Public Health because it is a vector of St. Louis Encephalitis Virus (SLEV) so that its control is important. *Heterophyllaea pustulata* is a native species with considerable biological properties. In view of the importance of *Cx. quinquefasciatus* and the antecedents of *H. pustulata*, we decided to evaluate the toxic effect of extracts of this species against *Cx. quinquefasciatus* larvae. Four extracts were obtained from stems, leaves and roots of *H. pustulata* (hexane, Hex; benzene, Ben; ethyl acetate, EtOAc and ethanol, EtOH), which were tested at 0, 5; 0, 3; 0, 1 and 0.01 mg/ml and applied to cohorts with 30 larvae (III state) on trays with 100ml of distilled water. Mortality rate was estimated at 24 hours from 3 replicates. The significant differences among treatments were evaluated through ANOVA and the Lethal Dose 50 (DL<sub>50</sub>) was estimated by the bivariate probit. Although all extracts produced a high mortality rate, only the ones from the stems and roots exceeded 90%. The stem extracts showed a significant effect of the interaction concentration-solvent (F=5.25; p<0.05) with the highest recorded mortalities (Ben 100% and EtOAc 95.6%) at the highest concentration. Similar results were recorded for root extracts (F=2.88; p<0.05). The DL<sub>50</sub> to the stem extracts were 0.121mg/ml and 0.161mg/ml for Ben and EtOAc respectively. As to the root extracts, DL<sub>50</sub> was 0.168mg/ml for EtOAc and 0.198mg/ml for Ben. The results indicate that the extracts Ben and EtOAc from *H. pustulata* stems and roots could be potential botanical insecticides to deal with *Cx. quinquefasciatus*.

A174

**EFFECTS OF PHENYLPROPANOIDS ON STORED GRAINS PESTS: *Sitophilus zeamais* MOTSCHULSKY (COLEOPTERA, CURCULIONIDAE) AND *Fusarium verticillioides***

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Argentina is the third largest maize producer in South America and the sixth in the world. *S. zeamais* and *F. verticillioides* are the primary pests that attack maize grains. Phenylpropanoids are interest alternatives to synthetic pesticides. The aim of this study is to evaluate the insecticidal and antifungal activities of structurally related phenylpropanoids against maize pests through “in vitro” contact bioassays. The most bioactive compounds against insects and fungus were: cinnamaldehyde, α-bromo cinnamaldehyde and 4-phenyl-3-buten-2-one (LC<sub>50</sub>: 51.7 µg/cm<sup>2</sup>, MIC: 0.5 mM; LC<sub>50</sub>: 67.1 µg/cm<sup>2</sup>, MIC: <0.031 mM; LC<sub>50</sub>: 69.8 µg/cm<sup>2</sup>, MIC: 0.5 mM, respectively). These compounds present a carbonyl group that could increase toxicity. In conclusion, phenylpropanoids with carbonyl group could be used as potential tools against stored products pests.

**A175**

**BIOACTIVITY OF CUMIN ESSENTIAL OILS AGAINST GRAIN MAIZE PESTS**

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Stored corn grains are attacked by numerous pests such as *Sitophilus zeamais*, a primary pest and *Fusarium verticillioides*, the main maize plant fungal pathogen. Since these pests cause qualitative and quantitative losses, there is a pressing need to protect stored grains from insects and fungi. Essential oils (EOs) are an alternative to synthetic pesticides. The objectives of this study were to evaluate the *in vitro* bioactivities of seven EOs of cumin plants, collected from different departments of Catamarca province, against *S. zeamais* and *F. verticillioides*. Insecticidal activity was evaluated by fumigant toxicity assay, and antifungal activity using the agar dilution method. The most active EOs against *S. zeamais* (LD<sub>50</sub> <42 µl/L air) were those that presented a similar composition: cuminaldehyde (20%), p-cymene (20%), α-terpinene (20%) and β-pinene (15%). These EOs inhibited the insect acetylcholinesterase enzyme. On the other hand, the most effective EOs on fungal growth were those with high concentration of cuminaldehyde (50%) at the three concentrations tested (250, 500 and 1000 ppm). In conclusion, EOs of cumin plants could be used to control the insect population and fungal growth, as a natural alternative for the integrated management of maize pests.

**A176**

**Macrocystis pyrifera ALGA EXTRACT IMPROVES IN VITRO SURVIVAL OF PGPRs BACTERIA**

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Application technology of bacterial inoculants on seeds is a practice that has been used in the productive sector for about 20 years. On the other hand, they have long been sold on the market as a natural extract of algae that can provide multiple benefits, including improved inoculation technique, preserving the viability and the overall physiological state of microorganisms to incorporate into the soil, using the virtues of both towards ecological and sustainable agriculture. Our objective was to evaluate *in vitro* microorganism survival in *Macrocystis pyrifera* algae extract (AE) as a precedent for the subsequent study of the bacterial recovery and use of seed extract in a mixed inoculation with PGPR. Bacterial inocula of strains Az39 and E109 in combination with four doses of AE were placed in test tubes. Survival of each strain was evaluated three times at 0, 9 and 28 days after the combination. Results showed that Az39 survival was enhanced in combination with low doses of AE unlike E109, whose survival increased with the concentration of the extract. The results indicate that the extract of the alga *Macrocystis pyrifera* is a product with high technological potential that could replace the products that are used today as protective / bacterial adhesives for application to seeds.

**A177**

**PARTICIPATION OF LACTATE DEHYDROGENASE IN THE IN VITRO CAPACITATION OF FRESH AND CRYOPRESERVED PORCINE SPERMATOZOA**

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Cryopreserved porcine sperm utilization in biotechnological processes is very limited and there are few studies on the relation between enzyme activity and the processes that lead to the acquisition of fertilizing capacity. The aim of this study was to determine the activity of lactate dehydrogenase (LDH; 1.1.1.27) in fresh and cryopreserved porcine spermatozoa and study its participation in the process of *in vitro* capacitation. LDH activity was determined spectrophotometrically and the enzyme unit (EU) was defined as the amount of LDH enzyme that oxidizes 1 µmol of NADH/minute. Sperm capacitation percentages were determined in the presence or absence of different concentrations of sodium oxamate (a competitive inhibitor of LDH) by the chlortetracycline technique. Viability and motility were also evaluated by the eosin/nigrosin technique and optic microscopy in thermal stage, respectively. The activity of LDH was 3.40±1.21 EU/10<sup>10</sup> spermatozoa in fresh sperm and 0.92±0.67 EU/10<sup>10</sup> spermatozoa in cryopreserved sperm. The addition of the competitive inhibitor of the enzyme significantly diminished capacitation and motility, at different concentrations in fresh or cryopreserved sperm. Our results demonstrate that lactate dehydrogenase participates in the *in vitro* capacitation in fresh and cryopreserved spermatozoa and its activity decreases in cryopreservation. The determination of the activity of LDH could be used as a marker of congelability in this species.

A178

**PHOSPHATIDYLCHOLINE: AN IMPORTANT LIGAND IN THE BIOSORPTION OF ALUMINIUM BY  
*Pseudomonas putida* INACTIVE BIOMASS**

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Biomasses of *P. putida* A (ATCC 12633), viable or nonviable (inactivated by heat treatment), were capable of adsorbing  $Al^{+3}$  to cell surface ligands, mainly to phosphatidylcholine (PC). The adsorption process is rapid, stable over time, agitation-independent and efficient at pH 4.3 and in a broad temperature range (15-42 °C). In order to confirm the importance of PC in the biosorption process, assays dependent on  $Al^{+3}$  concentration using as bioadsorbents nonviable biomasses of *P. putida* A (ATCC 12633) and *P. putida* PB01 (a mutant strain lacking PC) were conducted. 6 mg  $ml^{-1}$  of biomasses resuspended in aqueous solution of HCl (pH 4.3) were exposed to increasing concentrations of  $AlCl_3$  (0-300  $nmol\ ml^{-1}$ ). The samples were incubated for 1 min at room temperature without agitation and the free  $Al^{+3}$  was measured by spectrophotometry. The results showed that *P. putida* A (ATCC 12633) bioadsorbent was saturated with 130  $nmol\ ml^{-1}$  of the  $Al^{+3}$ , while only 60  $nmol\ ml^{-1}$  of the metal were necessary to achieve saturation of *P. putida* PB01. The maximum number of adsorption sites per microorganism was determined through adsorption isotherms. Biomass of *P. putida* A (ATCC 12633) showed about 30 times more binding sites ( $36 \times 10^5$  sites  $microorganism^{-1}$ ) than the biomass of *P. putida* PB01 ( $1.2 \times 10^5$  sites  $microorganism^{-1}$ ). These results demonstrated that although the mutant is capable of adsorbing  $Al^{+3}$  in other ligands, the absence of PC significantly affects the availability of the binding sites and thus the capacity of this biomass to adsorb  $Al^{+3}$ .

A179

**IMPORTANCE OF FINAL CONCENTRATION OF ETHANOL FROM YEASTS IN RELATION TO VINASSE LEVELS**

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The main liquid waste generated during ethanol production is vinasse. This residue is highly polluting for water bodies, where it can cause the collapse of aquatic life due to the large amount of organic matter it has, so it is necessary to handle it properly. Increasing the fermentative power of yeast for industrial use from 10 to 12% ethanol in musts would reduce the amounts of vinasse obtained. Traditionally, yeasts are the main microorganisms used in alcohol production and better yeast strains would increase bioethanol production at lower distillation costs and ensure a renewable and economically profitable biofuel. We isolated yeast strains for ethanol production; A2, A10 and A11 strains were selected, which produced 11.36; 11.13; 11.40% of ethanol respectively. A non flocculent strain A2 was chosen for further study of scaling because is compatible with the technology currently used in the industry. The use of *Saccharomyces cerevisiae* A2 would decrease the vinasse levels generated by 30%, from an average of 13 Lvinasse / L alcohol to 9 Lvinasse / L alcohol. The introduction of *Saccharomyces cerevisiae* in the milling factories would benefit production, energy consumption and environmental advantages.

A180

**EVALUATION OF HYDROLYTIC ENZYMES IN THERMOPHILIC BACTERIA ISOLATED FROM  
HIGHLAND ECOSYSTEMS, PUNA ARGENTINA AND SHRUBLANDS OF BRASÍLIA**

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Thermophilic microorganisms represent a valuable source of enzymes capable of catalyzing biochemical reactions at high temperatures. The purpose of this work was to select thermophilic bacteria with hydrolytic activity of amylase, xylanase, protease, cellulase and lipase from 22 isolates previously made from soil samples and lagoons of highland ecosystems. A first selection of bacteria was performed based on hydrolysis halos on selective agar media with soluble starch, carboxymethyl cellulose, skim milk powder, Birchwood xylan, and olive oil to visualize the presence of amylase, cellulase, protease, xylanases, and lipases respectively. Growth kinetics was performed at different temperatures to select thermophilic bacteria. Total protein and amylase activity in liquid medium at 45 °C with the selected bacteria was quantified. Three bacteria were selected: *Bacillus licheniformis* L2, *Bacillus amyloliquefaciens* L3 and *Alicyclobacillus acidocaldarius*. These bacteria had a higher hydrolysis halo for amylase, xylanase, protease and cellulase at 45 °C than at 37 °C. No significant lipases activity was observed. The maximum amylase activity obtained for *B. licheniformis* L2, *B. amyloliquefaciens* L3 and *A. acidocaldarius* was 9.65, 9.07 and 8.37 U/ml respectively. It is concluded that these enzymes can be used in bioprocesses where high temperatures are required for chemical reactions in order to improve the efficiency of the production stages.

**A181**

**CLONING AND EXPRESSION OF BULL CALTRIN PROTEIN IN *Escherichia coli*.**

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Bovine Caltrin (calcium transport inhibitor) is a secreted protein expressed by the bull seminal vesicles. It is known to bind to sperm during ejaculation and to inhibit sperm intake of extracellular Ca<sup>2+</sup>, preventing spontaneous acrosome exocytosis and increasing *in vitro* fertilization potential of epididymal sperm. Purification of bovine Caltrin from seminal plasma is rather complex. We designed a method to produce recombinant bovine Caltrin (Caltrin-rec) in bacteria. Total RNA isolated from bull seminal vesicles was purified using a standard protocol. Complementary DNA was synthesized using a retro-transcriptase and amplified by PCR using specific primers for directional cloning into a prokaryotic expression plasmid. The plasmid with/without (control) the Caltrin-rec encoding insert was used to transform *E. coli* BL21 (DE3) competent bacteria. Four recombinant clones were selected to evaluate the expression of Caltrin-rec after induction of liquid bacterial cultures with IPTG. Using SDS-PAGE, a protein band of 5.5 kDa was detected, wherein Caltrin bovine peptides were identified by HPLC-MS/MS. To optimize expression of rec-Caltrin, a set of protein expression induction protocols were assayed; bacteria cells were lysed and analyzed by SDS-PAGE. The use of Caltrin-rec will contribute to further characterize its role during mammalian fertilization.

**A182**

**EFFECTIVENESS OF MINIMUM VOLUME VITRIFICATION IN BOVINE OOCYTES MATURED IN VITRO, EVALUATED THROUGH MORPHOLOGICAL PARAMETERS**

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The standard method of cryopreservation of embryo and oocytes is slow freezing, although it has poor success in bovine oocytes, while the results obtained through vitrification in humans represents a possible alternative. It is not yet commonly used in bovines and has variable results with current methods. The objective of this work was to evaluate the morphology and viability of vitrified and defrosted matured bovine oocytes. We obtained oocytes-cumulus complex (COC'S) through aspiration of antral follicles of slaughtered bovine ovaries. COCs were matured in 199 solution with 50µg/ml of gentamicin sulphate, 5% of fetal bovine serum, 0.2 µg/ml of FSH and 2µg/ml of LH, under mineral oil at 39°C, 5% CO<sub>2</sub> and 100% of humidity for 24hs. The matured oocytes were denuded and vitrified through Cryotech, using permeable cryoprotectants (ethylene-glycol, DMSO) osmotically active components (sucrose) and a thin film as support. They were thawed using decreasing concentrations of sucrose solution in subsequent passages up to isotonic solutions. Later the oocytes were evaluated morphologically through stereomicroscope and their viability was proved through fluorochrome fluorescein diacetate technique. Up to now we have vitrified/defrosted 58 *in vitro* matured oocytes, out of which 55 remained viable (94%), with normal morphology, preserving their plasmatic integrity, a reconstructed perivitelline space and a uniform cytoplasm. The results would suggest that the proposed method is highly effective compared to slow freezing and to other vitrification methods in the bibliography, thus appearing as a viable alternative for oocyte preservation.

**A183**

**TECHNOLOGICAL EVALUATION OF SURFACTIN PRODUCTION BY BATCH FERMENTATION WITH *Bacillus subtilis*, *Bacillus licheniformis* AND *Bacillus amyloliquefaciens*.**

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Production media design for biosurfactants production with low cost non-conventional substrates is an important parameter for the technical evaluation and commercial feasibility of the microbial bioprocess. Batch fermentation under controlled ambient conditions allows the increase in yield and productivity of the bioprocess. Biosurfactants lower viscosity and increase oil emulsion in water. Another relevant property of surfactin is its antimicrobial activity against a wide range of microorganisms. In this work the capacity of surfactin production of three *Bacillus* strains: *subtilis*, *amyloliquefaciens* and *licheniformis* in batch fermentation was evaluated. The main substrate used was saccharose 25 g/L and salts, without yeast extract in order to decrease costs. Work volume was 1L and fermentation was carried out for 24 h with agitation. Surfactin was collected by extraction of foam during the first 15 h of fermentation. Surfactin was partially purified by acid precipitation with chloridric acid and extraction with chloroform-methanol. In all cases, surfactin production was within the range of 0.3 to 0.4 g /L and 88% of produced surfactin was present in the foam. This methodology allowed the simplification of the downstream process with a positive impact on the recuperation costs. The results obtained show that the production medium without yeast extract that can be utilized for surfactin production with *Bacillus* strains and represents a lower cost alternative.

A184

**BONE BIOCOMPATIBILITY OF HYBRID BIOACTIVE GLASS-CHITOSAN-POLYVINYL ALCOHOL 3D  
MATRIXES FOR TISSUE ENGINEERING.**

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Bone biocompatibility of hybrid polymer compounds bioactive glass (MHVB) 20% polymerized with different chitosan ratios (Q) and polyvinyl alcohol (PVA) for bone tissue engineering scaffolds was evaluated. MHVB with chitosan-PVA 3:1 ratio made up Group A (Ga), Group B (Gb): 1:1 ratio and Group C (Gc): 1:0 ratio, respectively. In femoral defects, 6 mm Ø, 15 matrices were implanted for 3 months into 1-week-old New Zealand rabbits, Samples were obtained, fixed in buffered formalin 10, with and without decalcification. Serial sections were stained with H&E or Masson-Goldner. Two areas were assessed: 1) matrix and interface and 2) surrounding bone. Zone 1: Ga) new bone sections surrounded by fibrous and bone tissue. Gb) little bone formed and mineralization cores surrounded by fibrous and bone tissues and Gc) mineralized matrix and new bone areas, granulation tissue and inflammation were found. There was non bone capsule. Zone 2: all groups showed bone trabeculae, compound and pagetoid type. Osteoid layer thickness was variable. Ga: cubic osteoblasts and other shapes, in multiple prominent layers. Gb and Gc: osteoblasts and lining cells were found. All groups showed foreign particles, bleeding and congestion. Inflammation. There was new bone formation in all MHVB-PVA-Q. Absence of bone capsule in Gc demonstrates its greater biocompatibility.

A185

**MEDICINAL PLANTS FROM KAZAKHSTAN AND ARGENTINA: ANTIFUNGAL ACTIVITY OF THEIR  
ESSENTIAL OILS**

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Plant essential oils are usually considered with low toxicity and with a reduced impact on the environment. Some of them are biostatics or biocides, with low ability to induce microbial resistance. The aim of this work was to evaluate the composition and antifungal activity of essential oils from native medicinal plants of Argentina and Kazakhstan. These oils were obtained by hydrodistillation from the aerial parts of *Acantholippia deserticola*, *Artemisia proceriformis*, *Achillea micrantha* and *Libanotis buchtormensis*. They were analyzed by gas chromatography coupled to mass spectrometry and the antifungal effect was evaluated by the microdilution method in YES medium. The main constituents of the essential oils were  $\beta$ -thujone (66.5 $\pm$ 0.2%), and *trans*-sabinyl acetate (12.1 $\pm$ 0.2%) in *A. deserticola*,  $\alpha$ -thujone (66.9 $\pm$ 0.4%) in *A. proceriformis*, 1,8-cineole (26.9 $\pm$ 0.5%), and camphor (17.7 $\pm$ 0.3%) in *A. micrantha* and *cis*- $\beta$ -ocimene (23.3 $\pm$ 0.3%), and *trans*- $\beta$ -ocimene (18.4 $\pm$ 0.2%) in *L. buchtormensis*. The fungal susceptibility was *Septoria tritici* and *S. glycines* (MIC<sub>100</sub>=0.70-2.70 mg/ml) > *Fusarium verticillioides*, *F. graminearum* and *Aspergillus carbonarius* (MIC<sub>100</sub>=2.70-10.60 mg/ml) and *A. niger* (MIC<sub>100</sub>=5.31-21.20 mg/ml). The essential oil of *A. deserticola* showed the highest antifungal activity. Further research is needed to establish the safety and real potential of the essential oils from *A. deserticola* as botanical fungicides.

A186

**EFFECT OF *Pseudomonas putida* ON THE GROWTH OF *Arabidopsis thaliana* IN THE PRESENCE OF HIGH  
LEVELS OF Al<sup>3+</sup>**

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The high levels of Al<sup>3+</sup> in the soil affect root growth, which influences the absorption of water and nutrients and disrupts normal plant development. *Pseudomonas putida* A (ATCC 12633) is capable of adsorbing Al<sup>3+</sup> through the formation of complexes with phosphatidylcholine (PC) of the membrane. The objective of this work was to study in *A. thaliana* the capacity of *P. putida* to mitigate the effects of Al<sup>3+</sup> on plant growth and development. 7 days after growth in MS medium or MS supplemented with 20 mM Al<sup>3+</sup>, the seedlings were inoculated with 10  $\mu$ l (1 x 10<sup>8</sup> ufc ml<sup>-1</sup>) of *P. putida* A (ATCC 12633) or *P. putida* PB01 (a mutant strain without PC). After 28 days of incubation, root length (A), leaf mass (B) and amount of protein (C) were determined. With respect to the absence Al<sup>3+</sup>, plants grown in the presence of the ion showed a decrease in A, B and C of 72%, 42% and 55%, respectively. In the presence of Al<sup>3+</sup> and when inoculated with *P. putida*, parameters A, B and C increased by 79%, 45% and 41%, respectively, while an increase of 33%, 22% and 19% for A, B and C, respectively, was detected in those inoculated with *P. putida* PB01. The analysis of plant roots using the fluorochrome 2,3,4,5,7-pentahydroxyflavone (morin reagent), which forms Al-morin complex, showed a lesser

amount of metal absorbed into the root when the plants were inoculated with *P. putida*. The results show that the ability of *P. putida* to form PC-Al<sup>3</sup> complexes decreases the availability of Al<sup>3+</sup>, allowing a mitigation of its effect on the growth of *A. thaliana*.

#### A187

### MICROWAVE-ASSISTED ENZYMATIC SYNTHESIS OF HOMOLOGOUS ESTERS OF 9-HYDROXYCINEOLE. STUDY OF ANTIMICROBIAL ACTIVITY.

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Previously we studied the differences between conventional heating and microwave radiation heating in esterification reactions. The results obtained then showed the efficiency of the latter by decreasing reactions times and costs. In this work the enzymatic esterification of 9-hydroxycineole with the homologous saturated monocarboxylic acid series (C2→C9) was performed and the antimicrobial activity of synthesized compounds was also evaluated. Experiments were carried out using a Philips MO-3446 household-type microwave oven at a power of 400 W. The immobilized enzyme Novozym 435<sup>®</sup>(CALB) was used as a catalyst. Synthesized compounds were identified by <sup>1</sup>H-NMR and <sup>13</sup>C-NMR. Antimicrobial activity was tested against: *Lactobacillus hilgardii* 5w, *L.plantarum*, *Saccharomyces cerevisiae*, *Kloeckera apiculata*, *Escherichia coli* ATCC 25922 and *Listeria innocua*, according to CLSI recommendations.

Eight homologous esters were obtained with yields of about 70% to 81%. Reaction times did not exceed 5 minutes, in contrast with reactions times obtained with conventional heating, which varies between 48 and 75 hours.

Antimicrobial activity assays revealed that increasing carbon chain length produced an increment in the inhibitory effect against all microorganisms assayed, probably due to the enhanced lipophilicity of compounds, C-9 being the most active ester (MIC= 100 µg/ml). These derivatives could be used as new antimicrobial agents in the food industry.

#### A188

### BIOCOMPATIBILITY EVALUATION OF *Sclerotium rolfsii* ATCC 201126 SCLEROGLUCAN IN SUBCUTANEOUS TISSUE OF RATS.

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Bone Tissue Engineering (BTE) combines three-dimensional scaffolds (3D), cells and signaling molecules to repair bone through **mature engineered tissue**. Our objective was to evaluate the in vivo biological response of a natural polymer scleroglucan called S. rolfsii ATCC 201126 implanted into rat subcutaneous tissues (ST) in order to consider its use combined with rhPTH scaffold for BTE. Polymer and zinc oxide eugenol pellets were implanted into the ST of 30 Wistar rats (150 ± 50 g). Animals were sacrificed at 7 and 30 days, and samples from implanted material and surrounded tissues were fixed in 10% buffered formalin. They were included in paraffin, serial cut and stained with H&E stain. At 7 days the polymer surrounded and colonized by polymorphonuclear neutrophils (PMN) was observed. A lympho-mononuclear infiltrate was also found. At the interface between polymer and ST granulation tissue, many fibroblasts and new vessels, surrounded by fibrous tissue were observed. Zinc oxide eugenol presented fibrin leukocyte exudation and coagulation necrosis, surrounded by loose connective tissue. At 30 days the polymer was resorbed and replaced by fibrous connective tissue. However, zinc oxide eugenol was surrounded by dense fibrous connective tissue-like capsule. The results demonstrated that scleroglucan S. rolfsii ATCC 201126 was biocompatible and bioresorbable in rat ST, supporting its application as a scaffold in BTE. In addition, the natural origin of this polymer makes it compatible with the environment.

#### A189

### POPULATION BEHAVIOR OF LACTIC ACID BACTERIA DURING ALCOHOLIC FERMENTATION OF WINES FROM MENDOZA

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Lactic acid bacteria (LAB) such as *Oenococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* are present throughout winemaking. Oenological practices, physico-chemical and nutritional factors influence their number and proportion. These bacteria may be responsible for malolactic fermentation and "lactic bite". Our objective was to determine the population kinetics during the alcoholic fermentation of Malbec and Petit Verdot wine varieties from Valle de Uco, Mendoza. Twenty and 10 samples of both varieties were taken respectively, starting from day 0 of the fermentation process until day 33 (Malbec) and day 18 (Petit Verdot). The cultures were grown in MRS medium supplemented with tomato juice and natamycin. Colony counts and phenotypic testing were performed. The following results (CFU / mL) were obtained: 1) Malbec: the initial values were 10<sup>3</sup>, followed by an increase of up to 10<sup>5</sup> on day 3, a

decline of  $10^2$  on day 8, remaining between the latter value and  $10^3$  until day 25, where it started to rise again up to  $10^5$  and then remained constant. 2) Petit Verdote: it started with values of  $10^4$ , followed by a decrease to  $10^2$  on day 8, with a final increase to  $10^5$  on day 17, this value being maintained until the end of the sampling. In both varieties, 4 types of colonies compatible with different LAB species were observed. The study with grapes from other areas of the province, of different varieties and with the identification of microbial species will continue. These preliminary results suggest a relationship between the population behavior of LAB with its *terroir*.

#### A190

##### ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM NATIVE SPECIES OF *Schinus*

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Bacterial diseases cause important economical crop losses every year, whose impact is only surpassed by fungal diseases. There is a narrow spectra of plant protectants able to control phytopathogenic bacteria. The aim of this work was to investigate the antibacterial activity of aerial parts from native plants of *Schinus*. Sequential extraction of leaves and stems from *S. fasciculatus* and *S. gracilipes* were extracted with dichloromethane, ethyl acetate and methanol, yielding fCH<sub>2</sub>Cl<sub>2</sub>, fAcet and fMeOH fractions, respectively. The antibacterial activity of the latter was evaluated by a microdilution method using Mueller-Hinton medium on *Pseudomonas corrugata*, *P. syringae*, *Xanthomonas campestris*, *Erwinia carotovora* and *Agrobacterium tumefaciens*. The concentration needed to inhibit 50% of bacterial growth (CI<sub>50</sub>) was calculated by probit analysis. fAcet fractions from leaves and stems of *S. fasciculatus* were active against *Pseudomonas syringae*, *X. campestris* and *A. tumefaciens* (IC<sub>50</sub>: 800-1500ppm). The growth of *P. corrugata*, *E. carotovora*, *X. campestris* and *A. tumefaciens* was inhibited by fAcet (IC<sub>50</sub>: 1200-1700ppm) and fMeOH fractions (IC<sub>50</sub>: 1700-1000 ppm) of *S. gracilipes*. The *Schinus* species seem to contain antibacterial compounds of high and intermediate polarity, which will be isolated and identified.

#### A191

##### TECHNOLOGICAL EVALUATION OF LACTIC ACID BACTERIA FOR THE DESIGN OF A GOAT CHEESE STARTER SYSTEM

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Nowadays, the goat cheese industry in Argentina uses starter and adjunct cultures obtained from bovine ecosystems. However, these natural biological resources are also available in caprine ecosystems, which are very different from bovine ones. The objective of this work was to study native lactic acid bacteria (LAB) for goat cheese culture design. 6 LAB strains isolated from goat milk of Arid (AV) and Temperate (TV) valley dairy systems from Salta province were analyzed. F7 *E. faecium*, F29 and G113 *E. durans* were collected from AV and H68 *E. faecium*, I19 and I35 *E. durans* from TV. Biochemical characterization: F7 and G113 diacetyl (d) and citrate (c) were positive (+), F29 d+, c negative (-), F7 and F29 caused clots after 16h of fermentation, G113, H68, I19 and I35 at 24h; H68 and I35 were d and c+ and I19 was d+ and c-. Each strain by itself and their combinations were tested as cultures (in 2% skim milk powder, 37°C). Acid production, clot formation (h), lipolysis development and LAB growth were evaluated for 140h. Results showed that maximum individual and combined strain growth occurred at 80 and 48h of incubation time respectively; individual strain concentrations were higher than combined ones in every case. Acidification range at 24h of incubation was 52-73%, F7 (individual strain) and H68-I19 (combined ones) produced the highest values. Maximum acidification rate was found at 24h for the F7 strain. Lipolysis production was important in every case (6.89-8.89 ueq/ml), although highest value was produced by I35 at 140h. Among the combined strains tested, F7-G113 (AV) and H68-I19 (TV) showed the highest rate of acidification and growth (2 orders of magnitude), so these two last strains were considered of interest for culture design.

#### A192

##### ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS FROM THE NORTH OF ARGENTINA AGAINST *Aspergillus section flavi* STRAINS.

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At present, different chemical preservatives are used to combat the growth of food contaminating toxigenic fungal strains, The growing interest in replacing them by natural products less hazardous to human health has led to the search for secondary plant metabolites able to fulfill this role. The objective of this work was to determine the antifungal effect of different plant extracts from the north of Argentina against *Aspergillus section flavi* strains. Aerial parts of *Prosopis ruscifolia* Griseb, *Bidens pilosa* L. *Cercidium australe* Jonhst and *Phorandendron liga* Eachler, collected in Tucuman and Santiago del Estero, were dried and crushed to conduct sequential extraction with solvents with increasing polarity (hexane, ethyl acetate, dichloromethane and methanol). Antifungal

activities of these extracts against six *A. section flavi* strains were analyzed by spot planting bioautography in Silica gel 60 F<sub>254</sub>. Also, a phytochemical analysis of biologically active extracts was performed in thin layer chromatography with Silica gel 60 F<sub>254</sub> and the bands obtained were identified with specific chemical groups for different developers, as a first approximation to identifying the biologically active substances. Antifungal activity was verified in methanol extracts of *P. ruscifolia* Griseb, dichloromethanic, ethyl acetate extract of *B. pilosa* L. and hexane and dichloromethanic extract of *C. australe* Jonhst, the most effective being the methanol extract of *P.ruscifolia* Griseb in which all strains tested showed sensitivity. The study of these extracts will continue in order to identify, isolate and purify these antifungal compounds.

#### A193

##### **SEED PROTECTANT POTENTIAL OF *Zuccagnia punctata* TINCTURE ON *Fusarium verticillioides***

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*Fusarium verticillioides* is a toxigenic fungus often found in maize grains. Its control is performed before sowing with commercial fungicides, which are not safe for humans or animals. New chemical controls are required. The aim of this work was to evaluate the seed protectant potential of *Zuccagnia punctata* tincture on *F. verticillioides*. Maize grains “diente de caballo” were disinfected with NaClO 0.02 % v/v and then embedded in an aqueous suspension of a leaf tincture of *Z. punctata* for 50 min. Vendaval Thi-carb fv was used as positive control. Germinative power and root and shoot elongation were evaluated in the treated grains. These grains were subjected to a mycelial colonization assay which was measured with the following scale: 1 (no colonized grain), 2 (colonized grain 1-60%) and 3 (colonization of 61-100%). The tincture prevented 47% mycelial colonization of grains exposed to *F. verticillioides* and was 14% more effective than Vendaval. The germinative power of grains was 93% irrespective of the assays performed. The tincture increased by 50% the elongation of the embryonic roots and shoots. Secondary roots increased 60% to 75% by the tincture with respect to controls. The *Z. punctata* tincture showed a relevant seed protectant power which was slightly higher than that of the commercial fungicide. Neither grain germination nor further early seedling growth were affected.

#### A194

##### **ASSESSMENT OF INTRACELLULAR LEVEL OF REACTIVE OXYGEN SPECIES, VIABILITY AND NUCLEAR MATURATION OF BOVINE OOCYTES PRESERVED WITH DIMETHYLTHIOUREA.**

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After slaughter, ovaries suffer ischemia by occlusion of the blood flow. Re-oxygenation of ischemic tissue generates reactive oxygen species (ROS) that are toxic to cells. The aim of this study was to evaluate the intracellular levels of ROS, viability and nuclear maturation of bovine cumulus-oocyte complexes (COCs) obtained by follicular aspiration and conserved for 1.5 hours (T = 1.5 h) in the recovery medium supplemented with the antioxidant dimethylthiourea (DMTU). COCs were conserved in follicular fluid plus different recovery media: control (TCM-199 with no antioxidant), DMTU 2 mM and DMTU 20 mM. ROS levels and viability (propidium iodide) of immature oocytes were assessed. The control and DMTU 2 mM groups were matured in vitro (22 h) and the rate of nuclear maturation was determined by Hoechst 33342 staining. The level of ROS was higher in DMTU 20 mM group (n = 60) than control (n = 109) and DMTU 2 mM groups (n = 124) (p-value = 0.0008). There were significant differences between the rates of dead immature oocytes (control: 25.4%, n = 165; DMTU 2 mM 14.6%, n = 116 and 20 mM: 43%, n = 186) (p-value = 0.0001). Moreover, the rate of nuclear maturation in DMTU 2 mM group (80.6%, n = 124) was higher than in the control group (63.8%, n = 72) (p-value = 0.0095). Therefore, the addition of 2 mM DMTU to the recovery medium was beneficial for survival and oocyte nuclear maturation, while the addition of 20 mM increased ROS levels and reduced oocyte viability.

#### A195

##### **VITRIFICATION OF MOUSE EMBRYOS INTO EMBEDDING CAPSULES BEEM®: EFFECT ON VIABILITY AND IN VIVO DEVELOPMENT**

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At present, vitrification of embryos is a useful tool for Assisted Reproductive Techniques (ART) in mammals, especially in human beings. This method is based on an ultra-rapid cooling technique using a minimum volume of highly concentrated solutions of cryoprotectants to avoid ice crystals formation. Effects of vitrification are not well known in *in vivo* development. The aim of this study was to assess the viability of vitrified embryos and *in vivo* development after embryo transfer (ET). The embryos from 12 superovulated mice uterus were flushed, exposed to equilibration solution for 5 min and then to vitrification solution for 1 min. Finally, the embryos were placed into the embedding capsules with a minimum volume and plunged into liquid nitrogen. For warming, 50 µL of warming solution (WS) at 37°C was added to each capsule. After 3 min, successive dilutions were carried out and kept in washed solution (SW) until embryo assessment or ET was performed. Blastocysts viability was determined by propidium

iodide (IP) and Hoechst 33342 staining (n = 36). The *in vivo* development was carried out using nonsurgical ET (n = 24) in 2 synchronized females. The post-warming viability was 86%. Up to now, no full-term pregnancies were obtained. Data from these results could provide information about the effect of long-term vitrification, which are necessary when designing and implementing new devices and methodologies of embryo vitrification in ART.

#### A196

##### MOLECULAR ANALYSIS OF THE MSN2 GENE OF *Saccharomyces cerevisiae*

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The MSN2 gene encodes a transcription factor involved in the regulation of a gene set induced as adaptive responses to adverse culture conditions such as carbon source starvation, heat shock and severe osmotic and oxidative stresses. In this study, we cloned MSN2 and studied its expression in response to different osmotic stress conditions using a reference strain of *S. cerevisiae*. In this work we analyzed the MSN2 sequence in different organisms through the use of bioinformatic tools and studied the effect of osmotic stress generated by KCl and NaCl on the growth of *S. cerevisiae* ATCC 32051. In these culture conditions we evaluated the expression levels of MSN2 by semiquantitative RT-PCR. We identified putative MSN2 orthologs in other fungal species and found high similarity regions that could guide the homology cloning approach for other species of interest. Osmotic stress affected the normal growth of *S. cerevisiae*, showing better tolerance to KCl than to NaCl. As the concentration of NaCl or KCl in the medium increased, the generation time increased and a delay in the growth phases was observed. High concentrations of NaCl and KCl (>1.5M) were toxic to *S. cerevisiae*. The highest expression level of MSN2 was achieved at 0.5M NaCl and the expression levels remained high until 12 hours of culture. These results suggest that MSN2 has a key role in osmotic stress response and its expression is necessary for long periods of time to maintain the adaptation to adverse culture conditions.

#### A197

##### ACTIVITY AND REGULATION OF KEY ENZYMES OF GLUCOSE METABOLISM IN BOVINE CUMULUS OOCYTE COMPLEXES

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Glycolysis and pentose phosphate pathway (PPP) constitute the main destination of the glucose consumed by the bovine cumulus oocyte complexes during oocyte maturation. The key enzymes are phosphofructokinase 1 (PFK 1) for glycolysis and glucose 6-phosphate dehydrogenase (G6PDH) for PPP, it still being unknown whether the regulation mechanisms are the same as in somatic cells. The main aim of this work was to determine the activity of PFK 1 in the presence of the modulators ATP and AMP and also to determine the activity of G6PDH in the presence of modulators NADP and NADPH. PFK 1 and G6PDH activities were measured in immature COCs by spectrophotometer for 9 minutes. An enzyme unit (U) of PFK 1 was defined as the amount of enzyme that catalyzes the formation of 1  $\mu$ mol of fructose 1,6-bisphosphate/min, measured as the oxidation of 2  $\mu$ moles NADH/min. For G6PDH, U was defined as the amount of enzyme that catalyzes the reduction of 1  $\mu$ mol of NADP/minute. To assess the effect of physiological modulators of the enzymes, at 3 and 6 minutes of each determination ATP was added to a final concentration of 2 and 10 mM and AMP to 1 and 10 mM, and NADP to a final concentration of 0.15 and 12.5 mM and NADPH to 1.25 and 12.5 mM. PFK 1 and G6PDH activity was  $5.70 \pm 1.35 \times 10^{-5}$  and  $5.08 \pm 0.70 \times 10^{-5}$  U / COC, respectively. The addition of modulators (AMP, ATP / NADP, NADPH) significantly modified the activity of both enzymes. Enzymatic regulation was similar to that observed for other cell types, indicating that PFK 1 is regulated by the COC energy charge and G6PDH by its redox status.

#### A198

##### ANALYSIS OF gp74 OVIDUCTAL SECRETION INDUCED BY PROGESTERONE. ITS ROLE IN FERTILIZATION

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*Rhinella arenarum* oviductal pars convolute (PC) secretes protein and glycoprotein components that form the jelly coats surrounding the oocytes at the oviposition time. These components shows variations in the secretion profile along their productive cycle. It was shown that the 74 KDa glycoprotein (gp74), secreted mostly in the reproductive period, is involved in fertilization. However, it is not known whether this process would be under hormonal control. The objectives of the study are: 1 –to analyze the effect of progesterone (P) on profile gp74 secretion in ovariectomized animals and 2- to determine whether gp74 purified from animals treated with P, gp74 (P), induces the acrosome reaction (RA). The results demonstrate that P acts on the PC as a secretagogue hormone whose function is to release a dense, opalescent and sticky material which is stored in the ovisac. The electrophoretic profile reveals 11 protein bands with identical mobility to the proteins of the jelly coats that surround the oocytes at the time of fertilization, gp74 being the protein that

exhibits the highest secretion percentage. It was shown that addition of gp74 (P) to the fertilization medium determines changes on the oocytes surface similar to those induced by the acrosomal lysines that are physiologically released during RA. These changes occur only in the presence of sperm. These results demonstrate for the first time that the physiological action of this glycoprotein, whose secretion is induced by P, is to induce RA, allowing the release of the acrosomal content.

#### A199

### FUNCTIONAL AND MORPHOLOGICAL ANALYSIS OF FOLLICULAR CELLS OF THE CUMULUS-OOCYTE COMPLEX AND THEIR CORRELATION WITH OOCYTE QUALITY

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In Assisted Human Reproduction, the choice of oocytes to be inseminated is vital for the sole purpose of obtaining successful fertilization and embryo development, minimizing the cryopreserved gametes. In this sense, follicular cells have a close relationship with the oocyte and its morphological and metabolic study could be used to predict the egg quality, choosing optimal oocytes. The objective of this work is to study the functional relationship between follicular cells and oocytes, establishing a physiological parameter to define egg quality. Follicular cell samples from assisted reproduction cycles of the Fertilía Medical Center were used for our determinations. Cumulus-oocyte complexes (COC) were selected based on their size and morphology. Follicular cells were obtained by enzymatic digestion of the extracellular matrix and physiological parameters such vitality (0.05% eosin), concentration, morphology and oxygen consumption in O<sub>2</sub> saturated phosphate buffered saline (PBS) were studied. The oocytes were inseminated by intracytoplasmic injection (ICSI). The results show that COCs with percentages lower than 30% of their follicular cell vitality and oxygen consumption of less than 50% had fertilization rates below 60% with asymmetric segmentation and a high percentage of fragmentation of blastomeres compared with the physiological parameters assayed. This study represents a direct non-invasive application of biotechnology to the gametes with the possibility of establishing predictive egg quality standards.

#### A200

### CALMODULIN-LIKE PROTEINS REVEAL THE INVOLVEMENT OF CALCIUM SIGNAL IN THE STRESS RESPONSE IN STRAWBERRY.

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In plant cells, changes in intracellular Ca<sup>2+</sup> levels serve to signal responses to various stimuli. Ca<sup>2+</sup> signals are perceived through proteins that bind Ca<sup>2+</sup>, undergo conformational changes following Ca<sup>2+</sup> binding, and interact with target proteins. The *calmodulin-like* (CML) gene family encodes proteins that contain conserved Ca<sup>2+</sup> binding domains, named EF hands. In *Fragaria x ananassa* cv Pájaro, two proteins, FaCML1 and FaCML2, were identified in leaves in the incompatible interaction with the fungus *Acremonium strictum*. In the present work we describe the structure, the evolutionary relationships, and analyze the expression behaviors of these proteins in the fungal interaction. FaCML1 encodes a peptide of 125 residues of amino acids and has 3 EF-hand motifs; and FaCML2 is a peptide with 157 residues and 4 EF-hand motifs. A deletion of 96 nucleotides in the *FaCML1* transcript results in a lack of the fourth EF motif in the protein. Phylogenetic analysis revealed the evolutionary division in two lineages; one of these grouped the CMLs from *Fragaria x ananassa*, *Populus*, *Zea mays* and *Oryza sativa*. In a transcript profiling analysis performed in strawberry leaves, *FaCML1* and *FaCML2* were induced in response to both biotic and abiotic stresses. These results suggest that the pathways involved in the response share calcium sensing. Together, these data present evidence that *FaCML1* and *FaCML2*, genes expressed in leaves encoding proteins that bind Ca<sup>2+</sup> and are potential sensors that detect changes in cytosolic Ca<sup>2+</sup> levels and mediate appropriate cellular responses through the interaction with target proteins.

#### A201

### REGULATION OF THE GAPDH ACTIVITY BY ABA AND ACIDIC PHOSPHOLIPIDS IN BARLEY ALEURONE

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Aleurone tissue is a model that allows the study of the antagonistic effect of the GA and ABA hormones. Previous results of our laboratory demonstrated the involvement of phospholipids during the response to ABA and GA. ABA modulates the levels of DAG, PA, DGPP through the activities of PAP, PLD, DGK and PAK. PA and DGPP are key phospholipids in the response to ABA since both are capable of modifying the hydrolytic activity of aleurone. Nevertheless, little is known about the mechanism of action of these phospholipids during the ABA signal. DGPP is an anionic phospholipid with a pyrophosphate group attached to diacylglycerol. The ionization of the pyrophosphate group may be important for allowing electrostatic interactions between DGPP and proteins. To understand how DGPP mediates cell functions in barley aleurone, we used a DGPP affinity membrane assay to isolate DGPP-binding

proteins from *Hordeum vulgare* followed by mass spectrometric sequencing. A cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was identified as being bound to DGPP. To validate our method, the relatively abundant GAPDH was further characterized with respect to its lipid-binding properties, by fat western blot. ABA treatment increased GAPDH levels and activity while DGPP and PA treatment inhibited GAPDH activity. These data suggest that DGPP binds to GAPDH and that the DGPP and GAPDH interaction may provide a signaling link coordinating carbohydrate and lipid metabolism.

#### A202

### COMMITMENT OF PROTEIN KINASE A IN THE REGULATION OF Na<sup>+</sup>/H<sup>+</sup> EXCHANGE IN *Trypanosoma cruzi*

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Adaptation processes of *Trypanosoma cruzi* during their life cycle involve the Ca<sup>2+</sup> ion as an essential component. Ca<sup>2+</sup> signals play an important role in the differentiation of epi- to tripomastigotes and during invasion of host cells. The objective of this work was to study the involvement of internal reservoirs in cytoplasmic calcium increases in epimastigote forms of *T. cruzi* during hyperosmotic stress. Mannitol caused the alcalinization of acid vacuoles, while in parasites pre-incubated with the inhibitor of the Na<sup>+</sup>/H<sup>+</sup> exchange (EIPA), a decrease was observed and a significant increase in parasites treated with a competitive inhibitor of phosphodiesterases (IBMX) was noticed. Hyperosmotic stress induced Ca<sup>2+</sup> release from the acidocalcisomes determined by changes in fluorescence of Fura-2/AM while pretreatment with EIPA also decreased ion signal. Furthermore, the release of calcium induced by mannitol increased when parasites were treated with the activator of PKA (dibutylryl-cAMP). In contrast, it decreased calcium release when cells were treated with an inhibitor of PKA (KT520). Through the use of western blot assays on isolated acidocalcisomes, the presence of Na<sup>+</sup>/H<sup>+</sup> exchange of *T. cruzi* was demonstrated. Therefore, we infer that the hyperosmotic stress triggers signals that lead to the activation of PI-PLC-mediated Ca<sup>2+</sup> release of acidocalcisomes, which is favored by alkalization of the vacuole through the Na<sup>+</sup>/H<sup>+</sup> exchange. This would be positively regulated by phosphorylation by PKC and PKA. These biochemical events may contribute to the adaptation of the parasite to the conditions present in the rectum of the insect vector and could be key steps in the process of differentiation.

#### A203

### PHOSPHOLIPIDS SIGNALLING AND PROLINE ACCUMULATION DURING RESPONSE TO COLD IN *Hordeum vulgare*

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Low temperature is perceived by plasma membrane as affecting its fluidity and the accumulation of compatible osmolytes. Lipid signalling is regulated by the activities of phospholipases (PLC-PLD), phosphatases (LPPs) and lipid kinases (DGK-PAK) through the dynamic changes in signal lipids such as PA and DGPP. The aim of this study was to determine the lipid kinase activities in relation with proline accumulation during exposure to cold. Seedlings grown at 25°C for 4d were exposed to 4°C for 30-180 min. Cold seedling exposure for 180 min evoked an increase in PAK and DGK activities in root tissue. In contrast, PA and DGPP levels were not modified by cold treatment in young leaves. Seedling exposure to cold triggered proline accumulation in young leaves, while there were no significant changes observed in root tissues. This suggested a possible translocation of proline from the root to the young leaves. Proline accumulated because the balance between its synthesis and its catabolism was altered or because proline was translocated. To test this hypothesis, we impaired its movement through the use of physical methods. In relation to this, under this condition, no proline accumulation was observed in young leaves. The results obtained allow us to suggest that the changes in the lipid kinase activities with the corresponding levels of PA and DGPP could be modulating proline metabolism and its translocation to compensate for the effect provoked by low temperatures in *Hordeum vulgare*.

#### A204

### EFFECT OF AN INTRACEREBROVENTRICULAR INJECTION OF AMYLOID BETA PEPTIDE (1-42) ON THE 24h RHYTHMS OF LIPID PEROXIDATION AND GSH IN RAT HIPPOCAMPUS

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Alzheimer's disease (AD) is a neurodegenerative disorder that leads to memory loss, dementia, and death. Accumulation of Aβ peptide causes an increase in intracellular reactive oxygen species (ROS) and free radicals associated with a deficient antioxidant defense system. Besides oxidative stress and cognitive deficit, AD patients show alterations in their circadian rhythms. Previously, we showed that injection of Aβ(1-42) phase shifts CAT and GPx daily rhythms in rat hippocampus. Continuing with that study, the objective of this work was to investigate the effects of an i.c.v. injection of Aβ(1-42) peptide on temporal patterns of lipid peroxidation and glutathione (GSH) levels, as well as on Aβ protein levels, in rat hippocampus. Four-month-old males Holtzman rats were divided

into two groups defined as: control (CO) and A $\beta$ -injected (A $\beta$ ). Rats were maintained under 12h-light:12h-dark conditions and received water and food *ad libitum*. Hippocampus samples were isolated every 4 h during a 24h period. Lipid peroxidation and GSH levels were determined by colorimetric assays. A $\beta$  protein levels were analyzed by immunoblotting. We found that i.c.v. injection of A $\beta$ (1-42) increased A $\beta$  peptide content in the hippocampus and phase shifted A $\beta$ , lipid peroxidation and GSH daily rhythms. These changes follow the previously observed changes in daily patterns of antioxidant enzymes activity. Thus, elevated A $\beta$  peptide levels alter temporal patterns of oxidative stress-related parameters and, consequently, would negatively affect cellular clock activity in the hippocampus.

#### A205

### ENDOGENOUS RHYTHMS OF LIPID PEROXIDATION AND RC3 EXPRESSION ARE MODIFIED IN THE TEMPORAL CORTEX OF AGED RATS

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Age-associated memory impairment has been related to increased oxidative stress as well as to alteration of circadian rhythms in the brain. Neurogranin (RC3), a postsynaptic protein kinase C substrate implicated in synaptic plasticity, is expressed in different brain regions involved in cognitive functions, including the temporal cortex (TC). Previously, we showed that antioxidant enzymes activity and Bdnf expression follow a circadian rhythm in the rat TC. Interestingly, aging abolished those rhythms. Continuing with those studies, the objectives of this work were: 1) to investigate endogenous rhythms of lipid peroxidation, RC3 expression and the cellular clock transcription factor, BMAL1, protein levels in the rat TC, and 2) to evaluate up to which extent aging could affect those temporal patterns. Young (3-month old) and aged (22-month old) male Holtzman rats were maintained under constant darkness conditions for 10 days before the experiment. TC samples were isolated every 4 h during a 24h period. Levels of lipid peroxidation were determined by a colorimetric assay. RC3 transcript levels were determined by RT-PCR and BMAL1 protein levels by immunoblotting. Regulatory region of RC3 gene was scanned for clock responsive E-box sites by using a bioinformatic tool. We found that lipoperoxidation levels and RC3 expression oscillate on a circadian basis in the rat TC. As expected, E-box sites were found in the RC3 gene regulatory region; however, BMAL1 rhythm peak is in antiphase with the maximal levels of Rc3 mRNA. Consistently with what we previously observed, aging also abolished the circadian rhythmicity of lipid peroxidation and RC3 expression, probably by flattening BMAL1 endogenous oscillation.

#### A206

### ANG-1, ANG-2 AND TIE-2 IMMUNOLocalIZATION DURING PORCINE GESTATION

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Angiopoietins 1 and 2 (Ang-1 and Ang-2) are produced mainly in the placenta. Both bind to Tie-2, a specific endothelial receptor, and participate in the last phases of angiogenesis. The objective of this work was to detect the localization of Ang-1, Ang-2 and Tie-2 by immunohistochemistry in the porcine placenta of early ( $\pm 30$  days), mid ( $\pm 60$  days) and at term ( $\pm 114$  days) gestation. Immunostaining intensity was semi-quantitatively evaluated through High Score (HS) determination. There were not statistical differences for Ang-1 throughout gestation, although its HS showed a tendency to increase from mid to term gestation. This would be related to the involvement of Ang-1 in the development and maintenance of blood vessels during the last stages of placental angiogenesis. A notable decrease in Ang-2 HS was observed from early pregnancy to term ( $p=0.001$ ). Ang-2 has an active role in the destabilization of endothelial cells for vascular remodeling during early pregnancy, which is characterized by the fast growth and development of the uterus and the embryos. Moreover, Tie-2 showed high HS levels at early and at term periods, and a low HS in mid gestation ( $p=0.001$ ). Fluctuation of Tie-2 staining is related to both ligands, its signaling being conditioned by the Ang-1/Ang-2 relation, dependent on the context and the capability of Tie-2 to respond to other angiogenic signals.

#### A207

### DAILY RC3 AND GAP43 EXPRESSION IS MODIFIED IN THE HIPPOCAMPUS FOLLOWING AN INTRACEREBROVENTRICULAR INJECTION OF AMYLOID BETA PEPTIDE

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Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder. The accumulation of amyloid- $\beta$  (A $\beta$ ) peptides in the brain is associated with cognitive deficits. Neurogranin (RC3) and neuromodulin (GAP43) are protein kinase C substrates that play an important role in the neuroplasticity mechanism underlying learning and memory and are expressed in the hippocampus. Besides the cognitive deficit, AD patients also show alterations in their circadian rhythms. Previously, we showed that injection of A $\beta$ (1-42)

phase shifted BDNF daily rhythms in rat hippocampus. Continuing with that, the objective of this study was to investigate the effects of an i.c.v. injection of A $\beta$ (1-42) peptide on RC3 and GAP43 expression as well as on A $\beta$  protein levels, throughout a 24 h period, in rat hippocampus. Four-month-old male Holtzman rats divided into the control groups (CO) and A $\beta$ -injected groups (A $\beta$ ) were maintained under 12h-light/12h-dark conditions. RC3 and GAP-43 mRNA levels were determined by RT-PCR and A $\beta$  protein by immunoblotting, in hippocampus samples isolated every 4 h during a 24h period. Regulatory regions of RC3 and GAP43 were scanned for E-box sites. We found E-box sites on regulatory regions of RC3 and GAP43 genes and observed daily rhythms of RC3 expression in the rat hippocampus. Even though GAP43 expression varies significantly throughout the day, its temporal pattern is not adjusted to a cosine curve. The i.c.v. injection of A $\beta$  (1-42) increased A $\beta$  content, phase shifted RC3 daily rhythms and abolished the GAP43 mRNA levels variation. Elevated A $\beta$  peptide levels might modify the temporal patterns of cognition-related factors, probably by affecting the endogenous clock factors rhythms in the hippocampus.

#### A208

### EFFECT OF I.C.V. AGGREGATED $\beta$ -AMYLOID (1-42) ON DAILY PATTERNS OF COGNITION-RELATED FACTORS IN RAT TEMPORAL CORTEX

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Alzheimer's disease (AD) is the most common form of dementia. Elevated levels of  $\beta$ -amyloid peptide (A $\beta$ ) in different areas of the Central Nervous System (CNS), including cortex, and progressive cognitive impairment, are characteristics of AD. Brain-derived neurotrophic factor (BDNF) and its high-affinity receptor, (TrkB), play a central role in the CNS by regulating synaptic plasticity and memory. TrkB and BDNF signaling are impaired in AD. Besides cognitive deficit, AD patients also show alterations in their circadian rhythms. Previously, we showed that aging abolished Trkb circadian rhythms and phase shifted BDNF endogenous rhythms in the temporal cortex. Continuing with that study, our objective was to investigate the effects of an i.c.v. injection of A $\beta$ (1-42) peptide on the 24h rhythms of BDNF and TrkB expression, as well as on BMAL1 and A $\beta$  protein levels in rat temporal cortex. Four-month-old males Holtzman rats were used in this study. Groups were defined as: control (CO) and A $\beta$ -injected (A $\beta$ ). Rats were maintained under 12h-light:12h-dark conditions throughout the experimental period. Temporal cortex samples were isolated every 4 h during a 24h period. Transcript levels of BDNF and TrkB were determined by RT-PCR. Protein levels were analyzed by Western blots. We found that i.c.v. injection of A $\beta$ (1-42) increased A $\beta$  content and phase shifted daily variation of BDNF and TrkB expression in the rat temporal cortex, probably by altering the daily patterns of clock activator (BMAL1) as a result of A $\beta$  accumulation. These results may constitute, at least in part, a molecular and cronobiological basis for deficits in temporal organization of temporal cortex-related cognitive functions in AD.

#### A209

### COAGULASE-NEGATIVE *Staphylococcus* SPECIES ASSOCIATED WITH SUBCLINICAL BOVINE MASTITIS: VIRULENCE FACTORS.

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Coagulase-negative *Staphylococcus* (CNS) are emerging as potential bacteria responsible for bovine intramammary infections (IMI) in modern dairy farms. Different species of CNS can produce various toxins and enzymes that contribute to its virulence. Our objective was to determine the presence of virulence factors in CNS species with clinical significance in IMI. A total of 37 *S. chromogenes* and 34 *S. haemolyticus* isolated from milk samples with subclinical mastitis were investigated for their ability to produce biofilm, protease, lipase, DNase and  $\delta$ -hemolysin, in micro titer plates, plates with trypticase soy agar-2% skim milk, Baird-Parker supplemented with yolk, DNase agar and blood agar 5%, respectively. The presence of genes encoding superantigens (SAGs) *sea*, *seb*, *sec*, *seln*, *selq*, *seh* and *sej* was investigated by PCR. Six different combinations of SAGs genes were detected. The most prevalent combination of genes (SAGs *sea* and *sec*) was found in *S. chromogenes*. A higher percentage of strains of *S. chromogenes* than of *S. haemolyticus* showed activity of proteases, lipase and DNase, whereas the ratio was reversed for  $\delta$ -hemolysin production. High percentages of biofilm forming strains for both species were observed. The isolates analyzed showed the co-expression of several virulence factors, both genotypically and phenotypically, which is relevant for the design of control and prevention measures against CNS mastitis.

**A210**

**$\beta$ -ATPase AND ITS FUNCTION IN THE TRANSFER OF LIPIDS FROM LIPOPHORIN TO TARGET TISSUES IN *Panstrongylus megistus* (HEMIPTERA: REDUVIIDAE).**

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Lipophorin (Lp), a high-density lipoprotein, is the main lipid carrier in the hemolymph of insects. Lp plays a role as a “reusable shuttle”, cycling among the tissues by loading and unloading its lipid cargo without synthesis or degradation of its apolipoprotein matrix. In order to exchange lipids, Lp must interact with specific binding sites located in the plasma membrane of the target cells. Currently, there are few characterized candidates supporting the functioning of the docking mechanism of Lp-mediated lipid transfer. In this work, we employed a combination of ligand blotting and tandem mass spectrometry to characterize proteins with the property to bind Lp to the midgut and fat body membranes of the hematophagous insect *Panstrongylus megistus*, a Chagas disease vector. The  $\beta$  chain of ATP synthase complex ( $\beta$ -ATPase) was identified as a Lp binding protein and its role in lipid transfer was further assessed at the biochemical and cellular level in different organs. After subcellular fractionation,  $\beta$ -ATPase was detected by western blot in enriched membrane preparations free of mitochondria of midgut and fat body. By immunofluorescence assays,  $\beta$ -ATPase was found at the surface of enterocytes and trophocytes, partially co-localizing with Lp. *In vivo* functional studies injecting an anti-  $\beta$ -ATPase antibody demonstrated that blocking of  $\beta$ -ATPase partially impaired Lp binding to midgut and fat body. The blocking of  $\beta$ -ATPase also diminished Lp-mediated lipid transfer to fat body. Taken together, these findings strongly suggest that  $\beta$ -ATPase is a docking receptor that mediates Lp lipid transfer to the major lipid target tissues in *P. megistus*.

**A211**

**EFFECT OF GENISTEIN ON BOVINE OVIDUCTAL EPITHELIAL CELL PROLIFERATION**

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Soybean is a source of high quality protein for cattle feeding. It also contains phytoestrogens such as genistein which is one of the most abundant in soybean. It is of considerable interest due to the adverse effects observed in bovine reproduction: hormonal changes in the estrous cycle, reduced fertility, abortions, implantation and fetal development problems. Genistein is a potent inhibitor of protein tyrosine kinases involved in the activation of intracellular signaling cascades that regulate processes such as cell proliferation. The purpose of this research was to evaluate *in vitro* the action of genistein on cyclin B1 expression in bovine oviductal epithelial cell cultures. Genistein was added at different concentrations (0.2, 2 and 10  $\mu$ M) to these cell cultures for 24 hours. Then, samples were processed for total RNA extraction using TRI Reagent and finally cDNA was synthesized with MMLV reverse transcriptase and oligo dT. Cyclin B1 expression was analyzed by Real Time RT-PCR using GAPDH as a loading control. Cyclin B1 mRNA significantly decreased in cultures treated with genistein. This molecule has an inhibitory action on proliferation genes in bovine oviductal epithelial cell cultures. This effect suggests that genistein ingested by animals could cause modifications in the normal performance of bovine oviduct.

**A212**

**BIOCHEMICAL AND MOLECULAR ASPECTS OF VITELLOGENIN, LIPOPHORIN AND THEIR PUTATIVE RECEPTORS IN OOCYTES OF *Dipetalogaster maxima* (HEMIPTERA: REDUVIIDAE)**

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Vitellogenesis is a central event in insect reproduction that elicits uptake and deposition of yolk protein precursors (YPP) and lipids in developing oocytes. At this stage, vitellogenin (Vg), the main YPP, and lipophorin (Lp), the main lipoprotein of insects, are taken up from the circulation by the oocytes through specific receptors. The aim of this work was to achieve a better understanding of the biochemical and molecular aspects of Vg, Lp and its putative receptors in the oocytes of *Dipetalogaster maxima*, a vector of Chagas disease. The metabolic pathways of Vg and Lp in vitellogenic oocytes were evaluated *in vivo* by injecting females with fluorescently labeled lipoproteins to follow the fate of the entire particle. The results indicated that Lp and Vg were endocytosed by vitellogenic oocytes, co-localizing in yolk bodies. The convergence of endocytic and non-endocytic pathways for Lp in the oocytes was also shown. A putative Vg receptor in the ovarian tissue that was up-regulated during vitellogenesis was identified by RT-qPCR. The  $\beta$  chain of ATP synthase ( $\beta$ -ATPase), recently reported as an Lp docking receptor in the midgut of the triatomine *Panstrongylus megistus*, was detected in enriched ovarian membrane preparations by western blot. Moreover, immunofluorescence assays allowed the detection of  $\beta$ -ATPase in the plasma membrane of oocytes and follicular cells. Taken together, our results indicated that in *D. maxima*, the putative Vg receptor and  $\beta$ -ATPase play a central role in regulating the storage of nutritional resources in the ovarian tissue during vitellogenesis.

**A213**

**GERM CELL SPECIFICATION IN THE EMBRYO DISK OF *Lagostomus maximus* (RODENTIA, CAVIOMORPHA).**

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Primordial germ cells (PGCs) are germline stem cells that give rise to gametes in vertebrates. During early embryogenesis in mammals, PGCs are found in an extra-embryonic region near the proximal epiblast; then they migrate and colonize the gonadal ridges. This process is regulated by different genes whose expression depends on development through an egg-cylinder (EC) morphology as in rodent embryos or an embryonic disc (ED) in other mammals. *Lagostomus maximus* (*Lm*) is a basal rodent with ED development. The objective of this study was to analyze the gene expression of germ line specification and migration in this ED-developing rodent. The expression of OCT4, BLIMP1, STELLA, FRAGILIS, VASA, SOX2 and SOX17 was analyzed in early-postimplantation embryos (n=20) of *Lm* by immunohistochemistry. OCT4 was observed in the ED before gastrulation, while the remaining proteins were negative for this stage. At a more advanced stage, OCT4, SOX17, STELLA & FRAGILIS were detectable in a small number of cells in the mesoderm of the proximal epiblast (PGCs specification), while the other proteins were negative. Then, during mesenchyme migration, PGCs became positive for STELLA, VASA, FRAGILIS and OCT4. BLIMP1 and SOX2 were negative throughout the developmental stages analyzed. These results show that the specification of PGCs in *Lm* is comparable to non-rodent species like pig or human and it differs from mouse and other muroid rodents. The basal location of *Lm* in rodent evolution suggests that PGC-specification in the mouse could be a peculiarity of the myomorpha rather than a characteristic of the order Rodentia.

**A214**

**ROLE OF TCF15 TRANSCRIPTION FACTOR IN SOMITE DIFFERENTIATION**

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Vertebrates have a metameric bodyplan that is based on the presence of somites. These are paired blocks of tissue located on either side of the neural tube which are formed sequentially by a regular segmentation of paraxial mesoderm. Once somites are formed, they mature and differentiate into myotomal, sclerotomal and dermatomal tissue, which eventually give rise to striated muscles in the body, to the axial skeleton and to the dermis of the back, respectively.

Studies carried out in different animal models have demonstrated that transcription factor Tcf15 plays a crucial role in somite formation; however, its role in the differentiation of this mesodermal tissue has been little explored.

This work aims at determining the role of the *tcf15* gene in somite differentiation in *X. laevis* embryos. In gain and loss of function experiments, using inducible constructs and a specific antisense morpholino oligonucleotide, we found that an alteration in the level of Tcf15 protein affects the expression of muscular and chondrogenic differentiation molecular markers. Loss of function of *tcf15* in the somite provoked a drastic reduction in the expression of *MyoD*, *Myf-5* and *pax3* myogenic markers; and of *pax1*, *pax9*, *uncx* and *col2a* chondrogenic markers while *tcf15* overexpression induced ectopic expression of muscular genes. Taken together, these results enable us to conclude that *tcf15* gene is required for somite differentiation.

**A215**

**MICROSATELLITE POLYMORPHISM IN *Tritrichomonas foetus*.**

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Bovine trichomonosis is a sexually transmitted parasitic disease that causes great economic losses. In Argentine, where livestock is extensive and reproduction is mainly made by natural service, it is considered endemic and an important health problem. The causative agent is the protozoan flagellate *Tritrichomonas foetus*. While other markers (RFLP, RAPD, SNP) have been used to gain information about the genetics of this parasite, microsatellites (STR) have not yet been employed for this purpose. STRs are considered the most appropriate tool to characterize genotypes because of their multilocus nature, high diversity and sensitivity. They are useful in studies of genomic variation, association and detection of mixed infections. The intraespecies biological variability existing in *T. foetus* made diagnosis and treatment difficult. That is why the aim of this work was the study of STR for the first time in this parasite to determine its genetic variability. Since sequencing projects have not been completed yet, genome sequences of its evolutionary counterpart *Trichomonas vaginalis* were used. Six markers were amplified by PCR in DNA samples from six different *T. foetus* isolates. The products obtained showed size polymorphisms with respect to *T. vaginalis* and between individuals. Obtaining the sequence of these products allows the generation of specific oligonucleotides to provide a useful tool for population and epidemiological studies contributing to develop improvements in disease control and the design of more effective of diagnostic methods.

**A216**

**BIOMARKERS IN SALIVA OF DIABETIC PATIENTS WITH PERIODONTAL DISEASE**

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Saliva contains products of circulation and molecules synthesized at the glandular level. Different biomarkers have been detected in order to relate them to the periodontal state. Aim: To evaluate salivary components of patients with chronic periodontitis with and without type II diabetes mellitus. Methodology: 69 patients with moderate and severe periodontitis who attended the Periodontal Department of FOUNT were selected, 14 with type II *diabetes mellitus*. Periodontal diagnosis included: probing depth, clinical attachment level, bleeding on probing, gingival index and plaque index. Alkaline phosphatase, calcium, total proteins and hydroxiprolin were determined. Data were analyzed with the SPSS program. In total saliva of the patients with periodontitis and *Diabetes mellitus* the average concentration of the levels of alkaline phosphatase ( $1.34 \pm 0.37$  U/l), calcium ( $3.57 \pm 2.6$  mg/dl), total proteins ( $2.52 \pm 1.22$  mg/ml) and hydroxiprolin ( $1.10 \pm 0.43$  ug/ul) were higher with respect to patients without diabetes ( $0.14 \pm 0.91$  U/l), ( $0.14 \pm 4.6$  mg/dl), ( $1.12 \pm 0.38$  mg/ml) y ( $0.42 \pm 0.18$  ug/ul), statistically significant differences ( $p < 0.001$ ) being observed between both groups. Our results revealed that alkaline phosphatase, calcium, total proteins and hydroxiprolin in saliva could be useful biomarkers for the control of periodontal disease in patients with and without *diabetes mellitus*.

**A217**

**CAN THE ENDOSYMBIONT HOSTED IN THE DIGESTIVE GLAND OF *Pomacea canaliculata* CONTRIBUTE TO THE DIETARY PROTEIN DIGESTION?**

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The digestive gland of *Pomacea canaliculata* is the most voluminous organ of this snail and it lodges an intracellular endosymbiont with two morphotypes identified as C and K. Enzymes and endosymbionts are secreted into digestive ducts and then travel to the stomach. Previously, we found protease activity along the digestive tract of the animal (crop, style sac and coiled intestine contents) represented by serine-protease of 30, 125, 145 and 198 kDa. Here, we studied if the endosymbiont contributes to the dietary protein digestion of this snail. In situ zymography showed that the glandular protease activity was placed on the endosymbiont and that it was inhibited by aprotinin (a serine-protease inhibitor). When the endosymbionts were maintained in BG11 culture medium (adjusted to ~145 mOsm) for two hours, a protease of 30 kDa was found in the medium. Protease activity (fluorometric assay using DQ-gelatin as a substrate and gel zymography) in the culture medium decreased when the endosymbiont was isolated from the digestive gland in the presence of streptomycin. We conclude that the endosymbiont has a serine protease of 30 kDa and its synthesis can be inhibited by streptomycin.

**A218**

**M105I MUTATION IN  $\alpha$ SNAP LEADS TO MULTIFACTORIAL FERTILITY DEFECTS IN HYH FEMALE MICE**

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Hydrocephalus with hop gait (hyh) is a recessive inheritable mouse disease that arose spontaneously in C57BL/10J mouse strain. A missense mutation in the gene encoding for  $\alpha$ SNAP causes the substitution of a highly conserved Met at residue 105 for Ile (M105I). Since  $\alpha$ SNAP KO is lethal, hyh mice provide a unique in vivo model to study  $\alpha$ SNAP function. We previously reported that hyh male mice have a strongly reduced fertility due to a defective acrosome reaction. The aim of this work was to investigate the role of  $\alpha$ SNAP in female fertility, focusing on the oocyte and its *cumulus oophorus* cells (COO). After superovulation with gonadotropins, hyh ovaries produced fewer oocytes, indicating that the function of the hyh ovary is defective. Furthermore, in vitro fertilization (IVF) rates of hyh oocytes were decreased, and polyspermic rates were increased. These findings and our previous data demonstrating that  $\alpha$ SNAP has an active role in cortical granules exocytosis (CGE) strongly suggest problems associated with this process. To gain further insight, cortical granules (CG) quantification was performed after IVF, showing a partial failure in CGE of hyh female. On the other hand, hyh mutant females showed a drastic reduction in COO  $\alpha$ SNAP and N-cadherin protein levels that may lead to changes in N-cadherin-based junctions between COO in the growing cumulus-oocyte complex (COC). Interestingly, these changes were associated with an increase in the apoptotic rate in COCs, assessed by TUNEL. Additionally, some hyh oocytes showed abnormal CG polarity, suggesting that  $\alpha$ SNAP might have a function in CG migration. Taken together, these data provide evidence showing that  $\alpha$ SNAP plays an important role in female reproduction.

**A219**

**MYOFIBROBLASTS ARE INVOLVED IN DIABETIC INTESTINAL REMODELING**

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Diabetes is associated with metabolic and functional alterations in the gut. Using an experimental model of streptozotocin-induced diabetes in rodents, we analyzed whether diabetic intestinal dysfunction could be partly due to changes in the mucosa layer. Morphological analysis of the colon of rats after 4 weeks of diabetes showed a profound alteration in the mucosa layer. A marked increase in the deposition of extracellular matrix components was found and high levels of fibrillar collagen (I and III) and fibronectin mRNAs were observed. Accumulation of these extracellular proteins was detected along the lamina propria. In addition, a significant increase in cell proliferation of the mucosa layer associated with an increase in the population of myofibroblastic cells  $\alpha$ -SMA<sup>+</sup> / vimentin<sup>+</sup> was determined. Paracrine factors study showed that the expression of TGF $\beta$ 1 signaling pathway, TGFRI, TGFRII and the cytoplasmic effector Smad2 / 3, was increased in the mucosa layer of diabetic rats compared to controls animals.

The overall results showed that the deregulation of the TGF $\beta$ 1 pathway is associated with the appearance of myofibroblasts and the accumulation of extracellular matrix in the mucosa of diabetic colon. Diabetes causes an imbalance in the process of normal colonic mucosa remodeling, initiating a fibrotic process at early stages of the disease.

**A220**

**OLIVE OIL EFFECT ON CHOLESTEROL REGULATION IN HYPERCHOLESTEROLEMIC RABBIT TESTIS**

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Hypercholesterolemia is not only considered the main cardiovascular risk factor but also interferes with the normal functioning of the male gamete and could cause infertility. The intracellular regulation of this lipid depends on a fine balance between production and incorporation *de novo* (from diet) by specific receptors. This balance is achieved by activation or inhibition of a pathway regulated by proteins called SREBPs (Sterol Regulatory Binding Proteins element-) 1 and 2, which regulate the expression of enzymes involved in cholesterol and lipid synthesis such as HMG-CoA (hydroxymethylglutaryl coenzyme A) reductase, among others. We used a rabbit model of diet-induced hypercholesterolemia and one protected with olive oil (AO). Our interest lies in studying the intracellular pathway of cholesterol in testis (an organ specialized in handling this lipid) of rabbits under different experimental diets; specifically, changes in two proteins in the above pathway: SREBP and HMGCoA reductase. To validate the animal study model, we compared the weight of the animals with different diets and performed the determination of serum total cholesterol and triglycerides. Regarding molecular analysis, we quantified expression levels of mRNA and SREBP HMGCoA by relative-quantitative PCR from testes homogenates. We noticed that the intake (saturated and unsaturated lipids) had different effect on cholesterol metabolism, both SREBPs proteins as one of its target genes, HMGCoA reductase, changed its expression depending on the type of fat consumed. In addition, these proteins also changed their expression depending on the time period of the diet. These results show that the testis is sensitive to different ingested lipids, and that they are capable of modifying the intracellular cholesterol metabolism depending on both time of consumption and type of lipid consumed. This is a potential target in the protection mechanism by AO already studied in other organs.

**A221**

**CELL BIOLOGY: PARADIGM SHIFT IN THE FIGHT AGAINST CERVICAL CANCER (CC)**

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The fight against CC was based on the Pap smear to look for precursor lesions of malignant neoplastic disease caused by the human papilloma virus (HPV). Today that is complemented by the biotechnology test for HPV (T-HPV). The present experience of the application of biomolecular techniques in the public subsector of Tucuman for secondary prevention of cervical cancer is discussed. 11,200 samples from women without healthcare insurance, older than 30 and not pregnant, were studied. 2 collection methods were used: "Double Take" (DT), cytological for PAP test and biological sampling for T-HPV and "Self-sampling" (A) biological material for T-HPV. In cytology, Pap smears were stained with Papanicolaou and for the biological material, T-HPV Hybrid Capture was applied. 13 oncogenic HPV types (16,18,31, 35,39,45,51,52,56,58,59 and 68) were detected; it is highly sensitive (95%) and with negative predictive value (it allows spacing interval screening). Out of 9630 samples processed to date, 1239 (12.8%) women showed oncogenic HPV, 641 (51.7,4%) from DT and 598 (48.3%) from A. Women with DT, positive T-HPV with diagnosed Cytologies: 565 ; 521 were successful (92%) and 31.7% of them showed lesions: Low grade 66/565 (40%); High grade 44/565 (26.7%), suspected high grade (ASC-H) 14/565 (8.48%) and suspicious low grade 41/565 (24.8%). This methodology is a major paradigm shift in the fight against cervical cancer. It is the initial period of the learning curve (March 2015) with gradual adjustments as required.

**A222**

**EVALUATION OF THE HEMATOLOGICAL PROFILE OF WEANED PIGLETES UNDER INTENSIVE PRODUCTION**

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Piglets undergo important stress during weaning, so they should be placed in the rooms to avoid rapid weight loss and dehydration. Besides, hematological profile varies with: race-sex, growth rate, age, type of production-altitude climate, health and disease. Therefore the haematological profile of weaned piglets (D) under intensive production facilities in the province of Santa Fe was evaluated. Blood samples were taken from vena cava in 24 piglets weaned from a from Santa Fe pig farm in the winter and summer in 2014. The following variables were determined: leukocyte formula: N, E, B, L and M by May Grünwald-Giemsa staining; microtechnology for hematocrit; Neubauer chamber for WBC and GR and cyanmethaemoglobin method for the determination of Hb. The hematimetric indices were calculated. The serum levels of iron (Fe) were also determined by the colorimetric method. The ANOVA method was applied. The mean values and standard deviations of D in winter and summer were: GB/(mm<sup>3</sup>) 11.339 ±6.283 ; 9.900 ±545, N(%) 36 ±6 ; 30 ±8 , E(%) 1 ±0 ; 3 ±1 , B(%) 0 ±0 ; 1 ±0 , L(%) 62 ±6 ; 64 ±10 , M(%) 2 ±1 ; 2 ±1 , GR/(mm<sup>3</sup>) 6.111.786 ±246.792; 5.734.175 ±286.931, Hb(g/dL) 10.74 ±0.91; 8.41 ±0.34, Hto(%) 31 ±2 ; 32 ±3 , VCM(fL) 51.19 ±1.66; 56.29 ±2.66, HCM(pg) 17.55 ±1.12; 14.67 ±0.32, CHCM(g/dL) 34.35 ±2.5; 26.11 ±1.48, Fe(µg/dL) 121.54 ±61.4; 100.34 ±58.5 respectively. Statistical differences (p <0.05) were observed for the variables GB, N, GR, Hb, VCM, HCM, CHCM and Fe. Hb during both seasons and CHCM in summer they were below normal. The average values of the other variables were within the values reported in the literature.

**A223**

**SEROLOGICAL MACROMINERALS DETERMINATION OF PIGS IN CONFINEMENT CONDITIONS IN THE CENTRAL REGION OF SANTA FE**

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Good mineral nutrition is essential for profitable production. Phosphorus and calcium are macrominerals of major importance in pig nutrition. Our objective was to determine serum concentration of calcium and phosphorus at different production stages of pigs in confinement conditions IN the central region of Santa Fe- Argentina.

The study was performed in 50 animals of 4 categories, genetic Topic, Topi 20 line randomly selected during the winter of 2013. Blood samples were drawn from a venipuncture: serum was separated and stored at 2 °C. Chemistry analysis was performed using UV-visible spectrophotometry for serological determination of Ca and P. ANOVA, statistical method was applied for analysis of results.

Average values and standard deviations of concentrations: Newborn piglets: Ca (mg/dL) 8.84 ± 0.61; and P (mg/dL) 7.00 ± 0.32. Weaned: Ca (mg/dL) 9.53 ± 0.59; and P (mg/dL) 8.50 ± 0.68. Puppies: Ca (mg/dL) 8.77 ± 0.65; and P (mg/dL) 6.36 ± 0.65. Pregnant adults: Ca (mg/dL) 8.05 ± 0.88; and P (mg/dL) 5.97 ± 0.56. Biochemical variables were affected by age, sex, nutritional status, race, season, stress and health; these were taken into consideration in the interpretation of results. On the whole, average values of the minerals studied were within the reference range provided by the literature. In pregnant adult category calcium was at the lower limit. These results contribute to the study of regional reference values.

**A224**

**EFFECT OF MATERNAL NUTRITION ON INSULIN AND IGF-1 DURING EARLY PREGNANCY IN SHEEP**

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The objective of the study was to analyze the effect of maternal nutrition during early pregnancy on the concentrations of two metabolic hormones and its influence on reproductive performance in sheep. The experiment was conducted at the Laboratory of Reproduction of INTA Bariloche during the breeding season of 2014. Adult Merino ewes were housed in pens and water was provided *ad libitum*. The Supplemented group (SG) (n=62) and the Restricted group (RG) (n=64) received a daily diet equivalent to 1.5 and 0.5 times of maintenance requirements, respectively, from day 32 pre mating to day 35 post mating. The ewes were synchronized in their estrus by 2 doses of 125 µg of PF2alfa every 14 days. Mating was performed immediately after detection of estrus (Day 0). Body condition (BC) and body weight (BW) were registered weekly. Serum insulin and IGF-1 were determined weekly from day 0 to 35. Pregnancy diagnosis was performed by transrectal ultrasonography at day 35 of gestation. The estrus synchronization rate was higher in the SG (95%) compared to the RG (64%). However, no differences were found in pregnancy rates between groups (80% SG vs. 79% GR). The RG showed a decrease in BC and BW compared to the SG (P<0.05). Serum insulin concentration between weeks 2nd to 4th post mating was lower in the RG compared to the SG (P<0.05). The IGF-1 was lower at day 0

and day 35 post mating in the RG ( $P < 0.05$ ). Maternal undernutrition affects metabolic concentrations of insulin and IGF-1 during early pregnancy and reproductive efficiency due to a lower rate of estrus synchronization.

#### A225

### EFFECT OF THE INTAKE OF NATURAL OILS ON BEHAVIOR PARAMETERS IN ADULT MALE OFFSPRING OF DIABETIC RATS.

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Fetal development is directly affected by changes in the intrauterine environment during pregnancy. A hyperglycemic environment during gestation leads to damage in the nervous system of the offspring due to an increase in reactive oxygen species (ROS) levels. The inclusion of monounsaturated  $\omega 3$  fatty acids in the diet has shown beneficial effects on the reduction of ROS by its antioxidant properties. The aim of this study was to analyze the effect of corn oil (MZ), extra virgin olive oil (OL) and pistachio oil (PS) on the behavior parameters of adult male offspring of diabetic rats. The offspring of an experimental model of streptozotocin-induced gestational diabetics (30 mg/Kg iv) (DO) and control mothers (CO) were used. Supplementation was performed from 2 to 62 days of age (dose: 8  $\mu$ l/15g body weight). Open field test was performed at the age of 8 months. Numbers of cross lines, time of sniffing, and movement speed were analyzed. Results: There was no difference in these parameters between the offspring condition (CO-DO) ( $p > 0.05$ ). However, a significant increase in the number of cross lines, time of sniffing and movement speed was observed in PS supplemented offspring when compared with the other oils ( $p < 0.05$ ). Conclusion: PS intake led to an increase in the exploratory activity in the Open Field behavior test. This could be partially due to the antioxidant effect of monounsaturated fatty acids taken during early life. (CONICET PIP243, PICTO/UCCuyo 2009-0158- CICITCA UNSJ).

#### A226

### CHARACTERISTICS OF CANINE AND FELINE PATIENTS TREATED AT THE HOSPITAL SCHOOL OF VETERINARY OF THE FAZ-UNT

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The Veterinary Hospital of the FAZ treats different types of animals since 2014 to date. The characterization of these patients is an important indicator in managing the service. Therefore, our objective was to study the frequency of races, ages, sex and diseases, in canine and feline patients treated at this hospital for one year.

We worked with patients treated from August 2014 to June 2015. We included only animals with full medical records.

The results showed the following: species: 90% canines and 10% felines; dog breeds: most often (65%) made up of Mestizos, Poodles and Bulldogs, and cats by Mestizos; age: higher frequency in both species between 1-24 months; sex: males constituted 63% of the total in both species; oncology, infectious diseases, reproductive disorders and trauma were the most frequent problems in both species, totalling 85%. Based on the results, we identified the animal group at greater risk of morbidity, which would be related to the immunization period. The frequency of sex could be related to territorial and sexual behavior of the animals. As to pathologies, besides their etiology, it is thought that animal ambulation, improper diet, lack of information on animal health as well as some genetic considerations, are some of their causal factors.

#### A227

### REGULATION OF THE DEVELOPMENT OF BOVINE MAMMARY GLAND. GASTROINTESTINAL PARASITES EFFECT ON HOLSTEIN HEIFERS.

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Gastrointestinal parasites affect Holstein heifer development and reduce IGF-1 levels. In order to study the possible alteration of mammary gland development and its regulation, 40 female Holstein calves from the dairy farm of the Experimental School of Inchausti, 25 de Mayo, Province of Buenos Aires, Argentina, were randomly assigned to an untreated (U) or to a treated group (T) at birth. T received monthly different anthelmintic drugs, ivermectin (0.63 mg/Kg), fenbendazol (7.5 mg/Kg) and levamisol (10 mg/Kg) in order to minimize parasite burden. P remained untreated. Both groups were raised outdoors on infected pastures and they were fed milk, balanced supplement or corn, depending on age. At 20, 30 and 40 weeks of age mammary biopsies were taken from 6 heifers in each group. A portion of the biopsy was immediately fixed in buffered 4% formaldehyde for histological and immunohistochemical studies and the other part was conserved in Tris-EDTA buffer with protease inhibitors at -70°C for Western blot studies. Immunohistochemical studies showed that the percent of PCNA immunolabeling was higher in the parenchyma of glands from T than in those from U ( $P < 0.05$ ), indicating a higher rate of cell division. No differences were observed in the stroma ( $P > 0.1$ ). Western blot

studies showed receptor Notch-3 expression, in its active (NICD) and membrane forms, in both assayed groups and at all ages studied. Notch receptors are associated with stem cell maintenance, proliferation and cell differentiation. Their presence in this model would indicate that they may be involved in the regulation of prepubertal bovine mammary gland development.

#### A228

##### **A NEW TECHNIQUE FOR THE ANALYSIS OF PROTEINS THAT DETERMINE MEAT TENDERNESS**

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Meat quality is one of the most important factors for consumers. Out of all meat traits, tenderness is considered the most important with regard to eating quality. Meat tenderness is determined by myofibrillar, connective and cytoskeletal elements of the muscle structure. Many studies have shown that the calpain system plays a central role in postmortem proteolysis and tenderness. In skeletal muscle, the calpain system consists of two proteases: calpain 1 (CAPN1) and calpain 2 (CAPN2); and a specific inhibitor: calpastatin (CAST). Methods commonly employed to determine the activity of proteases are the Casein Assay, Colorimetry and Casein Zymogram. The casein assay and colorimetry require purification by chromatography. In the zymogram, the proteases are separated into polyacrylamide gels (with casein) under non-denaturing conditions, and visualized as clear bands on a dark blue background after staining due to the proteolysis of the casein. This is a very simple technique. However, until this study, it was not used to determine the activity of CAST. The aim of this work, was to develop a new technique that allows the determination of CAST effectively, reliably and easily. For the determination, the crude extract (CE) was heated, inhibiting protease activity but not the CAST because it is heat resistant. This CE was coincubated with fresh extract, and a conventional zymogram was performed showing the inhibition of the protease activity. The novel assay enables us to determine the CAST inhibitory activity on the protease activity, allowing a reliable evaluation of the association of protein activity with meat tenderness.

#### A229

##### **DIGESTIBILITY AND ENERGY INPUT OF SHRUB SPECIES TO GOAT FEEDING IN TUCUMAN SCRUBLANDS**

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Nutritional value is a combination of the nutrient composition of foods and of their optimum use by animals. Accurate knowledge of the nutritional value of food is necessary, so the quantification of the digestibility, digestible energy and metabolic energy contents of food are widely accepted criteria. The vegetation resources of the Tucuman scrublands are important for the feeding of goat herds in the area of Taco Ralo (Tucuman). The objective of this work was to determine digestibility and the digestible and metabolic energy quantification of six (6) shrub species. Percent digestibility (D) was quantified by ANKOM equipment, digestible energy (DE) and metabolic (ME) by AFRC. The results obtained were: mistol (*Ziziphus mistol*): %D: 75  $\pm$ 0.289; DE: 332 Mcal/kg DM  $\pm$ 1.26; ME 272  $\pm$ 1; jarilla (*Larrea divaricata*): %D: 73  $\pm$ 0.87; DE: 323 Mcal/kg DM  $\pm$ 3.82; ME: 264 Mcal/kg DM  $\pm$ 3.13; poleo (*Lippia turbinata*): %D: 50  $\pm$ 0.27; DE: 219 Mcal/kg DM  $\pm$ 1.21; ME: 180 Mcal/kg DM  $\pm$ 0.98; mistolillo (*Castela coccinea*): %D: 72  $\pm$ 2.18; DE: 317 Mcal/kg DM  $\pm$ 9.61; ME: 260 Mcal/kg DM  $\pm$ 8; Chañar (*Geoffroea decorticans*): %D: 56  $\pm$ 1.97; DE: 247 Mcal/kg DM  $\pm$ 8.69; ME: 202 Mcal/kg DM  $\pm$ 7.12; atamisqui (*Caparis atamisquea*): %D: 72  $\pm$ 0.4; DE: 315 Mcal/kg DM  $\pm$ 2.06; ME: 258 Mcal/kg DM  $\pm$ 1.69. It is concluded that the digestibility of the species considered is high, considering that the animals only eat the leaves of shrubs and the metabolic energy input is important. The energy retained in the body covers the requirements of the animals, but it is necessary to consider the energy waste in activity that is about 3.35 J/kg of WL.

#### A230

##### **ASSESSMENT OF THE CONTENT OF CRUDE PROTEIN, FIBER AND TANNINS IN FIVE SPECIES OF THE TUCUMÁN SCRUBLANDS.**

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Goat farmers using the bushes of the scrublands to feed their goat herds. The bushes available can have a good content of crude protein (CP), acceptable levels of fiber and secondary metabolites such as tannins (T), which in moderate proportions can promote beneficial effects on the metabolism of proteins favoring by-pass protein. The objective of this work was to assess the crude protein, fiber and tannins content in plants at the end of the winter season of Tucumán scrublands. Five species were analyzed. 1) Atamisqui (*Caparis atamisquea*), 2) Chañar (*Geoffroea decorticans*), 3) Mistol (*Ziziphus mistol*), 4) Mistolillo (*Castela coccinea*) 5) Jarilla (*Larrea divaricata*). We analyzed crude protein (CP) content by AOAC, neutral detergent fiber (NDF) by Van Soest, ANKOM technology and total content of tannins by AOAC. Our results were: 1) Atamisqui: %CP=4.25  $\pm$ 1.14, %NDF=49.11  $\pm$ 1.13, %T=1.24  $\pm$ 1.13; 2) Chañar: %CP=13.19  $\pm$ 0.16, %NDF=48.70  $\pm$ 0.14, %T=5.56  $\pm$ 0.06; 3) Mistol: %CP=6.07  $\pm$ 0.7, %NDF=34.65

$\sigma \pm 0.64$ , %T=4 $\sigma \pm 0.15$ ; 4) Mistolillo: %CP=4.32 $\sigma \pm 0.81$ , %NDF=17.2  $\sigma \pm 0.12$ , %T=9.05 $\sigma \pm 1.64$ ; 5) Jarilla: %CP=17.16  $\sigma \pm 2.50$ , %NDF=32.39  $\sigma \pm 0.28$ , %T=23.5  $\sigma \pm 0.5$ . A good body condition was observed in the goat herds during the winter period (seasonal drought). Goats graze all species, some with good contents of CP, but high amounts of tannin (jarilla). The animals vary shrubs with different content of tannins. Thus, they increase the forage intake as a way to reduce the toxic effects of the bushes with high tannin content (jarilla) and in the case of shrubs with moderate tannin content they get a positive effect with the protein bypass.

### A231

#### EFFECTS OF BREEDS AND MUSCULAR TYPE ON FATTY ACID COMPOSITION IN KIDS MEAT

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Fatty acid composition in caprine meat is affected by breed, muscular type, nutrition, weight and age at slaughter. In this study we analyzed the fatty acid composition in goats from two breeds: Criollo (C) and Anglo Nubian (AN), in two muscles: *longissimus dorsi* (LD) and *semitendinosus* (ST). Twenty male suckling kids of each genotype were slaughtered, with 80 days of age and 11 kg weight average, from traditional extensive farming systems. The content of intramuscular fat (IMF), saturated fatty acids (SFA), mono- and polyunsaturated fatty acids (MUFA and PUFA), cholesterol and conjugated linoleic acid (CLA) were determined. The results were analyzed by ANOVA. There were no significant differences in the content of IMF or cholesterol levels between breeds. C kids (meat biotype) showed, in both muscles, higher SFA and lower MUFA than AN kids (dairy biotype). AN kids had higher content of PUFA and more appropriate UFA/SFA relationship for human health. Meat from C kids showed higher levels in: total CLA, n-3 and n-6/n-3 ratio. The muscular type analysis showed that ST contains significantly higher values of IMF and lower levels of cholesterol and PUFA than LD muscle. In conclusion, caprine genotype affected the lipid profile and fatty acids composition of meat. The muscular type influenced IMF, PUFA and cholesterol content.

### A232

#### MINERAL DIETARY CONSUMPTION AND GALLSTONES DISEASE

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In order to evaluate the possible association between minerals consumption and gallstones disease (GD), and to compare the intake of these nutrients with nutritional recommendations (RDA), a nested case-control study was carried out in Rosario. Cases were defined as those subjects whose cholelithiasis was diagnosed at the time of the previous cross-sectional study, or had evidence of cholecystectomy for gallstones. Controls were selected by random sampling from all subjects without GD who have a current normal ultrasound study. In order to estimate average daily mineral intake, participants were interviewed using a 210-item food frequency questionnaire (approximate time of each interview: 45 minutes) and a food photography atlas. General linear models analysis was used to compare cases and controls mineral consumption, adjusted by daily total energy intake. A total of 114 patients were studied, 49 cases and 65 controls. No statistical significant differences were found for age, gender, socioeconomic status, body mass index, smoking, and use of vitamin and mineral supplements. Controls consumed more calcium and phosphorus than cases ( $p = 0.004$  and  $p = 0.0001$ , respectively). When comparing the mineral consumption with RDA, these were exceeded for all minerals, except for calcium. While cases did not achieve the RDA (1000 mg), controls exceeded this recommendation. We conclude that these results could provide the basis to perform an analysis of GD risk based on consumption of these minerals.

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