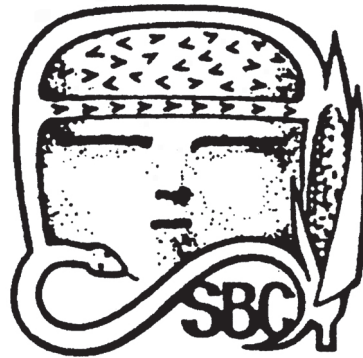


# **Cordoba Biology Society**

(Sociedad de Biología de Córdoba)



Abstracts from the

## **XVII Annual Scientific Meeting**

August, 2009  
Huerta Grande, Córdoba, Argentina

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**L-S1.  
NA,K-ATPASE REGULATED BY RECEPTORS ASSOCIATED TO G PROTEINS**

*Pedemonte CH.*

*University of Houston, Texas, USA.*

The dopaminergic and renin-angiotensin systems act coordinately to regulate blood pressure, in part, by affecting sodium transport in renal proximal tubules. In proximal tubule epithelial cells, the apical Na/H-exchanger and basolateral Na,K-ATPase are responsible for about 75% of the renal filtered sodium reabsorption. By maintaining a low intracellular sodium concentration, the Na,K-ATPase provides the driving force for renal proximal tubule sodium reabsorption. Both, the Na/H-exchanger and Na,K-ATPase are regulated by dopamine and angiotensin II. Angiotensin II stimulates and dopamine inhibits the Na,K-ATPase activity, and they do so by modifying the size of the plasma membrane pool of Na,K-ATPase molecules. We have been studying this hormonal regulation for many years and described many of the components of the signaling cascade induced by activation of the hormonal receptors. During our talk, we will discuss several aspects of the molecular mechanism by which dopamine and angiotensin regulate the activity of the proximal tubule Na,K-ATPase.

**L-S2.  
ORGANIZATION OF ACTIN FILAMENT NETWORK IN PERITUBULAR MYOIDE CELLS OF RAT TESTIS**

*Lopez LA.*

*IHEM. Mendoza.*

Testicular peritubular myoid cells (PMCs), the main cellular component of the seminiferous tubule (ST) wall, are contractile cells that express cytoskeletal markers of true smooth muscle, such as alpha-isoactin, F-actin, and myosin. The contractile activity of PMCs is responsible for the contraction of STs underlying the transport of spermatozoa and testicular fluid and, at least in part, for sperm release during spermiation. The actin cytoskeleton of PMCs from isolated segments VII-VIII of rat testis ST were analyzed by confocal and electron microscopy. PMCs of these segments contain two layers of actin filaments. One layer is localized underneath the nucleus and close to the basal membrane of the seminiferous epithelium and is oriented transversally to the major ST axis. The other layer is localized above the nucleus and oriented parallel to ST axis. The contraction induced by endothelin-1 reduced the area of PMCs and reoriented the parallel layer of actin filaments without changing the organization of the transverse layer. As result of the contraction, the parallel layer of actin filaments redistributed to the periphery of the cell. These results lead us to conclude that the particular organization of actin filaments in PMCs allow them to contract the cell in both parallel and transverse axes.

**L-S3.  
DYNAMICS OF BIOENERGETIC AND ELECTROMECHANICAL SYSTEMS: AN INTEGRATED COMPUTATIONAL-EXPERIMENTAL APPROACH**

*Cortassa S.*

*Johns Hopkins University, Baltimore, MD USA.*

Mathematical modeling represents a fundamental tool for integrating and interpreting events that participate in complex biological functions such as the matching of energy supply and demand in the heart. In this presentation, computational models that account for different aspects of cardiac muscle dynamics, will let me illustrate the trade-off between completeness and understandability in the interpretation of the collective behavior exhibited by complex systems. Cardiac function consists of three subsystems: electrical, mechanical, and energetic, interacting with each other mutually and reciprocally. The contractile activity of the cardiac muscle is the emergent result of multiple processes of different nature operating at different time-scales and in different cellular compartments.

In our work, mathematical models are tools for elucidating the underlying molecular mechanisms operating in organized networks which give rise to (dys)function. The usefulness and integrative power of mathematical models are based upon the following criteria for their formulation and validation: 1) sound physico-chemical basis; 2) capacity to reproduce experimental data; 3) explanatory power of the underlying mechanisms; 4) predictive power. These criteria will be illustrated through the modular and integrative approach followed in the build-up of computational models of the cardiac cell. Complex emergent behavior will also be illustrated such as mitochondrial oscillations that affect the excitability of the cardiac cell as an example of how a subcellular phenomenon can propagate toward higher levels of organization.

**L-S4.  
SYSTEMS BIOLOGY OF CARDIAC FUNCTION: NETWORKS, SELF-ORGANIZATION, AND CRITICAL PHENOMENA**

*Aon MA.*

*Johns Hopkins University, Baltimore, MD USA.*

At present, Biology is evolving from an *analytical* to an *integrative* period. During the analytical period, achieved throughout the 20th century, the main cellular components, metabolic pathways and molecular mechanisms were elucidated whereas the present period is characterized by an integrative aim, with a dominant role played by computational models, high throughput technologies, and their combination, grouped in what is called Systems Biology.

The Systems Biology of cardiac function represents a paradigmatic example in which theories as diverse as dynamics systems, self-organization, fractals, critical phenomena, and networks, take part. The exquisitely balanced physiology exhibited by the heart during its normal function is perturbed under stress. Under those conditions, the cardiac muscle becomes extremely sensitive to small changes showing a large susceptibility to external factors and a strong correlation among its different parts at all levels of organization. The generalized collapse of the electric membrane potential of the mitochondrial network, during oxidative stress or substrate deprivation, propagates to the cellular level, first, then to the tissue, and, finally, to the organ, originating potentially fatal arrhythmias. Complex systems of different nature, obtained either by natural selection or engineering, may collapse or rupture at critical states when stressed. Utilizing a combined theoretical-experimental approach I will illustrate these concepts as they apply to cardiac function.

#### L-S 5. ECO-EPIDEMIOLOGY OF LEISHMANIASIS IN ARGENTINA

*Salomón OD, Quintana M, Acardi S, Fernández MS, Rosa JR, Santini MS, Liotta DJ, Remondegui C, Szlag E, Fuenzalida D. CeNDIE-ANLIS Dr Carlos G Malbrán-Ministerio de Salud, REDILA-Red Investigación Leishmaniasis Argentina.*

Cutaneous Leishmaniasis: studied in the nine endemic provinces since the year 1990 (7,000 human cases, 250,000 phlebotomine). The scenarios of transmission were characterized as: 1) Sylvatic cycle/transmission. 2) Sylvatic cycle/peridomestic transmission, metapopulation structure-interface effect - environment modification. 3) Peridomestic cycle/transmission. The risk in each scenario and region was associated with the abundance, diversity and micro-scale distribution of *Lutzomyia neivai*, *Lu. whitmani*, and *Lu. migonei*. Based on the above scenario characterization the recommendations were stratified according to: 1) hyper-endemic to epidemic transmission: high to moderate risk, 2) moderate transmission-'common source' outbreaks, 3) receptive area- vectors without cases, 4) area vulnerable-adjacent/intense traffic with area 1. The proposed measures were: active search of cases, risk assessment, monitoring the trend of the eco-epidemiologic momenta (sentinel sites). Risk maps are developing.

Visceral Leishmaniasis (VL): The vector, *Lu. longipalpis*, was found in Corpus-Misiones 2000, Clorinda-Formosa 2004 and 2007 ('hot spots' and canine VL), Posadas-Misiones 5/2006 (canine VL and first VL human case). The evaluation of receptivity in Posadas (3/2007) showed the presence of the vector in 42% out of 314 sampled sites, 5.2% with > 31 vectors/trap. Santiago del Estero (10/2007-5/2008) reported 4 human cases and 6 canine cases associated with *Lu. migonei*. The expansion of vectors and canine VL to the province of Corrientes were observed in 12/2008. The following human VL cases were reported until June 2009: Misiones 44 (5 dead), Santiago del Estero 4 and Corrientes 1, with canine VL cases in almost all the country (> 7000 Posadas) due to the traffic of dogs from the endemic area. The risk scenarios were stratified, and so the recommendations for surveillance and control of vectors and reservoirs.

#### L-S 6. ECOLOGY AND CONTROL OF *Triatoma infestans* IN RURAL COMMUNITIES OF NORTHERN ARGENTINA

*Cecere MC.*

*Lab. Eco-Epidemiología. Depto EGE, FCEN-UBA. E-mail: carla@ege.fcen.uba.ar*

Chagas disease remains a public health priority to 100 years of the discovery. Control of this disease is based on the elimination of its main vector, *Triatoma infestans*, through the application of pyrethroid insecticides and horizontal actions involving the community. Identify the causes of incomplete elimination of *T. infestans* in isolated rural communities and preventing their restoration are key issues for the long-term control. In Argentina, the control of the disease has historically been more difficult in Moreno Department, Santiago del Estero. As part of a project on the eco-epidemiology of Chagas disease in rural communities in this province, we assessed the development of surveillance strategies against *T. infestans* with community participation (1993-2004), and identified the spatio-temporal patterns and sources of reinfestation after a massive spraying through the application of geospatial tools. Community-based surveillance strategies were based on the election of community leaders and the transfer of detection, notification and chemical control to the residents through workshops in schools, supervised by the research group and the Chagas disease control program. The spatial-temporal analysis of reinfestation was conducted by using geographic information systems, global positioning systems and spatial statistics. These tools helped to develop recommendations for scientifically based control and surveillance strategies. Sustained, supervised, community-based actions finally led to the interruption of local human *T. cruzi* transmission.

#### L-S 7. OIL PRODUCING FLOWERS. ADVANCES IN KNOWLEDGE ABOUT THEIR EVOLUTION

*Cocucci A, Sérsic A, Cosacov A.*

*Instituto Multidisciplinario de Biología Vegetal, Córdoba, Argentina. E-mail: aacocucci@gmail.com*

Recent molecular phylogenies let us draw conclusions on the origin and diversification patterns of *Nierembergia* (Solanaceae) and *Calceolaria* (Calceolariaceae), which are among the most speciose plant clades bearing oil-flowers in temperate South America. In both genera the acquisitions of elaiophores, which is traceable back to the earliest ancestors, represent key innovations associated with strong speciation relative to sister genera. Elaiophores have been presumably co-opted for pollination service from trichomes of vegetative organs or petal outside involved in other functions such as herbivore deterrent. Reports of extrafloral oil-collection further suggest a non-nuptial origin of the oil-based association. In *Nierembergia*, the transition to oil-flowers involved the loss of nectaries and the exaptation of the corolla tube from a nectar container to an exposition organ. Primary association was probably with Centridines in *Calceolaria* or with smaller Tapinotaspidines in *Nierembergia*. Shifts from large to small oil-collecting bees (*Calceolaria*) and vice versa (*Nierembergia*) have occurred. In both genera studies on multiple populations show that in a single species the locally predominant oil-bees changes between Centridines and Tapinotaspidines, suggesting that these shifts may still be occurring. Elaiophore loss, which is marked in tropical zones, is linked to shifts to selfing, pollination by pollen-collecting bees or (in *Calceolaria*) non-nectarivorous birds.

#### L-S 8. COGNITION AND INTERINDIVIDUAL INTERACTIONS IN INSECT SOCIETIES

*Farina WM.*

*Departamento de Biodiversidad y Biología Experimental, IFIBYNE-CONICET. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.*

Animal societies that have many individuals show a high number of interactions, a fact that facilitate the rapid spread of information. The cognitive skills of the agents involved on circulating information allow us to evaluate the scope of that spread to form a dynamic network. Interestingly, there are almost no experimental studies that combine multi-agent interactions occurring in animal societies with learning processes. We believe that a social insect like the honey bee *Apis mellifera* allow for this type of approach. The various mechanisms for communication in honeybees, and the division of labor based on the age polyethism facilitate the implementation of highly coordinated activities. In this context, the acquisition of information at early ages is important because the young worker bees, those subjects that only perform tasks within the hive, will begin to gather resources outside the nest at older ages. Through various experimental studies, we try to answer how information spreads in a large animal society and how that information remains on a collective level.



**1. HEXARELIN AFFECTS MEMORY ACQUISITION IN MICE**  
*Carlini V, Martini AC, Vincenti LM, Luque EM, Puechagut P, Ruiz RD, Rubiales de Barioglio S, Fiol de Cuneo M.*  
*Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba.*

In a previous work, we have demonstrated that ghrelin (Ghr) reverses the deleterious effects upon memory observed in chronic food restricted animals. The objectives of the present study were to evaluate the effects of a ghrelin analogue employed in human therapy, hexarelin (HEX), upon mice memory acquisition, employing the object recognition test, in two experimental designs: A) HEX administration (100 ó 200 µg/kg/día, sc) to adult mice for 28 days and B) HEX administration (200 µg/kg/día, sc) to pregnant females during different periods, evaluating memory acquisition in their litter at adulthood. In A, HEX diminished memory vs control group (C) in females with both doses assayed (% of time exploring novel object: C 1h: 68.5±2.3, n=14; HEX100 24h: 36.2±6.9, n=7; HEX200 1h: 39.2±4.7, n=8; HEX200 24h: 37.6±3.2, n=8, p<0.05) while in males, only with the mayor one (C 24h: 62.4±2.2, n=11 y HEX200 24h: 48.8±4.5, n=7, p< 0.05). In B, memory was altered (especially in females) only when pregnant females were treated for the whole pregnancy or during the first third. In contrast, this last treatment accelerated the neurobehavioral development (negative geotaxis, surface righting reflex and cliff avoidance, at 8 to 10 postnatal days). These discrepancies could be attributed to differential effects of HEX upon different central nervous system structures.

**2. EFFECT OF PRENATAL CHRONIC FLUORIDE ADMINISTRATION IN RATS**  
*Fontanetti PA, Choi ED, Vermouth NT.*  
*Facultades de Odontología y de Ciencias Médicas. Universidad Nacional de Córdoba. E-mail: pabfontanetti@gmail.com*

Fluoride (F<sup>-</sup>) is used to prevent tooth decay. However, it was demonstrated that excess of this ion in the organism can cause damage in several organs and tissues. Besides, F<sup>-</sup> can cross the placenta reaching the developing fetus. The aim of the present study was to determine if administration of sodium fluoride (NaF) to rats during gestation affects body weight and mandible growth of their pups. Pregnant rats were divided into two groups (control and treated). Treated group was administered with 20mg/kg or 50mg/kg of NaF (i.p.) since day 10<sup>th</sup> to 20<sup>th</sup> of gestation. Their pups were weighed and sacrificed at postnatal day 3, 15 or 20. Their mandibles were weighed and fixed in formaldehyde after sacrifice. The right hemimandible of each animal was radiographed and the left one was processed for histological analysis. Dose of 50mg/kg was lethal in 100% of the mothers. In the group treated with 20mg/kg, a significant decrease in food intake was observed. In addition, body weight and mandible growth of the pups were significantly lower compared with those of control (p<0.05). In conclusion, these results show that NaF administration to the mothers (20mg/kg) during gestation does not have a lethal effect, although it affects postnatal growth of their pups which may have a negative influence on normal development.

**3. CHANGES IN SUBMANDIBULAR SALIVA OF RATS CHRONICALLY EXPOSED TO CONSTANT LIGHT OR REPEATED IMMOBILIZATION**  
*Alterman A<sup>1,2</sup>, Mathison R<sup>4</sup>, Davison J<sup>4</sup>, Coronel C<sup>3</sup>, Bellavia SL<sup>1</sup>, Finkelberg A<sup>2</sup>, Gallarà RV<sup>1,2</sup>*  
<sup>1</sup>Cát. Bioq y Biol Mol, Fac. de Cs Méd; <sup>2</sup>Cát Fisiol, Fac de Odontol; <sup>3</sup>Lab. Bioq. Biol. Reproductiva-ICTA-FCEfyN, Univ Nac Córdoba, Argentina. <sup>4</sup>Dep of Physiology and Biophysics. School of Medicine. University of Calgary. Canada.

Sympathetic and parasympathetic systems are involved in the stress response and regulate saliva secretion. This study evaluated the effects of stress on trophic and functional changes in submandibular gland (SG). Male adult rats were divided in three groups and daily food intake and body weight were monitored. Group-I (IMO) were immobilized 2h per day for 7 days. Group-II (LL) was exposed to constant light (20 days). Group-III (C) was unstressed controls (14h light-10h dark). Saliva samples collected under anaesthesia following ip administration of isoproterenol and pilocarpine (5mg/Kg), were assessed for total proteins (TP), amylase activity and subject to SDS-PAGE. After 20 min glands were dissected and wet and dry weights determined. In IMO (n=11) rats SG wet (174.6±18.1) and dry (39.8±1.7) weights (mg) were significantly lower (p<0.05) than in C (n=13) (209.7±8.1 and 46.8±2.0) and LL (n=7) (205.5±9.4 and 52.2±1.9). In IMO, food intake (p<0.01) and body weight (p<0.01) were lower than in C and LL. The volumes of saliva secreted (µg/mg-dry-weight) in IMO (1470±198) and LL rats (1119±99) were significantly higher than in C (877±68) (p<0.05). No differences were observed in the amount of TP and amylase activity. The electrophoretic profile in LL rats lacked of a protein band of approximately 25KD. Peptide mass fingerprinting, using MALDI-TOF mass spectrometry, identified the two proteins absent in LL saliva as common salivary protein-1 and prolactin-induced protein. Stress modifies autonomic nervous control of saliva secretion. Immobilization stress induces trophic effects upon SG without changing saliva protein composition. Constant-light promotes changes in the protein composition of saliva without trophic effects upon SG. The function as well as their unexpression of common salivary protein-1 and prolactin-induced protein in animals exposed to constant light should be investigated.

**4. PROTECTIVE EFFECT OF MELATONIN IN PREGNANT RATS**  
*Ponce RH, Cisternas CD, Vaqué AM, Compagnucci MV, Abramor NF, Vermouth NT.*  
*Facultades de Odontología y de Ciencias Médicas. Univ Nacional de Córdoba. Córdoba. E-mail: nvermouth@odo.unc.edu.ar*

Melatonin (Mel) is known for its importance in circadian rhythms (CR) modulation and for its antioxidant, citoprotective and antiapoptotic properties. In mammals, the maternal melatonin circadian rhythm is considered the main synchronizing signal during gestation. It is known that prenatal chronic stress generates long term alterations over both hypothalamic-pituitary-adrenal axis regulation and neurophysiology development in pups. The aim of this study was to examine the possible protective role of melatonin administered to pregnant rats submitted to chronic varied stress (CVS). We analyzed the copulatory behaviour and the CR of drinking behaviour, α-amylase of salivary glands and malate and lactate dehydrogenase of testis in pups from mothers submitted to CVS from 10 to 20 day of pregnancy and treated with Mel (1 mg/Kg) or vehicle during the last 5 days of gestation. Melatonin administration to CVS mothers significantly prevented the CR disruption at 28 and 45 postnatal days and restored the normal patterns of latencies and number of mount, intromission and ejaculation at 120 postnatal days compared with pups from normal mothers. Results reinforce the Mel entrainment role over maternal programming. It is possibly, that melatonin through its different mechanisms exerts a neuroprotective action, restoring the behaviours studied.

5.

**Akodon azarae XY FEMALES**

*Ortiz M<sup>1</sup>, Marchal J<sup>2</sup>, Romero-Fernandez P<sup>2</sup>, Sánchez A<sup>2</sup>, Pinna-Senn E<sup>1</sup>, Lisanti J<sup>1</sup>.*

<sup>1</sup>Depto Cs Naturales, Fac. Cs. Exactas, Fís.-Quím. y Naturales, UNRC, Río Cuarto. <sup>2</sup>Depto de Biol. Experimental, Fac. Cs Exp. Univ. Jaén, Jaén. España.

In several rodent species, exceptional mechanisms of sex determination are seen, such as the XY fertile females of *Myopus schisticolor* and several species of *Akodon*. We have analyzed the transmission of the XY female condition in *A. azarae* taking advantage of the possibility of distinguishing, according to the C-banding pattern, male X chromosome (X<sub>1</sub>) from that of XY females (X<sub>2</sub> o X<sub>3</sub>). The study of F1 and F2 of different crossings confirmed the correlation between X type and sexual phenotype, and that the different X types are transmitted to the descendants. It was also observed that the male offspring of XY females receives the X from the father and the Y from the mother, which demonstrates that the Y chromosome of the above mentioned females is functional in a suitable context. On the other hand, we analyzed the *Sry* gene in two males and in three XY females by means of PCR amplification of a 823 bp fragment that includes the complete ORF of this gene. The obtained clones and the deduced proteins did not show significant differences between males and XY females. These results indicate the causal role of an X linked mutation in sexual reversion, and rules out the mutation of the *Sry* as the only reason of this phenomenon. On this base, we studied 653 bp of exón 1 of *Dax1*, obtained by PCR of two males, two XY females and three XX females. The resulting sequences did not show differences between males and XY females.

6.

**OBSERVATIONS ON AKODON BOLIVIENSIS XY FEMALES**

*Pinna-Senn E<sup>1</sup>, Marchal J<sup>2</sup>, Romero-Fernandez P<sup>2</sup>, Sánchez A<sup>2</sup>, Ortiz M<sup>1</sup>, Lisanti J<sup>1</sup>.*

<sup>1</sup>Depto Cs. Naturales, Fac. Cs. Exactas, Fís.-Quím. y Naturales. UNRC. Río Cuarto, Argentina. <sup>2</sup>Fac. Cs Exp. Univ. de Jaén. Jaén. España.

The mechanism of mammalian sex determination depends on the Y chromosome which, through the expression, at the correct time and level, of the *Sry* gene, leads to male development. However, in the populations of several rodents, including the South American genus *Akodon*, there are fertile XY females besides XX ones. With the objective of investigating the transmission of the XY female condition in *Akodon boliviensis*, the cytogenetics of the descendants of different laboratory crosses, including XX and XY females, was studied. The results confirmed the complete fertility of XY females. It was also observed that XY females appeared in the progeny of XX females, and not only in that of XY ones. Given the fundamental of the *Sry* gene, we amplified and sequenced a 832 nt fragment that includes the complete ORF (543 nt) and 5' and 3' segments. Sequencing of this gene in 5 *A. boliviensis* specimens did not reveal significant differences between this species males and XY females, which indicates that sex reversal would not be due to a mutation of this gene. Supposing that, analogously to our finding in *A. azarae*, sex reversal could be caused by the mutation of a sex-linked gene, we began to study this possibility by amplifying and sequencing a 654 nt fragment corresponding to great part of exon 1 (the largest) of *Dax1* gene in 6 *A. boliviensis* specimens (2 males, 2 XX and 2 XY females). This fragment sequence was the same in males and in both types of females.

7.

**Sry COMPARISON IN THREE SPECIES OF Akodon (RODENTIA, SIGMODONTINAE)**

*Marchal J<sup>2</sup>, Romero-Fernandez P<sup>2</sup>, Sánchez A<sup>2</sup>, Ortiz M<sup>1</sup>, Pinna-Senn E<sup>1</sup>, Lisanti J<sup>1</sup>.*

<sup>1</sup>Depto Cs. Naturales, Fac. Cs. Exactas, Fís.-Quím. y Naturales. UNRC. Río Cuarto, Argentina. <sup>2</sup>Fac. Cs Exp. Univ. de Jaén. Jaén. España.

*Sry* gene expression is necessary and sufficient to initiate male development, at least in the great majority of eutherian and metatheria mammals. The only *Sry* exon encodes a protein that binds DNA through an HMG domain. Comparative studies of the *Sry* gene in different mammalian species showed that only the HMG box region would be preserved. In order to determine the degree of conservation of the above mentioned gene in the genus *Akodon*, the *Sry* sequence of *A. boliviensis*, *A. azarae* and *A. dolores* was compared. Nucleotide alignments showed 96,8 % to 97,9 % identity between the species. *A. azarae* differs from *A. boliviensis* in 9 bp, an insertion of 2bp in the N-terminal region and a 11bp deletion in the C-terminal region. On the other hand, *A. boliviensis* differs in 13 pb and a 2bp deletion from *A. dolores*. The alignment of the deduced protein showed a range of identity between 97,7% - 98,33%. The comparison of the HMG box determined a similarity of 100% between *A. azarae* and *A. boliviensis*, and 98,79% between *A. dolores* and the other two species. The comparison of the deduced amino acids sequence of the three species studied with that of other mammals showed that the HMG domain is the most preserved region. The wide difference that exists outside this region would indicate that the SRY protein is functional only in terms of the HMG block or that there has been a divergent coevolution in other regions of the gene.

8.

**ADSORPTION OF AFLATOXIN B<sub>1</sub> (AFB<sub>1</sub>) by Kasachstania bovine SHOWING A COOPERATIVE EFFECT THAT INCREASE THEIR EFFICIENCY**

*Pizzolitto RP<sup>1</sup>, Armando R<sup>2</sup>, Combina M<sup>3</sup>, Cavaglieri L<sup>2</sup>, Dalcero AM<sup>2</sup>, Salvano MA<sup>1</sup>.*

<sup>1</sup>Dpto. Biología Molecular. <sup>2</sup>Dpto. Microbiología e Inmunología. Fac. de Cs. Exactas, Fco-Qcas y Naturales. Universidad Nacional de Río Cuarto. Ruta 36 Km. 601. Río Cuarto (Cba). <sup>3</sup>Laboratorio de Investigación de Biología Molecular (INTA), Lujan de Cuyo, Mendoza.

Many microorganisms, mainly yeasts and lactic acid bacteria are able to eliminate mycotoxins from liquid medium. In our laboratory, we developed a model which quantifies the efficiency of the process ( $M \times K_{eq}$ ) by determining the total number of sites for adsorption (M) and the equilibrium constant ( $K_{eq}$ ) involved. The purpose of this study was to evaluate the ability of yeast detoxifying *Kasachstania bovine* isolated from chicken feces.  $10^7$  cells ml<sup>-1</sup> were incubated at 37°C with increasing concentrations of AFB<sub>1</sub> (0.3-20 ug ml<sup>-1</sup>) for 10 min. Centrifugation at 5000 g 15 min, allowed us to determine AFB<sub>1</sub> concentration in supernatant by HPLC. Adsorption isotherms showed a biphasic curve whose inverse values corresponds to M and  $K_{eq}$ . When the concentration of AFB<sub>1</sub> in the medium exceeded 2.5 ug ml<sup>-1</sup>, M increased 6-fold and the  $K_{eq}$  declined only 40% so that the efficiency increased by almost 4 times. The results indicate that *Kasachstania bovine* is potentially a very effective bioeliminator AFB<sub>1</sub> in liquid medium, as in the presence of high concentrations of the toxin, showed a cooperative effect at the level of its cell wall that increases the number of adsorption sites and hence their efficiency.

**9. DECOMPOSITION OF DOMINANT SPECIES OF MONTANE WOODLAND (CORDOBA): EFFECT OF THE COMPOSITION OF SPECIES IN DOUBLE MIXTURES**

*Cuchiatti A, Pérez-Harguindeguy N, Gurvich DE, Cingolani AM. Cátedra de Biogeografía, Facultad de Ciencias Exactas, Físicas y Naturales. UNC. E-mail: anibal.cuchiatti@gmail.com*

Decomposition is a fundamental ecosystem process in which nutrients contained in vegetable material are liberated to the soil. There are evidences that decomposition of species in litter mixtures can differ from that of individually incubated species due to diverse mechanisms of interaction among the different species within the mixtures (priming effects, mining effects, among others). We analyzed if some of this mechanisms underlying synergic and antagonistic effects are operating in litter mixtures of dominant species from Córdoba montane woodlands. We found that the synergic and antagonistic effects did not relate significantly to the initial chemical quality of the species in the mixture, neither to the initial decomposition of the species of the mixtures. In relation to the mechanisms underlying these effects, some evidences indicate an influence of chemical secondary compounds in particular species. Other evidences reject the influence of the initial concentration of nitrogen in these effects (priming effects). For the analyzed species, and in the first stages of decomposition, the effects in litter mixtures would be idiosyncratic and dependent to presence of particular species more than of the initial decomposition, or the initial nitrogen concentration.

**10. ECOLOGICAL NICHE MODELS OF THREE SNAKE SPECIES (*Bothrops*, VIPERIDAE)**

*Di Cola V, Chiaraviglio M. Laboratorio de Biología del Comportamiento, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba. Vélez Sarsfield 299, (5000) Córdoba, Argentina. E-mail: valeriadicola@gmail.com*

Evolution of ecological niches, in their various guises, has become a central theme in organismal and evolutionary biology. One of the faunal zones in South America which has the biogeographic unity and the significance proposed for snakes is the relatively extensive xeric belt of the East and South of the continent. It is interesting to note that this xeric belt holds the highest diversity for the *Bothrops* genus. Three of its species, *B. alternatus*, *B. diporus* and *B. ammodytoides* occupy a large distribution range, being sympatric in some areas. We hypothesize that species will respond differently to environmental requirements according to the lack of resemblance of their distribution in the xeric band. We used ecological niche based models to examine the environmental factors correlated with species distribution and to identify potential areas for their occurrence and sympatry. From presence data and 23 environmental variables we perform Logistic Regression Analyses to obtain the models. The distribution of species is determined by different environmental variables. However, it is possible to identify common variables which explain the areas of sympatry found. In conclusion, several factors, acting as ecological barriers, limit the distribution of these species to spatially non-overlapping ranges, whereas other factors facilitate sympatry by allowing overlapping between their ecological niches.

**11. DISTRIBUTIONAL MODELING AND ENVIRONMENTAL REQUIREMENTS OF LIZARD (*Tupinambis rufescens*) AND ITS RELATION WITH POPULATION PARAMETERS**

*Naretto S, Lanfri S, Di Cola V, Cardozo G, Chiaraviglio M. Laboratorio de Biología del Comportamiento. Facultad de Ciencia Exactas, Físicas y Naturales. Universidad Nacional de Córdoba. E-mail: naretto sergio@gmail.com*

The analysis of species–environment relationship has always been a central issue in ecology. The quantification of such relationships represents the core of predictive geographical modeling in ecology. These models are generally based on various hypotheses as to how environmental factors control the distribution of species. Considering that species are sensitive to environmental heterogeneity, the study of distributional spatial patterns from an ecological perspective allows predicting their influence on population processes. The aim of this study is to determine the environmental variables that model the distribution of red tegu lizard (*Tupinambis rufescens*) in Córdoba and to analyze its relationship with population parameters. Using presence records of the species and considering biogeophysical variables, environmental requirements that determine the distribution were identified. From these results areas with different environmental conditions were identified to evaluate population parameters such as sex ratio and body size distributions. The variables that model and explain the distribution of *T. rufescens* are temperature and precipitation, linking the species to arid zones with high temperatures and seasonal climate. The populations exhibit sexual size dimorphism, with males attaining larger sizes than females. The differences between populations are reflected in sex ratio and body size distributions. The knowledge of the environmental requirements of species and the response of populations to changes in the landscape provide an opportunity to understand how environmental changes affect populations.

**12. QUANTIFICATION OF ALGAL GROUPS BY MEANS OF FLUORESCENCE TECHNIQUES**

*Gsponer N<sup>1</sup>, Rodriguez C<sup>2</sup>, Chesta C<sup>1</sup>. <sup>1</sup>Departamento de Química. <sup>2</sup>Departamento de Ecología. Universidad Nacional de Río Cuarto. Río Cuarto, Córdoba, Argentina. E-mail: ngsponer@exa.unrc.edu.ar*

*Microalgae* are unicellular organisms that contain *chlorophyll* (Chl) and photosynthetic pigments. The Chl is a fluorescent pigment with maximum at 685 (PSII) and 700 nm (PSI). The excitation spectrum of fluorescence at 685 nm strongly depends on the algal group. Each group shows a *norm* characteristic excitation spectrum which can be used to determine the presence of this algal group in water samples and to quantify their relative population. *Norm* spectra for some algal groups have been already reported (Chlorophyta, Bacillariophyta, etc.). The aim of this study was to obtain the *norm* spectrum for the genera *Anabaena* sp. (ITEP-024) and *Microcystis* sp. (NPLS-04/MPCD-01) (Cyanophyceae). These strains were cultured in BG<sub>11</sub> under controlled of light intensity and temperature conditions. The *norm* spectra were obtained from the *in vivo* excitation spectra of the samples containing different concentrations of the strains. Subsequently, the contents of Chl were quantified for each sample. We found an excellent correlation (R: 0.99) between fluorescence intensity and contents of Chl. The *norm* spectra thus obtained may be used now to determine the cyanobacteria populations by measure in the excitation spectrum fluorescence of surface water samples.



**13. POULTRY MANURE IN THE MICROBIOLOGICAL PRODUCTION AND QUALITY OF LETTUCE (*Lactuca sativa* L.)**

*Viale S, Guevara E, Willnecker A.*

*Vegetal Production Department, FAV-UNRC, Río Cuarto, Córdoba. E-mail: sviale@ayv.unrc.edu.ar*

The fruit and vegetable area is responsible for taking safe and qualitative products to the Argentinian table every day. Lettuce is a vegetable whose production needs aviary manure added to the soil to improve the structure and the contribution of nutrients. If wrongly handled, they may cause risk of pollution by *Salmonella* and *Escherichia coli*. The aim of the work is to analyze the effect of aviary manure in the production of lettuce and in the microbial pollution in the soil and the product. The work was done in the UNRC with the Rapidmor cv of loose leaf, sown 3/26/08 and harvested 6/5/08. It was scattered leaving 20x25 cm per plant. Chicken and hen manure were used (5kg.m<sup>-2</sup>) applied 30 days before the seed. The design was in BCA with 4 repetitions. When harvested, the microbiological recounts were evaluated in soil samples with manure and lettuce and green and dry weight per plant. The information was compared with ANOVA ( $\alpha=0.05$ ). Results: the recounts of *E. coli* in soil and lettuce were below the maximum limit admitted for consuming (10<sup>3</sup> ufc). *Salmonella* wasn't detected. The green weight average was of 89, 145 and 160 and the dry weight of 8, 11 and 12 g.pl<sup>-1</sup> for the witness paddies, soil with hen and chicken manure respectively. There are meaningful differences in statistics between the witness and the manure, but not between them. Manure increases the production of lettuce and if they are correctly handled, they do not present risk of pollution.

**14. GERMINATION OF *Acacia atramentaria***

*De Luca N<sup>1</sup>, Cantero JJ<sup>1,2</sup>, Fernandez EM<sup>2</sup>.*

*<sup>1</sup>Ministerio de Ciencia y Tecnología, Gobierno de la Provincia de Córdoba. <sup>2</sup>Facultad de Agronomía y Veterinaria. UNRC. Río Cuarto. Córdoba.*

The establishment of *Acacia atramentaria* "Espinillo negro" usually can fail due to problems in germination process caused by seed tegument impermeability which limit seed water imbibitions. Seed treatments to pass the germination were evaluated. Fruits were harvested from plants located on hillside of Uritorco hill in "Capilla del Monte" (30°41'53"SL and 64°31'33"WL), ~ 900 m over sea level (march/2006). Seeds obtained from those fruits were put in glass container and stored in freezer during 12 months and refrigerator during another 18 months. Water content was determinate as a percentage of wet weight. Treatments were seed scarification in humid conditions via seed immersion in water at 80°C which after get down to 20°C (24 hours) (EH); mechanical scarification with sandpaper N° 120 (EM); and control (T). Seed germination (G) was evaluated over paper at 25°C. After that seedlings were classified in normal (seedlings with all structures and 5 to 25 cm of length); weak (seedlings smaller than 10 cm); and abnormal (seedlings with fungal disease); other categories were hard seed (SD), fresh and dead seeds (SM). Counts were made at 5, 11 and 16 days. Germination is epigeal, cotyledons are green intensive and seedlings achieved 20 cm at 16 days. Differences among treatments were found; EM reached 98,3% of G and 5% of SM, meanwhile EH obtained 8,3% of G and the control 100% of SD.

**15. LIMNOLOGICAL ASPECTS AND ICHTHYOFAUNA IN A PAMPEAN SHALLOW LAKE OF THE RIO QUINTO SYSTEM (CORDOBA, ARGENTINA)**

*Mancini M<sup>1</sup>, Morra G<sup>1</sup>, Bethular A<sup>1</sup>, Salinas V<sup>1</sup>, Haro JG<sup>2</sup>.*

*<sup>1</sup>Acuicultura. FAV-UNRC, Río Cuarto. <sup>2</sup>Div. Animal II. FCEFYN. UNC, Córdoba. E-mail: mmancini@ayv.unrc.edu.ar*

The southern region of Cordoba Province presents an important surface covered by shallow lakes connected to Río Quinto through a series of canals. Many of these water resources are used as recreational fisheries of silverside *Odontesthes bonariensis*. Preliminary results on different limnological variables and ichthyofauna of the Onagoity lake (34°46'S, 63°38'W) are reported. In May 2009, in situ water measurements and laboratory analyses were performed. Fishes were captured with trawlnets and floating gillnets. The water had oligohaline characteristics (3.39 g/L) with a (Ca+Mg)/(Na+K) relationship of 0.18 and hardness of 392 mg/L CaCO<sub>3</sub>. The average temperature, pH and transparency (Secchi disk) were 15.5°C, 8.74 and 0.40 m respectively. Zooplankton abundance was 303,540 (± 96,160) org/m<sup>3</sup>. Oxygen concentration showed oversaturation values. Related to ichthyofauna, 10 species were captured being *O. bonariensis* the dominant one in numerosity (53.4%). Shannon-Wiener diversity index was 2.03 bits and evenness index was 0.61. The presence of new species in the region was confirmed. According to previous year's reports, hydrochory through canal systems man-made appears to be the main cause for increasing the number of species.

**16. SOYBEAN GERMLASM DEVELOPMENT FOR HUMAN CONSUMPTION**

*Bologna S<sup>1</sup>, Rojas E<sup>1</sup>, Soldini D<sup>2</sup>, Martínez Alvarez D<sup>1</sup>, Cattaneo N<sup>1</sup>.*

*<sup>1</sup>UNSL. <sup>2</sup>INTA.*

Soybean has an excellent nutritious value, but it presents trypsin inhibitors that reduce its nutritional quality and lipoxigenase that produce a bitter flavor. With the objective of generating genetic variability in characters for grain quality, in INTA Marcos Juárez (2006) biparental crosses were carried out between the progenitors BRM92-6600 (conventional line without lipoxigenase) y PI 542.044 (conventional line without trypsin inhibitors). In 2007 the F<sub>1</sub> population was sowed in greenhouse and the F<sub>2</sub> population was obtained. In the Department of Agricultural Sciences of the UNSL the F<sub>2</sub> was sowed using bulk method and was conducted by the Single Seed Descent Method. 938 plants were harvested and the number of pods per plant (NP) number of seeds per plant (NS) and plant yield were determined. The phenotypic, genotypic and environmental variances were estimated throughout an Analysis of Variance and the Coefficient of Heritability (h<sup>2</sup>) was estimated. The h<sup>2</sup> resulted more than 50%, showing the highest coefficients of heritability for NP and NS (78%). 81 plants were selected and in 2008 F<sub>3</sub> families were opened, selecting among and in families. The germplasm developed constitutes an important source of variability to select soybean genotypes with differential quality for human consumption.



**17. PHOTODYNAMIC INACTIVATION OF *Candida albicans* BY A PORPHYRIN POLYMERIC FILM**

Funes MD, Álvarez MG, Fungo F, Otero LA, Durantini EN.  
Dpto. de Química, Universidad Nacional de Río Cuarto, Argentina.

Photodynamic inactivation (PDI) of microorganisms is a new therapeutic modality that involves the treatment with a sensitizer, which it accumulates in cells. The exposure to visible light leads to the generation of reactive species of oxygen, which produces photodamage of cells. In these systems, the use of a sensitizing film favours the removal of the agent after treatment.

In the work, we synthesized a porphyrin substituted for four groups derived of carbazole, which allow the electrochemical polymerization. The spectroscopic studies indicate that the film high absorbs in the visible region and produces efficiently singlet molecular oxygen. *In vitro* investigations of PDI were realized in *Candida albicans*. The film exhibits a photosensitizing activity causing a decrease of *C. albicans* cellular survival in ~2 and 3 log after 30 min and 60 min of irradiation with visible light, respectively. However, in anoxic condition the cellular viability is not affected, indicating that an oxygen atmosphere is required for the mechanism of yeast photodynamic inactivation. Thus, the main advantage of use sensitizers chemically immobilized on a surface is producing the inactivation of microorganisms, without affecting the media with the agent.

**18. EVALUATION OF TOXICITY OF NANOFIBERS POLYANILINE IN LARVAE OF *Rhinella (Bufo) arenarum* FOR ITS APPLICATION IN PHOTO-ASSISTED THERAPY**

Yslas EI, Ibarra L, Ticle M, Peralta D, Barbero C, Rivarola VA, Bertuzzi M.

<sup>1</sup>Dpto Biología Molecular, Facultad de Ciencias Exactas Físico Químicas y Naturales U.N.R.C. <sup>2</sup>Dpto Química, Facultad de Ciencias Exactas Físico Químicas y Naturales U.N.R.C. E-mail: mbertuzzi@exa.unrc.edu.ar

The photothermal therapy (PTT) is based on the irradiation of certain nanomaterials with pulses of near infrared light, which absorb light and released as heat, causing the regression tumor. The objectives of this study were: Develop the polyaniline nanostructures and dispersed, by assessing the toxicity test in *Rhinella (Bufo) arenarum* larvae to determine median lethal concentration, internalization and excretion. The nanofibers were synthesized by interfacial polymerization. At physiological pH the nanofibers precipitate thus solubilized in the polyvinylpyrrolidone. The toxicity studies indicated that at concentrations between 0-400 mg / L did not cause lethal damage in larvae. Moreover, it was the internalization of the drug through uv-visible absorbance and IR. Also there were observed differences in excrement's coloration between larvae incubated with nanofibers with respect to their controls, indicating its transit through the digestive system. The nanofibers are interesting because they are biocompatible nanomaterial, uptake and no signs of toxicity in larvae. It therefore might be suitable for application in the PTT.

**19. ANTIFUNGAL ACTIVITY OF EXTRACTS FROM *Ouratea hexasperma* AND *Ouratea parviflora* ON *Candida albicans***

Álvarez MG<sup>1</sup>, Francisco de Araújo M<sup>2</sup>, Aragão Catunda Júnior FE<sup>2</sup>, Geraldo de Carvalho M<sup>2</sup>, Durantini EN<sup>1</sup>.

<sup>1</sup>Dpto. de Química, UN Río Cuarto, Argentina. <sup>2</sup>Dpto. de Química, UFR de Río de Janeiro. Brasil.

The search for compounds with antimicrobial activity is important to the treatment for infection diseases. Because of the flavonoids have very important properties in medicine, they have been proposed like antimycotic drugs against yeasts (*Candida albicans*) and human pathogen filamentous fungi. These compounds may be useful for controlling fungal growth and mycotoxin production.

In this study, we have evaluated the antifungal activity of different extracts from *Ouratea hexasperma* and *Ouratea parviflora* on *C. albicans*. The cultures were incubated with different concentrations of extracts (0.064-3.0 mg/ml), which were obtained by different solvents from leaves, roots and branches of *O. hexasperma* y *O. parviflora*. After 48 h of incubation at 37°C, the minimal inhibitory concentrations (MIC) were determined. Extracts obtained from *O. hexasperma* roots by solvents partition methanol:water ethyl acetate and *O. hexasperma* leaves by solvents partition methanol:water butanol showed a higher antifungal activity. Furthermore, the antifungal activity was compared by growth delay of *C. albicans* cultures. The studies indicate that these extracts have potential applications as therapeutic agents in the inactivation of yeasts.

**20. REMEDIATION OF PHENOL: ANALYSIS OF REACTION PRODUCTS TOXICITY**

Paisio C, González P, Bertuzzi M, Agostini E.

Dpto. de Biología Molecular, FCEFQyN. UNRC. E-mail: cpaisio@exa.unrc.edu.ar

A great quantity of phenolic compounds are discharged from different industries, producing several environmental impacts. So, the treatment of these effluents is strictly necessary. We have employed hairy root cultures (HR) from wild type tobacco (wt) and transgenic (DT) plants, to remove phenol with high efficiency. However, it is important not only to evaluate the decrease of a contaminant in the reaction medium but also to determine the potential toxicity of reaction products, which could be done using a bioassay. The aim of the present work was to evaluate the toxicological impact of post removal solutions, derived from treatments performed with tobacco HR (wt and DT), on *Rhinella arenarum* embryos. Acute test (96 hs) was carried out by exposing larvae to phenol solutions (25-250 mg/L) previously treated with HR. Phenol solutions with concentrations up to 150 mg/L, treated with wt and DT HR, produced 100 % mortality. Percentages of mortality for solutions initially containing 25, 50 and 100 mg/L were: 2.5, 15 and 37% for wt and 10, 57.5 and 65 for DT, respectively. CL50 were 84.5 and 54 for treatments with wt and DT HR, respectively. DT HR showed higher efficiency than wt HR, in removal assays. In conclusion, phytoremediation performed with tobacco HR was effective and reduced significantly the toxicity of post removal solutions, initially containing phenol in concentrations below 150 mg/L.

**21. PLANT REGENERATION THROUGH ANTHHER CULTURE IN APOMICTIC CULTIVARS OF BUFFEL GRASS**

*Carlioni E, Grunberg K, Griffa S, Ribotta A, López Colomba E, Tommasino E, Luna C, Biderbost E. Instituto de Fitopatología y Fisiología Vegetal (IFFIVE-INTA), Camino 60 cuadras Km 5°. CP X5020ICA. Córdoba, Argentina.*

Buffel grass is a grass used as forage in the Argentinian North West. It reproduces by apomixis thus generate variability by conventional breeding methods is difficult. Various biotechnology techniques to reduce or increase the level of ploidy such as anther culture can be combined to develop strategies for improvement. The aim of this study was to evaluate the response to androgenesis through anther culture in three cultivars of Buffel grass. To induce embryogenic calli, anthers containing microspores at the uninucleate stage of cvs. Biloela, Americana and Bella were cultured in darkness in Petri dishes, with basal medium Murashige and Skoog (MS), 3% (w/v) sucrose and 0.7% (w/v) of agar. The medium was supplemented with different combinations of 2,4-dichlorophenoxyacetic (2,4-D) 2, 4 and 6 mg / l 2 +0.5 4 +0.5 6 +0.5 mg / l 2,4-D +6 -benzylaminopurine (BAP), respectively, at 25°C, pH 5.8. Moreover, the inflorescences were subjected to different pretreatments: cold (5 and 7 days) and heat shock (40°C 30" + 10°C 30"). Embryogenic calli were transferred to germination medium (MS +1 mg / l (BAP) +0.1 mg / l naftalenacetic acid (NAA), 0.7% (w / v) agar (pH 5.8). Green plant regeneration from anther-derived embryogenic calli was achieved in Biloela and Bella. The highest percentage of plants was obtained on cv. Bella in cold pretreatments with 5 days (1.7%) and heat shock (1.7%). Plant regeneration was linked closely to number of produced embryogenic calli and has allowed regenerating green plants in two of the three cultivars of grass Buffel used.

**22. CHRONIC ADVERSE STIMULI DURING PREGNANCY: POSTNATAL CONSEQUENCES**

*Conti NR, Ponce RH, Vermouth NT. Facultades de Odontología y Ciencias Médicas, UNC. 5000 Córdoba, Argentina. E-mail: rhponce2006@gmail.com.ar*

In mammals, prenatal stress during pregnancy reprograms neuroendocrine and behavioural responses in offspring, inducing long-term consequences in the regulation of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonad axis. The objective of the present study was to compare the influence of prenatal constant light (LL) or chronic variable stress (EE), applied to the mother during pregnancy, on the anxiety behaviour and fertilizing capacity in adult male rat offspring. Mothers were exposed to LL or EE from day 10 to the 20 of pregnancy and their offspring studied. Offspring from undisturbed mothers were used as control. The elevated plus-maze test was used to investigate anxiety-related behaviour at 60 days old. The fertilizing capacity was tested at 90 days old and the testis histology studied. The offspring coming from LL or EE mothers were more anxious compared to control rats ( $p < 0.05$ ). In addition, 27.3 and 40% of males (LL and EE, respectively) were able to make pregnant a receptive female. No spermatogenesis changes were observed. In summary, these results indicate that prenatal exposure to LL or EE produce similar effects in the offspring. Those chronic adverse conditions would produce alterations on offspring behaviour on later life.

**23. EFFECT OF POST-WEANING DISTRESS ON IMMUNE PARAMETERS IN INTENSIVE SYSTEM RAISED SWINISH**

*Sacchetta R, García G, Mayer N, Gauna HF, Berardo D, Poloni L, Alustiza F, Alive T, Ashworth G, Greco C, Rodríguez N. Inmunología y Fisiología Animal. UNRC. Río Cuarto. Córdoba. E-mail: nmarioli@exa.unrc.edu.ar*

The stress factor affects the homeostasis of hypothalamic-pituitary-adrenal axis (HPA) in intensive systems raised pigs, which could induce long-term changes in offspring immune function. The objective of this work was to investigate the effect of weaning stress on lymphocyte proliferation at 14 days of age of vaccinated and control mothers. Hybrids Landrace x Large White raised under conventional management practices coming from 10 mothers (5 for litter) were used. The experiment was carried out in spring and summer stations. Blood samples were taken before and after weaning. Mononuclear cells were collected for use in lymphocyte proliferation assay stimulated with Concanavalin A (Con A). The data of offspring groups of vaccinated post-weaning (V-POST) and not vaccinated post-weaning (NV-POST) mothers were evaluated by analysis of variance (ANOVA). The offspring NV-POST group shows a significant decrease in the lymphocytes proliferation if we compare it with the V-POST group, in spring as in summer. In conclusion, the post-weaning stress diminished the cellular immunity functional response in spring and summer stations.

**24. EFFECT OF POSTNATAL STRESS IN PRENATALLY STRESSED RATS ON HYPOTHALAMIC-PITUITARY-GONADAL AXIS AND LYMPHOCYTES IMMUNE FUNCTION**

*Falcone E, Bertuzzi M, Ashworth G, Berardo D, Huck G, Poloni L, Rodríguez N, Bosch P, Gauna HF, Mayer N. Fisiología Animal. UNRC. Río Cuarto. Córdoba. E-mail: nmayer@exa.unrc.edu.ar*

Alterations induced by prenatal stress in hypothalamic-pituitary-adrenal axis (HPA) could change in long-term on hypothalamic-pituitary-gonadal axis (HPG) and in offspring immune function. The objective of this work was to investigate the effect of postnatal stress on luteinizing hormone (LH) and testosterone (TES) levels and cellular immune activity in adult rats prenatally stressed (EP). Males of three months of age were used, offspring of mothers stressed (EP) by immobilization (IMO) during pregnancy, and their control (CP). All groups were under acute postnatal IMO stress (20 minutes). Corticosterone (COR), LH and TES levels were determined before and after that stress were realized. Also the lymphocytes proliferation was studied. The TES and LH levels decreased in EP animals. This effect was negatively correlated with the COR levels. Postnatal stress diminished the lymphocytes proliferation *in vitro* in the animal EP. In conclusion, alterations induced by prenatal stress in HHA activity decreased HHG axis activity. Postnatal stress in EP animal diminished the cellular immunity functional response.

**25. PARTIAL CHARACTERIZATION OF CATHEPSIN D-LIKE PROTEASES DURING OVARIAN REGRESSION IN *Dipetalogaster maxima*, A VECTOR OF CHAGAS' DISEASE**  
*Fruttero L<sup>1</sup>, Carlini C<sup>2</sup>, Rubiolo E<sup>1</sup>, Canavoso L<sup>1</sup>*

<sup>1</sup>Dpto. de Bioq. Clínica, CIBICI-CONICET. Fac. Cs. Químicas, Univ. Nac. de Córdoba, Argentina. <sup>2</sup>Dpto. de Biofísica-Ctro. de Biotecnología. Univ. Federal do Rio Grande do Sul, Porto Alegre, Brasil.

Ovarian regression is a gradual process whereby insects and other oviparous animals regulate vitellogenesis according to several environmental and physiological factors. In this context, cathepsins, acid hydrolases of lysosomal origin, seems to be relevant to degrade vitellin stores. The purpose of this work was to analyze the activities of different cathepsin-like enzymes (EC 3.4.22-23) in regression ovaries of *Dipetalogaster maxima*. The enzymatic assays were carried out using ovarian homogenates and specific fluorogenic substrates for cathepsin B, D and L. The characterization of the involved enzymes was made using specific inhibitors and analyzing the degradation fragments by tandem mass spectrometry (LC-ESI-QTOF). The activity of cathepsin D-like proteases was confirmed by inhibition tests with pepstatin A and cleavage fragments analysis. The optimum pH for the activity was 3.5 and the estimated  $K_m$  was 24.56  $\mu$ M. These results contribute to our knowledge about physiological and biochemical events that regulate the ovarian regression in triatomines.

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**26. RENIN-ANGIOTENSIN SYSTEM (RAS) AND IMMOBILIZATION (IMO)-INDUCED HYPERTENSION IN OLD RATS**

*Binotti S, Boccolini A, Scoppa G, Torres C, Bensi N, Gauna H, Niebylski A.*  
*Fisiología Animal, Facultad de Ciencias Exactas Fco-Qcas y Naturales - UNRC, Córdoba. E-mail: sbinotti@exa.unrc.edu.ar*

Aging has been associated with increase of free radicals. Moreover, IMO stimulate RAS and this was associated with oxidative stress increment, which could be induce hypertension. RAS participation in the hypertension induced by IMO in aging rats was investigated. Oral Losartan (10mg/Kg) (L) or the solvent of the drug (s/L) was administrated to 8 months age male Wistar rats. One group was subjected to IMO 1h/day/14 days and another no stressed remained as control (C). At 1 (AS) and 14 (CS) days of IMO, systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure in response to stress and later of 6 h recuperation (R) were measured. In other group of rats plasma aldosterone (ALDO) and renal malonildialdehyde (MDA) were determined. SBP and MBP were transiently augmented in response to AS and CS, remaining high in CS rats in R. The augment of SBP and MBP in L with CS was lower than s/L and return at C values in R. Similar effects are observed in the DBP. ALDO increase in response to AS and CS in L and s/L rats, being lower this values in L. MDA increase in s/L and L rats with CS, being lower in L than s/L. The blockade of RAS mitigate the blood pressure, ALDO and MDA increase induced by IMO. The increase in the sodium renal excretion and the decrease of the free radicals formation could explain the minor increase of blood pressure in response to stress in aging rats.

**27. MECHANISMS INVOLVED IN ANTINATRIURESIS IN RATS SUBMITTED TO CHRONIC SOCIAL STRESS**

*Boccolini A, Binotti S, Hansen C\*, Torres C, Bensi N, Gauna H, Niebylski A.*

*Molecular Biology. UNRC. \*LACE lab.*

*E-mail: aniebylski@exa.unrc.edu.ar*

It has reported that social confrontations increase adrenaline, noradrenaline (NA) and corticosterone (CORT) levels besides other effects. Previously we have demonstrated a decrease of sodium renal excretion in subordinated rats. The objective was investigated the possible mechanisms involved in the antinatriuretic effect in rats submitted to chronic social stress (CSE). Wistar male rats were submitted to six sessions of CSE, following the resident-intruder paradigm. The experimental units were subordinated intruders.  $Na^+$ , creatinine (CREAT), NA and dopamine (DOPA) were measured in urine. Plasmatic levels of CORT, aldosterone (ALDO), CREAT and  $\gamma$ -glutamyl-transferasa-activity (GGT) were determined. The CREAT clearance (CREATCL) was calculated. Hepatic and renal malondialdehyde (MDA) concentrations were determined. CORT, ALDO, NA and DOPA were higher in stressed (E) animals. Antinatriuresis without changes in CREATCL was observed. The hepatic and renal levels of MDA and GGT activity were higher in E animals. The antinatriuresis not involucred changes in glomerular filtration rate and could be related to the increase in the sympathetic activity and ALDO secretion. In other side the increase in renal lipid peroxidation evidence an enlargement in reactive oxygen species (ROS). The ROS could decrease the biodisponibility of nitric oxide. This also may influence the  $Na^+$  renal tubular excretion.

**28. SOY BEAN OIL SUPPLEMENT IN A STRESS EXPERIMENTAL MODEL**

*Bianco M, Scoppa G, Bensi N, Niebylski A, Gauna H.*

*Fisiología Animal. FCEFYN. UNRC. Río Cuarto, Córdoba. E-mail: mbianco@exa.unrc.edu.ar*

Inadequate nourishing habits could be a risk factor of cardiovascular disease. The objective was to evaluate the soy bean oil supplement effect over lipidic profile, the osmotic fragility of erythrocytes and hemostasia in chronic stressed rats. Two groups of male Wistar rats were used, one with oil supplement for 4 weeks (OS) and the other without supplement (WS). Half of the animals of each group were subjected to immobilization (IMO) stress 2h/day/14 days and the other half remain as control rats (C). At the day 14, blood samples were obtained and activate partial thromboplastine time (APTT), coagulation time (CT), maximal (maxOF) and minimal (minOF) osmotic fragility of erythrocytes, fibrinogen (F) corticosterone (COR), glycemia (GL), total cholesterol (TC), triglycerides (TAG), HDL and LDL cholesterol levels were determined. COR and GL were higher in IMO rats. TC and LDL-cholesterol were higher only in WS stressed rats but TAG and HDL-cholesterol increased in both groups with IMO. Short CT and higher F levels were observed in all IMO rats, without changes in APTT levels. No changes in minOF with diminished maxOF were observed in oil IMO rats. The increase in HDL-cholesterol without changes in TC and LDL-cholesterol and the higher resistance of erythrocytes to an osmotic solutions show a protective effect of soy bean oil supplement in stressed animals. However, stress-induced hypercoagulability was no revert with soy bean oil supplement.



**29. LIPOPROTEINS AND OXIDATIVE STRESS BIOMARKERS IN MALE AND FEMALE RATS EXPOSED TO CHRONIC IMMOBILIZATION**

*Scoppa HG, Binotti S, Bianco M, Bensi N, García M, Niebylski A, Gauna HF.*

*Fisiología Animal, FCEFQyN, UNRC, Río Cuarto, Córdoba. E-mail: hscoppa@exa.unrc.edu.ar*

Plasmatic lipoproteins (PL), free radicals, vascular endothelium and subendothelium are vinculated to generate atherosclerosis pathology. In this work the plasmatic levels of PL and oxidative stress were evaluated in male (M) and female (F) rats stressed by chronic immobilization (IMO). Two groups of M and F Wistar rats were formed: controls (C) and stressed (S) by IMO 2h/day (three times a week) during 180 days. Blood and aortic samples were obtained. Plasmatic lipoproteins were separated by ultracentrifugation and the fractions obtained, Lp (a), apo B y MDA were measured. Aortic nitrotyrosin was determined. Triacylglycerides (TAG) and cholesterol (CHOL) were higher in SM than CM rats, in VLDL, LDL and HDL fractions. SF rats showed higher CHOL in VLDL and LDL and TAG in VLDL than CF rats. Otherwise, CHOL and TAG increase in lipoproteic fractions was minor in SF than SM rats. Apo B, Lp (a) y MDA levels were higher and aortic nitrotyrosin positive in S rats of two sex respect to the no stressed controls. Chronic IMO induced change in the plasmatic atherogenic pattern, in the lipoproteins and oxidative stress markers in both sex. The attenuated effects on F rats indicated the protector effects of ovarian steroids.

**30. ANTIOXIDANT EFFECTS OF VITAMIN E OVER THE ERYTHROCYTES AND COAGULATION IN CHRONIC STRESSED RATS**

*Torres C, Bianco M, Boccolini L, Binotti S, Bensi N, Gauna HF, Niebylski A.*

*Fisiología Animal, Dpto Biología Molecular, UNRC, Córdoba. E-mail: aniebylski@exa.unrc.edu.ar*

The increase in the free radicals has been associated to health disturbs as atherosclerosis. The inhibition of oxidative damage with the antioxidant administration prevents the lipid peroxidation of cellular membranes. Hence, Vit E supplementation could be an important therapeutic strategy. The objective was to evaluate the antioxidant effect of several doses of Vit E to stress-induced hemostatic and erythrocyte membrane stability changes. Male Wistar rats without supplement or with 6, 12, 50 or 100 UI of Vit E, via oral, for 4 weeks were used. Half animals of each grup (n=6) was subject to immobilization stress (IMO) 2 h/day/14 days and the remainder was the control rats. Blood and hepatic samples were obtained. IMO increase the hepatic malondialdehyde (MDA) and globular resistance. Moreover, the coagulation time (CT) was reduced. All Vit E doses reduced the MDA levels. A higher, doses dependent, coagulation time in rats with Vit E was observed. The dose of 100 UI increased the maximal globular resistance and decreased the minimal globular resistance. The lower lipid peroxidation and CT prolonged in rats with supplementation could be diminishing the thrombogenic risk, make evident the protective effect of Vit E. However, the effect of the higher dose over the erythrocyte fragility makes doubtful the application of this supplement for prolonged time.

**31. REDUCING CARBOHYDRATES AND ORGANIC ACIDS INDUCED BY NaCl AND Na<sub>2</sub>SO<sub>4</sub> IN A NATIVE HALOPHYTE**

*Llanes A, Palacio G, Luna V.*

*Lab. Fis. Vegetal, Fac Cs Exactas, UNRC, Río Cuarto, Córdoba. E-mail: allanes@exa.unrc.edu.ar*

Carbohydrates accumulation in plants undergoing salinity or drought has been widely reported despite the significant decrease in the net rate of CO<sub>2</sub> assimilation under these conditions (Murakeosy *et al.*, 2003). The aim of this study was to determine reducing sugars, sucrose and organic acids content in roots and leaves of *Prosopis strombulifera* seedlings hidroponically grown in Hoagland solutions with addition of 50 mmol/l NaCl for NaCl treatment, 38 mmol/l Na<sub>2</sub>SO<sub>4</sub> for Na<sub>2</sub>SO<sub>4</sub> treatment and the isoosmotic mixture of both salts (bisalinal treatment) every 48 h until final  $\Psi_o = -1, -1,88, \text{ and } -2,6$  MPa were reached. Control plants were grown in Hoagland 25%. Glucose and fructose content in roots and leaves were not affected by any treatment; roots showed highest levels of reducing carbohydrates at moderate and high salinity, possibly because of polyols formation and lignin synthesis. Sucrose decreased in sulphate treated roots showing the deleterious effect of this salt affecting transport and synthesis. High concentrations of citric and succinic acid in salinized roots could be related to an increased respiratory rate in these organs due to the demand for ATP and ion incorporation from the surrounding medium (osmorregulation). Highest content of malic acid in roots and leaves of treated seedlings, mainly under Na<sub>2</sub>SO<sub>4</sub> treatment, is not related to the possible induction of crassulacean acid metabolism in this species.

**32. FOLIAR AREA, STOMATAL CHARACTERISTICS AND WATER RELATIONS IN THE HALOPHYTE *Prosopis strombulifera* UNDER SALINITY**

*Reginato M, Llanes A, Reinoso H, Moschetti E, Luna V.*

*Fac. Cs. Exactas, UNRC, Río Cuarto, Córdoba. E-mail: mreginato@exa.unrc.edu.ar*

Plant leaves respond to stress modifying their foliar area, stomatal density and water relations. In this study foliar area, stomatal density (SD), epidermal cell density (ECD), stomatal index (SI), stomatal area, and water relations ( $\Psi_w$ ,  $\Psi_o$ , relative water content (RWC) and transpiration) were analyzed in seedlings growing hidroponically in Hoagland solutions with addition of 50 mmol/l NaCl for NaCl treatment, 38 mmol/l Na<sub>2</sub>SO<sub>4</sub> for Na<sub>2</sub>SO<sub>4</sub> treatment and the isoosmotic mixture of salts for bisalinal treatment every 48 h until final  $\Psi_o = -1, -1,88, \text{ and } -2,6$  MPa were reached. Control plants were grown in Hoagland 25%. Leaves of NaCl-treated plants showed a RWC higher than controls, with a decrease in SD (lower number of larger stomata). These characteristics maybe a mechanism of injury alleviation in response to excessive Na<sup>+</sup> accumulation in tissues, diminishing the transpiration rate and favoring higher water use efficiency. In the other hand, leaves of Na<sub>2</sub>SO<sub>4</sub> treated plants showed low values of  $\Psi_w$  and  $\Psi_o$  and the higher transpiration rate, which cause hydric disturbances in correlation with the decrease in total and specific foliar area and the increase in SD (highest number of smaller stomata) in these plants. Therefore, all these settings in the epidermal morphology of *P. strombulifera* under salinity are considered adaptive modifications specific to each salt and related to water use efficiency and photosynthesis control.



**33. SUNFLOWER NATIVE BACTERIA WITH ABILITIES TO PRODUCE PHYTOHORMONES *IN VITRO***

*Castillo P, Fantino A, Masciarelli O, Alemano S, Abdala G. Lab. de Fisiología Vegetal. Dpto. Cs Naturales. Fac. de Cs. Exactas, Físico-Químico y Naturales. UNRC. Río Cuarto, Córdoba. E-mail: paula\_v\_c@yahoo.com*

Numerous bacteria stimulate plant growth; they are named Plant Growth Promoting Rhizobacteria (PGPR), and one of their mechanisms involved in plant growth stimulation is phytohormones production. In this study we evaluated the *in vitro* production of indol acetic acid (AIA) and salicylic acid (SA) by different bacterial strains isolated from the sunflower rhizosphere. Strains *Achromobacter sp.* (SF2) and *Bacillus pumilus* (SF3 and SF4), were cultivated in GY medium for 48 h at 28°C under shaking. Hormones were extracted and purified following Durgbanshi *et al.* (2005) modified protocol, and identified and quantified by LC-ESI-MS/MS, equipped with double mass spectrometric and triple quadrupole. For quantification, deuterated internal standards ( $^2\text{H}_5$ -AIA and  $^2\text{H}_4$ -SA) were used. The three bacterial strains produced AIA and SA. SF4 showed the highest AIA production. This fact could contribute, at least in part, to the growth promotion of sunflower seedlings.

**34. EFFECT OF THE OIL OF NANDU ON THE CICATRIZATION IN SKIN OF EQUINE**

*Salvi M, Zubeldía D, Sereno M, Sereno J, Rotondo J, Cocco R, Bertone P. UNRC. Río Cuarto, Córdoba. E-mail: msalvi@ayv.unrc.edu.ar*

The aim of this work was to evaluate if the oil of nandu favors the cicatrization of the skin of the equine. The oil of nandu (AÑ) is obtained by warming and pressing of the fat. There were in use 3 horses. They were realized 2 wounds to every side of the neck of circular form and of 20mm of diameter and 5mm of depth, previous local anesthesia. It placed in one of the wounds, once a day and up to the total cicatrization of the physiologic solution, in another wound one proceeded to place AÑ. One came to the measurement and obtaining biopsy from the edges up to the cicatrization of the faults. The speed of centripetal contraction and the decrease of the volume was for the wounds treated with AÑ and the control of 10,33 mm / day and 3,10 mm / day and of 302,08 mm<sup>3</sup>/días and 78,51 mm<sup>3</sup>/días respectively. The wounds treated with AÑ to 4 days post surgery were appearing pink yellowish, dry and with granulation tissue, which was observed to 9 days in the controls. To 10 days were observed signs of chronic inflammation and to 21 days slight fibrosis reparative with the total cicatrization of the wounds treated with AÑ and in the test it happened to 26 days. It is possible to conclude that the oil of nandu accelerates the cicatrization process of the skin in equine.

**35. COMPARISON OF THE EFFECT OF THE OIL OF NANDU AND OIL WITH GARLIC ON THE HEALTH WOUND IN THE SKIN OF THE EQUINE**

*Zubeldía D, Salvi M, Sereno J, Rotondo J, Bertone P, Cocco R, Sereno M. UNRC, Río IV, Córdoba. E-mail: dzubeldia@ayv.unrc.edu.ar*

The aim of this work was to compare the healing effects of the oil of nandu (AÑ) and the oil with garlic (AA) on the skin of equine. The oil of nandu is obtained by warming and pressing of the fat and that oil with garlic by boiling 250 grs of garlic in 500 ml of sunflower oil. 3 horses of the FAV-UNRC were in use. They were realized 3 wounds to every side of the neck of 20 mm of diameter and 5 mm of depth, previous local anesthesia. There was placed in one of the wounds, once a day and up to the cicatrization, physiological solution, in another AÑ and in the third AA. Measurements of the wounds were realized and biopsies were obtained of the edges. The speed of centripetal contraction and the decrease of the volume was for the wounds treated with AÑ and AA of 2,05 mm / day and 2,33 mm / day and of 84,96 mm<sup>3</sup>/día and 98,62 mm<sup>3</sup>/día respectively. On the 3<sup>rd</sup> days the wounds treated with AÑ and AA they presented acute inflammation with ulceration and exuded purulent, from the 10<sup>th</sup> day they were showing signs of chronic inflammation and abundant presence of granulation tissue. To the moment of the cicatrization they demonstrated cicatrization tissue in ripeness. It is possible to conclude that the oil of nandu and the oil with garlic they behave of similar form in the cicatrization of the skin of the equine.

**36. TOOTH ENAMEL PATTERNS IN HUMAN PREMOLARS**

*Abal A, Durso G, Batista S, Tanevitch A, Anselmino C, Llompart G, Llompart J, Martinez C, Licata L. Facultad de Odontología. UNLP.*

Enamel type combinations called patterns have not yet been studied in human enamel. The aim of this work was to identify enamel patterns in premolars (Pm). Methodology: sections from 12 upper and 12 lower Pm crowns were obtained, embedded in epoxy resin, grinded, etched with acid, metallised and observed under a scanning electron microscope (SEM). Micrographs were identified in the free faces in the occlusal, medial and cervical thirds, as well as in the cusps in the inner and outer thirds. Results showed enamel with Hunter Schreger bands (inner) in the upper Pm free faces and radial enamel (outer) in the occlusal, medial and cervical thirds. In lower Pm, irregular enamel (inner) was found, whereas radial enamel (outer) was found in the occlusal, medial and cervical thirds. In Pm cusps, both upper and lower, the enamel pattern found was irregular enamel in the cusp inner third and radial enamel in the other two thirds. Differences were found between irregular enamel types present in the free faces and the cusps. In the free faces there are orientation changes of prisms that do not form bands and in the cusps there is a prism intercross compatible with the knot-like enamel shown by optical microscopy. We conclude that there exist several patterns in the same tooth group and that the combination of enamel types is a biomechanical adaptation.

**37. LOCALIZATION OF COLLAGEN THROUGH PICROSIRIUS RED IN PORCINE PLACENTA**

*Merkis C, Cristofolini A, Sanchis G, Chanique A, Moschetti E, Campos M, Taglialegna A, Alessio A, Allende F, Koncurat M. Area Microscopía Electrónica. FAyV. UNRC.*

During porcine gestation, the placenta carries out an essential role in implantation, materno-foetal recognition and maintenance of pregnancy. The collagen system, constituted by fibrillar proteins, is a component of the placental extracellular matrix. Our aim was to determine its localization in materno-embryonic tissue, since that will allow evaluating its contribution to the maintenance of placenta structural integrity. Placentas of  $\pm$  35, 60, 70, 80 and 114 days of gestation were processed. Picrosirius red and polarized light microscopy was used for localization of collagen fibers in the different gestational periods. The collagen fibers stained as green, yellow, orange and red, in sequence of increasing thickness, were expressed as semiquantitative, determining for each colour: (-): negative, (+): poor, (++): abundant and (+++): high expression. In porcine placental villi expression of collagen fibers was negative. As pregnancy progressed higher amount of heavy collagen fibers in connective tissue and myometrium was observed. In conclusion an increase of placental fibrousnesses was detected as pregnancy advanced, agreeing with the structural remodelling and physiological maturation that the placenta must undergo during the gestation to successfully accompany the embryonic-foetal development.

**38. PLACENTAL STRUCTURE AND ULTRASTRUCTURE IN ANGLO-NUBIAN GOATS. PRELIMINARY STUDY**

*Merkis C<sup>1</sup>, Turiello P<sup>2</sup>, Cristofolini A<sup>1</sup>, Sanchis G<sup>1</sup>, Chanique A<sup>1</sup>, Campos M<sup>1</sup>, Pastorino F<sup>3</sup>.*

<sup>1</sup>Area Microscopía Electrónica, <sup>2</sup>Cátedra Nutrición Animal, <sup>3</sup>Cátedra Embriología. FAyV. UNRC.

Maternal diet as well as nutritional transport through the placenta determines the growth and development of the *conceptus*, affecting the foetal weight and post-natal survival. In goat placentation the foetal cotyledons fusion with uterine caruncles giving rise to placentomes functional feto-maternal units. Our purpose was to study the structure and ultrastructure of placentae from Anglo-Nubian goats by means of optical and transmission electron microscopy. Young primiparous Anglo-Nubian goats (n=5) were subjected to a nutritional plan according to the physiological state of pregnancy. Gestation lasted on average 150 days. Obtained placentae were weighted ( $437 \text{ g} \pm 32,45 \text{ g}$ ) and the number of placentomes was determined ( $91,66 \pm 15,37$ ). In the analysed histological samples chorionic villi were observed, expressing numerous giant binucleate trophoblastic cells and a chorionic stroma highly vascularised. Ultrastructure examination allowed to detect binucleate foetal metabolically active cells showing numerous cytoplasmic organelles. Such cells were found immersed in cell debris due to the rupture suffered during delivery at the level of trophoblastic mononuclear cells, providing evidence of a contra-decidual condition. The present study will allow us to extent our knowledge about the morphological events taking place during the placentation of Anglo-Nubian goats subjected to a controlled diet.

**39. OSTEOPONTIN AND  $\alpha_v\beta_3$  INTEGRIN IMMUNOEXPRESSION IN PORCINE PLACENTAL TISSUES. PRELIMINAR STUDY**

*Sanchis G<sup>1,3</sup>, Williamson D<sup>2,3</sup>, Cristofolini A<sup>1,3</sup>, Merkis C<sup>1</sup>, Koncurat M<sup>1</sup>. <sup>1</sup>Área Microscopía Electrónica, FAyV, UNRC. <sup>2</sup>Dpto. Cs Básicas, FCV, UNLPam. <sup>3</sup>Becaria CONICET.*

The osteopontin is a glycoprotein present in the extracellular matrix of porcine placenta that binds integrins, as  $\alpha_v\beta_3$ , promoting cell-cell binding and communication, and changes in cytoeskeleton. The objective was to determine the expression of osteopontin and  $\alpha_v\beta_3$  integrin of porcine placental sections of early, intermediate and at term gestation. Detection was performed through immunohistochemistry. The results were expressed as semiquantitative, determining: (-): negative, (+): positive, (++): abundant and (+++): high marcation. Both proteins were detected in chorion and endometrium along pregnancy, making evident their role in cell adhesion. At Day  $\pm$ 30 both molecules were present in uterine and trophoblastic epithelial cells, implying their involvement in early pregnancy. As pregnancy progressed only  $\alpha_v\beta_3$  integrin was detected in both epithelia, suggesting the participation of other integrin receptors different from osteopontin involved in pregnancy maintenance. At Day  $\pm$ 30 osteopontin was expressed in glandular epithelium and lumen, indicating maternal contribution trough areolas in early periods of pregnancy to ensure materno-fetal contact. The present work would allow to improve our knowledge about certain mechanisms involved in the maintenance of gestation and placentation, indispensable for pregnancy to succeed.

**40. SEED SIZE OF PEANUT GENOTYPES**

*Girardi VN, Cerioni GA, Giayetto O, Fernandez EM. Facultad de Agronomía y Veterinaria. UNRC. Río Cuarto. Córdoba.*

Peanut breeding had produced lines and cultivars with high proportion of large seed (sieve (S) > 7,5 mm) in response to market request keeping unknown if the seed distribution in different size categories is similar between them. Size distribution and 100-seed weight of each size category were evaluated in 14 genotypes growing in non-limited conditions at CAMDOCEX of FAV-UNRC. Crop season spread from 26/10/06 to 20-25/04/07 and 782 mm of rain were registered together with maximum and minimum temperature between those adequate to crop growth. At harvest maturity, size distribution of each genotypes (seed retained in S with elongated holes of 10, 9, 8, 7,5, 7, 6,5 and 6 mm) and 100-seed weight of each size category were measured. Utre UNRC and Uchaima UNRC cultivars had the greater proportion of seed in S with hole of 10 and 9 mm and LF42 line the lowest. The genotypes were different in 100-seed weight of each size categories, being Utre UNRC cultivar which achieved the bigger values in most of them. Highest differences in this characteristic were registered in S with holes of 8 and 9 mm. These results verify peanut genotypic variability in seed size distribution and 100-seed weight of each size categories.

**41. RHIZOBACTERIA RESPONSE TO PEANUT RHIZODEPOSITION**

*Dardanelli MS, Albornoz A, Medeot D, Paulucci N, Fumero V, Bueno M, García M.*

*Departamento de Biología Molecular, FCEFQyN, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba.*

*E-mail mdardanelli@exa.unrc.edu.ar*

Bacteria able to colonize plant root systems are referred to as rhizobacteria and include plant growth promoting rhizobacteria and the N<sub>2</sub>-fixing rhizobio.

Most rhizobacteria living in the rhizosphere and can be defined as any volume of soil specifically influenced by plant roots and/or in association with roots and hairs, and plant-produced material. Plant root-soil-microbe interactions are an important aspect of plant nutrient uptake strategies.

The object of the present work was to contribute with the knowledge of physiological and biochemical response of rhizobacteria to peanuts under peanut rhizodeposition, in the presence and absence of saline stress (NaCl 50 mM). Rhizobacteria strains used were *Azospirillum brasilense* Cd and *Bradyrhizobium* SEMIA6144 and TAL1000. Viability, biomass, proteins, oligosaccharides profile and cellular trehalose were measured. Lipids were obtained and phospholipids were identified by TLC. Results indicated differences in rhizobacteria growth and profile proteins for the different experimental conditions. On the other hand, oligosaccharides periplasmic and trehalose were affected under saline peanut rhizodeposition and lipids pattern showed were similar. Results suggest that oligosaccharides and protein may be implicated in response to peanut rhizodeposition.

**42. EFFECT OF SALINITY AND GROWTH TEMPERATURE ON PHOSPHOLIPIDS AND FATTY ACIDS BIOSYNTHESIS OF BRADYRHIZOBIUM TAL 1000, ARACHIS HYPOGAEA (PEANUT) NODULANT**

*Paulucci NS, Medeot DB, Dardanelli MS, García MB.*

*Dpto. Biología Molecular. FCEF-QyN, UNRC, Argentina. E-mail: npaulucci@exa.unrc.edu.ar*

Many organisms alter the composition of their membrane lipids and fatty acids (AG) in response to environmental changes, to maintain the membrane fluidity at constant values, (Soltani *et al.*, 2005; Donato *et al.*, 2000). However there have been only a few studies on lipid biosynthesis. In this study we analyze the effect of salinity and growth temperature on the biosynthesis of phospholipids (PL) and (AG) in B. TAL 1000. B TAL 1000 was grown in B medium for 24-h at 28°C or 37°C with the addition of 300 mM NaCl. [1-<sup>14</sup>C] sodium acetate (0.5 µCi) was added at the time of inoculation. Lipids were extracted (Bligh and Dyer, 1959), the PL was separated by TLC and AG was separated according to insaturation degree by AgNO<sub>3</sub> TLC. The radioactivity of each fraction was then quantified. We observed an increase of 22% in phosphatidylcholine and a decrease of 30% in phosphatidylethanolamine under the combination of both stresses. The incorporation of radioactivity in the AG was modified by the stress. The ratio U/S changed from 11.5 to 3.5 for salt stress, 11.5 to 2.5 for temperature stress and from 11.5 to 1.6 for the combination of both. These results suggest that in B TAL 1000, the AG and PL biosynthesis is modified to counteract the effects that could cause stress in the plasmatic membrane.

**43. PATH COEFFICIENT ANALYSIS FOR DRY MATTER PRODUCTION IN WEEPING LOVEGRASS**

*Dallo M, Ibáñez M, Bonamico N, Borghi L, Di Renzo M.*

*FAV, UNRC. 5800 Río Cuarto, Córdoba.*

The importance of weeping lovegrass (*Eragrostis curvula*) in Argentina relies on its value as forage, adaptability to semiarid conditions and contribution to soil conservation. The direction and magnitude of the associations between a dependent variable and a series of independent variables, as well as the relative importance of each of them on a particular trait determine the efficiency of an improvement program. The objectives of the study were: i) to estimate genotypic and phenotypic correlation coefficients among dry matter (MS) and crown diameter (DI), plant height (HPI), panicle height (HP) and panicle number (NP) and ii) to apply path coefficient analysis to determine how these variables affect MS production. Eighteen weeping lovegrass hybrids were evaluated during an agricultural cycle and conducted in a RBD with three replicates in Río Cuarto. The path coefficient analysis was applied to separate the total correlation into direct and indirect effects and to make a cause-effect model. Due to the fact that the environment does not influence at a genetic level, the genotypic correlations turned out to be higher than the phenotypic ones. The path coefficient analysis showed that DI had the highest direct positive effect on MS either in genotypic or phenotypic levels. In addition, the highest indirect effects were observed for most traits via NP at a genotypic level and HP at a phenotypic level. The trait DI is useful as indirect selection criterion to improve dry matter production in weeping lovegrass.

**44. MAL DE RÍO CUARTO DISEASE IN MAIZE: INHERITANCE OF RESISTANCE**

*Borghi L, Ibáñez M, Bonamico N, Dallo M, Di Renzo M.*

*Facultad de Agronomía y Veterinaria. UNRC. Río Cuarto. Córdoba. E-mail: letibor@hotmail.com*

The genetic resistance may reduce the damages of 'Mal de Río Cuarto' (MRC) disease in maize, which causes great economic losses. The goal of this study was to estimate the heritabilities of severity grade, severity and incidence to MRC disease. One hundred and eighty recombinant inbred lines (RIL) originated from a cross between a resistant line and a susceptible line, were tested in six field environments. Each year-location combination was treated as an environment. The experimental design was a randomized complete block design with two replicates. A large range in disease reaction was observed and within each environment the heritability was high, however the across environmental analysis showed significant genotype -environment interaction and consequently low heritability. Some RILs showed greater disease than susceptible parent, therefore there is a transgressive segregation to MRC reaction. Knowledge of the inheritance of resistance to MRC disease could contribute to design efficient strategies for the improvement of MRC resistance.

**45. PATH COEFFICIENT ANALYSIS FOR TOLERANCE TO “MAL DE RÍO CUARTO” DISEASE IN MAIZE**

*Dallo M, Ibáñez M, Bonamico N, Borghi L, Di Renzo M. FAV, UNRC. 5800 Río Cuarto, Córdoba.*

Productivity of maize is affected by several diseases. The “Mal de Río Cuarto” (MRC) is the most important viral disease in Argentina. The severity of MRC symptomatology depends of the plant phenological state when the virus is acquired, the genotype and the environmental conditions in which the culture develops. The objective of this study was to interpret genotypic and phenotypic correlation coefficients between disease severity degree and disease related traits. A recombinant inbred line population derived from a cross between a tolerant line and a susceptible line was evaluated in four environments in an endemic disease area. The path analysis was applied to separate the total correlation coefficient into direct and indirect effects. Generally, genotypic correlations were higher than phenotypic ones showing that the environment effect does not influence trait associations at genetic level. The path analysis showed that enations and internodes had the highest direct positive effects on MRC severity degree. The highest indirect effects were observed for most traits via enations and internodes. The relationship between disease severity degree and disease related traits were consistent across the environments.

**46. ESSENTIAL OIL COMPOSITION AND TOTAL PHENOLIC CONTENTS OF OREGANO FROM ARGENTINA**

*Dambolena J, Zunino MP, Lucini EI, Olmedo R, Banchio E, Bima P, Zygodlo JA. Cátedra de Química Orgánica - UNC. FCEFYN. IMBIV-CONICET-ICTA.*

Oregano plays a primary role among temperate culinary herbs in world trade. Of the species commercially known as oregano, most of the production comes from species of the genus *Origanum*. *Origanum vulgare* L. is the most variable species of the genus and the only one commonly known as ‘oregano’ in most countries. Six subspecies have been recognised within *O. vulgare* based on the basis of morphological and chemical characters. The commercial oregano of Argentina comes from *O. vulgare* ssp. *vulgare*, *O. vulgare* ssp. *virens*, *O. x applii* and *O. x majoricum*. There is no report on the yield and composition of the essential oil (EO) and total phenol contents (TPC) of these cultivated populations in literature. Our objective was to analysis the EO, TPC and radical-scavenging properties from 3 populations of *Origanum* from Argentina. Results and discussion. The main components of EO were trans sabinene hydrate (>27.7%) and thymol (>17.7%). The main sesquiterpenes were beta caryophyllene and germacrene D. TCP of the samples ranged from 353.6 to 774.3 µg/ml. In conclusion, the results of this study demonstrated that the different *Origanum* cultivated in Argentina contained different levels of TPC and antioxidant properties by environmental conditions. The EOs of oregano species studied in this work showed variability in the relative percentage of the two main components, while no geographical pattern could be observed.

**47. EFFECT OF QUAIL FEED SUPPLEMENTATION WITH ESSENTIAL OIL COMPONENTS ON FLY PRODUCTION FROM POULTRY MANURE**

*Lynch Ianniello J<sup>1,2</sup>, Labaque C<sup>2</sup>, Battan M<sup>3</sup>, Marin RH<sup>2</sup>, Gleiser RM<sup>1</sup>.*

*<sup>1</sup>CREAN FCA, <sup>2</sup>ICTA and <sup>3</sup>Diversidad Animal I FCEFYN, UNC. E-mail: rgleiser@crean.agro.uncor.edu*

Functional foods are products that provide a health benefit beyond basic nutrition. Several essential oils (EO) have bioactivities on animal physiology and metabolism (antioxidants, anticholesterolemics), are antimicrobials, insecticides, among other. The aim of this research was to evaluate if the modification of quail diet (supplementing it with EO thymol and isoeugenol) could have an effect on another sanitary and economic poultry related problem: the production of flies in poultry litter. Samples of manure deposited in the previous 0 to 48 hs by quail that were fed with a supplementation of 400 mg thymol, isoeugenol or butylated hydroxytoluene (control) per kg of feed were collected. Each 200g sample (6 replicates per treatment) was incubated in a glass container inside an emergence cage. Cages were inspected daily and adult flies that emerged were collected for 40 days. The effect of quail feed supplementation on the total number of emerging flies and on the number of specimens from the 3 most frequent species (*Ophyra aenescens*, *Muscina stabulans* and *Musca domestica*) were assessed. Significantly less fly emerged from manure from the isoeugenol treatment, suggesting that the modification of the quail diet with this component may have a moderate insecticidal effect against these insects.

**48. Azospirillum brasilense SP 245 INCREASE GROWTH AND ALLEVIATE WATER STRESS IN Arabidopsis thaliana BY ABSCISIC ACID PRODUCTION**

*Cohen AC, Moreno D, Dominguez JE, Lopresti N, Bottini R, Piccoli PN.*

*Cátedra de Qca. Org y Biol, Fac de Ciencias Agrarias-CONICET, Universidad Nacional de Cuyo, Argentina. E-mail: acohen@fca.uncu.edu.ar*

*Azospirillum brasilense* is a plant growth promoter bacteria (PGPB) that increase grain yield in cereals and plant growth in different species. Its presence may also alleviate plant water stress. Among the mechanisms proposed to explain these beneficial effects is the production of phytohormones by the bacteria, including indole-3-acetic acid (IAA), gibberellins (GAs) and abscisic acid (ABA). The aim of this work was to evaluate different mechanisms involved in *A. thaliana* plants inoculated or not with *A. brasilense* in both drought stress and well watered conditions. *A. thaliana* was cultivated in plastic pots with sand:vermiculite (1:1) at 24 °C with 16 h light and 8 h dark.

The well watered plants inoculated with *A. brasilense* anticipated the phenological stages respect non inoculated plants, with a significant increase in rosette growth and rod length. Interestingly, *A. brasilense* inoculation enhanced percentage of survival in well watered plant as well as drought stress and ABA content in leaves measured by GC-MS. The results obtained suggested that *A. brasilense* promote growth and alleviate of water stress in *A. thaliana* plant by ABA production.



49.

**IN VIVO EFFECTS OF FRACTIONS OBTAINED FROM *Larrea divaricata* ON MACROPHAGES***Martino R, Davicino RC, Casali YA, Mattar MA, Micalizzi B. Cát. de Inmunología. Control de Calidad. UNSL.*

*Larrea divaricata* Cav is a plant known in Argentina for its use on folk medicine to treat different diseases. The aim of this work was to evaluate *in vivo* effect of three fractions (F1, F2 and F3) obtained from an aqueous extract on murine macrophages. Mice were injected intraperitoneally twice in a 48 hs period with 0,5; 5 and 15 mg/kg of each fraction. Peritoneal cells were obtained 96 hs after first injection and were harvested during 2 hs at 37°C. The following determinations were carried out: 1) cellular viability by MTT test; 2) phagocytosis by NBT reductions and 3) apoptosis by: a) Giemsa staining and b) ethidium bromide/ acridine orange staining. The results showed that a significant increase ( $p < 0.05$ ) of the cellular viability occur with F2 (15 mg/kg) compared with control. A no significant difference was observed with the other fractions. A significant increase ( $p < 0.05$ ) of the phagocytosis with F2 (15 mg/kg) and F3 (5 and 15mg/kg) was observed. A significant increase of the apoptosis ( $p < 0.05$ ) was observed when Giemsa was used, with F1 (15mg/kg), F2 (0.5mg/kg) and F3 (15mg/kg). In conclusion, F2 at 15 mg/kg showed a cellular viability and phagocytosis increased, and an anti-apoptotic effect too, as well as F3 increase la NBT reduction at high concentrations, possibly for its NDGA content. No effects were observed with F1 *in vivo* on macrophages, only an anti-apoptotic activity.

50.

**CHARACTERIZATION OF HUMORAL IMMUNE RESPONSE IN NATURALLY INFECTED CALVES WITH BOVINE RESPIRATORY SYNCYTIAL VIRUS***Raviolo J<sup>1</sup>, Zielinski G<sup>2</sup>, Giraud J<sup>1</sup>, Lovera H<sup>1</sup>, Navarro F<sup>1</sup>, Trotti N<sup>1</sup>, Mouguelar H<sup>1</sup>, Raviolo JE<sup>1</sup>, Aguilar J<sup>1</sup>.**<sup>1</sup>Facultad de Agronomía y Veterinaria. UNRC. <sup>2</sup>INTA. EEA, Marcos Juárez. E-mail: jraviolo@ayv.unrc.edu.ar*

Humoral and cellular immunity against Bovine Respiratory Syncytial Virus (BRSV) has a major role in prevention and establishment of infection. The aim of this study was to characterize the humoral response induced by BRSV in naturally infected calves. The immune response was studied by serum neutralization (SNV) and immunowestern blot (IWB) using serum from 4 calves (8 months of age) from the same farm where the RC-98 viral strain had been isolated. Titers of neutralizing antibodies were determined in the four sera (1/64, 1/64, 1/128 and 1/256). Major viral surface glycoproteins Gp G of 84kDa, F0 of 77kDa, F1 of 46 and F2 of 23kDa which induced neutralizing antibody were detected by IWB in the same sera. Nucleocapsid protein N of 43 kDa, P of 34 kDa and L of 200 kDa and matrix proteins M of 29kDa were also identified. These findings show that the natural infection stimulates the production of neutralizing antibodies in animals exposed to the virus, which is of vital importance in preventing the disease. Glycoproteins G and F of the viral envelope, the M protein associated with the matrix and nucleocapsid proteins N, P and L stimulated a humoral immune response in naturally infected cattle.

51.

**BIODISTRIBUTION AND ACUTE TOXICITY EVALUATION OF NATURAL ANTRAQUINONES IN VIVO***Comini L<sup>1</sup>, Fernandez P, Nuñez S<sup>1</sup>, Cabrera J<sup>1</sup>, Rivarola V<sup>2</sup>.**<sup>1</sup>Fcognosia, FCQ-UNC IMBIV-CONICET. Cba., Arg. <sup>2</sup>Dept. Biol. Mol. Fac. Cs. Exs. FQca and Nat. UNRC. River IV, Arg.*

Photodynamic therapy (PDT) is based on the toxicity induced in tumor tissue for combined action of a photosensitizer and light. Rubiadin (**1**), anthraquinone isolated from *Heterophyllaea pustulata*, exhibit photosensitizing properties, which suggested it could be considered a drug for PDT. Therefore, we evaluated the photodynamic activity of **1** *in vitro* on a human carcinoma cell line, obtaining promising results, which motivate to achieved studies *in vivo* (mice). This work intends to determine the biodistribution of **1** and evaluate their acute toxicity in possible target tissue, with the aim to determine the non-toxic dose that will be used in phototherapeutic studies. The biobdistribution was studied by means of a quali-quantitative HPLC analysis from liver, kidney, spleen, lung, muscle, intestines and skin tissue, extracted from BALB/c mice at 24-48 hs and 7 days after intraperitoneal (i.p.) administration of **1** (80 mg/kg). Acute toxicity was established by a histopathological analysis of the same tissue, after i.p. injection of **1** (20, 40, 80 mg/kg) at the times above mentioned. Results showed that levels of **1** are important at 24 hs in liver, kidney, skin, muscle and spleen, declining to 48 hs and 7 days. However, no alterations are observed in the analyzed tissues at doses and times tested. Therefore, **1** would fulfill with the primary requirements for future phototherapeutic studies.

52.

**EFFECTS OF PERINATAL EXPOSURE TO DIETS WITH DIFFERENT n6:n3 RATIOS ON MICE NEUROBIOLOGIC DEVELOPMENT***Santillán ME, Vincenti LM, Martini AC, Fiol de Cuneo MH, Ruiz RD, Stutz G.**Instituto de Fisiología Humana. FAC. CS. MÉDICAS, UNC.*

*Introduction:* n-6 and n-3 polyunsaturated fatty acids (PUFA) play an important role on early development of pups.

*Objective:* to investigate in mice the effects of diets enriched with soy (low n-6:n-3 ratio) or sunflower oil (high n-6:n-3 ratio), upon physical maturation and neurobiological development of the pups.

*Methods:* Dams were assigned, throughout gestation and lactation, to different groups: commercial diet (C), soy oil (S) or sunflower oil (SF) enriched diets. Measurements in the offspring: physical parameters (body weight, length and body mass index, fur appearance, pinna detachment, incisor eruption, eye opening and puberty onset); behavioral pre weaning tests (surface righting reflex, negative geotaxis and cliff avoidance). Physical (PS) and neurobiological scores were calculated with the previous data.

*Results:* S and SF pups became shorter than C ( $p < 0.05$ ) at weaning. PS in S and SF was higher than C and simple reflexes occurred earlier in both oil enriched diets. Male and female puberty onset was delayed in SF.

*Conclusion:* the maintenance of an adequate n-6:n-3 ratio is necessary for the optimal growth and development of murine offspring. Consumption of PUFA would be advisable during gestation and lactation in populations which do not have sufficient provision of PUFA in the diet.

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**53. EFFECTS OF ENERGY RESTRICTION ON METABOLIC PROFILE AND BODY MEASUREMENTS IN PREPUBERAL DOES**

Forchetti O, Cufre G, Turiello P, Coniglio V, Godio L.  
Fac de Agronomía y Veterinaria UNRC.

Ruminants have the ability to cope with periods of food shortage. Of particular interest is to understand undernutrition physiology under long-term feed restriction, to determine the limits of metabolic adaptation. Twelve prepuberal does were randomly assigned to two groups: fed ad libitum (C) or restricted 50% (R) during 250 days. Feed was offered in individual cages. Diets were formulated to contain 2.3 Mcal/kg DM and 15% CP. Restriction decreased gain and body measurements in R does compared with C. Liveweight at the end of the experiment were  $29 \pm 1.7$  y  $15 \pm 1.0$  for C and R, respectively. Serum non-esterified fatty acids concentration markedly increased following intake reduction in restricted does, decreasing to basal levels after a period of 30 days, demonstrating the adaptation of does to under nutrition. Glucose concentration was always within the range of reference value, although it was higher in C. Restriction depressed serum alkaline phosphatase (ALP), concentration increased gradually and exhibited a 60 days delayed lower peak, compared to control does. At the end of the study IGF-I levels, were depressed the most showing a 80% of reduction in concentration values. It is possible to conclude that under a long-term feed restriction in growing animals, ALP and IGF-I are accurate indicators that explain restricted growth in underfed goats.

**54. HISTO-MORPHOLOGICAL AND ULTRA-STRUCTURAL CHANGES AND CELL DEATH DURING FOLLICULAR REGRESSION OF *Dipetalogaster maxima***

Aguirre S<sup>1</sup>, Pons P<sup>2</sup>, Frede S<sup>1</sup>, Fruttero L<sup>1</sup>, Rubiolo E<sup>1</sup>, Canavoso L<sup>1</sup>.  
<sup>1</sup>Dpto de Bioquímica Clínica, CIBICI-CONICET, Fac. Cs. Químicas; <sup>2</sup>Centro de Microscopía Electrónica, Fac. Cs. Médicas. UNC, Córdoba, Argentina.

In hematophagous insects, a poor nutritional status of female induce the decrease of vitellogenin (Vg) synthesis and uptake, and a cellular remodelling of ovaries. In this process, known as follicular regression, atrophy of follicles and resorption of oocytes also occurred. The aim of this work was to analyze the morphological and ultra-structural changes as well as cell death events occurring during early and late regression of ovarian tissue of the hematophagous *D. maxima*, a vector of Chagas' disease. The approach included fluorescent and electron microscopy, immuno-histochemistry, TUNEL, *in vivo* and *in vitro* assays with vitellin (Vt) and monodansylcadaverine (MDC). In vitellogenesis, ovaries exhibited a typical asynchronous development and showed a differential storage of Vt. During regression, Vg uptake decreased and follicles were of small size. In early regression, follicular cells showed nuclei with an apoptotic pattern. By contrast, late regression displayed cytoplasm vacuolization of nurse cells, phagocytic activity and cell death by apoptosis, necrosis and autophagy. We concluded that in *D. maxima*, follicular regression is physiological and gradual to promote oocyte resorption and distribution of energy resources to sustain the development of younger follicles.

**55. PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF VITELLOGENESIS AND FOLLICULAR REGRESSION IN VECTORS OF CHAGAS' DISEASE**

Leyria J, Aguirre S, Fruttero L, Rubiolo ER, Canavoso L.  
Dpto. Bioquímica Clínica, CIBICI-CONICET. Fac. Cs. Químicas, UNC. Córdoba, Argentina, CP 5000.

In the hematophagous insects, reproduction is strongly associated with the nutritional status of females. To ensure an oviposition cycle, these species need a physiological compromise between the process of vitellogenesis and follicular regression. In Chagas' disease vectors, these events have been scarcely explored. Therefore, we have analyzed the main biochemical changes occurring during the progress of vitellogenesis (post-blood feeding) and follicular regression (days 15 and 30 post-vitellogenesis) in *Dipetalogaster maxima*, a vector of Chagas' disease. The study included: (a) measurement of vitellogenin (Vg) and vitellin (Vt) in hemolymph and ovary; (b) activity of acid phosphatase (PA), total cathepsins and cathepsin D in ovary; (c) lipids (LT) y carbohydrates (CH) in ovarian tissues. Results showed that transition to follicular regression was characterized by a significant decrease of circulating Vg and its storage in ovary. During early regression, there was a gradual activation of PA and cathepsin D, an aspartic protease involved in Vt degradation. In advanced regression, stores of LT and CH were very small. These findings evidenced that ovarian tissue adjusted its metabolic status along the regression period. Its exploration will be useful to improve our understanding of biology of reproduction of Chagas' disease vectors and to define strategies for their control.

**56. ALTERNATIVE METABOLIC PATHWAYS OF LIPOPHORIN INTERACTION AND UPTAKE IN DIFFERENT ORGANS OF *Panstrongylus megistus***

Fruttero L<sup>1</sup>, Stariolo R<sup>2</sup>, Rubiolo E<sup>1</sup>, Canavoso L<sup>1</sup>.  
<sup>1</sup>Dpto. de Bioq. Clínica, CIBICI-CONICET. Facultad de Cs. Químicas, Univ. Nacional de Córdoba and <sup>2</sup>Coord. Nac. de Control de Vectores, Ministerio de Salud. Córdoba, Argentina.

In insects, lipid transport is mediated by lipophorin (Lp), the main hemolymphatic lipoprotein. Lp acts as a "reusable shuttle", carrying several lipids between target tissues without being internalized and degraded. The aim of our work was to analyze the interaction between Lp-midgut and Lp-ovary in *Panstrongylus megistus*, an important vector of Chagas' disease. The biochemical analysis included binding assays with <sup>125</sup>I-Lp and ligand blotting experiments. In addition, cellular approaches were carried out by immunofluorescence and *in vivo* assays using fluorescent probes (Lp-Oregon Green). The results showed that Lp-midgut interaction was specific, displaying a high affinity constant ( $K_d = 5,1 \times 10^8$  M). Ligand blotting revealed that two midgut membrane proteins (61 and 45 kDa) were involved in such interaction. In this tissue, Lp interacted reversibly with the sub-epithelial layer. In contrast, the studies in ovary using confocal laser microscopy evidenced internalization of Lp by endocytosis. This is the first report in triatomines that provides evidence of alternative lipid transport pathways for different tissues.

57.

**CONTRIBUTION OF TOXINS ISOLATED FROM *Bothrops alternatus* (YARARÁ DE LA CRUZ) VENOM ON THE MYOTOXICITY OF C2C12 CELL LINE**García Denegri ME<sup>1</sup>, Bustillo S<sup>1</sup>, Gay C<sup>1</sup>, Gorodner JO<sup>1</sup>, Ponce-Soto LA<sup>2</sup>, Leiva L<sup>1</sup>, Acosta O<sup>1</sup>.<sup>1</sup>Universidad Nacional del Nordeste. Corrientes. Argentina.<sup>2</sup>Universidad Estadual de Campinas. San Pablo. Brasil.

Previous *in vivo* and *in vitro* assays demonstrated the myotoxic activity from *B. alternatus* crude venom. The aim of this work was to evaluate the contribution of a phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and a metalloproteinase (baltergine) to the *in vitro* myotoxicity in murine myoblast cell culture. Both toxins were purified from *B. alternatus* venom. Then, the cytotoxic activity displayed by each enzyme and also of the whole venom pre-incubated with anti-baltergine (a-balt) in different dilutions was studied. Cells (into DMEM - 5% of SFB - 5% CO<sub>2</sub>, 37°C) were treated with different concentrations of the isolated enzymes (up to 200 µg/ml) and crude venom (200 µg/ml) pre-incubated with different amounts of a-balt. The cell viability was measured, after 3 h of exposure, by colorimetric method (crystal violet staining). Both the PLA<sub>2</sub> and the venom pre-incubated with a-balt do not show myotoxic activity, whereas baltergine reduced the cell viability by about 30 %. Due to the exposure of cells to the whole venom (100 µg/mL) produced 100% cytotoxicity and the a-balt antibodies neutralized completely the myotoxic activity showed by the venom, we can conclude that the metalloproteinase is capable, by itself, of damage on muscular fibers. In spite of the lack of *in vitro* myotoxic activity by PLA<sub>2</sub>, a synergic action of both enzymes could develop an acute muscle lesion characteristic for *B. alternatus* intoxications.

58.

***Pseudomonas* ADAPTATION TO DIFFERENT ECOLOGICAL NICHEs: Phosphorylcholine phosphatase approach**

Otero L, Oyola M, Kilmurray C, Sanchez D, Beassoni P.

Dpto. Biología Molecular; UNRC, Río Cuarto, Córdoba. E-mail: l\_otero@exa.unrc.edu.ar

On the basis of 16s RNA, *Pseudomonas* genus belongs to group I, in the  $\gamma$ -branch. This group includes mammal's pathogens (ie. *P. aeruginosa*), plant's pathogens (ie. *P. syringae*) and non-pathogens (ie. *P. fluorescens*, *P. putida*). When *P. aeruginosa* grows in choline-containing media, synthesizes phospholipase C (PlcH) and phosphorylcholine phosphatase (PchP), two enzymes involved in the pathogenesis of this bacterium. The absence of PlcH in *P. syringae*, *P. fluorescens* and *P. putida* led us to focus in PchP properties. Motifs I and III of PchP are identical in the four species analyzed. But in motif II, PchP of *P. aeruginosa* and *P. putida* have a serine residue while *P. fluorescens* and *P. syringae* have a threonine residue in the same position. All these enzymes have a K<sub>M</sub> value for *p*-NPP in the same range. Nevertheless, with Zn<sup>2+</sup> or Mg<sup>2+</sup> as cofactors, the affinity was similar but with Cu<sup>2+</sup>, it was ~ 2.5 times lower for PchP of *P. syringae* and *P. fluorescens*, and ~ 4 times lower in *P. aeruginosa* and *P. putida*. All the enzymes showed preference for these ions in the order Zn<sup>2+</sup> > Cu<sup>2+</sup> > Mg<sup>2+</sup>. On the other hand, in *P. aeruginosa* y *P. putida*, PchP showed a higher K<sub>A</sub> for the three cations with respect to *P. syringae* y *P. fluorescens* enzymes. For this, the serine/threonine residue in motif II might be one of the factors involved in the affinity of PchP for Zn<sup>2+</sup>, Cu<sup>2+</sup> and Mg<sup>2+</sup>. This change seems to be an indicator of PchP function in bacteria colonizing different ecological niches.

59.

**HYDROLYSIS OF POLYPHOSPHATES IN *Pseudomonas aeruginosa* PAO1: MORE THAN ONE ENZYME RESPONSIBLE**

Vicario J, Gallarato L, Serra AL, Beassoni P, Garrido M.

Dpto. de Biología Molecular, FCEFQyN, UNRC, Río Cuarto, Córdoba. E-mail: juliovicario@gmail.com

In bacteria, inorganic polyphosphates (PPs) are involved in various physiological functions and in virulence of pathogens organisms such as *P. aeruginosa*. In our laboratory we are studying the *P. aeruginosa* PAO1 exopolyphosphatase (PPX) encoded by the gene PA5241. The aim of this study was to obtain a mutant strain unable to express the *ppx* gene to investigate the possible existence of more than activity responsible for the hydrolysis of PPs.

A deletional mutant strain was constructed according to Choi and Schweizer (Microbiol. 2005, 5: 1-11). Hydrolysis of PPs was measured by Pi release from substrates PP10, PP25 and PP65. The measurement of PPX activity in cytoplasmic extracts from stationary phase cells showed a specific activity decrease of only 30-35% for the mutant strain compared with that of the wild type (WT). Isoelectric focusing studies performed with extracts of WT and mutant strain showed three and two PPX activity areas respectively. The missing zone corresponded with PPX (pI 6.25). Enzymatic activities differing from PPX showed pI of approximately 7.1 and 5.2. Bioinformatics background suggests that the last protein could correspond to the survival protein SurE (PA3625).

60.

**OSMOTIC AND SALINE STRESS EFFECT ON LIPID METABOLISM AND ACCUMULATION OF PROLINE IN COLEOPTILE AND ROOTS OF BARLEY (*Hordeum vulgare*)**

Usorach J, Meringer V, Machado E, Racagni G.

Depto. de Biología Molecular. FCEFQyN. U.N.R.C. E-mail: javierusorach14051985@hotmail.com

Developing seedlings are subject to adverse environmental conditions. The salt stress is one of the most important abiotic factors that limit the normal development and activate a variety of signaling pathways in different organs of the plant. In response to salinity and drought stress, plants accumulate proline mainly. It was raised as to determine the biochemical and morphological changes at the level of lipid signals and proline accumulation in early germination of seeds under conditions of osmotic and salt stress. Morphological changes were observed in seeds germinated 4 days in sterile conditions and in darkness with different concentrations of NaCl and mannitol. We assessed lipid kinase activity in coleoptile and roots. Phospholipids identified were phosphatidylinositol 4.5 bisfosfato (PIP<sub>2</sub>), phosphatidylinositol 4-phosphate (PIP), diacylglycerol pyrophosphate (DGPP), lisofosfatidic acid (LPA) and phosphatidic acid (PA). Both types of stress caused reduction in seedling growth and increases in proline accumulation and PA levels in roots. Moreover, the osmotic stress also induces an increment in DGPP levels. In summary, the root as the first organ that senses the stimulus triggers the response, modulating lipid metabolism and levels of proline, and then sends the signal to the rest of the plant and enables it to respond appropriately.



**61. ABSICISIC ACID SIGNAL IN BARLEY ALEURONE (*Hordeum vulgare*): ROLE OF PHOSPHATIDIC ACID AND DIACYLGLYCEROL PYROPHOSPHATE**

*Di Palma MA, Villasuso AL, Aveldaño M\*, Pasquare S\*, Giusto N\*, Racagni G, Machado E.*

*Depto. de Biol. Molecular. FCEFQyN. UNRC. \*IIBBB, CONICET-UNS. E-mail: aldipa12@yahoo.com.ar*

Aleurone tissue is a unique and versatile system to study the effect antagonistic abscisic acid (ABA). During the ABA signal, phosphatidic acid (PA) generated by the phospholipase D (PLD) activity is the major phospholipid involved. Our objective was to determine the regulation of PA and DGPP levels in the ABA pathway. To analyze that, cells were incubated with [<sup>32</sup>P]Pi at different times incubations. We determined the fatty acid composition and PAP and DGPP phosphatase activities in stimulated aleurone with GA and ABA. The kinetic of labeling of PA and DGPP was similar in both. Stimulation with ABA resulted in a rapid and transient increase in [<sup>32</sup>P]PA and [<sup>32</sup>P]DGPP levels at 5 min. The analysis of fatty acids of different phospholipids fractions revealed that the major fatty acid was 18:2. ABA decreased the ratio of unsaturated/saturated in PA and also increased the activity of PAP1. These results suggest that ABA modulates the turnover of PA and DGPP via PLD and PAP1 activities.

**62. POTATO STOLONS: LIPID MOLECULAR SPECIES**

*Cenzano A<sup>1</sup>, Racagni G<sup>2</sup>, Abdala G<sup>2</sup>.*

*<sup>1</sup>Unidad de Investigación Ecología Terrestre. Centro Nacional Patagónico, Puerto Madryn, Chubut. <sup>2</sup>Fac. de Cs. Exactas, Fco-Qcas y Nat. UNRC, Río Cuarto, Córdoba.*

*E-mail: cenzano@cenpat.edu.ar*

Phospholipids, galactolipids and fatty acids esterified are involved in signaling pathways triggered by biotic and abiotic stress. The participation of these compounds in the process of plant development, such as tuberization of *Solanum tuberosum* L., is still limited. Therefore, we evaluated by ESI-MS/MS the lipid composition and molecular species of potato stolons, controls and stimulated with jasmonic (JA), abscisic and gibberellic acids. In control stolons, phosphatidic acid (PA) was the major compound, followed by digalactosyldiacylglycerol (DGDG) and phosphatidylinositol (PI). Monogalactosyldiacylglycerol (MGDG), phosphatidylcholine (PC), phosphatidylglycerol (PG) showed intermedia levels, and phosphatidylethanolamine (PE), phosphatidylserine (PS) and the lysophospholipids exhibited the lowest levels. No significant changes were found by the hormonal treatment. The presence of 34:2 in PE, PC, PI and PA supports the hydrolysis of PC and PE by phospholipase D and the hydrolysis of PI by phospholipase C, leading both to 34:2 PA production. In MGDG and DGDG 36:6 was abundant and their hydrolysis could release the substrate (18:3) for the JA biosynthesis. The identity of the molecular species suggest that lipid metabolism pathways are activated at the beginning of tuber formation, and shows the prevalence of PA and DGDG as constituents of photosynthetic membranes and nonphotosynthetic plastid.

**63. CHARACTERIZATION OF PchP FROM *Pseudomonas aeruginosa* IN ORGANIZED MEDIA**

*Challier C<sup>1</sup>, Beassoni P<sup>2</sup>, Lisa AT<sup>2</sup>, Biasutti MA<sup>1</sup>.*

*<sup>1</sup>Dpto. de Química, <sup>2</sup>Dpto. de Biología Molecular, UNRC.*

*Pseudomonas aeruginosa* is an opportunistic pathogen responsible for acute and chronic infections and it represents an important problem for patients with severe burns, cystic fibrosis, and immunocompromising diseases. We proposed a mechanism that would explain the pulmonary infection through the coordinated action of hemolytic phospholipase C and phosphorylcholine phosphatase (PchP). Therefore PchP is a potential pharmaceutical target. PchP catalyzes the hydrolysis of phosphorylcholine in choline and phosphate, and this reaction was previously studied by our group, in homogenous media. The aim of the present contribution was perform a comparative study of PchP behaviour in homogeneous and organized media. The kinetic of the enzymatic reaction was monitored by pNP formation following the absorbance at 410 nm after pNPP hydrolysis at pH 5.0 in presence of Mg<sup>2+</sup>. Two different surfactants were used: BHDC for reverse micelles and triton X-100 for direct micelles. In homogeneous media, PchP has a Michaelis-Menten behaviour and the parameters K<sub>m</sub>, k<sub>cat</sub> and the catalytic efficiency (k<sub>cat</sub>/K<sub>m</sub>) were obtained. In BHDC we observed that this surfactant is a competitive inhibitor of the enzyme. On the other hand, we found that Triton seems to activate PchP in submicellar concentrations. We also studied the fluorescence lifetimes and anisotropy (r) of the intrinsic Trp of the PchP. The results indicate the different micropolarity and microviscosity of the media.

**64. INTERACTION OF GABAERGIC PHENOLS WITH LANGMUIR FILMS**

*Reiner GN, Perillo MA, García DA.*

*Depto de Química, FCEFYN, Universidad Nacional de Córdoba. E-mail: gabrielareiner@gmail.com*

The GABA<sub>A</sub> receptor, the main inhibitory receptor from central nervous system, has different recognition sites for specific ligands. Our group demonstrated that several phenolic compounds, from the family of propofol analogues, are active ligands of this receptor. In order to define the structure-activity relationship and to improve previous pharmacophoric models, in the present work we study the interaction of five phenolic compounds (propofol, thymol, carvacrol, eugenol and chlorothymol) with model membranes (dpPC Langmuir films). At low pressure, all compounds were able to expand the films. The more hydrophobic phenols produced a displacement of the phase transition toward smaller molecular areas, while eugenol showed an inverse effect. At higher pressures, all compounds induced the decrement of the minimum area and the collapse pressure, which indicates an interface destabilization. The compound interfacial concentration analysis indicated that they penetrate and keep in the interface as increasing the phospholipid molecular packing, being squeezed at high pressures (propofol, thymol, carvacrol) or after the LE-LC phase transition (eugenol, chlorothymol). The penetration studies suggest that, in spite of these compounds can be introduced in the membrane until high pressures, the penetration rate diminishes as the interface is more packed.



65.

**INTERFASIAL ACTIVITY OF  $\beta$ -GALACTOSIDASE FROM *E. coli* FUSED TO AN HISTIDINE HEXAPEPTIDE***Sanchez JM<sup>1</sup>, Barra JL<sup>2</sup>, Argaraña C<sup>2</sup>, Perillo MA<sup>1</sup>.*<sup>1</sup>Depto. Química. ICTA. FCEFYN y <sup>2</sup>CIQUIBIQ-Depto. Química Biológica FCQ. UNC.

Previously we showed that the activity of the soluble wild-type *E. coli*  $\beta$ -galactosidase ( $\beta$ -Gal<sub>wt</sub>), against the soluble substrate ortho-nitrophenyl-galactopiranoside (ONPG), increased in the presence of multilamellar vesicles (MLVs) of a zwitterionic phospholipids. The purpose of this study was to compare the activity of a  $\beta$ -Gal<sub>wt</sub> (Sigma-Aldrich) with a recombinant  $\beta$ -Gal containing 6 histidine residues (His-tag) fused to the terminal carboxyl ( $\beta$ -Gal-His6), which was overexpressed in *E. coli*. This modification facilitates the protein purification by metal ion affinity chromatography (IMAC). The enzyme activity was measured by visible spectrophotometry, in the absence or presence of MLVs composed either pure egg phosphatidyl choline (EPC, zwitterionic interface) or a binary mixture of EPC and dioleoyl phosphatidylglycerol at a 80:20 molar ratio (doPG, negative interface). The kinetic parameters were determined by fitting the Michaelian model to the experimental data by a nonlinear regression. Our results showed that  $\beta$ -Gal-His6 presented lower affinity for the substrate with respect to  $\beta$ -Gal<sub>wt</sub>. Although the interfacial overactivity was retained, it was smaller than that of  $\beta$ -Gal<sub>wt</sub>. Moreover, the catalytic efficiency ( $k_{cat}/K_M$ ) increased in the presence of EPC:doPG MLVs, suggesting a higher sensitivity of  $\beta$ -Gal-His6 for the negatively charged lipid-water interface than  $\beta$ -Gal<sub>wt</sub>.

66.

**FLUCTUATIONS OF IONIC CURRENTS THROUGH BLACK LIPID MEMBRANES***Corvalán NA, Kembro JM, Perillo MA.**Química Biológica, Dpto. Química / ICTA, FCEFYN, Universidad Nacional de Córdoba.*

Some aspects of the membrane dynamics may be encoded in the temporal fluctuation of ionic currents. From this perspective, in the present work we studied the conductance of K<sup>+</sup> and Cl<sup>-</sup> through black lipid membranes (BLM) formed from poPC/POPE 3:7 dissolved in n-decane (~25 mg/ml) on a 150  $\mu$ m of diameter hole, carved in a wall that separated two chambers filled with solutions of identical composition (HEPES 10 mM, 150 mM KCl, pH 7.4). Electrical transmembrane potential ( $\Delta V$ ) within  $\pm 200$  mV was applied using Ag/AgCl electrodes connected through a saline 200 mM KCl bridge. The electrical current intensity (I) was recorded for 10 s at 10 kHz. Within the range 0 to  $\pm 100$  mV, a single conductance state ( $G=6.4 \pm 0.6$  fs) was observed. The magnitude, dispersion and amount of conductance states (2-4) increased at  $|\Delta V| > 100$  mV. These results could be associated with the formation of transient pores induced by electrostriction of the membrane. A detrended fluctuation analysis (DFA) of the temporal pattern of electric current amplitude recorded between  $\pm 100$  mV showed no autocorrelations (white noise). Beyond  $\pm 160$  mV a transition to 1/f type noise was observed which is characteristic of critical states acquired by biological systems far from equilibrium. In our system this would correspond to the appearance of reversible pores that precede the electroporation of the bilayer.

67.

**HIGH HYDROSTATIC PRESSURE EFFECTS ON STRUCTURE AND ACTIVITY OF  $\beta$ -GALACTOSIDASE***Caruso B<sup>1</sup>, Bonafe FS<sup>2</sup>, Perillo MA<sup>1</sup>.*<sup>1</sup>Química Biológica, Dpto. Química/ICTA, FCEFYN, UNC.<sup>2</sup>Instituto de Biología. UNICAMP, SP, Brasil.

This study evaluated the effect of hydrostatic pressure (P = 1-171 MPa) on the conformation and aggregation of  $\beta$ -galactosidase enzyme ( $\beta$ -Gal), through analysis of the intrinsic fluorescence of the protein and light scattering. Pressure induced a bathochromic displacement on the center of spectral mass (CSM), whose magnitude increased with P and with the [urea] ( $\Delta$  CSM<sub>max</sub> was -75 and -700 cm<sup>-1</sup> in the absence and presence of urea in 2.5 M, respectively). In the absence of urea, the pressurization to 171 MPa did not affect the kinetic parameters of the enzyme against an artificial substrate ( $V_{max} = 0.022 \pm 0.002$  and  $0.019 \pm 0.002$ ;  $K_M = 0.2 \pm 0.06$  and  $0.12 \pm 0.05$ ,  $P \approx 8$  or 171 MPa, respectively). For all the [urea], the return to  $P = 5.5$  MPa was accompanied by a partial recovery in the fluorescence signal while the activity is irreversibly lost and depending on the [urea] ( $V_{max}$  decreased and  $K_M$  remained constant) indicating that it was an "all or none" phenomenon. Light scattering showed an initial decline with P, reaching a minimum and then a recovery of the initial values with a slope that increased as a function of [urea], suggesting an early stage, at low P, dissociation monomer and a drop in the native structure in the second stage. While the half-inactivation of  $\beta$ -Gal in H<sub>2</sub>O occurs in  $P_{1/2} \approx 300$  MPa (BBA 129, 61-68, 1996), in the presence of 0.4 M urea,  $P_{1/2}$  was reduced to 171 MPa, with its consequent technological advantage.

68.

**STUDY OF CYTOTOXIC ACTIVITY AND RESISTANCE TO ANTIFUNGAL AND ANTIBIOTIC COMPOUNDS IN TWO PROBIOTIC *Lactobacillus* STRAINS FROM VAGINA***Caffaratti E, Ruiz F, Pascual L, Barberis L.**Dpto. Microbiología e Inmunología. UNRC. Ruta 36 Km 603, Río Cuarto (5800), Córdoba. E-mail: lpascual@exa.unrc.edu.ar*

*Lactobacilli* constitute the indigenous or common flora in human vagina, where they exercise resistance to colonization by pathogenic microorganisms. The objectives of the present work were to *in vitro* evaluate *Lactobacillus fermentum* L23 and *L. rhamnosus* L60 susceptibility to antifungal and antibiotic compounds of clinical use, as well as to study the hemolytic activity of bacteriocins produced by these strains. Bauer and Kirby's technique was performed (NCCLS, 2003). The inhibitory activity of the fractions obtained from purification was assayed by using the wells test, and a sensitive strain was used. The cytotoxic effect of bacteriocins on red globules was studied by means of the test of tubes containing both human and ram eritrocites. *L. rhamnosus* showed resistance to eight antibiotics and *L. fermentum* was resistant to 6. None strain was antifungal resistant. No bacteriocin hemolytic activity was detected in the species of eritrocites tested. DISCUSSION. The importance of these results should be highlighted in reference to the resistance to antimicrobial compounds, which represents an advantage in these probiotic strains because they would not be eliminated from the vaginal environment.

**69. DETECTION OF A BIOLOGICALLY ACTIVE SUBSTANCE PRODUCED BY *Lactobacillus fermentum* THAT INHIBITS *Streptococcus agalactiae* AND *Neisseria gonorrhoeae***

Ruiz F, Asurmendi P, Caffaratti E, Gerbaldo G, Pascual L, Barberis L. Dpto. Microbiología e Inmunología. UNRC., Río Cuarto (5800), Córdoba. E-mail: lpascual@exa.unrc.edu.ar

*S. agalactiae* (GBS) is a human pathogen that colonizes the female genital tract and it acquires relevance because of perinatal infection. *N. gonorrhoeae* causes a sexually transmitted disease, and it has developed resistance to antibiotics. Lactobacilli are the main microorganisms of the healthy female vaginal environment, and produce antibiotic compounds such as bacteriocins. In the present work, sensibility of GBS and *N. gonorrhoeae* to the *L. fermentum* L23 bacteriocin was evaluated. The bacteriocin was obtained from a culture with 18 h of incubation at 37°C and centrifugation at 8000 rpm for 20 min at 4°C. 20 *S. agalactiae* strains and 12 *N. gonorrhoeae* strains were tested. The wells technique was performed in Thayer Martin and Mueller Hinton Plates. 60% of the GBS strains and 100% of the *N. gonorrhoeae* strains were inhibited by the *L. fermentum* bacteriocin. The obtained results did not coincide with those of Juárez Tomas (2003), since inhibition was due to acids and not to the action of a bacteriocin. In summary, the results are highly encouraging since the L23 bacteriocin was able to inhibit the tested strains. These promissory findings would allow the use of the *L. fermentum* L23 as a biocontrol agent or as a therapeutic alternative in the future for suppression or prevention of genital infections.

**70. *Giardia lamblia*: PHOSPHOLIPID-BASED SIGNALLING AND ITS INVOLVEMENT IN THE ENCYSTMENT**

Palandri A, Gesumaria MC, Racagni G, Machado EE. Dpto. de Biología Molecular. FCEFQyN-UNRC. E-mail: anabelapalandri@hotmail.com

In order to adapt to their environment *Giardia* alternates between two different forms, trophozoite and cyst. The role of kinases and acidic phospholipids has been established in regulation of cell differentiation and adaptations of the lower eukaryotes under conditions of stress. However, the current understanding of intracellular signalling mechanisms that regulate *Giardia* differentiation is limited. The aim of this work was to determinate the involvement of acidic phospholipids metabolism during the encystment process. We analyzed phospholipid pattern in [<sup>32</sup>P]Pi labelled trophozoites during different periods of time. Moreover, we assayed lipid kinase activities phosphatidylinositol kinase (PI-K), phosphatidylinositol phosphate kinase (PIP-K), diacylglycerol kinase (DAG-K) and phosphatidate kinase (PA-K), by phosphorylation of endogenous substrates with [<sup>γ</sup><sup>32</sup>-P] ATP. An increase of PI-K and PIP-K activity was observed when trophozoites were induced to encyst suggesting a role for these enzymes in encystation process. Our results demonstrate the existence of acidic phospholipids and lipid kinase activities belonging to phosphatidylinositol pathway in *G. lamblia*. Furthermore, we suggest the involvement of this pathway in encystment process.

**71. *Achyrocline satureioides*: EVALUATION OF DIFFERENT EXTRACTS AGAINST *Paenibacillus larvae*, ETIOLOGICAL AGENT OF AMERICAN FOULBROOD**

González MJ<sup>1</sup>, Villanova J<sup>1</sup>, Agnese M<sup>2</sup>, Finola M<sup>1</sup>, Marioli JM<sup>3</sup>  
<sup>1</sup>Dpto. de Microbiología e Inmunología. <sup>2</sup>Dpto. de Química. UNRC. <sup>3</sup>Dpto. de Farmacia. UNC. E-mail: mgonzalez@exa.unrc.edu.ar

*Achyrocline satureioides* (Lam.) D.C. (Asteraceae), commonly known as “marcela”, is a medicinal herb widely used in Argentina for its digestive, anti-inflammatory, antioxidant, antitumoral, antibacterial and antiviral properties. American Foulbrood (AFB), a disease caused by *P. larvae*, affects the honey bee larval stage. Its control is made with antibiotics, but their irrational use may lead to antibiotic resistant microorganisms and to the presence of residues in honey. **Aims:** To evaluate the antibacterial activity of different extracts of *A. satureioides* against *P. larvae*. **Methodology:** *P. larvae* strains were isolated from hives with symptoms of AFB from Río Cuarto and INTA Balcarce. *A. satureioides* was collected in Córdoba province. Hexane (EH), benzene (EB), ethyl ether (EE) and ethyl acetate (EAE) extracts were obtained sequentially by using a liquid-liquid extractor for less dense than the water solvents. Antibacterial activity was analyzed by the well diffusion technique. **Results:** The values of Minimal Inhibitory Concentrations were in a range from 0.016 to 0.125 mg/ml for EH; from 0.063 to 0.125 mg/ml for EB; from 0,5 to 1 mg/ml for EE, and from 4 to 8 mg/ml for EAE. **Discussion:** All the strains of *P. larvae* were inhibited by the different extracts of *A. satureioides*. EH showed the highest antibacterial activity. Resistant strains were generated by the use of antibiotics, and residues remained in honey. Vegetal extracts which are considered appropriate for the “in vivo” testing in the control of AFB disease.

**72. ANTI-FUNGAL AND ANTI-ALLERGIC EFFECTS OF *Achyrocline satureioides* FLOWERS DECOCTION**

Alaniz E, Andrada M, Giordana M, González Pereyra M, Demo M, Cariddi L, Maldonado A. Dpto. Microbiol. e Inmunol. UNRC.

*Achyrocline satureioides* has been used in popular medicine by its anti-inflammatory properties. Objectives were to evaluate the effects of *Achyrocline satureioides* flowers decoction (FD) on basophils from allergic patients to environmental fungi and to investigate mycelia growth in cultures of isolated fungi from house of each. Prick test and enzyme β-hexosaminidase release (β-H) test from basophils challenged with the allergen were assayed. The basophils were challenged with the allergen alone (100 PNU/ml); or added of FD or antihistamine drugs. Aeroallergens fungi were isolated and identified by optical microscopy and characterized by sort. The anti-fungal effect of FD was determined on the growth of mycelia. The liberation of β-H was decreased by FD (optimal dose 4mcg/ml), p<0.01 and each one of the drugs: dexamethasone, theophylline, disodium cromoglycate and ipratropium bromide p<0.01. The isolated fungi sorts were: *Penicillium* spp., *Aspergillus* spp. Section Nigri, *Cladosporium* spp. y *Alternaria* spp. The development of the colonies was partially inhibited and diameter, sporulation, coloration, exudates and pigments were modified by FD. In addition, form, number and aspect of conidia were altered. Anti-allergic properties *in vitro* of FD were corroborated. Doses of the order of mcg/ml demonstrated effects similar to ipratropium bromide, the drug of greater effect. The anti-fungal properties of FD were verified on the different isolate sorts.

73.

**PCR STUDY OF PATHOGENIC *Escherichia coli* IN CALF FROM VILLA MARIA (CÓRDOBA) DAIRY BASIN**

*Picco N<sup>1</sup>, Alustiza F<sup>1</sup>, Bellingeri R<sup>1</sup>, Tiranti K<sup>1</sup>, Vissio C<sup>1</sup>, Lombardelli J<sup>1</sup>, Moreira A<sup>2</sup>, Louge E<sup>2</sup>, Luchetti C<sup>1</sup>, Larriestra A<sup>1</sup>, Terzolo H<sup>2</sup>, Vivas A<sup>1</sup>*

<sup>1</sup>Faculty of Agronomy and Veterinary Medicine (UNRC), <sup>2</sup>EEA INTA Balcarce.

In Córdoba, the bovine herd is the first production. Diarrhea caused by *E. coli* generates economic loss around 20%. Some bovine bacterial strains could infect humans. There is no recent information about neonatal diarrhea in Córdoba. The aim of this study was to obtain current data about pathogenic *E. coli* strains causing diarrhea in neonatal calves from Villa Maria. Samples of rectal swabs of calf less than 45 days old (n=61), were transported in Stuart and cultured on Mc Conkey agar. Lac + colonies compatible with *E. coli* were biochemically characterized and DNA was extracted by heating. Virulence factors (LT, STa, F17, F5 and eae) were identified by PCR. Results and Discussion: There was a 36.1% pathogenic strain: 16.42% EHEC, 9.84% ETEC, 1.64% EPEC and only 8.20% were F17+. A 63.9% of non-pathogenic *E. coli* was found. The predominance of EHEC strains could impact on public health. Furthermore the currency of ETEC and EPEC could affect the productive capacity of artificial bringing up calves.

74.

***Escherichia coli* PATHOGENIC STRAINS IN A NEONATAL PIGLET FROM PIG INTENSIVE BREEDING PLACE OF CORDOBA**

*Alustiza F<sup>1</sup>, Picco N<sup>1</sup>, Bellingeri R<sup>1</sup>, Ashworth G<sup>2</sup>, Berardo D<sup>2</sup>, Moreira A<sup>3</sup>, Louge E<sup>3</sup>, Luchetti C<sup>1</sup>, Greco C<sup>2</sup>, Terzolo H<sup>3</sup>, Vivas A<sup>1</sup>.*

<sup>1</sup>Facultad de Agronomía y Veterinaria (UNRC), <sup>2</sup>Fac. de Cs. Exactas, Fco-Qcas y Naturales (UNRC), <sup>3</sup>EEA INTA Balcarce.

Neonatal porcine diarrhea is a major cause of economic losses in pig production. One of the main etiologic agents is *Escherichia coli*. Both enterotoxigenic (ETEC) and enteropathogenic (EPEC), associated with immunosuppression were the cause of stress management. The aim of this work was to characterize by PCR the strains of *E. coli* that produce diarrhea in neonatal piglets of pig intensive breeding in Córdoba. Samples of rectal swabs (n=58) transported in Stuart were analyzed, cultured on Mc Conkey agar. Lac + colonies compatible with *E. coli* were biochemically characterized and DNA was extracted by heating. Virulence factors (LT, STb, STa, F18, eae) were identified by PCR. Result and Discussion: The findings showed a 1.72% of EPEC (eae+) and 24.14% of ETEC, which were 50% STb+, 21.4% LT+, 14.3% STa+; 7.15% F18+ and 7.15% F18+ STb+. A 74.14% of non-pathogenic *E. coli* was found. It shows a predominance of ETEC currency. These results show that, in this intensive breeding, with controlled environment and good prophylaxis (vaccination), the presence of pathogenic strains *E. coli* remains possibly by stress management.

75.

**FUNCTIONAL DIVERSITY AMONG ORTHOLOGS GLYCOSYL TRANSFERASES FROM BACTERIAL SYMBIONTS AND PATHOGENS**

*Ferrari W<sup>1</sup>, Fischer S<sup>1</sup>, Mori G<sup>1</sup>, Lagares A<sup>2</sup>, Jofré E<sup>1</sup>.*

<sup>1</sup>Dpto. Cs. Nat. FCEFQyN, UNRC; <sup>2</sup>IBBM-UNLP. E-mail: ferrariwalter@gmail.com; ejofre@exa.unrc.edu.ar

LpcC of *Rhizobium leguminosarum* and LpsB of *Sinorhizobium meliloti* are orthologs proteins sharing 58% and 72% of identity and similarity, respectively. LpsB and LpcC are glucosyl/manosyl transferases involved in the biosynthesis of Inner Core of LPS. Previous studies have shown that the gene *lpcC* not complemented *S. meliloti lpsB* mutants, while *lpsB* complemented *R. leguminosarum lpcC* mutants. Orthologs of LpsB and LpcC are present in other  $\alpha$ -proteobacteria including *B. japonicum*, *M. loti*, *A. tumefaciens* and the human pathogens *Brucella melitensis* and *Bartonella henselae*. To determine if this high conservation correlates with the functionality of these proteins, *lpsB* and *lpcC* mutants were complemented by their orthologs enzymes. The results showed that *lpcC* of *R. leguminosarum*, *A. tumefaciens*, *B. melitensis*, and *waaC* of *M. loti* complemented *B. japonicum lpcC* mutants, restoring the LPS profile to its wild-type phenotype. On the other hand, *lpsB* of *S. meliloti*, *lpcC* of *R. leguminosarum* and *B. japonicum* complemented *A. tumefaciens lpcC* mutants. In contrast, *S. meliloti lpsB* mutant was not complemented by any of its orthologs, suggesting that there is a functional diversity despite the high degree of conservation of these proteins.

76.

***Achyrocline satureioides*: VIRUCIDAL AND ANTIVIRAL ACTION OF AQUEOUS EXTRACTS**

*Sabini C<sup>1</sup>, Escobar F<sup>1</sup>, Zanon S<sup>1</sup>, Contigiani M<sup>2</sup>, Sabini L<sup>1</sup>.*

<sup>1</sup>Dpto. Microb. e Inmunol. FCEFQyN, UNRC, Río Cuarto, Cba.

<sup>2</sup>Inst. de Virología Dr. Vanella. UNC, Córdoba.

E-mail: csabini@exa.unrc.edu.ar

**Introduction:** The Western equine encephalitis virus (WEEV) (Togaviridae) is a pathogen very important transmitted by mosquitoes. This virus causes epizootics in Argentina and major economic losses. Numerous investigations have reported that medicinal plants have antiviral properties, *Achyrocline satureioides* (Asteraceae) deserves to be studied in this property. **Objective:** To evaluate virucidal and antiviral activities of aqueous extracts obtained from *A. satureioides* against WEEV. **Methodology:** Aerial vegetal parts were submitted to extraction with cold (4°C) and hot water (70°C) sequentially for two days. These two solutions were identified as Cold Aqueous Extract (CAE) and Hot Aqueous Extract (HAE). Virucidal and antiviral activities of CAE and HAE, used at different non cytotoxic concentrations (200-800 and 150-400 µg/ml respectively) were evaluated by plaque reduction assay using Vero cells and WEEV Ag 80-646 enzootic strain. **Results and Discussion:** Both extracts inhibited viral replication in 100%. The virucidal effect was dose-dependent, reaching 82% (HAE) and 91% (CAE). The aqueous extracts of *A. satureioides* have exerted greater antiviral activity than virucidal. Subsequent studies were designed to determine the selectivity index, mode of action and chemical characterization of extracts.



77.  
**MICROBIOLOGICAL QUALITY OF WATER AND ANTIBIOTICS RESISTANCE OF *Escherichia coli* STRAINS ISOLATED FROM KNUTZEN STREAM BASIN, CÓRDOBA**  
*Gambero L, Bettera S, Lombardo D, Frigerio C, Blarasin M, Giuliano Albo J.*  
*Fac. de Cs. Exactas Fco-Qcas y Nat. U.N.R.C.*

The supply of water in Knutzen stream basin is sustained by the aquifer and subordinate by the stream. An agroecosystem is developed livestock rearing with use of antibiotics (medicinal and promoter of growth). The aim of this work was to analyze the microbiological quality of water, its suitability of use and assess the antibiotics resistance of *Escherichia coli* strains isolated. Twenty six samples were extracted (4 of surface and 22 groundwater). Count of mesophilic aerobic bacteria (MA), total coliform (TC), fecal coliforms (FC), *Escherichia coli* and *Pseudomonas aeruginosa* and tests for antibiotics resistance were carried out. Surface water was more affected by contamination, all samples show high counts of CT in a range between 210-900 NMP/100 ml and presence of *E. coli*. The 54.5% of groundwater samples were unfit for human consumption to overcome some of the limits set in Argentina. The 92.9% (n=14) of the strains of *E. coli* was resistant to antibiotics ERY and PEN, the 35.7% to AM and the 28.6% a TET. All were sensitive to CIP, AMC and AMC and the 92.9% CEF. The results show that water is in bad microbiological quality, being the superficial most affected. The use of antibiotics in livestock, the *E. coli* isolates from samples associated to farm animal activity, and the pattern of antimicrobial resistance, let us to think that fecal contamination could be from cattle, in this basin.

78.  
**ANTIBIOTICS RESISTANCE STRAINS OF *Escherichia coli* ISOLATED WATER DAIRY HERDS OF THE VILLA MARIA, CÓRDOBA**  
*Gambero L, Bettera S, Lombardo D, Frigerio C.*  
*Dpto. de Microbiología e Inmunología. U.N.R.C.*

The bacteriological quality of water in the dairy herds can influence the hygienic quality of milk. Use of antibiotics incorporated into the food from bovine animals as prophylaxis and growth promoters, is cause of selection resistant bacteria to antibiotics. The objective of this study was to examine the microbiological quality of water according to the criteria established by the CAA, in 50 establishments dairy and assess antibiotics resistance of *E. coli* strains isolated. Two water samples are collected; one from source (SW) and another from wash (WW). Count of mesophilic aerobic bacteria (MA), total coliform (TC), *E. coli* and *P. aeruginosa* detection and testing to antibiotics resistance were carried out. The 46% WW and the 24% of SW presented MA exceeding 500 CFU/ml. TC of counting exceeded the value admitted in the 70% SW and in the 80% WW. *E. coli* was isolated in the 20% both origins of water samples *P. aeruginosa* was detected in the 36% SW and in the 42% WW. All of *E. coli* strains were resistant to the antimicrobials PEN and ERY, 46.7% and 26.7% to AM and TET respectively, the 20% a AMC and the 6.7% CEF. The 100% the isolated was sensitive to CIP and CMP. The results show that a high percentage of dairy herds have water of poor bacteriological quality. The high percentages of resistance were observed in antibiotics used for applications not therapeutic, administered in cattle diet.

79.  
**TRANSCRIPTIONAL REGULATION OF PHOSPHORYLCHOLINE PHOSPHATASE AND GENES INVOLVED IN THE UTILIZATION OF CHOLINE IN *Pseudomonas aeruginosa***  
*Llanos A, Sánchez DG, Serra AL, Lisa AT.*  
*Depto. de Biología Molecular. Fac. Cs. Exactas, Físico-Quím. y Nat. UNRC.*

Phosphorylcholine phosphatase (PchP) of *P. aeruginosa* is induced when choline is used as a source of carbon and/or nitrogen and it is codified by the gene *pchP*. Its promoter has been localized, and the +1 transcriptional start site and the -12/-24 elements of  $\sigma$ -54 dependent promoters have been identified. The transcriptional regulators, NtrC and GbdR are involved in the utilization of choline as a source of energy and in the regulation of *pchP* gene. The aim of this work is to elucidate in which way this regulators are involved in the metabolism of choline and in the induction of enzymes related to it. The second objective is to find the optimum conditions to obtain regulatory proteins in a soluble and active form. Firstly, the genes *gbdR* and *ntrC* amplified by PCR were cloned in the plasmids pET15b and pTOPO, respectively. The plasmid pET15b::*gbdR* was transformed in *E. coli* BL21 and the overexpressed protein appeared in inclusion bodies. This lead us to change the induction and overexpression conditions and to overexpress the protein in an homologous host, using another expression vector (pET1.6P). To obtain an active NtrC, it was necessary to make a site-directed mutagenesis in the gene *ntrC*, to replace S161F. The modified gene (*ntrC*<sup>constitutive</sup>) was subcloned in the plasmid pMPO321 and the protein was overexpressed in an heterologous host. Then, the soluble proteins were purified through an affinity column or by ammonium sulphate precipitation, showing a MW that corresponds to the size of the genes. *In vitro* and *in vivo* experiments will be performed, in order to study the interaction protein-DNA, and to elucidate the hierarchy between the regulatory proteins NtrC and GdbR in the metabolism of choline and enzymes induced by its presence in the culture medium of *P. aeruginosa*.

80.  
**PHOTODYNAMIC ACTION AND LOCALIZATION OF ANTHRAQUINONES IN CANCEROUS CELLS**  
*Fernandez I<sup>1</sup>, Comini L<sup>2</sup>, Nigra A<sup>1</sup>, Nuñez S<sup>2</sup>, Rumie Vittar B<sup>1</sup>, Cabrera J<sup>2</sup>, Rivarola VA<sup>1</sup>.*  
<sup>1</sup>Dpto. Biol. Mol. FCEFQyN-. UNRC. (5800) Río IV, Arg.  
<sup>2</sup>Fcognosia, FCQ-UNC, IMBIV-CONICET (5000) Córdoba, Argentina. E-mail: ivimar2002@yahoo.com.ar

Anthraquinones (AQs) rubiadin (1), 1-rubiadin methyl ether (2), soranjidiol (3), 1-soranjidiol methyl ether (4) and 5-5'-bisoranjidiol (5) isolated from *Heterophyllaea pustulata* are type I and/or II photosensitizers. Previously, we have demonstrated that (1), (2), (3) have photodynamic activity (PA) on human cancer cells. In this work, we evaluated the uptake of AQs into cancerous cells and its subcellular localization. Indeed, the PA of (4) and (5) was assessed. To determine the incorporation and localization of (1), (2) and (3) the cells were incubated with each AQ. For uptake, the analysis of AQs in each fraction (extracellular/ intracellular) was realized by HPLC whereas the intracellular pattern was observed by fluorescence microscopy. To evaluate the PA, cells were loaded with the AQs (4) and (5) and immediately exposed to 1 J/cm<sup>2</sup>. The cellular viability was analyzed by MTT. Controls without AQs were processed under same conditions. The results showed that AQs (1), (2), and (3) were incorporated in the cancer cells and localized mainly in the cytoplasm. The AQs (4)-(5) caused outstanding PA. According to the obtained, these AQs tested might be possible candidates for future studies in photodynamic therapy.



**81. EXPRESSION OF  $\alpha 3$ ,  $\beta 3$  AND  $\beta 1$  INTEGRIN SUBUNITS AND  $\alpha v \beta 3$  DURING EARLY PORCINE GESTATION**

*Williamson D<sup>1,3</sup>, Sanfilippo S<sup>2</sup>, Koncurat M<sup>1</sup>.*

<sup>1</sup>*Biología General;* <sup>2</sup>*Inglés, Depto de Ciencias Básicas. Facultad de Ciencias Veterinarias, Universidad Nacional de La Pampa. Calle 5 esq. 116. (6360).* <sup>3</sup>*Becaria CONICET.*

*E-mail: dmw@vet.unlpam.edu.ar*

Integrins are membrane glycoproteins composed of  $\alpha$  and  $\beta$  subunits necessary for adhesion between trophoctoderm and maternal uterine epithelium. The aim of the present study was to determine the presence of  $\alpha 3$ ,  $\beta 3$ ,  $\beta 1$  and  $\alpha v \beta 3$  in placental tissue in sows at different early gestational periods and understand the success implantation and adhesion have in this species. Porcine placentae of  $\pm 5, 15, 17$  and 37 days of gestation and empty uterus are used. The presence of  $\alpha 3$ ,  $\beta 3$  and  $\beta 1$  subunits and  $\alpha v \beta 3$  are determined by indirect and direct immunohistochemistry respectively. At day 5 the uterine epithelium showed the presence of all integrins with different grades of positivity. Integrins are not detected in maternal villi and uterine glands until 37 days of pregnancy. The maternal connective tissue exhibited high expression during the study. In conclusion, integrins would be involved in the molecular structure of implantation since they appear both in maternal and fetal villi at 37 days of pregnancy.

**82. MODULATION OF SOME ENDOCANNABINOID SYSTEM (ECS) COMPONENTS AND CELL PROLIFERATION BY EXOGENOUS FATTY ACIDS IN HUMAN CANCER CELL LINES**

*Repossi G, Das UN, Eynard AR.*

*Inst. de Biol. Celular, UNC. CONICET.*

**Introduction:** dietary unsaturated fatty acids (UFA) may modulate cancer development and ECS. **Aim:** to analyze the effects of UFAs 20:5n3 (EPA), 20:4n6 (AA), 18:1 (OA) and saturated 16:0 (PA) on MCF7, T24 and T98G cancer lines proliferation and ECS receptors. **Methodology:** the cells lines MCF7 (breast cancer), T24 (urinary bladder carcinoma) and T98G (neuroblastoma) were cultured and supplemented with each FA essayed. Cell proliferation was evaluated by MTT method, CB receptors by ELISA and FA cell profiles in membranes by GLC. Data obtained were statistically analyzed. **Results:** MTT: AA and EPA suppress cell proliferation in T98G and MCF7 respectively; whereas PA and OA stimulate cell growth. ELISA: slight increases on the immunodetection of CB1 receptors in MCF7 and T98 were observed. GLC: we observed a fairly reasonable correspondence between exogenous UFAs and the enrichment of FA in membranes composition. **Discussion:** this results show a differential modulation by FA supplementation on cell proliferation, CB receptors expression and membrane FA profiles in different cell cancer lines. The effects of FA have been associated with ERK kinase activation (Soto-Guzman *et al.*, 2008) and eicosanoids and endocannabinoids metabolism, metabolites FA derived, capable of modify growth in cancer cells (Repossi *et al.*, 2009; Pasqualini *et al.*, 2008).

**83. RESISTANCE TO PHOTODYNAMIC THERAPY WITH ME-ALA IN SQUAMOUS CELL CARCINOMA**

*Milla L<sup>1</sup>, Rodriguez E<sup>1</sup>, Zamarrón A<sup>2</sup>, Juarranz Á<sup>2</sup>, Rivarola VA<sup>1</sup>*

<sup>1</sup>*Facultad de Ciencias Exactas, Físico-Químicas y Naturales. Dpto. Biología Molecular, UNRC. Argentina.* <sup>2</sup>*Facultad de Ciencias. Dpto de Biología. UAM. España.*

One of the photodynamic therapy (PDT) problems is the apparition of resistant (R) cell populations. The objective of this work was study the mechanisms that confer resistance to PDT in squamous cell carcinoma (SCC-13).

The specific objectives were: 1) To obtain R populations to PDT with Me-ALA. 2) To characterize morphology and molecularly the R populations. The original population (parental) was submitted to 10 repetitive cycles of PDT and thus 10 R generations were isolated. Cell morphology was analyzed with toluidine blue stain. Employing Western Blot, expression levels of cell adhesion proteins (E-cadherin,  $\beta$ -catenin,  $\beta 1$ -integrin and vinculin) were determine. Through indirect immunofluorescence, expression patterns of these adhesion proteins, cytoskeleton proteins ( $\alpha$ -tubulin and F-actin) and inhibitor apoptosis proteins (survivina) were analyzed. R cells showed a more fibroblastic morphology than parental, more expression of cell-substrate adhesion proteins and F-actin and changes in F-actin distribution. The results permitted conclude that SCC-13 might involucrate alterations in the expression and/or distribution of the studied proteins, as a strategy to PDT resistance.

**84. MATERNAL EXPOSURE TO TRICLOSAN IMPAIRS THYROID HOMEOSTASIS AND FEMALE PUBERTAL DEVELOPMENT IN WISTAR RAT OFFSPRING**

*Rodríguez PEA, Sanchez MS*

*Subsecretaria CEPROCOR, Ministerio de Ciencia y Tecnología, Córdoba, Argentina. E-mail: msanchez@ceprocor.uncor.edu*

The present study examines the effect of maternal exposure to Triclosan on thyroid homeostasis and the development of the reproductive tract in female Wistar rat offspring. Female rats were exposed daily to Triclosan (0, 1, 10, or 50 mg/kg/day) from 8 days before mating to day 21 of lactation. Some offspring also were exposed directly after weaning. Maternal and reproductive outcome data were assessed. An uterotrophic assay was performed to screen *in vivo* estrogenic activity of Triclosan. Vaginal opening (VO), first estrus, and uterus and ovary weight were analyzed in female offspring. Blood samples were collected from the tail vein every 5 days during pregnancy and lactation of treated or control rats to determine the effect of Triclosan on the pattern of thyroid hormone secretion. Triclosan caused no external clinical signs of toxicity in mothers. Mean number of corpora lutea and implantation sites did not differ in treated groups compared to their respective controls. Female pup body weight significantly decreased in all treated groups on PND20. Fetal viability significantly decreased at doses of 100 mg/kg/day and sex ratio decreased at all doses. Exposure to Triclosan caused VO delay and uterotrophic assay indicated the absence of estrogenic activity. Triclosan significantly decreased total serum T<sub>3</sub> in a dose-dependent manner. The results show that Triclosan promotes hypothyroidism and reproductive toxicity in adult rats and produces fetal toxicity in offspring exposed in uterus, lactation, and after weaning. The NOEL for reproductive and teratogenic effects was less than 1 mg/kg/day.

**85. DEVELOPMENT OF A IMMUNOLOGIC PARANEOPLASTIC SYNDROME IN A LUNG ADENOCARCINOMA MODULATED BY DIETARY LIPIDS**

Piegari M, Soria EA, Eynard AR, Valentich MA.  
*Instituto de Biología Celular. FCM-UNC.*

Tumour and immune parameters were analyzed by linear regression in mice with lung cancer under diets with corn, fish or olein oils. Although diet affected the T lympho-proliferation (TLP) and survival, the found paraneoplastic syndrome (anaemia, neutrophilia, splenomegaly, thymus atrophy and cachexia) behave independent of the diet. The tumour diameter (TD) was indirect to spleen weight (SW), and to basal and ConA-stimulated TLP, whereas tumour weight (TW) was direct. TLP was positively associated with the SW/body weight (BW) ratio and negatively with the thymus/body weight ratio. Spleen and thymus weights were indirectly associated. BW decreased with disease progression, being splenomegaly a negative factor and thymus weight a positive one. Thus, TD was a better prognostic marker than the TW (may relate to histological differences), being linked to reduced survival and T response. The thymus associated with decreasing TD was a marker of the general status. Summing up, the lung cancer had a strong immunomodulating activity, of which parameters mutually interacted, giving identity to a paraneoplastic syndrome, where the thymus-dependent immunity may play a critical role.

**86. "EL MORRO" HILL FLORULA: THE ASTERACEAE FAMILY SPECIES (SAN LUIS, ARGENTINA)**

Rosa EB, Mercado SE, Scappini EG.  
*Departamento de Ciencias Agropecuarias. Facultad de Ing. y Cs. Econ. Sociales U.N.S.L. E-mail: erosa@fices.unsl.edu.ar*

El Morro Hill, as well as Los Morrillos and Yulto hills are situated at the NE of the province of San Luis. The climate is moderate, continental; rainfall varies from 500 to 600 mm annually. The soils are erodible litosols with low fertility and water retention. The physiognomic types of vegetation are: Forests, Forestry and Range foothills, mountain forests, grassland and shrublands called "romerillales", growing over non developed, drained soils, exposed to winds between 1100 - 1250 m. above sea level. Where dominant species are *Heterothalamus alienus* "romerillo" and *Eupatorium buniifolium* "romerito". The introduced invasive weed, *Heterotheca latifolia*, occurs in cultivated areas. In order to contribute to the development of the Sierra del Morro Florula, the species of the family Asteraceae in different physiognomic types were surveyed. They were identified and incorporated to the Ciencias Agropecuarias, UNSL, (VMA) Herbarium. At present, 43 entities were reported, grouped in 29 Genera: *Baccharis*, *Thelesperma*, *Porophyllum*, *Vernonia*, *Senecio*, *Heterothalamus*, *Eupatorium*, *Stevia*, *Chaptalia*, *Hysterionica*, *Bidens*, *Tagetes*, *Flaveria*, *Gaillardia*, *Viguiera*, *Trichocline*, *Zinnia*, *Schkuhria*, *Hetheroteca*, *Noticastrum*, *Ophryosporus*, *Churquiraga*, *Conyza*, *Gamochoeta*, *Taraxacum*, *Picris*, *Perezia*, *Hyalis*, *Gnaphalium*. The most common is the *Baccharis* genus with 10 species.

**87. ANTIGENIC CHARACTERIZATION OF STRAIN RC-98 OF BOVINE RESPIRATORY SYNCYTIAL VIRUS BY DOT BLOT**

Raviolo J<sup>1</sup>, Grosso M<sup>1</sup>, Zielinski G<sup>2</sup>, Trotti N<sup>1</sup>, Giraudo J<sup>1</sup>, Lovera H<sup>1</sup>, Bagnis G<sup>1</sup>, Navarro F<sup>1</sup>, Raviolo JE<sup>1</sup>.

<sup>1</sup>Facultad de Agronomía y Veterinaria. UNRC. <sup>2</sup>INTA. EEA, Marcos Juárez. E-mail: jraviolo@ayv.unrc.edu.ar

Bovine respiratory syncytial virus is a pneumovirus involved in bovine respiratory disease complex. Antigenic studies done by means of monoclonal antibodies (Mabs) based on viral glycoprotein G (Gp G) have determined existence of four antigenic subgroups. Two major A and B, one intermediate AB and the group of strains not classifiable. The aim of this study was antigenic characterization of the strain RC 98 with Mabs by dot blot technique. The viral antigens were obtained from strain RC-98 and applied by drops on polyvinidene fluoride membranes. Once dry, the membrane was blocked and incubated with each of the Mabs 48, 52, 54, 56, 59, 60, 67 and 70 (diluted 1:500) overnight at 4°C in constant shake. The antigen-antibody complexes were detected by indirect immunoperoxidase technique using commercial kits (Vectastain ABC and DAB Kit), according to the manufacturer's instructions. Seven Mabs (numbers 48 to 67) reacted to strain RC-98 Gp G antigens in dot blot, although Mab 70 did not. These results permit to characterize the strain RC98 within antigenic subgroup A. This will allow the design of future prevention and more effective control schemes, because neutralizing antibodies produced against the G glycoprotein of subgroup A are not effective in fighting the infection of subgroup B.

<b>A</b>		Cariddi L	72	Fischer S	75
Abal A	36	Carlini C	25	Fontanetti PA	2
Abdala G	33, 62	Carlini V	1	Forchetti O	53
Abramor NF	4	Carlioni E	21	Francisco de Araújo M	19
Acardi S	L-S 5	Caruso B	67	Frede S	54
Acosta O	57	Casali YA	49	Frigerio C	77, 78
Agnese M	71	Castillo P	33	Fruttero L	25, 54, 55, 56
Agostini E	20	Cattaneo N	16	Fuenzalida D	L-S 5
Aguilar J	50	Cavaglieri L	8	Fumero V	41
Aguirre S	54, 55	Cecere MC	L-S 6	Funes MD	17
Alaniz F	72	Cenzano A	62	Fungo F	17
Albornoz A	41	Cerioni GA	40	<b>G</b>	
Aleman S	33	Challier C	63	Gallarà RV	3
Alessio A	37	Chanique A	37, 38	Gallarato L	59
Alive T	23	Chesta C	12	Gambero L	77, 78
Allende F	37	Chiaraviglio M	10, 11	García DA	64
Alterman A	3	Choi ED	2	García Denegri ME	57
Alustiza F	23, 73, 74	Cingolani AM	9	García G	23
Álvarez MG	17, 19	Cisternas CD	4	García M	29, 41
Andrada M	72	Cocco R	34, 35	García MB	42
Anselmino C	36	Cocucci A	L-S 7	Garrido M	59
Aon MA	L-S 4	Cohen AC	48	Gauna H	26, 27, 28
Aragão Catunda Júnior FE	19	Combina M	8	Gauna HF	23, 24, 29, 30
Argaraña C	65	Comini L	51, 80	Gay C	57
Armando R	8	Compagnucci MV	4	Geraldo de Carvalho M	19
Ashworth G	23, 24, 74	Coniglio V	53	Gerbaldo G	69
Asurmendi P	69	Conti NR	22	Gesumaría MC	70
Aveldaño M	61	Contigiani M	76	Giayetto O	40
<b>B</b>		Coronel C	3	Giordana M	72
Bagnis G	87	Cortassa S	L-S 3	Girardi VN	40
Banchio E	46	Corvalán NA	66	Giraud J	50, 87
Barberis L	68, 69	Cosacov A	L-S 7	Giuliano Albo J	77
Barbero C	18	Cristofolini A	37	Giusto N	61
Barra JL	65	Cristofolini A	38, 39	Gleiser RM	47
Batista S	36	Cuchiatti A	9	Godio L	53
Battan M	47	Cufre G	53	González MJ	71
Beassoni P	58, 59, 63	<b>D</b>		González P	20
Bellavía SL	3	Dalcero AM	8	González Pereyra M	72
Bellingeri R	73, 74	Dallo M	43, 44, 45	Gorodner JO	57
Bensi N	26, 27, 28, 29, 30	Dambolena J	46	Greco C	23, 74
Berardo D	23, 24, 74	Dardanelli MS	41, 42	Griffa S	21
Bertone P	34, 35	Das UN	82	Grosso M	87
Bertuzzi M	18, 20, 24	Davicino RC	49	Grunberg K	21
Bethular A	15	Davison J	3	Gsponer N	12
Bettera S	77, 78	De Luca N	14	Guevara E	13
Bianco M	28, 29, 30	Demo M	72	Gurvich DE	9
Biasutti MA	63	Di Cola V	10, 11	<b>H</b>	
Biderbost E	21	Di Palma MA	61	Hansen C	27
Bima P	46	Di Renzo M	43, 44, 45	Haro JG	15
Binotti S	26, 27, 29, 30	Dominguez JE	48	Huck G	24
Blarasín M	77	Durantini EN	17, 19	<b>I</b>	
Boccolini A	26, 27	Durso G	36	Ibáñez M	43, 44, 45
Boccolini L	30	<b>E</b>		Ibarra L	18
Bologna S	16	Escobar F	76	<b>J</b>	
Bonafe FS	67	Eynard AR	82, 85	Jofré E	75
Bonamico N	43, 44, 45	<b>F</b>		Juarranz Á	83
Borghi L	43, 44, 45	Falcone E	24	<b>K</b>	
Bosch P	24	Fantino A	33	Kembro JM	66
Bottini R	48	Farina WM	L-S 8	Kilmurray C	58
Bueno M	41	Fernandez EM	14, 40	Koncurat M	37, 39, 81
Bustillo S	57	Fernandez I	51, 80	<b>L</b>	
<b>C</b>		Fernández MS	L-S 5	Labaque C	47
Cabrera J	51, 80	Ferrari W	75	Lagares A	75
Caffaratti E	68, 69	Finkelberg A	3	Lanfri S	11
Campos M	37, 38	Finola M	71		
Canavoso L	25, 54, 55, 56	Fiol de Cuneo M	1		
Cantero JJ	14	Fiol de Cuneo MH	52		
Cardozo G	11				

Larriestra A	73	Otero L	58	Salvano MA	8
Leiva L	57	Otero LA	17	Salvi M	34, 35
Leyria J	55	Oyola M	58	Sánchez A	5, 6, 7
Licata L	36			Sanchez D	58
Liotta DJ	L-S 5	<b>P</b>		Sánchez DG	79
Lisa AT	63, 79	Paisio C	20	Sanchez JM	65
Lisanti J	5, 6, 7	Palacio G	31	Sanchez MS	84
Llanes A	31, 32	Palandri A	70	Sanchis G	37, 38, 39
Llanos A	79	Pascual L	68, 69	Sanfilippo S	81
Llompart G	36	Pasquare S	61	Santillán ME	52
Llompart J	36	Pastorino I	38	Santini MS	L-S 5
Lombardelli J	73	Paulucci N	41	Scappini EG	86
Lombardo D	77, 78	Paulucci NS	42	Scoppa G	26, 28
López Colomba E	21	Pedemonte CH	L-S 1	Scoppa HG	29
Lopez LA	L-S 2	Peralta D	18	Sereno J	34, 35
Lopresti N	48	Pérez-Harguindeguy N	9	Sereno M	34, 35
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Lovera H	50, 87	Picco N	73, 74	Sérsic A	L-S 7
Luchetti C	73, 74	Piccoli PN	48	Soldini D	16
Lucini EI	46	Piegari M	85	Soria EA	85
Luna C	21	Pinna-Senn E	5, 6, 7	Stariolo R	56
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Machado EE	70			Terzolo H	73, 74
Maldonado A	72	<b>Q</b>		Ticle M	18
Mancini M	15	Quintana M	L-S 5	Tiranti K	73
Marchal J	5, 6, 7			Tommasino E	21
Marin RH	47	<b>R</b>		Torres C	26, 27, 30
Marioli JM	71	Racagni G	60, 61, 62, 70	Trotti N	50, 87
Martínez Alvarez D	16	Raviolo J	50, 87	Turiello P	38, 53
Martínez C	36	Raviolo JE	50, 87		
Martini AC	1, 52	Reginato M	32	<b>U</b>	
Martino R	49	Reiner GN	64	Usorach J	60
Masciarelli O	33	Reinoso H	32		
Mathison R	3	Remondegui C	L-S 5	<b>V</b>	
Mattar MA	49	Repossi G	82	Valentich MA	85
Mayer N	23, 24	Ribotta A	21	Vaqué AM	4
Medeot D	41	Rivarola V	51	Vermouth NT	2, 4, 22
Medeot DB	42	Rivarola VA	18, 80, 83	Viale S	13
Mercado SE	86	Rodríguez C	12	Vicario J	59
Meringer V	60	Rodríguez E	83	Villanova J	71
Merkis C	37, 38, 39	Rodríguez N	23, 24	Villasuso AL	61
Micalizzi B	49	Rodríguez PEA	84	Vincenti LM	1, 52
Milla L	83	Rojas E	16	Vissio C	73
Moreira A	73, 74	Romero-Fernandez I	5, 6, 7	Vivas A	73, 74
Moreno D	48	Rosa EB	86		
Mori G	75	Rosa JR	L-S 5	<b>W</b>	
Morra G	15	Rotondo J	34, 35	Williamson D	39, 81
Moschetti E	32, 37	Rubiales de Barioglio S	1	Willnecker A	13
Mouguelar H	50	Rubiolo E	25, 54, 56		
		Rubiolo ER	55	<b>Y</b>	
<b>N</b>		Ruiz F	68, 69	Yslas EI	18
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Navarro F	50, 87	Rumie Vittar B	80	<b>Z</b>	
Niebylski A	26, 27, 28, 29, 30			Zamarrón A	83
Nigra A	80	<b>S</b>		Zanon S	76
Nuñez S	51, 80	Sabini C	76	Zielinski G	50, 87
		Sabini L	76	Zubeldia D	34, 35
<b>O</b>		Sacchetta R	23	Zunino MP	46
Olmedo R	46	Salinas V	15	Zygadlo JA	46
Ortiz M	5, 6, 7	Salomón OD	L-S 5		