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1. PHOTOPERIOD INFLUENCE ON SEXUAL MATURITY OF OBESE β RATS

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Changes in the natural photoperiod – 12 h light / 12 h darkness – may affect the reproductive ability and the sexual maturity of rats. Obese β and non-obese b female rats were exposed to three different photoperiods: I) 12 h light / 12 h darkness (n_{β} :7; n_b :7) II) 24 h light (n_{β} :8; n_b :7) III) 24 h darkness (n_{β} :8; n_b :5).

Age and body weight values were gathered at vaginal opening moment and at the beginning of the first oestra cycle. Statistical analysis was performed using two-way ANOVA, followed by Tukey's test.

Related to age, results show that in β rats, vaginal opening occurred earlier than in b ones when under I, II and III conditions ($p<0.001$) and so did the oestra cycle under I and II ($p<0.05$). There was no difference between lines under treatment III.

Related to body weight, significant differences between lines were observed only under treatment II - being higher in β - at vaginal opening moment ($p<0.05$) and at first oestra cycle ($p<0.01$).

Obese β rats became pubertal earlier than non-obese b ones probably because those could reach speedily a critical body / adipose tissue weight in accordance with its genetically lower capacity to dissipate energy.

Such as in other laboratory animal models, the exposure to 24 h light affected more deeply the reproductive processes than that to 24 h darkness, supporting the theory that proposes that increasing periods of light cause diminished melatonin production and by this way anticipate puberty.

2. GLUCOLIPIDIC PROFILE IN SURGICALLY STRESSED β RATS

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The aim of this work was to analyze the variations of serologic glucolipidic patterns in surgically stressed 250 day obese and diabetic male IIM/ β rat. This line develops basal hiperglycemia and hipertiglyceridemia since puberty. Three groups were set: insulin only(i), insulin and surgery(i+s) and basal lipidic values(b). Basal glycemia(g0) was obtained from (i)n:10 and (i+s)n:10, then regular insulin (1U/kg SC) was given to both groups. Fifty minute surgery was performed to (i+s). The operation consisted of analgesia, antibiotic single dosis, anesthesia, left subcostal laparotomy and physiologic solution injection (0.1ml) into pancreatic tissue. Glycemia was measured again to (i)n:10 and (i+s)n:10 at 60(g60), 105(g105) and 180(g180) minutes. Colesterolemia and trygliceridemia were determined to (b)n:10 at beginning as (i+s)n:8 and (i)n:6 were measured 180 minutes (c180);(t180) after treatment beginning. Analysis made with t test and Anova/Tukey (mean \pm SEM). (g0) in (i) and (i+s) were >1.10 g/l being compatible with glucose intolerance. Comparing g0, g60, g105 and g180 between (i) and (i+s) differences were ($p>0.05$). Glycemia maintains low 180 minutes after treatment (i)g0 1.50 ± 0.062 vs (i)g180 1.09 ± 0.144 ; (i+s)g0 1.65 ± 0.085 vs (i+s)g180 0.95 ± 0.126 ($p<0.05$). Comparing t0, c0 to t180 and c180 among (b), (i) and (i+s) differences were ($p>0.05$). Insuline does not only decrease glycemia while keeping triglyceridemia and colesterolemia, but also maintains effects for two-hour period after surgery ending, minimising surgical stress effects on glucolipidic profile on β rats.

3. VARIABLES CONNECTED WITH FERTILITY IN TWO LINES OF INBRED RATS

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Line IIM/b was incorporated to the bioterio of Cátedra de Biología in November 2001. A contrast with line IIM/ β obese and diabetic line was made with the purpose of validating b as a control eumetabolic line. Variables that had to do with 120 day female rat fertility were evaluated in two moments: when first b generation (1^aG, n:11) with β (51^aG, n:8) simultaneous cohort was produced and when sixth b generation (6^aG, n:9) and the β (56^aG, n: 14) simultaneity was produced. Values were analysed through ANOVA and Tukey post test (media \pm SD). **Time from joined for mating up to birth time(ds)** does not differ ($p>0.05$) among the groups but it lasts longer in b. **Total biomass increase during pregnancy(g)** is always higher in β than in b ($p<0.05$), not differing ($p>0.05$) inside the b generations nor inside the β ones. **Litter birth size and litter weaning size:** in both variables ($p<0.05$). **Breeding average weight at birth time(g)** ($p>0.05$) and **breeding average weight at weaning(g)** ($p<0.05$). **Fertility coefficient:** with differences ($p<0.05$). **Survival coefficient:** without differences ($p>0.05$). **Combined coefficient:** with differences ($p<0.05$) b(1^aG)= 0.480 ± 0.430 ; b(6^aG)= 0.947 ± 0.305 ; β (51^aG)= 1.170 ± 0.370 ; β (56^aG)= 0.973 ± 0.360 . In line b females, get less biomass during pregnancy and the breeding production is somewhat less. With the combined coefficient the new environment effect over b is proved. β breeding has higher weight, not at birth time but at weaning. In this way obesity is shaped. In line β a phenotypic stability is confirmed for all variables. Line b stability must be verified in future generations.

4. RED BLOOD CELLS SHAPE AND OSMOTIC RESISTANCE IN PRESENCE OF ARSENATE (As V)

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The object was to study the effect of As V on the osmotic response and shape of human red cells. We worked with healthy red cells washed with PBS, which were separated in three fractions and re-suspended in PBS (control), PBS-As (100 and 200 ug/l) They were incubated during 30 minutes at 37°C. Osmotic response was measured by Dacie method and of each hemolysis curve, X_{50} was obtained. Besides, a drop of red cells suspension was analyze by microscopy by setting a morphological index according to Bessis. Statistical Analysis: t Student for pair groups. Data are presented as media \pm SEM. * $p<0.02$ Osmotic response: X_{50} (mM): control: 77.84 ± 0.86 ; + 100 ug/l As^V: $82.17\pm 1.38^*$; + 200 ug/l As^V: $81.24\pm 0.84^*$. Shape: control: biconcave disc; + 100 ug/l As^V: equinocytes type I and II; + 200 ug/l As^V: equinocytes type I and II. The results prove that As^V reduces osmotic response and it causes shape changes, both alterations at significantly form. The shape changes would attribute at modifications of anionic gradients, leading to conformational change of Banda 3 protein. In other way, the high osmotic fragility could be explained by union of As^V to structural protein sulphhydryl groups of cellular membrane. In consequence it increases the sensibility to osmotic changes.

5. HEMORHEOLOGICAL ASSESSMENT IN ALLOXAN-INDUCED DIABETIC RATS

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The aim of this communication was to widen previous clinical researches using induced diabetic rats as models for hemorheological assessments. 10 male "m" rats (25 days old and mean body weight 56 ± 3.16 g) were divided into two groups. 6 were intraperitoneally injected with alloxan (24 mg / 100 g body weight) and 4, intraperitoneally injected with normal saline, vehicle of alloxan (1 cc / 100 g body weight) (controls). Glycemia reached 2.90 ± 0.80 g/dl 48 hours after the alloxan injection, and kept that range along the experiment. Rats were sacrificed by ether overdose 17 days after alloxan administration. The index of erythrocyte deformability was studied in blood samples obtained from the heart and anticoagulated with heparin, through a diffractometric method employing the erythrodeformeter. No significant differences were revealed between alloxanized rats and controls (0.66 ± 0.02 and 0.66 ± 0.04 , respectively). Besides, the microscopic analysis of the blood samples allowed detecting the absence of "rouleaux" and "clusters". These results could point out that the erythrocyte deformability in the studied rats was not affected by a short-term alloxan-induced diabetes and that the red blood cells did not show aggregating trends, exceeding the referred hyperglycemia. The study of hemorheological parameters in long-term alloxan-induced diabetes is considered of interest.

6. GENITAL DUCTS (WOLFF AND MÜLLER) DURING THE EARLY FETAL DEVELOPMENT

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This communication explores the morphogenesis and differentiation of the genital ducts (Wolff and Müller) as well as the relationships reached by both structures during the early fetal development. Serial 7 μ m thick sections from the abdominal-pelvic zone of 9th and 10th - week-old six human fetuses, obtained from spontaneous abortions in public hospitals, were sequentially exposed to several histological and histochemical procedures. Then, they were planimetrically processed and superposed for spatial reconstruction. In the studied ages, neither morphological nor histochemical differences between both ducts, a close relationship between the mesonephric duct and the celomic epithelium and a high cellularity in the peritubular stroma were revealed. The aforesaid relationship could be related with a possible inductive interaction between the Wolfian duct and the celomic epithelium whilst the high cellularity could be referred to the embryological origin of the stroma (lateral mesoderm) as well as to its steroidogenic potentiality.

7. FAT DEPOTS IN 1 YEAR-OLD TYPE 2 DIABETIC RATS

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The endocrine role conferred to the fat tissue in the association overweight / obesity and type 2 diabetes led us to quantify renal, epididimal and abdominal fat depots in spontaneously type 2 diabetic rats (obese β , non obese eSS and its overweighed hybrid eSMT). Six one year-old male rats of each line were weighed and sacrificed by ether overdose and the fat depots, excised and weighed. Data, registered as $x \pm$ standard error, were analyzed through the Kruskal- Wallis test. Results were: **Body weights (β):** 508 ± 9.95 g; **eSS:** 335 ± 5.11 g; **eSMT:** 357.60 ± 26.09 g (β vs. eSS and eSMT, $p < 0.01$; eSS vs. eSMT, $p > 0.05$); **Absolute weights of abdominal (β):** 41.09 ± 2.96 g; **eSS:** 9.09 ± 0.82 g; **eSMT:** 21.93 ± 4.79 g); **renal (β):** 26.24 ± 2.22 g; **eSS:** 4.67 ± 0.62 g; **eSMT:** 11.75 ± 3.17 g) **and epididimal fat depots (β):** 14.85 ± 0.85 g; **eSS:** 4.41 ± 0.36 g; **eSMT:** 10.18 ± 2.02 g). **Relative weights of abdominal (β):** 8.09 ± 0.49 ; **eSS:** 2.71 ± 0.45 ; **eSMT:** 6.13 ± 0.84), **renal (β):** 5.16 ± 0.39 ; **eSS:** 1.39 ± 0.30 ; **eSMT:** 3.28 ± 0.62) **and epididimal fat depots (β):** 2.92 ± 0.11 ; **eSS:** 1.31 ± 0.25 ; **eSMT:** 2.85 ± 0.33) In relation with absolute and relative fat depots weights: $\beta > eSMT$ ($p < 0.05$) $> eSS$ ($p < 0.01$). Differences between eSMT and eSS body weights were attributed to the loss of weight of eSMT at this age, due to its more severe dysmetabolism Results obtained in β and eSMT matched with the relevance of the fat depots in type 2 diabetes development and those in eSS pointed out its minor influence in their diabetic expression. To sum up, β y eSMT rats could be adequate models for studying the referred association.

8. LIPID COMPOSITION AND ERYTHROCYTE MEMBRANE FLUIDITY IN DIFFERENT ANIMAL SPECIES

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Erythrocyte aggregation manifestly changes in different mammals, samples from equines shows intense aggregation, bovines do not form rouleaux and humans have intermediate tendency. We analyzed the lipid composition differences of the erythrocyte membrane from that three species and estimated the fluidity of the external layer of the double lipid layer. Measures were made in 20 humans, 10 equines and 10 bovines by fluorescence polarization method. Red blood cells were treated with TMA-DPH (Trimethylammonium-Diphenylhexatriene). The fluorescence intensity of the component in parallel and perpendicular direction of the excitation beam was determinate. The anisotropic coefficient (r) was calculate, which is inversely related with the membrane fluidity. Results were: $r_{\text{horse}} = 0,1657 \pm 0,0267$, $r_{\text{human}} = 0,1873 \pm 0,0257$, $r_{\text{cow}} = 0,2313 \pm 0,0368$. Statistic analysis with t of Studens showed a significantly difference in membrane fluidity, bigger from equines ($p < 0.05$) and minor from bovines ($p < 0.0005$) than humans. The external lipid layer with a larger proportion of sphingomyelin –with only one lateral acid chain and richer in 20 carbons saturated fat acids- are less "fluid" than those with relatively larger proportion of phosphatidilcoline –with two shorter insaturated acid chains-. Erythrocyte membrane from equines have half and bovines double amount of sphingomyelin that humans, while the phosphatidilcoline amount shows an inverse relation. This results support the hypothesis that there is a relation between lipid composition and freedom grade of molecules that constitutes the membranes and the tendency to associate in erythrocyte rouleaux.

9. OPPOSITE EFFECTS OF LOW DENSITY LIPOPROTEINS AND HIGH DENSITY LIPOPROTEINS ON ERYTHROCYTE MEMBRANE FLUIDITY. PRELIMINARY STUDY

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The objective of this job was to study the correlation between membrane fluidity and seric levels of cholesterol (C), low density lipoproteins (LDL-C) and high density lipoproteins (HDL-C) in healthy women in their middle age (50±5 years old). Measures of erythrocyte fluidity were made by fluorescence polarization method. Red blood cells from blood samples were isolated by centrifugation and treated with TMA-DPH (Trimethylammonium-Diphenylhexatriene) that allows measuring the freedom grade of phospholipidic molecules. Using a polarize ray of light the fluorescence intensity of the component in parallel and perpendicular direction of the excitation beam is determinate. These measures allowed the calculation of anisotropic coefficient, which is inversely related with the membrane fluidity. Seric levels of LDL-C, HDL-C and C were obtained using the colorimetric enzymatic method. When the values were correlated, these results were obtained: anisotropic coefficient is positively associated with C ($r = 0,55$, $p < 0,005$) and LDL-C ($r = 0,71$, $p < 0,001$), and negatively associated with HDL-C ($r = -0,37$, $p < 0,05$). Our results show that plasmatic lipids interact with cellular membranes and affect lipid fluidity. Subsequent studies will be needed for establish the molecular mechanism of that effect.

10. MODIFICATIONS OF ERYTHROCYTE MEMBRANE FLUIDITY BY AMPHIPATHIC DRUGS EFFECT. PRELIMINARY STUDY

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Pharmacological active agents can interact with the double lipid layer of the cellular membrane, increase the lateral and transversal lipid movement and modify the surface potential altering the phospholipids heads disposition. In this job the effect of amphipathic drugs on membrane fluidity from red blood cells were studied. The red blood cells were from 35 middle age women, 20 of them were not medicated, 5 were medicated with Ibuprofen, 5 with Acetylsalicylic Acid and 5 with Diclofenac. The three drugs had in common a hydrophobic ring associated with a hydrophilic chain. None of the women presented systemic diseases history that could modify the hæmorheology of the membrane. Measures of fluidity were made by fluorescence polarization method. Red blood cells are treated with TMA-DPH (Trimethylammonium-Diphenylhexatriene) that allows measuring the freedom grade of phospholipidic molecules. The fluorescence intensity of the component in a parallel and perpendicular way of the excitation beam is determinate. These measures allowed the calculation of anisotropic coefficient (r), which is inversely related with the membrane fluidity. The r values from non medicated women were related with some of the three amphipathic drugs, using the t of Studens. Results showed that r from non medicated women ($r = 0,1845 \pm 0,0142$) was significantly different ($p < 0,05$) than the r from women medicated with amphipathic drugs ($r = 0,1708 \pm 0,0121$). With these results it can be conclude that amphipathic drugs could interact with membrane phospholipids by a modification of their behavior and determining a fluidity increase.

11. NADPH NEURONS FROM RAT AUERBACH'S PLEXUS WITH LOW REACTIVITY TO ALLOXAN

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Alloxan is a well known toxic for β cells of Langerhans islet. We report results obtained with NADPH histochemical study of myenteric neurons. 10 male rats 25 days old, weight 56.00 ± 3.16 g were separated into the following experimental groups. Group A: 6 rats injected with alloxan (24 mg/100 g CBW) (survived 4); group B: 4 rats injected with distilled water 1cc/100 g CBW. Glycemia was performed after 48h and at autopsy. After sacrificed with ether overdose autopsy was performed when 40 days old. Small intestines were removed and washed with chilled PBS, fixed in 4% paraformaldehyde in PBS and procesed for NADPH histochemistry. Results are expressed as media \pm SEM and were compared with the test 't' of Student. Group A corporal body weight: 119.33 ± 11.56 g <group B: 172.25 ± 6.27 g ($P < 0,05$). Group A small intestine weight: 6.37 ± 0.29 g <group B: 9.14 ± 0.23 g ($P < 0,05$). Group A neuronal count: $41.00 \pm 1.79/\text{mm}^2$; group B: $51.14 \pm 4.08/\text{mm}^2$ (n.s.). We could observe in rats injected with alloxan a smaller increase of corporal and intestinal weight, in spite of that histological reticular structure of the plexus was conserved. Neuronal count did not show significative differences, that could be attributed to the moderated glycemia level or the short period of time between the injection and animal autopsy.

12. STRUCTURAL DIFFERENCES OF MYENTERIC PLEXUS STUDIES BY NADH AND NADPH TECHNIQUES IN A LINE OF YOUNG AND AGED DIABETIC OBESE RAT

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Auerbach's plexus are formed by nerves, neurons and glias that constitute ganglia conforming a reticular image characteristic of each gut segment. Neurons from small intestine and distal colon in 4 and 18 month-old beta rats have been analyzed. Male beta rats (obese and diabetic), 4 and 18 months of age were sacrificed with ether overdose. Gut was washed with PBS. Segments of small intestine and distal colon were filled with PBS and both ends tightened. Such segments were processed for NADH histochemistry. Other segments fixed in 4% paraformaldehyde in PBS, incubated in nitroblue tetrazolium and NADPH: NADPH-positive neurons were counted under graticulated grid attached to ocular. With NADH there was a partial disruption in the reticular image in the 4-month rats which became total in the 18-month animals. With NADPH the reticular structure was preserved in both, young and adult animals. Neurons were counted: 1) small intestine: 4-month rats: $56.64 \pm 0.31/\text{mm}^2$; 18-month rats: $32.50 \pm 2.05/\text{mm}^2$, 2) distal colon: 4-month rats: $66.02 \pm 2.14/\text{mm}^2$; 18-month rats: $35.05 \pm 2.91/\text{mm}^2$. The beta rat is an animal model of spontaneous obesity and diabetes. NADH method showed that the reticular structure is disrupted in beta rats, increasing with ageing. With NADPH, the reticular structure is preserved, neurons decreased with age, and probably, have gotten worse because of diabetes.

13. HISTOLOGICAL STUDY OF THE GASTROESOPHAGEAL JUNCTION IN FETUSES OF THE THIRD TRIMESTER

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Classic descriptions of the gastro-esophageal mucosa assert that it is composed by branched tubular glands, lined with clear cells. The histological study of this junction is interesting due to the lesions produced in reflux esophagitis and Barrett's esophagus, a pre-neoplastic condition. At present, some authors state that this structure does not exist; if it does, it would be an initial metaplastic process leading to Barrett's esophagus.

Objective: to observe and describe the cardial mucosa of the gastro-esophageal junction in fetuses of the third trimester.

Material and methods: twenty specimens were studied, obtained from the files of different services of pathology from several hospitals, where the fetal autopsies had been performed. The samples were embedded in paraffin, sections cut 5µm thick and stained with H-E, P.A.S. and Alcian Blue.

Results: in 15 out of the 20 cases, the presence of cardial mucosa was assessed. In the other 5, gastric body mucosa followed the esophageal mucosa directly.

Comments: 75% of the cases exhibited the presence of cardial mucosa. External pathogenic causes, such as environmental factors and *Helicobacter pylori*, cannot operate on fetuses of the third trimester. This finding suggests that this structure might be regarded as normal. The other 25% indicates that there may be individual variations in a smaller number of cases.

14. BRONCHIECTASIAS IN TWO LINES OF DIABETIC RATS. STUDY IN ANIMALS OF BOTH SEXES AT 6, 12 AND 18 MONTHS OF AGE

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Laboratory rats often present bronchiectasias (BE). eSS and eSMT lines are models of spontaneous type 2 diabetes; having observed BE associated with other neumopathies in eSS males, it was interesting to investigate modifications of lung histopathology in both lines. Animals of 6, 12 and 18 months (mo) were used (eSS: 39 males and 26 females; eSMT: 28 males and 29 females). The rats were euthanised with ether and autopsies were performed. Samples were obtained from both lungs, fixed in formaline, embedded in paraffin, cut 5µm thick and stained with H-E and PAS. At 6 mo, five eSS males (26%) showed BE, one with abscedation. One eSMT male presented an abscess in a pulmonary lobe and two females had sacular BE. At 12 mo, 4 eSS males (40%) exhibited fusiform and sacular BE with goblet cell hyperplasia, areas of squamous metaplasia and alveoli filled with mucus. BE were found in 6 eSS females (75%). Few eSMT rats showed BE. At 18 mo, 8 eSS males presented abscedated BE and large areas of consolidation; 8 females showed similar lesions, but less extended. 8 eSMT males had abscedated BE and one showed bronchopneumonia; one eSMT female presented an abscedated BE. Pulmonary lesions were detected from 6 mo on; they increased in frequency and severity with age and are more conspicuous in eSS. This fact stressed a characteristic of this line, even though the expression of the diabetic syndrome is more marked in eSMT.

15. DIFFERENCES THROUGH GENETICS AND INTERNAL ENVIRONMENT IN PANCREATIC FEATURES OF MURINE MODELS OF DIABETES, EVIDENCED BY MULTIVARIATED CLUSTER

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Insular structure was analyzed in rats of eSS and eSMT lines, that showed different expression of their diabetic syndrome. In 261 animals, genetic line, sex, and weight (TW) were recorded. Absolute (AW) and relative weight (RW) of pancreas, the amount of small (SPI) and big pancreatic islets (BPI) per microscopic field and insular area (IA) were registered, as well as presence of round (R), polycyclic (P) or star-shaped (SS) islets, fibrosis (F), lymphocytic infiltrates (LI), nesidioblastosis (N) and hemosiderosis (H). An analysis in main components was carried out and a typology of individuals was constructed considering the coordinates of such in the factorial axes, interpreting the classes based on the original variables. The most characteristic modalities were hierarchized by means of value tests; the averages by classes of the continuous variables were compared with the general average. The statistical processing took place with software SPAD version 5,41, 2000. A partition in three clusters was defined. CLASS 1: contained mainly eSMT animals, of lower age with R or P islets, without H, with higher BI and IA and lower RW and TW; CLASS 2: mostly older eSS animals, with R islets, N or H and higher BPI and IA; CLASS 3: integrated by older males with no P, SS nor R islets, higher TW and RW, minor amount of SPI, BPI and IA. This configuration allowed to distinguish differences between lines, stages of the diabetic syndrome, as well as age and sex.

16. MORPHOLOGY OF ISLETS OF LANGERHANS IN TWO LINES OF SPONTANEOUSLY DIABETIC RATS: eSS AND eSMT

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The eSS rat is a model of type 2 diabetes of moderate intensity, showing early glucid intolerance that progresses with age; eSMT rat present overweight and earlier onset of hyperglycemia. Both lines show an age-dependent reduction of insular number and size. The aim of this work is to study their morphological modifications. Islets with neat borders, round and oval, were considered well preserved; abnormal shapes included those with polycyclic contours, star-shaped and disrupted islets. 144 eSS and 132 eSMT rats of both sexes from 3 to 26 months of age and 8 eumetabolic Wistar males of 12 months were studied. The majority of the animals, with no significant differences between sexes, had, at least, some well-preserved islets. Abnormal shapes were detected mostly in males of over 1 year, especially in eSMT. Islets with polycyclic contours represented the most frequent feature in eSS as well eSMT, with no differences between males and females. However, there were variations with age; this was observed in 10 out of 11 6-months-old eSMT females. Star-shaped islets were scarce. The presence of disrupted islets was similar in both sexes, but the frequency in eSMT males was higher than in eSS females. Insular lesions were more marked and appeared earlier in eSMT animals; the lapse from contour distortion to insular disruption was shorter. Islets in Wistar controls were morphologically undamaged. Insular modifications in rats of these two diabetic lines may be associated with the course of their metabolic alterations.

17. WEIGHT AND INTAKE IN DIABETIC MURINE MODELS. AGE, GENDER, LINE AND DIET EFFECTS

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eSS and eSMT rats, two spontaneously diabetes models, could be appropriate for the study of genotype-internal (gender and age) and external (diet) environment interactions. 10 animals of each genotype and gender, 4 and 9 months of age, were used to characterize the *ad libitum* ingestion of a diet (D) with the same components but with different physical form from the previously used (A). Biomass and *ad libitum* ingestion were measured, calculating the average weight (W), the absolute and relative daily average ingestion (DAI and DAIR). ANOVA showed significant effects of each one of the four factors (diet, line, gender and age) in the three variables, with the exception of line in DAI. Out of the 6 interactions of two factors, 5 were significant in W and 4 in DAI and DAIR; out of the 4 of three-factors, 2 had statistical significance in W and DAI and 3 in DAIR, whereas the quadruple interaction only in DAI. These results indicate that with diet A, males obtain higher W, even though with diet D the difference between ages is higher. DAI is similar with diet A in eSMT, whereas with diet D DAI is smaller in females. Inversely, eSS females reduce the intake of diet A at 9 months, but males increase it. In both genders diet D intake increases with age. Females show higher DAIR than 4-months-old males with A, and at 9 months with D. Intake of both diets and weight increase with age in both genders, except in eSMT males. These animals achieve a biomass increase with similar intake of diet D, evidencing their greater efficiency of food conversion. The difference in the physical form and/or in the quality of some components of diet D affects the adult biomass of both murine models.

18. ANTIMICROBIAL SUSCEPTIBILITY OF NASOPHARYNGEAL *Streptococcus pneumoniae* ISOLATES RECOVERED FROM HEALTHY CHILDREN IN ROSARIO

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Pneumococci are a part of the normal microbial flora of the nose and pharynx, particularly among young children, and are easily transmitted, usually by droplet secretions, from person to person. Resistance of microbes became a major concern at the end of the last century. The aim of this study was to assess resistance rates of *Streptococcus pneumoniae* to antimicrobial agents. Between 2004 and 2005 in a healthy children community in the Hospital de Niños Víctor. J. Vilela in Rosario, 48 *Streptococcus pneumoniae* strains were isolated from the nasopharynxes of 130 children. The antibiotics tested were erythromycin, cotrimoxazole, rifampicin, chloramphenicol, tetracycline, vancomycin, clindamicine, oxaciline, ciprofloxacin. Susceptibility to these drugs was determined by disk diffusion (NCCLS). In oxaciline resistant clones, minimum inhibitory concentration (MIC) was estimated. (n:16)=33,3% of 48 isolates were sensitive to all drugs. (n:17)= 35,4% were resistant to one drug. Multidrug resistance was detected in (n:6)=12,5% of the isolates. The highest resistance rate observed was to trimethoprim-sulfamethoxazole. All strains were sensitive to vancomycin. The carriage of resistant *Streptococcus pneumoniae* in healthy children, is an important risk factor for invasive diseases.

19. NASOPHARYNGEAL CARRIAGE OF *Streptococcus pneumoniae* BY HEALTHY CHILDREN IN ROSARIO, ARGENTINA

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Streptococcus pneumoniae frequently colonizes the upper respiratory tract asymptotically; particularly children's nasopharynx. It's leading bacterial cause of paediatrics infection (common to serious invasive infections). Carriage can be influenced by age, immune state, seasonal variation, antibiotic therapy, cohabiting with other children, respiratory diseases, hospitalizations, and other factors. It was evaluated the prevalence and potential risk factors of pneumococcal carriage in healthy children aged 0-10 years. Nasopharyngeal cultures and information about risk factors were obtained from 130 infants at Hospital de Niños Víctor J. Vilela's vaccination centre. (n:68)=52,3% samples were collected from children <2 years and (n:62)=47,7% from 2-10 years old children. The carriers prevalence was (n:48)=36,9% ; (n:22)=45,8% of them were <2 years and (n:26)=54,2% were 2-10 years old children. Among carriers, (n:9)=18,7% attended day-care centers, (n:30)=62,5% had upper respiratory infections antecedent, (n:1)=2,1% had meningitis antecedent, (n:11)=22,9% had been previously hospitalized and (n:4)=8,33% received pneumococcal vaccines. Nasopharyngeal pneumococcal carriage was associated with: cohabiting with <5 years old children (Odds Ratio [OR]: 2,7; p= 0,008), antibiotic therapy in last 6 months (OR: 1,3; p>0,05), acute otitis antecedent (OR: 1,5; p>0,05) and pneumonia antecedent (OR: 2,5; p=0,09). Conclusions: The high rate of pneumococcal carriers in Rosario's healthy children population was mostly influenced by cohabiting with <5 years old children and pneumonia antecedent.

20. STUDY ON THE DISTRIBUTION AND IRRIGATION OF THE AXILLARY ARTERY AND THEIR COLLATERAL BRANCHES

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It is considered of importance, all the vascular patterns knowledge of the axillary artery, and its collateral branches to prevent surgery damage in different invasive maneuvers during the arrival to the axillary region. This is important for the vital continuity of myocutaneous tags, and it also provides arguments of interest for different medical specialties as Image Diagnosis, Vascular Surgery and Reconstructive myocutaneous, between others. A descriptive anatomical analysis has been carried out, by studying 10 foetus cadavers preserved with formalin according to MAR II technique, and 4 foetus cadavers using the same method, but fresh channelled and then injected with Latex RV 620, red coloured. In this analysis, the number of collateral branches, birth order, the face of the origin, the irrigation zone, the external diameter and length of all the vessels, were told in detail. After analysing the results obtained in this work, the following discoveries are outstanding: variety in the birth order of the collateral branches of the analysed axillary artery; differences in the external diameter and the length of the collateral vessels were observed, and variations on the irrigation zone. Several branches presented truncus in common. The most frequently founded, according to the classic bibliography, was the circumflex arteries, anterior and posterior. Although, the associations given by a truncus in common were extremely different, among other discoveries.

21. LEVOSULPIRIDA: ANTIDOPAMINERGIC AND NON-SEROTONINERGIC EFFECT ON AGGRAVATED GASTRIC LESIONS INDUCED BY ETHANOL AND STRESS IN RATS
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Levosulpirida (LSUL) is a D2 dopaminergic selective antagonist and 5HT4 serotonergic. Objective: to study LSUL as gastric mucosa protector against ethanol (ETOH) and stress injury, since as an antidopaminergic agent is aggressive and as serotonergic is gastric protector. Randomized Wistar rat groups (n=7), 200 gr, 24 hr fasting, provided with water ad-libitum, were submitted to the following experiments: **I.** 1) 1ml saline orogastric gavage, in bolus, 60 min wait. 2) 1ml 96% ETOH, 60 min wait. 3) 10 mg/kg OG LSUL, 60 min and then ETOH. 4) 20 mg/kg LSUL and then ETOH. **II.** 1) 1 ml saline, OG and stress by immobilization and water immersion at 18°C during 6 hr, 2) 10 mg/kg OG LSUL, 60 min, then Stress. 3) 20 mg/kg OG LSUL, 60 min, then stress. The rats were sacrificed by ether overdose. Laparotomy and gastrectomy were performed, and percentage (%) of macroscopic gastric lesion area was assessed by planimetry. Tissue samples were obtained for histological examination (H-E). Statistics: Student's t test and ANOVA. Results: **I.** gastric lesion area %: 1) 1.0 ± 0.1 , 2) 35 ± 5 , 3) 57 ± 7 (<0.02). 4) 61 ± 8 (<0.01). **II.** 1) 65.1 ± 7 , 2) 80 ± 6 (<0.01), 3) 90 ± 7 (<0.01). Conclusion: LSUL is a gastric mucosa aggressive agent in presence of ETOH and stress due to its predominant antidopaminergic mechanism.

22. SOCIAL SUPPORT, HOPELESSNESS AND FEARS IN ADULT PATIENTS WITH CONNECTIVE TISSUE DISEASES

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The present work attempts to evaluate the search of social support in patients with connective tissues diseases, identifying the relationship between control and hopelessness, analyzing coping strategies utilized and assessing main fears. Adult patients (n=33), males and females, attending the Dermatology Service in a Public Hospital (Rosario), aged 39.8 ± 15.2 yr were recruited. Time since diagnosis: 9.3 yr. Collecting data instrument applied: hopelessness index in arthritis (Nicassio 1985), modify for the present work, list of coping strategies (Lazarus and Folkman, 1984) and a questionnaire constructed for the current research, in order to explore patients fears and worries. Results show that most patients search actively social support both from the instrumental and informative point of view and none from the emotional one. There is no evidence of correlation between internal locus and hopelessness ($r=0.13$). They use frequently close up coping strategies to solve the problem and less frequently avoiding or negative ones. No relevant differences were observed from symptoms onset up to now. Most of them are afraid of disease progression, pain, disability or image worsening. In many of them is present fear to be abandoned by their close relatives and friends, which might explain the lack of emotional support search on them.

23. M CELLS IN CECAL PATCHES FROM OVALBUMIN SENSITIZED RABBITS

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Cecal patch is one of the great immuno-inductive sites in rabbit gut, and they are covered by a simple epithelium where M (membranous) cells are found. In rabbits, Vimentin is a reliable marker of M cells and allow us to classify M cells into two morphological categories: mature ones with membranous pockets full of leukocytes or immature ones lacking basolateral infoldings. In previous studies we demonstrated that sensitization induces morphological changes. The aim of the present study was to determine if the subcutaneous sensitization produces quantitative modifications in the M cells populations and associate these changes to the histopathological status. Rabbits were divided into two groups (G). G1: normal control and G2: ovalbumin subcutaneously sensitized. In each animal cecal patches were immunostained with Vimentin, and mature M cells/immature M cells were counted. Results were referred as the arithmetic mean \pm standard error of cells per dome. Results showed for mature M cells $6,33 \pm 0,92$ in G1, and $15,5 \pm 1,29$ in G2 ($p < 0,001$). Immature M cells: $5,5 \pm 0,22$ in G1 and $6,27 \pm 1,03$ in G2 (ns). The total M cell number was $11,5 \pm 0,77$ in G1 and $22,44 \pm 2,22$ in G2 ($p < 0,001$). Cecal patches from G2 presented oedema, lymphangiectasias and eosinophils leukocyte infiltration. We conclude that OVA sensitization induces inflammatory changes, associated with an increase in M cell number, specially in the mature population, with lymphocytes inside membranous pockets. We can assume the hypothesis, that the more mature M cells, the more macromolecular traffic, to induce and immune response rich in IgA.

24. STUDY OF THE VASCULAR ACCESES OF THE PISIFORM BONE (Os pisiforme)

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The aim of this work is the get the best of the knowledge of the vascularization of the pisiform bone (os pisiforme), taking into consideration the quantity and the distribution of the vascular acces, expressing its results whit its arithmetic media and standard desviation. The pisiform is a bone of the first line of the carpo, it presents four faces and two extremes, the from face, the external face, the internal face, the superior extremes and inferior extremes. The back face as it is an articular area it does not present vascular acces. We used seventeen bones of both hands taken from adults carpes whitout any specific killness and whitout knowingh the sexes, the bones here macerated and later on the fatness was taken with tricoloroetileno. For the estudy the bone a stereoscopic lens was used. The lens has got form de six to forty widerness of Riechert make. We count the vascular acces taking into consideration each face: external face $3,29 \pm 3,70$; internal face $2,64 \pm 3,84$; inferior extremes $2,17 \pm 2,55$; from face $1,11 \pm 1,61$; superior extremes $0,88 \pm 2,17$. Acording with the analysis of the results we get to the conclusion that the external face presents the greader number off vascular acces.

25.

SODIUM THIOPENTAL INTRAPERITONEAL SUDDEN EFFECT OVER BLOOD VISCOSITY AT SPRAGUE DAWLEY RATS

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It has been a fact that in vitro Sodium Thiopental interacts with double shaped lipid red cells membrane enhancing his deformability.

On blood viscosity of Sprague Dawley rats it was attempted to know the immediate effect of a single Intraperitoneal shot of Sodium Thiopental (2.5%) (IST).

It had been used adult male Sprague Dawley rats divided in Group A (GA) (n=8) IST (0.2ml/100g. weight) and Group B (GB) (n=8) (0.2ml/100g. weight) 0.9% Intraperitoneal Sodium Chloride. Blood had been treated with EDTA becoming not clotted and viscosity measured with viscosimeter conical/plated at 46(a) 115(b) 230 (c) shear rates per minute, corrected at 37°C to 45% hematocrite. Results had been expressed as mean values of Viscosity (mPa.s) and were analyzed with T Student's test.

GA a 5.54 vs. GB a 6.34 p=0.0253; GA b 4.5 vs. GB b 4.85 p=0.0345; GA c 4.25 vs. GB c 4.17 p= 0.5738.

According with the analysis of results we can conclude that Sodium Thiopental Intraperitoneal Via, diminish significantly blood viscosity at 46 and 115 shear rates per minute, not being noticed differences at 230 shear rate per minute. After this results it may be infer that Sodium Thiopental diminish predominantly interaction in between red cells.

26.

TESTICULAR HISTOLOGY IN RATS INJECTED WITH ALLOXAN AFTER WEANING

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A single dose of an alloxan injection could be followed by different levels of hyperglycemia or death. We have already reported that wíeh high hyperglycemia (more than 300mg/dl) changes in testicular histology are observed. We report changes in testicular histology due to the effects of moderated hyperglycemia after a single dose of alloxan. 10 male rats, ageing 25 days old were separated in: 1.- 6 rats injected with alloxan (A) (24mg/100gBW), only 4 survived. 2.- 4 rats injected with distilled water (C)(1cc/100gBW). After 48 hs and at autopsy glycemia was determined. At autopsy (40 days old) alloxan injected animals' glicemia was 186.67 ± 0.45 mg/dl. Testes were dissected out and fixed in Carnoy's fluid. Specimens were processed with routine histological techniques. Slices were stained with H-E, Giemsa, PAS. As results we could report: 1.- Body weight: (A) 119.33 ± 11.56 g < (C): 172.25 ± 6.57 g (P<0.05); 2.- Testicular weights: (A): 1.15 ± 0.03 g/100 g BW < (C): 1.44 ± 0.09 g/100 g BW (P<0.05) 2.- Histologically, at this age in tubular testes, germinal epithelia reach up spermatids, also, some few spermatozoa could be described. Some tubular lumen is filled up with spermatocytes and spermatids. In testes from alloxan injected rats no differences could be seen. In this model testicular histology of rarts with moderated hyperglycemia show no differences with controls'. This fact differed from tstes of rats with higher hyperglycemia. These findings suggest that testicular histology depends upon hyperglycemia levels.

27.

TEACHING HISTOLOGY SOFTWARE

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Experts Workshop admits worldwide the importance of a collective effort in methodology and strategies update to encourage the practical learning and promotion of self learning in pre-graduate medicine training. The present investigation focus on an interactive method attempting to reproduce Histology problems, hence designing a software of simulated histology problems for first year students of medicine. Methodology: software design considering the mechanics of a creative process with two aims: a) knowledge issues to transfer, and b) tools for presentation. The software is characterized by realism, friendly performance and low-cost, with a flexible structure that enables reception and display of histological slides in shortest time. The software was applied to four cohorts of first year students of Medicine (2002-2005), evaluating their performance regarding the problem. The student performance was evaluated through a problem, and information was simultaneously provided to aid self learning. Conclusion: A support software in teaching activity, simulating problems, that could be used by people without previous experience as PC users, enabling interaction, and student evaluation regarding problems and information in self learning tasks and, feedback and motivating training in the process of evaluation.

28.

SIALOPROTEINS CONTENT IN BONE MATRIX OF RATS OF DIFFERENT AGE

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Its known that when mammalian bone is being remodeled, resorption is followed by deposition of new bone on either old bone or calcified cartilage. The calcification process is always associated with the deposition of several organics, non collagenous, compounds which enhance the mineral deposition being sialoproteins the principal ones in this process. In order to investigate whether the amount of sialoproteins present in the matrix is related to bone growth, sialic acid associated to protein was measured in groups of different age animals. Twenty-five male rats, IIM/FcM subline m, were divided into 4 groups: G₁: 10 rats; G₂: 6 rats; G₃: 6 rats and G₄: 3 rats (31, 85, 180 and 270 days old respect). Powered bone (femur) from these animals was processed in order to isolate sialoproteins. As these proteins represent less than 1%o of dried bone weight and the method sensibility for sialic acid determination is as low as 20 ug/ml, pools of powered bone from the animals of each group should be processed. Each pool was extracted with guanidine clorhidrate (pH 5.8), supernatant run down and sediment extracted with EDTA (0.25 M, pH 7.5). The EDTA-extracts were the samples analysed for sialic acid. Diminished values were issued as the age of the animals increase till they reach adulthood. The following results were obtained: Sialic ac.mg/g dried bone: G₁: 2.9; G₂:1.5; G₃:0.94;G₄: 0.92. Statistic analysis were made by the Spearman coefficient and a value for r_s = -1, p< 0.05 were obtained. These results express an inverse relation between age and amount of sialoproteins in bone matrix. It was assumed that the decrease in sialoproteins concentration may be linked to the decreased osteogenesis activity.

29. INTESTINAL ALKALINE PHOSPHATASE (IAP) ACTIVITY AND KINETIC OF INTESTINAL CALCIUM (Ca) ABSORPTION IN RATS

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Both expression of IAP and intestinal Ca absorption decrease from duodenum to jejunum. The role of IAP on Ca absorption is partially known, but its activity is Ca-dependent. The objective of this work is to investigate the kinetic of duodenal Ca absorption and expression of IAP in a model of *in situ* isolated duodenum, in rats. Duodenum was isolated by one ligature in the pylorous and another 4 cm in the caudal direction, where a catheter was inserted to instill 2 ml of the following solutions: Treated (n=4): glucose 160 mM, CaCl₂ 50 mM, 4 μ Ci de ⁴⁵Ca⁺⁺, Tris 25 mM, pH 7, Controls (n=4) the same buffer without calcium. The catheter was also used to obtain samples of lumen at the beginning and every 10 min. Luminal IAP activity was measured with p-nitrophenylphosphate and amount of protein by Dot Blot. At times mentioned above duodenum was biopsied for histochemical assessment of brush border IAP. Ca absorption was measured by ⁴⁵Ca⁺⁺ decrease in the luminal solution. Ca concentration was fitted by a biexponential function (r= 0.903). When [Ca⁺⁺] > 25 mM, t_{1/2} = 4.7 min and when [Ca⁺⁺] < 25 mM, t_{1/2} = 254 min. Amount of IAP in brush border of controls decreased, simultaneously with an increase in activity and amount of IAP in lumen. In treated, the phenomenon was observed when [Ca⁺⁺] decreased below 25 mM, without significant increase in luminal activity.

Conclusions: 1- Brush border retention of IAP is Ca dependent. 2- High expression of IAP in brush border is associated with fast Ca transport. These results indicate that IAP would participate in fast Ca transport.

30. MEASUREMENT OF IONIC (F), ACID-LABILE (FAL) AND ACID-RESISTANT (FAR) FLUORINE

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Potentiometry of F is only possible in aqueous samples at levels greater than 0.5 μ M. After a dose of monofluorophosphate (MFP) there are non ionic fluorine, undetectable with the fluoride selective electrode (FSE). Total fluorine (FT) of a sample is the sum of F-, FAL and FAR. Both FAL and FAR are not directly detected with FSE. FAL distills as F from compounds by treatment with strong acids, and FAR distills as F after incineration and treatment with strong acids. The aim of this work was to develop a technique to measure potentiometrically F, FAL and FAR.

F measurement (F): with the FSE directly on the sample with the addition of 10% V/V of buffer pH 5.5 to adjust ionic strength and to complex trivalent metals. Measurement of F post-isothermic distillation (μ DI): 100 μ l of sample are mixed with 100 μ l H₂SO₄ in a 1.5-ml tube with a microtube containing NaOH, capped and maintained at 37°C for 6 days. F and FAL distillates from the sample to the alkali solution, where F is measured with the FSE. FT measurement post-incineration and isothermic distillation (FT): 100 μ l of sample are absorbed on CaSO₄, incinerated at 500°C for 6 hs, and subjected to μ DI.

Calculations: FAL = μ DI - F, FAR = FT - FAL.

Calibration curves with aqueous NaF were performed. Nernst equation fitted significantly for the different forms of fluorine (p < 0.05). F: r = 0.98, μ DI: r = 0.94 and FT: r = 0.91. As an example, this methodology was applied to bone from rats treated with MFP, F = 237 ± 25, FAL = 64 ± 11 and FAR = 770 ± 37 nmoles.

Conclusion: This methodology allows measurement of fluorine not detected directly with the electrode in biological samples.

31. ANTITUMORAL EFFECT OF CRONIC ORAL ADMINISTRATION OF CYCLOPHOSPHAMIDE (CY) IN MAMMARY ADENOCARCINOMA-BEARING MICE

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The administration of Cy in high doses and repeated cycles induces reduction of tumor mass along with renal, cardiac and/or bone toxicities. We have previously demonstrated that metronomic chemotherapy (chronic administration of low doses without extended rest periods) with Cy have a strong antitumoral effect on rat lymphoma and sarcoma and is devoid of toxicity. Our aim was to study the therapeutic activity and the toxicity of metronomic therapy with Cy on M-406 mouse mammary adenocarcinoma. Inbred CBi mice were s.c. challenged with M-406 (Day 0) and distributed in three groups on day 10: I) Controls and II) and III) Treated: which received \approx 20 and 30 mg/kg/day, respectively, Cy in the drinking water. Tumor size in group I was higher than in group III on days 13 and 17 (p < 0.05), 27 and 31 (p < 0.01). The survival increased in group III compared to group I (Kaplan-Meier). No body weight losses were observed in the treated groups. Similar results were obtained with other murine mammary adenocarcinoma. The mechanism of action was evaluated with immunohistochemistry for the vascular marker CD34. The antitumoral effect and the lack of toxicity of metronomic therapy with Cy p.o. suggest its future application as a non-invasive intervention strategy in the treatment of human tumors which could be potentiated by combination with antiangiogenic drugs.

32. KINETICS OF GALECTIN-1 (GAL-1) EXPRESSION AND ITS RELATIONSHIP WITH THE IMMUNE RESPONSE IN MURINE TUMOR MODELS

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We have previously demonstrated that Gal-1 expression increases during the progression of rat lymphoma L-TACB and that it would participate in the tumor escape from the immune response (IR). Our aim was to study the kinetics of Gal-1 expression in a rat sarcoma (S-E100) and a mouse mammary adenocarcinoma (M-234p) and to compare to that obtained for L-TACB. Inbred *m* rats and Balb/c mice were challenged s.c. with S-E100 and M-234p, respectively (Day 0). Animals were sacrificed periodically and 1^{ary} tumors and spleens were excised. Protein homogenates were obtained and Gal-1 expression was determined by Western Blot. No significant modifications of Gal-1 expression were obtained during S-E100 growth. Spleen cells showed a lower expression of Gal-1 on days 14 and 21 than on day 0 (p < 0.05). During M-234p growth the level of Gal-1 was lower on day 30 than on day 10 (p < 0.05), while no modifications were observed in spleen cells. Considering that Gal-1 is overexpressed in L-TACB in order to abolish the IR, in the case of S-E100, a tumor with very low immunogenicity, there would be no need to modulate Gal-1 expression because it does not exist the threat of an immune attack. On the other hand, the decrease of Gal-1 in M-234p could be explained by the phenomenon of tumor immunoestimulation, as happens in certain mammary human tumors. Gal-1 would serve as a potentiometer in a servomechanism with the tumor as the motor and the IR as the actuator arm.

33.

PHAGOCYTOSIS IN PERITONEAL AND ALVEOLAR MACROPHAGES OF *Toxocara canis* INFECTED BALB/c MICEEchenique C¹, Tosello M², Biasoli M², Luque A², Magaró H¹.¹Área Parasitología. Dto. Microbiología. ²CEREMIC. Fac. de Cs. Bioq. y Farm. UNR. E-mail: cgechenique@yahoo.com.ar

The phagocytosis and the microbial death are important functions of the accessory cells, needed to control protozoos, bacterias and fungi. In helminths, the action of parasitological secreted products, modify the functions of accessory cells. *Toxocara canis* larva produces secretion-excretion antigen (TES). The objective of this work was to evaluate the phagocytosis (F) of alveolar (MA) and peritoneal macrophages (MP) of *T. canis* infected BALB/c mice, with yeasts: *Candida albicans* (CA) and *Candida tropicalis* (CT). Adult BALB/c mice infected orally with 1000 embryonated eggs of *T. canis* and necropsied 5 animals for each group at 7, 14 and 28 days post infection (dpi). MP and MA were cultivated at 37°C and 5% of CO₂ during 24 hs. A suspension of 5 x 10⁶ yeasts was added: collection CEREMIC CA(CCC:112/2000) and CT (CCC:121/2000), opsonised with normal mouse serum. MP, MA with yeasts were incubated 37°C in 5% of CO₂ during 60 minutes. The % of phagocytated yeasts was determined: MP+CA without TES: 40 ± 2.1; with 100 µg/ml of TES, 7dpi: 31 ± 1.3; 14 dpi: 26 ± 0.8; 28 dpi: 19 ± 0.3. The % phagocytated yeast in MP+CT without TES: 46 ± 3.2; with TES, 7 dpi: 30 ± 1.1; 14 dpi: 21 ± 0.8; 28 days: 18 ± 0.7. The % in MA+CA without TES: 43 ± 0.7; with TES, 7 dpi: 35 ± 0.7; 14 dpi: 22 ± 0.5; 28 dpi: 18 ± 0.2. In MA+CT without TES: 38 ± 1.3; with TES, 7 dpi: 29 ± 0.5; 14 dpi: 23 ± 1.1; 28 dpi: 17 ± 0.3. We observed a reduced F of accessory cells could be due to *T. canis* infection and to the action of TES.

34.

DETECTION OF *Toxocara canis* LARVAE IN BALB/c MICE BY ARTIFICIAL DIGESTION, HISTOLOGICAL STUDIES AND PCREchenique C¹, Nocito A², Serra E¹, Magaró H¹¹Área Parasitología. Dpto. Microbiología. Fac. de Cs. Bioq. y Farm. UNR. ²Anatomía Patológica. Fac. de Cs. Médicas. UNR. E-mail: cgechenique@yahoo.com.ar

Murine model is adecuated to study *Toxocara canis* infection in the man. In paratenic hosts, *T. canis* larvae reach the liver and the lungs (hepato-pulmonary phase), then migrate around the body and they accumulate in the carcass and brain (myo-tropic-neurotropic phase). The objective of this work was to detect *T. canis* larvae in liver, lung, brain, heart and kidney of BALB/c mice, by histological studies (EH), artificial digestion (DA) and PCR. Adult males BALB/c mice (n=60) were infected orally with 1000 embryonated eggs of *T. canis*. By EH eosin hematoxylin stain were made in liver, heart, lungs, kidney and brain of control and infected mice in 7, 14, 21, 28, 40 and 60 days post infection (dpi). By DA the organs were exposed in a pepsina treatment and incubated at 37°C, 18hs in constant agitation, then centrifuged and observed in the microscope. To purify tissue DNA and larvae DNA (positive culture of 50000 larvae) a treatment with proteinase K was carried out during 8 hours to 60°C, followed by extractions with fenol, chloroform, isomalic alcohol and then with sodium acetate and absolute ethanol. For PCR we used ITS1 and ITS2 like species-specific markers for *T. canis* genetic identification. Amplification conditions were: 5 min at 94°C, 35 cycles for 1 min at 94°C, 1 min at 63.3°C and 1 min at 72°C, a final elongation step of 5 min at 72°C. PCR products analyzed by electrophoresis in agarose gels. By HE we found larvae in liver (7 dpi). By DA larvae were found in liver (7 dpi), lung (14 dpi) and brain (21dpi). By PCR, *T. canis* larva DNA was detected in all organs and is the elected technique.

35.

IMPLEMENTATION OF A TECHNIQUE FOR THE EXTRACTION OF RAT BONE MARROWSgro MV¹, Alet N², Blanco ML², Gomez JA¹, Passerini F¹, Culasso M¹, Navoni S³, Eichhorn MC³, Caraballo E del C³.¹Instituto de Cirugía Experimental "Dr J.J. Boretti" Hosp. Esc. Eva Perón, Fac. Cs Médicas, UNR; ²Cát. Biología Fac. Cs. Médicas; ³Servicio Anatomía Patológica, Hosp. Esc. Eva Perón.

This study, part of a larger one, looks for a technique implementation to obtain bone marrow by tibia puncture in obese and diabetic β rats. Twenty 250 days male rats were anaesthetized with sodic thiopental (20-40 mg/kg) through intraperitoneal passage, and ether through inhalatory passage. Nalbufina was used as analgesic and Cefalotina as prophylactic antibiotic. One mm hole was made in tibia proximal edge, 0,3 ml physiologic solution was instilled and aspirated.

Microscope preparations were made. Samples were considered:

representative: with mature hematopoietic tissue "represented" by three blood lineage cellular elements,adequate: only if at least 70% surface was integrated by hematopoietic tissue with three blood series without limitation for observation.

Twenty samples in all were evaluated resulting in: 11(eleven) representative and adequate, and 9(nine) neither representative nor adequate.

Results revealed positive achievements: -1) the obtaining of hematopoietic material though the rat bone reduced dimension,

-2) the animal survival after surgery. However further studies will be necessary to improve this technique as 45% of samples have been no representative.

36.

SUSCEPTIBILITY TO NATURAL INFECTION WITH INTESTINAL FLAGELLATES IN YOUNG MICE OF THE CBI STOCKVasconi MD², Font MT^{1,3}, Di Masso RJ^{1,3}, Hinrichsen LI^{1,3}.¹Instituto de Genética Experimental, Facultad de Cs. Médicas; ²Cátedra de Parasitología, Facultad de Cs. Bioquímicas y Farmacéuticas; ³CIC-UNR; Universidad Nacional de Rosario.

Study of host susceptibility to parasitic infections is important to understand the host-parasite relationship, particularly the way in which the host genetic constitution allows the parasite to establish. Previously, we reported differences due to genotype in the quantity and diversity of natural enteroparasitosis in adult mice of 4 lines of the CBI stock of the Instituto de Genética Experimental. To determine whether age influences genetic susceptibility to intestinal protozoa infection, young mice of the CBI-IGE stock were examined. Thirty days old mice from lines CBI+ (+), CBI- (-), CBI/L (L), CBI/C (C) and CBI were sacrificed, their intestine was excised and its content microscopically examined. Parasite load was quantified by crosses (1-5+). Genotype and sex effects on the proportion of positive mice were evaluated with the χ^2 test; quantitative variables were analyzed with the Kruskal-Wallis test. The flagellates *Trichomonas muris* and *Spiroplasma muris* were found. High *T. muris* loads were observed in all (C) mice and in a small proportion of (+) males and females and CBI males (p<0.01); these protozoa were not observed in the other lines (p<0.001). All (-) and CBI mice showed a high *S. muris* burden, differing from (+), (L) y (C) which had a smaller proportion of positive mice (p<0.01). These results suggest that genetic differences in the host-parasite relationship are expressed early in this model. Genotype (L) was the more resistant, as already demonstrated in natural and experimental infections in adult mice.

37. ALKALINE PHOSPHATASE (ALP) ACTIVITY IN LONG BONES OF MICE WITH DIFFERENT POST-INFLECTION EXPONENTIAL GROWTH RATE

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Alkaline phosphatase plays an important role in the formation and mineralization of the osteoid; its level of activity reflects an individual's current bone remodelling. Tibial morphometry and osteogenic potential, estimated by ALP activity, were studied in adult mice of both sexes belonging to a F₂ segregating population obtained by crossing two lines with different body conformation and long bone morphometry (CBI/L and CBI/C). Mice were selected from each tail of the frequency distribution of the exponential post-inflection growth rate (tail I: low rate; tail II: high rate). Body weight (BW, g), tibia absolute (TW, mg) and relative weight (TWr, mg/g) and length (TL, mm), and absolute (ALPa, mU) and relative (ALPr, mU/mg) ALP activity in tibia extracts, were measured at 150 days of age. Comparisons between tails, within sex, were done with Student's t test. All variables showed sexual dimorphism. BW only differed between females (0±SE) (I: 42±1.1; II: 37±0.7; p<0.0005). TW and TL were similar between tails, within sex; TWr was higher in tail II in both sexes (%I: 112±2.3, %II: 119±2.3, p<0.05; &I: 115±2.2, 132±3.5, p<0.0001). ALPa and ALPr did not differ in males while females I had higher means than II (ALPa, I: 304±34.4, II: 148±16.4; ALPr, I: 6.3±0.68, II: 3.1±0.39; p<0.01). The high ALP activity observed in the heavier &I mice suggests an actual bone remodelling, since tibial morphometry was similar to that of &II. The larger adipose tissue deposit in &I, resulting in a higher estrogenic activity, could partly explain this differential behavior in ALP activity.

38. SKIN BASAL CELL CARCINOMA AND SQUAMOUS CELL CARCINOMA RISK FACTORS. PRELIMINARY RESULTS

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Skin basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common cancers. We carried out a case-control study to evaluate risk factors for BCC and SCC. Cases were both sexes individuals with clinical and histologic diagnosis. Controls were patients that attended the same institutions for other pathologies. Age, gender, education, skin sensitivity to sun exposure, sunburns history, skin cancer positive family history, instruction level, and occupational history were obtained applying a standardized questionnaire. A full-body skin examination was done to detect naevi and actinic keratosis. A total of 16 cases and 34 controls (mean age 67,8 ± 13,1 and 64,1 ± 12,3), were studied. Twenty-one tumors were diagnosed, 19 in face and 2 in trunk. Histologic diagnosis were BCC in 17 and SCC in 4 cases. When analyzing skin sensitivity to sun exposure, reaction was type 1 in 69% of cases and 27% of controls; type 2 in 19% and 39%, type 3 in 12.5% and 21% and type 4 in 0% and 12%, respectively (p=0.04). The proportion of patients with intermittent sun exposure was higher in cases than in controls (p=0.02). Forty percent of cases and 34% of controls had sunburns antecedents. When analyzing full-body skin examination, SCC or BCC were found to be more common in patients with 10 or more naevi (OR=19; p=0.008), and in those with actinic keratosis (OR=3,6; p=0.04). Risk of developing SCC or BCC was also higher in the more educated group (p=0,07), and in those with a positive skin cancer family history (p=0.08). These preliminary results emphasize the need to identify patients at risk.

39. ANALYSIS OF BIOLOGICAL DATA WITH MISSING INFORMATION USING SPSS SOFTWARE

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The statistical analysis of data sets obtained from designed experiments and observational studies result inappropriate when missing information is present. It can lead to serious biases and the precision is related to the amount of missing data and to the characteristics of the missing data mechanism. Nowadays, this problem is considered crucial for the inference due to its permanent presence, and it has determined the flourishing of many statistical methods that intend to solve it. As the decision for their use should be done taking in account the pattern of the missing data, the kind and number of variables affected and the aim of the analysis, in many procedures the missingness is simulated according to the characteristics of the different mechanisms that could produce them. Many statistical software have used only the complete units, others allow for a previous treatment before using the classical methods. In this project we present a module for the analysis of data sets with missing information from SPSS software, using an application to a study of children and adolescents with diabetes from private and public hospitals of Rosario, Argentina. The missing data has been created in one of the variable in an approximate 25% of the units with two missing data mechanism: missing completely at random (MCAR) using a random seed restricted to the established percentage and not missing at random (NMAR) simulating the missingness in the higher values. The analysis has been done in each of the data sets obtained, with the purpose of visualize the incidence of the different mechanisms in the data structure and the estimates. The simulation procedure allows the analysis under different scenarios.

40. CORRELATION BETWEEN EXTERNAL GLOMERULAR DIAMETER AND WEIGHT IN REMAINING KIDNEY OF "L" LINE RATS UNDERGOING UNILATERAL NEPHRECTOMY

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In previous studies we demonstrated that the weight (W) and the external glomerular diameter (EGD) of the remaining kidney (RK) in "L" line rats (LLR) undergoing unilateral nephrectomy is different depending on sex and age. The aim of this work was to know the possible correlation between W and EGD of RK in LLR undergoing a nephrectomy depending on sex and age. We used 64 LLR classified according to: 1) Sex: 1a- Females (F) (n=32) and 1b- Males (M) (n=32); 2) Age: 2a- Young (Y) 50 days (n=32) and 2b- Adults (A) 85 days (n=32) and 3) Treatment: 3a- Nephrectomized (N) and 3b- Control (C). The animals of group N underwent a left nephrectomy. 45 days later the animals were euthanased and the right remaining kidney was removed. The animals of the group C were treated under the same conditions of group N. 45 days later the animals were euthanased and the right remaining kidney was removed. The kidneys were weighed with an electronic balance and fixed in neutral 10% formaldehyde solution. 5µm sections were obtained from the paraffin and stained with hematoxylin-eosin. The observations were performed with a microscope equipped with a Shimadzu eyepiece calibrated for µm and we measured the EGD at 100X going through the cortex counting in each kidney 10 glomeruli presenting vascular pole in the section shaped. We calculate the correlation coefficient between W and EGD (Spearman's test). The correlation is p=0,85. So, when the W increase, the EGD increase, too. When we check this correlation with the studied variables we observed similar results to the obtains without controlling.

41. CORTISOL AND DEHYDROEPIANDROSTERONE FAIL TO MODULATE THE INDUCTION OF APOPTOSIS IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM TUBERCULOSIS PATIENTS

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The immune response to *Mycobacterium tuberculosis* (*Mtb*) not only control infection but can also mediate the accompanying tissue injury. Within the context of Cortisol-Cort- and Dehydroepiandrosterone-DHEA-mediated immunoregulation we previously demonstrate that Cort inhibition of specific proliferation was more evident in advanced TB being not reverted by DHEA. To ascertain whether this inhibition was related to a rise in the apoptotic levels (Apo) of immunocompetent cells (PBMC), 10⁶ cells from adult TB patients (N=6) and healthy controls (HCo, n=7) were cultivated with whole sonicated *Mtb* (8µg/ml-Tso), Cort 10⁻⁶ M and/or DHEA (10⁻⁷, 10⁻⁸, 10⁻⁹ M) in RPMI media. Apoptotic cells were determined by flow cytometry (Coulter Epics), using Annexin V/ Propidium Iodide -AV/PI- (Bender MedSystems) and the incorporation of Propidium Iodide to measure the content of DNA (hipodiploid cells). The results were presented as % of apoptotic cells [median (rank)] AV/IP₂ cells with no stimulus -Basal- HCo:18,5(14,4-53,4), TB:26,1(13,5-29,6); cells stimulated with Tso -Tso- HCo:18,7(12,6-22,0), TB:28,4(24,8-29,8) p<0,01; Tso + Cort 10⁻⁶M, HCo: 21,3(16,3-28,3), TB:29,8(25,8-34,9) p<0,05; Tso + DHEA 10⁻⁷M, HCo:19,7(13,1-22,0), TB:27,7(25,1-30,0) p<0,01; Treatment with both hormones ej.: Tso + Cort 10⁻⁶M + DHEA 10⁻⁷M, HCo: 20,9(16,5-31,0), TB:28,5(26,3-33,2) p<0,05. Similar results were observed measuring the DNA content. *Mtb* stimulation induces higher levels of Apo in patient cells, this effect being not modulated by Cort and/or DHEA.

42. INTERLEUKIN-6 (IL-6), GROWTH HORMONE (GH) AND INSULIN LIKE GROWTH FACTOR-1 (IGF) LEVELS IN PATIENTS WITH LUNG TUBERCULOSIS

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The release of proinflammatory cytokines like IL6 during the immune response stimulates the neuroendocrine system to promote the activation of several axes, one of them, the hypothalamus-pituitary. With the release of GH and IGF. Beyond the metabolic effects, both hormones exert immunomodulatory activities. Given the remarkable systemic repercussion of Tuberculosis and the substantial immune response underlying disease development, we decide to study the systemic levels of this hormones and IL-6 (commercial immunoassays). Patients (n=37) with a recent diagnosis of TB (mean age 45.9 years, rank 23-65 years, 81% men) were classified into three groups: mild (Mi; n=11), moderate (Mo; n=13) and advanced (Ad; n=13). The control population was composed of 22 age- and sex-matched healthy volunteers (HCo). Results were expressed as mean ± SEM; **IL-6**, HCo=27.1±1.83 (p<0.0001, with respect to patients), Mi=113.4±31.8, Mo=162.4±38.8, Ad=244.4±42.8 (Mi vs. Ad, p<0.025); **GH**, HCo=2.71±1.64, Mi=5.07±1.93, Mo=9.8±3.19, Ad=6.5±1.64 (HCo vs. patients, p<0.001); **IGF-1**, HCo=53±5.18, Mi=71±14.4, Mo=52.3±4.7, Ad=46.8±3.5; **Insulin**, HCo= 25.09±2.54, Mi=18.9±5.9, Mo=24.7±4.42, Ad=28.3±7.42. The chronic inflammatory response during TB coexists with a significant rise of IL-6 and GH levels. The absence of a subsequently increment of insulin and IGF suggest a peripheral resistance state to the action of GH in this patients.

43. ANTIBIOTIC SENSITIVITY OF ISOLATED MICRO-ORGANISMS FROM KERATITIS AND ENDOFTALMITIS IN TWO PUBLIC HOSPITALS OF ROSARIO, ARGENTINA

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The Endoftalmitis and Keratitis are severe ocular infections caused by a series of microorganisms. Keratitis treatment is generally topical which allows concentrations that exceed the minimum bactericidal doses. In the case of Endoftalmitis the procedure is the intravitreo injection. In general the antibiotic are effective in the treatment of intraocular infections; nevertheless the microorganisms can develop resistance. The purpose of the present paper is to understand and communicate the antibiotic sensitivity of the isolated microorganisms in our hospitals. The antibiotic sensitivity was determined through diffusion discs according to the norms of the NCCLS.

We studied 41 isolations of samples from Hospital Provincial of Centenario and Hospital Provincial, both from the city of Rosario from July 2003 to July 2005. The percentage of sensitivity in gram positive coccus (n:25) was the following: OXA 76%, OFL 100%, CIP 100%, GEN 80%, ERY 76%, RIF 92%, VAN 100%.

The percentage of sensitivity in gram negative bacilli (n:16) were the following: CAZ 100%, CTX 100%, IMP 100%, OFX 100%, CIP 100%, GEN 87.5%, AKN 100% and NN 100%.

These results suggest that the sensitivity to the antibiotics frequently used is greater than in the microorganisms isolated from other clinical samples. These results are similar to other published results.

44. ASSOCIATION BETWEEN HIP FRACTURES AND RISK FACTORS FOR OSTEOPOROSIS: MULTIVARIATE ANALYSIS

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The goal of this study was to explore the association between **risk factors for osteoporosis (RF)** and hip fracture in hospitalized patients; to investigate the influence of RF related to low bone mass and to trauma; and to evaluate the existence of **protective factors (PF)**. In 376 inpatients (151 hip fracture cases and 225 controls), a case-control study was carried out. Data were obtained from a RF questionnaire and central medical records. Variables, related to low bone mass studied were: coffee, alcohol, and tobacco intake, sun exposure, past and family fracture history, physical activity, intake of dairy products, BMI, diabetes, chronic obstructive pulmonary disease, urolithiasis, hyperparathyroidism, connective tissue disorders, gastrectomy, chronic renal and liver diseases, hyperthyroidism, malabsorption syndrome, intestinal resection; in women, gynecological history was obtained. Those related to trauma: falls sustained during the previous year, visual and hearing impairment, neurological diseases, mechanical aid for ambulation, functional capacity parameters. Significant **RF** in the multivariate logistic regression analysis were: history of falls (OR: 1.68, CI: 1.34-2.12, p < 0.0001), cognitive impairment (OR: 1.74, CI:1.25-2.43, p = 0.001), and **PF**: Ca intake during young age (OR: 0.48, CI: 0.33-0.70, p < 0.0001), present Ca intake (OR: 0.41, CI: 0.27-0.62, p < 0.0001), and mechanical aid for walking (OR: 0.45, CI: 0.29-0.71, p < 0.0001). These results suggest that a careful evaluation of RF and PF could contribute to diminish the risk of hip fracture, through the change of some personal habits, and actions to decrease the risk of falls among old persons.

45. EFFECT OF TWO ANESTHETIC REGIMENS ON QTc INTERVAL DURING ANESTHETIC INDUCTION

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The QTc interval is a marker for clinical risk of severe arrhythmias, such as ventricular fibrillation. QTc length correlates with the intensity of the sympathetic response during the induction of general anesthesia. Present work compared QTc values in two different anesthetic induction regimens, evaluating both the pre and post intubation period. QTc was evaluated in three successive beats, in ECG DII derivation. Twenty surgical patients, aged 21-60, ASA I-II were randomly allocated to Group M-R (Midazolam-Remifentanyl) or to Group M-F-K (Midazolam-Fentanyl-Ketamine). In control group, QTc was obtained from 20 healthy age-matched subjects.

Hemodynamic variables (HR, SBP and DBP) and ECG were registered before anesthetic administration (Ba), preintubation (PreI), and 1, 3, 5, 10, 15, 20, 25 and 30 min postintubation (Pi)

QTc values in M-F-K group were significantly higher than in M-R. Eg: (mean±sd, msec): PreI: M-R: 440±50; M-F-K: 460±50 (p=0.165); Pi1: M-R: 430±40, M-F-K: 480±20 (p=0.005); Pi5: M-R: 420±30, M-F-K: 470±40 (p=0.011); Pi10: M-R: 420±30, M-F-K: 470±40 (p=0.019); Pi20: M-R: 420±30, M-F-K: 450±40 (p=0.089); Pi30: M-R: 430±40, M-F-K: 460±30 (p=0.063). Also, in M-F-K group, QTc values were higher than the ones registered in the control group (420±40 msec, p<0.05).

In the Midazolam-Fentanyl-Ketamine regimen higher values than the limit (440 msec) were consistently found. Hence, the Midazolam-Remifentanyl regimen might offer a better protection against the risk of severe arrhythmias.

46. MOLECULAR CHARACTERIZATION OF RHD HAPLOTYPES

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Determination of RHD zigosity is commonly inferred from serological testing. RHD is flanked by the homologous 5' and 3' Rhesus boxes. The RHD deletion, found in most RhD negative Caucasians, was due to recombination of the 5' and 3' Rhesus boxes resulting in the formation of a hybrid Rhesus box. The aim of this work was to study the RHD zigosity by amplification of the hybrid Rhesus box and determine the haplotypes in the population of Rosario. Blood samples from 106 white trios (father, mother, child) were studied. The Rh phenotype was performed by hemmagglutination. The RHD deletion was determined by PCR-ARMS with primers that selectively amplify a 1981 bp segment of the hybrid Rhesus box that is present in RhD negative and RhD positive heterozygous samples. We phenotyped 179 (84,4%) RhD positive samples, 75 (41,9%) were RHD homozygous and 104 (58,1%) were RHD heterozygous according to PCR. These results did not differ from those obtained by the most probable genotype method. However, the homozygosity indexes were significantly higher in the DccEe (20,0% vs 6,6%) and Dccee (16,6% vs 3,3%) phenotypes. In all samples with the Dce haplotype the RHce^s allele, frequent in individuals of African descent, was investigated by PCR-SSP. This allele was found in 36.4% of the Dce haplotypes. The analysis of hybrid Rhesus boxes in family groups allowed the determination of RHD zigosity and exact haplotypes in different Rh phenotypes. The homozygosity indexes in the DccEe and Dccee phenotypes and the frequency of the RHce^s allele obtained tend to that found in Africans. These findings will contribute to estimate the genetic admixture of our population.

47. PRELIMINARY EVALUATION OF ATB-FUNGUS 2 AND NEOSINTAB METHODS TO DETERMINE YEASTS SUSCEPTIBILITY TO ANTIFUNGAL AGENTS

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The aim of this work was to evaluate the ATB FUNGUS 2 (ATBF) technique and Neosensitab (NST) diffusion method for the determination of sensibility of 18 strains of *Candida* spp to anphotericin B (AMB), fluconazole (FCZ) and itraconazole (ITZ) in comparison with macrodilution reference method (MR).

Six *C. albicans*, 3 *C. dubliniensis*, 2 *C. tropicalis*, 2 *C. parapsilosis*, 2 *C. glabrata* and 1 *C. krusei*, isolated from clinical specimens, and two reference strains were studied. The yeasts were identified throughout by micromorphology in corn meal agar-Tween 80 at 1%, CHROMagar Candida and API 20 Aux (bio-Mérieux). The MR was performed according to M27 A2 macromethod in RPMI 1640 (NCCLS) with 2% of glucose and ATBF and NST according to manufacturer instructions.

The reading was carried out after 24 or 48 hours according to the development of the control. Mc Nemar test was used to compare the results. The concordance percentages for AMB respect to MR were 72% with ATRF method and 55% with NST method. The concordance percentages for FCZ respect to MR were 66% with ATBF method and 55% with NST method. For ITZ the concordance percentages respect to MR were 66% with ATB method and 36% with NST method.

Only for AMB there were not significant differences between ATBF and MR methods; in the other cases there were significant differences (p < 0,005). The unequal results among ATBF, MST and MR methods allow us to conclude that though the two first methods are faster and more practical can be used in reliable form in substitution of the MR method.

48. DEVELOPMENT OF A CHEMOMETRIC METHOD FOR DOSE CONTROL OF PHARMACEUTICAL POLYSULFA PRODUCTS OF USE IN VETERINARY

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Polysulfa drugs are habitually employed in veterinary therapeutics for small and big animals. Sulfas show little structural variations; therefore, HPLC is usually required for their quantification. We report the development of an alternative, non-chromatographic method for the quantification of sulfa drugs in veterinary products, based on the chemometric (partial least squares, PLS) analysis of spectrophotometric data in the ultraviolet region. Calibration models were simultaneously prepared for each analyte, employing synthetic samples with different concentrations of the analytes. The models were internally validated employing the leave one out procedure within spectral regions selected for each analyte by use of the variable size-moving window algorithm with a minimum prediction error (PRESS) search. An external analytical validation with synthetic samples, designed to evaluate accuracy and precision of the determinations, demonstrated the suitability of the calibration models. The validated models were applied to the simultaneous quantification the amounts of sulfaquinolaxine, sodium sulfamethazine and sodium sulfathiazole in a veterinary pharmaceutical powder currently sold in the local market. Results were accurate, reproducible and relative standard deviations of the determinations were acceptable, in view of the important superimposition of the spectra of the three drugs.

49. HLA CLASS II DRB1 POLYMORPHISM IN PATIENTS WITH CHRONIC *TRYPANOSOMA CRUZI* INFECTION

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Infection with the protozoan parasite *Trypanosoma (T) cruzi* causes Chagas' disease, a major health problem in the American continent. The factors underlying the establishment of chronic myocardial lesions are not fully understood. However, it is clear that chagasic pathology is associated with the immune response elicited against the parasite. The aim of the present study was to investigate the contribution of the *HLA-DRB1* alleles in determining the susceptibility to *T. cruzi* infection as well as in the development of chagasic heart disease in subjects that were born in endemic areas of Chagas' disease from Santa Fe Province, Argentina. The study included 35 unrelated individuals serologically positive for *T. cruzi* (IIF, IHA and ELISA). Additionally, a group of 41 unrelated healthy individuals with neither symptoms nor previous diagnosis of Chagas disease was studied as control group. DNA typing of *HLA-DRB1* alleles was performed. *DRB1*0409* allele was significantly more prevalent in seropositives with a trend being also observed for the *DRB1*0701*. A greater occurrence of the *DRB1*1503* was found in patients with cardiomyopathy. Molecular typing allowed the identification of increased frequencies of *DRB1*0409*, *DRB1*1503* and *DRB1*0701* in infected individuals respect the seronegative controls, suggesting that these antigens could be related with the infection by *T. cruzi*. Increased frequency of *DRB1*1503* allele was found among cardiomyopathy patients suggesting that this antigen could be associated with the genetic susceptibility to cardiac damage in these individuals.

50. PHAGOCYTOSIS OF ANTI-D SENSITIZED SENESCENT ERYTHROCYTES

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The determination of erythrocyte lifespan is a complex process affected by many cellular parameters. Recently, we observed an increased Rh(D) antigenic expression in the population of red blood cells (SeRBC). The aim of this work was to study the phagocytosis of anti-D sensitized (S) SeRBC. Blood samples were drawn by venipuncture from 12 volunteer donors. Density separation was effected by short-duration high speed centrifugation of concentrated RBC at 10000g for 15 minutes. Following centrifugation the top and bottom 10% fractions were removed and designated as the young (Y) RBC and SeRBC. The monocyte monolayer assay was performed with: 2% suspensions of a) SeRBC; b) YRBC; c) SSeRBC d) SYRBC. Negative (NRBC) and positive (SRBC) controls were performed. The % of active phagocytic cells (APC) was determined:

- a) % APC with SeRBC: 17.1 ± 1.5
- b) % APC with YRBC: 3.1 ± 0.9
- c) % APC with SSeRBC: 34.4 ± 1.8
- d) % APC with SYRBC: 28.5 ± 1.7
- e) % APC with NRBC: 2.7 ± 0.8
- f) % APC with SRBC: 30.1 ± 1.9

The % APC with SSeRBC were significantly higher than that found with SeRBC (p < 0.001) and with SYRBC (p < 0.05). This results may be related to conformation modifications of Banda 3 protein during the erythrocytes aging that would allow an increased interaction between antibodies and Rh antigens.

51. REFERENCE VALUES FOR SERUM ANTI-OXIDIZED LOW DENSITY LIPOPROTEINS ANTIBODIES DETECTED BY ELISA. A PRELIMINARY STUDY

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The oxidation of low density lipoproteins (LDL) leads to the formation of highly immunogenic molecules (LDLox), related to the development of atheromatous plaque. The antibodies against LDLox (a-LDLox Ab) are considered as atherogenesis markers. The aim of the study was to establish a reference value interval for a-LDLox Ab levels in serum of healthy blood donors, using a sandwich-type ELISA developed by us. Thirty-one serum samples were provided by the Servicio de Hemoterapia from the Hospital Centenario. Total cholesterol level, high density lipoprotein cholesterol level and triglyceride level were assessed by enzymatic methods, while low density lipoprotein cholesterol level was determined by the Friedewald equation. Only normolipemic individuals (ATP III) were included. Polystyrene strips coated with either LDL or LDLox were used for the determination of a-LDLox Ab. Then, a peroxidase-labeled a-human immunoglobulins was added, followed by the chromogenic substrate TMB-H₂O₂. Concentration of a-LDLox Ab was expressed as the difference between the absorbance value obtained with LDLox and the corresponding value obtained with LDL. The results were then referred to the value of a control sample exposed to LDLox and expressed as a-LDLox Ab units. An intra-assay variation of 8.32% and an inter-assay variation of 8.10% were found and the analyzed data resulted in a reference interval of 0-22 a-LDLox Ab U. The setting of a reference interval for these antibodies in the healthy population will provide a useful tool for the identification of patients with elevated atherogenic risk.

52. EFFECTS OF THE GABA_B AGONIST, BACLOFEN (BAC), ON THE RENAL TUBULAR TRANSPORT OF GLUCOSE

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In previous experiments, we observed that BAC induces modifications in renal tubular function, as increment in the fractional excretion of water, sodium and glucose. In this work, the effects of BAC on glucose transport were further investigated. Rats were surgically prepared for clearance techniques, injected with BAC 1 mg/kg i.v. (BAC, n=5) or vehicle (C, n=4) and infused with 25 % glucose solution. Infusion was performed at increasing rates (1,75; 2,1; 2,6; 3; 4 and 5 ml/hour). A 20-min stabilization period was allowed prior to the two consecutive 10-min urine collection periods at each rate. At the end of each period, arterial blood samples were obtained. Inulin and glucose concentrations in plasma and urine were determined in order to estimate glomerular filtration rate (GFR) and glucose transport (Tglu). Obtained values of Tglu/GFR versus plasma glucose concentration were adjusted to Michaelis-Menten kinetic. Double reciprocal analysis showed that BAC evokes a significant change in 1/Km (C= 0.1766 ± 0.0359; BAC= 0.0329 ± 0.0121 ml/mg, p<0.05) and 1/Vmax (C= 0.1167 ± 0.0129; BAC= 0.0324 ± 0.0104 ml/mg, p<0.001). These results indicate that BAC modifies renal glucose reabsorption, increasing the maximum transport and decreasing the affinity. Different carriers are involved in glucose reabsorption. The use of other experimental models might be useful to identify the kind of protein affected by this treatment.

53. COVERAGE AND FACTORS ASSOCIATED WITH ANTITETANUS VACCINATION STATUS AMONG ADULTS. REGIONAL STUDY

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The aim of this study was to estimate the coverage and determine the factors associated with antitetanus vaccination status among adults. The place and duration of the study was: Villa Constitución city, province of Santa Fe, Republic of Argentina, from January to July 2005. Data for 402 adult volunteers aged 25-82 years were collected. Questionnaires were applied at Callegari Pharmacy. This Pharmacy office is located in the vicinity of the Public Hospital of Villa Constitución, so it is visited by persons from different socio-economical conditions. Volunteers were found out about: age, sex, complete antitetanus vaccination schedule (CAVS), knowledge of vaccination schedule, possession of vaccination certificate, health insurance and educational level. From this study, only 30% of adults has presented CAVS. The following factors were associated with CAVS: possession of vaccination certificate ($p < 0.0001$), knowledge of vaccination schedule ($p < 0.0001$), educational level ($p < 0.0001$) and age ($p < 0.0001$). In logistic regression analysis, the variables included into the model were age ($p = 0.0008$) and educational level ($p = 0.03$). This study indicates the influence of educational level on CAVS, probably due to a higher access to information and better comprehension of its importance. CAVS diminished in elderly people, so extra precautions in this age group should be taken into account.

54. STABILIZATION OF SEMISOLID SYSTEM CONTAINING 3-BENZOPHENONE

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It was assayed different systems containing 3-benzophenone, which acts as solar filter, with the goal to obtain a semi solid emulsion with 15 FPS (protector solar factor). The system were under stress (at two temperatures); the size and distribution of the particles and the rheological shape were studied.

The emulsifiers used were: Trietanolamine stearate, Tween 80, Glycerol stearate, Glycerol stearate citrate and.. The other substances used were: Cetostearyl alcohol, Carboxymethyl cellulose and Glycerol.

The systems were prepared by heat, adding the aqueous phase on the oil phase with a light stirring until of a temperature of 25°C.

The systems with Gyceryl stearate, Tween 80 and Trietanolamine stearate didn't support the temperature stress, making each other phases separation. The others remained stable and showed good size of internal phase particles.

The rheological shape of these system showed to be plastic with tixotropy, and the optimal system was the one with Dimeticon and caprylic/capric triglyceril, who showed a lower size of internal phase (5 micras).

55. STUDY OF THE DISSOLUTION AND DELIVERY OF NON SOLUBLE DRUGS IN AQUEOUS MEDIUM IN SEMI SOLID SYSTEMS OF TOPIC USED

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To day the focus of the galenic developpe in the semi solid systems are: a) the penetration of drugs in the statum corneus layer, and the increase of the efficiency by the use of enhancers, and b) the use of biopolymers and other substances of ecology importance which allow the solubilizacion and delivery the optimal amount of a drugs. The aim of this work is to compare (in vivo) different semi solid systems of topic use made by sintetic and natural polymers. It was analysed the incorporation and delivery of drugs with low absorption characterist. The following semi solid system were used: **System 1:** CBP 934 2% + Plantaren 1200 2% + KTO 2%, **System 2:** CBP 934 2% + Oliven 700 3% + KTO 2%, **System 3:** CBP 934 2%+PEG 1500 3% + PEG 400 6% + KTO 2%. The same systems were studied by Chitosan addition (0,8%). Active drug: ketoconazol (KTO), flexible chain polymers, polyethylen glycol 1500 and 400 (PEG), poly acrylic acid derivatives, carboxy polymethylen (CBP), partially desacetilated derivatives of poly (B (1-4)-2 acetamide-2-desoxygluco-pyranose, (Chitosan), non ionic ecology surfactants, olive derivatives (*Olea europaea*), PEG 7 Oleate (Oliven 700), and corn derivatives (*Zea mais*) alkil polyglycosides, (Plantaren 1200). The systems 1 and 2 showed the best capacity to solubilize in the presence of 0,8% of chytosan, increasing the time stability. The mass of drug released vs. time curve allows to determine a process with a kinetic of orden one, pH independently and the drug initial concentration. The system 1 and 2 with chitosan showed a high delivery of KTO.due to the moleculars characteristics and the excellent hydrations properties of the chitosan, makes an offer of innovatories semisolids systems of topical applications.

56. GELYFIED SYSTEMS: DELIVERY OF TOCOPHEROL IN SYSTEMS OF DEMIC USE AND ITS RELATION WITH THEIR RHEOLOGICAL SHAPE

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The elucidation of the biochemical composition and the diffusive function of the corneum stratum layer has origin the investigation of many forms to optimizace the penetration of poorly absorption drugs. In previous woks, we have selected systems which contains carboxypolymethylen (2% w/v) to develop systems with high efficient of drugs delivery. To obtain this goal we have used vitamin E as drug model because decrease the lipidic peroxidation and the erythema made by actinism eradiation. exposition.

To solubilize the vitamin E it was selected: ecology surfactants: Oliven (700 and 900) and alkil polyglucosides (Plantaren 1200) and flexible chain polymers: such as polyethylene glycols. We formulate several terapeutics systems of topical application using those emulsifiers, and with the aim to optimise drug delivery we modified the original systems, using propilen glycol in all cases (3% w/v). First, we obtained the rheological profiles of each system, and then we determined the alfa tocopherol delivery, from the same systems, with optics proff. At least, we found that the higher delivery was at two hours for all systems. It was found that all the systems lost 80% or higher of the vitamin E, being the optimal delivery in the system with Oliven 700. The rheological shape was similar for all the systems with tixotropic shape plastic, the systems with the best delivery had the plastic and apparent viscosities lower than the other systems.

57. NEW PEROXIDASE AND Mn-CATALASASE MIMICS

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Mn catalases are a particular class of catalases of bacterial origin that possess a diMn active site and disproportionate H_2O_2 by cycling between the Mn^{II} and Mn^{III} oxidation states. Peroxidase is a unique heme peroxidase that uses Mn^{II} as its primary substrate and, together with ligninase, catalyses the oxidation of lignin by H_2O_2 . The obtention of efficient mimics of these biosites requires to find structure/activity relationships and to understand the factors that control the catalytic activity. With this in mind, we have obtained three new diMn complexes with 1,3-bis(2-hydroxybenzyl-2-methylpyridylamino)propan-2-ol (H_3L^1), 1,4-bis(salicylidenamino)butan-2-ol (H_3L^2) y 1,5-bis(2-hydroxybenzyl-2-methylpyridyl-amino)pentan-3-ol (H_3L^3). With these ligands differing in the length of the $-(CH_2)_n-$ spacers- it was possible to modulate the $Mn^{III}-Mn^{II}$ distance and the symmetry of the metal centre and analyse the influence of these structural features on the catalytic activity. The catalase activity was determined polarographically by measuring the $[O_2]$ in $H_2O_2 +$ catalyst mixtures with a Clark-type electrode. The peroxidase activity was evaluated spectrophotometrically on $H_2O_2 +$ catalyst + 4-aminophenazone/phenol mixtures, in buffer $HPO_4^{2-}/H_2PO_4^-$. In dimethylformamide, the three complexes catalyse the disproportionation of more than 1000 equivalents of H_2O_2 . The complex of H_3L^1 is the less efficient at binding the substrate, while that formed with H_3L^2 is the most reactive. In protic solvents, the complex of H_3L^1 inactivates after ≈ 200 cycles. The high peroxidase activity but low catalase activity shown by the complex of H_3L^3 in acetonitrile, makes this compound a potential catalyst for organic oxidations with H_2O_2 .

58. ANTIFUNGAL PROPERTIES OF COMPLEXES OF COBALT WITH SULFANILAMIDE (SA) AND WITH PHTHALYLSULFATHIAZOLE (PhST)

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During the last two decades there was a large increment of fungal infections. Complexes of silver and zinc sulfonamides have successfully been used for the last 20 years for the prophylaxis and treatment of microbial and fungal infections in burns and wounds. Previously we had evaluated the antifungal properties of the complex Co(II)-sulfathiazole, Co(II)-ST, and we found that two species of the *Aspergillus* genus were susceptible (*A. fumigatus* and *A. flavus*). Now we inform the antifungal properties of the following cobalt-sulfa complexes, which were previously obtained and analyzed: Co(II)-SA; Co(III)-SA; Co(II)-PhST and Co(III)-PhST. We determined the minimum inhibitory concentration for these complexes, for the following human opportunistic pathogenic fungi: *C. albicans*, *C. tropicalis*, *S. cerevisiae*, *C. neoformans*, *A. fumigatus*, *A. flavus*, *A. niger*, *M. gypseum*, *T. rubrum*, *T. mentagrophytes*. All the fungi were susceptible to both PhST complexes (except *M. gypseum* and *T. mentagrophytes*), suggesting that the effect could be due to PhST. Co(II)-SA inhibited: *S. cerevisiae*, *C. neoformans*, *A. fumigatus*, *A. flavus*, *A. niger* and *T. rubrum*, while no one was susceptible to Co(III)-SA. This difference in activity could be due to the metal in the complex which, similarly to Co(II)-ST and according with the literature, might exert its antifungal action by inhibition of enzymes involved in biosynthesis of fungi cell walls.

59. DETECTION OF MUTAGENICITY IN SULFADRUG-NITRITE MIXTURES IN ACIDIC MEDIUM BY MEAN OF THE AMES TEST (SULFAS: Co(II)-SULFATHIAZOLATE, Co(II)-ST; PHTHALYLSULFATHIAZOLE, PhST)

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The Ames test is an economic and effective bacterial mutagenicity assay that uses *Salmonella typhimurium* as indicator bacteria. The *S. typhimurium* strains employed cannot synthesize histidine, so they need this aminoacid in order to grow. A mutagenic substance can overflow this lack, making colonies grow (revertant colonies). According to Ames, a compound should be considered mutagenic if the reversion coefficient, R.C., exceeds 2.0. (R.C. = revertant number per tested plate/revertant number per control plate -spontaneous-). Besides primary drugs, it is necessary to consider interactions that might occur into the organism, like the nitrosation of substances with amine/amide function by the nitrite added into food products (cured meats, fried bacon, etc). Sulfadruugs are common antibiotic, with amine and amide functions in their molecules. Mutagenicity of PhST and Co(II)-ST, in the presence and absence of $NaNO_2$ in acidic medium was evaluated with the Ames test, employing *S. typhimurium* TA98 y TA100 strains. It was detected mutagenicity in both mixtures, at different concentrations. UV-Vis spectra and the detection of mutagenicity in absence of S9 let us suggest that the amide functions could be involved. Nitrosation were positive for both mixtures at pH similar to the gastric juice one, so, it would be convenient, as a caution, to not eat food with added nitrites in combination with sulfadruugs.

60. ADHESION MECHANISMS IN INFLAMED HUMAN DENTAL PULP TISSUE

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The human dental pulp is a special connective tissue, that presents some anatomic and functional characteristics that make it different from another connective tissues of the body, because it is in a cavity with hard walls, it doesn't have collateral circulation and it have a poor nutritive blood through the apical foramen. As a consequence of the aggression caused by caries, there are changes in the dental pulp that give origin to inflammatory processes and then some adhesion molecules are expressed such as the CD44. The purpose of this study was to prove the presence of soluble molecule CD44 (CD44s) in healthy human dental pulp and compare with inflamed tissue by a simple immunohematologic technique. The CD44s in the supernatant of dental pulp was studied from pulp samples obtained from 66 different human adult donors; clinically normal pulp tissue (n=10), from the Chair of Surgery, pulps with inflammation (n=46) with a diagnosis of acute irreversible pulpitis, from the Chair of Endodontics. It was used Panel ABH from the Blood Center, Hyaluronic acid sodium salt from human umbilical cord (H 1786) and PBS pH7.4. The samples were divided into 3 groups: healthy, open processes and close processes. The results obtained from the sensitivity parameter (α) show significant statistical differences between the three groups (p=0.001). The sensitivity of this technique allows to demonstrate the presence of hyaluronan-CD44 in inflamed human pulp tissue. The presence of CD44 in healthy teeth was minimal or absent agree with the published researches in other tissues. This model can be applied to healthy and inflamed human pulp tissue.

61. EFFECTS OF LOSARTAN ON RENAL SODIUM AND WATER EXCRETION AND ON THE EXPRESSION OF Na⁺, K⁺ ATPase (NKA) α SUBUNIT AFTER ISCHEMIA-REPERFUSION

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In previous studies we reported that after 40 min of unilateral renal ischemia followed by 24 h of reperfusion (IR24), postischemic kidneys showed marked tubular dysfunction. We also reported an increment in NKA α subunit abundance after 40 min of ischemia without reperfusion (I40) and in IR24 renal cortex and medulla. Losartan pretreatment, an AT1 angiotensin II (ANGII) receptor blocker, did not prevent this increment in I40 group. The aim of this work was to study the effects of losartan pretreatment on tubular function and on expression of NKA α subunit after an ischemic-reperfusion (IR) insult. Male adult Wistar rats were treated with losartan (80 mg/kg/day, i.p., n=6) during 3 days before ischemia followed by 24 h of reperfusion (IR24+L). Tubular functions of postischemic kidney were studied by clearance techniques. NKA α subunit abundance was evaluated by Western Blot in cortex and medulla. Sham operated rats without (C) and with (C+L) losartan pretreatment were used as controls. Losartan improved fractional excretion of Na and water: FE_{Na} (%) C: 1.5±0.4; C+L: 1.4±0.1; IR24: 78±16*; IR24+L: 3.8±0.7*[#], FE_{H₂O} (%) C: 0.9±0.2; C+L: 0.7±0.03; IR24 71±20*; IR24+L: 4.1±0.5*[#], *p<0.05 vs C, [#]p<0.05 vs IR24. NKA α subunit abundance in IR24+L was not different from C in cortex and medulla. Our results suggest that ANGI, through AT1, would play a key role in the evolution of tubular dysfunction and in the alterations in NKA α subunit expression after an IR insult.

62. STUDY OF BIOCHEMICAL PARAMETERS IN ROSARIO PEDIATRIC POPULATION. ESTIMATE OF RISK SITUATIONS

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Values of reference for biochemical parameters in Rosario pediatric population were established. 1100 female and male children and adolescents, aged from 5 to 18 were studied. They were divided into three groups according to their age: Group I from 5 to 9; Group II from 10 to 14 and Group III from 15 to 18. Glucose, urea, proteins, calcium, phosphorus and the lipidic profile were determined by usual laboratory methods. The body mass index (BMI) weight / height², was calculated and results were tested in the following chart

Age/	5 - 9 n= 250			10 - 14 n= 473			15 - 18 n= 377		
	X	d.s	VR	X	d.s	VR	X	d.s	VR
GLU	84	9,0	70-100	85	9,1	70-102	84	10	70-106
URE	24	5,8	14-36	25	7,2	14-42	21	5,1	14-40
PRO	7,0	0,4	6,2-8,0	7,0	0,5	6,1-8,0	7,3	0,4	6,4-7,9
CA	9,4	0,5	8,5-10,4	9,4	0,6	8,3-11	9,3	0,5	8,5-10,4
PHO	5,2	0,6	4,0-6,3	4,6	0,5	3,5-5,8	4,5	0,3	4,2-4,7
BMI	16,	3,2	11-24	19	3,2	14-27	22	3,5	17-29
CHO	163	21	129-208	162	22,	130-210	165	24	126-215
TRY	71	31	30-154	74	32	31-159	67	28	35-135
HDL	53	6,3	40-65	56	6	45-69	59,	6	40- 65
LDL	96	21	60-136	91	21	57-144	94	26	50-144

The values obtained do not differ significantly from those found in were taken. 200 mg/dl for CT; 130 mg/dl for TG; <50 mg/dl for C-HDL the references, the lipidic profile was found to be higher than expected. The following cut-off cardiovascular risks and 110 mg/dl C-LDL.

5-9 years: CT 4,3% TG: 22,9% C-HDL: 11,5% C-LDL 31%
 10-14 CT 4,8%, TG 24% C-HDL: 14% C-LDL 19%
 15-18 CT 8,8%, TG 16% C-HDL: 8% C-LDL 25,8%

The detection of these risks factors at an early age emphasizes importance of promoting healthier dietary habits to avoid CVD in adult age

63. PRESENCE OF T-2 TOXIN IN HENS AND CHICKS FEED

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T2 is a trichotecen found in crops in several countries. This is a secondary metabolite produced by certain species of genus *Fusarium*. Bleeding diseases, oropharyngeal injuries and neurotoxic effects in hens are associated to this toxin, though the most significant effect is the immunosuppressive activity.

The aim of this work was to evaluate the presence of this toxin in hens feed used mainly in breeding place where egg producing hens are laying in the south of Santa Fe Province.

The study of 131 samples from raw material of hens feed was carried out. T2 detection was performed with the ELISA method: AGRAQUANT[®] T2 according to the manufacturer's instructions. The standard solutions used to construct the calibration curve were tested under the same condition.

Absorbance data by interpolation from the samples allowed us calculate the concentration of the toxin in each sample.

From all the tested samples, 32% reached lower values than 35 ppb which is the limit of detection to this method, 26% showed values between 35 and 100 ppb. Finally a 42% showed higher values than 100 ppb, maximum allowed amount in countries that have a legislation referring to the presence of this toxin. It carried primary oral lesions in animals ingesting it. Besides, oropharyngeal, stomach and liver damages were detected, avoiding them a right assimilation of the nutrients, with a consequently loss of weight. In the breeding place of egg producing hens, a lower production of eggs, and failure in them, was found. In all the cases high levels of mortality were detected by infections as from the effects of this toxin.

64. PREVALENCE OF CANDIDA SPECIES IN OROPHARYNGEAL CAVITY AND ITS PHILOGENETIC RELATIONSHIP

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Infections due to opportunistic fungi like species of genus *Candida*, have increased during the last decade. The identification of the different species of *Candida* is important, due to their ability in developing diseases and their different susceptibility to antifungal drugs. The aim of the present work was to study the isolated *Candida* species from oropharyngeal cavity and analyze the phylogenetic relationship among these species through pattern bands generated by PCR fingerprinting. Samples of 130 patients were obtained from Odontology Faculty, UNR. The yeasts were isolated and identified for traditional procedures. Chromogenic CHROMagar-*Candida*, API 20 C, and PCR fingerprinting using the M13 core sequence, were performed from positive samples.

The similarity coefficient Nei & Li was used to calculate the evolutive distances among isolated strains and the Neighbor Joining Method to perform the dendrogram. The phylogenetic tree was visualized through the Tree View Program.

It could be verified from 100 positive samples that in the physiological, morphological and genetic criteria, the isolation corresponded to *C. albicans* 63%; *C. parapsilopsis* 21%; *C. tropicalis* 8%, *C. krusei* 6% and *C. glabrata* 2%.

Phylogenetic analysis showed quantitative evidence that PCR fingerprinting allowed the total differentiation among the different species of *Candida*, obtaining a minimum distance value between two of them, 029771.

65. COMPARISON BETWEEN THE *IN VITRO* EFFECT OF CATECHIN- AND PROANTOCYANIDINE-ENRICHED EXTRACTS OF *Ligaria cuneifolia* (*Lc*) ON ERYTHROCYTE SHAPE, DEFORMABILITY AND OSMOTIC RESISTANCE
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We showed that both *Lc* crude extract (containing quercetin, proanthocyanidin and catechin) and methanolic fraction (enriched in non-glycosylated quercetin) decrease erythrocyte deformability and increase osmotic resistance. Objective: To compare between *in vitro* effect of catechin- and proanthocyanidine-enriched extracts of *Lc* on erythrocyte shape, deformability and osmotic resistance. Methods: Blood samples from male adult Wistar rats were divided into 3 groups: Control (C) (n=5), catechin-treated (Tcat) (n=6) and proanthocyanidin-treated (T_{plc}) (n=6), concentration: 2.3 mg% (0.60 mg/100g bw), incubation: 30 min, 37°C. Results (mean ±SE): Rigidity index (filtration through nucleopore membranes): C: 5.67±1.19; Tcat: 7.20±0.91; T_{plc}: 6.20±0.34 (p>0.01 vs. C). Morphological index (MI, microscopy and Bessis classification, Σ[shape index x number of cells/total number of cells]): C: -1.00±0.08; Tcat: -2.93±0.13*; T_{plc}: -0.89±0.08 (*p=0.003 vs. C). Stomatocytes III (%): C: 15.67±2.81; Tcat: 58.27±2.4*; T_{plc}: 14.44±2.42 (*p<0.001 vs. C). No alteration in osmotic resistance (X50) occurred (C: 0.575±0.032). Conclusion: While catechin-enriched extract of *Lc* induces a discocyte-to-stomatocyte change, proanthocyanidin-enriched extract of *Lc* causes no alteration of red blood cell shape.

66. EFFECTS OF SOY BRAN ON OBESE DIABETIC RATS
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In view of the reported beneficial physiological effects of soy components, potential actions of soy bran – an oil industry waste- on the prevention and therapy of obesity and type 2 diabetes were evaluated in this study. In order to assess soy fiber effects on food intake (FI), biomass (B), fasting glycaemia (FG), plasmatic triacylglycerides (TAG) and cholesterol (CHOL), 200 days old β obese diabetic rats were fed diets containing casein as protein source and 10% of soy bran (C group n: 9) or 10% microcrystalline cellulose (S group n: 9). (FI) and (B) were registered every other day. (TAG), (CHOL) and (FG) were measured on days 0, 14 and 28. Statistical analysis was performed using ANOVA and Tukey's test. Results are expressed as media ± standard error. FI(g): lower in S (day 14: S: 334.4 ± 15.2-C: 405.8 ± 34.7, p> 0.05; day 28: S: 687.5 ± 35.7- C: 748.0 ± 40.7, p>0.05). B progress (g): in S lower and slowly C (S: day 0: 385.4 ± 13.5 - day 28: 397.1 ± 11.5, p>0.05; C: day 0: 382.2 ± 15.6 - day 28: 398.7 ± 16.6, p< 0.05). TAG (g/l): both groups maintained high line β characteristic values. CHOL (g/l): significant difference on day 28° (S: 1.26 ± 0.05 - C: 1.51 ± 0.06, p < 0.01). FG (g/l): an abrupt and marked diminution in S (S: day 0: 1.62 ± 0.03 - day 14: 1.23 ± 0.03 - day 28: 1.26 ± 0.04, p<0.05; C: day 0: 1.51 ± 0.07 - day 14: 1.41 ± 0.09 - day 28: 1.34 ± 0.06, p >0.05). Confirmed effects of soy bran on parameters involved in the onset and evolution of obesity and type 2 diabetes, suggest its use in *functional foods* might be considered. *This study was supported by Alberto J. Roemmers Foundation.*

67. EFFECTS OF SOYBEAN BRAN ON PROTEIN DIGESTIBILITY AND BODY FAT IN IIM *Beta* RATS
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Eventual effects of soybean bran on protein digestibility and size and distribution of body fat were evaluated. Adult IIMBeta obese, diabetic male rats, were fed during 28 days with a diet containing sodium caseinate as protein source and 10% of two different fibers: soybean bran S (n=9) and microcrystalline cellulose C (n=9). Protein digestibility (PD) was determined at days 14 and 28. Animals were sacrificed, liver and fat depots were removed and relative weights calculated (g/100g biomass). Total lipids (LTL), cholesterol (LCHOL) and triacylglycerides (LTAG) were determined in liver. Results are expressed as mean ± SD. PD was not significantly different. Day 14: S: 0.96 ± 0.03; C: 0.98 ± 0.01 (p>0.1). Day 28: S: 0.98 ± 0.01; C: 0.97 ± 0.02 (p>0.1). Fat depots relative weights were slightly lower in S. Perigonadal: S 1.90 ± 0.25; C 2.03 ± 0.33 (p>0.05); retroperitoneal: S 2.17 ± 0.45, C 2.27 ± 0.31 (p>0.05). Liver relative weight was significantly lower in S. S 3.64 ± 0.37; C 4.32 ± 0.38 (p< 0.001). LTL (g/100g liver) were significantly lower in S. S 0.99 ± 0.11; C 1.52 ± 0.14; (p< 0.01). LCHOL (mg/100g liver) was significantly lower in S. S: 38.66 ± 1 8.43 C: 64.03 ± 15.67 (p<0.05). LTAG did not differ between groups. Similar PD values in both groups as well as reduction of fat depots and lower LTL and LCHOL in group S confirm beneficial effects of soybean fiber on lipidic profile. *This work was supported by Alberto J. Roemmers Foundation.*

68. VARIATION IN THE EXPRESSION OF THE ABO BLOOD ANTIGENS IN CANCER PATIENTS
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It has previously been observed that the expression and the structure of cell membrane antigens undergo certain modifications in some types of cancerous cells. Such changes can also occur in blood cells, mainly in erythrocytes. In the present study, the expression of the ABO blood group antigens on erythrocytes from cancer patients was assessed and compared to that of healthy controls, using a simple photometric method of agglutination kinetics. Whole blood samples from 140 individuals with blood group A and B were collected, 118 samples came from patients with solid cancers, 18 from healthy adult donors and 4 from umbilical cords. Specific monoclonal antibodies, in conjunction with indirect photometric techniques, were used to measure the expression of the ABO blood antigens on the cells. Using the relative percentage of optic extinction in function of the time, the initial speed for each agglutination reaction was obtained. The samples from healthy adults were used to determine the normal range of antigenic expression. We found that the expression of the blood group A antigen is 86% below the normal range and 76% for the blood group B on the erythrocytes of cancer patients. The analysis of these results shows a statistically significant difference between the cancer patients and the healthy adult values. In conclusion, the photometric method of agglutination kinetics used in this study is a simple indirect way to measure the expression of A and B antigens in patients with pathologies that affect the expression of cell membrane antigens. This could be a complementary, simple and fast method to assess the patient's evolution during treatment.

69. PROGRESSION OF P-AMINOHIPPURATE (PAH) RENAL ELIMINATION IN RATS WITH OBSTRUCTIVE UROPATHY (BUO)

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It has been described renal function alterations in rats with BUO. Organic Anion Transporter 3 (OAT3) is a tertiary active carrier located in renal basolateral membranes. OAT3 is involved in renal PAH uptake. The aim of this study was to evaluate the time evolution of PAH renal elimination in rats with BUO. Adult male Wistar rats were used. Both ureters were ligated during 24 h, then they were released and the studies were done after 24 h (T24, n=5) and 7 days (T7, n=5). A parallel group of sham rats was employed (S, n=5). Plasma urea levels (U) and PAH renal clearance (Cl) were determined. Na,K-ATPase (NK) activity and the abundance of OAT3 were assayed in homogenates from kidney cortex. Statistical analysis: ANOVA plus Newman-Keuls test ($[a]P < 0.05$ vs S, $[b]P < 0.05$ vs T24, $[c]P < 0.05$ vs T7). U(g/L); S: 0.56 ± 0.03 ; T24: $2.91 \pm 0.30_{a,c}$; T7: $0.60 \pm 0.09_b$. Cl(mL/min/100gbw) S: 3.59 ± 0.43 ; T24: $0.49 \pm 0.18_{a,c}$; T7: $2.79 \pm 0.23_b$. NK(umolPi/h/mgProt.); S: 15.93 ± 0.81 ; T24: $11.92 \pm 0.69_{a,c}$; T7: $15.53 \pm 1.08_b$. OAT3(%); S: 100 ± 6 ; T24: $48 \pm 11_{a,c}$; T7: $88 \pm 10_b$. The diminution in OAT3 abundance and in NK activity might explain, at least in part, the diminished Cl observed in T24. Seven days after the release of obstruction, no modifications were observed in PAH renal elimination in parallel with no changes in OAT3 abundance and in NK activity as compared with sham values.

70. ISOLATION AND PARTIAL CHARACTERIZATION OF PIG, *Sus scrofa domestica*, SPERM SURFACE MEMBRANE PROTEINS ABLE TO INTERACT WITH SBG ("SPERM BINDING GLYCOPROTEIN")

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In mammals millions of sperm enter the female reproductive tract, but only thousands arrive to the oviductal isthmus, where most of them bind to form a reservoir. This provides a controlled release of sperm competent for fertilization at the moment of ovulation. We have purified a glycoprotein from pig oviductal epithelial cells capable of binding to sperm, SBG ("Sperm Binding Glycoprotein"). SBG shows Gal β 1-3GalNAc terminal groups. Upon deglycosylation a polypeptide of apparent molecular mass 85,0 kDa is formed. The potential sperm molecules involved in its binding to the oviduct are surface membrane proteins (SMP) called spermadhesins, which are able to recognize and bind to specific carbohydrate residues. Objective: to isolate and partially characterize the SMP capable of interacting with SBG. SMP were isolated from sperm and those able to interact with SBG were purified by affinity chromatography on an SBG-Sepharose column. These proteins were separated and partially characterized by 2D-gel electrophoresis. At list ten protein spots of isoelectric points between 6.6 and 8.4 and apparent molecular masses from 6 kDa to 66 kDa were detected. The interaction of several sperm SMP with SBG indicates that the binding of sperm to the oviduct is a complex process in which a set of molecules from both cell types are involved.

71. VASCULAR FLORA OF SANTA FE PROVINCE: *Liliaceae*

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The family *Liliaceae* comprises 220 genera and 3500 species of all temperate regions of the world. In Argentina the family *Liliaceae* includes 14 genera, though only three of them are present in Santa Fe: *Asparagus* L., *Herreria* Ruiz & Pav. and *Nothoscordum* Kunth. The present work contributes to the knowledge of this family providing keys to the identification of different taxa based on morphological characters and a map of their ecological and geographical distribution. The methods consist of a thorough bibliographical review, consultation of national herbaria with important collections of the province (SF: Esperanza; SI: San Isidro; UNR: Rosario), field work experience of the authors and lab work to confirm their identity. As a preliminary result only two adventitious species of the genus *Asparagus* L. were found: *Asparagus densiflorus* (Kunth) Jessop and *Asparagus setaceus* (Kunth) Jessop, both new citations for the province. The genus *Herreria* Ruiz & Pav. includes three native species: *Herreria bonplandii* Lecomte, *Herreria montevidensis* Klotzsch ex Griseb. and *Herreria ophiopogonoides* (Kunth) Niederlein. Finally, the genus *Nothoscordum* Kunth. comprises two native herbaceous species: *Nothoscordum gracile* (Dryand. ex Aiton) Stearn var. *gracile* and *Nothoscordum montevidense* Beauverd subsp. *montevidense*. Their geographical distribution is concentrated mainly in the north-central and eastern departments of the province. Taxonomic information, illustrations and a distribution map are provided.

72. ELIMINATION OF CHROMIUM (VI) IN WATERS BY THE USE OF WASTE AGRICULTURAL BIOMATERIALS

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Cr (VI) is accepted to be a carcinogenic and mutagenic agent and its concentration may be elevated in industrial effluents. Biosorption has been accepted as an effective method to the reduction of contamination by metals in industrial effluents. Maize is one of the major farming in our country. One of the harvest byproducts of maize is the grain less stalk of corn. The goal of this project is to find low cost biomaterials able to retain chromium species in an insoluble matrix.

The adsorption studies in batch are performed at different dose of adsorbent to obtain the equilibrium isotherm. Also it is performed a pH dependent adsorption study.

On changing pH of the reaction mixture it was observed that chromium adsorption diminished on increasing pH at a given grain less stalk of corn mass.

The adsorption isotherm was performed at pH = 0,8 and T = 20°C. Isotherm parameters were evaluated by non lineal regression applying Freundlich isotherm model. The greatest chromium quantity adsorbed by unit of grain less stalk of corn mass was $2,61 \pm 0,01$ mg/g. This value is equivalent to that obtained using other biomaterials therefore grain less stalk of corn would be a good biomaterial in order to be used in remediation of chromium contaminated waters.

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73. STABILITY OF O/W EMULSIONS PREPARED WITH CASEINS AND DIFFERENT CALCIUM CONCENTRATIONS STUDIED BY DIGITAL ANALYSIS OF IMAGES

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Sodium caseinates are a mixture of sodium salts of milk caseins which is broadly used in the food industry. The presence of mono and divalent cations affects the caseins capacity as emulsifiers and emulsion stabilizers. Previous studies have shown that Ca²⁺ modifies the rheological behavior and the stability to creaming of O/W emulsions stabilized with caseins. The aim of this work was to study the action of Ca²⁺ by digital analysis of microscopic images of emulsions obtained at different Ca²⁺ concentrations. Emulsions were prepared from 0,1% sodium caseinate in imidazol buffer, pH 6,8, with pure corn oil and desired Ca²⁺ concentration. Samples of the emulsions were observed with an inverse microscope interfaced to a digital camera, and the drop and floccule dimensions were measured. The size distribution of the isolated drops of oil remains uniform, while the number and size of the floccules increased with the Ca²⁺ concentration, remaining constant the size of the drops inside them. The Ca²⁺ ions can bind to caseins phosphoserine residues decreasing the net charge and modifying the self-assembly behavior of the caseins, changing the relative proportions and promoting conformational changes in the proteins adsorbed on the O/W interface. This could cause a modification of the electric double layer that surrounds the drops and a decrease of the electrostatic repulsions among them. A reduction of the adsorbed layer would diminish the mechanism of steric stabilization, increasing the instability of the drops and leading to the formation of bigger floccules.

74. EFFECT OF POLYCATIONS ON THE ERYTHROCYTE AGGLUTINATION MEDIATED BY ANTI-GLYCOPHORINES USING MICROSCOPIC IMAGE DIGITAL ANALYSIS

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Polycations are oligomers or polymers, which bear a great number of positive charges at pH 7,4. They are used in medical and veterinary purposes. Their application as vectors in genetic therapy or as an agent capable of increasing drug diffusion is now being studied. The aim of this work was to study synthetic polycations effects on erythrocyte agglutination mediated by anti-glycophorin using image digital analysis. The interaction on the red blood cell membrane of three synthetic polycations (B₂₂, B₈₆ and B₁₂₄) from polyetilenglicol segments with branched structures and positive charged sequences was studied. Treated erythrocytes agglutinated by monoclonal anti-glycophorin A, B, C were labeled with fluorosphere Alexa488TM and the digital images obtained by confocal microscopy were analyzed. The number of cells and agglutinate distribution in each visual field were determined. The perimeter and surface of each agglutinate were used to obtain their agglutinated shape parameter (AgSP). Results show different behaviors in the polycation effect on the erythrocyte agglutination produced by anti-glycophorin monoclonal antibodies. These findings could provide more information about the mechanisms of polycation interaction on erythrocyte membrane. This work could be useful to improve the use of polycations in future biocompatibility and hemocompatibility in Clinical Applications.

75. ERYTHROCYTIC CHARGE STUDY IN HIPERGLICEMIC SAMPLES

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The purpose of the present investigation was to study the variation of the erythrocytic surface charge (ESC) in hyperglycemic blood samples through the Partition Coefficient (PC). The CES was estimated through the partition assay, which is a sensitive technique that separates cells with slightly different surface properties. Fresh blood samples from healthy subjects with glucose concentration glucose ≤ 110 mgr/dl (n=30) and hyperglycemic patients with glucose > 140 mg/dl levels (n= 30) were studied. The aqueous two-phase system containing 5%(w/w) dextran 500.000, 4% polyethylene glycol 6000 (w/w 6000) in 0.11 M PBS (phosphate buffer saline) was used. Negatively charged RBC move to the top phase (positively charged polyethylene glycol-rich). The PC was defined as the percentage of hemoglobin content in the top face to the total hemoglobin.

The glycosilated hemoglobin (HbA1C) was measured by chromatographic-spectrophotometric ION EXCHANGE method. The hyperglycemic patients showed values of HbA1C > 7 % and PC values 0.51 ± 0.1. Healthy subject showed PC values 0.90 ± 0.13. Higher than normal glucose concentration produce no-enzymatic glycosylation of proteins. This process could modify the erythrocyte membrane properties, affecting the surface charge of RBC and consequently the microcirculation properties.

76. EFFECT OF *Streptomyces* spp. ON *Macrophomina phaseolina*, *Rhizoctonia solani* AND *Sclerotinia sclerotiorum*

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During early growth stages soybean (*Glycine max* L. Merr.) seeds and seedlings can be affected by soil borne pathogens like *Rhizoctonia solani* (Rs), *Macrophomina phaseolina* (Mp), *Sclerotinia sclerotiorum* (Ss), *Fusarium* spp., *Sclerotium rolfsii*, *Pythium* spp. and *Phytophthora* spp. Antagonistic effect of *Streptomyces* C33-6, C202 and C208 (local strains previously selected as biological agents) was evaluated on Mp, Rs and Ss. *Streptomyces* strains were applied by the slurry method (3.5 x 10¹² CFU of each strain/ 100 kg seeds). The effect of the seed fungicide Maxim XL (fludioxonil+metalaxyl, 100 ml/100 kg seeds) was used as the best chemical control. Treated soybean seeds were placed on potato dextrose agar (PDA) in Petri dishes simultaneously with the pathogen culture which developed from 6 mm disk of PDA. Four replications of each combination *Streptomyces* – pathogen were incubated at 25°C during 6 days under natural light. Diameter (mm) of colonies was measured at 2, 4 and 6 days after sowing. The colonization of the seed coats and cotyledon tissues, necrotic lesions on cotyledons and esclerotia production were registered. *Streptomyces* strains C33-6, C202 and C208 reduced the pathogens growth at two days old; they also reduced the production of microsclerotia by Mp on seed coats (p<0.05) in 93.75%, 81.25% and 75.00%, and the necrosis on cotyledons by Rs in 25.00%, 54.16% and 58.34% respectively. This study suggests biocontrol with *Streptomyces* strains C33-6, C202 and C208 as a promissory tool in controlling soil borne pathogens in a competitive and sustainable agriculture system.

77. SPERMATOGENIC ARREST IN INFERTILE SMOKERS PATIENTS

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The seminiferous epithelium is a rich medium in androgens, indispensable requirement for the development of the spermatogenesis. The hormonal lack produces immaturity in the ejaculate with maturative arrest which is showed with high concentrations of immature germinal cells (GC) in the semen. The tobacco has toxic substances such as the nicotine that decreases the serum levels of gonadotrophins. Our objective was to relate the presence of GC in semen with the consumption of tobacco (CT) in men with idiopathic infertility. We have worked with 108 men without present or past pathologies able to alter the seminal quality. All of them have been carried out sperm study on functional tests according to WHO. The GC was determined with Papanicolaou stain, considering as reference value (RV) $<0.5 \cdot 10^6$ /ml (Gatti V et al, SAA 1999. 8(2):19) The studied population was divided in 4 groups: S1 (n=18) smokers more of 20cig/d; S2 (n=30) smokers of 6 to 20 cig/d; S3 (n=20) smokers up to 5 cig/d; S4 (n=40) non smokers. The smokers had had the habit for over a year. From the 40 of S4 only 13 (32.5%) had increased GC. In the S3 there were 7 (35%) with altered GC. From the 30 of S2 there were 17 (56.6%) with GC higher to RV. In the S1 there were 12 (66.6%) that had increased GC. The Chi Square Test (χ^2) was applied to value statistically and significant association was found between CT and spermatogenic arrest in S1 and S2 ($p < 0.05$). Significant association wasn't observed in S3. These results show that smoking induces anomalies during spermatogenesis due to a incomplete process of maturation. The CT alters the seminal quality and this is a factor to take into account to carry out the integral study of infertile men.

78. QUALITATIVE ANALYSIS OF A MATHEMATICAL MODEL APPLICABLE TO THE GASTRIC ABSORPTION OF ONE DRUGS

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The sodium monofluorophosphate (MFP) given orally is hydrolyzed into fluoride (F) in the intestine. At gastric level, the MFP is fully absorbed without undergoing hydrolysis. This determines the existence of two different forms of fluorides in plasma: the ionic fluoride and the MFP. The gastric MFP_g is absorbed with a rate absorption k_g . The analysis is restricted to the variable MFP concentration at plasma. The following differential equation system is set out with initial conditions $dMFP_p/dt = k_g MFP_g - k_c MFP_p$; $dMFP_g/dt = -k_g MFP_g$; $MFP_g(0) = MFP_0$; $MFP_p(0) = 0$ where MFP_0 is the orally given dose and $MFP(0)$ is the MFP concentration at plasma at 0 time

The qualitative analysis of the system has been accomplished determining the point of balance and the trajectories of the phase plane. The phase portrait show the declination of the MFP_g concentration as well as the variation of the MFP_p. It is also observed that the disturbed system tends to retakes its estate of balance as time passes. In this cases, in which the system solutions are already known, its main features are verified through the qualitative analysis of equations.

In complex pharmacokinetic or biological processes mathematical scheduled by equations systems whose solutions can't be expounded, the qualitative analysis allows us to get information about the behaviors of the variables involved in this process.

79. ADAPTATIVE OXIDATIVE STRESS RESPONSE OF *Candida dubliniensis*

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The objective of this work was to characterize the *Candida dubliniensis* response to the hydrogen peroxide (H₂O₂) and superoxide and to compare it, with that of *Candida albicans*. We used the reference strains: *C. albicans* ATCC 10231 and *C. dubliniensis* NCPF 3949. The assays were carried out with exponential growth yeasts incubated 1 hour at 30°C, with and without 0,4 mM H₂O₂, and then the cells were exposed to increase levels of H₂O₂, being determined the survival percentages. Superoxide resistance was determined with a cycling redox agent -metil-viológeno (MV)- incubating the yeasts with and without 0,5 mM MV, under the same conditions. The results indicate that *C. dubliniensis* possesses a smaller resistance level (17,15% of survival to 100 mM of H₂O₂; 33% of survival to 50 mM MV) than *C. albicans* (44,88% of survival to 100 mm of H₂O₂; 100% of survival to 50 mM MV). This suggests that *C. dubliniensis* responds to both oxidants with an adaptive response which protects yeasts from the lethal effects of a subsequent challenge with higher concentrations. We have also seen an increase in protective enzymatic activities of superoxide dismutase, catalase and glucose 6-P dehydrogenase (100% increase in *C. dubliniensis* and 50% in *C. albicans*) on cytosolic extracts of the pretreated reference strains. MV pretreatment protects the yeasts of cell killing with H₂O₂, being a crossed protection toward this oxidizer. An adaptive response and crossed protection to these oxidants suggest that *C. dubliniensis* would have mechanisms that would allow to respond to generators of peroxide and superoxide.

80. ERYTHROCYTES DEFORMATION: WAVELET ENTROPY AND DYNAMICAL NATURE

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By using nonlinear methods and time-frequency analysis we characterized the cell behaviour under shear stress. 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. 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, using delay coordinates we generate two different phase space with creep and recovery process to be used in the correlation. We used: time delay suggested by Takens, May algorithm, false nearest neighbours proposed by Abarbanel, and modified Grassberger and Procaccia dimension. Here we report studies on 20 donors: 3 α /thalassemic, 7 β /thalassemic and 10 healthy donors non smokers and non alcoholic individuals. 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. On calculating wavelet entropy the mean values for healthy donors was 0.2243, for β thallemic 0,2415 and for α thallemic 0.3012, which could reflect a loose of viscoelasticity from the sample cells. The results also suggests that the time series from β thallemic the correlation decrease with the steps, while the series from healthy donors and also from α thallemic 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.

81. EXPERIMENTAL MODEL FOR THE STUDY OF THE INTERACTION BETWEEN INTRACELULAR PARASITES AND THE HOST IMMUNE SYSTEM

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The study of the interaction between the host immune system and intracellular parasites allows the development of vaccines, immunomodulators and strategic control measures. Our aim was to gain a deeper knowledge about the interaction between porcine mononuclear phagocytes (MNP) and *B. suis* and to study their role in infections with intracellular bacteria. We analyzed the expression of cell surface antigens and functional activity of "in vitro" cultivated MNP of seropositive (S⁺) pigs for brucellosis and seronegative (S⁻) ones from a herd naturally infected with *B. suis* and brucellosis free pigs as controls. Our results showed that S⁺ and S⁻ pigs differed in phagocytic activity, secretion and activity of TNF- α and phenotype of MNP (p<0.05). The animals modulated differently the expression of surface antigens when they interact with virulent or attenuated *Brucella* spp. Moreover, the experimental model was successfully extrapolated to another species, being able to be adapted and utilized for the study of the interactions between *N. caninum* and bovine MNP. The phagocytic activity of the MNP of S⁻ bovines for *N. caninum* was higher than that of the S⁺ ones (p<0.05). With the results obtained we developed a theoretical model which suggests that the different behavior against *in vivo* infection with *Brucella* spp of the groups of animals studied herein would allow the bacterium survival in S⁺ animals and its early elimination in S⁻ ones.

82. EFFECT OF HEPARIN ON SPERM CAPACITATION IN PIGS: DIFFERENT COMPOSITION OF THE INCUBATION MEDIUM

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Sperm capacitation may be promoted *in vitro* by the incubation in media that mimic the oviductal fluids. Although a universal medium does not exist, the presence of Ca²⁺, HCO₃⁻ and BSA (bovine seroalbumin) seems to be essential. Previously, we observed an increase in the capacitation values of sperm incubated during 120 min and at 39°C, with 10 μ g/ml of heparin. To determine the possible interaction between heparin and the mentioned components (Ca²⁺, HCO₃⁻ and BSA), sperm samples from 6 fertile boars were incubated in capacitating media: TALP (CM), non capacitating media: (NC) TALP-BSA-Ca²⁺-HCO₃⁻ and combinations: NC+Ca²⁺, NC+HCO₃⁻ and NC+Ca²⁺+HCO₃⁻, in the presence or absence of heparin. Capacitation was assessed by fluorescent chlortetracycline staining (CTC). Statistics: ANOVA. The rate of capacitation between NC, NC + Ca²⁺ and NC + HCO₃⁻ did not differ significantly regardless of the presence of heparin (p>0.05). Sperm capacitation in CM and NC + Ca²⁺ + HCO₃⁻ showed significant differences with the other media tested (p<0.01). Also, the presence of heparin in these media augments the capacitation (p< 0,05). Conclusions: heparin enhances the *in vitro* capacitation of pig spermatozoa in the presence of Ca²⁺ and HCO₃⁻. The absence of BSA did not affect the capacitation, supporting other reports which suggest it is not essential for sperm capacitation in pigs. Cyclic variations in the number of heparin - like glycosaminoglycans in the pig oviduct have been demonstrated, suggesting the possibility of an important role of these compounds in fertilization.

83. IDENTIFICATION OF HEMATOPOYETIC CELLS IN SWINE HEMOLYMPHATIC NODES

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In reference to the structure and ultrastructure of haemal and hemolymphatic nodes, we found out that the pig has a typical "hemolymph gland". The results allowed for an identification of different haematopoietic cell types in the three categories that were sampled. Cells with eosinophilic cytoplasm, lax chromatin rounded nucleus, evident nucleoli that could be eosinophilic granulocytes were localized. These cells were found disseminated throughout the parenchyma of the organ, its population density being higher in the cortical zone. These cellular elements were seen during mitosis. Megakaryocytes / megakaryoblasts, were observed both in the periphery and the interior of blood vessels. A lobulated nucleus, in central position and lax chromatin with evident nucleoli was also observed. Cellular elements with cytoplasmatic and nuclear characteristics that might be considered polichromatophilic erythroblasts and proerythroblasts, precursor cells of the red series in different maturation stages were also detected. These cells were grouped forming erythroblastic nests or small islands similar to those observed in the marrow bone. The conclusion is that the results confirm the structure and ultrastructure of swine hemolymphatic ganglia suggested by Weller (1938), Ceccarelli *et al.* (1981) and Fagioli *et al.* (1981). The immunologic role detected for these organs coincides with that suggested by Thorp *et al.* (1981) and Ceccarelli *et al.* (1982). The finding of myelopoietic, erythroblastic cells and megakaryoblasts in the parenchyma of these organs has no preceding literature though it coincides with the results obtained by Salazar (1984) for bovine haemal nodes.

84. THE ANSWER TO TWO ENDOMETRITIS TREATMENTS ABOUT PRODUCTIVE AND REPRODUCTIVE INDICATORS IN MILKING COWS

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The objective of the present work has been the assessment of two treatments about productive and reproductive indicators in milking cows. The trial was made between the years 2002 and 2004 in premises of the Iriondo Department, in the province of Santa Fe. The animals with endometritis (G2: flocculus of pus, G3: purulent and G4: bloody and smelly) were randomly assigned to two groups: 1) ceparifin group 500 mg as benzatinican salt (*Mitricure*®) and 2) prostaglandin group: D (+) cloprostenol 0,75 mg (*Prelobán*®), treating them till their cure. The following variables were studied: milk production at 150 days (pl 150), birth-conception interval (ipc), number of services per pregnancy (s), number of deliveries (np) and physical state (1-to-5-ranking) at the first checking. It has been studied the minimum average quadratics and their standar errors for pl, ipc and s per group (t) per each grade through tests of Variacin Analisis and average comparison (Duncan's test, p < 0.05). For np and ec the medians and the range were estimated and the test of Kruskal-Wallis hypothesis was applied. The results have been: ceparifin group for grade 2 and grade 3 there were no significative differences (p \geq 0.05) for the analysed variables, prostglandin group for grade 2 and grade 3 there were no significative differences (p \geq 0,05). There were no significative differences (p \geq 0,05) about np and ec. The results show that there are no differences between the productive and reproductive indicators for both treatments used, although for Prostaglandin there are significative differences in the number of services despite the fact that, for this result the low number of G3 cows should be taken into account.

85. LYME DISEASE IN ARGENTINA: RESERVOIRS AND VECTORS

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An epidemiological study has been done in Funes, Santa Fe, Argentina, to describe tick species described as reservoirs and vectors of Lyme diseases in small mammal. This place was selected according to medical information about human cases of Lyme disease. In 2002 we interviewed ten people which have suffered the Lyme disease in order to check the risk. Small mammals were trapped in the years 2000, 2001, 2002, during a week, three trapings were conducted in the spring of each year and one in the autumn of 2001. Ticks were identified according to stage and species. Rodent species removed were *Rattus rattus*, *Rattus norvegicus* and *Mus domesticus* and the cricetids: *Akodon azarae* and *Oligoryzomys flavescens*. The ectoparasites found were fleas, ticks, and larvae, nymphs, and adults of *Ixodes loricatus* which were collected in *Akodon azarae* and *Oligoryzomys flavescens*. By the cotton sheet method dog's ticks *Rhipicephalus sanguineus* were collected. In the year 2000, cysts of *Taenia taeniformis* whose adults are cats' parasites were diagnosed in the liver of *Akodon azarae* and *Rattus rattus*. The epidemiological research was important because we identified small mammals like *Akodon azarae* and *Oligoryzomys flavescens* infested with the ticks *Ixodes loricatus*. *Ixodes spp* ticks are the most important vectors in countries where Lyme disease is endemic. Other important result was the diagnosis of cats' parasites, because it can show the nexus between rural and urban area, and how can be transmission between domestic and wild animals.

86. PREVALENCE OF HYPERGLYCEMIA IN CANINES FROM CASILDA

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Diabetes mellitus is characterized by a chronic hormonal disorder of fat, protein and carbohydrate metabolism due to a reduction in the production or release or use of insulin. It is early seen with a level higher than 140 mg% in basal conditions. The aim of this study was to know the prevalence of hyperglycemia in dogs from Casilda, analyzing its relationship with sex and age. One hundred and twenty nine blood samples were randomly taken. Glucose determination was done by the quantitative enzymatic method of glucose oxydase working on plasma obtained with EDTA-fluoride. Samples were grouped according to sex and age. From 129 analyzed canines, 23 showed hyperglycemia with a prevalence of 17.8% higher than that of bibliography. Data were analyzed using χ^2 test and there was not association among sex, age and hyperglycemia. This study will continue on a larger population.

87. CORNIFICATION INDEX VARIATION DURING OESTRUS IN DIFFERENT CANINE BREEDS

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Exfoliative vaginal cytology (EVC) is a simple and economic method to predict ovulation 48 to 72hs before its occurrence by measuring the cornification index (CI) periodically. Several authors coincide in that the expected cornification value is highly variable, surpassing, in some cases, 80%, 90% and even reaching 100% as early as 9 days before ovulation or as late as two days after. With the aim of getting deeper into the differences between breeds, the highest CI reached and that detected during female breeding days were calculated. Twelve female dogs (Beagles, Cocker, Labrador, Bull Terrier) were used. Samples for EVC were periodically taken starting on the first day a bloody vaginal secretion was detected. Samples were stained with haematoxylin and eosin stain. The breeding day was registered and pregnancy confirmed for each female. Results show that the highest CI and the moment it is reached (D: days since the start of bleeding) vary between the studied breeds. Results also show that some breeds do not reach the stated cornification index that the literature mentions: CI: CI Beagles: 69.67% - D 13; CI Spanish Cocker: 67% - D 6; CI Labrador: 75.03% - D 15, CI Bull Terrier: 82.77% - D 8.67. This peak indicates that ovulation would occur between 24 and 48 hs after, so mating should be planned two days after its detection. Sub-fertility is the result of the lack of knowledge of the physiology and manifestation of the oestrus cycle for each breed. A serial control of each female dog during the cycle would efficiently render the appropriate moment for breeding.

88. INFLUENCE OF ERYTHROCYTE DEFORMABILITY AND PLASMATIC MACROMOLECULES IN EQUINE LAMINITIS

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Laminitis is associated with a primary process involving other systems, mainly the digestive tract. Is an acute/subacute disease affecting the hoof capsule and soft tissue between hoof and 3rd.phalanx. Objective: to study the influence of some plasma molecules and erythrocyte deformability on blood flow in anticoagulated blood samples in 6(six) horses bearing laminitis and 6(six) healthy controls were analyzed. Sample obtains at 2(two) days disease-onset.

Methods: Erythrocyte deformability by means of erythrocyte rigidity index (RI), relating filtration time of red blood cells (RBC) suspension through a polycarbonate membrane (5 μ m diameter) to filtration time of medium suspension was assessed?

Total proteins (TPr) with autoanalyzer (Hitachi 902), fibrinogeno (Fb,mg/dl). IgG (IgG,g/dl), ceruloplasmine (Cp,mg/dl) and haptoglobine (Hp, mg/dl), by immuno-turbidimetry. Statistic: Wilcoxon test for paired data was applied. Results expressed as median and range show significant modifications ($p < 0.05$) in TPr [8.10,7.8-8.4], Cp [28,25-32], IgC [282,279-285]and Hp[178,172-182]. No significant modifications were observed in RI ($p > 0,05$). We can conclude that in the laminitis the increase in proteins and consequently in plasma viscosity could be one of the factors wich difficult microcirculation flow.

89. SENSITIVITY TO ANTIBACTERIAL AGENTS IN *Staphylococcus aureus* ISOLATES THAT CAUSE BOVINE MASTITIS
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S. aureus is the main agent associated to bovine mastitis. This disease is important for its incidence in economy and in public health as milk could be the source of pathogenic strains for man, some of them resistant to antimicrobial drugs. The aim of this work was to study the sensitivity to antibacterial drugs of *S. aureus* that cause bovine mastitis. Four hundred samples of mastitis milk were analysed and 72 isolates of *S. aureus* were obtained and identified with standard biochemical tests. Susceptibility essays were performed according to the diffusion test through plates and to NCCLS norms. Susceptibility to penicillin, oxacillin, cephalothin, erythromycin, azithromycin, clindamycin, neomycin, gentamycin, streptomycin, rifampicin, vancomycin, chloramphenicol, tetracycline, trimethoprim-sulfametoxazole, ciprofloxacin and enrofloxacin were analysed. Results showed high percentages of sensitivity to cephalothin (100%), oxacillin (98%), trimethoprim-sulfametoxazole (100%), rifampicin (98%), enrofloxacin (97%) and gentamycin (87%). A 14% of strains with a multiple resistance was observed as well as an increase in the percentage of strains with an intermediate resistance to neomycin (42%) and to macrolides. The highest resistance percentages were observed for streptomycin (90%) and penicillin (30%). The data on susceptibility of *S. aureus* to antibacterial can be used together with other parameters as a guide for the selection of drugs for the treatment of bovine mastitis.

90. VASCULAR FLORA OF SANTA FE PROVINCE: *Ranunculaceae*
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The family *Ranunculaceae*, belonging in the order Ranales, comprises some 1500 species of wide world distribution, mainly herbs with numerous stamens and free carpels. In Argentina the family *Ranunculaceae* includes 52 species and 8 genera, though only three genera are present in Santa Fe: *Anemone* L., *Clematis* L. y *Ranunculus* L. The present work contributes to the knowledge of this family providing keys to the identification of different taxa based on morphological characters and a map of their ecological and geographical distribution. The methods consist of a thorough bibliographical review, consultation of national herbaria with important 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 preliminary results show that in Santa Fe only three genera, seven species and three varieties are found. For the genera *Anemone* L. and *Clematis* L. only one species each was found: *Anemone decapetala* Ard. var. *foliolosa* Eichler and *Clematis montevidensis* Spreng. The genus *Ranunculus* L. includes five species (one with three varieties): *Ranunculus apiifolius* Pers., *R. bonariensis* Poir. var. *bonariensis*, *R. bonariensis* Poir. var. *phyteumifolius* (A. St.-Hil.) Hicken ex Lourteig, *R. bonariensis* Poir. var. *trisepalus* (Gillies ex Hook. & Arn.) Lourteig, *R. cymbalaria* Pursh, *R. flagelliformis* Sm. y *R. platensis* Spreng. Taxonomic information, illustrations and a distribution map are provided.

91. VASCULAR FLORA OF SANTA FE PROVINCE: *Araceae*
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The family *Araceae*, belonging in the Order Arales, comprises 120 genera and 1800 species of tropical and subtropical regions of the world. In Argentina the family *Araceae* includes 13 genera and 18 herbaceous species (16 native and two introduced). The present work contributes to the knowledge of this family providing keys to the identification of different taxa based on morphological characters and a map of their ecological and geographical distribution. The methods consist of a thorough bibliographical review, consultation of national herbaria with important collections of the province (SF: Esperanza; SI: San Isidro; UNR: Rosario), field work experience of the authors and lab work to confirm their identity. As a preliminary result only two species were found in Santa Fe: *Pistia stratiotes* L., known as 'repollito de agua', and *Spathicarpa hastifolia* Hook., called 'pata de loro'. It is also likely that *Anthurium paraguayense* Engl. grows in the province, given that it appears in the neighbouring provinces of Chaco and Corrientes in forest environments very similar to those of northeastern Santa Fe. Individuals of 'oreja de elefante' (*Alocasia odora* Roxb.) thriving spontaneously in Rosario city have also been reported. Taxonomic information, illustrations and a distribution map are provided.

92. VASCULAR FLORA OF SANTA FE PROVINCE: *Typhaceae*
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The family *Typhaceae* comprises aquatic herbs belonging in only one cosmopolitan genus, *Typha* L., with about 15 species of all regions of the world. In Argentina the family *Typhaceae* includes three species distributed in wetlands all over the country except for southern Patagonia (Santa Cruz and Tierra del Fuego). The present work contributes to the knowledge of this family providing keys to the identification of different taxa based on morphological characters and a map of their ecological and geographical distribution. The methods consist of a thorough bibliographical review, consultation of national herbaria with important collections of the province (SF: Esperanza; SI: San Isidro; UNR: Rosario), field work experience of the authors and lab work to confirm their identity. As a preliminary result only two species of *Typhaceae* were found in Santa Fe: *Typha domingensis* Pers. y *Typha latifolia* L., both known as 'totoras', growing in shallow pond environments of all natural regions of the province (Chaco, Espinal, Pampa and Paraná River Valley). Taxonomic information, illustrations and a distribution map are provided.

93. CHARACTERIZATION OF SHORT - SEASON SOYBEAN CULTIVARS IN ZAVALLA, ARGENTINA

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A field trial was carried out in Zavalla, Argentina (33° 01' S lat.) in the 2004/05 crop growing season on a Typic Argiudoll soil. Seeds of eight cultivars of Maturity Group II; III and IV were sown on 21 Oct (SD1); 23 Nov (SD2) and 27 Dec (SD3). Lodging, plant height, main stem node number, phenology, photothermic sensibility, and foliar diseases at the end of crop cycle were evaluated. Experimental design was a randomized complete block with 3 replications. DM4800 had the highest lodging score in SD1 and SD2. Plants were taller and with more nodes in SD2 in most cvs., except for DM4800, ADM4870 y A4910RG that had higher values in SD1. Crop cycle decreased from SD1, but there was a different response in the duration of VE-R1; R1-R5 and R5-R7 stages, depending on the photothermic sensibility. AW2886RR and ADM4870 had the highest sensibility to temperature and photoperiod, respectively and A4910RG had the lowest responsive to photoperiod and temperature for the VE-R1 stage. ADM4870 and A4910RG had the highest sensibility to temperature and photoperiod, respectively and AW2886RR and ADM3100 had the lowest photothermic sensibility for R1-R5 stage. ADM3700, ADM4800 and ADM4600 had the highest photothermic sensibility for the R5-R7 stage. In the three sowing dates *Cercospora kikuchii* and *Septoria glycine* were present along with an early incidence of *Pseudomonas syringae*. AW2886RR in SD3 showed a severe anticipated leaf senescence caused by *C. kikuchii* but ADM4200 had the lowest leaf damage in the 3 sowing dates. As cultivar yield rankings differ between sowing dates, new cultivars must be tested yearly to make a detailed description in photothermal sensibility and disease tolerance for predicting cultivar performance.

94. LEAF/STEM RATIO AND IN VITRO ORGANIC MATTER DEGRADABILITY OF ALFALFA (*Medicago sativa* L.) CULTIVARS OF DIFFERENT WINTER DORMANCY

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The objective of this study was to determine leaf/stem ratio (L/S) and *in vitro* organic matter degradability (IVOMD%) of alfalfa cultivars at three stages of development (Vegetative, V; Early Bloom, EB; Full Bloom, FB). The hypotheses were (i) L/S ratio and IVOMD vary with the phenological stage (PS) and the cultivar (cv) and this would be associated with the level of winter dormancy (WD); (ii) differences among cv vary with the PS. The experiment was carried out in Zavalla, Santa Fe, Argentina. Seven cv (Aconcagua (AC)-WD 5; Tahoe (TA) and Victoria (VI) - WD 6; Mayacó (MA)-WD 7; Monarca (MO)-WD 8-9; Araucana (AR) and Reina (RE)-WD 9) were sown in autumn. The experimental design was a complete randomized block design with three replications. In each sample, the L/S ratio was determined on a subsample of 30 stems. The rest of each sample was oven-dried at 60°C and ground to determine the IVOMD (Menke's method). Data were analyzed using a split plot design with complete randomized blocks where the main plot was the cv and the subplot was the PS. Means were compared by Tukey's test. The significance level used was 10%. Results show that L/S ratio and IVOMD decrease with the maturity of the crop and this is particularly associated with each cv but not with the level of WD. L/S differences among cv state in V an EB whereas in FB all cv are similar. Even though IVOMD ranking among cv vary with the PS, AR state a high IVOMD in all PS.

95. WHEAT DRY BIOMASS PRODUCTION IN DIFFERENTS NITROGEN AND TILL CONDITIONS

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The objective of this study was to evaluate the wheat dry biomass aboveground produced with three N levels and two S level under two till production systems, no till (NT) and conventional till (CT) management. The field trials were carried out in Zavalla, Argentina (33° 01' L.S. - 60°53' L.W.) in the 2004/05 growing seasons on a typical Argiudoll soil. The experimental design was a complete randomized block with two replications for each tillage system. Two levels of N fertilizer (check and 120 kg N ha⁻¹) and two level of S level (check and 18 kg S ha⁻¹) were applied at tillering. The dry biomass aboveground were measured at 3.1 (first node detectable), at 6.0 (flowering) and harvest ripe (HR) (Zadoks *et al.*, 1974). Were evaluated spike.m⁻² (SN), dry biomass spike (DBS), dry biomass kernel (DBK), dry biomass stover (DBST) and dry biomass aboveground (DBA), at 6.0 and HR. Data were analyzed using ANOVA method and Duncan's Test. The tillage systems and the treatments were not significant at 3.1. At 6.0 stage, were significant differences for N level for DBA, DBS (p<0.01), DBST and SN. Level S differences were significant (p<0,10). At HR, were significant differences among tillage systems for DBS (p<0,10) and DBK (p<0,01). The N level were significant for DBST, DBA (p<0,10) and DBK (p<0,05). In conclusions, N level had more impact on DBA and DBK than the tillage systems.

96. EFFECT OF SEEDING DATA ON THE PHYSIOLOGICAL QUALITY OF SOYBEAN SEED (*Glycine max* (L.) Merrill)

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The most intensity seed aging process is produced during the latest period of grain filling, R₅-R₇. So, a delay in the sowing date, SD, expose that period to unfavorable climatic conditions that may cause the loss of seed viability, V, and vigor, Vg. With the object to describe the type of damage that can affect seed quality when the production ambient is modified, an assay was made with the cultivar A 4910 RG in two SD 21/10/04 and 27/12/04. The occurrence of a strong hail produced, besides, damage on the seed structures in the second SD. To determine the alterations produced by diverse agents during the ontogenic cycle that affected seed quality, there were studied the V. and Vg by the Tetrazolium Test. In the first SD were observed a 72% of viable seeds without damage; 94 % of V and 89% of Vg. In the second SD there were a 28% of seeds without damage; 80% of V and 57% of Vg, as a consequence mostly to ambiental damage (44,5%) and by sting attack (42%). Besides, it was detected in that SD, the presence of green grain (8%) and mechanic damage (6%), produced by the impact of hail in the pod and seed. The results showed significative differences between SD in the variables studied. The conclusions were: a) The selección of the SD for each cultivar must be considered on the base of the genotype response to ambiental conditions, b) The most important damages registered were attributed to the ambiental damage during seed filling and to the sting damage.

97.

ASSESSING THE PENETRATION INSTANCES OF *Fusarium graminearum* IN CORN SILKS (*Zea mays* L)Incremona M¹, González M del P¹, Pioli R¹, Salinas A².¹Cát. de Fitopatología. Fac. de Cs Agrarias. CIUNR U.N.R. ²Cát. de Fisiología Vegetal. Fac de Cs Agrarias. CIUNR U.N.R.

The objective of this work is to assess the effective penetration instances of *F. graminearum* through the silks. Two hybrids with two replications were inoculated in two instances: a) before pollination and b) after pollination. In both cases the pollinating method was artificial. The silks were extracted during 48 hs and were separated in two samples at the laboratory. Each sample had 12 silks / replication / hybrid. One of the samples was clarified in a solution of 5% Na OH containing 0.2 g of trypan blue, the silks were clarified in fresh lactofenol. So as to observe the advance of the fungal infection, the silks were then examined in fresh glycerol with an optical microscope. The other sample was submerged in a medium containing potato-glucosade-agar (PGA) with lactic acid. After a seven days incubation of 12 hs (light/darkness) the colonies of *F. graminearum* were identified according to their macro and micro-morphology. The presence of hyphae were observed in the silks extracted before pollination and 32% *F. graminearum* colonies in PGA. After pollination the presence of hyphae were observed and 32% *F. graminearum* colonies in PGA. The penetration of *F. graminearum* in the corn silks was produced both before pollination when the silks were not pollinated and after pollination.

98.

INCIDENCE OF THE FUNGIC PATHOGENS ON EMBRYO, COTYLEDONS AND TEGUMENTS OF ARTICHOKE (*Cynara scolymus* L.) SEEDSAcuña L¹, Incremona M¹, Gonzalez M¹¹Estudiante de Licenciatura en Biotecnología. ²Cát. Fitopatología. Fac. Cs. Agrarias. UNR. C.C. 14. (S2125ZAA) Zavalla. E-mail: mgonzale@unr.edu.ar

The object of the present work was to determinate the incidence of fungic pathogens on embryo, cotyledons and teguments of artichoke seeds. Until the incubation, the seeds were dissected on embryo (E), cotyledons (C) and teguments (T). These parts were put in Petri dishes with potato dextrose agar 2% and were incubated with alternating light (ISTA, 2003). The cultivars were Oro Verde, Imperial y Estrella del Sur. The 100 seeds of each cultivar were evaluated. The fungi were identified with light and stereoscopic microscopes. The infection percentage was established. On Oro Verde cultivar were made the identification of: *Alternaria* sp 2% (E); *Aspergillus niger* 1% (E) and 1% (C); *Chaetomium* sp 14% (E), 52% (C) and 100% (T) and bacteria 17% (E) and 50% (C). On Imperial: *Alternaria* sp. 3% (T); *Aspergillus niger* 17% (E) and 31% (C) and 15% (T); *A. flavus* 2% (E), 4% (C) and 2% (T); *Chaetomium* sp. 1% (C); bacteria with 35% (E), 70% (C) and 1% (T); yeast with 4% (E), 22% (C) and 1% (T). On Estrella del Sur: *Fusarium* sp. 2% (E), 6% (C) and 16% (T); *F. verticilloides* 4% (E) and 1% (C); *F. graminearum* 1% (T); *Alternaria* sp. 3% (C) and 7% (T); *Chaetomium* sp. 1% (T); *Verticillium* sp 1% (T); *Rhizoctonia* sp. 1% (T); bacteria 41% (E), 71% (C) and 6% (T). All of the seeds of Oro Verde were infected at least one of these tissues. The other two cultivars presented 9% and 14%, without infection at least one of these parts.

99.

VIRUS FREE PLANTS OF ARTICHOKE (*Cynara scolymus* L.) FROM MERISTEM TIP CULTUREPigozzi L¹, Rotondo R, Cravero V, García S, González M, Cointry E.

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The objective of this work was to micropropagate artichoke plants of high level of health. On March were obtained 119 meristem tips of shoots of Frances cultivar. The explants were disinfected with sodium hypochlorite (2%), alcohol 70% and tween 20. The meristems were placed in a medium with Murashige y Skoog (MS) macro and micronutrients, 0,1 mg/l of giberelic acid, 0,1 mg/l of kinetine, 20 g/l of sacarose and 7 g/l of agar, pH 5.8. After a month of culture the explants were put in a MS medium with 0,1 mg/l of naftalene acetic acid, 0,1 mg/l de benzilaminopurine, 30 g/l de sacarose and 8 g/l of agar and pH 5.8. Considering the total of the explants 91,6% were free of contamination on the sown. Five months later 44% of the explants were lost by the contamination, the other 56% grew and gave 1 or more shoots. The proliferation rate by subculture (number of shoots / meristem / 30 days) on proliferation medium was 1,09 on the first month, 1,28 on the second, 2,23 on the third and 6,07 on the fourth month. The tests to determine the sanity were made with: *Chenopodium amaranticolor*, *Datura stramonium*, *Nicotiana benthamiana*, *Nicotiana clevelandii*, *Gomphrena globosa* and *Phaseolus vulgaris*. The results show the healthy of the plants obtained through meristem culture. The identification of viruses will be complete with ELISA test.

100.

ASSESSMENT OF GERMINATION CAPACITY OF *Phakopsora pachyrhizi* UREDINIOSPORES IN LABORATORY CONDITIONS, IN ARGENTINAMaumary RL¹, Pioli R², Ivancovich A³, Astegiano E⁴, Herzog L⁵.¹Beca Postgrado CONICET/INTA, ²Fitopatología, FCA.UNR, ³EAA INTA Pergamino, ⁴Director Progr. Riia FCA-UNL, ⁵Fitopatología UNL. Argentina. E-mail: roxilm@yahoo.com.ar

Several hypothesis on dispersion and survival capacity of *Phakopsora pachyrhizi* had been formulated after the introduction of the pathogen in Argentina. Some studies reported that urediniospore viability can be preserved for 50 days but the information about conditions that affect this process is minor. The objective was to evaluate the germination capacity and viability of the urediniospores during 10 days after uredia maturity and release of urediniospores (damr) under different temperature regimes, in laboratory conditions. The experiment was carried out on five to eight uredinia formed on the same petiole and development stage, with 4 replicates. Combined samples of 750-1000 to 1200-1600 urediniospores were evaluated. Germination was promoted placing the urediniospores into pendulous drop in excavated glass, under appropriated temperature regimes and humid chamber. The germination and viability of urediniospores was determined as the proportion of spores with a developed germination tube / total number of spores for each evaluation moment. Mean germination expressed in percentage values, were: 90.8 ± 8.7; 87.4 ± 3.8; 70.7 ± 10.9; 31.4 ± 5.6 y 17.2 ± 2.8%, registered with 23°C and humidity, at 2, 4, 6, 8 y 10 damr, respectively. According to our result the urediniospore viability decreased 0.6, 4.0, 20.7, 60.0 y 74.2% during evaluation period under our environmental conditions.

101. FIRST REPORT ON PATHOGENIC VARIABILITY IN THE INTERACTION *Glycine max* – *Diaporthe phaseolorum* var. *caulivora* IN ARGENTINA

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Soybean stem canker (SSC) is caused by two fungal varieties: *D. phaseolorum* var. *caulivora* (DPC) and *D. phaseolorum* var. *meridionalis* (DPM). During 2001/02, an outbreak of SSC caused by DPC was reported in the main soybean producing area of Argentina. Morphologic and molecular identification of 19 isolates, collected in 2003/04 from 12 locations of Santa Fe and Buenos Aires Provinces, determined that DPC is the predominant variety of *D. phaseolorum*. The objective of this work was to evaluate the reaction of 35 soybean genotypes inoculated with three local DPC isolates: DPC-S, DPC-ZA and DPC-ES, and one DPM isolate: CE109, in order to detect pathogenic variability among distinct DPC isolates. Greenhouse inoculations were done by inserting a mycelia portion in the hypocotyls and covered with vaseline. Thirty-six replicates per interaction were used. At 15, 30 and 47 days after inoculations (dai) the percentage of death plants was measured. At 50 dai, the soybean genotypes were grouped into six categories, according to the result of the plant-pathogen interactions: i) resistant to all isolates assayed, ii) resistant to the three DPC isolates but susceptible to the DPM isolate, iii) resistant to DPM and to two DPC isolates but susceptible to one DPC isolate, iv) resistant to DPM and to one DPC isolate but susceptible to two DPC isolates, v) resistant to DPM but susceptible to the three DPC isolates, and vi) susceptible to all isolates assayed. These results constitute the first report about differences in the aggressiveness and existence of local physiological races of DPC.

102. JUVENIL GENE INCORPORATION IN SOYBEAN AND ITS EFFECT ON TEMPERATURE RESPONSE DURING THE REPRODUCTIVE STIMULUS PROPAGATION

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Photoperiod, P, and temperature, T, are the main environmental factors that synchronize soybean flowering with seasonal variations. Reproductive stimulus propagation, RSP, (*i.e.* transformation to reproductive way of all meristems) delays one or several weeks. Days elapsed among first flower apparition, and first flower apparition in apical node, are considered like a all plant RSP time measure. Evidences exist that juvenile gene does not only modify P response but also affects T response in flowering control. This work was done to quantify the effects of juvenile gene incorporation on soybean PER, in T function. Three pairs of isolines with (J) and without (N) the juvenile gene were used. Two experiments applying 5 different T covering a total rank of 12°C was developed. T smaller (sub) or greater (supra) that the T optimum was applied [mean T, (T_m), from 20,5 to 32,5°C]. P was 12 and 8 h d⁻¹ respectively. First flower anthesis (IF) and main stem apex first flower anthesis (Ra) were registered. In T sub-optimum rank, not differences in IF-Ra interval duration between J and N was observed. IF-Ra mean values between J and N were 2,2; 2,3; 2,9; 2,3; 2,9; 2,7 and 3,2 d for T_m of 20,5; 23,1; 24,6; 25,6; 26,6; 27,8 and 28,5°C respectively. Differences between J and N were significant in T supra-optimum rank (P<0,0001). IF-Ra increased with T_m increase. From 30,4°C so much in J as in N, with values of 9,7; 14 and 17 d for J and 3,2; 4,3 and 4,3 in N for 30,4; 31,2 and 32,5°C T_m respectively. Supra-optimum T have a greater impact in isolines J that in N on RSP.

103. DENITRIFICATION CAPACITY OF *Bradyrhizobium* sp. ISOLATES FROM SOILS CULTIVATED WITH SOYBEAN

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Denitrification is the microbial process in which nitrogen oxides are used as alternative electron acceptors for energy production. Two criteria must be met for an organism to be called a denitrifier: N₂O and N₂ are products of nitrate reduction and this is coupled to a growth yield. The objective of the present work was to determine the phenotypic characteristic of denitrifying from *Bradyrhizobium* sp. isolates. They were obtained from 2 silt loam soils cultivated with soybean from Pergamino and Manfredi. Eleven isolates were analyzed, out of 41 which have been described previously (another work) as being qualitatively denitrifiers. The isolates were grown in tubes with septa for gas extraction containing Bergensen medium with 10 mM KNO₃ (3 replicates) and incubated at 28°C. The atmosphere was replaced with filter-sterilized N₂ and C₂H₂ (10%). After 7 days of incubation, N₂O production was quantified with gas chromatography and cells were harvested by centrifuging. Bacterial protein concentration was determined by the method of Bradford. Out of the 11 qualitatively denitrifiers analyzed, only 2 isolates grew to a limit extent and N₂O was not detected. Denitrifying ability was confirmed in 9 isolates. The N₂O production varied from 0.02 to 0.57 μmoles ml⁻¹. The protein concentration varied from 48.22 to 153.54 mg l⁻¹. Significant differences (p<0.05) were observed between the 9 isolates with respect to N₂O production. Therefore, we conclude that: (i) a great proportion of *Bradyrhizobium* sp. isolates significantly contribute to the denitrification capacity of the studied soils, (ii) the denitrifying ability differs among isolates from the cultivated soybean soils.

104. RELATIONS BETWEEN CALCIUM EXCRETION AND URINARY PHOSPHORUS TO THE NUTRITIONAL STATE OF A UBN (UNFULFILLED BASIC NEEDS) POPULATION

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The aim of our work was to evaluate parameters related to metabolism, such as urinary and seric calcium (Ca) and phosphorus (P), and relate them to the nutritional state of children who live in the poor outlying areas of the city of Santa Fe. We worked with 100 children aged between 2 and 6, selected at random from the UBN population. Anthropometric parameters (weight and size), which allowed us to classify the nutritional state of these children, were determined. Three nutritional states were identified: acute malnutrition 32%, chronic malnutrition 30%, and in risk 38%. The blood samples determined the seric Ca and P concentrations. The isolated urine samples showed the renal excretion of Ca and P in relation to Creatinine (Cr)(mg/mg). The obtained data were compared to the following cut rates from a population of well-nourished children: Ca/Cr 0.090 and P/Cr 1.06. A higher excretion in the Ca/Cr and P/Cr rates was observed in these children, this being highly meaningful (P < 0.0001). Variations in the seric concentrations of calcium and phosphorus were not observed. After analyzing the Ca/Cr and P/Cr rates in each of the three groups, it was proved that in the chronic malnutrition group 86% presented Ca/Cr rates in the excretion which were significantly superior to the 8% in relation to the cut rate. The phosphorus excretion showed a significant increase in the acute malnutrition group in 79% of the children (P < 0.10). According to obtained data the children would present a minor Ca and P retention in the organism, possibly related to proved malnutritional state.

105. EFFECTS OF TWO ALUMINUM (Al) SPECIES ON DIFFERENT PHYSIOLOGICAL SYSTEMS IN ADULT MALE RATS

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Our goal was to study and compare the toxic effects of 2 Al species on biological systems during chronic intoxication. Male Wistar rats were distributed in 4 groups: treated (Lac, n=7; Al₂, n=8) and controls (C₁ y C₂ respectively, both n=5). Lac and Al₁ were injected IP with 0,5ml of Al lactate (Lac₃Al) and Al hydroxide ((OH)₃Al), respectively, and C₁ and C₂ with 0,5ml of sodium lactate and saline, 3 times per week, for 3 months. At the end of the 1°, 2° and 3° month, water and Na balances and osmolarity were measured, urine concentration tests were realized, and blood was extracted from the tail for hematological parameters. At the 3° month, we measured seric aldosterone (Ald), glutation-SH (GSH) and degree of lipoperoxidation (LPO) in liver and kidney, and seric and tisular Al. Results: seric Al concentration* was 1200 in Lac and 210µg/L in Al₂ (C₁:7,9; C₂:5,5µg/L). RBC concentration* increased in both groups from the 1° month, (higher significance in Lac: Lac:7,94, C₁:6,75; Al₂:7,39, C₂:5,97M/L^a). The Hct* was reduced only in Lac (Lac:31, C₁:37; Al₂:36, C₂:36%^a). Both treatments reduced MHC* (Lac:37; Al₂:13%^{ab}) and MCV* (Lac:36; Al₂:10%^{ab}), with more effects in Lac. RDW increased with both treatments. There was a decrease in urine osmolarity*, accentuated in Lac in the 2° and 3° month (Lac:1862, C₁:2484; Al₂:2461, C₂:2356mOsm/kg^a). Na excretion* increased with Lac₃Al (Lac:106; Al₂:10%^{ab}), and so did Ald* (Lac:129%, Al₂:123%^{ab}). Oxidative stress in liver and kidney increased in both groups, with higher levels of LPO and lower levels of GSH*, (significant difference in GSH in kidney with Lac₃Al: Lac:57; Al₂:26%^{ab}). Discussion: Lac₃Al, due to its major stability and solubility, could induce greater effects on tubular renal functions, metabolic functions and on the hematopoietic system. (*p<0,05; ^a values at 3° month, ^b percentages regarding control values).

106. IN-VITRO REGENERATION OF ASPARAGUS ANTHER CULTURE

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The F₁'s hybrids shown a higher yield than traditional populations due to heterosis but the conventional production techniques of these hybrids are a slow process. The anthers culture produce homozygous females (mm) and male plants named supermales (MM) depending on the physiological condition of donor plants and the genotype used. The aim of this work was to identify from a group of 14 elite males genotypes, those with the ability to regenerate plantlets from anthers. The anthers were placed on MS medium supplemented with 2 mg.l⁻¹ of ANA and 1 mg.l⁻¹ of BAP and transferred to a MS medium with 0,1 mg.l⁻¹ of ANA, 0,1 mg.l⁻¹ of KIN and 0,65 mg.l⁻¹ of ancymidol for their regeneration. The androgenetic response (AR) was evaluated as the relation between the number of regenerated callus and the total of anthers placed and the regeneration rate (RT) as the relation between the number of shoots regenerated and the total of anthers placed. It was also estimated the proportion of rooting (TE). Statistical analyses were performed using Chi-square test for AR and it was done a Cluster's Analysis to assemble homogeneous genotypes towards a similar AR, RT and TE. Significant difference for AR between genotypes ($\chi^2=1026.9$; p<0.001) were found. 32% of callus produced were yellow; 49,6% were green and the rest brown. Only yellow callus regenerate plantlets in a proportion of 29,1%, shown significant differences between genotypes ($\chi^2=186.8$; p<0.001). The rooting percentage was 82,1. Four groups with different characteristics was established. The efficiency of the method was 7% for the genotypes 461, 664, 700 and 894. 40% of the regenerated plantlets were haploids, which let us conclude that from them we can derivate lines for the production of simple hybrids.

107. PROTEICS PROFILES IN PEA (*Pisum sativum L.*)

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The pea seed has high proteins levels. The proteins are synthesized during grow, then are stored and finally are hydrolyzed for the developing embryo during germination so they are call storage proteins. Differences between genotypes can permit characterizer the germplasms as potentials parentals for later hybridizations. Genetics studies have previously been reported that storage proteins are inherit with discretos characteristics and in codominance. The storage proteins have low influence of environment so are an important biochemical marker and they allow a quick and cheap assay of varieties compared to other techniques.

We study the genetic diversity of 14 commercial variety of pea by separation of storage proteins in polyacrilamide gel (12%), (SDS-Page). The proteics profiles were compared by conglomerates assay using Jaccard distance. Genetics distances were analyzed through Nei index (NI). The analysis showed four groups (G). G₁ was composed for four cultivars: Canada B, Eric 2, Marina and Amarilla; G₂ for C2001 and DDR 11 cultivars, the G₃ only by Cometodo cultivar and G₄ for Turf, Sprut, Inca, Aparecida, Viper, Explorer and B2001 cultivars. The higher values of NI corresponded to Cometodo (NI=0,15), Marina (NI=0,14), C2001 (NI=0,13) and DDRII (NI=0,12). The biggest genetic distance were found between Marina and Cometodo (NI=0,23). The results show that in these materials the genetic variability available was of 36 %. The used method allowed the characterization of materials at divergent groups which is important to planning the hybridizations.

108. MOLECULAR CHACTERIZATION OF THE IN VITRO CULTURE RESPONSE IN TOMATO (*Lycopersicon spp.*)

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Polymorphism in the total protein patterns is an indicator of variability for genes associated to regeneration ability in various plant species. The objective was to characterize the early expression of the proteins involved in tomato organogenesis from explants of genotypes with different regeneration abilities. Cultivar Caimanta of *L. esculentum* was the highest regenerating genotype, while accession LA722 of *L. pimpinellifolium* was the lowest regenerating genotype. *In vitro* culture was performed according to the standard protocol for tomato. Ten samples from each genotype were analyzed at different incubation periods (among 1 to 10 days), considering the explants without being cultured as the experimental testers. Total proteins were extracted from these samples in the phosphate buffer and then separated in SDS-PAGE. Polymorphism was found for polypeptides having molecular weights of 76.6, 52.1, 49.7, 44.5, 27.9, 24.7, 24.1, and 19.5 kDa. These polypeptides accounted for the 57 % of the total protein patterns. Polymorphic polypeptides between incubation periods were those of 76.6, 49.7, 44.5, and 24.7 kDa. Polymorphic polypeptides between genotypes were those of 52.1, 27.9, 24.1, and 19.5 kDa. Polymorphism in the total protein patterns between early incubation periods and between genotypes of different regeneration abilities indicates variability for genes associated to the *in vitro* culture response in tomato.

109. ANALYSIS OF THE PROTEIN COMPOSITION OF GERMOPLASM OF SOYBEAN

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The soybean grain has two types of proteins, storage proteins (mainly globulins), and biologically active proteins like lipoxigenases and the tripsins inhibitors. The globulins can be classified in two fractions, the β -conglucinin composed by the subunits: α (~67 kDa), α' (~71 kDa) and β (~50 kDa), and the glycinins composed by two subunits: one acidic and another basic. Several authors have observed a negative correlation between the subunit β and the methionin content, as well as a positive correlation among the acidic subunits at globulins and the stability of the soybean noodle. The objective of this work was to characterize the protein composition of exotic soybean germoplasm. Extractions of proteins fraction from exotic genotypes were carried out using standard protocols and separated by SDS-PAGE. In most of the tested genotypes no differences in the size of the main proteins were observed. Nevertheless in the PI 88302-1, showed a 44 kDa fragment that was not detected in the rest of genotypes and PI 200485 was the only genotype lacking 74 kDa fragment. A smaller relative amount of 47 kDa fraction was observed in genotypes PI 123439, PI 165896, PI 227687, PI 542045 and PI 408251. BRM92-6600, PI 408251, PI 506817, BAG527 were high lighted as they showed differential expression of 96 kDa fraction. The polymorphic fractions (74, 47 and 96 kDa) showed molecular weight similar to subunits α' , β at β -conglucinin and the lipoxigenases. This data suggest that the genotypes that possessing fractions could contribute in the development at differential quality in soybean germoplasma for the consumption and industrial use.

110. CHARACTERIZATION OF TOMATO GERMOPLASM BY MORPHOLOGICAL DESCRIPTORS

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To characterize different tomato genotypes by morphological descriptors and to estimate their genetic divergence were used: ToUNR1, ToUNR18, ToUNR2, ToUNR4, ToUNR5, ToUNR6, ToUNR7, ToUNR8, ToUNR9 (recombinant inbreeds lines obtained from an interspecific cross between the cultivar Caimanta of *Lycopersicon esculentum* and the accession LA722 of *L. pimpinellifolium*) and the cultivar Caro Sel. INTA La Consulta. The analyzed morphological descriptors were: number of leaves to first cluster (D1); number of leaves between first and second cluster (D2); number of flowers in the first cluster (D3) and number of flowers in the second cluster (D4). A randomized design was used (Number of plants=129). Means values were compared by a one way ANOVA and a multivariate analysis was used to cluster the genotypes for the analyzed descriptors. Highly significant differences ($p < 0.01$) were found among the genotypes for D1 ($F=2.57$), D2 ($F=4.91$), D3 ($F=3.40$) and D4 ($F=4.29$). The clustering analysis showed the genotypes in two clusters being the D3 and D4 descriptors those that presented significant differences. The cluster N°1 was constituted for: ToUNR18, ToUNR2, ToUNR4, ToUNR6, Caro Sel. INTA La Consulta and ToUNR1 and the cluster N°2: ToUNR5, ToUNR7, ToUNR8 and ToUNR9 (genotypes with bigger values for D3 and D4). As a conclusion, the genotypes were different and could be characterized for the analyzed morphological descriptors. In the clustering analysis, D3 and D4 were the discriminatory descriptors.

111. IMMUNOLOGICAL STIMULUS TO INCREASE LAMB PREWEANING WEIGHT

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Different infection agents could reduce the productivity level of animals reared in range conditions because they cause stress and may retard growth. Nevertheless they are essential for the learning process of the immune systems. It is known that the maturity of the immunological system begins very early in the life of the animals and it could be enhanced if animals are immunized with saprophyte agents. This improvement could also produce an increase in lamb weight. In this experiment lambs of one day of age were immunized by bacteria killed by heat: *Tsukamurella inchoensis* (Ti), *Gordonia bronchialis* (Gb) and *Rhodococcus coprophilus* (Rc) (subcutaneous doses: 1 mg of protein in 0.1ml of buffer borate (pH8). Testers (T) only inoculated with 0.1ml of buffer borate). Lamb weights were registered at different ages: at lambing, one month of life and at weaning. Lamb Feed Efficiency (FE) was estimated in the first month of life by the relative Average Daily Gain (ADG/ mean weight in the period). Ewe weight (EW) at lambing was always registered. No significant differences for weight lamb in this preweaning period were found among treatments and T but a significant correlation was found between EW and FE ($p < 0.10$). Apparently mother's corporal state at parturition could affect the weight of the lamb and provoke an increase in the error variance that make unable to detect differences among treatments. Maternal environment conditions could be another factor to be control in this kind of experiment.

112. IN VITRO CULTURE OF SUNFLOWER MATURE EMBRYOS FOR THE SELECTION OF IMAZAPYR RESISTANCE

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The objective of this work was to determine the expression of imidazolinone resistance of mature sunflower embryos cultivated *in vitro*. The genotypes evaluated were three inbred lines differing for their degree of imidazolinone resistance: susceptible (S), intermediate (I) and resistant (R). Culture media was a half-strength MS (Murashige y Skoog, 1962) supplemented with different doses of imazapyr: 0 (control) - 1,25 - 2,5 - 5 - 7,5 and 10 μ M. For each treatment two replications of 10 seeds each were grown in sterility conditions and incubated at $25 \pm 2^\circ\text{C}$ with a 12-h photoperiod for 8 days. The variables hypocotyl (HI) and root length (RI) were analysed through ANOVA. The S genotype showed short, tip-necrosed roots in presence of herbicide. Principal roots of I genotype elongated on media containing herbicide, but secondary roots were poorly developed. R inbred line root growth for all herbicide doses did not differ from the control. Both S and I genotypes showed significant differences between control and herbicide treatments for RI. Aerial biomass of S inbred line diminished as imazapyr doses increased, while HI of R and I genotypes were slightly affected. It is concluded that different resistance degrees can be discriminated by *in vitro* culture of mature embryos with imazapyr as selective agent. This *in vitro* screening method could be useful to assist conventional sunflower breeding programs.

113. REGENERATION ABILITY FROM DIFFERENT COTYLEDONARY REGIONS IN SIX SUNFLOWER GENOTYPES

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The aim of this work was to evaluate the regeneration ability from three different regions of cotyledonary explants in six sunflower inbred lines. The inbred lines K-3225, HA89B, N-834, GP762B, N-855 y HA300B were cultivated *in vitro* on the basal medium MS (Murashige y Skoog, 1962) supplemented with 1 mg l^{-1} IAA, 2 mg l^{-1} KIN. Proximal (P) middle (M) and distal (D) cotyledonary sections of two-days-old seedlings were used as starting materials. Cultures were grown at $25 \pm 2^\circ\text{C}$ with a 12-hr photoperiod for 30 days. The experimental design was a complete randomised with three replications of 20 explants each. The regeneration capacity was evaluated as the *average number of shoots per total explants* (A_v). Transformed data ($(x+0.5)^{1/2}$) were subjected to an analysis of variance. Significant differences were observed among genotypes ($F=16.85^{**}$) and cotyledonary regions ($F=12.99^{**}$). Inbred line N-834 showed the highest regeneration capacity ($A_v = 2.57$). For all genotypes P explants showed higher regeneration potential, and a decreasing organogenic response was observed from P to D sections. Mean values for A_v were 1,52, 0,76 y 0,26 for P, M and D sections respectively. Regeneration ability from cotyledonary explants in this species is strongly influenced by the genotype and by the region from which the explant was obtained. The distance to the cotyledonary node plays a preponderant role in the expression of shoot forming capacity.

114. MORPHOMETRICAL CHARACTERIZATION OF THE CLAW CLOSER MUSCLE OF *Chasmagnathus granulatus*

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All crustaceans muscle fibers are striated with a wide diversity of morphological characteristics, mainly in their sarcomere length. There are fibers with short, long or intermediate sarcómeros (SS, LS and IS respectively). The claw or cheliped is a multifunctional organ, which is used during feeding, mating, agonistic interactions and burrowing. It is composed by two antagonistic muscles: the closer and the opener. The aim of the present study was to characterize, based on sarcomere length, the different types of fibers in the claw closer muscle of *Chasmagnathus granulatus*. Adult male crabs in intermoult (N=3) were caught off the Mar Chiquita Lagoon (Bs. As., Argentina) and acclimated in aquarias for ten days. The animals were cold anesthetized and their claws were removed. The closer muscles were fixed with Bouin's watery solution during 24 hs. The sarcomere length of muscle fibers were measured from dorsal, medial and ventral regions of both claws. The claw closer muscle exhibited a heterogeneous composition of fiber types (LS: 62.9% and 65.8%; IS: 27.8% and 21.6%; SS: 9.3% and 12.5% for right and left claws, respectively). Fiber types frequencies of each region showed the same order observed in the whole claw (LS>IS>SS). The LS fibers ratio decreased from dorsal area toward ventral zone of the muscle, whereas the IS and SS fibers ratio increased, following a similar distribution for both claws. The claw closer muscle of *C. granulatus* presents different types of fibers, prevailing the LS fibers. The morphology of right and left claws and the closer muscle composition would indicate similar functions for both claws in *C. granulatus*. This differs from observations made in aquatic and semi terrestrial heterochelous crustaceans.

115. PROTEIN PROFILE OF MUSCLE OF EURYHALINE CRABS *Cyrtograpsus angulatus* AND *Chasmagnathus granulatus*

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We have previously shown differential responses of alkaline phosphatase activities and an increase of Na^+/K^+ ATPase activity in chela muscle of *C. angulatus* and *C. granulatus* upon hypo-osmotic stress. The aim of this work was to study the protein profile of chela muscle of both crabs in different salinities. Male adults were acclimated for 15 (t15) or 33 (t33) days to 35‰ salinity (S) and 15 days at 10‰S (*C.angulatus*) or 38-40 days to 35‰ or 10‰S (*C.granulatus*). An aliquot (20µg proteins) of muscle homogenates (0.25M Sucrose/0.5mM EGTA-Tris pH 7.4) was loaded into native polyacrilamide gels and PAGE-SDS gels (β mercaptoethanol). (Molecular weight (MW) standards: carbonic anhydrase for native gels; Sigma standards mixture for PAGE-SDS). Proteins were stained with Brilliant Blue Coomassie R-250. In *C. angulatus* upon osmoconforming conditions (35‰S) occurred a decrease by t33 in several bands (Rf=0.045; 0.134; 0.239; MW:180-84 kD) and an increase in bands with MW lower than 37 kD. In 10‰S a band of Rf= 0.239 and bands with MW 180-84 kD were increased by t33. In *C.granulatus* in 10‰S occurred an enhancement in bands with Rf= 0.022-0.134 and Rf=0.239 and MW between 180-84 kD and 48-37 kD. Our results show the occurrence of variations in protein profile in chela muscle upon hypo-osmotic stress which could be related to adjustments (i.e. cell volume regulation, mobilizations of energy substrates) secondary to osmo-ionoregulation. Whether increases in high molecular proteins are related with a synthesis *de novo* of Na^+/K^+ ATPase remains to be investigated.

116. IN VITRO EFFECT OF SEROTONIN ON BRANCHIAL Na^+/K^+ -ATPase ACTIVITY OF THE EURYHALINE CRAB *Cyrtograpsus angulatus*

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Biogenic amines appeared to regulate Na^+/K^+ -ATPase activity of posterior gills of some euryhaline crabs. We have previously shown a *in vivo* effect of serotonin on Na^+/K^+ -ATPase activity of anterior gills of *C. angulatus* from Mar Chiquita coastal lagoon in reduced salinity. The aim of this work was to study the effect *in vitro* of serotonin on Na^+/K^+ -ATPase activity in anterior and posterior gills of *C. angulatus* in reduced salinity. Male adult crabs were acclimated at 10 ‰ S for at least 10 days. Branchial gills sections were incubated at 30°C in saline (400 mM NaCl, 10 mM KCl, 20 mM imidazole (pH 7.4)) ($2 \text{ ml} \times 100 \text{ mg tissue}^{-1}$) or in saline + 10^{-4} or 10^{-5} M serotonin. At 15 or 60 min tissue was separated. Na^+/K^+ -ATPase activity was determined in 10000xg supernatant from anterior gill 4 and posterior gill 6 homogenates (0.25 M sucrose/0.5 mM EGTA-Tris pH 7.4) by measuring ATP hydrolysis in the presence of (mM): 20 imidazol (pH 7.4)/100 NaCl/30 KCl/0.5 EGTA (control: without KCl, with 1 mM ouabain). Three independent experiments were carried out. ANOVA was used as statistical analysis ($p < 0.05$). At 15 min 10^{-4} or 10^{-5} M serotonin did not affect Na^+/K^+ -ATPase activity in either gill 4 or gill 6. At 60 min 10^{-4} M serotonin increased (26%) Na^+/K^+ -ATPase activity in gill 6 whereas no effect was found in gill 4. Our results show the direct effect of serotonin on posterior gill 6 and a differential response of Na^+/K^+ -ATPase activity in anterior and posterior gills suggesting the occurrence of differential mechanisms of regulation mediated by this biogenic amine in individual gills of *C. angulatus*.

117. LECTIN HISTOCHEMICAL STUDY OF THE GERMINAL EPITHELIUM OF *Genypterus blacodes* (PINK CUSKEEL)

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The ovary of *Genypterus blacodes* is typical of teleost fishes. The germinal epithelium borders upon the ovarian lumen. Epithelial cells show morphological changes depending on the maturity stage. During the spawning season the flat squamous epithelium becomes columnar shaped and evidences glicoproteins (GPs) secretion activity. GPs probably form a mucous envelope surrounding the spawned oocytes. The aim of this work is the characterization of the lectin histochemical pattern of GPs and its distribution in the germinal epithelium during spawning stage. Samples were collected from fishes captured in San Jorge Gulf, fixed in 10% formaldehyde and embedded in parafin. Sections were stained with a battery of biotinilated lectins: Con-A, WGA, DBA, SBA, PNA, RCA-1 and UEA-1. Diaminobenzidine was used as a cromogen, ABC complex as an amplification system and hematoxylin as a counterstain. The glicocalix of epithelial cells showed a strong staining with Con-A (which binded mannose) and WGA (N-acetyl- glucosamine), but a moderate reaction with N-acetyl-galactosamine binding lectins (DBA, SBA) and fucose binding lectins (UEA-1). Apical cytoplasm showed moderate reaction with Con-A and SBA. Basal cytoplasm showed not reaction. The composition and distribution of GPs secreted by the germinal epithelium during spawning would be related to the physical-chemical properties of the mucus. Moreover they would be also related to functional aspects of the epithelium in oocytes maturation.

118. ORAL HEALTH AND DIET HABITS IN THREE SUB-POPULATIONS OF ROSARIO

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In Rosario City, like in the rest of the country, oral health attention is offered in private and public services. There are no oral preventive health programs to avoid dental loss in adults. The objective of this study was to describe oral health epidemiologic profile and dietetic habits in three young adults sub-populations. Applying a standardized questionnaire, 517 patients (15-45 years; 65% women, 35% men) were included. They went to the services in order to carry out dental pieces extractions. Statistical comparisons were made applying t-student, ANOVA and chi-square tests. Patients attention was done at a private (G1, 19%) and at two public services (81%: G2 is down town situated, and G3 in a marginal area). Decayed teeth average was $1,7 \pm 2,0$ in G1, and $4,1 \pm 2,8$ in G2 and G3, $p < 0.0001$. Absent teeth average was $5,4 \pm 5,2$ in G1; $7,0 \pm 5,4$ in G2, and $6,6 \pm 5,5$ in G3 ($p = 0.04$). Obturated teeth average was $4,4 \pm 4,2$ in G1, $1,2 \pm 2,0$ in G2, and $1,5 \pm 2,4$ in G3 ($p < 0.0001$). Daily milk consumption was 52% in G1, 26% in G2, and 27% in G3 (difference between private and public services, $p < 0.001$). Cookies, cakes, jam and honey consumption were greater in G1, soft candies and "dulce de leche" in G2 and G3. Smaller risk food consumption in G1 were ice creams, tablets, soda waters and chocolates, and in G2 and G3, caramels and bubble gums. Conclusions: The number of lost dental pieces was greater in public services, whereas the number of obturated pieces was higher in the private one. Daily milky products consumption was higher in the private sector. Candies and sweet foods consumption is extended to all of the services, but differences were found in the type of ingested foods.

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