



**ROSARIO BIOLOGY SOCIETY**  
(SOCIEDAD DE BIOLOGÍA DE ROSARIO)

Abstracts from the  
**VIII CONGRESS – XXVI ANNUAL MEETING**

December, 5-7, 2006

Sede de Gobierno de la Universidad Nacional de Rosario  
Rosario, ARGENTINA

**Abstracts were revised by a Scientific Committee**

**Board of the Rosario Society Biology 2006**

President *Stella M. Pezzotto*  
Vicepresident *Bibiana Riquelme*  
Secretary *Viviana Rozados*  
Prosecretary *Patricia Silva*  
Treasurer *Héctor Dávila*

**Members**

*Roxana Zorzoli*  
*Claudia Biondi*  
*Roberto Figallo*  
*Guillermo Pratta*  
*Dora Dapino*  
*Tomás Tellez*

**Governmental sponsors**

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)  
Agencia Nacional de Promoción Científica y Tecnológica  
Fondo para la Investigación Científica y Tecnológica (FONCYT)

**Private sponsors**

Fundación Josefina Prats, Fundación del Gran Rosario

**Collaborators**

Wiener Lab, Editorial Corpus, Café La Virginia SRL, Establecimiento La Cumbre, Alfajores Gayalí

### 1. LUNG CANCER AND ALCOHOL CONSUMPTION

Pezzotto SM<sup>1,2</sup>, Dagatti MS<sup>2</sup>, Bertola Compagnucci A<sup>2</sup>, Poletto L<sup>1,2</sup>.  
<sup>1</sup>Consejo de Investigaciones, <sup>2</sup>Instituto de Inmunología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario.

A case-control study was carried out. Cases (n=87) were men with lung cancer (LC) histological diagnosis. Controls (n=153) were patients attending at the same institutions for pathologies unrelated to LC risk factors. Age, smoking habits (non smokers were excluded), and occupation were investigated. Alimentary habits were studied applying a food-frequency-quantitative questionnaire. Daily mean alcohol grams ingested were calculated to analyze its possible association with LC risk. A generalized linear model, adjusted by total energy consumed (Kcal), was applied to evaluate mean alcohol consumption differences between cases and controls. Odds Ratios (OR) and 95% confidence intervals (CI) were also calculated by logistical regression analysis. Alcohol was not consumed by 10.3% of cases and 19.6% of controls (p=0.06). For consumers, alcohol averages ( $\pm$ standard error) daily grams were 94.1 $\pm$ 9.1 for cases and 86.9 $\pm$ 7.3 for controls (p=0.53). However, an increased LC risk was found in high consumers, after controlling for cigarette consumption, occupation and energy consumed.

Daily grams of alcohol	OR	IC	P
Non consumers	1.0	-	-
< 6	0.8	0.1-4.8	0.137
6-130	2.1	0.9-4.8	0.103
> 130	5.3	1.7-16.4	0.004

Although smoking habit and occupational exposure are the main LC risk factors, a high alcohol consumption also increases this risk.

### 2. SKIN CANCER NON MELANOMA: RISK FACTORS

Dagatti MS<sup>1</sup>, Cesarios GMP<sup>1</sup>, Poletto L<sup>1,3</sup>, Pezzotto SM<sup>1,3</sup>.  
<sup>1</sup>Instituto de Inmunología, Fac. Cs. Médicas, UNR. <sup>2</sup>Policlínico PAMI I, Rosario. <sup>3</sup>Consejo de Investigaciones, UNR.

To evaluate Basal Cells (BCC) and Squamous Cells Carcinomas (SCC) risk factors, a case-control study was carried out. Cases were histologically confirmed, and controls were patients with other diseases. Information on socio-demographic data, pheno-typic characteristics, skin cancer family history, sun exposure habits, sunburns history, previous skin lesions, and dietary habits, were obtained by interview. 22 cases and 35 controls were studied (mean age: 65.1 $\pm$ 15.1 and 64.5 $\pm$ 12.3). 31 tumors were diagnosed (BCC:24, SCC:7), only 4 were in sun unexposed areas. Skin color types: I 50%, II 31.8%, III 18.2%, IV 0% for cases, and 28.6%, 34.3%, 22.9%, and 14.3% for controls. Hair color: clear 54.6%, red 4.5%, dark 40.9% for cases, and 48.6%, 0% and 51.5% for controls. Eyes color: clear 45.5%, dark 54.5 for cases, and 37.1% and 62.9% for controls. 45% of cases and 42.7% of controls had sunburns antecedents. 72.7% of cases and 37.1% of controls had actinic keratosis, and 27.3% of cases and no controls had more than 10 naevi. 22.7% of cases and 5.9% of controls had skin cancer family history. Vitamin A consumption was high in 18.2% of cases and 28.6% of controls. Vitamin E consumption was high in 18.2% of cases and 34.3% of controls. 27.3% of cases and 31.4% of controls had high vitamin C consumption. When adjusting a multiple logistic regression model to estimate odd ratios, the possibility to have BCC or SCC was higher in patients with 10 or more naevi (p=0.03), actinic keratosis (p=0.05), vocational sun exposure (p=0.04), and smaller in those high vitamin C ingestion (p=0.04).

### 3. GENERALIZED MIXED LINEAL MODELS USE FOR HIP FRACTURE INCIDENCE RATES ESTIMATION

Hachuel L, Boggio G, Pezzotto SM.  
 CIC, Consejo de Investigaciones, UNR.

Osteoporosis is the most frequent bone-metabolic disease, and hip fractures are the most significant consequences of this disorder. A survey was carried out in Rosario to collect all hip fracture cases in the period August 2001-July 2002, with the purpose of determining the incidence of this pathology (Osteoporosis International 2005, Morosano, Masoni, Sanchez). During this period, 576 cases were diagnosed. They were classified according to sex (male: n=95, female: n=450), age group (50-54: n=6; 55-59: n=7; 60-64: n=18; 65-69: n=47; 70-74: n=78; 75-79: n=120; 80-84: n=166;  $\geq$ 85: n=134) and municipal district (North: n=105; Center: n=245, West: n=38; South-west; n=34; North-west: n=71; South: n=83). With these cases and the population at risk, also classified according to sex, age group and district, a contingency table was formed. This table was sparsed, because certain combinations of categories of the considered variables had null frequency. The purpose of this work was to replace this observed distribution with another that preserves the main aspects of the data avoiding the attributable irregularities due to the sampling method. This "softened" estimation involves the adjustment of a mixed generalized lineal model supposing Poisson distribution for the number of cases, and taking as "offset" the population of risk. Age, sex and municipal district were considered as main effects, and a specific random component for each cell of the table was used. This method allowed to obtain no null specific hip fracture incidence rates for those covariables combinations where the observed number of cases was zero. Agreement between observed and expected rates was high (r= 0.96).

### 4. MAXIMUM LIKELIHOOD ESTIMATION IN BIOLOGICAL DATA SETS WITH MISSING INFORMATION

Badler C, Alsina S, Puigsubirá C, Vitelleschi MS.  
 Inst. de Investigaciones Teóricas y Aplicadas de la Escuela de Estadística. Fac.de Cs. Económicas y Estadística.UNR. E-mail: cpuigsu@fcecon.unr.edu.ar

The problem of missing and/or messy information in data sets obtained from experiments or observational studies in the biological area, has become a constant worry for the researchers and is known the lack of rigorosity if the work is done only with the complete observations when the percentage of loss is high and/or when they were not lost completely at random. If the purpose is to estimate the mean and covariance matrix of the variables of a data set with these characteristics, a strategy is the maximum likelihood estimation that incorporates all the available information. Nowadays the implementation of this methodology is facilitated by the availability of many softwares. In this work is performed the maximum likelihood estimation of the mean and covariance matrix of the variables of a data set of diabetes children and young adults, that have been assisted in public and private institutes of Rosario city, during the year 2003. These estimations were obtained by the «Sweep» operator with a macro of the SAS software and the Expectation-Maximization algorithm (EM) with the PROC MI module of the same software. As the original data set was completely observed and the methodology presented is for those data sets with missing and/or messy information, missing observations were generated at random in three variables in approximately a 10, 15 and 30% of the units of the data set. The estimations obtained were compared with those from the complete data set.

## 5. CARE PROVIDED TO PRESERVE DENTAL HEALTH IN THREE ROSARIO CITY SUBPOPULATIONS

Kohli A<sup>1</sup>, Pezzotto SM<sup>2</sup>, Poletto L<sup>2</sup>.

<sup>1</sup>Cát Histología, Fac. Odontología. <sup>2</sup>Consejo de Investigaciones. UNR. E-mail: alicia kohli@hotmail.com

Odontological procedures to recommend the patients to carry out are: a control visit every six months, to restore damaged dental pieces, to delay posterior pieces extraction, and to perform the tooth daily hygiene. Our aim was to know odontological care fulfilled for dental pieces conservation in three young adults subpopulations of our city. A standardized anamnesis was applied: age, sex, service type, time passed from last visit, piece extracted the day of interview, treatments fulfilled to preserve it, and brushing habit. Tests applied were: Student's test, analysis of the variance and exact probability of Fisher, with a 5% significance level. A total of 517 patients were incorporated, 65% females and 35% males, from a private center (G1, 19%) and two publics (81%) one from a central area (G2) and the other from an extremely poor one (G3). Time passed from last visit was shorter in G1 ( $p=0.04$ ), whose patients did a higher proportion of endodontic treatments, restaurations and protheses trying to prolong tooth life ( $p<0.001$ ). Premolars and molars were the most extracted in all of the three services, but in a minor proportion in G1 ( $p=0.004$ ). The daily brushing habit was 84% in G1, 67% in G2 and 69% in G3 ( $p=0.04$ ). The only once a day brushing was 27% in G1 and G3, and 22% in G2; from these patients 68% in G1, 47% in G2 and 51% in G3 do it before breakfast, which is a not correct modality. In the private service time elapsed between visits was shorter and a higher proportion of restorations was done to preserve the dental pieces. The daily brushing habit, in the correct frequencies and schedules was similar in all of the three studied services.

## 6. CYTOLOGICAL STUDY OF THE ORAL MUCOSA IN SMOKERS ADULTS

Kohli A, Postiglione G, Pilafis M, Espejo T, López C, Escovich L.

Cytological study of oral cells is a non-aggressive technique that is well accepted by the patient, and is therefore an attractive option for the early diagnosis of oral cancer, including epithelial atypia and squamous cell carcinoma. The aims of this study were to verify the existence of cytomorphological changes in the epithelial cells of the oral mucosa as well as *Candida*'s presence and bacteria in the exfoliative cytology in smoker patients. The samples were obtained from smokers patients that voluntarily came to be examined during the Campaign for early Detection of Oral Cancer, organized by Oral Medicine Service, School of Dentistry, Rosario University, Argentina, selected and that gave the assent informed to be included in this study. The cells were fixed and stained with hematoxylin-eosin and PAS. They were observed with optical microscope to 450 increases. Results: 74 smoking patients were included, with an average of 46 years of age (range 21-81 years); 42% of the male sex and 58%, female. The superficial cells appeared in all the samples, where as the intermediate, parabasals and basals cells were observed in 77%, 42% and 30% respectively. It was observed 34,6% nuclear in cellular division, 13% with nuclear atypias, 7,7% with out nucleus cells and 3,8% smaller nuclei. 87% of the cells had cytoplasm with granules. It was also observed a) blood cells, b) fungi and bacteria. They were detected lymphocytes in 25 % of the samples. The yeasts appeared in a major frequency (36%) with regard to the bacteria (5%). The patients with nuclear atypias (10/74), were *Candida*+ 40% ( $p<0.05\%$ ). Conclusion: The cytological study of oral cavity is suitable method for routine application in population screening programmes, for early analysis of suspect lesions, and for pre-and post-treatment monitoring of confirmed malignant lesions.

## 7. THE IMPACT OF CHEST THERAPY ON VENTILATORY MECHANICS IMMEDIATELY POSTOPERATIVELY OF CARDIOVASCULAR SURGERY

Forcher A, Converso G, Manago M, Vadell D.

E-mail: aforcher@fgr.org.ar

This study analyses the changes in mechanical ventilation correlating the forced vital capacity (FVC) with the forced expiratory volume in the first second ( $FEV_1$ ), the coughed peak flow (CPF) and the maximum inspiratory and expiratory pressures (MIP and MEP) in the preoperative (PreO) and immediate postoperative (PosO) of cardiovascular surgery and following chest therapy (CT). Twenty patients (14 men; mean age 61.1; SD= 8.2) who had undergone programmed cardiac surgery, signed informed consent upon entry to the trial. Exclusion criteria: patients with severe COPD, asthma, pulmonary fibrosis and previous thoracic surgery. Measurements were taken of FVC,  $FEV_1$ , CPF, MIP and MEP in the PreO and first day PosO, in sitting position and following a CT session (respiratory exercises, sniffing, forced inspiration with inspiratory stimulation maneuvers, assisted expiration, assisted cough, huffing). Neither sedation nor bronchodilators between measurements in the PosO were administered. The Pearson's Correlation coefficient ( $r$ ) was calculated. In the PreO significant correlations between FVC vs.  $FEV_1$  ( $r=0.842$ ;  $p<0.001$ ); CPF ( $r=0.416$ ;  $p=0.034$ ); MEP ( $r=0.421$ ;  $p=0.032$ ) were observed. In the sitting position (PosO), significant correlations were registered between FVC vs.  $FEV_1$  ( $r=0.899$ ;  $p<0.001$ ); MIP ( $r=0.49$ ;  $p=0.014$ ) and MEP ( $r=0.455$ ;  $p=0.022$ ). Following CT, significant correlations were found between FVC vs.  $FEV_1$  ( $r=0.783$ ;  $p<0.001$ ); MIP ( $r=0.412$ ;  $p=0.035$ ) and MEP ( $r=0.602$ ;  $p=0.003$ ). These data suggest that the impact of CT on mechanical ventilation is primarily related to its greater correlation between FVC and MEP.

## 8. ALTERED CORTISOL/DHEA RATIO IN TUBERCULOSIS PATIENTS AND ITS RELATIONSHIP WITH THE SPECIFIC IN VITRO IMMUNE RESPONSE (IR)

Bozza V<sup>1</sup>, D'Attilio L<sup>1</sup>, Mahuad C<sup>1</sup>, Didoli G<sup>1</sup>, Giri A<sup>2</sup>, Bottasso O<sup>1</sup>, Bay M<sup>1</sup>.

<sup>1</sup>Instituto de Inmunología, FCM; <sup>2</sup>Area Virología, (IBR-CONICET) FCByF; UNR.

Tuberculosis (TB) is the main cause of death induced by an infectious agent. We have shown that Cortisol (GC) inhibited blastogenesis and interferon gamma ( $IFN-\gamma$ ) production in cultured peripheral blood mononuclear cells (PBMC) from TB patients; these effects being not reversed by dehydroepiandrosterone. (DHEA). We have now studied the relationship between GC and/or DHEA plasma levels and *in vitro* IR of PBMC to *Mtb* sonicate (ST), in HIV (-) adult males with pulmonary TB ( $n=25$ ) and age- and sex-matched healthy controls (Co  $n=14$ ). PBMC were cultured for 36 h or 96 h with ST (10 $\mu$ g/ml) for measurement of  $IFN-\gamma$  and IL-10 in culture supernatants (ELISA, pg/ml). Proliferative response was determined as <sup>3</sup>Hthymidine incorporation (cpm: count per minute). TB patients showed a greater IL-10 production (means $\pm$ sem, (152.7 $\pm$ 22.2), but lower proliferation (cpm: 7331 $\pm$ 1790) and  $IFN-\gamma$  synthesis (72.7 $\pm$ 14.9) respect to Co (IL-10: 71.3 $\pm$ 14.2,  $p<0.025$ ; cpm: 21067 $\pm$ 6178,  $p<0.01$ ;  $IFN-\gamma$ : 314.5 $\pm$ 127,  $p<0.05$ ). Plasma levels of GC and DHEA were found increased and decreased in TB patients (233.1 $\pm$ 15.1 and 3.54 $\pm$ 0.36 nM, respectively), with an augmented Cortisol/DHEA ratio (78.7 $\pm$ 6.9) if compared to Co (GC: 182 $\pm$ 14.9,  $p<0.05$ ; DHEA: 10.9 $\pm$ 1.2,  $p<0.0001$ ; Cortisol/DHEA: 20.37 $\pm$ 3.6,  $p<0.001$ ). Plasma DHEA levels were positively correlated with  $IFN-\gamma$  values ( $r=0.44$ ,  $n=20$ ,  $p<0.05$ ), whereas an inverse correlation was found between the cortisol/DHEA ratio and  $IFN-\gamma$  levels ( $r=-0.65$ ,  $n=20$ ,  $p<0.01$ ). The balance between GC and DHEA may partly account for the immune perturbations seen in TB.

**9. INTERLEUKIN-6 (IL-6), CORTISOL (GC) AND DEHYDROEPIANDROSTERONE-SULPHATE (DHEA-S) LEVELS IN HEALTHY CONTACTS OF PATIENTS WITH PULMONARY TUBERCULOSIS**

Bozza V, Rico M, Marcipar F, Rascón A, D'Attilio L, Dídoli G, Bottasso O, Bay ML.

Instituto de Inmunología, Facultad de Ciencias Médicas, UNR.

In earlier studies we demonstrated that peripheral blood mononuclear cells (PBMC) from patients with progressive TB displayed a reduced proliferation in relation to controls (Co). At the plasma level, TB patients had increased and decreased GC and DHEA concentrations, this imbalance being significantly correlated with IL-6 values. To ascertain whether these abnormalities were also present during sub-clinical infection, healthy subjects heavily exposed to *Mycobacterium tuberculosis* (Mtb), -healthy contacts, HC- were studied. The sample comprised 10 HC, 8 TB and 6 Co, sex and age matched. PBMC were stimulated with whole sonicated of Mtb during 5 days. Plasma levels of GC, DHEA-S (Chemiluminescence, nM) and IL-6 (ELISA, pg/ml) were measured, as well. Results: IL-6, median(rank), TB: 21.2(11-39); HC: 10.0(6-19), Co 13.1(4-24),  $p < 0.05$ ; GC: TB 743.6(312-1120), HC 491.1(221-905),  $p = 0.05$ , Co 560.1(513-701); DHEA-S TB 221.1(39-404) HC 279.1(69-655),  $p > 0.05$  Co 452.5(158-615). GC/DHEA-S ratio: TB 3.4(1-11); Co 1.3(0.9-4),  $p < 0.03$ , HC 1.5(0.5-13),  $p < 0.05$ . HC displayed the highest mycobacteria-driven proliferation (counts per minute -cpm- of stimulated cultures/ cpm of cultures without stimulation) 33.4(13-167) if compared to TB 21.6(1-73), or Co 14.5(5-69),  $p < 0.01$  vs. HC. These results indicate that HC presents a proper cellular specific immune response with a preserved immunoendocrine relationship.

**10. OBTAINING AND PRELIMINARY STUDIES OF A SULFATHIAZOLE NITROSO DERIVATIVE**

Pontoriero A<sup>1</sup>, Trossero C<sup>1</sup>, Hure E<sup>1</sup>, Monti L<sup>1</sup>, Mosconi N<sup>1</sup>, Gorr C<sup>1</sup>, Azum Y<sup>1</sup>, Caffarena G<sup>2</sup>, Rizzotto M<sup>1</sup>.

<sup>1</sup>Área Inorgánica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531; <sup>2</sup>Cátedra de Microbiología, Facultad de Ciencias Médicas, Av. Francia y Santa Fe. UNR, 2000 Rosario. E-mail: mrizzott@fbioyf.unr.edu.ar

Nitrosoderivatives, formed in stomach by reaction between amines or amides -from diet- and nitrites -food preservative-, might damage DNA leading to mutation and/or cancerous processes. Sulfa drugs, common use antibiotics, have such functions in their molecules. We have previously found mutagenic behavior in sulfa drug-nitrite mixtures in acid medium. An orange solid was obtained by adding an aqueous solution of NaNO<sub>2</sub> to a chloridric solution of NaST ([NaNO<sub>2</sub>]/[NaST] = 5/1; [HCl] ≈ 1 M). At 230°C the compound breaks down, carbonizing itself completely at ≈ 260°C, so, it was not possible to determine melting point. Spectroscopic (<sup>1</sup>H-RMN, IR, UV-Vis) and mutagenic (Ames' Test) studies were carried out with the isolated solid. All <sup>1</sup>H-RMN signals were modified respect to the sulfathiazole one. IR signals for -SO<sub>2</sub> groups remain the same (1283 y 1146 cm<sup>-1</sup>), while new ones appear which could be attributed to the N-N and NNO groups. Isolated compound's and mutagenic mixtures' electronic spectrum patterns were similar, suggesting that such compound would be responsible for the mixture spectrum. Ames' Test: with the *Salmonella typhimurium* TA98 strain the nitrosocompound showed mutagenic behavior from 6 ug/plate. Results found so far suggest that the isolated solid might be one of the responsables for the mutagenicity of the sulfathiazole-nitrite-HCl mixture.

**11. ANTIFUNGAL ABILITY OF PHTHALYL-SULFATHIAZOL-COBALTO COMPLEXES: TOXICITY AND ACTION MECANISMS' TESTS**

Monti L<sup>1</sup>, Hure E<sup>1</sup>, Trossero C<sup>1</sup>, Pontoriero A<sup>1</sup>, Mosconi N<sup>1</sup>, Gorr C<sup>1</sup>, Azum Y<sup>1</sup>, Zacchino S<sup>2</sup>, Juárez S<sup>2</sup>, Rizzotto M<sup>1</sup>.

Áreas <sup>1</sup>Inorgánica y <sup>2</sup>Farmacognosia, Fac. de Ciencias Bioquímicas y Farmacéuticas, UNR, Suipacha 531, 2000 Rosario. E-mail: mrizzott@fbioyf.unr.edu.ar

During last decades there was a large increment of human fungal infections. Several antifungals show toxicity and resistance reducing the therapeutic options. In general, metal complexes of sulfa drugs enhance biological properties of the free ligand. Several sulfa-cobalt complexes were assayed in our laboratory showing moderate antifungal ability, *minimum inhibitory concentration* (MIC): 62.5-250 ug/mL. [Co<sup>II</sup>(HFST)Cl(H<sub>2</sub>O)<sub>4</sub>] and [Co<sup>III</sup>(FST)Cl(H<sub>2</sub>O)<sub>3</sub>] complexes were selected to test mechanisms of action. (FST: phthalylsulfathiazolate). Human opportunist pathogens used were: *C. albicans*, *C. tropicalis*, *S. cerevisiae*, *C. neoformans*, *A. fumigatus*, *A. flavus*, *A. niger*, *T. rubrum*. Assays of *N. crassa*, sorbitol and ergosterol performed were negative so it is possible to disregard all mechanisms that have to do with inhibition of fungal cell walls' biosynthesis and ergosterol binding. Toxicity for human cells was tested by the erythrocyte hemolysis assay. No toxicity was detected at concentrations in which they are active. It is important to remark that none of the fungi tested was sensitive to the free ligand (phthalylsulfathiazolate) and that for each fungus we obtained the same MIC value with both complexes. This suggests that activity could be due to the cobalt been complexed regardless of its oxidation state.

**12. DIFFERENT WAYS OF CHALLENGE INDUCED SIMILAR CHANGES IN CD25-POSITIVE CELLS POPULATION IN LUNG FROM AN ANIMAL MODEL OF OVALBUMIN SENSITIZATION**

Chaparro S, Ruiz MI, Fontanella GH, Bassan N, Vinuesa MA.

Cátedra de Histología y Embriología. Fac. de Cs. Médicas. UNR.

Most of foreign challenges confronting mammals, including microbes and soluble antigens, make contact with mucosal surfaces. To carry out this function, the mucosal immune system has developed complex and dynamic mechanisms involving many structurally different tissue compartments. In previous works we demonstrated that rabbit is a useful animal model to study local allergic response in respiratory tract. The aim of the study is to analyze modifications in the distribution and number of CD25+ cells population in lungs. Adult male New Zealand rabbits were divided in 4 groups (n: 6 each). G1: control; G2: subcutaneous sensitized; G3: subcutaneous sensitized and oral challenged; G4: subcutaneous sensitized and inhaled challenged. Animals were subcutaneously sensitized twice, with 15 days interval, with 2 ml of 70 ug ovalbumin (OVA) in 30 mg ALUM/ml. G3 rabbits were challenged fifteen days after the second sensitization by an intragastric cannula with 150 mg OVA in 20 ml saline. G4 rabbits received a single nebulization of OVA (1%). Sensitization was determined by Passive Cutaneous Anaphylaxis Test (PCA). Samples were obtained 4 hours (inhalation route) and 24 hours (oral via) after challenge and processed for immunohistochemistry using anti CD25 monoclonal antibody to IL-2 receptor  $\alpha$ -chain, considered as an activation receptor. 20 microscopic fields at 400 X per animal were counted, and expressed as the median (range) of positive cells. Results: G1: 8 (13-4); G2: 7 (11-4); G3: 32 (40-1) and G4: 37 (44-25), G3 and G4 vs G2  $p < 0.005$ . These results showed a functional interface between respiratory and gastrointestinal tract.

### 13. EFFECTS ON NEUROENDOCRINE CELL POPULATION OF RESPIRATORY TRACT FROM OVALBUMIN SENSITIZED AND ORAL CHALLENGED NEW ZEALAND RABBITS

Fontanella GH, Krumrick GE, Bassan ND, Vinuesa MA.  
Cátedra de Histología y Embriología. Fac. de Cs. Médicas. UNR.

In previous works on digestive tract ovalbumin (OVA) administration (2 ml 70 ug OVA + 30 mg ALUM/ml, twice, subcutaneous route) induced high levels of specific anti-OVA IgE antibodies (PCA test). Subsequent contact with orally administered antigen (15 days after second sensitization: 150 mg OVA in 20 ml saline) produced an acute anaphylactic inflammatory response with mast cell degranulation, edema, lymphocyte and eosinophil infiltration. Here we analyzed a possible compromise of neuroendocrine cell population in response to sensitization and later oral challenge with OVA in non related digestive tract organs such as lungs, larynx, and trachea. Rabbits were divided in 3 groups (n=6, each): G1: control; G2: sensitized; G3: sensitized and orally challenged. Samples were obtained 24 hs after challenge and processed for HE and histochemistry (Pascual technique). Neuroendocrine cells were counted as number of positive cells per 100 epithelial cells. Lung: G1: 0(2-0), G2: 3(6-2), G3: 1(2-1); larynx: G1: 1(3-1), G2: 4(9-3), G3: 2(3-0) and trachea: G1: 1(2-0), G2: 3(5-2), G3: 1(2-0). In all cases G2 vs. G1, G3  $p < 0.005$ . Neuroendocrine cell increase in G2 would be related to intracellular granules enhance and posterior degranulation in G3 or differentiation or replication of stem cells. This let us establish a functional interface between immune mucosal system of the respiratory and gastrointestinal tracts.

### 14. MODIFICATIONS OF MAST CELL AND EOSINOPHIL POPULATIONS IN TURBINAL MUCOSA FROM OVALBUMIN (OVA) SENSITIZED AND CHALLENGED RABBITS, PREVIOUSLY TREATED WITH THE ANTILEUKOTRIENE MONTELUKAST (Mk)

Caviglia N, Prestigiovanni G, Fontanella G, Bassan ND, Vinuesa MA.  
Cátedra de Histología y Embriología. Fac. de Cs. Médicas. UNR.

It is proven that subcutaneous sensitized rabbits with OVA (70 ug + 30 mg ALUM/ml, two times, 15-day interval) and challenged orally with the same antigen (15 days after the second sensitization), induced quantitative modifications on lymphoid cellular populations, mast cells (Mc) and eosinophils (Eo) distributed on the respiratory mucosa. Mk is a leukotriene D4 receptor antagonist that has been found to be an inhibitor of the immune allergic response in different human and animal models. The aim of this study was to quantitatively evaluate the recruitment of Mc and Eo on turbinal mucosa from sensitized and orally challenged rabbits with OVA after treatment with Mk. Adult male New Zealand rabbits were divided in 4 groups (n=6 each): Group 1 (G1): control, G2: sensitized, G3: sensitized and challenged and G4: idem G3 plus treated with single dose of Mk 4 hours before challenge. Samples of turbinal mucosa were processed and stained with HE, Alcian blue PH<1 (Mc) and Chromotrope II-R (Eo). Mc: [median (range)] G1: 0 (1-0); G2: 1 (3-1); G3: 0(1-0) and G4: 1(1-0) (G4 vs G3  $p > 0.005$ ). Eo: G1 1(1-0); G2: 2 (6-1); G3 6(11-4) and G4: 2(5-1) (G4 vs G3  $p < 0.005$ ). We conclude that previous treatment with Mk did not modify Mc response to the oral challenge with OVA. However, the number of Eo on treated animals (G4) was similar to those found on sensitized animals without challenge (G2). This finding would be related to a partial inhibition of the allergic inflammatory response produced by the antagonist D4.

### 15. HEMATOCRIT VALUES IN EXPERIMENTAL HYBRIDS OF FREE RANGE BROILERS WITH CORNISH GENES: A DYNAMIC APPROACH

Fernández R<sup>1</sup>, Rozados VR<sup>1</sup>, Font MT<sup>2,3</sup>, Dottavio AM<sup>1,3</sup>, Di Masso RJ<sup>1,2,3</sup>.  
<sup>1</sup>Cátedra de Genética. Facultad de Ciencias Veterinarias; <sup>2</sup>Instituto de Genética Experimental, Facultad de Ciencias M; <sup>3</sup>CIC-UNR.

Meat type chickens have been intensively selected for rapid growth without a concomitant increase in the rate of development of respiratory and cardiovascular systems, causing changes in other physiological parameters. High yielding meat lines often experience metabolic stress in form of hypoxemia, resulting from a high blood oxygen demand by the rapid developing tissue mass. In response to such metabolic demands, broilers usually have higher values for packed cell volume or hematocrit (PCV). Campero INTA chickens grow slower than commercial broiler strains. As White Cornish is a breed used as paternal genotype in commercial broilers, the aim of this work was to measure the PCV in two experimental hybrids with White Cornish paternal genotype and Barred Plymouth Rock (CP) or Rhode Island Red (CR) maternal genotype and in Campero INTA (reference genotype). Six males of each genetic group were weekly sampled from 7 to 70 days of age. Statistical significant genotype ( $P = 0.03$ ) and age ( $P = 0.0003$ ) effects jointly with a non significant genotype x age interaction effect ( $P = 0.12$ ) were evident for PCV while all the effects were significant ( $P < 0.0001$ ) for pre-slaughtered body weight. Age effect on PCV was not associated with a particular trend. On the other hand, PCV was higher in Campero INTA ( $31.6 \pm 0.61$ ) vs CP ( $30.6 \pm 0.46$ ) and CR ( $30.6 \pm 0.24$ ). Campero INTA birds showed a higher body weight than both experimental hybrids during the experiment, a difference that can explain their higher PCV value. It is concluded that the inclusion of Cornish genes would not produce a metabolic stress compatible with a modification in PCV values.

### 16. BREAST PROPORTION IN TWO EXPERIMENTAL HYBRIDS OF FREE RANGE BROILERS WITH CORNISH GENES: A TRANSVERSAL ANALYSIS

Romera BM<sup>1</sup>, Librera JE<sup>1</sup>, Varela D<sup>1</sup>, Font MT<sup>2,3</sup>, Dottavio AM<sup>1,3</sup>, Di Masso RJ<sup>1,2,3</sup>.

<sup>1</sup>Cátedra de Genética, Facultad de Ciencias Veterinarias; <sup>2</sup>Instituto de Genética Experimental, Facultad de Ciencias Médicas; <sup>3</sup>CIC-UNR.

The improvement of the final product quality is one of the main objectives in poultry meat production. That is the reason why breeders try to increase not only growth rate but also the percentage of prime parts like breast meat. White Cornish is a breed used as paternal genotype in commercial broilers to improve daily body weight gains and body conformation. The aim of this study was to evaluate the contribution of this breed to breast muscle yield in the carcass of slow-growing free range broilers. Percentage breast meat of two experimental hybrids with White Cornish paternal genotype and Barred Plymouth Rock (CP) or Rhode Island Red (CR) maternal genotype was compared with that of a reference genotype (Campero INTA). Six males of each genetic group were weekly slaughtered from 7 to 70 days of age. The statistical significance of genotype, age and genotype x age interaction effects was assessed by ANOVA. Significant age ( $P < 0.0001$ ) and genotype ( $P = 0.047$ ) effects jointly with a non significant ( $P = 0.66$ ) genotype x age interaction effect were observed. The age effect indicates a growing contribution of breast muscle weight to body weight when birds grow to maturity. This response was higher in both CP and CR birds than in the reference genotype showing a positive contribution of Cornish genes introgression in experimental hybrids of free range chickens proposed as a productive alternative to Campero INTA chickens which is independent of birds' age.

# 17. **ABDOMINAL FAT IN TWO EXPERIMENTAL HYBRIDS OF FREE RANGE BROILERS WITH CORNISH GENES: A TRANSVERSAL ANALYSIS**

Martínez A<sup>1</sup>, Advínculo S<sup>1</sup>, Dottavio AM<sup>1,3</sup>, Font MT<sup>2,3</sup>, Di Masso RJ<sup>1,2,3</sup>.

<sup>1</sup>Cátedra de Genética, Facultad de Ciencias Veterinarias; <sup>2</sup>Instituto de Genética Experimental, Facultad de Ciencias Médicas; <sup>3</sup>CIC-UNR.

In poultry meat production abdominal fat is being regarded as one of the main sources of waste in the slaughterhouse. This depot is a good estimator of total fat weight and represents approximately 3.5% of live body weight. Commercial selection of meat type chickens increased both body weight gain and carcass fat content. As White Cornish is a breed used as paternal genotype in commercial broilers the aim of this study was to evaluate its contribution to the relative content of abdominal body fat in slow-growing free range broilers. Percentage abdominal fat of two experimental hybrids with White Cornish paternal genotype and Barred Plymouth Rock (CP) or Rhode Island Red (CR) maternal genotype and Campero INTA (reference genotype) was compared. Six males of each genetic group were weekly slaughtered from 7 to 70 days of age. The statistical significance of genotype, age and genotype x age interaction effects was assessed by ANOVA. A significant ( $P < 0.0001$ ) age effect consistent with the experimental evidence of increasing fatness as chickens grow to maturity, jointly with non significant effects of genotype ( $P = 0.1511$ ) and genotype x age ( $P = 0.2076$ ) effects were observed. These results show that the introgression of Cornish genes in experimental hybrids of free range chickens proposed as a productive alternative to Campero INTA birds, does not carry on undesirable effects in terms of body fat content.

# 18. **GROWTH CURVE PARAMETERS IN EXPERIMENTAL HYBRIDS OF FREE RANGE BROILERS WITH CORNISH GENES**

Álvarez M<sup>1</sup>, Dottavio AM<sup>1,4</sup>, Canet ZE<sup>1</sup>, Antruejo AE<sup>2</sup>, Font MT<sup>3,4</sup>, Di Masso RJ<sup>1,3,4</sup>.

Cátedras de <sup>1</sup>Genética y <sup>2</sup>Producción Animal I, Facultad de Ciencias Veterinarias; <sup>3</sup>Inst. Genética Experimental, Facultad de Ciencias Médicas; <sup>4</sup>CIC-UNR.

Broilers are slaughtered at a target body weight being the main difference among available commercial genotypes their early growth rate and, consequently, their age at slaughter. Non linear growth curve models can be used to fit weight-age data so growth pattern can be described by growth curve parameters. Free range broilers are characterized by their lower daily weight gain if compared to commercial broilers. In this study growth of two experimental hybrids with White Cornish as paternal genotype and Barred Plymouth Rock (CP) or Rhode Island Red (CR) as maternal genotype was compared to a reference genotype (Campero INTA) in terms of two parameters of the Gompertz function: asymptotic weight (A) and maturing rate (k). The expected negative relationship between both parameters was corroborated. Campero INTA showed a higher A value ( $3690 \pm 89$ g) and a lower k value ( $0.2619 \pm 0.0052$ ) than CP ( $2782 \pm 75$ g;  $0.3071 \pm 0.0088$ ) and CR ( $2716 \pm 42$ g;  $0.3066 \pm 0.0064$ ). No difference was evident between experimental hybrids. Although CP and CR broilers required two additional weeks to reach the prefixed slaughter weight, their growth pattern was compatible with the conditions established in the protocol for free range broiler production. Notwithstanding, as mean asymptotic body weights of CP and CR chickens are very close to the target mean slaughter weight this delay could be important in terms of feed conversion ratio.

# 19. **MURINE MAMMARY TUMOR VIRUS (MMTV) INVOLVEMENT IN SUSCEPTIBILITY TO SPONTANEOUS MAMMARY CARCINOGENESIS IN CBI MICE**

Suárez C<sup>1</sup>, Mundiñano J<sup>3</sup>, Piazzon F<sup>3</sup>, Hinrichsen L<sup>1,2</sup>.

<sup>1</sup>Inst. de Genética Experimental, Facultad Cs. Médicas, <sup>2</sup>CIC-UNR, U.N.R., <sup>3</sup>Div. Medicina Experimental, Academia Nacional de Medicina.

MMTV is a common cause of development of breast tumors in mice and both the exogenous and endogenous variants induce deletion of specific clones of V $\beta$ +T cells in the host. MMTV-like gene sequences have recently been found in human breast cancer tissue; they would be involved in breast cancer aetiology and associated to more aggressive tumors. To determine if the differences in susceptibility to spontaneous breast carcinogenesis observed in lines CBI+ and CBI/C of the CBI-IGE colony were due to exogenous MMTV variants, its presence was evaluated by flow cytometry. T-cell clone deletion was determined in Balb/c litters nursed by CBI+ or CBI/C. Data were expressed as percentage of CD4+ V $\beta$ x+ cells within the CD4+ population of T cells; virus-free Balb/c cells were used as controls. Deletion of V $\beta$ 14+ cells was observed in mice nursed by CBI+ while none was seen in Balb/c nursed by CBI/C, suggesting that the exogenous MMTV variant is absent in this strain. Also, several traits of tumor development were analysed in CBI+ females nursed by virus-free Balb/c (CBI+/s) and compared to those of CBI+, CBI/C and the F1 reciprocal crosses. CBI+/s tumor latency was similar to that of CBI+; the F1s differed between them and from their maternal lines. CBI+/s showed a small but significant decrease in the percentage of mice developing tumors as compared to CBI+; the F1s showed a maternal effect in this trait. Overall, the results suggest that MMTV would partially explain the different susceptibility to spontaneous mammary carcinogenesis observed in CBI+ and CBI/C mice.

# 20. **ANTIANGIOGENIC AND IMMUNOMODULATORY EFFECT OF THE METRONOMIC THERAPY WITH CYCLOPHOSPHAMIDE (CY)**

Rozados VR\*, Mainetti LE\*, Rico MJ, Zacarías Fluck MF, Matar P, Scharovsky OG<sup>1</sup>.

Instituto de Genética Experimental, Facultad de Cs. Médicas, <sup>1</sup>CIC-UNR. \*Contributed equally.

Antitumoral metronomic therapy is characterized by the chronic administration of equally spaced low doses of chemotherapeutic drugs. Conventional chemotherapy with Cy utilizes high doses administered between rest periods and its antitumoral action goes together with high renal, cardiac and hematologic toxicity. We have demonstrated the antitumoral effect without toxicity of the metronomic therapy with Cy. As low dose Cy stimulates the immune response in the rat lymphoma L-TACB model, we decided to investigate the confluency of immunopotentiality and antiangiogenic effects of the metronomic therapy with Cy. L-TACB was inoculated s.c. (day 0) in euthymic rats (e line) and in nude mice. Cy was administered i.p. (10 mg/kg, 3 times/week) from day 7 until day 20. Tumors were measured and on days 0 and 20, the serum concentration of VEGF was determined by ELISA. In e rats, after a slight increase in tumor volume, tumors regressed [mean  $\pm$  SE (mm<sup>3</sup>), day 7:  $641 \pm 189$  vs day 20:  $643 \pm 218$ , NS] On the contrary, in nude mice, tumors grew all over the experiment (day 7:  $138 \pm 66$  vs day 20:  $6855 \pm 532$ ,  $p < 0.001$ ). At the end of the experiment (day 20), VEGF serum concentration in e rats treated with Cy (mean  $\pm$  ES:  $145 \pm 26$  pg/ml) was significant lower than that of control e rats ( $644 \pm 148$ ) ( $p < 0.05$ ). Both preliminary results suggested that immunopotentiality combined with a probable inhibition of angiogenesis would be the mechanism of action of the antitumoral metronomic therapy with Cy. Future experiments will give us more information about these mechanisms.

**21. THE TREATMENT OF MAMMARY ADENOCARCINOMA (M-406) OR LIMPHOMA (L-TACB) BEARING ANIMALS WITH LOVASTATIN (LOV) AND DOXORUBICIN (DOX) MODULATES THE EXPRESSION OF THE PROAPOPTOTIC PROTEIN BAX**

Gervasoni SI\*, Rozados VR\*, Rossa A, Roggero E, Scharovsky O.G. Instituto de Genética Experimental, Facultad de Ciencias Médicas, U.N.R. Rosario, Argentina. \*Contributed equally.

We have demonstrated the antitumoral effect of the combined treatment with LOV + DOX on mouse M-406 and rat L-TACB tumors. The treatment induced an increase in tumor cells apoptosis. Our aim was to study if Bax and Bcl-2 molecules were involved in that process of cellular death. Two experiments were designed: A) M-406 bearing Balb/c mice received: I) Controls without treatment; II) LOV 25mg/kg, 3 times/week, from day 5 on; III) DOX 1mg/kg, twice/week from day 11; IV) Idem II + III. B) L-TACB bearing rats received: I) Controls without treatment; II) LOV 25mg/kg from day 0 through day 4; III) DOX 0.5mg/kg on day 10; IV) Idem II + III. Tumors were measured; on day 21 animals were sacrificed, tumors excised, fixed and paraffin embedded. Bax and Bcl-2 expression was determined by immunohistochemistry. A) Tumor size in group IV on day 21 was lower than in the other groups ( $p < 0.05$ ). Apoptosis increased in groups II, III and IV with respect to group I ( $p < 0.05$ ). B) The results were similar to A. Bax expression was higher in groups A-IV and B-IV compared to their respective controls. No modifications on Bcl-2 expression were observed. The combined treatment with LOV + DOX administered to M-406 and L-TACB bearing animals induced an increase of Bax expression which would be responsible, at least in part, of the enhanced apoptosis observed.

**22. KINETICS OF GALECTINS 1 AND 3 (GAL-1, GAL-3) EXPRESSION IN MURINE LYMPHOMA L-DGE AND ITS MODULATION BY CYCLOPHOSPHAMIDE (CY)**

Zacarias Fluck MF<sup>1,3\*</sup>, Rico MJ<sup>1\*</sup>, Rabinovich GA<sup>2</sup>, Scharovsky OG<sup>1</sup>.

<sup>1</sup>Instituto de Genética Experimental, Facultad de Ciencias Médicas, UNR, <sup>2</sup>Laboratorio de Inmunogenética, Hospital de Clínicas "José de San Martín" UBA. \*Contributed equally.

<sup>3</sup>E-mail: marianozacarias@yahoo.com.ar

We have previously demonstrated that Gal-1 expression increases during rat lymphoma L-TACB progression and that low dose Cy restores tumor and spleen Gal-1 basal levels. Gal-1 and Gal-3 are immunomodulatory glycoproteins. Our aim was to study the kinetics of Gal-1 and Gal-3 expression in tumor and spleen in the murine lymphoma L-DGE model and their modulation by Cy. Balb/c mice were s.c. challenged with L-DGE (day 0). On day 14, half of the animals were inoculated with Cy (25 mg/kg i.p.). Tumor volume was determined and mice were sacrificed and their tumors and spleens excised on days 0, 7, 14 and 21. Gal-1 and Gal-3 expression was determined by Western Blot. <sup>1</sup>ary Tumor: Gal-1 expression increased from day 7 to day 21 (7 vs 21  $p < 0.05$ ); correlation tumor volume-Gal-1:  $p < 0.05$ . Gal-3 expression was similar on days 7 and 21 and lower than on day 14 (7 vs 14 and 14 vs 21,  $p < 0.05$ ). Spleen: Gal-1 expression decreased from day 7 to day 21 ( $p < 0.05$ ) and it was not modulated by Cy. On days 7 and 21, Gal-3 expression was similar and significantly lower than on day 14 (7 vs. 14,  $p < 0.01$ ; 14 vs 21,  $p < 0.05$ ). Conclusions: I) Gal-1 could be considered as an estimator of tumor evolution on lymphomas. II) Gal-1 expression, as previously found in L-TACB, showed in L-DGE a mirrored behavior between tumor and spleen. III) Cy treatment down-regulates Gal-1 expression in lymphomas and do not modifies Gal-3 expression.

**23. MARGINAL SPHINCTER OF TWO SPECIES OF SEA ANEMONES FROM INTERTIDAL OF MAR DEL PLATA: MORPHOLOGICAL AND FUNCTIONAL STUDY**

González Olivera E<sup>1</sup>, Patronelli DL<sup>1</sup>, Zamponi MO<sup>2</sup>.

<sup>1</sup>Lab. Fisiología Animal. Depto. Biología. <sup>2</sup>Lab. Biología Cnidarios. Depto. Cs. Marinas. Fac. Cs. Ex. y Nat. UNMdP. Funes 3250. (7600) Mar del Plata. E-mail: golivera@mdp.edu.ar

The aim of this work is to make the characterization of marginal sphincter to the species *Phymactis clematis* and *Aulactinia marplatensis* from intertidal through their morphological and functional study. **Morphological study:** The specie *P. clematis* has a circumscrip sphincter of palmate type which is between 1,06 to 1,67 mm long and between 0,76 to 1,21 mm wide. This muscle is constituted by a mesogloal axis and several mesogloales subaxis. Axis as well as subaxis give a support to the endoderm which border is smooth. The specie *A. marplatensis* has a circumscrip sphincter pinnate type which length is between 1.21 to 1,76 mm and its wide between 1,06 to 1,36 mm. The axis has a truncated cone shape while in *P. clematis* change of cylindrical to bifurcate shape. **Physiological study:** Both species experiments were carried out using the isolated muscles in sea water. They were stimulated at increasing KCl concentrations ranging from 20 to 200 mM. The results were analyzed in the form of dose-response curves expressed in tension in grams force (gr) vs concentration. Contractil force increases in a sigmoid form to increasing KCl concentrations. In *P. clematis* the maximal developed force obtained with 200 mM KCl was  $2,68 \text{ gr} \pm 0,20 \text{ gr}$  ( $n=22$ ), while in *A. marplatensis* was  $1,04 \text{ gr} \pm 0,10 \text{ gr}$  ( $n=20$ ) at the same dose. The correlation between morphology and function and the differences shown in both species would be related to their intertidal distribution. The results lead to deep systematic and evolutive knowledge about two species of Actiniaria order which has a higher population frequency in intertidal from Mar del Plata.

**24. IDENTIFICATION OF MICROORGANISMS IN SALT USED IN PROCESS OF FOOD CONSERVATION**

Felix M<sup>1</sup>, Amezttoy P, Yeannes MI<sup>1,2</sup>.

<sup>1</sup>UNMDP. <sup>2</sup>CONICET. Preservación y Calidad de Alimentos. Dto Química. FCEyN. UNMDP. Funes 3350, 7600 Mar del Plata. Argentina. E-mail: mfelix@mdp.edu.ar

Extreme halofilic bacteria are microorganisms that growth in high salt concentration. In the osmotic dehydration of anchovy (salted-maturated) to achieve their microbiological stability, ClNa is used being a process that overcomes 5 months. These slow processes allow the growth of these bacteria. The aim of this work was to know the initial load of salts and to determine the genera present to predict the possible changes in the final product. Six lots of washed and centrifuged salt (Sample A) and six lots of the salt without treatment (Sample B) were analyzed. The spread in surface (0.1ml) was done in H&L for moulds and yeast (incubated at 25°C) and for bacteria with Gibbon agar and incubated to 37°C for 12 days. Both studies were done by triplicate. The colonies were characterized macroscopically and microscopically by means of coloration of Gram. The following biochemical tests were carried out: oxidase, catalase, TSI (iron triple sugar), carbohydrates, oxidation-fermentation, citrate, nitrates production, sulfate-indol-mobility (SIM). Test of lipolisis and proteolisis were carried out. In sample A was obtained averages count of  $2,0 \times 10^4$  UFC/g of red colonies identified as *Halococcus* spp. In sample B the average count was of  $3,1 \times 10^5$  UFC/g of red colonies identified as *Halococcus* spp. and *Halobacterium* spp. It is not observed growth of yeasts neither moulds. The reddish coloration of these bacteria producing the food product rejection for sensorial aspect. *Halobacterium* spp. causes a deterioration of the product for their lipolitic and proteolitic activity shortening its shelf life. This could indicate a benefit in the use of washed salt.



## 25. BODY MASS RECOVERY IN OBESE LINE $\beta$ RATS TREATED WITH OLEOYL ESTRONE (OE)

Posadas M<sup>1</sup>, Olguin C<sup>2</sup>, Gayol M del C<sup>1</sup>, Marinozzi D<sup>2</sup>, Leiva R<sup>1</sup>, Zingale F, Labourdette V<sup>1</sup>.

<sup>1</sup>Cát. de Biología, Fac. de Cs Médicas; <sup>2</sup>Bromatología, Fac. de Cs Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. E-mail: martaposadas@hotmail.com

Estrone / (OE) ratio may regulate ponderostate and its value adjusted at different points by synthetic OE administration. OE induces a decrease in food intake maintaining energy out put, thus promoting a body weight decrease. OE administration may be considered as an alternative therapy for obesity treatment.

To evaluate an eventual body mass recovery a month after a standard OE treatment, 200 days old male  $\beta$  rats (n=10) were given a daily oral gavage of OE (15 nmol/g biomass) for 10 days. Both groups, control (C: n=8) and experimental were fed *ad libitum*. On days 11 and 40, body weight and food intake values were registered; half of the animals in each group were sacrificed and retroperitoneal fat depots dissected and weighed.

Results: Day 11: biomass (g): C:  $403.8 \pm 10.7$  vs OE:  $356.4 \pm 4.3$  ( $p < 0.001$ ); food intake (g): C:  $278.4 \pm 9.8$  vs OE:  $167.1 \pm 7.1$  ( $p < 0.001$ ); retroperitoneal fat depots weight (g/100 g biomass): C:  $2.66 \pm 0.25$  vs OE:  $1.91 \pm 0.14$  ( $p < 0.05$ ). Day 40: biomass: C:  $428.1 \pm 8.1$  vs OE:  $386.6 \pm 15.9$  ( $p > 0.05$ ); retroperitoneal fat depots weight: C:  $2.58 \pm 0.16$  vs OE:  $2.40 \pm 0.12$  ( $p > 0.05$ ).

Although a month after treatment, OE group recovered body mass, registered values were 10% lower than controls. This fact is auspicious since post treatment body mass values might be new initial ponderal points.

## 26. THE UROGENITAL SINUS: A CROSSROAD IN THE EARLY FOETAL DEVELOPMENT

Téllez TE<sup>1</sup>, Carrera LI<sup>1</sup>, D'Ottavio AE<sup>1,2</sup>.

<sup>1</sup>Cátedra de Histología y Embriología, Medicina, UNR. <sup>2</sup>CIUNR.

Considering that the final structure and function of the urogenital sinus depends on the differentiation of the external genitalia and the sexual internal ducts, this report explores its structural components. Serial 7  $\mu$ m thick sections from the abdominal-pelvic zone of 9<sup>th</sup> and 10<sup>th</sup> - week-old six human fetuses, obtained from spontaneous abortions in public hospitals, were sequentially exposed to several histological and histochemical procedures and subsequently planimetrically processed and superposed for spatial reconstruction. This last procedure revealed three portions: cephalic or vesical, intermediate or pelvic and caudal-ventral or phallic. Microscopically the sinus appeared covered by a stratified epithelium with small basal cells, big and clear polygonal intermediate ones and flattened cells in the periphery possibly generating an impediment to the luminal liquid absorption. The remaining wall showed a high cellularity into a thin III collagen stroma where a few peripheral cells evidenced signs of myocytic differentiation probably obstructing its flux (propulsion-retention).

## 27. CORPUSCULAR SIALOCONJUGATES AND WGALECTIN IN ADVANCED MURINE DIABETIC NEPHROPATHY

D'Ottavio GE<sup>1</sup>, Frontini AV<sup>1</sup>, Hisano N<sup>1</sup>, D'Ottavio AE<sup>1,2</sup>.

<sup>1</sup>Cátedra de Histología y Embriología. Medicina, UNR. <sup>2</sup>CIUNR.

Among others, sialoconjugates (SCG) are relevant in the renal filtration barrier. *Triticum vulgaris* lectin (WGA) put it specifically into evidence. Continuing previous histochemical studies, this report analyzes SCG specificity for WGA in superficial (SC) and juxtamedullary (JMC) corpuscles of advanced nephropathic male SMT rats. Wistar rats were used as controls. Rats were euthanized at 12 months of age. Kidneys were dissected and fixed in Bouin's liquid (45') and 10% buffered formalin (3h). Specimens were included in paraffin wax and cut at 6  $\mu$ m. Endogen peroxidase was blocked by 3% H<sub>2</sub>O<sub>2</sub> (100V)/methanol. Subsequently, slices were exposed to bovine serum albumin and WGA (all night long at 4°C). The next day, sections, treated with avidine-biotin complex and diaminobenzidine-hematoxylin, were visualized in a Zeiss microscope. Complementarily, optical densities (OD) were studied in digital photos through the Image-pro Plus Program version 1.1. With the aforesaid procedures, both types of corpuscles showed a diminished reactivity for WGA in eSMT rats (OD = 1 versus 3). These results, revealing a sialic acid decrease in SC and JMC –congruent with reported in bibliography for other sialo-binding lectins– could indicate a histochemical disturbance in the filtration barrier, apt to be deepened.

## 28. STUDY OF THE FIBRINOGEN EFFECT ON HEMORHEOLOGICAL VARIABLES IN MENOPAUSE

Carrara P, Bertoluzzo S, Mengarelli G, Spengler MI.

Cátedra de Física Biológica, Facultad de Ciencias Médicas; Univ. Nacional de Rosario, Argentina. E-mail: npablont@hotmail.com

The menopause is associated with a thrombosis risk and the erythrocyte hiperaggregability is perjudicial for the microcirculation. The lipidic fluidity of the red blood cell membrane (f) is associated with its aggregation. Objective: analyse plasmatic fibrinogen effect on the RBC tendency for aggregating and on f. The erythrocyte aggregation (EA), plasmatic fibrinogen concentration (F) and f in a group of healthy no menopausal women (n=20) and in menopausal women (n=43) with previous written consent were measured. In the last group the possible correlation between the studied variables was analysed. The EA was measured by optic methods and two parameters were obtained:  $s_0/n_0$  (mean aggregate size), and  $2k_2n_0$  (initial speed of the process). F was measured by gravimetric method and f by fluorescence polarization method. TMA-DPH (Trimethylammonium-Diphenylhexatriene) was used as fluorescence marker. The anisotropy coefficient (r) is inversely relates with f. The results shown that menopausal women presented significant higher values than the no menopausal ones:  $2k_2n_0$  ( $0.56 \pm 0.15$  vs  $0.32 \pm 0.09$ ;  $p < 0.05$ ),  $s_0/n_0$  ( $1.86 \pm 0.05$  vs  $1.82 \pm 0.02$ ;  $p < 0.01$ ), F ( $351 \pm 82$  mg/dl vs  $286 \pm 31$  mg/dl;  $p < 0.0001$ ) y r ( $0.18 \pm 0.03$  vs  $0.15 \pm 0.02$ ;  $p < 0.01$ ). In menopausal women, a positive correlation between F and  $2k_2n_0$  ( $r=0.66$ ;  $p < 0.0001$ ), between F and  $s_0/n_0$  ( $r=0.27$ ;  $p < 0.05$ ), between F and r ( $r=0.23$ ;  $P < 0.05$ ), were found. F is a possible responsible of increased EA and lower f in menopause. These hemorheological events could explain, in part, the rising cardiovascular risk present in menopause.

### 29. EVOLUTION OF IIMb RAT LINE IMITATING DIABETES AND OBESITY $\beta$ MODEL

Alet N, González D, Posadas M, Gayol MdelC.

Cátedra de Biología. Facultad de Ciencias Médicas. UNR.

IIMb rat line entered as eumetabolic control, but early showed phenotypic instability. 1<sup>o</sup>, 6<sup>o</sup> and 11<sup>o</sup> generations IIMb glucolipidic profile variables were contrasted with simultaneous diabetic, obese model  $\beta$  cohorts, at 50, 100, 200 and 300 days old. 60 rats in total were processed. ANOVA and Tukey test (media $\pm$ SD): Biomass(g): 50ds: b1<sup>o</sup>:158.37 $\pm$ 21.04 vs  $\beta$ :212.45 $\pm$ 11.48(p<0.05); b11<sup>o</sup> vs  $\beta$ (p>0.05). 100ds: (p>0.05). 200ds: b1<sup>o</sup>:352.10 $\pm$ 23.34 vs b11<sup>o</sup>:405.04 $\pm$ 48.53 (p<0.05); b11<sup>o</sup> vs  $\beta$  (p>0.05). 300ds: b1<sup>o</sup>:374 $\pm$ 40 vs b11<sup>o</sup>:474 $\pm$ 42 (p<0.05); b11<sup>o</sup> vs  $\beta$ (p>0.05). Basal glycemia(g/l): 50ds: b1<sup>o</sup>:0.99 $\pm$ 0.13 vs  $\beta$ :1.20 $\pm$ 0.10 (p<0.05); b11<sup>o</sup> vs  $\beta$  (p>0.05). 100ds: b1<sup>o</sup>:0.83 $\pm$ 0.09 vs  $\beta$ :1.32 $\pm$ 0.09 (p<0.05); b11<sup>o</sup> vs  $\beta$  (p>0.05). 200ds: b1<sup>o</sup>:0.94 $\pm$ 0.28 vs  $\beta$ :1.39 $\pm$ 0.14 (p<0.05); b11<sup>o</sup> vs  $\beta$  (p>0.05). 300ds: b1<sup>o</sup>:0.75 $\pm$ 0.05 vs  $\beta$ :1.50 $\pm$ 0.16(p<0.05); b11<sup>o</sup> vs  $\beta$  (p>0.05). Glycemia 120'(g/l): 50ds: (p>0.05). 100ds: b1<sup>o</sup>:0.86 $\pm$ 0.08 vs. b11<sup>o</sup>: 1.14 $\pm$ 0.29(p<0.05). 200ds: b1<sup>o</sup>: 0.91 $\pm$ 0.27 vs. b11<sup>o</sup>:1.30 $\pm$ 0.41 (p<0.05); b11<sup>o</sup> vs.  $\beta$ (p>0.05). 300ds: b1<sup>o</sup>: 0.94 $\pm$ 0.11 vs. b11<sup>o</sup>:1.50 $\pm$ 0.30 (p<0.05); b11<sup>o</sup> vs.  $\beta$ (p>0.05). Triglyceridemia(g/l): 300ds: (p<0.05) b1<sup>o</sup>:1.05 $\pm$ 0.41 vs  $\beta$ :3.40 $\pm$ 1.48; b6<sup>o</sup>:0.65 $\pm$ 0.17 vs  $\beta$ : 2.86  $\pm$ 1.35; b11<sup>o</sup>:0.78 $\pm$ 0.28 vs  $\beta$ :2.86  $\pm$ 1.20. Cholesterolemia(g/l) normal in  $\beta$  and b. Fatty panicle weight(g): b1<sup>o</sup>:21.96 $\pm$ 2.57 vs b11<sup>o</sup>:30.42 $\pm$ 6.25(p<0.05); b11<sup>o</sup> vs  $\beta$  (p>0.05). B model phenotypic stability is shown. Nowadays, line b behaves as  $\beta$  in its origins, evidences biomass and fatty panicle weight increase and glucose intolerance. Only  $\beta$  is hypertriglyceridemic. Obesity and diabetes are heterogeneous syndromes with polygenic controls and susceptible to environmental influences that modulate expression.

### 30. VARIABLES CONNECTED WITH FERTILITY IN AN ORIGINALLY EUMETABOLIC AND LATELY DIABETIC RAT LINE

Sgro MV, Spies J, Gioiella L, Gayol MdelC.

Cátedra de Biología. Facultad de Ciencias Médicas. UNR.

Fertility and 120 day old female  $\beta$ (n:10) and IIMb(n:12) offspring production variables were evaluated with the object of characterizing IIMb (b) eumetabolic in its origin and afterwards becoming glucidic intolerant. The values were analyzed through student test and ANOVA (media  $\pm$  SD). Time from joined for mating up to birth time(ds): without difference (p>0.05):  $\beta$ = 29.20 $\pm$ 4.89, b=30 $\pm$ 5.82. Total biomass increase during pregnancy(g): without difference (p>0.05):  $\beta$ =122.3 $\pm$ 15.31, b=113.8 $\pm$ 19.83. Litter birth size: without difference (p>0.05):  $\beta$ =8.50 $\pm$ 1.72, b=7.00 $\pm$ 2.70. Litter weaning size: with difference (p<0.05):  $\beta$ =8.20 $\pm$ 1.99, b=5.41 $\pm$ 3.09. Breeding average weight at birthtime, middle lactancy and weaning(g): without difference (p>0.05). At birth time:  $\beta$ =6.19 $\pm$ 1.13, b=6.36 $\pm$ 0.70. Middle lactancy:  $\beta$ =21.26 $\pm$ 2.19, b=22.04 $\pm$ 4.51. Weaning:  $\beta$ =44.54 $\pm$ 5.10, b=46.21 $\pm$ 4.98. Fertility coefficient: with difference (p<0.05):  $\beta$ =1.18 $\pm$ 0.19, b=0.94 $\pm$ 0.32. Survival coefficient: with difference (p<0.05):  $\beta$ =0.96 $\pm$ 0.10, b=0.72 $\pm$ 0.33. Combined coefficient: with difference (p<0.05):  $\beta$ =1.14 $\pm$ 0.23, b=0.72 $\pm$ 0.36. Pregnant female average:  $\beta$ =75%, b=100%. Litter sex average:  $\beta$ = 50% of each sex, b= 40%: males; 60 %: females.  $\beta$  and b lines take the same time to reach pregnancy. Female biomass, quantity and offspring weight, now, equal  $\beta$ . Born female % is bigger in b. Female productivity, survival and weekly-saved production is bigger in  $\beta$ . Nowadays, b imitates  $\beta$  model in glucose intolerance (basal 200ds(g/l) (p>0.05): b=1.30 $\pm$ 0.41,  $\beta$ =1.45 $\pm$ 0.20) and it could also be going to obesity.

### 31. EFFECT OF SODIUM FLUORIDE (NaF) AND DISODIUM MONOFLUOROPHOSPHATE (MFP) ON ERYTHROCYTE AGGREGATION (EA) *IN VITRO*

Hasenauer E, Di Loreto V, Moreno H, Hernández G, Rigalli A, Bollini A.

Cátedra de Biofísica y Laboratorio de Biología Ósea. Facultad de Ciencias Médicas. UNR.

MFP is used in the treatment of osteoporosis. It binds to alfa-2-macroglobulin ( $\alpha_2$ M), a conspicuous plasma antiproteinase, also involved in EA in rats and human beings. A decrease in EA in Dextran, in rats after an oral dose of MFP, indicates an alteration of mechanic properties of this cell. The objective of this work was to investigate the causes of the modifications of mechanic properties of red cells. Erythrocytes from 14 IIM/Fm "m" rats were incubated in NaF 100  $\mu$ M (group 1) or MFP 100  $\mu$ M (group 2) or without both drugs (controls). Plasma with high levels of  $\alpha_2$ M was obtained by treatment of rats with MFP, and the effect this plasma was studied. EA was measured in 2% Dextran 500 by optical method and  $s_0/n_0$  (estimation of size of aggregates) and  $2k_2/n_0$  (estimation of initial rate of the process) were determined.  $\alpha_2$ M ( $\mu$ M) was measured by Dot Blot. Results are expressed as mean $\pm$ SD and were compared with unpaired Student's t test, \*\* p<0.01, \* p<0.05. Results of  $s_0/n_0$ : controls: 1.90 $\pm$ 0.01, group1: 1.88 $\pm$ 0.01\*\*, group2: 1.89 $\pm$ 0.0004. Results of  $2k_2/n_0$ : controls: 1.59 $\pm$ 0.31, group1: 1.35 $\pm$ 0.16\*\*, group2: 1.39 $\pm$ 0.24\*. In spite of high levels of  $\alpha_2$ M in plasma, control: 10.8 $\pm$ 1.2, MFP: 17.66 $\pm$ 2.55\* there was no differences in aggregation in plasma, unpaired student's t test, \* p<0.05. These results indicate that fluoride would have effect on erythrocyte membranes by altering mechanic properties decreasing aggregation of red cells.

### 32. INFLUENCE OF THE CHRONIC TREATMENT WITH MONOFLUOROPHOSPHATE ON SANGUINEOUS VISCOSITY IN RATS

Cinara L, Moreno H, Bollini A, Rigalli A, Di Loreto V, Hernández G. Dpto Cs. Fisiológicas, Fac. Cs. Médicas, UNR.

The  $\alpha_2$ -macroglobuline ( $\alpha_2$ M) is a normal antiprotease of plasma that contributes to plasmatic viscosity (PV). Monofluorophosphate (MFP), is used in treatment of osteoporosis, binds to and disturbs its homeostasis to plasmatic  $\alpha_2$ M. Previous studies in rats have shown that one MFP oral dose (80 $\mu$ mol) increased PV and  $\alpha_2$ M concentration. In the present work, the consequences of chronic treatment with MFP on rheologic resistance to the flow, determined by sanguineous viscosity (SV) at high shear rate ( $\gamma$ ), were studied. Therefore SV, erythrocytes deformability (ED) and PV were measured. IIM/Fm rats substrain "m" (n=6) where treated with MFP (80 $\mu$ mol/day by gastric tube) during 30 days, six rats act as controls. PV and SV were measured with a cone-plate viscometer. ED was estimated upon the viscosity of erythrocytes suspensions in physiological solution (VESF) to Hto=40% and high  $\gamma$ . The [ $\alpha_2$ M] was measured by Dot Blot using an anti- $\alpha_2$ M antibody. Relative sanguineous viscosity (RSV) was calculated as SV/PV. The data were analyzed with Student's t-Test for non-apared data. According to results, chronic treatment with MFP increased PV due to higher plasmatic levels of [ $\alpha_2$ M] although it did not significant modify in VESF. Neither the changes in the RSV (estimated parameter of resistance red blood cells to flow) were observed. In conclusion, the chronic treatment with MFP would not produce significant changes in the rheologic resistance to high rate flow.

### 33. FEMALE FERTILITY COMPONENTS IN CBI/O MICE WITH SUNFLOWER AND SPINACH DIET ADDITION

Gayol MC<sup>1,2</sup>, Montenegro S<sup>2,3</sup>, Tarrés MC<sup>2,3</sup>.

<sup>1</sup>Bioterio Central. <sup>2</sup>Cátedra de Biología. Facultad de Ciencias Médicas, UNR. <sup>3</sup>CIC-UNR. E-mail: mgayol@hotmail.com

Since its origin in 1983, an excellent maternal attitude of the CBI/0 mouse inbred strain was evident. Besides, up to 2003, all bioterio animals had received "pellet" balanced food, now replaced by "extrusade" one, with different shape and composition changes: replacement of animal proteins for vegetal ones and lipid average reduction 4,2% real vs declared 6 % minimum (diet E). After some time, it was observed intake, hyperactivity, and muscular flaccidity increase in young adults. It was specially observed offspring and lactant mother death in CBI/0 strain. This work objective is an attempt to improve animal nutritional condition and its impact on CBI/0 female mouse fertility components, through diet E addition of whole sunflower seeds (5g/cage 1 male + 3 female mating or a pregnant or lactant mother) and spinach leaves (15g/cage) given twice a week together with diet E (diet E+G+E). The registers of 30 females from 55 to 57 generations, fed with diet E, were analysed and compared with females from generation 58 to 63 that received diet E+G+E. The results obtained (mean  $\pm$  SD in E and E+G+E respectively) show no significant differences in mating weight, time from mating to parturition, litter size and Fertility Coefficient. While significant statistical differences ( $p < 0,01$ ), in litter weaning number ( $9,64 \pm 2,21$  vs  $10,87 \pm 1,74$ ), Survival Coefficient ( $0,89 \pm 0,20$  vs  $0,97 \pm 0,0054$ ) and Combined Coefficient ( $1,51 \pm 0,36$  vs  $1,69 \pm 0,28$ ) were evident. The addition had high acceptance degree and profitable effects on outliving offspring. This can be attributed to spinach Ca, P, Fe, K and Na contribution and sunflowerseed fat, proteins and E vitamins.

### 34. COLON HISTOMORFOMETRY FROM DIABETIC RATS INDUCED BY ALLOXAN

Figueroa HE, Hisano N.

Cátedra de Histología y Embriología. Facultad de Ciencias Médicas. UNR. E-mail: emilianofigueroa\_77@hotmail.com

Colon histomorphometry was done in alloxan injected in Sprague-Dawley rats. Five Sprague-Dawley male rats 25 days old were injected i.p. with alloxan (A) (24mg/100gCW), four rats were injected with distilled water (C) (1cc/100gCW). At 115 days old, from tail vein, blood were collected for glycemia and euthanased with ether. From median laparotomy, colon was dissected out, washed with PBS, and weight and length were determined. Segments of 3-4 cm distal colon were immersed in Carnoy's fluid, histologically processed and semiserially cut at 6  $\mu$ m, stained with H-E. Wall, mucous and muscularis externa thickness were determined through a linear devise located in the eye-piece. Data are expressed as mean  $\pm$  SEM. Statistical analysis was done with Student "t" test. Body weight (g): C:  $563.00 \pm 18.52$ ; A:  $251.60 \pm 24.33$  ( $P < 0.05$ ). Colon weight: C:  $2.47 \pm 0.13$ ; A:  $2.60 \pm 0.46$  (n.s.). Relative colon weight (g/100gBW): C:  $0.44 \pm 0.03$ ; A:  $1.02 \pm 0.13$  ( $P < 0.05$ ). Colon length (cm): C:  $24 \pm 1$ ; A:  $25 \pm 0.4$  (n.s.). Colon wall thickness ( $\mu$ m): C:  $491.21 \pm 26.86$ ; A:  $643.43 \pm 95.09$  (n.s.). Mucous thickness ( $\mu$ m): C:  $250.16 \pm 15.95$ ; A:  $263.80 \pm 21.13$  (n.s.). Muscularis externa thickness ( $\mu$ m): C:  $150.18 \pm 14.45$ ; A:  $168.33 \pm 65.68$  (n.s.). In alloxan injected in postweaning rats resulted in a minor body weight, but, histomorphological parameters are similar to controls.

### 35. GASTRIC STRESS AND ANGIOTENSIN II AT1 RECEPTOR ANTAGONISTS

Vegetti R, Laudanno OM, Cesolari JAM, Godoy AR, Vasconcelos LA, Urteaga A, Calvi BJ, Villarruel R, Wandzik CI, Rojas P, Simó M. Experimental Gastroenterology. Histology and Embriology Department. Medicine University-Rosario National University.

The present study was undertaken to evaluate the angiotensin II At1 receptor antagonists in the protection of gastric mucosa under stress. In randomized groups of female Wistar rats (n: 7 in each group) 200 gr after a 24 h fasting, water ad-libitum and avoiding coprophagy the following experiments were carried out: 1-Stress was induced by immobilization of the animals and immersion in water bath at 23° C for 7 hours (S). (Witness). 2-Candesartan 1-10 mg/Kg, intraperitoneal (IP) and wait 60 min, and then S. 3-25-50 mg/Kg Losartan, IP and then S. 4-25-50 mg/Kg Valsartan, IP and then S. 7 hours later all the rats were sacrificed by ether overdose. Laparotomy, total gastrectomy, and opening along the greater curvature were performed. The percentage area of the gastric lesions was measured with a planimetric computerized method. Samples for histological studies were obtained, where fixed in formaldehyde and stained with hematoxiline-eosina (HE) and myeloperoxidase (MPO) for histochemical studies. The Student's "t" test, ANOVA was used. The percentage of gastric lesional areas showed  $1-48 \pm 7$ ;  $2-51 \pm 7$  (ns);  $3-44 \pm 7$  (ns);  $4-18 \pm 3$  ( $p < 0.01$ ). The MPO showed  $1-245 \pm 15$  mg/protein  $2-280 \pm 17$  mg/protein (ns).  $3-225 \pm 21$  mg/protein (ns)  $4-81 \pm 12$  mg/protein ( $p < 0,01$ ). It could be concluded that only Valsartan yielded gastric mucosa protection against stress.

### 36. CHRONIC GASTRIC ULCER DUE TO STRESS AND INDUCED BY NON-STEROID ANTIINFLAMMATORY AGENTS (NSAAs)

Urteaga A, Laudanno OM, Cesolari JAM, Vasconcelos L, Vegetti R, Villarruel R, Wandzik CI, Calvi B, Simó M, Rojas P.

Experimental Gastroenterology. Histology and Embriology chair, School of Medicine. UNR. E-mail: sbrunetto@ciudad.com.ar

Acute stress in rats causes gastric ulcer 24 h later, totally disappearing after 7 days. There are different cyclooxygenase 1 (COX-1), cyclooxygenase 2 (COX-2), and cyclooxygenase 3 (COX-3) non-steroidal antiinflammatory selective inhibitors and COX-1, COX-2, and COX-3 simultaneous inhibitors which could potentially lead to chronic stress ulcer. Objective: to study which NSAAs, lead to chronic gastric ulcer induced by stress through immobilization or immersion in water at 23°C for 7 h. Experiments were performed on 7 random groups of female Wistar rats (n=7 each group), 200gr, 24 h fasting, water ad-lib., avoiding coprophagia, all of them E (controls), administering either COX-1, COX-2, and COX-3 selective inhibitors or COX-1, COX-2, and COX-3 simultaneous inhibitors, or orogastric saline (OG), at different doses, every 24 h for 7 days. Rats were killed by ether overdose. Laparotomy, total gastrectomy, and gastric major curvature opening were performed. The percentage of the gastric lesion area (%) was measured through computerized planimetry. Gastric body samples, fixed in 10% formaldehyde and stained with HE, were processed for histological examination. Statistics: Student's t test and ANOVA. Conclusion: COX-1 and COX-2 through a simultaneous inhibitory mechanism due to Indomethacin aggravated stress ulcers.

### 37. EFFECT OF THE ADDITION OF SUNFLOWER TO THE DIET ON THE WEIGHT OF DIABETIC LINES eSS AND eSMT

López JP, Marani J, Daniele S, Martínez SM, Tarrés MC, Montenegro S.

Cátedra de Biología. Facultad de Ciencias Médicas. CIC-UNR.

A change in the usual food given to laboratory rodents in pellets with another preparation (P) induced a lesser weight in 4 month-old males and females of eSS and eSMT lines of diabetic rats.

P diet shows changes in comparison with the previous one, due to the replacement of animal proteins by vegetal proteins and a decrease of ethereal extract. The addition of sunflower seeds (S) was decided as a dietary supplement which contains about 60 % of oil in their fruit (diet P+S). To evaluate its effects, the weight of adult animals of the two lines fed since weaning was registered in males of two ages. The following values, expressed in grams, were obtained (average  $\pm$  DS) :

DIET	LINE	AGE (months)	
		8	12
P	eSMT	270.93 $\pm$ 51.8	483.29 $\pm$ 27.5
	eSS	242.47 $\pm$ 13.8	309.68 $\pm$ 41.1
P+S	eSMT	318.02 $\pm$ 93.8	412.05 $\pm$ 16.1
	eSS	204.8 $\pm$ 24.5	245.11 $\pm$ 34.2

The ANOVA confirmed effect *age* ( $p=0.000$ ), *line* ( $p=0.049$ ), interactions *age x diet* ( $p=0.000$ ), *line x age* ( $p=0.000$ ) and *line x diet* ( $p=0.026$ ), not showing the *diet* significant effect ( $p=0.533$ ). The probable substitution of part of P diet by sunflower and/or the rise in fiber intake would affect, at least partially, the animals' weight. The erratic effect of sunflower on the weight of the analyzed groups leads us to reconsiderate the addition of this seed as a supplement for the diabetic lines.

### 38. BRONCHIECTASIAS IN MALE AND FEMALE DIABETIC RATS OF eSS AND eSMT LINES AT 24 MONTHS OF AGE AND MORE

Joffré A, Buffo M, Galeano A, Montenegro S, Martínez SM, Tarrés MC, Picena JC.

Cátedras de Biología y de Anatomía y Fisiología Patológica. Facultad de Ciencias Médicas. CIC-UNR.

Bronchiectasias causing infections can be observed in laboratory rats as well as in humans. Evidences indicate that the lung is a target organ in diabetes mellitus. The eSS line develops type 2 diabetes, more intense in males, with progressive glucid intolerance, without obesity. The eSMT rat is more corpulent and its diabetic syndrome has an earlier onset and exacerbated evolution. Both lines showed lung pathology from 6 to 18 months, with increasing frequency and intensity as age advances, being more marked in the eSS line. It seemed important to analyze lung injuries in animals of both lines of 24 months of age and more.

18 eSS rats and 22 eSMT rats were euthanized following ethical standards. Autopsies were performed and samples taken from both lungs. Macro and microscopic studies revealed 6 eSS males with fusiform and saccular bronchiectasias with abscedation, ulceration and chronic peribronchitis. 8 eSS females showed bronchiectasias, 2 of them with squamous metaplasia; the other 6 with abscedation, one of them complicated with pleural empyema. As to eSMT animals, 4 males showed bronchiectasias, only one suppurated; none of the 8 females showed lung pathology.

In conclusion, lung pathology in both lines worsens with age. The incidence of bronchiectasias, as well as their infections, is higher in eSS, in spite that the expression of their diabetic syndrome is less marked than in eSMT. This suggests the existence of a especial susceptibility to neumopathies of the eSS line.

### 39. TRUE YOGHURT OBTAINED WITH PROBIOTIC BIOFLORA OF HUMAN INTESTINAL MICROBIOTICA

Rojas P, Laudanno O, Cesolari JAM, Simó M, Urteaga A, Vasconcelos LA, Vegetti R, Villarruel R, Wandzik CI, Calvi BJ.

Gastroenterología Experimental Gastroenterology. Cátedra de Histología y Embriología. Facultad de Ciencias Médicas. Universidad Nacional de Rosario. E-mail: paoliten@hotmail.com

The probiotic Bioflora is constituted by four bacteria: *Lactobacillus casei*, *Lactobacillus plantarum*, *Streptococcus fecalis* and *Bifidobacterium brevis*; obtained by cultures of human fecal stool, being a true probiotic, with antiinflammatory properties, and it also prevents the intestinal bacterial overdevelopment and the intestinal bacterial translocation. The objective was to produce a true yoghurt, with potential clinical use. In a yoghurt maker containing 10 glasses of 125 ml of whole pasteurised milk and in water bath to a constant temperature of 41°C, with a  $n=10$  of milk glasses and for each dose; they went away applying 0.1; 0.5; 1.0; 1.5; 3.0; 4.0; 5.0 and 7.0 ml of the probiotic Bioflora between 2 and 8°C (alive bacteria); where the culture of 1 mililiter showed  $1.9 \times 10^6 \pm 2.1 \times 10^5$  CFU counts. Controls of the formation of yogurth were made at 3, 6, 9, 12 and 14 hours and was stated from the dose of 3 ml of Bioflora and in all the following doses and to the 12 hours. In the statistical evaluation, the test of Fisher gave with 3, 4, 5 and 7 ml a  $p < 0.001$  and there were no differences between these doses. Conclusions: it was verified that the true bioflora probiotic forms yoghurt, with the dose of 3 ml and in the time of 12 hours, and that could be of clinical use.

### 40. BLEEDING GASTRIC ULCER AND ANTIDEPRESSANTS INHIBITORS OF SEROTONIN AND INDOMETHACIN RECAPTATION IN RATS

Wandzik CI, Laudanno OM, Cesolari JAM, San Miguel P, Villarruel R, Rojas P, Calvi BJ, Simó M, Urteaga A, Vasconcelos LA, Vegetti R. Experimental Gastroenterology. Histology and Embriology chair, School of Medicine. UNR. E-mail: corawandzik@yahoo.com

Aim: To study if antidepressants selective inhibitors of serotonin recaptation (ASISR) and tricycle antidepressants (TA) given with non-steroidal antiinflammatory agents (NSAA), caused hemorrhagic ulcers in two experimental models. Experiments were performed on random groups of female Wistar rats ( $n=7$ /group), 200gr, 24 h fasting, water ad-lib., avoiding coprophagia: Group I- 1) 24h fasting, solid meal-pellets (SM) for 2 h, Indomethacin (Indo) 30mg/kg, subcutaneous (SC) and SM 24h (witness). 2) Fluoxetine (Fluo), 20 mg/kg, intraperitoneal (IP), SM 2h, Indo and SM 24h. 3) Amitriptyline (Ami) 25mg/kg IP, SM 2h, Indo and SM 24h. 4) Fluo 20 mg/kg IP, SM 24 h. 5) Ami 25 mg/kg, SM 24h. Group II-1) 1ml physiologic saline SC, stress through immobilization or immersion in water at 23°C (E) for 7 h, waiting (w) 24h (witness). 2) Indo 10 mg/kg SC, E, w 24h. 3) Fluo 20mg/kg IP, E, w 24h. 4) Fluo+Indo, E, w24h. 5) Ami 25mg/kg IP, E, w 24h. 6) Ami+Indo, E, w 24h. 7) Tegaserod 25mg/kg orogastric+Indo, E, w 24h. Rats were killed by ether overdose. Laparotomy, total gastrectomy, and gastric major curvature opening were performed. The percentage of the gastric lesion area (%) and bleeding were measured. Histological cuts were fixed in 10% formaldehyde and stained with HE. Statistics: Student's *t* test and ANOVA. Conclusion: ASISR, and not TA, given with NSAA made gastric ulcers worse with hemorrhage.

#### 41. CELL – CELL INTERACTION, PLASMATIC PROTEINS AND BLOOD VISCOSITY

Bollini A, Hernández G, Bazzoni G, Rasia M.  
Cát. de Biofísica. Fac. Cs. Médicas. UNR.

We investigated the cell-cell and cell - proteins interaction influence upon human and bovine blood viscosity. Bovine RBCs do not aggregate in comparison with human ones. RBCs plasma and saline suspensions ranging from 5-60% were prepared.  $\eta$  measurements (plasma ( $\eta_p$ ), saline ( $\eta_{sf}$ ) and suspension media ( $\eta_s$ )) were performed at 37°C in cone-in-plate viscometer. (115 for both plasma and saline and 230s<sup>-1</sup> for suspensions shear rate) Relative  $\eta$  ( $\eta_r$ ) was calculated as  $\eta_s$  / medium  $\eta$ . Statistics: Student t test for unpaired data. In the whole hematocrit range studied,  $\eta_s$  exhibited by bovine RBCs was significant higher than human ones. Relative viscosity, that emphasises the flow properties of the suspending particles, show differences between species in a particular direction when the medium was autologous plasma, and in the opposite direction when the medium was saline: in plasma human  $\eta_s$  was higher than bovine one, while in saline it resulted in an inverse fashion since 40% hematocrit. We concluded that plasmatic protein interaction with cell surface impaired RBCs flow ability greater in human blood than in the bovine one. This effect is point out at physiologic and higher hematocrits, when cell – cell interaction is particularly important and it is possible related with differences in blood aggregating behaviour between both species.

#### 42. INTRACITOPLASMATIC CYTOKINES (IFN $\gamma$ , IL2, IL4, IL8) IN CD4+ and CD4- CELLS WITH INACTIVATED *M. tuberculosis* H37RV (iMtb) IN TUBERCULOSIS (TBP) PATIENTS

Martinel Lamas D, Fiorenza G, Selenscig D, Farroni M, Vogué C, Dlugovitzky D.  
Sección Inmunología. Cátedra de Microbiología. Facultad de Ciencias Médicas. Universidad Nacional de Rosario. Hospital I. Carrasco. Rosario. E-mail: cheferom@yahoo.com.ar

We have reported the functionality of mononuclear and polymorphonuclear cells involved in the immune response against *M. tuberculosis*. To know better the role of these cells in TBP immune response, we studied the intracytoplasmatic cytokine (In.Cy) production of IFN $\gamma$ , IL2, IL4, IL8 in CD4+ and CD4- unstimulated (Basal: B) or iMtb cells (S) stimulated. Twenty TBP, both sexes and variable age (35 $\pm$ 14.5 SD) from I. Carrasco Hospital and 12 normal persons (Co) same characteristics of sex and age, were studied. Tuberculosis was diagnosed by clinical, radiological and bacteriological exams. Intracytoplasmatic cytokine production by CD4+ and CD4- cells were evaluated in heparinized blood by Flow Cytometry (FACS-CALIBUR) with or without iMtb previous stimuli. The data showed that CD4- cells produced higher IFN $\gamma$ , IL2, IL8, IL4 levels than CD4+ cells (B: p<0.05, 0.01, 0.01, 0.01; S: p<0.05 in all cases) in both conditions. Stimulated CD4+ and CD4- cells increased the IFN $\gamma$ , IL2, and IL8 production in TBP cells S vs B: IFN $\gamma$ : p<0.0002, IL2: p<0.0001, IL8: p<0.0005, IL4: N.S. Cytokine increased production by CD4- cells observed in Co, that was lower than in TBP in B and S (B: IFN $\gamma$ : p<0.05, IL2: p<0.05, IL4: p<0.05, IL8: p<0.05) (S: IFN $\gamma$ : p<0.05, IL2: p<0.05, IL4: p<0.05, IL8: p<0.04). We conclude that specially in TBP, CD4- cells are the main producers of intracytoplasmatic IFN $\gamma$ , IL2, IL4 and IL8, and less frequently in Co, cytokine production was increased by the specific stimuli.

#### 43. JOINT LESIONS IN TWO LINES OF SPONTANEOUSLY DIABETIC RATS

Gorosito MD, Picena JC, Lembo GM.  
Cátedra de Anatomía y Fisiología Patológicas. Facultad de Ciencias Médicas. Universidad Nacional de Rosario. Rosario. Argentina. E-mail: gorositomario@hotmail.com

**Introduction.** The eSS rat is a model of spontaneous type 2 diabetes of moderate intensity, characterized by early and progressive glucose intolerance; the eSMT rat presents overweight and an earlier onset of the syndrome with an exacerbated evolution of hyperglycemia.

**Objectives.** To analyze and describe articular surfaces, synovial membrane, periarticular vessels and soft tissues from knees and ankles of adult diabetic rats.

**Materials and methods.** 9 eSS and 11 eSMT rats, of both sexes, aged 27 to 30 months, were employed. Animals were euthanised according to bioethical procedures. Knees and ankles were fixed in 10% formaline, decalcified in Bouin, embedded in paraffin and sections were stained with H&E. The presence of: synovial hypertrophy and hyperplasia, inflammatory infiltrates of synovial stroma, periarticular blood vessels of small and medium caliber, articular cartilagenous surfaces and bone trabeculae were investigated.

**Results.** Alterations in knees and ankles joints were assessed in both lines of rats; the lesions were more marked in corpulent animals. Ankles were more severely affected than knees.

**Conclusions.** Both lines of rats present lesions expressing more severity in eSMT –with overweight- ; ankles the most affected joints.

#### 44. MORPHOLOGIC FINDINGS IN INTERVERTEBRAL DISKS IN TWO LINES OF SPONTANEOUSLY DIABETIC RATS: eSS AND eSMT

Gorosito MD, Picena JC, Lembo GM.  
Cátedra de Anatomía y Fisiología Patológicas. Facultad de Ciencias Médicas. Universidad Nacional de Rosario. Rosario. Argentina. E-mail: gorositomario@hotmail.com

eSS and eSMT lines of rats are models of spontaneous type 2 diabetes. eSS develops a diabetic syndrome of moderate intensity that worsens with age, with no overweight; eSMT is corpulent and shows an exacerbated metabolopathy with earlier onset.

In the dorsal region of both lines of rats, degenerative changes of intervertebral disks have been observed. The aim of paper is to analyze and classify these morphologic findings.

9 eSS and 11 eSMT rats of both sexes, from 27 to 30 months of age, were studied. The animals were euthanised following bioethical criteria. The dorsal spine was fixed in 10% formalin, decalcified in Bouin solution, embedded in paraffin; sections were stained with H&E. Observation, analysis and classification were carried out systematically; findings were classed in 4 groups; disks with no lesions, disks with degenerative lesions of mild, moderate and severe intensity.

In the 9 eSS, 3 with no lesions, 1 with lesions of mild degree, 4 of moderate and 1 with intense degree were found. In the 11 eSmt, 7 with lesions, 1 with lesions of mild degree and 3 with moderate lesions -none with severe lesions- were found.

Both lines present discal lesions of different intensity; the majority of cases and the more severe lesions correspond to eSS rats. Paradoxically, eSMT rats –with overweight- showed a higher incidence of cases with no discal alterations, as well as absence of severe lesions.

#### 45. STUDY OF THE CRANEO-CERVICAL BOARDING ROUTES IN FETAL DISECTION

*Pellicciotti MC, Ortega MA, Lavooy LA, Gorosito MD, Calvo JE, Mignaco R.*

*Instituto Museo de Ciencias Morfológicas. Facultad de Ciencias Médicas. U.N.R.*

The study of the fetal anatomy has acquired importance due to advances in surgery and neonatal and first infancy interventionism. It is of extreme importance to remind the differences in surface anatomy, fetal morphology and the delimitation of the regions; and to recognize the differences that it poses compared with the adult regards the size and the possibility to the access to organs and superficial vascular structures.

The objective was to enlarge on the present theory marc, the analysis and description of the anatomical repairs, for the access to superficial anatomic regions in fetus.

It has been studied cráneo-cefalic and cervical regions of 10 fetus of both sexes without any specific pathology, between 23 and 33 weeks of gestational age obtained by fetal morphometry. They were fixed and conserved by the MAR II technique (Museo Anatomía de Rosario). It was proceeded to delimit the territories to study, dividing them in anterior and posterior, to realize micro and macro dissection of the cráneo-cervical regions, and to photograph for documentation.

It was concluded, the existence of significant differences between the superficial anatomy of the adult and the fetus; because in the fetus, the discrimination of the regions demands a mayor precision in order to exploit the cadaveric material. In the fetus, besides, the delimitation of the regions regarding the musculoskeletal repairs is difficult due to the fact that this structures are not completed conformed.

#### 46. STUDY OF THE DISTRIBUTION OF THE EXTRAOSSEUS IRRIGATION OF THE HALLUX

*Calvo JE, Pellicciotti MC, Lavooy LA, Ortega MA, Gorosito MD, Mignaco R.*

*Instituto Museo de Ciencias Morfológicas. Facultad de Ciencias Médicas. U.N.R.*

The vitality of the Hallux depends on an arterial system whose elements are: Metatarsal Dorsal Artery (MDA), Metatarsal Plantar Artery (MPA), and the superficial branch of the Metatarsal Plantar Artery (branch of the Tibial Posterior Artery).

The objective was to analyze and describe the different anatomical patterns of the vessels that nourish the Hallux, categorizing the more fragile and exposed to vascular damage zones.

It has been studied 20 cadaverical feet and pieces of amputation of adults and adolescents conserved by the MAR II technique (Museo Anatomía de Rosario). It was proceeded to realize micro and macro dissection and to photograph for documentation.

The following results were found: variations in the number of collateral branches and irrigation territory of the MDA, MPA and the superficial branch of the Metatarsal Plantar Artery, presenting a wide extraosseus net originated, mainly, in the plantar and lateral faces of the Hallux.

We conclude that is of extreme importance to respect the vessels that irrigate the Hallux, mostly in the plantar and lateral regions, places in where are possible injuries by errors in the technique during different surgical procedures (ex: Chevron osteotomy, first metatarsal transference or the second foot finger to the hand). Finally, in order to diminish the incidence of aseptic necrosis of the metatarsal head, it must be practice a precise dissection, respect the different irrigation patterns and preserve the plantar and lateral vessels.

#### 47. STUDY OF THE CAROTID ANGLES IN FETUS

*Ortega MA, Pellicciotti MC, Lavooy LA, Ruiz RI, Miralles DR, Calvo JE, Mignaco R, Gorosito MD.*

*Instituto Museo de Ciencias Morfológicas. Facultad de Ciencias Médicas. U.N.R.*

The vascular anatomy of the cervical region, the carotid irrigation territories or the angles formed by the artery bifurcations, are in continued study.

A critical point is the angle formed by the origin of the internal and external carotid arteries, due to the fact that is a region in where turbulent flow is produced. The objective was to analyze and describe the carotid angles, and group them according to the obtained values.

It has been studied the carotid regions of 10 fetus of both sexes without any specific pathology, between 23 and 33 weeks of gestational age obtained by fetal morphometry. They were fixed and conserved by the MAR II technique (Museo Anatomía de Rosario). It was proceeded to the dissection of the cervical regions and to photograph for documentation. The cases were grouped according the obtained angles.

The twenty investigated angles were totally grouped and there measure varies between 7.5° and 12°. Values of 7.5°, 8.5°, 9° y 9.5° were present in a 20% and the incidence of the values 11.5° and 12° appeared in a 10% each one.

It was concluded that because of the fact that all angles are acute and with scanty amplitude, they are less prone to the development of turbulent flow.

Confronting each one of the values found, we maintain that the angles near 12° are the ones that present a higher risk to develop turbulence in comparison with the ones that present minor values. It is our porpoise to continue with the present work to the aims to extend the statistic final study.

#### 48. EFFECT OF ASCORBIC ACID BASED AMPHIPHILES ON THE ERYTHROCYTE AGGREGABILITY

*Spengler MI, Rasia M.*

*Cátedra de Física Biológica, Facultad de Ciencias Médicas. UNR. E-mail: isabelspengler@hotmail.com*

The aim of the present paper was to study the effect of Ascorbic Acid based amphiphiles (ASCn (n=8, 10 y 12)) upon red blood cell (RBC) aggregability, in blood extracted from healthy donors. In order to perform the experiment, RBC were suspended (30% htc) in Dextran diluted at 2% in 1)PBS (control) (pH:7.4; 300mosm.) y 2)PBS added with the three kinds of ASCn. Two concentrations were tested for each ASCn: a) concentrations which prevent cells from haemolysis (AHc): 2mM for n=8, 0,2mM for n=10 y 0,02mM for n=12; and b) higher concentrations, at which a 10% haemolysis takes place (CH<sub>10</sub>): 9mM for n=8, 1,4mM for n=10 y 0,12mM for n=12, repeating the measurements on 6 different samples. After 30 minutes of incubation at 37°C, erythrocyte aggregation was determined by optical method, and process initial rate values (2k<sub>i</sub>n<sub>0</sub>) were determined in both concentration sets: at cAH values did not show significant variation from controls', whereas at CH<sub>10</sub> values were: 1,83±0,20 for control suspension; and 2,75±0,22, 2,86±0,25 and 2,97±0,23 for ASC<sub>8</sub>, ASC<sub>10</sub> and ASC<sub>12</sub> suspensions respectively. Statistical analysis proved that haemolytic concentrations of the three kinds of ASCn augmented the aggregation rate considerably (p<0,01, for all three cases). We claim that, even though these compounds do no affect erythrocyte aggregation at cAH; at CH<sub>10</sub> they do interact with RBC membrane, compromising their employment as drug carrier of pharmaceutical interest.

## 49.

**THE PLASMATIC FIBRINOGEN EFFECT ON HEMORHEOLOGICAL VARIABLES IN PATIENTS WITH SYSTEMIC SCLERODERMA**

Parente F, Spengler MI, Leroux B<sup>2</sup>, Svetaz MJ<sup>3</sup>.

<sup>1</sup>Cátedra de Física Biológica, <sup>2</sup>Cátedra de Dermatología, Facultad Ciencias Médicas. <sup>3</sup>Sección Inmunidad Celular, Departamento de Bioquímica Clínica, Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR. E-mail: flor\_bunny@hotmail.com

The aim of this work was to estimate quantitatively, in patients with systemic scleroderma, plasma fibrinogen (pf), erythrocyte aggregability (EA), plasma viscosity ( $\eta_p$ ) and the association between these variables, comparing the values obtained with those of the normal controls. 52 patients and 15 normal controls were studied, all of them were women with similar age. The  $\eta_p$  was measured with cone-plate rotational viscometer and pf by gravimetric method. The EA was measured by optic method and one parameter was obtained:  $2k_2n_0$  which estimate the indicial velocity of the process. For the analysis of the differences observed between the groups the t of student was utilised and the Pearson coefficient for the correlation between the variables was applied. The results shown that the patients presented significant higher values than the controls:  $2k_2n_0$  ( $0,749 \pm 0,085$  vs  $0,584 \pm 0,080$ ;  $p < 0,01$ ),  $\eta_p$  ( $1,57 \pm 0,02$  vs  $1,50 \pm 0,04$ ;  $p < 0,05$ ), and the pf ( $355 \pm 42$  vs  $299 \pm 35$ ;  $p < 0,05$ ). Besides a positive correlation between fp and  $\eta_p$  ( $r = 0,53$ ;  $p < 0,01$ ) and between pf and  $2k_2n_0$  ( $r = 0,27$ ;  $p < 0,05$ ) were found. These results point out that, in the scleroderma, the fibrinogen is the possible responsible of the augment of the velocity erythrocyte aggregability and the plasma viscosity and contribute in this way to the damned fluid in the microcirculation.

## 50.

**SODIUM THIOPENTAL INTRAPERITONEAL EFFECT OVER TOTAL WHITE CELLS RECOUNT AND LEUCOCITARY FORMULA AT SPRAGUE DAWLEY RATS**

Contesti JF<sup>1</sup>, Palmisano EM<sup>2</sup>, Catalá Schonfeld HG<sup>2</sup>, Bechara Baladi VS<sup>2</sup>, Diez GA<sup>2</sup>, Diez J de la C<sup>2</sup>, Bobrovsky EO<sup>2</sup>, Spengler MI<sup>1</sup>.

<sup>1</sup>Cátedra de Biofísica. FCM, UNR. <sup>2</sup>Instituto de Cirugía Experimental. "Prof. Dr. J. J. Boretti", HEPE, FCM, UNR.

In previous studies we evaluate the sodium thiopental effect over blood viscosity at Sprague Dawley rats (SDR). The aim was to know the immediate effect (IE) and after 12 hs (12E) of a single intraperitoneal shot of sodium thiopental (2,5%) (IST) over total white cells recount (TWC) and percentage (%) of segment neutrophily (SN) and lymphocyte (L) in SDR. It had been used adult male SDR divided in thiopental group (TG) (n=16): IST (0,2 ml/100g weight) subdivided in TGi (n=8): immediate euthanasia and TG12 (n=8) 12 hs euthanasia. Control group (CG) (n=16): 0,2 ml/100g weight of intraperitoneal NaCl 0,9% subdivided in CGi (n=8): immediate euthanasia and CG12 (n=8) 12 hs euthanasia. Blood had been treated with EDTA, processed in an automatic blood test for the recount of TWC and were prepared extended stained with May-Grünwald-Giemsa for manual recount of N and L. Comparisons were realized using t Student Test. It had been demonstrated for each group an increment in the % of N and a decrease in the % of L comparing the IE and 12E, without modifications in the TWC recount. It had not been demonstrated significant differences between TG and CG in the IE and 12E for TWC, N and L.

## 51.

**EFFECT OF VEGETABLE LYSATE ON EQUINE HEMORHEOLOGICAL PARAMETERS**

Dottavio ME<sup>1</sup>, Catalani G<sup>2</sup>, Miglietta MC<sup>3</sup>, Peroni M<sup>2</sup>, Rasia M<sup>1</sup>.

<sup>1</sup>Cát. Física Biológica. Fac. Cs. Médicas. UNR. <sup>2</sup>Cát. Fisiol. Fac. Cs. Vet. UNR. <sup>3</sup>Cát. Anatomía Vet. 1<sup>era</sup> parte. Fac. Cs Veterinarias UNL. E-mail: galena@arnet.com.ar

A crude 30% hydroalcoholic vegetable lysate (VL) prepared using *Portulaca sp* foliar parenchyma was administered to thoroughbred horses with different degrees of epistaxis (Exercise-Induced Pulmonary Hemorrhage) (EIPH). Animals show clinical improvement after treatment, such as bleeding decrease and rapid post-exercise respiratory recovery. Objective: To study rheological changes produced by treatment in 12 clinically healthy untrained adult horses, with same management and feeding. Treatment consisted on daily oral administration of 50ml VL. Horses were divided in 2 groups (n=6), with the following scheme treatments regarding duration: Group A: 7 days, and Group B 14 days. Anticoagulated (EDTA) whole blood samples (10ml) were analyzed. Assays: erythrocyte deformability through its inverse i.e., rigidity index (RI), relating RBCs filtration time through a polycarbonate membrane (5µm diameter pores) with saline filtration time. RBCs aggregation: time optical density variability in whole blood samples; 2 parameters estimating aggregate average size ( $s_0/n_0$ ) and initial formation velocity ( $2k_2n_0$ ). Assays performed at 40% hematocrit in autologous plasma previous and after treatment. Statistics: Wilcoxon's test for paired data. Results (median and range). Group A:  $s_0/n_0$  and  $2k_2n_0$  were lower after treatment [1.74, 1.70-1.78] and [0.99, 0.79-1.10] respectively,  $p < 0.05$ . Group B n.s. RI decrease in Group A [4.03; 3.6-10.8]  $p < 0.05$ . Group B n.s. Results: VL administration improves flow conditions in 7 days regime but not in 14 one, which is important since blood flow impairment is one of the causes of EIPH.

## 52.

**IMMATURE ELEMENTS IN THE RED BLOOD CELLS OF 12-MONTH-OLD MALE eSS RATS WITH RENAL DAMAGE**

Daniele SM, Picena JC, Rodríguez H, Montenegro SM, Tarrés MC, Martínez SM.

Facultad de Ciencias Bioquímica, Facultad de Ciencias Médicas, CIUNR. UNR.

The eSS rat displays type 2 diabetes more remarkable in males. Anaemia was verified with low red blood cell count and less amount of haemoglobin. At one year of age, May-Grünwald-Giemsa-stained peripheral blood smears showed immature erythrocytes, polychromatophilia, "rouleaux" formations and Howell-Jolly bodies. The presence of erythroblasts in peripheral blood was quantified in eSS ( $1.17 \pm 0.34$ ) but was never detected in Wistar eumetabolic controls (n=8). In diabetic animals glomeruli showed diffuse hypertrophy of mesangial tissue though a number of small glomeruli was also found; areas of markedly atrophic and dilated tubules containing acidophilic proteinaceous material were also prominent. In eSS, the finding of immaturity, polychromatophilia and Howell-Jolly bodies in peripheral blood erythrocytes would reflect an increase of erythropoiesis. Present results suggest that renal damage as well as hyperglycaemia could be responsible for the haematologic alterations of eSS.

### 53. NAPROXEN INDUCED GASTRIC ANTRAL ULCER: PREVENTION AND CHRONICITY

Villarruel R, Laudanno OM, Cesolari JAM, Rojas P, Calvi BJ, Simó M, Urteaga A, Vasconcelos LA, Vegetti R, Wandzik CI.

Experimental Gastroenterology. Histology and Embriology Chair; School of Medicine. National University of Rosario (NUR). E-mail: roro\_villarruel@hotmail.com

Aim: To check if Naproxen (NA), a cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) selective inhibitor, given subcutaneously (SC) after a solid meal, or orally (OG), caused gastric antral ulcer in rats in contrast to the Satoh method which uses Indomethacin (INDO) SC, having eaten a solid meal; to verify its prevention with Pantoprazol (PANTO), proton pump blocker, and to study its chronicity using INDO. Experiments: In randomized Wistar rat groups (n:7/group), 200g, 24 h fasting except water ad-lib., and avoiding coprophagia, 1) Solid Meal (SM), 2h, INDO, 30mg/kg, SC and then SM for 24h (witness); 2) SM, 2h, then NA SC and later SM 24h; 3) SM, 2h, NA, SC and SM 24h, then NA, SC every 24h for 3 days; 4) NA, 80mg/kg OG, then SM and waited 24h (witness); 5) PANTO, 5mg/kg SC, then NA, OG and SM 24h; 6) NA, OG, every 24h, for 3 days (witness); 7) NA, OG every 24h for 3 days, then INDO, 10mg/kg, SC every 24 h for 3 days. After an ether overdose Laparotomy, total gastrectomy, and gastric major curvature opening were performed. The percentage of the antral ulcer was measured through computerized planimetry. Cuts for histological study were fixed in formaldehyde at 10% and Hematoxylin-Eosin (HE) stained. Statistics: Student's *t* test and ANOVA. Conclusion: NA, OG electively showed erosion in the gastric antrum, prevented with PANTO. INDO caused induction to chronic antral ulcer.

### 54. PANCREAS MORPHOLOGIC EVALUATION IN $\beta$ LINE RATS SUBJECTED TO SURGICAL STRESS

Passerini F<sup>1,3</sup>, Gómez JA<sup>1</sup>, Cánaves MA<sup>1</sup>, Alet NA<sup>1,3</sup>, Blanco ML<sup>1,3</sup>, Sgro MV<sup>1,3</sup>, Eichhorn MC<sup>2</sup>, Navoni S<sup>2</sup>, Díez GA<sup>1</sup>, Caraballo E del C<sup>2</sup>, Gayol M del C<sup>3</sup>.

<sup>1</sup>Inst.Cirugía Exp. Prof Dr J Boretti ; <sup>2</sup>Servicio de Anat. Patol. del Hosp.Esc.E.Perón; <sup>3</sup>Cátedra Biología, Fac.Cs. Médicas, UNR.

The surgical act starts a systemic response and the affected tissue inflammation, with later possible residual injuries. Two 250 day old male IIM/ $\beta$  rat groups: control (GC) received no treatment and experimental (GE) received 50' surgical stress one. This consisted of insuline application, anaesthesia (Tiopental IP + inhalatory ether), analgesia (Nalbufina), antibiotic prophylaxis (Cefalotina), left subcostal laparotomy and physiologic solution injection (0.01 ml) into pancreas. Analysis made by *t* student (media  $\pm$  SEM) and Fisher test. Fifteen days after surgery, pancreas were obtained and fat removed. Macroscopically colour and aspect were the same GE and GC, except intraperitoneal adhesions in GE. Pancreas relative weight: GE(n=9): 2,204 $\pm$ 0,175 vs GC(n=9): 1,977 $\pm$ 0,169 (p>0,05). Histologically the presence(+) or the lack(-) were evaluated: Preserved morphology islets: GE+(n=5); GE-(n=2); GC+(n=7); GC-(n=5); Nesidioblastosis: GE+(n=2); GE-(n=5); GC+(n=4); GC-(n=8). Polycyclical edge islets: GE+(n=6); GE-(n=1); GC+(n=10); GC-(n=2); Dislocated islets: GE+(n=5); GE-(n=1); GC+(n=11); GC-(n=1). Insular fibrosis: GE+(n=5); GE-(n=1); GC+(n=12); GC-(n=0). Interstitial hemosiderosis: GE+(n=5); GE-(n=1); GC+(n=9); GC-(n=3). All (p>0.05). Linfocitary infiltration: GE+(n=6); GE-(n=3); GC+(n=1); GC-(n=11); (p<0,05), probably due to surgery inflammation. Insular Area: GE: 4,80 $\pm$ 0,291 vs GC: 4,00 $\pm$ 0,354 (p>0.05). Surgery only affected linfocitary infiltration and peritoneal adhesions.

### 55. DULOTEXINE EFFECTS ON THE RAT GASTRIC MUCOSA

Cesolari JAM, Laudanno OM, Calvi BJ, Vegetti R, Rojas P, San Miguel P, Vasconcelos LA, Villarruel R, Simo M, Urteaga A, Wandzik CI.

Gastroenterología Experimental. Cátedra de Histología y Embriología. Facultad de Ciencias Médicas. Universidad Nacional de Rosario. E-mail: ceso@cimero.org.ar

Duloxetine (Dulo) is a new antidepressant inhibiting serotonin reuptake. Objective: To study Dulo in two experimental models: I) gastric cytoprotection and II) gastric antral ulcer produced by indometacine and stress by immersion in 23°C water for 7 hours. Randomized female rat groups (n=7 each), 200g weight, 24h fasting, water ad lib and avoiding coprophagy were submitted to the following experiments: Group I-1) 1 ml saline by orogastric gavage (OG), 60min wait (control); 2) 96% Ethanol (ETOH), 1 ml OG, wait 20 min (control); 3) 10-75 mg/kg Dulo, intraperitoneal (IP), 60 min, then ETOH Group II-1) 1ml saline, subcutaneous followed by stress by immobilization and immersion (water 23°C) for 7 h, wait 14h. (control) 2) 50 mg/kg SC Indo, followed by e AND 24H WAIT. 3) 10 mg/kg IP Dulo, 60 min, followed by E wait 24 h. 4) Dulo + Indo, wait 24hr. 5) Dulo + Indo, then E wait 24 h. Animals were sacrificed by ether overdose. Laparotomy, total gastrectomy, opening through major gastric curvature And percentage quantification of the lesion gastric area (%) were performed. Samples fixed in 10% formaldehyde and stained with H-E were obtained for histological examination. Student's *t* test and ANOVA were calculated. % was: Group I-1) 0.1  $\pm$  0.1; 2) 35.5  $\pm$  5.5 (p<0,001); 3) 65.5  $\pm$  1 (p<0,01); Group II-1) 15  $\pm$  3,2; 2) 23  $\pm$  5 (p<0.05); 3) 17.2  $\pm$  4.5 (ns); 4) 30  $\pm$  5 (p<0.05); 5) 85.5  $\pm$  6.5 (p<0.001). Conclusion: Duloxetine aggravated lesions produced by 96% ethanol, and adding Indometacin aggravated gastric ulcer produced by stress.

### 56. MASKED MALNUTRITION IN SCHOOL CHILDREN IN THE CITY OF ROSARIO

Marchegiani M, Cesolari JAM, Calvi BJ.

Instituto Gral. San Martín. Facultad de Ciencias Médicas, U.N.R. E-mail: marcelamarchegiani@yahoo.com.ar

Masked malnutrition affects children although having access to healthy feeding, due to an inadequate diet. Lack of nutrients is produced by a high calorie intake (providing energy), but deficient in other substances important for growth, such as Fe, Ca, P and vitamins A and C. Objective: to study the existence of hidden malnutrition, namely specific lack of micronutrients, mainly Fe and Ca in school children Rosario (S.F.), considering the transitional nutritional process, present feeding behavior, and little information about micronutrients deficiency, in a school population with middle-high socio-economic status, in our country. Descriptive, transversal study (93 school children, both sexes), 1st to 5th year, 1st and 2nd EGB cycle, Mariano Moreno Public School No. 60, Rosario, S.F. Food intake was evaluated administering a structured anonymous and voluntary, questionnaire, with open and closed questions, about patterns of food intake, in school children of high-middle class, to be completed by parents or surrogate parents, to obtain more precise and reliable data. Recommendation tables for Fe and Ca were used, comparing the estimated intake for each child and classifying the adequate %. Results: mean total Fe intake was 4,32 $\pm$ 1,89 mg/day and total Ca 717.19 $\pm$ 314.63 mg/day. Categorizing the individual intake in 5 consumption items according to nutritional recommendations, results were as follows (%), Fe: <50: 73.1; 50-75 21.5; 76-100 3.2; 101-120 1.1; >120 1.1; Ca: <50 15; 50-75 21.5; 76-100 29; 101-120 20.5; >120 14%. There were no significant differences in both micronutrients considering schooling. Conclusion: Children showed a low Fe intake, lower for Ca, evidencing a need to implement nutritional education campaigns to increase Fe and Ca intake, and optimize their absorption, through flour fortification which is one of the strategies to prevent Fe lack.



### 57. SUBCUTANEUS BIOFLORA PROBIOTICS PREVENTING THE GASTRIC ULCER

Vasconcelos LA, Cesolari JA, Laudanno OM, Urteaga A, Vegetti R, Villarruel R, Wandzik C, Godoy A.  
Experimental gastroenterology, Histology and Embriology, School of Medical Sciences of Rosario, U.N.R.

Aim to study the bioflora probiotic with intestinal bacteria to prevent gastric ulcers in the models of gastric lesions that are induced by indomethacin (indo) or by stress. The following experiment were performed in aleatory groups of wistar rats (n=7 in each group) 200 gr... 24 hours fasting, water ad libitum and avoiding coprophagy. I. Antral gastric ulcer induced by indo 1) 24 hs. Fasting, then solid food (S F) - 2 hs., then subcutaneous indo 30 mg./kg.- (S F) then (SF) follows for 24 hs. (witness). 2) 24 hs. Fasting, SF for 2 hs. Indo and simultaneously bio 1ml., SF, then SF for 24 hs. II) Stress by immobilization or immersion in water. 1) stress in a 23°C water - 7 hs. (S) and the oral administration follows for 24 hs. (F) (witness) 2) Bio 1ml. Solid food- 60 minutes then (S) and (F)- at the end the percentage of the gastric ulcerous area was tabulated, measured by computed planimetry, sections for histologic studies fixed by formol(10%) were obtained and then stained with hematoxylin eosin (HE). Twenty four hours later the rats were sacrificed. Laparotomy, gastrectomy, complete enterectomy and the stomach opening on the greater curvature were performed. **Results**: The percentage of the gastric ulcerous area was I. Modelo I: 1)  $25 \pm 3$ . 2)  $1.5 \pm 0.2$  ( $p < 0.01$ ). Modelo II: 1)  $17 \pm 1.5$ . 2)  $1.3 \pm 0.4$  ( $p < 0.01$ ). The stomach, ileum and colon's. Histochemical method of Lymphocytes-CD.20-(B) and CD4(T) just showed a pronounced growing of the lymphocytes in the ileum and colon. In conclusion: the bioflora probiotic avoids the formation of gastric ulcers by indomethacin and stress.

### 58. M CELLS AND LYMPHOID FOLLICLES IN SACculus ROTUNDUS WITH INFLAMMATORY CHANGES

Pérez F<sup>1</sup>, Roma S<sup>1,2</sup>.

<sup>1</sup>Cát Histología y Embriología. Fac. Ciencias Médicas. UNR. <sup>2</sup>Cát. Genética Humana. Facultad Medicina. UAI.

The sacculus rotundus is one of the main immuno-inductive sites in the rabbit gut. This small organ constitutes the lymphoid tissue associated to the ileo-cecal valve with many lymphoid follicles covered by a simple epithelium where specialized M (membranous) cells are found. In previous studies we demonstrated that ovalbumin (OVA) sensitization induces an inflammatory response with edema, lymphangiectasias and eosinophils infiltration. The aim of the present work was to analyze the modifications in the diameter of the lymphoid follicles and in the number of the M cells in sacculus rotundus from sensitized rabbits. Six New Zealand adult rabbits were divided into two groups (G). G1 (n=3): normal control and G2 (n=3): subcutaneously OVA sensitized. Slides were immunostained with Vimentin to detect M cells. The lymphoid follicles diameters were measured using a calibrated ocular. Results were referred as the arithmetic mean  $\pm$  standard error and statistically analyzed using the Student t test. The number of M cells was in G1:  $22,82 \pm 1,35$  and in G2:  $32,89 \pm 1,01$  ( $p < 0.0001$ ). The lymphoid follicles diameter was  $78,87 \pm 1,65 \mu\text{m}$  in G1 and  $101,63 \pm 3,59 \mu\text{m}$  in G2 ( $p < 0.0012$ ). We conclude that sensitization induces an increase in the M cells population and in the lymphoid follicles diameter. These changes probably reflect a more antigenic traffic through these cells and a secondary stimulation of the local immune response, specially of the lymphoid follicles, where the switching to IgA isotype takes place.

### 59. UPDATE OF CLINICAL AND PRODUCTIVE ASPECTS OF MANDIBULAR LYMPH NODE ALTERATIONS (MLNA) IN BOVINE

Costa A, Oyarzabal MI, Dasso L, Prat G, Décima M.

Fac. Cs. Veterinarias. UNR. E-mail: alcosta@fveter.unr.edu.ar

MLNA are disorders in the subparotid and retropharyngeal mandibular lymph nodes with manifestations that show from an increase in size and abscesses to suppurative liquefaction, and, on occasions, satellite nodes and abscesses. They are generally diagnosed as Actinobacillosis. The aim of this presentation is to report the studies carried out at the Faculty of Veterinary Sciences of the National University of Rosario in relation to the MLNA issue. A study done in a southern district of Santa Fe province (Argentina) reported a 0.66% (N= 10980) prevalence. In relation to clinical studies, an approach employing four MLNA characterising variables was carried out. A lower milk production and a higher inter-calving period were observed in sick cows, though no significant differences were found. Significant differences were observed in the sale price of sick animals compared with healthy animals of the same category and condition. Since 2004 work has been done at a local abattoir and the presence of *Actinobacillus*, *Corynebacterium*, *Streptococcus* and *Pseudomonas* was detected. The presence of other genera must also be highlighted; though not significant to buiatrics they so are to human medicine, such as the case of *Acinetobacter* and *Staphylococcus coagulase-negative*. The presence of genera different from *Actinobacillus* can help explain the difficulties in treatments as many of them are chemo resistant. No association was found between bacterial genera and lesion localization. Further studies on these MLNA would contribute to their treatment and prevention.

### 60. IgG<sub>1</sub> AND IgG<sub>2</sub> ISOTYPES IN PORCINES FROM A HERD NATURALLY INFECTED WITH *Brucella suis*

Arestegui MB<sup>1</sup>, Gualtieri CAS<sup>1</sup>, Torioni de Echaide S<sup>2</sup>, Aguirre N<sup>2</sup>, Delgado G<sup>3</sup>, Peralta L<sup>1</sup>, Besso R<sup>1</sup>, Gentile N<sup>1</sup>, Coca L<sup>1</sup>, Martineli F<sup>1</sup>, Scharovsky OG<sup>4</sup>.

<sup>1</sup>Cátedra de Sueros y Vacunas, <sup>3</sup>Cátedra de Obstetricia, Fac. Cs. Veterinarias, Casilda; <sup>2</sup>INTA EEA Rafaela; <sup>4</sup>Inst. Genética Experimental, Fac. Cs. Médicas, CIC-U.N.R., Rosario; Argentina.

During *Brucella* infection the main function of IgG<sub>2</sub> is opsonization of bacteria, leading to an increase in intracellular death, while IgG<sub>1</sub> is associated with chronic infection. Our aim was to study if the differences found in functional activity and phenotype of mononuclear phagocytes of seronegative (S<sup>-</sup>) and seropositive (S<sup>+</sup>) pigs for brucellosis were associated to the type of response to *Brucella* infection. The predominant IgG isotype was determined in S<sup>+</sup> and S<sup>-</sup> pigs from a farm with 85% brucellosis prevalence. The serologic diagnosis of brucellosis was determined in 320 female pigs through BPA, 2ME, FPA, C-ELISA and CF (complement fixation) analysis. Those animals that were positive to three tests were considered S<sup>+</sup> and those non reactive, S<sup>-</sup>. All the animals shared the same environment during the infected abortions. IgG isotype was determined by Indirect ELISA with mouse MoAb anti porcine IgG<sub>1</sub> and IgG<sub>2</sub>. IgG<sub>1</sub> title was higher in S<sup>+</sup> (n=207) [median (range): 60(4-116)] than in S<sup>-</sup> (n=113) [3 (2-49)] ( $p < 0.0001$ ). Also for IgG<sub>2</sub>, the differences [83 (3-174); 42 (2-139), respectively] were significant ( $p < 0.05$ ). The predominance of IgG<sub>1</sub> in S<sup>+</sup> animals would be related to the modulation towards a non-protective Th<sub>2</sub> immune response and the higher production of IgG<sub>2</sub> in S<sup>-</sup> animals would suggest the protection against the infection. This behavior would agree with the functional and phenotypic differences previously found in these animals.

#### 61. SELECTION OF BIOMEDICAL MODEL IN HUMAN AND ANIMAL BUCCAL HEALTH

Arango JF, Reynoso AC, Alfieri AE, Seghesso AB, Lapalma MA, Steeman R(h).

Cátedra de Higiene, Epidemiología y Ad. Sanitaria. Fac. de Cs. Veterinarias. U.N.R. Casilda. E-mail: aalfieri@fveter.unr.edu.ar

A selection and study were made to identify adequate animals for prosthodontics clinical practices, it was done by bibliographic investigation of the compatibility of anatomic, functional, physiologic and pathologic of man with those of non-human primates, dog, cat, pig, cow, horse, sheep, goat, rodents, rabbit, guinea pig, Wistar rat, fish and reptiles. Man buccal apparatus (heterodont, diphyodont and with definite growth) is compatible with enunciated animals, except fish and reptiles for being monophyodont; and rodents (incisors and molars) and boar (inferior canines) for having a continuous growth of their teeth. Man shares his dental formula with catarrhine non-human primates—gorilla and orangoutan-. Both man mouth and dog one have large opening, but it is not so in herbivores, rodents and lagomorphs. Man teeth act by pressing and cutting. Dog incisors gnaw and clean, premolars have cutting, serrate and discontinuous edges which cut while necklines hold food. First and second maxillary molars and second and third ones of mandible crush and cut. Pig premolars have blunt peaks allowing crushing and molars are secodont. Cow and horse molars have a structure of crowns of folded enamel and soft tissues, with wide scraping surface. Development, change and position pathologies are common in dog and horse. Pigs and non-human primates show caries, which are not frequent in dogs due to the diet and the lack of *Streptococcus mutans* and *salivarius*. Presented results suggest that rodents, lagomorphs and herbivores are not the most adequate animals as biomedic models for those aims of investigation.

#### 62. LOCALIZATION OF HEPARIN BINDING SITES IN PORCINE SPERM PLASMA MEMBRANE

Dapino D<sup>1,3</sup>, Cane F<sup>2</sup>, Marini P<sup>3</sup>, Cabada M<sup>3</sup>.

<sup>1</sup>Cát. Fisiología. Facultad de Cs. Veterinarias. <sup>2</sup>Instituto de Porcinoecnia. <sup>3</sup>Div. Biología del Desarrollo (IBR-CONICET) and A. Biología. Facultad de Cs. Bioquímicas y Farmacéuticas. U.N.R.

The physiological changes that sperm experiment in the female tract and give them the capacity to fertilize the oocyte are collectively called capacitation. Most of those changes have been related to modifications in the plasma membrane architecture in response to medium's components. Previously, we observed an increase in the capacitation values of sperm incubated in capacitating medium with heparin. To locate topographically the heparin binding sites on sperm during capacitation, indirect immunofluorescence was used. Sperm from 5 boars was incubated for 120 min in TALP. At 0, 60 and 120 min aliquots were taken and incubated with biotinylated heparin, followed by streptavidin-FITC. A control aliquot was competed by incubation with non biotinylated heparin. Capacitation was estimated by calcium ionophore A 23187 technique. Two stain patterns were observed: (A), fluorescence in postacrosomal area and (B), fluorescence in acrosomal area. An augment in B and a decrease in A were noted along the capacitation process. In the competed sample, no fluorescence was observed. Statistics: ANOVA. The rate of acrosome reaction before the addition of ionophore did not differ significantly from the sperm showing B pattern in each incubation time in the presence of biotinylated heparin ( $p > 0.05$ ). Conclusions: heparin binds to sperm plasma membrane in a specific and competitive manner and the heparin binding sites experiment a redistribution during the capacitation process. Both facts should be of utility for the development of a new capacitation stain technique and for the study of the specific role of heparin-like glycosaminoglycans in the capacitation process.

#### 63. PHYSIOLOGICAL PROFILE MODIFICATIONS OF SOIL MICROBIAL COMMUNITY ON AN TYPICAL ARGIUODOL SUPPLEMENTED WITH BIOSOLID INOCULATED WITH *Pseudomonas fluorescens* 92

Perotti EBR<sup>1</sup>, Fracchia L<sup>2</sup>, Pidello A<sup>1</sup>, Martinotti MG<sup>2</sup>.

<sup>1</sup>LQB-FCV-CIUNR Universidad Nacional de Rosario, Argentina.

<sup>2</sup>DiSCAFF, University of Eastern Piedmont, Novara, Italy. E-mail: eperotti@unr.edu.ar

The aim of this work was to study the functional microbial community changes of a typical Argiudol soil after inoculation with *Pseudomonas fluorescens* 92<sup>RTe-Gfp</sup>, a siderophore and indolacetic acid producer and phosphate solubilizer strain, using as support a biosolid derived from urban wastewater treatment sludge. Four treatments (soil, soil + *P. fluorescens*, soil + biosolid, soil + biosolid + *P. fluorescens*) were carried out in micro-cosmos and the bacterial physiological diversity was analysed 3 and 7 days after inoculation by means of the BIOLOG EcoPlate™, a system using 31 different carbon sources. The results show that the strain survival was high ( $10^5$  cells/g soil dry weight) and that the metabolic activity of the inoculated soil treatments were lower than those of the non-inoculated treatments. In particular, the metabolic profile changes were related to only 7 different carbon sources, these were: L-Threonine, L-Arginine, L-Phenylalanine, 2-Hydroxy Benzoic Acid, Glycyl-L-Glutamic Acid, Pyruvic Acid Methyl Ester, D-Galactonic Acid  $\gamma$ -Lactone.

This work was realised in the framework of the scientific Bilateral Co-operation between Argentina and Italy supported by SeCyT and the Italian Ministry of Foreign Affairs N°IT/PA05-BVII/085.

#### 64. DIAGNOSTIC OF CESTODES IN CANINE. COMPARISON OF TWO TECHNIQUES

Porto MLP, Negro PS.

Cátedra de Parasitología y Enf. Parasitarias: Fac. Cs. Veterinarias, UNR. E-mail: marialauraporto@hotmail.com

The laboratory diagnosis of the cestodes is based on the identification of proglottids, eggs or both, in the faeces. The technique for a definitive diagnosis is the arecoline diagnostic test, which requires biosecurity protocols to avoid infections to human beings or other animals. On the other hand, the sedimentation-filtration technique requires biosecurity, but is cheap and no drug is administered to animals. Our objective was to compare these techniques to determine the presence or absence of cestodes in canine faeces. The test of independence of attributes was used based on the distribution of the  $\chi^2$ . The drug was administered to 30 canines, 8 were from an urban environment and 22 from a rural one. The sedimentation-filtration technique was carried out for each dog before performing the arecoline test. After the administration of the drug, 12 canines (40%) eliminated cestodes. The found cestodes were of the Eucestoda class; the diagnosed species were: *Dipylidium caninum* (11 animals), *Echinococcus granulosus* (1 animal) and *Taenia hydatigena* (4 animals). By using the coproparasitological technique 7 canines (23.33%) presented eggs and/or cestodal oviferous capsules (these dogs had eliminated adult parasite with the other technique). A significant association between technique used and cestode presence was observed ( $p < 0.05$ ). Further research for an easy coproparasitological technique that presents low biorisk should be done, since the same antiparasitic drug is used to treating cestodal infestations in dogs (Praziquantel).

# 65. DETERMINATION AND CHARACTERIZATION OF DOG BITES TO HUMAN BEINGS IN AREQUITO

Pirles ME, Schiaffino LS, Pagni C.

Facultad de Ciencias Veterinarias. U.N.R.

Dog bites to human beings cause organic lesions of diverse type, physical perturbations and various patrimonial damages. Available publications show that dog bites are mostly caused on the thoroughfare by dogs with owners and not all are reported. The aim of the present study was to begin to characterize data related to dog bites caused to human population from Arequito, to generate preventive policies. During 2005 a retrospective study was done. Data related to dog bites to human beings were received from the Department of Bromatology of the Commune of Arequito, having as source reports from SAMCO Hospital and its own records. Number of attended cases caused by bites, total number of attended cases, gender and ages of victims, localization and severity of lesions, animal possession (with owner or not), place of aggression and aggressor habits were analyzed. Of 22 reported cases before the Ministry of Public Health and Environment of Santa Fe, it represents 0.2% of total attended cases in the same period in the Hospital, an average of 1.83 cases per month, victims were both men and women, from children to managers, all the bites were done on the public thoroughfare. Of the total of biting dogs, 90% have a well known owner a 100% of them roam freely about the public thoroughfare. It is highly probable that the number of cases exceeds the analyzed ones, as there is no information from other sources which can add more data. A better knowledge on ages, gender, lesions, circumstances of bites and victims will allow a punctual focussing on the design of preventive policies, so that, there will be an advance on the redefinition of the information to be requested.

# 66. METHANE PRODUCTION IN RUMEN. RELATION WITH FORAGE CHEMICAL COMPOSITION

Smacchia AM, Figallo RM, Pidello A.

Lab. de Química Biológica. Fac. Cs.Veterinarias. CIUNR. UNR.

The aim was to study forage CH<sub>4</sub> production and to determine its relation with the chemical composition. Samples from a wide range of ruminant food forages were chosen. Oats (*Avena sativa*) OP and bromus pastures (*Bromus unioloides*) BP; alfalfa (*Medicago sativa*) HA, and setaria hays (*Setaria italica*) SH and Corn Silage (*Zea mays*) CS were incubated *in vitro* in rumen in closed systems 24h at 39°C. Final gas production and CO<sub>2</sub> and CH<sub>4</sub> were measured by gaseous chromatography. The CH<sub>4</sub> produced referred to the degraded dry matter DDM in rumen at 24h incubation *in sacco* and expressed in l/kgMSD<sub>24h</sub>. Chemical composition was measured as: OM, NSCS, lignin, CP, NDF and ADF. Data obtained were analysed through correlation and regression (P ≥ 0.05).

	Chemical Composition						<i>in vitro</i> Studies		
	OM	NSCS	Lig.	CP	NDF	ADF	Gas	CH <sub>4</sub> <sup>(1)</sup>	CH <sub>4</sub> <sup>(2)</sup>
OP	89.0e	11.9 e	4.6b	18.4c	47.8b	30.5a	18.7e	22.70e	28.6 b
BP	81.3a	2.8 a	7.4d	21.7e	59.2d	40.9d	17.3d	21.91d	25.2 a
AH	87.6c	5.7 c	4.4a	19.5d	45.7a	32.6b	16.9c	20.85c	28.6 b
SH	86.4b	2.9 b	6.9c	6.9a	68.2e	51.3e	14.5b	19.55b	33.7 c
CS	88.3d	9.5 d	4.4a	7.6b	54.4c	39.2c	14.0a	19.43a	33.6 c

<sup>(1)</sup>l/kgMS; <sup>(2)</sup>l/kgMSD<sub>24h</sub>; different letters indicate differences (P<0.05).

OP and CS showed the highest NSCS and SH and BP the lowest. SH showed the highest NDF and ADF values. BP showed the highest lignin and CP. CS and SH showed lower production of CH<sub>4</sub> at 24 h incubation as a consequence of a lower DM degradation. At equal degraded DM, CH<sub>4</sub> production was higher in CS and SH, intermediate in AH and OP and lower in BP. CH<sub>4</sub> in l/kgMSD<sub>24h</sub> related inversely with gas production r: -0.828, p<0.0829 and with the CP concentration, r: -0.973, p<0.053. The equation that represents it = 37.45 (±1.11)- 0.506 (± 0.0615).x; r<sup>2</sup>: 94.70. Results show that the CP concentration of forages determined CH<sub>4</sub> production referred to equal quantity of degraded DM and challenges further studies.

# 67. DRY MATTER RUMINAL DEGRADATION AND CHEMICAL COMPOSITION OF PASTURE HAYS

Figallo RM, Faienza HL, Pidello A, Smacchia AM.

Lab. Química Biológica. Fac. Cs. Veterinarias. CIUNR. UNR. E-mail: rfigallo@fveter.unr.edu.ar

The aim was to study the relationship between the parameters that describe the kinetics of dry matter ruminal degradation (DMRD) with the chemical composition of pasture hays. The samples employed included Setaria (*Setaria italica*), festuca (*Festuca arundinacea*) and alfalfa hays (*Medicago sativa*). They were dried at 60°C, grounded and sieved through a 2mm mesh. DMRD was determined according to Meherz and Orskov (1977). Chemical characterization consisted in: OM, lignin, CP (N x 6.25), NDF and ADF according to AOAC (2000). DMRD data were fitted to Orskov and McDonald (1979) model: DMRD = a + b (1 - e<sup>-ct</sup>); where a: soluble fraction, b: slow degradable fraction, and c: degradation rate. Data obtained were studied through correlation. The observed correlations were the following for the soluble fraction: -0.91; -0.457; -0.938; -0.94 and -0.938; for show degradable fraction: 0.3134; 0.0785; 0.5733; 0.4252 y 0.4053, and for degradation rate: -0.8674; -0.6506; -0.9819; -0.9235 and -0.8889 with OM, CP, Lignin, ADF and NDF respectively. These results show that as the soluble fraction degradability and degradation rate increase, the concentration of the parameters of the chemical composition decrease especially in OM Lignin, ADF and NDF in hays. The show degradable fraction showed an inverse behaviour but not as marked, increasing as the chemical concentration parameters increase. Cdude protein showed less close relations in all degradable fractions in rumen.

# 68. KINETICS OF GAS PRODUCTION IN RUMEN. RELATION WITH CHEMICAL COMPOSITION IN FORAGES

Smacchia AM, Figallo RM, Pidello A.

Lab. Química Biológica. Fac. Cs.Veterinarias. CIUNR. UNR.

The aim of this research was to study the kinetics of gas production of forages and determine its relation with its chemical composition. A wide variety of forages employed in ruminant diets have been selected: Oats (*Avena sativa*) OP and bromus pastures (*Bromus unioloides*) BP; alfalfa (*Medicago sativa*) AH, and setaria hays (*Setaria italica*) SH and corn silage (*Zea mays*) CS, were incubated at 1%, *in vitro* systems at 39°C, 28 h. Production was recorded at 0.5; 2; 6; 9 and 24 h. Data were adjusted to Orskov and Mc Donald (1979): DMRD = a + b (1-e<sup>-ct</sup>); where a stands for soluble fraction, b for slowly fermented fraction, c for fermentation rate. Chemical characterization consisted in: MO, NSCS, lignin, CP, NDF and ADF. Data obtained were analysed employing simple ANOVA and correlation (P ≥ 0.05).

	Chemical Composition						Gas Production			
	OM	NSCS	Lig.	TP	NDF	ADF	ml <sub>24h</sub>	Fractions		
								a	b	c
OP	89e	11.9e	4.6b	18.4c	47.8b	30.5a	18.7e	5.21e	49.9d	0.092c
BP	81.3a	2.8a	7.4d	21.7e	59.2d	40.9d	17.3d	4.73d	49c	0.092c
AH	87.6c	5.7c	4.4a	19.5d	45.7a	32.6b	16.9c	3.61c	48.8b	0.092c
SH	86.4b	2.9b	6.9c	6.9a	68.2e	51.3e	14.5b	1.08a	52.9e	0.073b
CS	88.3d	9.5d	4.4a	7.6b	54.4c	39.2c	14a	3.47b	47.3a	0.048a

Note: the different letters in a column indicate that there are differences (P<0.05).

The OP and BP produced more gas with higher a and c fractions; as opposed to SH and CS. Between the studied variables, gas production was directly related to CP, r: 0.8853 (p=0.0458); rapid fermentable fraction, inversely with ADF r: -0.8131 (p= 0.0942); and fraction c: directly with CP r: 0.8605 (p=0.0612). Gas production was related with c r: 0.8746 (p= 0.0523). Gas production and fermentation rate were related with CP. Fraction a was inversely related with ADF. These results show the influence of the chemical composition of food on gas production kinetics in rumen *in vitro* and challenge the study of other materials.

**69. DOMINANCE RATIO OF IMPORTANT AGRONOMIC TRAITS IN TOMATO CROSSES**

Liberatti DR<sup>1</sup>, Mahuad SL, Scarafiocca MJ, Rodríguez GR<sup>1</sup>, Pratta GR<sup>1</sup>, Zorzoli R<sup>2</sup>, Picardi LA<sup>2</sup>.

Cátedra de Genética. Facultad de Ciencias Agrarias. Universidad Nacional de Rosario. CC14. (S2125ZAA) Zavalla. <sup>1</sup>CONICET, <sup>2</sup>CIUNR. E-mail: dliberat@unr.edu.ar

Seven recombinant inbred lines (RILs, ToUNR1; ToUNR5; ToUNR6; ToUNR8; ToUNR9; ToUNR15; ToUNR18) obtained by the Cátedra de Genética, Facultad de Ciencias Agrarias (UNR) from an interspecific cross between a cultivated parent and a wild genotype, and thirteen hybrids among the RILs were used to estimate the dominance ratio for important agronomic traits in tomato (*Lycopersicon esculentum* Mill). The traits evaluated were: number of leaves to first inflorescence (V1); number of leaves between the first and second inflorescence (V2); stem diameter at the basal part (V3), the middle part (V4) and the apical part (V5), and number of flowers by inflorescence (V6). Field design was randomized (N=200). One way ANOVA was used to compare genotypes and the dominance ratios were estimated. Significant differences were found for all traits ( $p < 0.05$ ). Complete dominance and additivity were involved in the determination of V1, V2 and V5 whereas complete dominance and overdominance were found for V4 in the different crosses. Complete dominance was just detected for V3 and V6. For V6, RILs ToUNR8; ToUNR9 and ToUNR18 behaved as the dominant genotypes while ToUNR1 and ToUNR15 behaved as the recessive ones. The predominant dominance ratio involved in the determination of the traits analyzed in this set of genotypes was the complete dominance.

**70. PROTEIN PROFILES FROM FRUIT PERICARP AT RED RIPE STAGE TO CHARACTERIZE TOMATO GENOTYPES**

Pereira da Costa JH, Tomat DD, Rodríguez GR<sup>1</sup>, Liberatti D<sup>1</sup>, Pratta GR<sup>1</sup>, Zorzoli R<sup>2</sup>, Picardi LA<sup>2</sup>.

<sup>1</sup>CONICET. <sup>2</sup>CIUNR. Cátedra de Genética, Fac. de Ciencias Agrarias (UNR), CC N° 14, (S2123ZAA) Zavalla, Argentina. E-mail: rzorzoli@unr.edu.ar

The objective of this work was to characterize different tomato genotypes by protein profiles from fruit pericarp at red ripe stage. The genotypes were: nine recombinant inbred lines (RILs) obtained by an interspecific cross between the cultivar 'Caimanta' (Cai) of *L. esculentum* and the accession LA722 of *L. pimpinellifolium*, two accessions of *L. pimpinellifolium* (LA1246, LA2181), three accessions of *L. esculentum* var. *cerasiforme* (LA1673, LA1320, LA1385) and two genotypes of cultivated type: 'UCO' and 'NOR'. Three fruits for genotype were harvested at red ripe stage. A phenol extraction followed by methanolic ammonium acetate precipitation was made. Proteins were separated by SDS-PAGE and genotypes were compared by the presence or absence of bands. Specific protein profiles were found for each genotype. A multivariate analysis was used to cluster the genotypes by protein profiles. Two clusters were observed when the RILs, Cai and LA722 were analyzed: 3 RILs grouped with LA722 while 6 RILs grouped with Cai. When the 18 genotypes were analyzed, the distribution in 2 groups was kept: 3 accession of *L. esculentum* var. *cerasiforme* and LA1246 of *L. pimpinellifolium* grouped with Cai and the 6 RILs, and UCO and LA2181 grouped with LA722 and the 3 RILs. Protein profiles from fruit pericarp at red ripe stage allow the characterization of different tomato genotypes.

**71. LAMBS REARED UNDER TWO PREWEANING ENVIRONMENTAL CONDITIONS**

Mahuad S, Pafundi L, Ghirardi G, Kerps G, Segatori S, Martín B<sup>1</sup>, Picardi LA.

Cátedras de Genética y <sup>1</sup>Forrajes, Ciencias Agrarias. UNR-CIUNR. Campo Experimental Villarino. (S2125ZAA) Zavalla. E-mail: sabinamahuad@mailcity.com

Magrario is a new genotype that was obtained by backcrossing of the Ideal breed to Texel breed. Lamb weaning weight could be influenced by the rearing conditions and the corporal stage of ewes at lambing as well as the genetic background. Magrario lambs were reared under two different conditions: on Pasture (RP) and on Grassland (RG). Lamb weight was obtained at Lambing (LW), one month of life (MW) and at weaning (WW). Also ewe weight (EW) at lambing was registered. One of the pastures were composed by *Lotus sp*, *Medicago sativa*, *Festuca arundinacea*, *Bromus catharticus* and the other basically with *M. Sativa* and *L sp*, *F. arundinacea*, *B. catharticus*. The grassland conditions were composed by *Paspalum dilatatum*, *Stypa brachychaeta*, *Trifolium repens* and *Cichorium intybus*. A one way ANOVA was performed to compare lamb weight mean values between groups. Correlation (r) was estimated between EW and LW and between EW and MW in each group. Significant differences ( $p < 0.05$ ) were found between the mean values of WW (RP=23.9 kg and RG= 26.6 kg). In RG group, the association between EW and LW was significant ( $p < 0.01$ ). These results suggest that under RG condition lambs had a higher weaning weight than the others on RP. Perhaps due to the selective behaviour of this species to get their food the grassland conditions could be a better habitat to improve their weaning weight.

**72. VASCULAR FLORA OF SANTA FE: *Elatinaceae***

Lusardi MB, Galetti LA, Oakley LJ, Prado DE.

Botánica, Dpto. Biología y Recursos Naturales, Fac. Cs. Agrarias, U.N.R., C.C. N° 14, S2125ZAA Zavalla. E-mail: mlusardi@unr.edu.ar

The Family *Elatinaceae* comprises small annual or perennial aquatic herbs, belonging in two genera only: *Elatine* and *Bergia*, of cosmopolitan distribution. These plants show opposite or verticillate leaves with interpetiolar stipules. Small hermaphrodite axilar flowers, isolated or fascicled, sessile or pedicellate. Free imbricate petals 2-5. Stamens free, iso- or diplostemonous. Ovary superior, carpels 2-5, united. Axilar placentation, ovules anatropous; free short styles. Fruit capsular. The present work provides a key for the taxa identification based on morphological characters, and a geographical distribution map. The methods consist of bibliographical review, consultation of herbaria with collections of the province (SF: Esperanza; SI: San Isidro; UNR: Rosario), field work experience of the authors and lab work to confirm their identity. The family is represented by one genus and only two species in the Santa Fe province: *E. lorentziana* Hunz. and *E. triandra* Schkuhr var. *triandra*.

73.

**VASCULAR FLORA OF SANTA FE: *Alismataceae*, *Limnocharitaceae* AND *Juncaginaceae***

Galetti LA, Oakley LJ, García RCA, Prado DE.

Botánica, Dto. Biología y Recursos Naturales, Fac. Cs. Agrarias, U.N.R., C.C. N° 14, S2125ZAA Zavalla.

The Families *Alismataceae*, *Limnocharitaceae* and *Juncaginaceae* comprise small annual or perennial aquatic herbs, belonging in the order *Helobias* in the old classification systems. Their plants show perfect flowers, trimerous and with dialicarpelar gynoecious. The *Juncaginaceae* are distinguished by their very small homochlamydeous or achlamydeous flowers, whereas the other two families have showy heterochlamydeous flowers. The present work provides a key for the taxa identification based on morphological characters, and a geographical distribution map. The methods consist of bibliographical review, consultation of herbaria with collections of the province (SF: Esperanza; SI: San Isidro; UNR: Rosario), field work experience of the authors and lab work to confirm their identity. The Families *Juncaginaceae* and *Limnocharitaceae* are represented in the Santa Fe province by one species each: *Triglochin striata* Ruiz & Pav. and *Hydrocleys nymphoides* (Willd.) Buchenau, respectively. The *Alismataceae*, in change, present two genera: *Echinodorus* Rich. ex Engelm. with three species: *E. aschersonianus* Graebn.; *E. bolivianus* (Rusby) Holm-Niels. and *E. grandiflorus* (Cham. & Schltdl.) Micheli subsp. *grandiflorus*; and *Sagittaria* L. with one species: *S. montevidensis* Cham. & Schltdl.

74.

**IMIDAZOLINONE RESISTANCE (IMI-R) EVALUATION OF SUNFLOWER SEEDLINGS IN EARLY STAGES OF DEVELOPMENT**

Breccia G, Vega T, Calderari M, Giorgi D, Nestares G, Picardi L. Cátedra de Genética. Facultad de Ciencias Agrarias. UNR. CC14 (S2125) Zavalla. E-mail: gabrielabreccia@yahoo.com

IMI-R was incorporated in sunflower using conventional breeding methods and represents a great advantage for controlling weeds. The objective of this work was to evaluate the expression of IMI-R during germination and development of sunflower plantlets. The genotypes evaluated were three inbred lines differing for their IMI-R: susceptible (S), intermediate (I) and resistant (R). Achenes were seeded in speedlings filled with perlite and watered by capillarity with a 25% strength MS saline solution (Murashige and Skoog, 1962) with different doses of imazapyr: 0 (control)-1,25-2,5-5-7,5-10 uM. For each treatment 2 replications of 30 seeds each were incubated at  $25 \pm 2^\circ\text{C}$  with a 12-h photoperiod for 8 days. The variables hypocotyl and root length, aerial and root fresh and dry weight were analysed through ANOVA. R and I lines did not present significant differences among control and herbicide treatments for any variable. Conversely, for S genotype mean values significantly diminished as imazapyr doses increased for all variables. Moreover, S plantlets grown in presence of herbicide showed short tip-necrosed roots and no secondary roots. Principal root of I genotype elongated but secondary roots were poorly developed in the presence of imazapyr 10 uM. It is concluded that different resistance degrees can be discriminated through this method. This test could be considered an alternative to field screening in sunflower breeding programs.

75.

**MORPHOLOGICAL CHARACTERS, FIBER CONTENT AND DRY MATTER PRODUCTION OF ALFALFA CULTIVARS WITH DIFFERENT WINTER REST**Verdura L, Spiller L<sup>1</sup>, Cechetti S, Ceconi F, Calvo F, Acebal M, Figallo R<sup>2</sup>.Animal Nutrition and <sup>1</sup>Forages, Agricultural Sci.; <sup>2</sup>Biological Chemistry, Veterinarian Sci. University of Rosario; <sup>3</sup>KWS SA.

Animal production systems response depends on the amount and quality of provided forage. In the south of Santa Fe Province, the most utilized alfalfa (*Medicago sativa* L.) cultivars belong to intermediate rest (IR) and short rest (SR). The objective of the work was to study number of stems, height, forage production and Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) content of alfalfa cultivars in vegetative state during 2006 autumn-winter growing cycle. The experiment was carried out in the Faculty of Agricultural Sciences (UNR), Zavalla (Argentina) (33° 01' S). Alfalfa cultivars were GAPP 969; Cikum; Bárbara; Araucana and Reina (SR); and Victoria; Mayacó; Patricia and 5681 (IR). Experimental design was a Randomized Complete Block with three replications. Samples were obtained with mechanical scissors using 0.6 m<sup>2</sup> marks to quantify forage production (DM kg/ha) and to determine number of stems/m<sup>2</sup>. Subsamples of 30 stems were utilized to determine maximum and medium heights. NDF% and ADF% were estimated according to Hintz and Albretch, (1991); Sulc and col (1999); Cechetti and col (2003). Means were compared by Tukey Test (P<0.05). SR medium height (60.40 cm) was greater than IR. Results suggest that greater production tendency of SR cultivars must be due to height and number of stems whereas nutritional fractions associated to animal feed intake and forage quality are similar in all cultivars.

76.

**VIRULENCE OF THE POPULATIONS OF *Puccinia sorghi* TO THE RESISTANCE RP GENES IN THE ARGENTINEAN CORN BELT**González M<sup>1,2</sup>, Incremona M<sup>1</sup>, Ghio A<sup>1</sup>.<sup>1</sup>Fitopatología. Fac. Cs. Agrarias. UNR. <sup>2</sup>CIUNR.

The objective of this work was to determine the variability of the population of *P. sorghi* at the level of virulence on the isolines with Rp resistance genes in the Argentinean corn belt. To identify factors of virulence on the pathogens population, plots with the 30 isolines of corn with the Rp genes were grow. These genes have specific resistance to races of *P.sorghi*. The isolines were grown in Pergamino and Zavalla. From 1999 to 2000 isolines with one single gene was sowed. In 2001 and 2002 isolines with one, two or three bigger genes were sown. A random block design with 10 plants/isolines and 2 repetitions was used. The isolines were provided by the Department of Crop Sciences at the University of Illinois, US. The natural infection was allowed by *P. sorghi*; severity and infection type were evaluated. For the evaluation of severity the visual scale was used to determine the percentage of affected foliar (0 to 100). The infection type was determined with a scale from 1 to 4 made by González in 1997. Being: 1 - absence of symptoms or necrotics or chlorotic points, 2 - small uredinia with or without necrotics points 3 - big uredinea and 4 - big uredinia with necrotics areas. According to the evaluations of the lines with a single Rp gene, only 3 (Rp1-N, Rp 3-TO and Rp3-C) to be effective against the population of *P.sorghi*. In the isolines with several resistance genes, the effectiveness of the lines was shown with the genes Rp 1 - GFJ and Rp1 - IG. The rest of the lines showed half to high severity and high infection types.

**77. USE OF WATER WASHER NOZZLES FOR DECONTAMINATION OF PHITOSANITARY PRODUCTS CONTAINERS**

Sarubbi C, Bulacio L, Panelo M, Giuliani S.  
Facultad de Ciencias Agrarias UNR, CC14 (S2125ZAA) Zavalla,  
Pcia. Santa Fe, Argentina. E-mail: lgb@tower.com.ar

Fifteen million containers of phitosanitary products are used annually in Argentina. For their safe final destination one alternative is to recycle them, previously decontaminated. The objective of this work is to compare the technique of the triple washing by hand to pressure washing of the containers, using three models of hydraulic sprinkler nozzles: A) Teejet D25468-POM; B) Teejet VSM 44 and C) Arag Code 510 110. Unused phitosanitary containers, each of 20 L of capacity and containing 50% of phenolphthaleine solution ( $12,5 \text{ g.l}^{-1}$ ) and 50% alcohol 96% v/v to ensure the solubility of the colorant, were kept 60 days in impregnation. The hydrowashing was performed taking the volumen of water used for the triple washing as a constant, changing the time of exposition according to pressure and volume of flow provided by the different models of nozzles. Samples of wash broth were collected at the end of each operation in clean 50 ml plastic containers. Colorant concentration in the samples was determined in a spectrophotometer at 560 nm. Data as percentage of cleaning were analyzed according to a completely randomized design, with 4 repetitions. Not all the pressure washing devices had the same cleaning capacity. With nozzles (A) and (C) the level of residue was reduced to less than 1% in regard to the original colorant concentration, (A) behaving similarly to triple washing. Therefore, this technique, if properly applied, represents a valid and more practical alternative in its execution to replace triple washing by hand.

**78. EVALUATION OF PERSONAL CONTAMINATION DURING THE APPLICATION OF PHITOSANITARY PRODUCTS IN HORTICULTURAL MEDIUM HEIGHT CROP**

Panelo, M.; Bulacio, L.; Giuliani, S.; Giolito, I.; Saeta, J. Facultad de Ciencias Agrarias UNR, CC 14 (S2125ZAA) Zavalla, Pcia. Santa Fe, Argentina. E-mail: msp@tower.com.ar

The use of phitosanitary products in Horticulture, when improperly performed, may result in serious disorders in the health of the operators. The objective of the work was to determine the areas of deposition of the product on the operator's body at the time of performing a foliar treatment with phitosanitary products, in a medium height crop. On artichoke plants (*Cynara scolymus* L.) spaced out  $1,40 \text{ m} \times 0,70 \text{ m}$ , an application was simulated using a 20 L manual backpack sprinkler and replacing the phitosanitary product for a  $0,5 \text{ g/l}$  solution of phenolphthaleine colorant. Four colorant applications were performed, covering a distance of 100 m each time along crop lines. Previous to each treatment, white  $10 \times 10 \text{ cm}$  cotton cloth patches were stitched over different areas of the operator's body. The residue of phenolphthaleine was recovered from each patch with sodium hydroxide  $0,1 \text{ N}$  and it was valued in a spectrophotometer at 540 nm. Data were analyzed according to a completely randomized statistical design, with 4 repetitions. On the front of the operator's body,  $980 \text{ µg/cm}^2$  of colorant were detected (7,48% on the head; 14,80% on torso and abdomen; 20,22% on upper limbs; 57,50% in lower limbs). On the back of the body,  $540 \text{ µg/cm}^2$  were detected, also with greater deposition on the lower limbs (62,30%). Results validate the need of using personal protection equipment that must cover all parts of the body at the time of applying phitosanitary products in conditions similar to those of the experiment.

**79. FLUCTUATIONS IN KIWI FRUITS QUALITY (*Actinidia deliciosa*), DURING THEIR COMMERCIALIZATION**

Fusiglieri D, (ex-equo) Flores P, Poggi D.  
Cátedra C. Intensivos-Área. Fruticultura, UNR.

In postharvest of horticultural products, the visual quality is a high-priority objective to complete. Any objective in postharvest, has as purpose the reduction of losses. Ripening includes processes like coloration, loss of stability, increase in the solids soluble concentration, descent of starch and reduction of the acidity. They fruits climatéricos are usually harvested with firm pulp. It is essential to determine the opportune moment of crop using indexes ripening. The stability of the pulp decreases, as the fruit matures. The objective of this work has been to know the development of variations in the quality of kiwi fruits in fresh markets. The essay took place with samples of thirty fruits that were acquired in the same trade, weekly and during a period of 2 months. Of each sample, of 15 fruits, weight was measured, TSS, stability of the pulp and tituable acidity the day of acquired. To determine damage, they were evaluated the same parameters after 6 days to ambient temperature, to the other 15. The experimental design was in totally randomized blocks, with analysis of the Variance to two classification approaches. Treatments were each date (factor), with two levels (n.1: the day of the purchase and n.2: after six days). There were highly significant differences for the variables weight and stability. For weight of the fruits differences were observed among crop dates. The weight of the fruit increased as the gathering advanced. The stability varied considerably in the different dates, between its two levels. It could be observed that the fruits that are left during six days at  $20^\circ\text{C}$  to ambient temperature increase their weight. The stability of the fruits at  $20^\circ\text{C}$  decreases during days, like it happens with the fruit in fresh markets ripening. The concentration of SS didn't increase. Tituable acidity, significant differences were not presented between different treatments and their corresponding levels.

**80. LETTUCE (*Lactuca sativa* L.) AND BROCCOLI (*Brassica oleracea* var. *italica* L.), THEIR PRODUCTIVITY IN SOILS WITH THREE ORGANIC AMENDMENTS AND FERTILIZER APPLICATION**

Firpo I, Rotondo R, Fernández E, Toresani S, Gomez E, Ferreras L.  
Cult Intensivos, Edafología y Microbiología Agrícola. Fac de Cs. Agr. UNR. E-mail: firpoit@fibertel.com.ar

Organic amendments are an alternative to improve soil conditions in Rosario, allowing a higher crop yield potential. The effect of 3 organic amendments and fertilizer applications on lettuce and broccoli productivity was evaluated. The trial was carried out in Fac. de Cs. Agrarias in RCBD. Amendments were vermicompost of household solid waste (RD), and horse and rabbit manure (CC), chicken manure (CP), and a control. In 2001/2002, 1 and  $2 \text{ kg.m}^{-2}$  (dry basis) were incorporated. In 2003,  $2 \text{ kg m}^{-2}$  and Urea  $120 \text{ kg ha}^{-1}$ . Lettuce harvest was in December and broccoli in August. In lettuce, crop yield (R;  $\text{kg.m}^{-2}$ ), plant average weight (PM; g) and number of commercial leaves (NHC) were determined. In broccoli, crop yield (R;  $\text{kg.m}^{-2}$ ), principal bud average weight (PPP; g) and bud diameter (DP; cm) were evaluated. Organic carbon (CO) and saturated flow hydraulic conductivity (Ks) were evaluated in soil. Results were analyzed by ANOVA. In lettuce, differences were found among the amendments in R and PM; CP showed the higher values. In 2004, all the amended plots were higher than the control. In broccoli, there were significant differences among the amendments in DP, R and PPP, showing CP the higher value. R, PM, and DP were higher in the fertilized plots in 2005. CO increased after the three years, showing the higher values RD and CC. Ks was higher in CC and RD than in the controls. Annual amendment applications for a longer period are needed to maintain their effect on lettuce and broccoli productivity.

### 81. GREEN STEM DISORDER IN SOYBEAN IS ASSOCIATED WITH ENVIRONMENTAL STRESS

*Tuttolomondo G, Rosbaco I, Romagnoli M, Bisaro V, Martignone R. Facultad Ciencias Agrarias, Univ. Nac. de Rosario, 2123 Zavalla.*

Green stem disorder (GSD) causes the stems to remain green, nonsenescent, and moist, although pods and seeds are fully ripe and dry. The GSD is a nuisance for producers because it complicates harvesting of soybeans by significantly increasing the difficulty in cutting the affected plants during harvest. The cause of GSD is unknown; but differences in relative sensitivity to GSD have been observed. The objective of this paper was to establish the relationships between GSD and thermal and water stresses. Field trials were carried out in Zavalla, Argentina (33° 01' S lat.) in the 2005/06 growing season on a Typic Argiudoll soil. Seeds of DM 3100 RR and DM 3700 RR (MG III); DM 4200 RR and RA 418 (MG IV); RA 514 and TJ 2055 RR (MG V) and A 7336 RG (MG VII) were sown on 3/10/05 (SD1) and 1/12/05 (SD2) in a randomized complete block design with 3 replications. Phenological dates, Ve, R1; R3; R5 and R7, rainfall and mean daily temperature and GSD incidence were recorded. Analysis of variance was performed and genotype x environment interactions were evaluated with standard Principal Components Analysis in order to associate GSD with Ve-R1, R1-R3, R3-R5 and R5-R7 daily mean temperature and rainfall. In SD1, GSD was present in all MG III and IV cultivars with 80% of incidence and absent in the other cultivars. In SD2, only RA418 had 20,7% of incidence. Principal Components explained the 98 and 91% variability in SD1 and SD2, respectively. Rainfall between R3-R5 had the highest and significant association with GSD. GSD was present when R3 – R5 rainfalls were lower than 21 mm. At this moment, the early pod formation stage had the highest sensibility to GSD.

### 82. MUTAGENESIS IN PEA (*Pisum sativum* L)

*Espósito MA<sup>1</sup>, Martín E<sup>1</sup>, Cravero V<sup>2</sup>, Cointry E<sup>1</sup>.*

<sup>1</sup>Cátedra de Genética y Mejoramiento Vegetal-UNR. CC14 (S2125ZAA). Zavalla-Santa Fe. <sup>2</sup>CONICET.

E-mail: [espositoandrea@yahoo.com.ar](mailto:espositoandrea@yahoo.com.ar)

In Argentina, pea is cultivated in Buenos Aires and Santa Fe predominantly. Market requirement and decrease of cultivated areas caused a reduction of genetic diversity in this species. So then, mutagenesis appear such as tool to generate new and superior genotypes. The aim of this work was to determine the ability of chemical mutagens to generate genetic variability. Seeds of two varieties (Sprut and Erik II) were imbibed in water overnight. Then were treated with ethyl methane sulfonate (EMS) 4mM for 5 hours at room temperature and decontaminated with sodium tiosulphate 0.2 M. Plants obtained were harvested and seeds were sowed per families. To determine the mutagen effect, six quantitative traits were evaluated: number of pods, seeds per plot, seeds per pod, weight of 100 seeds; days to flowering and yield. Furthermore, molecular markers, SRAP (Sequence-Related Amplified Polymorphism) were used. Gels were stained with AgNO<sub>3</sub> and SRAP fragments were scored as present or absent. Cluster analysis were performed with morphological and molecular data and genetic distances (Nei index) were calculated. Three clusters were obtained for Sprut and two for Erik II. Nei index values ranged from 0.74 to 0.79 between mutants and original plants in Sprut and from 0.49 to 0.93 between two different mutants in Erik II. Through mutagenesis latter plants, with higher yield and higher number of seeds were obtained. In conclusion, new genotypes were generated in both cultivars by EMS action, extending their genetic bases and facilitating new cultivars obtainment.

### 83. SOYBEAN: INCIDENCE OF TILLAGE SYSTEM AND RESIDUAL NITROGEN AND SULFUR FERTILIZATION ON CROP YIELD

*D'Angelo M, Franzoni R, Romagnoli MS.*

*Facultad Cs. Agrarias. U.N.R. CC N° 14, 2125 Zavalla, Argentina.*

The objective of this study was to evaluate the effects of both tillage systems (TS) – no tillage (NT) versus chisel plow (CP) – and of residual nitrogen and sulfur fertilization on crop yield. The trial was carried out on a vertic Argiudolls soil, in Zavalla (33° lat. S - 60°53' long W) on 31/10/05. The previous crop of the plot used was corn fertilized with different rates of nitrogen and sulfur: N0S0 (T0), N60S16 (T1) and N120S16 (T2). The cultivar (cv) used was DM 4600 RR. The following determinations were made: phenological stages occurrence (Fehr and Caviness, 1977), total aerial biomass (g/m<sup>2</sup>, BAT) in the R3, R5, R7 and R8 stages. At maturity, the following information was determined: yield (kg/ha, YLD), seed number/m<sup>2</sup> (S), 1000-seed weight (g, PGR) and harvest index (HI). Data were subjected to ANOVA. Low water availability from the emergence to the beginnings of R5 had influence on the low YLD obtained. The results showed that there were only significant differences between TS for TAB (CP greater than NT) and for HI (NT greater than CP). As regards the response to residual fertilization, T2 had greater YLD, S and BAT in R7 and R8 than T0. Biological nitrogen fixation is a process very sensitive to environmental disturbance, particularly to occurrence of water stress. The low water availability the crop submitted during the nodular system development period and the subsequent fixation made edaphic nitrogen the main source of provision, which would explain the responses observed as regards residual fertilization.

### 84. PLASMATIC SIALIC ACID LEVEL IN DIABETIC PATIENTS: ITS POSSIBLE HEMORHEOLOGIC IMPACT

*Carrera LI, Etchepare RG, Lebensohn N, D'Arrigo M, Valverde JR, Foresto PG, D'Ottavio AE.*

*Laboratorio de Inmunohemorreología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Cátedra de Histología y Embriología. Facultad de Ciencias Médicas. CIUNR. Universidad Nacional de Rosario.*

A diminution in sialic acid bound to plasmatic membranes may be related with formerly reported disorganization and increase of erythrocytic aggregates in diabetic patients. Plasmatic sialic acid level (PSAL) was studied hypothesizing its possible plasmatic increase in congruence with its membranous decrease. Respecting ethical rules, PSAL was analyzed in 66 diabetic patients and 25 wealthy controls assisting during 2005 to the Hospital Provincial (Rosario). PSAL was determined by a colorimetric method requiring serum acid hydrolysis in presence of Erlich's reactive. Statistical analysis was performed through ANOVA test. Significant differences were revealed when comparing PSAL in diabetic patients ( $9.40 \pm 0.36 \mu\text{mol/l}$ ) with controls ( $7.30 \pm 0.30 \mu\text{mol/l}$ ) ( $p=0,004$ ). Obtained results, compatible with reported in murines and humans, corroborated our hypothesis, provided data for the probable association between sialic acid bound to plasmatic membranes and erythrocytic aggregation and certified the existence of microvascular complications in our studied diabetic patients since PSAL is a feasible marker for its presence.

**85. DETECTION OF MICROMETASTASIS IN PATIENTS WITH BREAST CARCINOMA: DETERMINATION OF MAMMAGLOBIN A (MGA)**

Ceballos P<sup>1</sup>, Zumoffen CM<sup>1</sup>, Capitaine Funes C<sup>2</sup>, Cipulli G<sup>2</sup>, Tozzini R<sup>2</sup>, Ghersevich S<sup>1</sup>.

<sup>1</sup>Área Bioquímica Clínica. Facultad Cs Bioquímicas y Farm.

<sup>2</sup>Cátedra de Ginecología. Facultad de Cs Médicas. UNR.

The expression of mammaglobin A (MGA) is restricted to the normal breast epithelium and most of breast carcinomas. In this study the expression of MGA in peripheral blood from 16 patients diagnosed with breast cancer (without treatment) was investigated for the detection of tumour circulating cells (micrometastasis). It was also analyzed the correlation between MGA detection and the presence of node metastasis. The nucleate cells were isolated from the peripheral blood with a density gradient and total RNA was isolated with TriZol. The expression of MGA was detected by RT-PCR, using a nested PCR to amplify a specific sequence (201 bp) of the cDNA of MGA. PCR products were analyzed on 2% agarose gels and stained with ethidium bromide. The cDNA obtained from a non-malignant breast tissue was used as positive controls and the cDNA from blood samples of healthy volunteers was used as negative controls. The correlation analysis was performed with the use of contingency tables based on Fisher's exact test. A value of  $p < 0.05$  was considered to be statistically significant. The expression of MGA was detected in 44% of the analyzed samples and in 42% when only were considered the patients with node metastasis. It was detected in 50% of the samples in absence of node metastasis. A lack of significant correlation between the detection of the MGA and the presence of node metastasis was found. The detection in blood of MGA might be an independent marker to establish the advance of the disease and to evaluate the type of treatment to provide to patients in the absence of node metastasis.

**86. MORPHOLOGICAL AND MOLECULAR TAXONOMY OF *Fusarium* SPECIES**

Barosso I<sup>1</sup>, Biasoli M<sup>1</sup>, Colletto A<sup>1</sup>, Scandiani MM<sup>1,2</sup>, Luque A<sup>1</sup>.

<sup>1</sup>CEREMIC. Fac. Cs. Bioq. y Farm., UNR, Rosario, <sup>2</sup>Lab. Agrícola Río Paraná. San Pedro. E-mail: ibaros@gmail.com

The *Fusarium* genus includes pathogens of plants, mycotoxigenic species and opportunistic pathogens in humans and animals. Conventional identification is carried out by colony morphology and conidial characteristics. The objective of the present study was to use PCR fingerprinting, to identify species of *Fusarium*. Eighty two *Fusarium* strains corresponding to 17 different species were used, 65 isolated as plant pathogens and 17 reference strains. The morphologic studies were carried out on PDA (potato dextrose agar) and SNA (synthetic nutrient to agar) incubated at 28°C. A minipreparation for DNA extraction was used. In the PCR fingerprinting a single primer, (GACA)<sub>4</sub> was used. The amplification products were separated by electrophoresis in 1.4% agarose gels photographed after staining with ethidium bromide. Of the 17 references of *Fusarium* strains used, 12 presented specific species profiles. The remaining 5 species, responsible for soybean sudden death syndrome (SDS) (of great phylogenetic proximity), presented the same profile that allowed to differentiate them from non SDS-causing species. In most of the cases the morphological identifications corresponded with the profiles of bands of the reference strain. This technique of PCR is a simple, rapid and sensitive assay, and has been of utility to confirm the phenotypic identification, specially in those species of *Fusarium*, that by their morphologic similarity, are difficult to differentiate.

**87. OLIGOMERIC STRUCTURE OF CarO, A CARBAPENEM SENSITIVE-ASSOCIATED PROTEIN OF THE *Acinetobacter baumannii* OUTER MEMBRANE**

Relling V, Mussi MA, Limansky AS, Viale AM.

IBR (CONICET), Facultad de Cs. Bioq. y Farm., UNR. Suipacha 531. 2000 Rosario, Argentina. E-mail: reling@ibr.gov.ar

*Acinetobacter baumannii* (*A. baumannii*), an aerobic Gram-negative opportunistic pathogen, accounts for a large percentage of infections affecting immunocompromised patients in particular. Nosocomial strains of *A. baumannii* are generally multidrug-resistant (MDR) and it is due to an outstanding ability to rapidly respond to the challenge of new antibiotics that the emerging resistance to the last generation of  $\beta$ -lactams, the carbapenems, represents a major concern in the actuality. We previously demonstrated that acquisition of resistance to carbapenems among MDR isolates of *A. baumannii* from local hospitals is associated to the loss of an outer membrane protein of 29 kDa designated CarO. Membrane fractions were isolated from these bacteria and outer membrane vesicles were purified by different methods and analyzed by 1-D or 2-D SDS-PAGE and immunoblotting. Both analyses indicated that CarO forms oligomeric structures in the *A. baumannii* outer membrane, a property distinctive of channels for specific hydrophilic solutes present in the outer membranes of gram-negative bacteria.

**88. RAPID MICROBIOLOGICAL CHARACTERIZATION OF CARBAPENEMASES IN GRAM-NEGATIVE BACTERIA**

Marchiaro P<sup>1</sup>, Spalding T<sup>1</sup>, Mari R<sup>1</sup>, Cera G<sup>1</sup>, Ballerini V<sup>2</sup>, Vila A<sup>1</sup>, Viale A<sup>1</sup>, Limansky A<sup>1</sup>.

<sup>1</sup>Dep. de Microbiología, IBR (CONICET), Fac. Cs. Bioq. y Farm., UNR, Suipacha 531, 2000 Rosario; <sup>2</sup>Dep. Bioq. Municipal, Municipalidad Rosario. E-mail: limansky@ibr.gov.ar

Resistance to  $\beta$ -lactam carbapenems such as imipenem and meropenem among Gram-negative pathogenic bacteria due to the acquisition of wide-spectrum  $\beta$ -lactamases constitutes an acute problematic in the actuality, and demands the implementation of assays for their rapid identification and characterization. We report here the optimization of both synergy (SA) and microbiological (MA) assays for this purpose. Modifications of SA included the incorporation of  $\beta$ -lactamase substrates such as imipenem (I), meropenem (M), and ceftazidime (C) as well as the use of chelator agents including EDTA, 2-mercaptopropionate (MP), mercaptoacetate/EDTA (MA-EDTA) and etilendinitrilotetracetate (T) to identify metallo- $\beta$ -lactamases (MBL). For the MA we evaluated different cell-disruption methods including: (i) sonication (gold standard); (ii) freeze-thawing; (iii) mechanical rupture with zirconium/silica beads, glass beads, or quartz sand. Best combinations inhibitor/substrate were MP/M-C (sensitivity, S=1); T/M-I (S=1) and EDTA/M-C (S=0.92). Best disruption methods were sonication and zirconium/silica. The combined assays were highly useful for both the routine practice and to alert on the dissemination of pathogens possessing new MBLs.



89.

**INTRASPECIFIC VARIABILITY IN *Streptococcus agalactiae* CAUSING INFECTIONS IN HUMANS AND CATTLE**

Toresani I, François S, Sutich E, Ebner G, Viale A, Limansky A.  
Departamento de Microbiología, IBR (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Suipacha 531, 2000 Rosario. E-mail: limansky@ibr.gov.ar

*Streptococcus agalactiae* (Group B *Streptococcus*, GBS) is the causing agent of diseases affecting both humans and cattle. We analyzed in this work whether different GBS populations were responsible of infections in these different hosts by characterizing a total of 62 isolates (16 from humans and 46 from bovines) obtained from local sources. These isolates were characterized by type-specific agglutination, capability to metabolize lactose and galactose, clonal relationships determined by degenerate oligonucleotides-PCR (DO-PCR), and presence of the *lacG* gene of the tagatose phosphate pathway of lactose catabolism. Bovine GBS isolates were mostly non-serotypeable and capable (100%) to metabolize lactose. On the contrary, most human GBS isolates were serotypeable and incapable (94%) to use lactose, suggesting the existence of two distinct GBS populations affecting these different hosts. In agreement, DO-PCR analyses showed two divergent groups which grouped the above isolates in 15 and 9 distinct clones of bovine and human origin, respectively, suggesting a significant genetic distance between both populations. Amplification of the *lacG* gene could be obtained only in strains that metabolized lactose, i.e., all 15 different clones of bovine origin and 1 clone of human origin. Our results thus uncovered a large degree of GBS intraspecific variability, and suggested strongly dichotomy between GBS from human and bovine origins.

90.

**ALTERATIONS OF THE ERYTHROCYTE SUPERFICIAL CHARGE IN GLYCOSYLATED SAMPLES *IN VITRO***

Lebensohn N, Re A, Radcliffe S, D'Arrigo M, Valverde J, Foresto P.  
Lab. Inmunohem. Y Hemorreol. Depto. Bioquímica Clínica. Fac. Cs. Bioq. y Farm. UNR.

The purpose of the present investigation was to study the variation of the erythrocyte superficial charge, using the Dx/PEG aqueous two-phase system in glycosylated samples *in vitro*. Fifteen blood samples from healthy donors were studied. They were glycosylated *in vitro* through the incubation of the erythrocytes in PBS containing glucose (0.5 g/dl), 24 hs at room temperature. Then, the cells were washed three times with PBS and after that they were subjected to separation in an aqueous two-phase system containing 5% (w/w) Dx and 4% (w/w) PEG in PBS. The Partition Coefficient (PC) was defined as the percentage of haemoglobin in the top phases regards the total haemoglobin. Glycosylated samples showed  $0.53 \pm 0.15$  PC values whereas no treated samples showed  $0.94 \pm 0.06$  PC values. This decrease observed in PC values for glycosylated samples suggests membrane related to the non-enzymatic glycosylation process. This methodology could contribute to the study of the erythrocyte aggregation alterations found in diabetic patients with abnormal metabolic control.

91.

**FINDING OF *STRONGYLOIDES stercoralis* EGGS, LARVAE AND ADULT IN A DUODENAL PROBE IN A HIV ( + ) PATIENT**

Bertorini G, Indelman P.

Área Parasitología. Departamento de Microbiología. Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR. Suipacha 531. Rosario.

Even though *Strongyloides stercoralis* (S.s.) is a helminth mainly located in the intestine, it can spread out and cause death in immunosuppressed patients.

Its diagnosis may be difficult in the fecal examination. The objective of this work was to study the parasite which causes respiratory and gastrointestinal infections in a HIV ( + ) patient. The patient was a 42-years-old male admitted in the Centenario Hospital.

The duodenal histopathological examination had shown hemorrhagic edema of the intestinal folds compatible with duodenitis. Both a duodenal liquid sample and a fecal sample were received. The duodenal probe was carried out using an endoscopy technique and centrifuged during 5' at 1500rpm. The sediment was observed in a light microscope at 100x and 400x. A microscope and macroscopic fecal examination was also carried out. S.s. rhabditiform larvae, eggs (embryonic and larvate) were found in both samples. Adult specimens of this nematode were also found in the duodenal liquid. The result of the fecal macroscopic examination was negative. Strongyloidosis diagnosis is based on the detection of rhabditiform larvae in feces. In most of the patients, the parasite load is low, except in those who show massive dissemination. This hyperinfection causes severe damage in the intestine epithelium which is coincident with the patient's duodenitis. The detection of eggs and adult specimens of this helminth is rare, but in this case evidence, the parasite's invasion and dissemination in the host. The duodenal liquid is highly sensitive to S.s. diagnosis. The main disadvantage of this technique is the invasive character than feces collection. As Strongyloidosis is such a severe disease in immunosuppressed patients, it would be convenient to investigate the presence of this parasite in serial fecal samples using enriching techniques and feces culture to enhance diagnosis sensitivity.

92.

**EFFECT OF FIBRONECTIN (Fn) ON REACTIVE OXYGEN SPECIES (ROS) AND COMPLEMENT (C) ACTIVATION INDUCED BY MERCURY**

Salvarrey M, Pérez G, Moreno J, Abraham N, Elías M, Saball E.  
Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR.

We have previously proven partial Fn protection in acute renal failure induced by  $\text{HgCl}_2$  in rats. In this work we study *in vitro* if Fn is able to protect from ROS production and C activation induced by  $\text{HgCl}_2$ . Cellular viability and ROS were measured on isolated PMN. Average number of lytic sites per cell (Z) generated on blood red cells (RC) by C3-classical (CC) and alternative (AC) convertases were determined. Three independent duplicate experiments employing  $\text{HgCl}_2$  (0-50  $\mu\text{M}$ ) and Fn (100  $\mu\text{g/ml}$ ) were performed. PMN or RC+ buffer (BC) and PMN or RC + Fn (FnC) were included as controls. Data was analyzed by ANOVA. Viability of PMN exposed to 15  $\mu\text{M}$   $\text{HgCl}_2$  ( $78.0 \pm 1.2\%$ ) was lower than BC ( $88.5 \pm 2.3\%$ ) and FnC ( $89.0 \pm 1.4\%$ ); Fn exert a protective effect taking it to  $92.0 \pm 0.8\%$ , which does not differ significantly from BC nor FnC;  $p < 0.05$ . Dose-dependent ROS generation reached its maximum at 5  $\mu\text{M}$   $\text{HgCl}_2$  and was inhibited by Fn. Z produced by AC+Hg+Fn ( $1.38 \pm 0.18$ ) < AC+Hg ( $2.03 \pm 0.13$ ), does not differ from BC ( $0.87 \pm 0.10$ ) nor FnC ( $0.85 \pm 0.10$ );  $p < 0.05$ . Z CC+Hg+Fn ( $1.95 \pm 0.07$ ) does not differ from CC+Hg ( $2.05 \pm 0.03$ ) both differing from BC ( $0.88 \pm 0.07$ ) and FnC ( $0.88 \pm 0.03$ );  $p < 0.05$ . Our results give additional evidence on inflammatory nature of  $\text{HgCl}_2$  mediated cytotoxicity and demonstrates that Fn is capable of reversing such a process at cellular viability, ROS production and alternative C activation level.

**93. EVALUATION OF C-REACTIVE PROTEIN (CRP) AS AN INFLAMMATORY BIOMARKER IN THE ACUTE CORONARY SYNDROMES (ACS)**

Arriaga S<sup>1</sup>, Pellizzón O<sup>3</sup>, Pelusa H<sup>1</sup>, Daniele S<sup>1</sup>, Valdés M<sup>1</sup>, Viso M<sup>2</sup>, Griot E<sup>3</sup>, Pezzotto S<sup>2</sup>, Jacobi P<sup>3</sup>, Ponzio S<sup>3</sup>, Almará A<sup>1,2</sup>.

<sup>1</sup>Área Bioq. Clínica. Fac. de Cs. Bioq. y Farm. <sup>2</sup>CIUNR. UNR.

<sup>3</sup>Servicio de Cardiología, Hospital Provincial del Centenario.

We analyzed the possible existence of differences in the CRP levels between acute myocardial infarction (AMI) and unstable angina (UA). Patients who entered in the coronary unit, within 6 hours of the symptom onset, either with diagnosis of AMI (n=16) or UA (n=17) were selected. Patients with cardiac resuscitation, infectious and/or inflammatory chronic diseases, neoplasias, recent traumatism, major surgery, pregnancy, cerebrovascular accident or cardiovascular interventionist procedures in the last 6 months were excluded. The levels of CRP (mg/dl; immunoturbidimetric assay), creatine kinase (CK; U/l; kinetic method) and creatine kinase MB fraction (CK-MB; U/l; kinetic method) were evaluated on admission. The CPR level was also measured every 6 hours during the first 48 h and then daily until hospital discharge. The results for CK and CK-MB (mean±SD) were respectively: AMI=925±205 and 71±14 vs UA=107±15 and 15±2; p=0,001. The CRP levels (median/range) at the admission were similar in both groups: AMI=0,6/0,2-7,0 and UA=0,3/0,1-7,0; p>0,2. The CRP maximum value (median/range) tended to be higher in AMI than in UA: 5,9/0,6-40,0 and 2,3/0,1-9,9; respectively, p=0,087. Although the % of patients with AMI that showed elevated CRP values at the admission (>0,5 mg/dl) was greater than those with UA: 50% vs 29%, respectively; this difference was not significant suggesting the participation of inflammatory mechanisms in the development of both ACS. The increase of the maximum value of CRP in the AMI would be a consequence as much of the underlying inflammatory process like of the myocardial necrosis.

**94. ISOLATION AND CHARACTERIZATION OF HYALURONIC ACID OBTAIN FROM HUMAN UMBILICAL CORD**

Gerhardt N, Panozzo Zénere E, Lebensohn N, Valverde J, Foresto P. Laboratorio de Inmunohematología y Hemorreología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario.

Hyaluronan (HA) is an anionic polysaccharide found in the extracellular matrix. It has a repeating disaccharide structure of 1- and 4-linked β-D-glucuronate and 1- and 3-linked 2-acetamido-2-deoxy-β-D-glucose. HA has an extremely wide variety of proposed functions: mediates cell-cell and cell-matrix adhesion, maintains tissue hydration by binding water, participates in inhibition or stimulation of cell proliferation, cell migration, angiogenesis and chondrogenesis, it's involved in modulating tissue cicatrization and has anti inflammatory properties. The objective of this work was to isolate and quantificate HA obtained from human umbilical cord. The cords were treated with a sodium chloride solution under controlled temperature and agitation. The polysaccharides were precipitated by adding a dissolution of cetyltrimethylammonium 1%. The sediment obtained in a sodium chloride solution was resuspended and purified from insoluble products at that ionic force by centrifugation. The product of interest was precipitated by adding ethanol to the supernatant. This pellet was used to determine the amount of protein present by the pyrogallol-red technique. The presence of HA was evaluated by erythrocyte agglutination and adhesion assays, based on the specific binding of the seric and membrane receptor CD44 and was quantified by a colorimetric reaction with carbazole. The amount of HA obtain was 4.5% in the pellet.

**95. MYCOTOXINS AND QUALITY OF SOYBEAN SEEDS**

Fulgueira CL<sup>1</sup>, Scandiani MM<sup>1,3</sup>, Amigot S<sup>1</sup>, Bottai H<sup>2</sup>, Leiva M<sup>2</sup>, Ruberti D<sup>3</sup>, Bulacio L<sup>1</sup>, Luque A<sup>1</sup>, Ramos L<sup>1</sup>, Biasoli M, Ramadán S<sup>1</sup>, Tosello ME<sup>1</sup>, Sortino M<sup>1</sup>, López C<sup>1</sup>.

<sup>1</sup>CEREMIC, <sup>2</sup>Cát. Estadística. Fac. Cs. Bioq. y Farm., UNR,

<sup>3</sup>Lab. Agrícola Río Paraná. S. Pedro, Argentina. E-mail: cfulgueira@yahoo.com.ar ; labagricola@sanpedro.com.ar

The germinative power (GP) and the health's test are two of the most important indexes to evaluate the quality of the soybean like seed. Recent studies showed the contamination of soybean seeds with mycotoxins. In this work it was evaluated the quality as seed of 20 of soybean samples and the presence of Fusarium's mycotoxins. The GP was determined by ISTA rules (2006) and percentage of infected seeds, by sowing in blotter test, accepting for seed the samples with a GP > 75% and a percentage of healthy seeds > 80%. The mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin (T-2) were determined using ELISA AGRAQUANT® (Romer Labs). Only a 25% of the samples would be acceptable to be used like seeds, destining the rest to human and animal feeding. Ninety five % of the samples presented Fusarium spp. with an incidence average of 2% (0 - 7). In all the samples the presence of storage pathogens (*Aspergillus spp.*) was causing of GP decrease. The incidence of DON, T-2 and ZEA was 30%, 90% and 25%, respectively with an average of 415 µg/kg, 109,05 µg/kg and 75,86 µg/kg. Eight samples were positive for 2 or more mycotoxins. All the acceptable samples to be used as seeds presented mycotoxins. Although most of the values of DON, T-2 and ZEA they would be acceptable in the countries with regulation, is important to consider the chronic effects produced by the ingestion of these grains on the human health and animal and the potential additives or synergic effects.

**96. EPIDEMIOLOGY OF *Malassezia* SPECIES THAT CAUSE PITIRIASIS VERSICOLOR IN ROSARIO CITY DURING THE PERIOD 2003-2005**

Ramadán S, Ramos L, Sortino M, Bulacio L, López C.

Centro. de Referencia de Micología (CEREMIC) Fac. de Cs. Bioquímicas y Farmacéuticas. UNR. Rosario. Argentina. E-mail: sramadan@fbiof.unr.edu.ar

Genus *Malassezia* is composed of twelve species of yeast, whose clinical importance has increased due to the advances in their isolation and identification. This has allowed us to reveal its role in human and animal pathologies. One of these diseases is the Pitiriasis Versicolor (PV), a chronic superficial infection characterized by hypo or hyperpigmented lesions. The aim of this work was to isolate, identify and analyze the distribution of the different species of *Malassezia* in patients with presumptive diagnosis of PV, who came to the CEREMIC from 2003 to 2005. A number of 135 patients (89 females, 46 males) were studied. The samples were cultivated in Sabouraud-Glucose Agar adding 2-3 drops of sterile olive oil, and in a modified Dixon Agar, and incubated at 32°C for 7 days. From the 135 studied patients, 84 (62%) positive microscopic examinations were obtained. We obtained 24 positive cultures (18%) that were identified by their phenotypic characteristics in: 8 *M. sympodialis* (50%); 6 *M. furfur* (37%) and 2 *M. slooffiae* (12%). In 34 % of the cases (8) the isolations could not be identified by their lack of viability. We agree with the results obtained in Canada and Buenos Aires (INEI-Malbrán) where *M. sympodialis* was isolated in the first place. It is important to carry out this kind of studies in our region in order to deepen the knowledge of ecology, epidemiology and the role of the different species of *Malassezia* genus like pathogenic agents in other pathologies of the skin to which it seems to be related, such as seborrheic dermatitis, folliculitis and atopic dermatitis.

97.

**VIABILITY OF *Malassezia* STRAINS CONSERVED UNDER STERILE MINERAL OIL**

Ramadán S, Ramos L, Sortino M, Bulacio L, López C.  
 Centro de Referencia de Micología (CEREMIC) Fac. de Cs. Bioq. y Farmacéuticas. UNR. E-mail: sramadan@fbioyf.unr.edu.ar

Yeasts included within *Malassezia* genus are humas and animal skin saprophytic microbiota that under certain predisposing conditions in host, can cause dermatological disorders or systemic diseases. At the moment the genus includes twelve species. The aim of this work was to study the viability of strains of *Malassezia* preserved under column of sterile mineral oil (petroleum jelly) at room temperature during a three years period. The yeasts used were 4 strains of reference: *M. furfur* (CBS 1878), *M. sympodialis* (CBS 7222), *M. slooffiae* (JG 554 = CBS 7956) and *M. obtusa* (INEI) and 52 isolated clinical samples of patients who came to the CEREMIC. These last ones were identified by physiological tests obtaining: 37 strains of *M. sympodialis* (71,15%), 7 of *M. slooffiae* (13,46%), 6 of *M. furfur* (11,54%), and 2 of *M. obtusa* (3,85%). They were recovered from the primary culture and inoculated in Sabouraud-Glucose Agar in column with 2 or 3 drops of sterile olive oil; they were incubated for 5-7 days at 32°C. When they were grown, 3 ml of sterile petroleum jelly was added to each tube. Equal treatment received the 4 strains of reference. Every year, during three years, viability and conservation of the phenotypic characteristics were analyzed. This analysis included catalasa and  $\beta$ -glucosidase activity, and thermotolerance at 37 and 42°C. Viability and characteristics tested were successfully preserved. The low cost and accessibility, make this method useful for its implementation in mycology laboratories. Although studies will be carried out in longer periods, we propose this method for preservation and storage of *Malassezia* species.

98.

**VIABILITY OF FILAMENTOUS FUNGI AND YEAST IN THE CULTURE COLLECTION OF CEREMIC**

Ramadán S<sup>1</sup>, Biasoli M<sup>1</sup>, Sortino M<sup>1</sup>, Racca L<sup>2</sup>, Luque A<sup>1</sup>.  
<sup>1</sup>CEREMIC, <sup>2</sup>Cat. de Estadística. Fac. Cs. Bioq. y Farm. UNR. E-mail: sramadan@fbioyf.unr.edu.ar

Techniques of fungal preservation must keep their viability, and their morphologic and biochemical characteristics. The aim of this work was to study viability of 498 filamentous fungi (F) and 177 yeast (Y) deposited in the collection of CEREMIC, in agar under sterile mineral oil layer. Strains were transferred to Potato Dextrose Agar and incubated at 28°C. Macro and micromorphologic examinations from the recovered cultures were carried out. The test of Mantel-Haenszel was used to analyze the association between the recovery and the years of storage of F and Y and also to study the association between the viability and the years of storage of both kinds of fungi. The test of Cochran-Armitage was used to evaluate the existence of a tendency in the recovery through the years of storage. The periods of time analyzed in the study were 24, 15 and 8,5 years. For F, the association between recovery and the time of storage was significant,  $p=0.019$ . In addition, there is an increasing tendency in the proportions of recovery when the years of storage diminish,  $p=0.0189$ , the recovery after 8,5 years is significantly greater than for 15 and 24 years of storage that do not differ from each other. For Y, the association between the viability and the time of storage is very significant,  $p<0.0001$ . The increase in the recovery is still stronger in Y when the years of storage diminish,  $p<0.0001$ . In addition, the recovery after 24 years of storage is significantly smaller than for 8,5 and 15 years of storage, that do not differ from each other. There are significant associations between viability and time of storage for both kinds of studied fungi,  $p<0.0001$ . This method of storage has the advantage of its low cost and the possibility of its implementation in the laboratories without an expensive infrastructure.

99.

**HUMAN OVIDUCTAL SECRETION: ISOLATION OF PROTEINS THAT BIND TO SPERM MEMBRANE**

Zumoffen CM<sup>1</sup>, Caille AM<sup>1</sup>, Munuce MJ<sup>1</sup>, Cabada M<sup>2</sup>, Ghersevich S<sup>1</sup>.  
<sup>1</sup>Área Bioquímica Clínica; <sup>2</sup>Área Biología, FCByF. UNR. E-mail: czumoffen@fbioyf.unr.edu.ar

Previously we have detected numerous proteins in conditioned media (CM) from human oviductal tissue cultures. The aim of this study was to isolate proteins from CM of human oviductal culture with capacity to bind to the sperm membrane. Membrane extracts were prepared from motile sperms obtained from normozoospermic donors. These protein extracts were coupled to Sepharosa 4B which was used to prepare a column of chromatographic affinity. Twenty human oviducts were obtained from pre-menopausal patients, without cancer disease, who were scheduled for hysterectomies. The tissue was cultured in DMEM/Ham's F-12 at 37°C, in atmosphere of 5% of CO<sub>2</sub>. Then, some of the tissues were incubated with [<sup>35</sup>S]-Met (30  $\mu$ Ci/ml), to obtain the proteins secreted *de novo*. CM was dialysed, protein concentration was determined and later they were seeded in the affinity column mentioned above. The [<sup>35</sup>S]-proteins in the fractions eluted from the column were detected measuring radioactivity. The protein fractions were analyzed by SDS-PAGE (7%, 10% and 15%). The protein patterns of [<sup>35</sup>S]-proteins were detected by autoradiography. At least five protein bands were observed (127 kDa, 94 kDa, 79 kDa, 17 kDa and 15 kDa). The detection of these oviductal proteins that interact with sperm membrane would support the hypothesis that some oviductal proteins could modulate the capacitation or fertilization process through their binding to the spermatozoa.

Project PICT 0115092 FONCYT.

100.

**GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD) ERITHROCYTE ENZYMIC ACTIVITY VARIABILITY IN PREGNANT WOMEN**

Acosta I<sup>1</sup>, Pérez S<sup>1</sup>, Milani A<sup>1</sup>, Amigot S<sup>2</sup>, Arciero G<sup>2</sup>, Hourquesco MC<sup>2</sup>, Molina C<sup>2</sup>, Cocconi E<sup>2</sup>.

<sup>1</sup>Cát. de Hematología. Fac. de Cs Bioquímicas y Farmacéuticas. U.N.R Suipacha 531. S2002 LRK. <sup>2</sup>Servicio de Microbiología del CEMAR. Rosario. E-mail: iacosta@fbioyf.unr.edu.ar

G6PD is the most important enzyme concerning the pentose shunt; essential to produce nicotinamide adenine dinucleotide phosphate reduced form (NADPH), necessary to maintain the reduction potential in red blood cells (RBCs). Objective: to detect G6PD significant enzymatic activity (EA) in normal and in asymptomatic urine infection (urine asymptomatic bacteremia: UAB) pregnancy. Thirty pregnant women without UAB (G1); thirty women with 12-32 wk gestation pregnancy and UAB (G2) and 38 healthy age-matched non-pregnant women (G3), were studied. All groups were submitted to urine culture and antibiogram by microbiological methods, and G6PD's EA by Glock and McLean kinetic method. G1 EA (IU G6PD/g Hb): 8.4 $\pm$ 1.3, G2: 9.5 $\pm$ 1.8 and G3: 7.8 $\pm$ 2.0. There is EA significant difference between G1 and G2 ( $p<0.01$ ), and between G2 and G3 ( $p<0.001$ ), while there was no difference between G1 and G3 ( $p>0.05$ ). Conclusion: Pregnancy does not affect G6PD's EA, since values are not different from non pregnant women. There is G6PD's EA in pregnant women with UAB due to a greater RBC NADPH production. This response to Gram negative UAB could be related to oxidative stress stimulated by generalized tissue infection and present in RBCs also.

**101. EVALUATION OF TWO CHEMOMETRIC METHODS FOR THE CLASSIFICATION OF GENERIC MEDICINES THROUGH THEIR DRUG DISSOLUTION PROFILES**

Maggio RM<sup>1</sup>, Castellano PM<sup>1</sup>, Kaufman TS<sup>1,2</sup>.

<sup>1</sup>Área Análisis de Medicamentos and <sup>2</sup>Instituto de Química Orgánica de Síntesis (CONICET-UNR). Facultad de Ciencias Bioquímicas y Farmacéuticas, U.N.R. Suipacha 531, (2000) Rosario, Argentina. E-mail: tkaufman@fbioyf.unr.edu.ar

The dissolution rate is an important parameter in quality control of tablets, because this test evaluates the bio-availability and the possibility of exchange between different brands. Chemometric methods are valid means for pharmaceutical quality control. In this work we disclose the results of the application of two chemometric methods [SOM (self-organized maps) and PCA-3D (3D-principal components analysis)] to the evaluation of dissolution profiles of acetaminophen. Dissolution profiles for three commercial brands were obtained by determination (243 nm) of the amount drug dissolved at 11 times, in 900 mL of phosphate buffer medium pH 5.8 at 37.5°C, with a dissolutor configured as USP apparatus II, at 30 rpm. The application of SOM showed differences between profiles of all brands, but these were only qualitative. On the other hand, PCA-3D resulted to be more efficient than SOM, allowing to classify the profiles with confidence intervals ( $p < 0.10$ ) using three dimensional ellipsoids of confidence and calculating multivariate statistical distance.

**102. LEVELS OF SERUM NITRIC OXIDE ALTERED IN MEN WITH DIAGNOSIS OF HYPOGONADISM**

Pituelli NE, Paparella C, Zumoffen C, Bouvet B, Feldman R.

Área Química Analítica Clínica Fac. Cs. Bioq. y Farm. <sup>2</sup>Servicio de Reproducción. Fac. Cs. Médicas. UNR Suipacha 531. (2000) Rosario. Argentina.

Nitric oxide (NO) is synthesized from L-arginine by action of nitric oxide synthase. NO spreads from the nervous completions and acts like neurotransmitter. It was demonstrated that NO stimulates secretion of gonadotrophin-releasing hormone (GnRH) in the hypothalamus. It has been postulated that plays an important role in the erectile dysfunction and other reproductive endocrine disorders related to androgenic synthesis. The aim was to study a population of infertile men with increased levels of serum nitric oxide and its relation with hypogonadism. Among 76 men (18 - 45 years), who were attended at Reproduction Service of Hospital Provincial del Centenario from Rosario, 12 men presented hypogonadism and 24 men idiopathic infertility. Spermograms were made according to WHO's norms. Serum NO concentrations were determined using the Griess reaction. Seven men with hypogonadism (58,0%) presented levels increased of serum nitric oxide, whereas 2 men with single idiopathic infertility (8,3%) presented high levels of serum nitric oxide. We used the chi-square test to analyze the data. We found a statistically significant association between hypogonadism and increased serum levels of nitric oxide ( $p = 0,03819$ ). The results obtained in this population of men with idiopathic infertility, suggest that the high serum levels of nitric oxide could be a risk factor in the clinical manifestations of hypogonadism.

**103. IDENTIFICATION OF SEMINAL STAINS EXPOSED TO HIGH TEMPERATURE AND DIFFERENT POLLUTING AGENTS**

Pavesi A, Bouvet B, Paparella C, Pitueli N.

Q. A. Clin. Fac. Cs Biochemistry and Farm-UNR.

The high index of sexual crimes request the analysis of seminal stains en the laboratories. The most samples are clothes, rises realized en the place of the fact and the body of the victim. These stains will be exposed to different climacteric conditions. Our objective was to use a technique for to determine prostatic acid phosphatase, after to exposed the seminal stains to high temperatures and to study the enzymatic behavior front to biological and no biological polluting agents. The stain were realized in hydrophilic gauze pieces, 20µl of seminal plasma were deposited, they let to dry and they were exposed to a temperature of 54°C+/- 2°C and the study was realized in biological and no biological polluting agents with and without semen. The prostatic acid phosphatase was determined with reagent of kinetic prostatic acid and L+ tartrate 0,4 M was added to him. The biological samples were blood, urine, vaginal fluid and the no biological vegetables and legumes. In all the exposed analyzed seminal stains, the enzymatic activity was detected , it didn't detect in the biological and no biological agents. The results were analyzed using the chi-square test. A significant statistically association was found between the positive results of prostatic acid phosphatase en seminal stain with biological and no biological substances ( $p < 0,01$ ). The author of sexual abuses will leave, the smaller sign possible, this determination is important, mainly in azospermics and oligospermis subjects and exposed samples to climacterics factors.

**104. G894T POLYMORPHISM OF ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE IN AN ABORIGINAL POPULATION**

Gerrard G<sup>1</sup>, Zumoffen C<sup>1</sup>, Lioi S<sup>1</sup>, Pituelli N<sup>1</sup>, Corbera M<sup>1</sup>, Turco M<sup>1</sup>, Beloscar J<sup>1</sup>, D'Arrigo M<sup>1</sup>, Rosillo I<sup>1</sup>.

<sup>1</sup>Área Química Analítica. Dpto de Bioquímica Clínica. Fac. de Cs. Bioquímicas y Farm. <sup>2</sup>Carrera de Cardiología. Fac. de Cs. Médicas. UNR. Argentina.

In G894T polymorphism of the gene of endothelial nitric oxide synthase (eNOS), a G→T substitution at position 894 takes place with a resulting reduction in its activity because of a Glu→Asp substitution that produces a conformational change in the polypeptide. The result of this mutation becomes a genetical risk factor for coronary disease and myocardium infarct. The object of this work was to study the frequency of G894T polymorphism in an aboriginal population. A total of 41 patients were studied: 21 aborigines (PA) and 20 non-aborigines (PnoA). The genotypification was made by using the PCR-RFLP technique, with a previous DNA extraction from blood leukocytes. The analysis of the genotypes distribution was in PA (GG 66,6%, GT 23,8%, TT 9,5%) and in PnoA (GG 65%, GT 35%, TT 0%). In order to compare the allelic frequency of both populations, a test of hypothesis of proportion under normal theory was carried out. This test proved that there are no significant differences between both groups. Since the usual risk factors give a partial explanation, new etiological factors are being searched for in the molecular genetics area looking for the detection of patterns related to pathogenic effects. The addition of unfavorable polymorphisms and a favorable environmental frame could facilitate the development of typical polygenetic and multifactorial pathologies such as coronary disease.

**105. ALTERATION OF THE SPERM FUNCTION ASSOCIATED WITH THE OCCUPATIONAL EXPOSITION TO SOLVENTS**

Bouvet B<sup>1</sup>, Paparella C<sup>1</sup>, Feldman R<sup>2</sup>.

<sup>1</sup>Area Qca. A. Clin Fac. C. Bioq y. Far. <sup>2</sup>Serv Rep. Fac. Cs. Méd. UNR Rosario. Argentina.

The environmental is a global problem. The incorporation of new industrial products is permanent, the knowledge of acute or chronic damage that they produce to the human health and specially in the reproductive health is insufficient. Within these products are the solvents, largely used in the industry of the picture and wood. Our objective was to study the relation of occupational exposition to solvents with alterations in the sperm morphology and viability, in semen samples from men with idiopathic infertility. Semen samples of men with idiopathic infertility between 18 and 45 years old were analyzed, and were divided in two groups: G1 (n = 22) men occupationally exposed to the use of solvents and G2 (n = 29) men that performed works of no spermatogenic risk (students, teachers, merchants). A sperm study and functional tests according to OMS have been carried out to all samples. The sperm morphology was evaluated with Papanicolaou and micrometric ocular, the integrity of nuclear AND with acridine orange, condensation state of chromatin with blue aniline and functionally of the sperm membrane with the Hiposmotic Test. In the groups of exposed men, decreased sperm functional parameters were found. The obtained results in the evaluation of nuclear condensation were: G1 (54,5 +/- 18,7) vs G2 (82,0 +/- 7,07); in the integrity G1 (50,3 +/- 15,4) vs (90,3 +/- 5,9) and in the functionality of the sperm membrane G1 (53,6 +/- 14,5) vs G2 (79,3 +/- 11,5). With respect to sperm morphology was observed under percentage of normal forms in the exposed G1 (5,2 +/- 1,4) vs G2 (9,4 +/- 2,5). Significant statistical difference was found between both groups (p<0,001). According to the obtained results; In workers exposed to solvents, there is a potential risk for the human reproductive health.

**106. NH<sub>3</sub>/NH<sub>4</sub> TRANSPORT AND Rh COMPLEX IN THE RED BLOOD CELL MEMBRANE**

Munini G, Biondi C, García Borrás S, Racca L, Racca A<sup>1</sup>, Cotorruelo C<sup>2</sup>.

Area Inmunología. Fac. Cs. Bioquímicas y Farmacéuticas. <sup>1</sup>CIUNR. <sup>2</sup>CONICET. UNR.

The RhD, RhCcEe, RhAG and the accessory proteins LW, CD47, GPB and Band 3 are assembled in the Rh membrane complex. The core of the complex is a tetramer composed of two RhD or RhCcEe and two RhAG molecules to which the accessory chains are associated by non-covalent linkages. Rh polypeptides are related to the family of ammonium transporters, although it has not been established their function in the red blood cell (RBC) membrane. The aim of this work was to analyze the participation of the Rh complex in NH<sub>3</sub>/NH<sub>4</sub> transport. Untreated (U), bromelinated (B) and anti-D sensitized (S) RBC suspensions (n=12) were prepared. A kinetic ammonium lysis assay was performed in each suspension with an isotonic buffer containing NH<sub>4</sub>Cl, KHCO<sub>3</sub> and EDTA. Equal volumes of suspensions and buffer were mixed and free haemoglobin was measured by optical density at 405nm during 40 minutes. The percentage of haemolysis was calculated relating the results with total lysis. The results obtained at the time when the URBC showed the maximum hemolysis were: URBC: 0.642±0.117, BRBC: 0.447±0.107 and SRBC: 0.730±0.129. The percentage of hemolysis of BRBC was significantly lower than that observed with URBC (19.5%, IC 95%: 16.5%-22.5%) while SRBC reached values marginally higher than URBC (8.9%, IC 95%: 7.3%-10.5%). The findings with BRBC indicate that NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> transport is mediated by membrane proteins. Moreover, anti-D and RhD proteins interaction could modify the conformational structure of the Rh complex resulting in an increased accumulation of NH<sub>4</sub><sup>+</sup> y Cl<sup>-</sup> in the RBC cytoplasm.

**107. DECREASE IN THE ANTIOXIDANT RESPONSE DURING ERYTHROCYTE AGING**

Rucci A, Luján M, Cotorruelo C, García Borrás S, Racca L, Milani A, Biondi C, Acosta I, Racca A.

Fac. Cs. Bioquímicas y Farmacéuticas. UNR.

Red blood cell (RBC) aging is a complex process affected by immunological and biochemical parameters. Lipids and proteins oxidation by oxygen reactive species (ORS) generated by cellular metabolism is the most frequent membrane biomolecules modification. The antioxidant defenses consist of an enzymatic and a non enzymatic system. The aim of this work was to study the antioxidant response during RBC aging studying the metahemoglobin-NADH reductase (Hbi-R) and glucose-6-phosphate dehydrogenase (G6PD) activities. Blood samples were drawn by venipuncture from 15 O RhD+ volunteers' donors. Senescent (Se) RBC and young (Y) RBC were obtained by differential centrifugation. The Glock and McLean method was used to determine the G6PD activity and the Board method was used to analyze the Hbi-R activity in soluble and membrane bound fractions. The G6PD activity in SeRBC (4.55 ± 1.46 UI G6PD/Hb) was significantly lower than that observed in YRBC (7.11 ± 1.44 UI G6PD/gHb; p < 0.0001). The activity of the membrane bound Hbi-R in SeRBC (441.27 ± 97.79 UHbi-R/mgProt) was significantly lower than that found in YRBC (491.73 ± 84.02 UHbi-R/mgProt; p < 0.005). No differences were found in the soluble fractions of both groups (SeRBC: 20.50 ± 2.64 UHbi-R/mgHb vs YRBC: 19.80 ± 2.22 UHbi-R/mgHb; p > 0.05). The results indicate that the antioxidant capacity during RBC aging is decreased due to a lower production of NADPH by G6PD that allow the regeneration of Hb from Hbi.

**108. STR MARKERS POLIMORPHISM IN A POPULATION OF ROSARIO**

García Borrás S, Cotorruelo C, Racca L, Biondi C, Racca A\*.

Area Inmunología, Facultad de Ciencias. Bioquímicas y Farmacéuticas. \*CIUNR. UNR.

Genetics markers analyses allow the ethnic characterization of populations. In our country, the distribution of ABO phenotypes varies in the different regions, but the allelic frequencies of STR systems, which are highly polymorphic, have not been well established. The aim of this work was to study ABO phenotypic frequencies and the polymorphism of 9 STR loci (CSF1PO, TPOX, TH01, F13A01, FESFPS; vWA, D16S539, D7S820 and D13S317) in a population of Rosario. Blood samples from 429 unrelated individuals were studied. ABH antigens were detected by hemagglutination. STRs loci were amplified by multiplex PCR strategies using DNA obtained from peripheral blood with a salting out technique. PCR products were analyzed by polyacrylamide gel electrophoresis and stained with silver nitrate. All loci studied were in Hardy-Weinberg equilibrium. The results obtained for the ABO system did not differ from those reported for Caucasians. The comparative analysis of the allelic frequencies found for the STRs systems differed significantly (p<0.05) from those reported for Caucasian Americans in CSF1PO, TH01, F13A01, vWA and D13S317 loci. The genetic diversity found in our population reflects the utility of these markers for population studies and human identification.

## 109.

**ANALYSIS OF THE *FUT2* GENE POLYMORPHISM**

Valdez V, García Borrás S, Racca A, Biondi C.

Area Inmunología. Fac. Cs. Bioquímicas y Farmacéuticas. UNR.

The human Lewis histo blood group system comprises two main oligosaccharides antigens Le<sup>a</sup> and Le<sup>b</sup> which were first discovered in red blood cells, but were later also identified in plasma and exocrine secretions. The secretor status is determinate by the secretor gene (*Se*) responsible of the antigens expression in human secretions. The Lewis phenotype is influenced by the secretor status, although the *Le* (*FUT3*) and *Se* (*FUT2*) genes are inherited independently. The aim of this study was research the allelic varieties of the *FUT2* enzyme by a PCR reaction. We worked with peripheral blood samples of volunteers (n=60) with negative serology that attended to the Service of Hemotherapy of the Centenary Provincial Hospital. We determinated the secretor status in plasma with the hemagglutination inhibition technique. The genomic DNA was extracted by an enzymatic digestion method and was analyzed by ASA-PCR with specific primers for the G428A allele and for the wild type allele of the *FUT2* gene. The PCR products (132 bp) were analyzed in 3-percent agarose gel containing ethidium bromide. The obtained results by serology and molecular methods showed that the 75 percent of the investigate individuals were secretors. The G428A polymorphism had present in a 13 percent, smaller value to report in the bibliography for the Caucasian population. The allelic varieties of the non-secretor individuals different to the G428A might to correspond to other mutations. The molecular analysis of the *FUT2* gene confirm the genetic diversity of the investigated population and might to be utilized the same as a population marker.

## 110.

**SECRETOR STATUS AND ABH ANTIGENS EXPRESSION IN PATIENTS WITH ORAL LESIONS**

Campi C\*, Escovich L\*, Racca L, García Borrás S, Racca A, Cotorruelo C, Biondi C.

Fac. Cs. Bioquímicas y Farmacéuticas. \* Fac. Odontología. UNR.

ABH antigens, may be located not only in the red blood cells but also in almost all cells in the human body. Changes of blood group determinants and modification of carrier carbohydrate chains are characteristic of essentially all common human cancers. The aim of this work was to investigate the secretor status of patients with oral pre-cancerous and cancerous lesions and ABH antigens in fixed tissue sections. To reveal A, B and H antigens in the tissue sections (n= 34) we used a modified specific red cell adherence technique (SRCA), and to analyze the secretor status in saliva we applied an haemoagglutination inhibition test. The SRCA test results were graded from negative adherence to very strongly positive adherence. Negative adherence was defined as a complete absence of adhered indicator erythrocytes. In 21 of the 34 samples analyzed the SRCA test showed slightly positive results on atypical areas, and there was a complete antigen deletion in areas affected by neoplasia. Eight samples showed a total absence of ABH antigens in both histologically normal and pathological areas. The 52.3% of the patients with oral precancerous and cancerous lesions was no secretors, RO= 2.44, IC 95%, in contrast with the healthy population (19.5%). We observed a marginal association between secretor status and these lesions. Further it is suggested that areas of blood group isoantigen negative epithelium showing atypia, or in some instances near normal histology, may give rise to relatively low grade carcinomas. Considering these results we suggest the use of this method to monitor probable preneoplastic lesions in risk population, especially in those with no secretor status.

## 111.

**Rh COMPLEX AND BAND 3 PROTEIN IN SENESCENT RED BLOOD CELLS**

Ensinck A, Biondi C, García Borrás S, Racca L, Cotorruelo C, Racca A.

Area Inmunología. Fac. Cs. Bioquímicas y Farmacéuticas. UNR.

Red blood cell (RBC) membrane Band 3 protein presents a strong interaction with the Rh complex. It takes part in the formation of the senescent antigen that induces the binding of autologous antibodies and the selective removal of senescent (Se) RBC from circulation. The aim of the present work was to study the interaction between the Band 3 protein and the Rh complex in SeRBC. Blood samples were drawn by venipuncture from 20 O RhD+ volunteers' donors. SeRBC and young (Y) RBC were obtained by differential centrifugation. Title and score of the interaction between IgG anti-D and whole blood, SeRBC and YRBC suspensions were obtained. The monocyte monolayer assay was also performed with all suspensions and with sensitized (S) SeRBC. Negative and positive controls using normal (N) RBC and anti-D SRBC respectively were performed. We determined the % of active phagocytic cells (APC). The titles obtained in the study of the reactivity of the D antigen were the following: whole blood:  $448.0 \pm 115.8$ ; YRBC:  $245.3 \pm 101.5$ ; SeRBC:  $853.3 \pm 252.1$ . The values found in the samples of whole blood were significantly higher than those observed in YRBC ( $p < 0.001$ ) and lower than those obtained with SeRBC ( $p < 0.05$ ). No differences were found in the score values in all suspensions. The % of APC found were: SeRBC:  $17.1 \pm 1.5$ ; YRBC:  $3.1 \pm 0.9$ ; NRBC:  $2.7 \pm 0.8$ ; SRBC:  $30.1 \pm 1.9$ . The % of APC with SSeRBC was significantly higher ( $p < 0.05$ ) than that observed with SeRBC and SRBC. These findings may be related to conformational modifications of the Band 3 protein during the aging process that would allow an increased interaction between antibodies and Rh antigens.

## 112.

**STUDY OF THE ALTERATION IN THE ERYTHROCYTE AGGREGATION PRODUCED BY SYNTHETIC POLYCATIONS USING IMAGE DIGITAL ANALYSIS**

Fontana A<sup>1</sup>, Alessi A<sup>1</sup>, Relancio MF<sup>2</sup>, Foresto P<sup>1,2</sup>, Grandfils C<sup>3</sup>, Valverde J<sup>1,2</sup>, Riquelme B<sup>1,2</sup>.

<sup>1</sup>Faculty of Biochemical and Pharmaceutical Sciences Natural UNR. <sup>2</sup>Optics applied to Biology, IFIR (CONICET-UNR). <sup>3</sup>Centre Interfacultaire des Biomateriaux. ULB, Belgium.

The effect of synthetic polycations B86 and B124 on human erythrocyte aggregation was studied. For this purpose, human red blood cells (RBC) suspensions having different polycation concentrations were compared and the microscopic images of erythrocyte aggregates were obtained using a light inverted microscope (Unions Optical, 40x) connected to a CCD camera. Images were recorded corresponding to four fields of the same sample for both polycations at 2, 50 and 250 µg/ml concentrations. These images were digitally processed and the geometrical dimensions of the aggregates were measured (Digital Image Processor, IPPLUS System, IPLAB). The aggregation shape factor (ASP) was determined according to the perimeter and the area corresponding to each aggregate. Were its usefulness in erythrocyte aggregate morphology has been proved. Results show a not significantly increase in the ASP value due to polycations' activity. Microscopic images show that RBC aggregates treated with polycation B124 at the highest concentration (250 µg/ml) differ from the rouleaux shape, which corresponds to untreated RBC. We conclude that they're not significantly alterations in the aggregation state observed by a macroscopic and microscopic level, ASP being the quantifying parameter. We believe that these findings could be very useful to study the biocompatibility of synthetic polycations not only for future clinical uses but also in blood transfusions.

113.

**DETERMINISTIC CHAOS AND SENSITIVITY TO INITIAL CONDITIONS ON SHEAR STRESSED ERYTHROCYTES**Korol A<sup>1</sup>, Riquelme B<sup>1</sup>, Perez S<sup>1</sup>, Rosso O<sup>2</sup>, Valverde J<sup>1</sup>.<sup>1</sup>Facultad de Cs. Bioquímicas, UNR. <sup>2</sup>Facultad Cs. Exactas, UBA.

We analysed the problem of characterize the cell deformation on the basis of fractal approximation for ordinary (OBM) and fractionary Brownian motion (FBM), because the data set contains deterministic and stochastic components, so it could be modelled as a system of bounded correlated random walk. Here we report studies on 20 donors: 2  $\alpha$ -thalassemic, 7  $\beta$ -thalassemic, 1 haemolytic anaemia patients and 10 healthy, non smoker and non alcoholic donors. The photometrically recorded series were obtained by Ektacytometry during creep and recovery process over several millions of cells, using for this purpose a home made device called Erythrodeformeter. We have the time series but we have not the differential equations for the process so it could be modelled by a trajectory in phase space. We used: time delay suggested by Takens, May algorithm, and false nearest neighbours proposed by Malat. On calculating wavelet entropy the mean values for healthy donors was 0.2243, for  $\beta$ -thalassemic 0.2415 and for  $\alpha$ -thalassemic 0.3012, which could reflect an alteration on the viscoelastic properties of erythrocyte membrane on thalassemic patients. On calculating false nearest neighbour %FNN=%21.793 for healthy donors, for  $\alpha$ -thalassemics and haemolytic anaemia % FNN = %1.271; and for  $\beta$ -thalassemics % FNN = %14.019 The results also suggests that the time series from  $\beta$ -thalassemic the correlation decrease with the steps, while the series from healthy donors and also from  $\alpha$ -thalassemic it is not function of the step. This information could be used in evaluating clinical aspects of erythrocyte rheological properties. Further studies with larger and well defined patient populations are in process.

114.

**RE-ESTABLISHMENT OF STORED RBC CHARACTERISTICS STUDIED BY FLOW CYTOMETRY**Di Tullio Budassi L<sup>1</sup>, Relancio F<sup>2</sup>, Delannoy M<sup>1</sup>, Riquelme B<sup>1,2</sup>, Foresto P<sup>1,2</sup>, Valverde J<sup>1,2</sup>.<sup>1</sup>Fac. Cs. Bioquímicas y Farmacéuticas (UNR). <sup>2</sup>Instituto de Física Rosario, (CONICET-UNR), Argentina.

Red blood cells (RBCs) show characteristic shape changes, especially discocyte–echinocyte transformation when they are stored. Even though this behavior has a potential reversibility, these alterations may alter transfusion effectiveness. The aim of this study was to observe the re-establishment of the stored erythrocyte characteristics after incubation in autologous fresh plasma by flow cytometry. Concentrated RBCs prepared from whole blood of a healthy donor collected and stored under blood bank conditions during 45 days. A sample was taken from the concentrated RBC storage bag to be analyzed every week. Equal volumes of RBCs and autologous fresh plasma were mixed and incubated during 60 minutes, then analyzed by flow cytometry. We have observed that SS and FS values of the RBC decrease along the storage period time. The SS value tends to recover the initial value with fresh frozen plasma treatment in terms of the incubation time but the same behavior was not observed in the FS values, where all values tend to decline. Data obtained by means of flow cytometry became useful to evaluate the RBCs behavior and their reversibility properties as the storage time increases, since it is possible to count a great number of cells in a few minutes and evaluate them in a multi-parametric way independently from the operator's subjectivity.

115.

**A CALIBRATION METHOD FOR THE MEASUREMENTS OF FLUORESCENCE CORRELATION SPECTROSCOPY**Castellini H<sup>1</sup>, Riquelme B<sup>2</sup>, Dumas D<sup>3</sup>, de Isla N<sup>3</sup>, Stoltz JF<sup>3</sup>.<sup>1</sup>Fac. Cs Exactas e Ingeniería, UNR; <sup>2</sup>Fac Cs. Bioq. y Farm. Óptica Aplicada a la Biología, IFIR, CONICET-UNR. <sup>3</sup>Lab. Mécanique et Ingénierie Cellulaire et Tissulaire, UHP, Nancy, France. E-mail: hcaste@ifir.edu.ar

The dynamics of biomolecules in suspension by means of the fluorescence correlation spectroscopy (FCS) is a noninvasive method that allows to study the free and bounded molecule diffusion within alive cells and in volumes of the order of the femtoliters. In this work, we developed a calibration method that allows to determine simultaneously in an only series of measurements, the dimensions of the effective volume and the diffusion coefficient of biomolecules in solution. The autocorrelation functions  $G(t)$  from the fluorescence intensity fluctuations of diluted suspensions of Fab2 fragments conjugated with Alexa Fluor 488 (Molecular Probes, A11017) were determined. The FCS measurements were made using a confocal system FCS (Analytical Extension of Leica TCS SP2 AOBs). The model of the phenomenon was made applying adapted mathematical approaches and considering that an only species in solution stops, the initial value of the autocorrelation function  $G(0)$  is inversely proportional to the number of molecules in the volume of excitation. From the analysis of the data, an effective volume  $V_{\text{eff}} = (0,85 \pm 0,03)$  fl and  $w_r = 0,48$   $\mu\text{m}$  and  $w_z = 0,65$   $\mu\text{m}$  dimensions were obtained. For the studied Fab2 fragments a diffusion coefficient of  $D = (164 \pm 6)$   $\mu\text{m}^2/\text{s}$  is obtained. These values are in correspondence with the obtained by other investigators with the traditional methods of calibration. Thus we can conclude that the methodology of propose calibration is correct.

118.

**STUDY OF THE EXPRESSION OF MOLECULAR CHAPERONES IN PLANTS SUBJECTED TO BIOTIC AND ABIOTIC STRESS**

Garofalo CG, Garavaglia BS, Orellano EG, Ottado J.

Instituto de Biología Molecular y Celular de Rosario (IBR), Área Biología Molecular, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. E-mail: cgarofalo@yahoo.com.ar; ottado@ibr.gov.ar

In plant-pathogen interactions, plant resistance is mediated by R proteins that allow the recognition of specific molecules of the pathogen, and induce the hypersensitive response (HR), characterized by a programmed cell death. Recent studies suggest that resistance mediated by R proteins depends on the molecular chaperon Hsp90, and that Hsp90 and Hsp70 would be essentials in the signal transduction pathway in the activation of the HR. *Xanthomonas axonopodis* pv. *citri* (Xac) is the bacteria responsible for citrus cancer (compatible interaction). Xac is also able to induce HR in non-host plants as pepper (incompatible non-host interaction). By western-blot using anti-Hsp70 antibodies, we analyzed the expression of Hsp70 in orange and pepper plants subjected to thermal stress and in the interaction with Xac. We observed that Hsp70 is induced during the treatment at 37 and 47°C while at temperatures lower than 25°C the level of expression of Hsp70 is similar to the controls. In Xac-orange interaction (evaluated by infiltration of  $10^7$  UFC/ml of Xac) no differences in Hsp70 expression was observed. In the incompatible interaction between Xac and pepper an increase in the expression of the molecular chaperon was observed at 24 hours post-infiltration. These results might be suggesting that resistant host plants and non-host plants responses could be sharing similar defense mechanisms.

## 119.

**PARTICIPATION OF *Xanthomonas axonopodis* pv. *citri* LIPOPOLYSACCHARIDE IN CITRUS CANKER**

Petrocelli S, Ottado J, Orellano EG.

Instituto de Biología Molecular y Celular de Rosario, IBR (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. E-mail: petrocelli@ibr.gov.ar/orellano@ibr.gov.ar

Plants have basal perception systems for characteristic patterns from different classes of microorganisms, named pathogen-associated molecular patterns (PAMPs). PAMPs are recognizable by the innate immune system via receptors in plant cells. One of the PAMPs that act as general elicitor in plants is the lipopolysaccharide (LPS), a cell surface component of Gram-negative bacteria. The LPS molecule consists of three major substructures: the O antigen, the core region and the membrane-anchored lipid A. *Xanthomonas axonopodis* pv. *citri* (*Xac*) is the phytopathogen responsible for citrus canker. Two genes, *wzt* and *rfb303*, present in *Xac* genome, code for an ATP-binding component of the O antigen ABC transporter and a core glycosyltransferase, respectively. *Xac* mutant strains defective in *wzt* and *rfb303* genes were constructed by conjugation with the mobilizable suicide vector pKmob. When these mutant strains were grown in SB solid media phenotypic and growth differences were observed between *Xac* wild type and  $\Delta wzt$  but these differences were not observed for  $\Delta rfb303$ . Infection of orange leaves with wild-type and mutant strains resulted in plant disease. However,  $\Delta wzt$  mutant showed reduced bacterial growth. By the other hand, infection of non-host plants (*Nicotiana tabacum*) with wild-type and mutant bacteria produced the typical hypersensitive response. These results suggest that LPS could participate in pathogenicity in host plants.

## 120.

**ISOLATION OF CYSTEINE PROTEINASES FROM *Carica papaya* BY PARTITIONING IN AQUEOUS BIPHASIC SYSTEM**

Bertoluzzo MG, Rigatuso R, Romanini D, Nerli B, Farruggia B, Picó G.

CONICET, FonCyT- Area Físicoquímica. Departamento de Química Física. Facultad de Ciencias Bioquímicas y Farmacéuticas. U.N.R. Suipacha 531 (2000) Rosario. E-mail: gpico@bioyf.unr.edu.ar

Latex of *Carica papaya* is a complex mix of cysteine endo-proteinases (CEP). Papain is one of them; its uses are diverse and thus it makes it an extremely valuable enzyme. Papain has been employed to treat ulcers, dissolve membranes in diphtheria, and reduce swelling, fever and adhesions after surgery, it is also used to tenderise meat, reduce viscosity in juices and eliminate turbidity in beer. Due to its properties it is necessary to count with isolation methods and purification processes in macro scale. The objective of this work was to study the partition profile of CEP obtained from the *Carica papaya* fruit, placing it in aqueous two phase systems of polyethyleneglycol and potassium phosphate, in order to apply them for enzyme isolation. It was found that CEP, has preference for potassium phosphate rich phase. Besides, the partitioning coefficient diminished with the increasing of polyethylene glycol concentration, probably due to the fact that CEP was partitioned with a mechanism guided by the exclusion volume of the polyethylene glycol. The increase in the molecular weight of the polyethylene glycol and the presence of NaCl induced a reduction in the partitioning coefficient. When this method was applied to an homogenate of skin, seed and pulp of papaya it was observed that the system formed by polyethylene glycol of molecular weight 4000 and 4% NaCl produced an enzyme yield of 40-60%, and a purification factor between 3,0 and 6,0.

## 121.

**VASCULAR FLORA OF THE SANTA FE PROVINCE: *Hydrocharitaceae*, *Potamogetonaceae* and *Zannichelliaceae***

Di Sapia O<sup>1</sup>, Mc Cargo J<sup>1</sup>, Oakley L<sup>2</sup>, García R<sup>2</sup>, Prado D<sup>2</sup>.

<sup>1</sup>Botánica, Fac. Cs Bioquímicas y Farmacéuticas, UNR, Suipacha 531, S2002RLK Rosario. <sup>2</sup>Botánica, Fac. Cs. Agrarias, UNR, C.C. N° 14, S2125ZAA Zavalla. Argentina.

The Monocots families *Hydrocharitaceae*, *Potamogetonaceae* and *Zannichelliaceae* comprise herbaceous, submerged, floating or marshy species. The first one is part of the order Hydrocharitales, while the rest are integrated in the order Najadales; previously the three families belonged in the order Helobias (=Fluviales). The present work provides keys for the taxa identification based on morphological characters, and a geographical distribution map. The methods consist of bibliographical review, consultation of herbaria with collections of the province (SF: Esperanza; SI: San Isidro; UNR: Rosario), field work experience of the authors and lab work to confirm their identity. The family *Zannichelliaceae* is represented by one genus and a single species: *Zannichellia palustris* L., cosmopolitan, usual in seasonal ponds south and north of the province. The family *Hydrocharitaceae* is represented by three genera of native aquatic grasses, with one species each: *Limnobium spongia* (Bosc.) Steud. subsp. *laevigatum* (Humb. & Bonpl. former Willd.) Lowden, floating plants distributed in center- northern departments; the submerged *Elodea callitrichoides* (Rich.) Casp., concentrated on the center-east; and possibly the submerged *Egeria densa* Planch. The family *Potamogetonaceae* comprises a genus and two taxa: *Potamogeton gayi* A. Benn., also submerged aquatic, native, distributed throughout the province, and *Potamogeton striatus* Ruiz & Pav., common in ponds in southern departments.

## 122.

**BEHAVIORAL AND DEVELOPMENTAL ALTERATIONS IN NEONATE WISTAR RATS EXPOSED TO VANADIUM THROUGH LACTATION**

Soazo M del V, Garcia GB.

Morphology Area, Faculty of Biochemical and Pharmaceutical Sciences, National University of Rosario. Rosario. Santa Fe. Argentina. E-mail: marinasoazo@yahoo.com.ar

Vanadium (V) is a major trace metal found in fossil fuels, and combustion of these materials provides a significant environmental source of this element. In V-induced developmental toxicity studies, several effects such as: body, liver and renal weights decrease and skeletal defects were observed. Since V was reported to be present in milk of V-treated nursing rats, the aim of this work was to study the effects of V on physical and neurobehavioral developmental parameters of neonate rats exposed through lactation. 10 litters were randomly assigned to the following groups: V-treated: offspring of dams i.p. injected with 3 mg/kgbw of sodium metavanadate from the 10<sup>th</sup> to the 21<sup>st</sup> post natal day (PND). Control: offspring of dams i.p. injected with an equal volume of saline solution. Surface righting reflex, negative geotaxis and hindlimb support tests were performed to neonates every 48 hours, from 8th to 18th PND. Open field test was performed on the 21<sup>st</sup> PND. Pups body weight and eye opening were recorded as physical developmental parameters. Righting times were significantly longer ( $p < 0.001$ ) on days 12, 14, 16 and 18; forelimb support latency ( $p < 0.05$ ) decreased on days 19 and 20 and a significant decrease of locomotor activity and rearing ( $p < 0.05$ ) were observed in V-exposed pups. A decrease in body weight ( $p < 0.05$ ) on days 12, 14, 16, 18 and 20 and a highly significant ( $p < 0.0001$ ) delay in the eye opening mean day were also detected in these pups. Results suggest that vanadium exposure through lactation would induce physical and neurological developmental alterations in Wistar rat pups.



### 123. INTERACTION OF HIPervalent CHROMIUM WITH PECTIN

Bellú SE, García SI, González JC, Signorella SR, Sala LF.  
Área Inorgánica, Fac. Cs. Bioq. y Farm. Suipacha 531, Rosario.

Chromium(VI) is a widespread environmental contaminant. Cr(VI) is mobile, and has a long residence time in surface water and ground-water. Many interactions of hipervalent chromium with low-mass reductants have been studied but a few with biopolymers. Pectins are a group of polysaccharides present in the plant primary cell walls of plants. The objective of this work was to study the ability of pectins to reduce and/or stabilize hipervalent chromium in order to ascertain the potential role of this plant component in the transport of Cr.

Kinetics and spectroscopic studies (UV-vis, EPR) of the reaction of Cr(VI) and apple pectin revealed that the reaction proceeded through a mechanism combining  $\text{Cr}^{\text{VI}} \rightarrow \text{Cr}^{\text{IV}} \rightarrow \text{Cr}^{\text{II}}$  and  $\text{Cr}^{\text{VI}} \rightarrow \text{Cr}^{\text{IV}} \rightarrow \text{Cr}^{\text{III}}$  pathways. The presence of Cr(IV) intermediates can contribute to the oxidative damage in plant cells. Free radicals formed in the  $\text{Cr}^{\text{IV}} \rightarrow \text{Cr}^{\text{III}}$  path were detected. Cr(V) species were characterized by EPR spectroscopy. The Cr(V)-pectin species showed  $g_{\text{iso}}$  values around 1.9785 typical of oxo-Cr(V) bound to two carboxylato and two alcoholato donor groups. These species were stable for at least 6 hours at pH = 4.66, suggesting that the pectin can stabilize this oxidation state of chromium.

HCOOH was found as one of the oxidation products of apple pectin when treated with Cr(VI).

Acknowledgments: FONCyT- ANPCyT (PICT 10625), CONICET PIP 03016.

### 124. NEW BIOMIMETIC ANTIOXIDANTS WITH CATALASE AND SUPEROXIDE DISMUTASE ACTIVITY

Daier V, Palopoli C, Biava H, Moreno D, Signorella S.  
Área Inorgánica, Fac. Cs. Bioq. y Farm. Suipacha 531, Rosario.

In a variety of pathological situations the production of reactive oxygen species overwhelms the activity of endogenous defence systems (catalases (CAT) and superoxide dismutases (SOD)). Renewed interest has recently been stimulated by reports of catalytic antioxidants as novel therapeutics based on their ability to sequester oxidants at a much higher rate than stoichiometric antioxidants. Particularly attractive are complexes exhibiting combined CAT and SOD activity because they offer a possible therapeutic advantage arising from their multiple mechanism of action. With this in mind, we have evaluated the SOD and CAT activity of new Mn complexes of 1,3-bis(2-hydroxybenzyl-2-methylpyridylamino)propan-2-ol ( $\text{H}_3\text{L}^1$ ), 1,4-bis(salicylidenamino) butan-2-ol ( $\text{H}_3\text{L}^2$ ), 1,5-bis(5-sulphonatesalicylidenamino)pentan-3-ol ( $\text{H}_3\text{L}^3$ ) and 1,5-bis(2-hydroxybenzyl-2-methylpyridylamino)pentan-3-ol ( $\text{H}_3\text{L}^3$ ). These ligands afford Mn complexes of different nuclearity, that are good structural mimics of MnSOD and MnCAT. The catalase activity was determined polarographically by measuring the oxygen concentration of  $\text{H}_2\text{O}_2$  + catalyst mixtures with a Clark-type oxygen electrode. The SOD activity was assayed by measuring the inhibition of the photoreduction of nitro blue tetrazolium in the presence of the catalyst. Complexes  $\text{Mn}_2\text{L}^1$  and  $\text{Mn}_2\text{L}^4$ , with Mn ions possessing saturated coordination spheres, showed moderate CAT activity. Complex  $\text{Mn}_2\text{L}^3$  was found to be highly efficient to disproportionate  $\text{H}_2\text{O}_2$  in aqueous basic medium and is the most efficient MnCAT mimic known up to date. Complex  $\text{MnL}^2$  was found to be an excellent catalyst with combined SOD/CAT activity.

### 125. INTERACTION OF D-GLUCOSAMINE WITH $\text{Cr}^{\text{VI}}$ REACTION INTERMEDIATES

Frascaroli M, Andreu V, Signorella S, Sala L.  
Área Inorgánica, Fac. Cs. Bioq. y Farm. Suipacha 531, Rosario.

Chitin is an abundant polysaccharide present in the exoskeleton of invertebrates including crustacean shells and cell walls of fungi. The natural degradation of chitin and chitosan has a significant role in recycling sources of C and N and in the production of useful chemical reagents. The N-acetylglucosamine y glucosamine (Nglc) are the products of the hydrolysis of chitin and chitosan, respectively.

The environmental pollution by metals is known. Compounds of  $\text{Cr}^{\text{VI}}$  are among the chemicals first classified as carcinogens for humans. In this work we focused our investigation on the interaction of  $\text{Cr}^{\text{VI}}$  with Nglc, the first isolated natural aminosugar. Previous studies on the oxidation of D-glucose and 2-deoxy-D-glucose with  $\text{Cr}^{\text{VI}}$  showed that the C1-OH hemiacetalic function reacts faster than the primary or any of the secondary alcoholic groups, giving aldonic acid as the only reaction product. The study of the interaction of this aminosugar with  $\text{Cr}^{\text{VI}}$  allows to determine the role of the C2-NH<sub>2</sub> on this reaction.

With the objective to detect reaction intermediates and obtain information on the mechanism of the metal-substrate interaction, the reaction was followed spectrophotometrically in the region of 200-700 nm, using appropriate conditions of concentration of reagents and acidity. These studies allowed to determine the participation of ligand- $\text{Cr}^{\text{VI}}$  species and the detection of  $\text{CrO}_2^{2+}$  (taken as evidence of the participation of the ion  $\text{Cr}^{\text{IV}}$  in the studied redox process). The detection of intermediate  $\text{Cr}^{\text{V}}$  was carried out by means of EPR spectroscopy and that of radical species by the acrylonitrile polymerization test.

### 126. ASCORBIC ACID IN SEMISOLIDS SYSTEMS WITH ECOLOGIC EMULGENTS: COMPARATIVE STUDIES OF INCORPORATION, RELEASE AND RHEOLOGICAL PROFILES

Lavaselli S, Marineli D, Orlandi S, Calafato R.  
Area Técnica Farmacéutica. Fac. Cs Bioq. y Ftcas. U.N.R.

Most of the dermatologic formulations are emulsive systems and the interactions between phases give their properties. The goal of this work is the correct incorporation and release of ascorbic acid from different semisolids systems with the rheological profiles, to chose the best for industrial scale. There were used high impact ecological emulgents like PEG (polyethyleneglycol) - 4 Oliviate, Lauryl Polyglucose and biopolymers  $\beta$  (1-4)-2-acetamide-2-desoxi-D-glucose. The reference system was the N° 1 prepared with non ionic emulsifying wax. In the N° 2 system was changed a part of the emulsifying wax, with PEG-4 Oliviate. The system N° 3 it was made the same with Lauryl Polyglucose. Otherwise the N° 4 and 5 systems are corresponding to the N° 2 and 3 systems plus *chitosan*. The ascorbic acid concentration used was 1%, the usually valor for topic via; its release was determined by titration. It was determined the following system parameters: pH, distribution and globule size and rheological shape.

All the systems were stable to the termic stress. System N° 5 is the most stable according the photography. The acid amount released was similar in the system. N° 1, 2 and 4, and higher in 3 and 5. The rheological shape N° 1, 3 and 5 systems are plastic and tixotropic, and systems N° 3 y 5 show minor viscosity while systems N° 2 and 4 are plastic without tixotropic behaviour and low viscosity than system N° 1. The results show a good correlation between the hydrophilic character of polymeric matrix and the nature of the active, which is efficiently incorporated by the emulsifying agents in relationship with the anfífilic nature of them. System N° 4 (*chitosan*) because has the smallest globule size in the internal phase and better rheological properties, showed major stability on time being a novel formula in the semisolid system at scale up.

- A**
- Abraham, N. 92
- Acebal, M. 75
- Acosta, I. 100, 107
- Advínculo, S. 17
- Aguirre, N. 60
- Alessi, A. 112
- Alet, N.A. 29, 54
- Alfieri, A.E. 61
- Almará, A. 93
- Alsina, S. 4
- Álvarez, M. 18
- Ameztoy, I. 24
- Amigot, S. 95, 100
- Andreu, V. 125
- Antruejo, A.E. 18
- Arango, J.F. 61
- Arciero, G. 100
- Arestegui, M.B. 60
- Arriaga, S. 93
- Azum, Y. 10, 11
- B**
- Badler, C. 4
- Ballerini, V. 88
- Barosso, I. 86
- Bassan, N. 12, 13, 14
- Bay, M.L. 8, 9
- Bazzoni, G. 41
- Bechara Baladí, V. 50
- Bellú, S.E. 123
- Beloscar, J. 104
- Bertola Compagnucci, A. 1
- Bertoluzzo, M.G. 120
- Bertoluzzo, S. 28
- Bertorini, G. 91
- Besso, R. 60
- Biasoli, M. 86, 95, 98
- Biava, H. 124
- Biondi, C. 106, 107, 108, 109, 110, 111
- Bisaro, V. 81
- Blanco, M.L. 54
- Bobrovsky, E.O. 50
- Boggio, G. 3
- Bollini, A. 31, 32, 41
- Bottai, H. 95
- Bottasso, O. 8, 9
- Bouvet, B. 102, 105
- Bozza, V. 8, 9
- Breccia, G. 74
- Buffo, M. 38
- Bulacio, L. 77, 78, 95, 96, 97
- C**
- Cabada, M. 62, 99
- Caffarena, G. 10
- Caille, A.M. 99
- Calafato, R. 126
- Calderari, M. 74
- Calvi, B.J. 35, 36, 39, 40, 53, 55, 56
- Calvo, F. 75
- Calvo, J.E. 45, 46, 47
- Campi, C. 110
- Cánaves, M.A. 54
- Cane, F. 62
- Canet, Z.E. 18
- Capitaine Funes, C. 85
- Caraballo, E. del C. 54
- Carrara, P. 28
- Carrera, L.I. 26, 84
- Castellano, P. M. 101
- Castellini, H. 115
- Catalá Schonfeld, H.G. 50
- Catalani, G. 51
- Caviglia, N. 14
- Ceballos, P. 85
- Cechetti, S. 75
- Cecon, I. 75
- Cera, G. 88
- Cesarios, G.M.I. 2
- Cesolari, J.A.M. 35, 36, 39, 40, 53, 55, 56, 57
- Chaparro, S. 12
- Cinara, L. 32
- Cipulli, G. 85
- Coca, L. 60
- Cocconi, E. 100
- Cointry, E. 82
- Colleto, A. 86
- Contesti, J.F. 50
- Converso, G. 7
- Corbera, M. 104
- Costa, A. 59
- Cotorruelo, C. 106, 107, 108, 110, 111
- Cravero, V. 82
- D**
- D'Angelo, M. 83
- D'Arrigo, M. 84, 90, 104
- D'Attilio, L. 8, 9
- D'Ottavio, A.E. 26, 27, 84
- D'Ottavio, G.E. 27
- Dagatti, M.S. 1, 2
- Daier, V. 124
- Daniele, S.M. 37, 52, 93
- Dapino, D. 62
- Dasso, L. 59
- de Isla, N. 115
- Décima, M. 59
- Delannoy, M. 114
- Delgado, G. 60
- Di Loreto, V. 31, 32
- Di Masso, R.J. 15, 16, 17, 18
- Di Sapia, O. 121
- Di Tullio Budassi, L. 114
- Dídoi, G. 8, 9
- Diez, G.A. 50, 54
- Diez, J. de la C. 50
- Dlugovitzky, D. 42
- Dottavio, A.M. 15, 16, 17, 18
- Dottavio, M.E. 51
- Dumas, D. 115
- E**
- Ebner, G. 89
- Eichhorn, M.C. 54
- Eliás, M. 92
- Ensínck, A. 111
- Escovich, L. 6, 110
- Espejo, T. 6
- Espósito, M.A. 82
- Etchepare, R.G. 84
- F**
- Faienza, H.L. 67
- Farroni, M. 42
- Farruggia, B. 120
- Feldman, R. 102, 105
- Felix, M. 24
- Fernández, E. 80
- Ferreras, L. 80
- Figallo, R. 75
- Figallo, R.M. 66, 67, 68
- Figueroa, H.E. 34
- Fiorenza, G. 42
- Firpo, I. 80
- Flores, P. 79
- Font, M.T. 15, 16, 17, 18
- Fontana, A. 112
- Fontanella, G.H. 12, 13, 14
- Forcher, A. 7
- Foresto, P.G. 84, 90, 94, 112, 114
- Fracchia, L. 63
- François, S. 89
- Franzoni, R. 83
- Frascaroli, M. 125
- Frontini, A.V. 27
- Fulgueira, C.L. 95
- Fusiglieri, D. 79
- G**
- Galeano, A. 38
- Galetti, L.A. 72, 73
- Garavaglia, B.S. 118
- García Borrás, S. 106, 107, 108, 109, 110, 111
- García, G.B. 122
- García, R. 121
- García, R.C.A. 73
- García, S.I. 123
- Garofalo, C.G. 118
- Gayol, M. del C. 25, 29, 30, 33, 54
- Gentile, N. 60
- Gerhardt, N. 94
- Gerrard, G. 104
- Gervasoni, S.I. 21
- Ghersevich, S. 85, 99
- Ghio, A. 76
- Ghirardi, G. 71
- Gioiella, L. 30
- Giolito, I. 78
- Giorgi, D. 74
- Giri, A. 8
- Giuliani, S. 77, 78
- Godoy, A.R. 35, 57
- Gomez, E. 80
- Gómez, J.A. 54
- González, D. 29
- González, J.C. 123
- González, M. 76
- González Olivera, E. 23
- Gorosito, M.D. 43, 44, 45, 46, 47
- Gorr, C. 10, 11
- Grandfils, C. 112
- Griot, E. 93
- Gualtieri, C.A.S. 60
- H**
- Hachuel, L. 3
- Hasenauer, E. 31
- Hernández, G. 31, 32, 41
- Hinrichsen, L. 19
- Hisano, N. 27, 34
- Hourquesco, M.C. 100
- Hure, E. 10, 11
- I**
- Incremona, M. 76
- Indelman, P. 91
- J**
- Jacobi, P. 93
- Joffre, A. 38
- Juárez, S. 11
- K**
- Kaufman, T.S. 101
- Kerps, G. 71
- Kohli, A. 5, 6
- Korol, A. 113
- Krumrick, G.E. 13
- L**
- Labourdette, V. 25
- Lapalma, M.A. 61
- Laudanno, O.M. 35, 36, 39, 40, 53, 55, 57
- Lavaselli, S. 126
- Lavooy, L.A. 45, 46, 47
- Lebensohn, N. 84, 90, 94
- Leiva, M. 95
- Leiva, R. 25
- Lembo, G.M. 43, 44
- Leroux, B. 49

- Liberatti, D.R. 69, 70  
 Libreria, J.E. 16  
 Limansky, A.S. 87, 88, 89  
 Lioi, S. 104  
 López, C. 6, 95, 96, 97  
 López, J.P. 37  
 Luján, M. 107  
 Luque, A. 86, 95, 98  
 Lusardi, M.B. 72
- M**  
 Maggio, R.M. 101  
 Mahuad, C. 8  
 Mahuad, S.L. 69, 71  
 Mainetti, L.E. 20  
 Manago, M. 7  
 Marani, J. 37  
 Marchegiani, M. 56  
 Marchiaro, P. 88  
 Marcipar, F. 9  
 Marí, R. 88  
 Marinelich, D. 126  
 Marini, P. 62  
 Marinozzi, D. 25  
 Martignone, R. 81  
 Martín, B. 71  
 Martin, E. 82  
 Martinel Lamas, D. 42  
 Martineli, F. 60  
 Martinez, A. 17  
 Martinez, S.M. 37, 38, 52  
 Martinotti, M.G. 63  
 Matar, P. 20  
 Mc Cargo, J. 121  
 Mengarelli, G. 28  
 Miglietta, M.C. 51  
 Mignaco, R. 45, 46, 47  
 Milani, A. 100, 107  
 Miralles, D.R. 47  
 Molina, C. 100  
 Montenegro, S.M. 33, 37, 38, 52  
 Monti, L. 10, 11  
 Moreno, D. 124  
 Moreno, H. 31, 32  
 Moreno, J. 92  
 Mosconi, N. 10, 11  
 Mundiñano J. 19  
 Munini, G. 106  
 Munuce, M.J. 99  
 Mussi, M.A. 87
- N**  
 Navoni, S. 54  
 Negro, P.S. 64  
 Nerli, B. 120  
 Nestares, G. 74
- O**  
 Oakley, L.J. 72, 73, 121  
 Olguin, C. 25  
 Orellano, E.G. 118, 119  
 Orlandi, S. 126  
 Ortega, M.A. 45, 46, 47  
 Ottado, J. 118, 119  
 Oyarzabal, M.I. 59
- P**  
 Pafundi, L. 71  
 Pagni, C. 65  
 Palmisano, E. 50  
 Palopoli, C. 124  
 Panelo, M. 77, 78  
 Panozzo Zénere, E. 94  
 Paparella, C. 102, 103, 105  
 Parente, F. 49  
 Passerini, F. 54
- Patronelli, D.L. 23  
 Pavesi, A. 103  
 Pellicciotti, M.C. 45, 46, 47  
 Pellizzón, O. 93  
 Pelusa, H. 93  
 Peralta, L. 60  
 Pereira da Costa, J.H. 70  
 Pérez, F. 58  
 Pérez, G. 92  
 Pérez, S. 100, 113  
 Peroni, M. 51  
 Perotti, E.B.R. 63  
 Petrocelli, S. 119  
 Pezzotto, S.M. 1, 2, 3, 5, 93  
 Piazzon, I. 19  
 Picardi, L. 69, 70, 71, 74  
 Picena, J.C. 38, 43, 44, 52  
 Picó, G. 120  
 Pidello, A. 63, 66, 67, 68  
 Pilafis, M. 6  
 Pirlles, M.E. 65  
 Pitueli, N.E. 102, 103, 104  
 Poggi, D. 79  
 Poletto, L. 1, 2, 5  
 Pontoriero, A. 10, 11  
 Ponzio, S. 93  
 Porto, M.L.P. 64  
 Posadas, M. 25, 29  
 Postiglione, G. 6  
 Prado, D.E. 72, 73, 121  
 Prat, G. 59  
 Pratta, G.R. 69, 70  
 Prestigiovanni, G. 14  
 Puigsubirá, C. 4
- R**  
 Rabinovich, G.A. 22  
 Racca, A. 106, 107, 108, 109, 110, 111  
 Racca, L. 98, 106, 107, 108, 110, 111  
 Radcliffe, S. 90  
 Ramadan, S. 95, 96, 97, 98  
 Ramos, L. 95, 96, 97  
 Rascón, A. 9  
 Rasía, M. 41, 48, 51  
 Re, A. 90  
 Relancio, M.F. 112, 114  
 Relling, V. 87  
 Reynoso, A.C. 61  
 Rico, M. 9  
 Rico, M.J. 20, 22  
 Rigalli, A. 31, 32  
 Rigatuso, R. 120  
 Riquelme, B. 112, 113, 114, 115  
 Rizzotto, M. 10, 11  
 Rodríguez, G.R. 69, 70  
 Rodríguez, H. 52  
 Roggero, E. 21  
 Rojas, P. 35, 36, 39, 40, 53, 55  
 Roma, S. 58  
 Romagnoli, M.S. 81, 83  
 Romanini, D. 120  
 Romera, B.M. 16  
 Rosbaco, I. 81  
 Rosillo, I. 104  
 Rossa, A. 21  
 Rosso, O. 113  
 Rotondo, R. 80  
 Rozados, V.R. 20, 21  
 Ruberti, D. 95  
 Rucci, A. 107  
 Ruiz, M.I. 12  
 Ruiz, R.I. 47
- S**  
 Saball, E. 92  
 Saeta, J. 78
- Sala, L.F. 123, 125  
 Salvarrey, M. 92  
 San Miguel, P. 40, 55  
 Sarubbi, C. 77  
 Scandiani, M.M. 86, 95  
 Scarafiocca, M.J. 69  
 Scharovsky, O.G. 20, 21, 22, 60  
 Schiaffino, L.S. 65  
 Segatori, S. 71  
 Seghesso, A.B. 61  
 Selenscig, D. 42  
 Sgro, M.V. 30, 54  
 Signorella, S.R. 123, 124, 125  
 Simó, M. 35, 36, 39, 40, 53, 55  
 Smacchia, A.M. 66, 67, 68  
 Soazo, M. del V. 122  
 Sortino, M. 95, 96, 97, 98  
 Spalding, T. 88  
 Spengler, M.I. 28, 48, 49, 50  
 Spies, J. 30  
 Spiller, L. 75  
 Steeman, R(h) 61  
 Stoltz, J.F. 115  
 Suárez, C. 19  
 Sutich, E. 89  
 Svetaz, M.J. 49
- T**  
 Tarrés, M.C. 33, 37, 38, 52  
 Tellez, T.E. 26  
 Tomat, D.D. 70  
 Toresani, S. 80  
 Toresani, I. 89  
 Torioni de Echaide, S. 60  
 Tosello, M.E. 95  
 Tozzini, R. 85  
 Trossero, C. 10, 11  
 Turco, M. 104  
 Tuttolomondo, G. 81
- U**  
 Urteaga, A. 35, 36, 39, 40, 53, 55, 57
- V**  
 Vadell, D. 7  
 Valdés, M. 93  
 Valdez, V. 109  
 Valverde, J.R. 84, 90, 94, 112, 113, 114  
 Varela, D. 16  
 Vasconcelos, L.A. 35, 36, 39, 40, 53, 55, 57  
 Vega, T. 74  
 Vegetti, R. 35, 36, 39, 40, 53, 55, 57  
 Verdura, L. 75  
 Viale, A. 87, 88, 89  
 Vila, A. 88  
 Villarruel, R. 35, 36, 39, 40, 53, 55, 57  
 Vinuesa, M.A. 12, 13, 14  
 Viso, M. 93  
 Vitelleschi, M.S. 4  
 Vogué, C. 42
- W**  
 Wandzik, C.I. 35, 36, 39, 40, 53, 55, 57
- Y**  
 Yeannes, M.I. 24
- Z**  
 Zacarias Fluck, M.F. 20, 22  
 Zacchino, S. 11  
 Zamponi, M.O. 23  
 Zingale, I. 25  
 Zorzoli, R. 69, 70  
 Zumoffen, C. 102, 104  
 Zumoffen, C.M. 85, 99

