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A1
STUDY OF ASPARAGUS DECLINE SYNDROME IN THE HORTICULTURE MODULE OF THE COLLEGE OF AGRICULTURAL SCIENCES (ZAVALLA, SANTA FE)

Peruzzo AM[1,4], Amato LD[3,4], Pioli RN[1,4,5], Martin EA[2,4]
[1]Cát. de Fitopatología, FCA, UNR. [2]Cát. de Mejoramiento Vegetal y Prod. de Semillas, FCA, UNR. [3]Cát. de Qca. General e Inorgánica, FCA, UNR. [4]IICAR, CONICET, Campo Exp. J. Villarino, Zavalla, Santa Fe. [5]CIUNR, Maipú 1065, Rosario, Santa Fe. E-mail: peruzzo@iicar-conicet.gob.ar

Asparagus (*Asparagus officinalis* L.) is a horticultural crop cultivated as a perennial alternative of productive diversification, highly valued by both producers and consumers. The edible part, called shoots, has important nutritional characteristics, including fiber, protein, vitamin, and mineral content. The main producing areas in our country include the northeast and southeast of the province of Buenos Aires, south of Santa Fe, San Juan, Mendoza, and Córdoba provinces. The crop presents yield losses due to fungal diseases that affect the plants, one of the main health problems being the Asparagus Decline Syndrome (ADS), whose most relevant disease-causing agent is the *Fusarium* complex. The disease is characterized by a progressive loss of vigor that can lead to the death of the affected plants. The symptoms are variable and can be observed at different stages of the crop. In our country, there are no exhaustive studies of this syndrome, so the objective of this work was to identify the pathogens associated with asparagus cultivation during the vegetative stage of the plants. As experimental material, an asparagus production batch implanted in 2017 in the Horticulture Section of the College of Agricultural Sciences-UNR (33º01' LS and 60º53' LO) was used. The survey was carried out in 2022, and stem samples were taken from at least three plants from four asparagus zones, coded as 12 bulk (A), 11 bulk (B), row 2 facing west (C), and row 2 east (D). Koch's postulates were applied to isolate the pathogens from the stem samples, disinfecting the plant tissue with 2% NaCl for 45 s. Subsequently, the stems were separated into aerial and underground parts, each one was divided into 1cm pieces that were seeded in Petri dishes with 2% APGA medium and incubated at 27 ± 2°C for 7 days. For the identification of pathogens, the monosporic culture technique was used based on macro and micro-morphological characters. The incidence of pathogens in the plant tissue samples was 66.7 to 100%, with zone D being the most affected; and no differences were observed between the aerial or underground part of the stem samples. The main genera detected were: *Fusarium*, *Phomopsis*, *Alternaria*, *Colletotrichum*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Botrytis*, *Drechslera*, *Phialophora*, *Pythium*, *Trichoderma*, *Sclerotium*, and *Verticillium*. A great diversity of *Fusarium* species was identified: *proliferatum*, *solani*, *chlamydosporum*, *ramigenum*, *semitectum*, and *oxysporum*; the latter being the most recurrent. This first survey allowed us to identify a correct sampling method for the phytosanitary study of asparagus, as well as to identify the main ADS-associated pathogens. A more detailed study of the *Fusarium* species associated with the asparagus cultivars produced in our region will allow us to advance in the development of adequate strategies tending to reduce the incidence of the disease in the crop.

A2
IN VITRO RESPONSE OF THE APICAL MERISTEM OF MATURE EMBRYOS OF *Eragrostis curvula*

Souza Canada ED[1], Bellido AM[2], Diaz AR[2], Echenique V[2], Permingeat H[1]
[1]Plataforma Agrotecnológica Biomolecular - Facultad de Ciencias Agrarias, UNR. [2]CERZOS (CONICET) CCT Bahía Blanca. Departamento de Agronomía, UNS. E-mail: souzadaniel8@hotmail.com

Eragrostis curvula, weeping grass, is a perennial, forage grass, appreciated for its low demand for irrigation and fertilization, which makes it suitable for conquering semi-arid regions. Its reproductive mode –apomixis– restricts the use of conventional breeding methods. Therefore, biotechnological techniques are used for this purpose, which implies the development of an efficient *in vitro* regeneration system, as well as a transformation protocol, associated with a selection mechanism. The shoot apical meristem (SAM) is an excellent explant because its main function is the continuous growth of the shoot and the production of reproductive organs, and it can also be genetically transformed, as has already happened in some cereals. Depending on the conditions used during *in vitro* cultivation of the SAM, including, most importantly, the induction medium, they can be stimulated to develop embryogenic callus and/or somatic embryos on the one hand or stimulate multiple shoot proliferation on the other. For this reason, the response of the SAM to different conditions during *in vitro* culture, such as salt composition, carbon source, type of phytoregulators in the culture medium, as well as different light intensities or darkness, was analyzed in this work. The extracted mature embryos were cultured on eleven different induction media, which can be divided into three groups. In the first group, the base medium consisted of Murashige and Skoog (MS) salts, B5 vitamins, 3% maltose, 10 µM 2,4-dichlorophenoxyacetic acid (2,4-D), from which media A to G were derived. Media A to C contained 0.5% Phytagel™. Medium B contained 25 µM 2,4-D and C 500 mg/L hydrolyzed casein. Media D to G, on the other hand, contained 0.04 µM 6-benzylamino purine (BAP), 5.13 mM glutamine, 1.30 mM proline, 0.76 mM asparagine, and 0.8% agar. Media E and G did not contain BAP, and F and G did contain 6% maltose. The second group consisted of media H to J, whose base comprised modified MS salts, N6 vitamins, 3% maltose, 0.85 mM glutamine, and 6.1 mM proline. Media H and I had 10 µM 2,4-D, 0.04 µM BAP; and J 8.9 µM BAP, and 2.5 µM 2,4-D. The third group,

consisting only of K medium, containing MS salts and vitamins, 3% sucrose, 8.9 µM BAP, 2.5 µM 2.4-D, and 0.3% Phytagel™. The light intensities were three 29.4 (low), 55.2 (medium), and 107.4 µmol/m^{2}s (high), with a photoperiod of 16/8h, and a temperature of 25 ± 2°C. For each medium, four replicates were made with 34 explants. The media with higher auxin concentration (A–I) induced somatic embryos, with a significant difference ($X_2 = 31.4$; $P < 0.001$) between them. The medium I showed the highest induction efficiency with 65% under high light intensity. The media with a higher concentration of cytokinin (J–K) developed multiple shoots. They also showed a significant difference ($X^2 = 5.3$; $P < 0.05$), where medium J with medium light intensity had the highest efficiency (74.2%). Thus, the SAM response was achieved by two alternatives, both of which are candidates for use in genetic transformation programs.

A3
KINETICS OF *IN SACCO* RUMEN DEGRADATION OF *Lippia alba*

Sciutto A[1], Smacchia ML[1], Figallo R[1,2]
[1]Cátedra de Química Biológica. Facultad de Cs. Veterinarias. Universidad Nacional de Rosario (UNR). [2]CIUNR. O. Lagos y Ruta 33. Casilda. Santa Fe. E-mail: anabelsciutto@fcv.unr.edu.ar

Lippia alba (Mill.) N.E.Br. ex Britton & Wilson, aromatic plant of the *Vervenaceae* family, commonly called "purple sage". Originally from the American continent, it grows from the south of the United States to the center of the Argentine Republic and is present in the islands of the Paraná River delta. Perennial shrub up to 2 meters high, with simple, opposite, and sometimes triple, ovate or elliptical, wrinkled leaves, very marked veins, rough hairiness on the upper face, toothed edge, and short petiole. The flowers are purplish-pink hermaphrodites, arranged in globose heads in the axils of the leaves. The chemical composition of the leaves can vary, depending on plant factors such as phenological stage, age and genetics, and environmental factors. In recent years, information on native food resources consumed by ruminant herbivores that graze the islands of the upper Paraná River Delta has been generated, however, it is still insufficient, especially regarding chemical composition, digestibility in ruminants, etc. This work proposes to determine the *in-sacco* dry matter ruminal degradability of *Lippia alba* (LA) during its vegetative growth period. We worked with leaf samples, obtained at regular intervals, in the spring–summer 2019–2020 growth period, on the islands located at km 430 of the Paraná River. Immediately after collection, the dry matter content (DM %) was measured, then they were dried at 60°C, ground, and sieved (2 mm). The ruminal degradability of dry matter (RDDM %) was determined for each sample at 0, 3, 6, 12, 24, and 48 h of incubation in the rumen *in sacco*, in four Pampinta breed sheep (Mehrez and Orskov, 1977). The data obtained were adjusted to the Orskov and McDonald (1979) model: RDDM % = a + b $(1 - e^{-ct})$, where: a, rapidly degradable fraction; b, slowly degradable fraction; c, rate of degradation of b; and a + b, potentially degradable fraction. The results obtained were studied by Analysis of Variance and Tukey's Test ($P > 0.05$). The average (SD) of the DM % of the LA samples was 25.5 (3.5) %. The mean (SD) values of the RDDM at 0, 3, 6, 12, 24, and 48 h of incubation were 22.24 (3.5), 31.72 (5.9), 41.06 (8.4), 56.01 (11.4), 71.05 (7.3), and 71.07 (6.1) %, respectively. The R^2 obtained from the adjustment to the proposed model were 97–99%, considered very adequate. The average values of the rapidly (a), slowly (b), and potentially (a + b) degradable fraction in the rumen of the LA samples were 20.05, 52.99, and 73.04 %, and the degradation rate (c) 0.09593 %/h. Although differences ($P < 0.05$) were observed between samples in the rapidly (a) and potentially degradable (a + b) fractions, the slowly (b) degradable fraction and the rate (c) degradation were similar. These variations indicate differences in the extent, but not in the degradation dynamics of the samples. The variations found were not related to the growth period of the plant. *Lippia alba*, with respect to previously studied cultivated forage species, can be considered a rapidly degradable food resource for ruminant herbivores with high potential degradability in the rumen *in sacco*.

A4
EFFECT OF INFECTION WITH THE NEMATODE *Trichinella spiralis* (Ts) ON THE DEVELOPMENT OF THE BREAST ADENOCARCINOMA M-406 IN CBi-IGE MICE, RESISTANT TO THE PARASITE

Perez J[1], Indelman P[2], Fernández MA[2], Codina AV[1,3], Pistelli R[1], Fussini M[1], Rico MJ[1,4], Hinrichsen LI[1]
[1]Instituto de Genética Experimental, Fac. Cs. Médicas, UNR. [2]Área Parasitología, Fac. Cs. Bioquímicas y Farmacéuticas, UNR. [3]CIC-UNR. [4]CONICET. E-mail: joacoperez06@hotmail.com

Helminths, as part of the animal evolutionary environment for millions of years, left an enormous imprint on the design and function of the mammalian immune system, especially the type 2 response. *Trichinella spiralis* can adapt to the host, creating the nurse cell. This immunologically privileged place allows it to orchestrate a long-term molecular dialogue with its host through excretion-secretion products, which successfully modulate immune responses against the parasite and responses to unrelated antigens such as tumor antigens. Infection with Ts may exert a regulatory effect on invasion, metastasis, and antiproliferative signals. The objective of this work was to study, in CBi/L-resistant mice, whether infection with Ts exerts this antitumor and antimetastatic effect on the development of the transplantable triple-negative breast adenocarcinoma M-406. Adult mice of both sexes were infected orally with 4 infective L1 Ts larvae/g BW 7 days before (**−7T**) or 7 days after (**+7T**) challenge with a subcutaneous tumor inoculum (treated groups). Uninfected animals inoculated with M-406 on the same date were used as controls (**C**). From day 4 post-challenge,

tumor growth was controlled by measuring its largest (DM) and minor (dm) diameters with a caliper three times a week, calculating the tumor volume (VT = $dm^2 \times DM \times 0.4$; mm^3). With this variable, the tumor doubling time (td, days) was estimated in each group using the exponential growth curve. During the experience, the general health status of mice was evaluated (N = 5 by sex and treatment); all were sacrificed by overexposure to CO_2 when the first mouse reached the maximum ethically allowed tumor size. After sacrifice, the lungs were removed and fixed in Bouin to determine, by direct observation and counting, the number of gross metastases. The presence of the parasite did not change the proportion of mice with lung metastases (C♀: 60%, −7T♀: 80%, +7T♀: 80%; C♂: 75%, −7T♂: 50%, +7T♂: 75%). The analysis of the average growth curves showed that males and females differed from each other in their response to treatment and compared to their respective controls ($P < 0.01$). The treatment did not show a significant effect on mean tumor volume 24 days post-tumor challenge (mean ± SEM, C♀: 748±553.0, −7T♀: 257 ± 71.0, +7T♀: 574 ± 202.6; C♂: 48 ± 34.2, −7T♂: 607 ± 202.7, +7T♂: 385 ± 133.3) but it did affect tumor doubling time (td average curve; C♀: 4.8 (2.8–15.2), −7T♀: 5.6 (4.0–8.9), +7T♀: 4.2 (3.2–6.0), $P = 0.025$; C♂: 8.5 (5.0–28.4), −7T♂: 5.9 (4.5–8.6), +7T♂: 1.9 (1.3–3.9), $P < 0.001$). These results suggest that, in this experimental model, Ts and its products promote a sex-dependent immunomodulatory network, allowing the host to perturb tumor development. Since the specific mechanisms induced by Ts infection on tumor growth are still unclear, the CBi-IGE + M-406 mouse system shows promise as an experimental model for discovering or using new molecules derived from these biological agents that could function as adjuvant therapy in the treatment of various types of cancer.

A5
EVALUATION OF THE INTERACTION OF MICROALGAL PROTEINS WITH BOVINE SODIUM CASEINATE BY FLUORESCENCE SPECTROSCOPY

Sanchez MF[1,3], Risso PH[2,3], Ingrassia R[2,3]
[1]Facultad de Cs. Veterinarias, UNR. [2]Facultad de Cs. Bioquímicas y Farmacéuticas, UNR. [3]CONICET. E-mail: romina_ingrassia@yahoo.com.ar

Spirulina protein derivatives (SPD) present antioxidant properties that may be stabilized by encapsulation by using biopolymers. The objective of this work was to study the interaction of SPD and sodium caseinate (NaCAS) to evaluate the use of the latter, derived from dairy casein, as wall material. Spirulina extract (10% W/V) was prepared in 1.5% $CaCl_2$, stirred for 7.5 h, and centrifuged at 1,000×*g* for 20 min. The antioxidant capacity of the SPD was measured by the 2,2'-azino-bis-(3-ethylbenzothiasoline-6-sulfonic acid) radical capture method ($ABTS^+$) at 0, 48, and 96 h after storing at −18°C or 3°C. The results were expressed as a function of the percentage of inhibition of the $ABTS^+$ radical. NaCAS was prepared at 3% (%W/W) in 10 mM Tris-HCl buffer (pH 7). The interaction between SPD and NaCAS was evaluated by measuring the fluorescence intensity (FI) in an Aminco–Bowman spectrofluorometer. The FI of NaCAS was measured in a range of 300–400 nm with an excitation wavelength (λ_{exc}) of 288 nm, in the absence and the presence of increasing concentrations of SPD (NaCAS+SPD). In the same way, the FI of SPD was determined in the absence and the presence of increasing concentrations of NaCAS (SPD+NaCAS). Also, the FI of SPD was measured in a range of 630–700 nm with $\lambda_{exc} = 620$ nm, in the absence and the presence of increasing concentrations of NaCAS. The SPD presented an antioxidant capacity of 48.0 ± 0.6%, but at 48 h, the samples stored at 3°C and −18°C decreased the inhibition percentage by 15% and 14%, respectively. At 96 h, the percentage inhibition was reduced by 9% and 28% for samples stored at −18°C and 3°C, respectively. Therefore, although the antioxidant capacity was more preserved at −18°C, it is demonstrated that it is essential to implement a methodology to achieve the stabilization of the SPD. For NaCAS/SPD interaction studies, in NaCAS+SPD and SPD+NaCAS assays, the FI increased as the added protein concentration increased, without evident changes in the maximum emission wavelength. This would be due to an increase in the concentration of intrinsic protein fluorophores (tyrosine and tryptophan), without appreciable changes in their environment. In the assays at $\lambda_{exc} = 620$ nm, where the prosthetic group of the major protein of SPDs is excited, a decrease in FI was observed as the concentration of NaCAS increased, along with a blue shift of the maximum emission wavelength. These results reveal a conformational change with the insertion of the prosthetic group in a more hydrophobic environment. Future studies with other complementary techniques are required to deepen our knowledge about these interactions, such as infrared spectroscopy and differential scanning calorimetry.

A6
EFFECT OF LACTOFERRIN ON GENOTOXIC DAMAGE IN HUMAN SPERMATOZOA

Vich E, Gola A, Pretto L, Tonutti S, Massa E, Ghersevich S
Área de Bioquímica Clínica-Facultad de Ciencias Bioquímicas y Farmacéuticas- UNR.
E-mail: sghersev@fbioyf.unr.edu.ar

Reactive oxygen species (ROS) would be involved in sperm capacitation in the female reproductive tract. However, ROS excess can negatively affect fertility causing oxidative damage. In previous studies, we have observed that the incubation of sperm under capacitating conditions affects the integrity of sperm DNA. We have identified an 80 kDa oviductal protein called lactoferrin (LF) that can affect sperm function parameters *in vitro*. Since LF is present in the

female reproductive tract and considering that one of its effects reported in other cell models is its antioxidant capacity, we propose to evaluate the effect of LF on sperm exposed to oxidative damage *in vitro*. Semen samples were obtained from 14 normozoospermic donors (WHO, 2010). Motile sperm were recovered using the swim-up technique and incubated under capacitating conditions in a culture medium supplemented with serum albumin (3.5 mg/mL) at 37°C, 5% CO_2 for 18 h, and in the presence or absence (control) of increasing doses of LF (1, 10, or 100 μg/mL). At the end of incubation, genotoxic damage to sperm DNA was investigated by the Comet assay. In other experiments, the protective action of LF on spermatozoa exposed to an oxidizing agent (H_2O_2) was evaluated. Spermatozoa were incubated under capacitating conditions, in the presence or absence (control) of increasing doses of LF (1, 10, or 100 μg/mL) and the presence or absence of H_2O_2 (50 μM) for 1 h. Genotoxic damage was assessed with the Comet assay. For the analysis of the results between the different experiments, the average score in the controls in the absence of treatment was considered as 100%, and the score of all treatments was relativized to the value in the control. The semen samples analyzed presented: a concentration of 85.2 ± 25.6 $\times 10^6$ spermatozoa/mL, progressive motility of 62.3 ± 17.5%, morphology of 7.3 ± 1.1% of normal spermatozoa, and viability ≥ 85%. Lactoferrin did not affect the level of sperm genotoxic damage related to incubation for 18 h [control: 196.2 ± 15.9%; LF(1): 200.6 ± 19.1%, LF(10): 198.4 ± 24.3%; LF(100): 211.5 ± 33.0%]. However, the protective effect of 100 μg/mL LF ($P < 0.01$) against the damage by an oxidizing agent could be observed [control: 100.0 ± 3.0%; LF(100): 94.3 ± 25.5%, LF(100) + H_2O_2: 102.9 ± 22.5%; H_2O_2: 165.9 ± 35.0%]. The presence of LF did not modify the genotoxic damage on sperm DNA related to incubation, but the highest concentration used decreased the damage caused by H_2O_2, demonstrating its protective action against an oxidizing agent. Since the concentration of LF in the female tract is maximal at the periovulatory period and increases in inflammatory processes, its presence could contribute to reducing sperm genotoxic damage.

A7

STUDY OF THE EFFECT OF ANAEROBIC DIGESTATE AND ITS COMBINATION WITH INORGANIC FERTILIZER ON THE AERIAL BIOMASS OF *Lolium perenne* L.

Allegrini M[1], Iocoli GA[1], Felitti SA[2], Zabaloy MC[1]
[1]Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Universidad Nacional del Sur (UNS)-CONICET. San Andrés 800 (8000) Bahía Blanca. [2]Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR), Universidad Nacional de Rosario (UNR)-CONICET. Campo Experimental Villarino. CC N° 14 (2125) Zavalla.
E-mail: mallegrini@cerzos-conicet.gob.ar

A considerable amount of waste is continuously generated from diverse agricultural and livestock productions in Argentina, particularly those of intensive origin (feedlots, effluents from large dairy farms and poultry farms). The appropriate management of these residues is critical to avoid or mitigate their negative impact on the environment. The anaerobic treatment of wastes generates a by-product called digestate. In this study, the effect of digestate (from poultry, dairy and swine productions; retention time: 25 days) was assessed on perennial ryegrass as indicator of fertility (*Lolium perenne* L.), relative to the effect of an inorganic fertilizer (urea 46% N) and a control without fertilizer (water). A combination of urea and digestate (50:50 ratio) was also tested as an alternative fertilization treatment. The assay was conducted in the greenhouse under a complete randomized design using a soil without history of organic amendments (0–20 cm) from a natural grassland in Colonia Napostá (38°25'39"S, 62°17'41"W). Five mowing events were applied (25, 42, 58, 85, and 114 days after sowing). The fresh and dry weights (oven at 70°C, 48 h) were measured at each mowing date as well as the cumulative weight. The same N rate (106.33 mg $N\text{-}NH_4^+$ kg $soil^{-1}$, equivalent to 140 kg/ha) was applied in all treatments, fractioned in 3 applications (3 days before sowing, and 25 and 42 days after sowing). Comparisons among treatments were conducted within each mowing date. In all cases, results were analyzed with a one-way ANOVA and Tukey HSD test. At the first mowing event, a significantly higher biomass weight was obtained with digestate relative to the inorganic fertilizer ($P < 0.05$), both in the uncombined application (fresh weight) as well as combined with urea (dry and fresh weight). At the second mowing event, significantly higher values were observed relative to the inorganic fertilizer (fresh and dry weight) but only with the uncombined digestate. In later mowing events, no significant differences were observed among fertilization treatments. Only the digestate showed a residual effect with a mean dry weight significantly higher than the control at the fifth mowing event. The cumulative values of dry and fresh weight were also significantly higher for the fertilization treatments relative to the control but without differences among them. Overall, the digestate alone or combined with the inorganic fertilizer results in a significantly higher aerial biomass than the inorganic fertilizer in the first mowing events maintaining the same cumulative biomass. Further studies should assess the response to digestate under field conditions with grazing managements and consider the effects of digestate in soil health.

A8

TEMPORAL DYNAMICS OF ADULTS OF *Drosophila suzukii* (MATSUMURA) AND *Zaprionus indianus* (GUPTA) IN FRUIT TREES ORCHARDS OF SOUTH SANTA FE

San Pedro P[1], Vega MS[1], Gonsebatt G[1], Seta S[1], Coniglio R[1], Díaz BM[2]
[1]Facultad de Cs. Agrarias. Universidad Nacional de Rosario. C.C N°14. S2125ZAA Zavalla, Santa Fe. [2]E.E.A INTA Concordia. Estación Yuquerí, Ruta Prov. 22 y vías del ferrocarril. 3200 Concordia, Entre Ríos.
E-mail: paulasanpedro@mi.unc.edu.ar

Drosophila suzukii (Matsumura) "spotted wing drosophila" and *Zaprionus indianus* (Gupta) "African fig fly" (Fam: Drosophilidae) are two pest species of fruit trees. Temperature (T°) influences distribution patterns and population growth of drosophila flies. For *D. suzukii*, the optimum T° is between 20–25°C. T° lower than 10°C and above 30°C reduce the activity and reproduction of adults, while 15°C or less causes sterility in *Z. indianus* males. Both species have been detected in the south of Santa Fe province (Argentina), characterized by a humid temperate climate and national relevance for fruit production. In order to determine the population evolution in relation to T°, adults of both species were captured using apple vinegar traps. From September 2020 to August 2021, once a month and for a 7-day period, 3 traps were placed on each fruit species in two commercial orchards. 18 spots were sampled in Rosario (32° 56' S; 60° 38'W) (pomegranate, persimmon, prickly pear, and figs, purple and white) and 9 in Piñero (33° 06' S; 60° 48 'W) (persimmon, kiwi, and orange). The average number of adults for each sampling period was calculated and the mean, maximum, and minimum T° data were taken from the nearest meteorological station, located in the Faculty of Agricultural Sciences of the National University of Rosario (33°01′ S, 60°52' E). 64.833 adults (86% Drosophilidae spp., 9% *D. suzukii*, 5% *Z. indianus*) were collected. Both species had a 1:1 sex ratio, *D. suzukii* 52% female and 48% male and *Z. indianus* 51% female and 49% male. From September to November, the fructification period of most fruit trees, there were no captures due to the systematic applications of pesticides. During other periods, collections of both species were recorded. The initial abundance (individuals/trap) was low: *D. suzukii* 3.1 (December), 1.3 (January), 3.6 (February), and 3 (March), and *Z. indianus* 0.9 (February) and 15.7 (March). The low abundance overlapped with the mean maximum T° recorded, exceeding the maximum threshold of 30°C, from which *D. suzukii* decreases its fertility. In January when the minimum number of individuals was recorded, the mean maximum T° during that sampling week was 33.4°C (26.8–36.9°C). The maximum peak for both species occurred in April, 104.6 for *D. suzukii* and 90.1 for *Z. indianus*. In that period, the mean maximum T° of 23.2°C (13.7–27.6°C) was recorded. After this maximum abundance, both species experienced a significant reduction, *D. suzukii* 17.2 and *Z. indianus*13.1, coinciding with the lowest mean minimum T° 2.6°C (0.4–7.2°C) (June). During July and August, when maximum and minimum T° increased, *D. suzukii* experienced an increase in abundance of 47.1 and 41.9, respectively, while *Z. indianus* did not recover from the decline. In conclusion, the favorable T° for both species occurs between the months of December–June with a peak of abundance in April. In future research, evasion strategies of both species and food resources to survive during autumn/winter should be studied.

A9

DETERMINATION OF THE NUTRITIONAL YIELD POTENTIAL OF *Lens culinaris* MEDIK

Maglia F, Palacios LT, Cointry E, Bermejo CJ
Instituto de Investigaciones en Ciencias Agrarias de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (IICAR-CONICET), Facultad de Ciencias Agrarias, UNR, CC 14, S2125ZAA Zavalla, Santa Fe, Argentina.
E-mail: fer.maglia@hotmail.com

The lentil (*Lens culinaris* Medik) crop is of great interest throughout the world, mainly in developing countries, since it has a high content of proteins, carbohydrates, and essential minerals to meet the needs of the human diet. However, it contains antinutrients that bind to proteins and minerals resulting in decreased absorption and bioavailability, likewise, its concentration in the plant increases when stressful environmental conditions occur. The objective of this work was to determine the nutritional yield potential of lentil varieties in three contrasting environments. As experimental material, 12 varieties of lentils (8 *macrosperma* and 4 *microsperma*) with good agronomic characteristics from the Grain Plant Breeding Program of the Faculty of Agricultural Sciences, UNR, including the commercial tester Silvina were used and were sown in the following environments: growth chamber (GC), field in 2020, and field in 2021. The experimental design for the three environments was a randomized complete block design (DBCA) with two replicates. The GC consisted of a hydroponic system with a photoperiod of 20 hours of light provided by LED tubes, a temperature of 23 ± 2°C, a constant supply of nutrients, and perlite as a substrate; each repetition consisted of a multipot with 30 plants per genotype. In the field, each repetition consisted of 2-m plots with 50 plants planted per genotype at the beginning of July. The year 2020 presented temperatures above 30°C and daily rainfall greater than 40 mm of water during the critical period of cultivation and grain filling. The following traits were evaluated: % protein (P), % phytic acid (AP), % phenols (PH), and % tannins (T). An ANOVA was carried out using the Infostat program, which showed a significant genotype-environment interaction ($P < 0.001$) for all traits. Its effect was studied using a GGE biplot, which explained 98.3%, 96%, 99.8%, and 100% of the accumulated variation in the first two CP for P, AP, PH, and T, respectively. It was possible to identify the presence of two mega-environments for all the traits, grouping the field

2021 and GC in the same mega-environment. In this mega-environment, the genotype 58-13 presented high values of P (31%), 42a presented lower values of AP (0.52%) and T (0.21%), and 43a presented lower values of PH (0.27%). These results indicated that when the cultivation environment is ideal, as occurs in the GC, and field conditions do not present significant limitations, as occurred in 2021, the genetic potential of lentil varieties is achieved.

A10
SUSCEPTIBILITY OF BACTERIA ISOLATED FROM DOGS AND CATS TO FREQUENT CLINICAL-USED ANTIMICROBIALS

Pereyra N[1,2], Freije J[1], Cane V[1,2], Olarreaga G[1], Barbero U[1], Cane J[2,3], Giacomelli P[4], Muchut M[4], Nina M[4], Rossi J[1], Ascini V[1]
[1]Servicio de Bacteriología, Cátedra de Microbiología, Facultad de Ciencias Veterinarias (FCV), Universidad Nacional de Rosario (UNR), Argentina. [2]Medax, Laboratorio de Diagnóstico Veterinario, Chañar Ladeado. [3]Cátedra de Parasitología, FCV, UNR; Actividad Privada. E-mail: normapereyra@fcv.unr.edu.ar

Laboratory diagnosis of dog and cat bacterial pathologies allows identifying bacteria that are causing diseases and selecting the most appropriate antimicrobials (AM) for each case. Carrying out antibiograms (ATBg) for isolated bacteria is essential to implement an adequate treatment. The use of AM in animals is seriously questioned today because growing antimicrobial resistance (AMR) that inadequate treatment generates. The aim of this work was to monitor the sensitivity (S) of bacteria isolated from dogs and cats to AM frequently used in the clinic, in the Argentine region of South of Santa Fe and Southeast of Córdoba. Fifty-one bacterial isolates recovered during 2022 from 18 ear swabs, 13 urine samples, 8 fistula/intramedullary nail/abscess samples, 7 nasal swabs, and 1 vaginal swab were analyzed. For each strain, ATBg was performed (agar diffusion method by Kirby–Bauer) testing all AM available for clinical use; ciprofloxacin (CIP), enrofloxacin (ENR), doxycycline (DOX), trimethoprim/sulfamethoxazole (TMS), gentamicin (GEN), clindamycin (CLIN), erythromycin (ERI), penicillin (PEN), amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), cephalexin (CEF), ceftazidime (CTZ), amikacin (AMN), and florfenicol (FLOR) discs were used; differences in AM use between dogs and cats were considered to choice AM for the test. General bacterial S: 100% of the strains were sensitive to AMN, 81% to GEN, 77% to CIP, 59% to ENRO, 57% to CTZ, 50% to AMX, 33% to DOX, 48% to TMS, 43% to AMC, 42% to CEF, 33% to CLIN and ERI, and 10% to PEN. But S presented variations for different bacteria and pathologies. For example, *Pseudomonas aeruginosa*, the main isolate from ear swabs, was 100% sensitive to CIP, 81% to GEN, and 0% to DOX. *Escherichia coli*, isolated mainly from urine, was 57% sensitive to CIP, 50% to ENR and 33% to CEF. *Staphylococcus pseudointermedius*, isolated almost as the only bacterium (besides a low proportion of coagulase-negative staphylococci) from otic and dermal swabs, was 52% sensitive to DOX and 50% to both ENR and AMC. Besides, ATBg results give clues of RAM mechanisms that are necessary to investigate: beta-lactamases in enterobacterales or resistance to methicillin in staphylococci. Bacteria S obtained for the CIP was generally the highest, despite the fact that the CLSI (USA) does not recommend its use in pets because it does not present cut-off points for veterinary medicine; in this work, standardized values for humans should be used for ATBg interpretation. This work's aim was to verify the current S of bacteria that are commonly isolated from cats and dogs in this region of Argentina, although it is known that the efficacy of the treatment is conditioned by numerous other factors.

A11
EFFECT OF BLUEBERRY EXTRACT ADDITION ON BOVINE SODIUM CASEINATE

Lanari G[1], Hidalgo ME[2], Risso P[2]
[1]Facultad de Cs. Veterinarias, Universidad Nacional de Rosario (UNR). [2]Facultad de Cs. Bioquímicas y Farmacéuticas, UNR-CONICET. E-mail: phrisso@yahoo.com.ar

Anthocyanin/protein complex formation can be used to overcome chemical instability, which affects health-beneficial properties and color stability of anthocyanins through confining this active compound within a polymeric matrix for its encapsulation. This work aimed to study, using fluorescence spectroscopy, the interaction between sodium caseinate (NaCAS) and a blueberry extract (BEX) that contains anthocyanins. Furthermore, it assessed the effect of temperature (25, 37, and 42 °C) on the system. BEX was extracted from blueberries using a 0.25 M citric acid aqueous solution. The anthocyanin concentration of BEX was determined by the differential pH method based on the colored oxonium form predominating at pH 1.0 and the colorless hemiketal form at pH 4.5. The maximum excitation (EX) and emission (EM) wavelengths (λ) of a 0.06% NaCAS solution in 10 mM Tris–HCl buffer (pH 6.5) were determined at room temperature. EX and EM spectra were performed on a thermostatic Aminco Bowman Series 2 spectrofluorometer, with 286 nm and 340 nm determined as the λ_{EX} and λ_{EM}, respectively. Then, EM spectra of 0.06% NaCAS were performed in the absence and the presence of different amounts of BEX (anthocyanin concentration: 56 ± 6 mg/L) in the named buffer and at a given temperature (in duplicate). In addition, at the same pH and temperature, the fluorescence intensity (FI) of the BEX was measured. It observed that, as the BEX concentration increased, there was a decrease in FI without

significant changes in the EM peak. This extinction of fluorescence (quenching) may be due to the collisions of the fluorophore (NaCAS) with the solvent molecules, decreasing the FI due to the loss of energy in a non-radiative way (dynamic quenching). Or it may be due to static quenching due to the formation of a non-fluorescent complex between the fluorophore and the BEX (quencher). To determine the quenching type, the Stern–Volmer constant (K_{SV}) obtain from an adjustment of the F/F_0 *vs.* BEX concentration graphics, where F is the FI in the presence of BEX and F_0 is the FI in the absence of BEX. The values of K_{SV} (mM^{-1}) at 25, 35, and 42 °C were (0.549 ± 0.001), (0.572 ± 0.001), and (0.711 ± 0.001), respectively. It observed that there was a significant increase ($P < 0.05$) of the K_{SV} with the increment in the temperature, which indicated a dynamic quenching. This is due to collisions between the sodium caseinate and the anthocyanins in the blueberry extract. In conclusion, the study should be deepened with other techniques, such as infrared spectroscopy, before ruling out the formation of complexes between NaCAS and anthocyanins.

A12
ANALYSIS OF ENVIRONMENTAL FACTORS AND STUDY ORGANIZATION IN BIOLOGY STUDENTS OF DENTISTRY

Basal RL, Paleo MA, Pilone LS, Bander MP, Astudillo L, Suarez S, Degaetano S, Dorati P, Serrano VC
Faculty of Dentistry. UNLP. E-mail: roxanabasal@gmail.com

The permanent challenges offered by the different fields of study, and especially the university in biological areas, require the application of effective habits that allow academic success to be achieved, being in the initial stages a necessary aspect to be analyzed. The Study Habits Inventory (IHE) by Fernández Pozar (1989) is an instrument that allows inquiring about different levels, organized into four scales. The first one asks about the environmental conditions of the study, scale II inspects the planning of the study, scale III asks about the use of the material, while scale IV asks about the assimilation of contents. The objective of this work has been to analyze the environmental conditions of study and study planning in first-year students of the Dentistry career. A quantitative, descriptive, observational correlation research was carried out. The sample consisted of 107 students enrolled in the General Biology Course of the UNLP Dentistry course, who responded to the Fernández Pozar Study Habits Inventory. The 18 returns corresponding to axis No. 1 and the 12 of axis II were studied in relation to the environmental factors of the study and study planning, respectively. The responses were analyzed based on the rating established by the same author (bad, unsatisfactory, good, normal, and excellent), in percentages. The results obtained show for axis 1: 48.5% (52) normal, 30.8% (33) good, 14% (15) excellent, 3.7% (4) unsatisfactory, and 2.8% (3) wrong; while for axis II: 40.18% (43) normal, 11.21% (12) good, excellent 6.54% (7), and 25.23% (27) unsatisfactory, bad 16.82% (18). The analysis of the results obtained allows us to conclude that among the participants those with relevant study habits predominate, while a minority group needs to reflect on their study planning.

A13
ENERGY DRINKS CONSUMPTION PATTERN IN STUDENTS OF THE FACULTY OF MEDICAL SCIENCES OF ROSARIO NATIONAL UNIVERSITY (UNR)

Ballistreri M[1], Ballerini A[2], Boglioli A[1], Calgaro G[1], Gaselli M[1], Guarda L[1], Ponce C[1], Rebecchini A[1], Bertola Compagnucci A[1]
[1]Facultad de Ciencias Médicas, Universidad Nacional de Rosario. [2]Facultad de Psicología, Universidad Nacional de Rosario. E-mail: agustina_bc@hotmail.com

Research studies that address substance use in university contexts have one of the strongest hypotheses that if the use turns into a problematic one, it can lead to dropouts or graduation delays during the course of studies and if they get to graduate, this type of consumption may interfere with their professional activity. The aim of this study is to evaluate the consumption of energy drinks in students of the course of studies of Medicine, Bachelor's in Speech and Hearing Therapy, and Bachelor's in Nursing from the Faculty of Medical Sciences of UNR and, if necessary, to implement interventions aimed at preventing such consumption. A quantitative, descriptive, cross-sectional study is being carried out, with a non-probabilistic and convenience sample (N = 713) composed of 1st- and 3rd-year students, who were present at the time of applying a survey, and after having given their written consent. Averages ± standard deviations (SD), absolute and relative frequencies were calculated, according to the type of variables. The differences between the students who consume and those who do not consume energy drinks were compared with the Chi-square test. The average age of the Bachelor's in Speech and Hearing Therapy students is 21.93 ± 5.62 years. For the Bachelor's in Nursing students, it is 27.41 ± 8.27 years, and for the Medicine students is 21.61 ± 4.26 years. 80% of the sample belongs to the female gender, 18% to the male, and 2% preferred not to answer. They stated that they were from Rosario (57%), other towns in the province (18.5%), other provinces (11%), and other countries (12%). Regarding work, 47% of the sample does not work, 21% does it sometimes, and 31% works. Of the sample, 74% stated that they had consumed energy drinks, 21% had never consumed them, and 5% did not answer this question. No significant differences were found regarding consumption based on their origin and whether they worked or not. However, a difference in consumption was found according to gender since 90% of those who consume energy drinks were male and 76% female

(P = 0.006). In reference to whether they mix energy drinks with alcohol, 58% stated that they do and the drinks they do it the most with are vodka, whiskey, and champagne. Moreover, 38% indicated that they do not drink more or less alcoholic beverages when they mix them, 15% drink a little more, 7% drink a lot more, 10% drink a little less, and 6% a lot less. As a preliminary result, it was found that even being students of health-related careers, the majority consume energy drinks and more than half do so mixed with alcohol.

A14

EFFECT OF *Ligaria cuneifolia* INFUSION *(*"Argentine mistletoe") ON PLASMA CHOLESTEROL, TRIGLYCERIDES AND ERYTHROCYTE AGGREGATION IN DYSLIPIDEMIC PATIENTS

Balmaceda F[1], Ferrero M[1], Urli L[1], Perez M[1], De Vuono D[4], Wagner M[5], Belloscar J[3], Carnovale C[2], Luquita A[1]
[1]Biofísica, Facultad de Ciencias Médicas. Universidad Nacional de Rosario – CIURN. [2]Fisiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR; IFISE-CONICET. [3]Servicio de Cardiología, Hospital Provincial del Centenario. Rosario, Santa Fe. [4]Laboratorio Central del Hospital Provincial del Centenario. [5]Cátedra de Farmacobotánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.
E-mail: florbalmaceda03@hotmail.com

Ligaria cuneifolia (*Lc*), popularly known as "Creole mistletoe" is a hemiparasitic plant whose infusion is used in folk medicine to increase blood fluidity and decrease plasma cholesterol levels. Objectives: to analyze the effect of *Lc* infusion on plasma cholesterol levels (Cho), triglycerides, and erythrocyte aggregation in patients with cholesterol > 200 mg/dL. Methods: blood samples were collected for basal determinations (C) from eight patients aged 50 ± 15 years old (male), attending the Cardiology Service at the Hospital Provincial Centenario (Rosario, Argentina). Patients received lyophilized extract of *Lc* in tea bags (2.6 g each) to be diluted in 100 mL of warm drinking water and to be taken three times/week for a month time. 31 days later, blood samples were again obtained from these same patients (T*Lc*). We assessed in serum: plasma Total Cho by the esterase–oxidase method, HDLCho and LDLCho by colorimetric methods, triglycerides (TG) and fibrinogen (FB) by enzymatic methods; all concentrations were expressed in mg/dL. Into blood:erythrocyte aggregation (AE), by optical densitometry, getting the average size (s) of the aggregates and the initial velocity (v) of the process, erythrocyte sedimentation rate. Statistical analysis was performed using the Wilcoxon test. Results: Median and confidence interval (CI 95%). Cho: C: 223.5 (197–257) *vs*. T*Lc*: 222.5 (206–261) ns; HDLCho: C: 49 (42–62) *vs.* T*Lc*: 48.5 (48–68) ns; LDLCho: C: 157.5 (155–168) *vs*. T*Lc*: 146 (138–166)*; TG : C: 182 (112–184) *vs*. T*Lc*: 136 (109–149)*; FB C: 396.5 (380–597) *vs.* T*Lc*: 334 (293–481)*; V: C: 0.44 (0.21–0.69) *vs.* T*Lc*: 0.52 (0.32–0.58) ns; S: C: 1.83 (1.78–1.86) *vs*. T*Lc*: 1.79 (1.7–1.86) ns. (*P < 0.01 *vs.* C; ns: non-significative *vs*. C). Conclusion: in the patients studied, treatment with *Lc* generated a significant decrease in LDL cholesterol, TG, and FB blood levels, without promoting alterations in blood viscosity or in the evaluated parameters of erythrocyte aggregation. In addition, considering that elevated values of plasma Cho LDL and TG are related to atherosclerosis development, the importance of these results lies in considering *Lc* feasible to be used for the prevention of cardiovascular diseases.

A15

PRELIMINAR STUDY OF NON-LINEAR DYNAMIC BEHAVIOUR OF RED BLOOD CELLS GLICATED AND INCUBATED WITH β-SITOSTEROL

Mancilla Canales M[1,2], Buszniez P[1], Mascaro Grosso HV[1], Riquelme BD[1,2], Korol A[2]
[1]Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). [2]Grupo de Física Biomédica, Instituto de Física Rosario (CONICET-UNR). E-mail: riquelme@ifir-conicet.gov.ar, briquel@fbioyf.unr.edu.ar

Phytosterols (PSs) are sterols obtained from plants, more than 250 PSs have been isolated, and each species contains a characteristic composition. Among them, β-sitosterol is effective for diabetes treatment. On the other hand, the analysis and quantification of the dynamics of the red blood cell (RBC) deformability process are crucial due to its application and immediate utility in biomedicine. This paper analyzes the alterations in the dynamics of erythrocyte deformation due to hyperglycemia in diabetic patients through *in vitro* glycation of red blood cells from healthy donors (gRBC). The possible effect of β-sitosterol on glycated red blood cells (gFRBC) for reversal of the indicated alterations is subsequently studied. The β-sitosterol was dissolved in benzyl alcohol/H_2O (71 mg/8 mL), also analyzing its effect on control red blood cells (SRBC) and glycated red blood cells (gSRBC). The untreated whole blood (SERBC) was also analyzed to evaluate the possible influence of red blood cell manipulation in the different stages of the experimentation. All samples, except the whole blood, were washed twice with phosphate-buffered saline (pH 7.4; osmolarity 295 mOsm/L). For this, a simple and new mathematical alternative based on ordinary Brownian motion and fractional Brownian motion for time series was used. This mathematical technique was applied to photometric time series corresponding to fluctuations in the deformation of red blood cells subjected to controlled shear stress. The photometric time series were obtained using the Erythrocyte Rheometer based on the laser diffractometry technique for the viscoelastic parameter determinations, using the time series corresponding to RBC under shear stress in the steady state.

Eighty-four time-photometric series corresponding to samples obtained in quintuplicate were evaluated: RBC, gRBC, FRBC, SRBC, gFRBC, gSRBC, and SERBC, in addition to evaluating 30 surrogate series samples obtained from the photometric measurements. A quantifier that provides a value for the inclusion dimension (D_e) of the process attractor was used, and a quantifier was applied to estimate the percentage of false neighbors based on purely geometric considerations (%FNN). In all the cases studied, %FNN and D_e were obtained and resulting in a high initial negative slope for %FNN that later becomes constant for %FNN = 1%, and $D_e \geq 6$. From these results and according to the Theorem of Takens, non-linear parameters are obtained within statistical mechanics that would provide new information on the viscoelasticity of the membrane of glycated and β-sitosterol treated RBCs. These results are expected to contribute to the elucidation of the possible antidiabetic activity of this phytochemical.

A16
DEVELOPMENT OF A PCR TECHNIQUE FOR SEXING BIRDS

Ringer S, Garré M, Schaer J, Alet A, Peralta L
Faculty of Veterinary Sciences (FCV), National University of Rosario (UNR). Spangenberg and Bv Colón (2170) Casilda. E-mail: leticiaperalta@fcv.unr.edu.ar

In the field of veterinary medicine, knowing the sex of an individual can be decisive when addressing a clinical case or making a situation diagnosis. In the case of birds, many species lack sexual dimorphism. It is known that the chromosomes of male birds are homogametic (ZZ), and those of female birds are heterogametic (ZW). Since specific DNA sequences for the W gene were identified in birds, such as the CHD1-W gene and its homologous copy on the Z chromosome (CHD1-Z), its amplification by polymerase chain reaction (PCR) became a frequent method of molecular sexing. This work aimed to develop a PCR technique to determine the sex of monomorphic birds to create, within the FCV-UNR, a sexing service for pet and wild birds of different species. This technique is based on the difference in the size of the introns of the CHDW and CHDZ genes, obtaining as amplification product two fragments in females and a single fragment in males. Given the universality of the presented technique, it was proposed to work with sexually dimorphic species such as pigeons (*Columba livia*). Previously developed primers for other species were used to achieve the aforementioned diagnostic method. To perfect the DNA extraction technique, in conditions similar to those that would occur in the referral from other professionals, blood samples were taken from birds by venipuncture with heparinized syringes and 22/23G needles, depositing four drops on filter paper and air drying them. For DNA extraction, a DNA PuriPrep-S kit® from Inbio Highway was used. Given certain drawbacks that it presented (low efficiency and degradation), a second paper extraction protocol was also carried out, proposed by Tomasulo (2008). Subsequently, degenerate primers (suitable for several different orders) published by Fridolfsson and Ellegren (1999: 2550F/2718R) were used for PCR under the conditions tested by them. PCR products were separated by electrophoresis on 1.8% agarose gels and visualized by SyberSafe® staining to reveal the presence of bands. As a result, two amplicons of different sizes were obtained in the case of females and a single amplicon for males. In this way, we corroborate that under the working conditions of the laboratory in which the activity was carried out, it was possible to replicate the technique, obtaining reliable results in pigeons, and achieving the objective of determining the sex by this methodology. Based on these advances, it is planned to validate the technique in the future in other non-dimorphic birds, such as *Amazona aestiva* (Chaco parrot) or *Myiopsitta monachus* (Argentine green parrot), and other native wild birds, such as *Caracara plancus* (carancho) and *Phalcoboenus chimango* (chimango).

A17
STUDY OF MUCORMYCOSIS CASES ASSOCIATED WITH DIABETES MELLITUS, DIAGNOSED IN ROSARIO IN 2022

Dalmaso H, Ramadán S, Sortino M, Tosello ME, Bertola D, Podestá MV, Bulacio L
CEREMIC (Centro de Referencia de Micología). Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR) Universidad Nacional de Rosario). E-mail: hernan78dalmaso@gmail.com

Mucorales are cosmopolitan fungi with a natural habitat in soil, wood, etc. *Rhizopus arrhizus* and *Rhizopus microsporus* are the species most frequently associated with human infections worldwide. Their speed to invade and damage tissues and disseminate, due to their angioinvasive ability, requires early diagnosis to reduce morbidity and mortality. So far, the visualization of hyphae in the direct examination (DE) of biological samples is the most specific diagnostic method. Mucormycosis comprises a group of opportunistic life-threatening invasive fungal infections, which are caused by fungi that present hyaline, wide, non-septate hyphae, branched at right angles. They are currently the third cause of invasive fungal infection after candidiasis and aspergillosis. Their most frequent clinical presentation is the form with rhinosinus involvement linked to diabetic ketoacidosis, whose mortality reaches 46%. This research consisted of a study of mucormycosis cases, which were diagnosed at CEREMIC. Samples were processed according to the mycological routine analysis protocol. In the period from January to September 2022, three cases were diagnosed. The first case was a 39-year-old patient with diabetic ketoacidosis, from whom a sinusoidal sinus and turbinate biopsy sample were submitted. In the DE, as well as the stains, non-septate hyaline fungal hyphae were observed, developing in the cultures, *Rhizopus arrhizus*, whose identity was confirmed by techniques of MALDI-TOF and by sequencing of

partial portions of rDNA (ITSI-5.8-ITS2) and LSU (D1-D2 region). The second case was a sample of bronchoalveolar and cerebral lavage of multiple lesions, from a 19-year-old patient from Entre Ríos, also with diabetic ketoacidosis, in which non-septate filaments were observed both in DE and permanent staining, developing the same species - identification confirmed by the previously mentioned techniques. The patient also presented malnutrition and died while hospitalized. Lastly, the third mucormycosis case was a 59-year-old patient with the same predisposing conditions, from whom a sinus aspirate and tissue samples from the nostril, nasal septum, turbinate, ethmoid were referred with positive DE and staining, being recovered also *Rhizopus arrhizus.* The patient received treatment with liposomal amphotericin for 4 weeks but who died while hospitalized. The great invasive capacity and mortality associated with this pathology make collaborative work by the health team necessary for early diagnosis and early initiation of treatment.

A18

DETECTION OF HEMOPATHOGENS IN DOGS AND CATS WITH SUSPECTED VECTOR DISEASE IN SOUTHWEST CÓRDOBA AND SOUTHEAST SANTA FE REGION

Cane V[1,2], Barbero U[1], Rossi J[2,3], Cane J[1], Freije J[1], Olarreaga G[1], Asciani V[1,2], Pereyra N[1,2]
[1]Servicio de Bacteriología, Cátedra de Microbiología, Facultad de Ciencias Veterinarias (FCV), Universidad Nacional de Rosario (UNR), Argentina. [2]Medax, Laboratorio de Diagnóstico Veterinario, Chañar Ladeado. [3]Cátedra de Parasitología, FCV, UNR. E-mail: valentinacane@fcv.unr.edu.ar

Hemopathogens (HP) detection represents a challenge for vets. Many HP cause vector-borne diseases (VBD) and their behavior and distribution are diverse. Clinical signs of VBD in dogs and cats are variable and nonspecific: hyperthermia, weakness, anorexia, and pale or jaundiced skin/mucous membranes. In Argentina, described HP are bacteria such as *Anaplasma platys*, *Ehrlichia canis*, (EC) and hemotrophic mycoplasmas (HM), and parasites such as *Dirofilaria immitis*, *Babesia vogeli*, *Babesia gibsoni*, *Hepatozoon canis*, and *Trypanosoma cruzi*. The aim of this work was to analyze the results of HP detection using microscopy, hematology, and serology in dogs and cats with suspected clinical VBD. Blood with EDTA from 33 dogs and 12 cats with the clinic of VBD was studied in the Argentine region of SW Córdoba and SE Santa Fe during January–October 2022 period. In all samples, HP were investigated using microscopical and hematological methods, and in a group of 21 dogs, also with a serological test. Hematological studies were carried out in an automated counter and were complemented with May Grünwald–Giemsa-stained blood smears, in which HP search was performed by microscopical observation at 1000×. For serology of the 21 dogs, immunochromatography kits were used to detect antibodies (Ab) against EC (Fastest *Ehrlichia canis*). HP could be detected by microscopy in only 4 canines (12.12%): *Anaplasma platys* morulae in 3 and MH (hemoplasmosis) in 1; in these dogs, anemia and thrombocytopenia were detected in anaplasmosis cases, and anemia, leukocytosis, and thrombocytopenia in HM cases. In dogs in which no HP was observed (29/33 or 87.88%), unique hematological alterations were found such as anemia in 5 dogs (17.24%), leukocytosis in 2 (6.90%), total leukopenia or in any leukocyte line in 5 (17.24%) and thrombocytopenia in 4 (13.80%), and also multiple alterations: anemia and thrombocytopenia (6.9%), anemia and leukocytosis (6.9%), anemia, leukocytosis and thrombocytopenia (6.9%), and anemia and leukopenia (3.45%). Six of 33 dogs had normal hematological values. In 4 cats (33.33%) MH were observed but only 1 animal presented anemia with leukocytosis, thrombocytopenia and megaloplatelets. Six dogs (6/21 or 28.57%) were positive for serological tests, but no EC morulae were observed; one dog with Ab had thrombocytopenia, another had activated monocytes, and 2 presented anemia and activated monocytes. Microscopy is a method that confirms suspicion but has low sensitivity. Serology is valuable if clinical is present. Hematologic abnormalities are highly variable. Confirmation of VBD by methods used in this work, available by most clinicians, was achieved in a few cases. Diagnostic algorithms should also include molecular methods.

A19

PHOTODYNAMIC ANTIFUNGAL CHEMOTHERAPY EFFECT, ON PSEUDOMYCELIUM AND/OR CHLAMYDOCONIDIA PRODUCTION ABILITY, BY GENUS *Candida* YEASTS

Bulacio L, Ramadán S, Dalmaso H, Luque A, Podestá MV, Sortino M
CEREMIC (Centro de Referencia de Micología. Facultad de Ciencias Bioquímicas y Farmacéuticas). UNR: Universidad Nacional de Rosario. E-mail: lcbulacio@hotmail.com

The genus *Candida* is made up of opportunistic yeasts that are part of normal microbiota in skin and mucous membranes of human being. Under certain physiological, pathological or iatrogenic conditions, they can invade and disseminate, causing generalized symptoms knows as Candidiasis. *Candida albicans* is the most frequently isolated species in these locations, followed by *Candida parapsilosis* and *Candida tropicalis*. Virulence factors are biomolecules, or metabolic strategies of these microorganisms, that improve invasion and resistance to host defenses. These factors have currently emerged as a new antimicrobial target. The virulence factors of *Candida* are: the ability for biofilm production, presence

of polymorphisms, such as pseudomycelium and chlamydoconidia, formation of germ tubes (GT), etc. The aim of this work was to evaluate the effect of photodynamic antifungal chemotherapy (PACT) with Toluidine blue O (TBO) on the ability of *C. albicans*, *C. parapsilosis*, and *C. tropicalis* isolates, to maintain certain virulence factors. The strains were evaluated for the persistence of the ability to produce GT, to generate pseudomycelium and chlamydoconidia. A number of 44 strains were studied: 24 strains of *C. albicans*, 10 of *C. parapsilosis* and 10 of *C. tropicalis*, all of them obtained from oral mucosa from the CEREMIC culture collection. PACT studies were performed with TBO, determining the minimum inhibitory concentration (MIC) after irradiation with white light for 60 min. Subsequently, the GT generation ability (% of yeasts with GT) in serum was tested, as well as the ability to maintain the production of pseudomycelium and/or chlamydoconidia for sub-inhibitory concentrations. Pseudomycelium production was tested in two natural media that stimulate its development: Milk Agar (MA) and Spider Agar (SA); chlamydoconidia was studied in MA. Percentage of GT formation of by *C. albicans* control (without treatment) ranged from 66.67 to 91.67%. Under PACT treatment, GT formation was totally inhibited at TBO concentrations corresponding to MIC/2-MIC/4. Meanwhile, at lower concentrations, a decrease of GT production ability was observed (range 5.00–76.19%). Regarding the production of pseudomycelium, the isolates of the different species, incubated in MA after treatment, did not present typical pseudomycelium production at the standardized reading time (48 h) and *C. albicans* isolates did not produce chlamydoconidia at 48 h. About production of macroscopically detectable pseudomycelium in SA medium, the time required for *C. albicans* and *C. tropicalis*, was double that expected in untreated strains. The *C. parapsilosis* isolates did not produce pseudomycelium in any of the culture media mentioned above, under the tested conditions. Taking into account that PACT is not invasive, and its adverse effects are minimal compared to other therapeutic strategies, and based on the results obtained, we could consider it a promising strategy in the treatment of superficially located mycosis.

A20
PROFILE OF SEMINAL MICROORGANISMS IN INFERTILE MEN

Brunori M[1,2], Grillo J [1,2], Raspo E[1], Colombo L[2,3], Orellano E[2,4,5,6], Brufman A[1,2,6]
[1]Reproduction Immunology Laboratory (LIR). [2]Cs. Biochemical and Pharmaceutical School, National University of Rosario. [3]Microbiology Service "Eva Perón" School Hospital – Bacteriology Area. [5]Molecular and Cellular Biology Institute (IBR-CONICET-UNR). [6]Research Council from National University of Rosario (CIUNR), UNR.
E-mail: magalibrunori@gmail.com

Male infertility has been linked with bacterial infections of the genital tract. They can cause tissue inflammation, obstruction of the genital ducts, epididymitis, and orchitis among other pathologies, but the true impact of bacterial infections on male fertility remains controversial. Like other sites in the human body, semen has a specific microbiota necessary for normal sperm function. The objective of this study was to evaluate if some of the microorganisms present in infertile men are associated with abnormal semen parameters. 280 semen samples from infertile men who attended the Reproduction Immunology Laboratory (LIR) in the period 2019–2021 were retrospectively studied. Semen parameters were analyzed according to the Manual of the World Health Organization (WHO 2010). Culture of microorganisms from the male genital tract was carried out in the Microbiology Service of the Hospital Escuela "Eva Perón" in Granadero, Baigorria, according to the methodology proposed by Santoianni et al. as "screening" (first stream of urine/urethral secretion and semen) based on the technique of Stamey and Meares (2002). To study the association between qualitative variables, the Chi-square test or Fisher's exact test was used, as appropriate. In cases where there was an association, the odds ratio (OR) was estimated punctually and by a confidence interval. The microbiological studies showed the absence of pathogenic microorganisms in 61.4% of the samples, the presence of at least one pathogen in 32.1%, and the usual microbiota (such as monoflora and a count of 104 or higher) in 6.4% of the samples. Based on these results, the samples were divided into three groups. No significant differences were observed in ejaculate volume, pH value, viscosity, and citric acid concentration between the different groups. In all three groups, the total sperm count was decreased. For this reason, in addition to the study of morphology with hematoxylin–eosin–phloxine staining, the teratozoospermia index (TI) was evaluated, which predicts sperm function both *in vivo* and *in vitro*. Although the TI determinations of all the infertile patients in the study showed a marked asymmetry to the right (RI = 0.43), the group of patients with at least one pathogen presented TI values higher than those of the groups of patients with usual microbiota. The scarcity of works on the subject justifies further research on the impact of the seminal microbiota on male fertility and infertility. Our results call for further study of bacterial colonization of the urogenital tract and, as with the female reproductive tract, opens up potential niches for probiotic therapeutic avenues.

A21
INTESTINAL TISSUE INTEGRITY ALTERATION IN HIGH FAT DIET-FED MALE C57BL/6 AND TNF-α RECEPTOR 1 KNOCKOUT MICE

Gioberti F[1], Singh MA[1], Barranco MM[1,2], Zecchinati F[3], Perdomo VG[2,4], Villanueva SSM[3], García F[1,2]
[1]Laboratorio de Fisiología Metabólica, Facultad de Ciencias Médicas, FCM-UNR. [2]CONICET-Rosario. [3]Instituto de Fisiología Experimental-CONICET. [4]Área Parasitología, Facultad de Ciencias Bioquímicas y Farmacéuticas, FCBF-UNR. E-mail: fgarcia@fmedic.unr.edu.ar

Obesity is a multifactorial disease characterized by a systemic chronic inflammatory status that disturbs several organs, including the small intestine. TNF-α receptor 1 (TNRF1) knockout mice have an altered inflammatory response and are commonly used to study the role this cytokine has in different inflammatory diseases. The aim of this study was to assess high-fat diet-fed male C57BL/6 and TNF-α receptor 1 knockout mice small intestine histological modification. Five-week-old male C57BL/6 (C57, N = 20) and TNF-α receptor 1 knockout (R1KO, N = 20) mice were randomly divided into 4 groups (each one with N = 10). For 16 weeks, C57-C and R1KO-C groups were fed a standard diet, while C57-HFD and R1KO-HFD groups were fed a high-fat diet (HFD; 40% kcal fat). Body weight was assessed weekly. Toward the end of the treatment, insulin resistance was evaluated through an insulin tolerance test curve (intraperitoneal injection, 0.75 UI/kg, 6 h fasting). Later the mice were anesthetized and sacrificed. The blood obtained was used to measure biochemical parameters (plasma cholesterol and triglyceride levels). Adipose tissue and small intestine were collected. Hematoxylin–eosin-stained jejunum histological sections were examined under light microscopy and assessed using the ImageJ software. Statistical analysis was performed using the Student's *t*-test (comparisons were considered significant for $P < 0.05$). There was a significant increase in body weight, fat mass, and plasma cholesterol and triglyceride levels in all HFD-fed mice as compared with controls. Only the C57-HFD group showed higher plasma glucose and insulin resistance levels. Regarding histological evaluation, HFD modified the intestinal structure both in C57BL/6 and TNFR1 knockout mice. Intestinal villi height decreased both in C57-HFD (−6%, $P < 0.0001$) and R1KO-HFD (−11.6%, $P < 0.0001$) as compared with their respective controls. Intestinal villi width increased +20.8% ($P < 0.0001$) in C57-HFD and +21.7% ($P < 0.0001$) in R1KO-HFD. Likewise, HFD increased the number of goblet cells per villus both in C57BL/6 (mean ± EE, C57-C: 1.73 ± 0.11 *vs.* C57-HFD: 4.02 ± 0.99 cells/villus, $P < 0.05$) and in TNFR1 knockout mice (R1KO-C: 1.57 ± 0.53 *vs*. R1KO-HFD: 2.98 ± 0.39 cells/villus, $P < 0.05$). The intestinal tract comprises the largest border between the host and the environment, maintaining intestinal homeostasis through a physical and chemical barrier. The high-fat diet altered barrier integrity, leading to villous blunting, as a characteristic sign of intestinal mucosa atrophy. This alteration, analyzed in this diet-induced obesity model, would not be influenced by the TNF-α receptor 1 pathway.

A22
LEPTOSPIROSIS IN DOMESTIC CAT: DETECTION OF INFECTION BY SEROLOGY, CULTURE AND REAL-TIME PCR

Poli G[1], Anthony L[1], Yaafar N[1], Chiani Y[2], Landolt N[2], Schmeling MF[1], Margenet L[2], Cane V[1], Francois S[1]
[1]Facultad de Cs. Veterinarias, Universidad Nacional de Rosario, Ruta 33 y Bv. Spangenberg s/n, (2170), Casilda, Santa Fe. [2]Lab. Nac. de Referencia de leptospirosis del Inst. Nac. de Enf. Respiratorias E. Coni. Blas Parera 8260 (S3006FTP) Santa Fe. E-mail: silvinafrancois@fcv.unr.edu.ar

Leptospirosis is a disease caused by pathogenic strains of *Leptospira.* Environmental contamination with pathogenic *Leptospira* occurs through urinary shedding from infected animals. Infected cats have been shown to shed *Leptospira spp*. in urine. The aim of this work was to detect anti-*Leptospira* spp. in sick cats, using the microscopic agglutination technique (MAT) and *Leptospira* spp. in the urine of seropositive cats by culture and qPCR. Sixty blood serum samples from domestic cats (*Felis silvestris catus*) of different breeds, ages, and sex, from Rosario and Casilda clinics, were analyzed with the consent of their owners. The pets were urban, had indoor/outdoor habits, and hunted rodents. One adult cat presented with hematuria, with suspicion of clinical leptospirosis. Blood samples were obtained by venipuncture and the sera were refrigerated at −20°C. In the MAT, the reference strains used were Pomona Pomona; Icterohaemorrhagiae Copenhageni M 20, Canicola Canicola Hond Utrech IV, Australis Bratislava Jez bratislava, Pyrogenes Salinem, Sejroe Hardjo type Prajitno Hardoprajitno, Autumnalis Autumnalis Akiyami A, Bataviae Bataviae Swart of *Leptospira interrogans*; Grippotyphosa Moskva V and Cynopteri Cynopteri 3522 C from *L. kirschneri*, and Ballum Castellonis Castellon 3 from *L. borgpetersenii*. The cut-off point was 1:25. The bacteriological culture of the urine of three seroreactive cats was carried out in EMJH medium, incubated at 30ºC, and observed weekly by dark field microscopy at 40×. *Leptospira* DNA was extracted from 200 µL of culture medium, using the QIAamp extraction kit, DNA mini kit (Qiagen, Basel, Switzerland). Real-time TaqMan PCR targeting the lipL32 gene was performed as described (Stoddard et al. 2009). A positive result was considered when the Ct was less than 40. In the MAT, 39 (65%) seroreactive cats were found, and 7 (17.94%) reacted against a serovar: 3 to Castellonis, 3 to Autumnalis, and 1 to Pomona, with titers of 1:25. The remaining 32 (82.05%) showed cross-reactions between Castellonis and Autumnalis and between Bratislava, Castellonis and Autumnalis. The highest titer was 1:400 for Bratislava and resulted from seroconversion in the second serum sample from the hematuric cat. From the bacteriological culture of their urine, a spirochete compatible with *Leptospira* spp. was isolated, which could be observed by dark field microscopy. In this

isolate, the lipL32 gene was identified by qPCR. The results suggest that there is a high percentage of cats seroreactive to *Leptospira* spp. and that the sick cat suffered from an infection caused by a pathogenic strain of *Leptospira* spp, presumably from the *L. interrogans* serogroup Australis. It is the first finding of this type obtained from the urine of naturally infected cats in Argentina.

A23
PYTHON INTERFACE DESIGN FOR MICROPHOTOGRAPHY ANALYSIS: APPLICATION TO EVALUATE THE HEMORHEOLOGICAL ACTIVITY OF PHYTOCHEMICALS

Gomez M[1], Buszniez P[1], Castellini HV[2], Riquelme BD[3,4]
[1]Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). [2]Facultad de Cs. Exactas, Ingeniería y Agrimensura (UNR). [3]Grupo de Física Biomédica, Instituto de Física Rosario (CONICET-UNR). [4]Consejo de Investigaciones de la UNR. E-mail: magomez@fbioyf.unr.edu.ar

A lot of digital images are obtained in the *in vitro* hemorheological study of the phytochemicals that could be used in diabetes treatment. These images correspond to red blood cells of healthy donors that were glycated and treated with phytochemicals. The red blood cells were incubated in media with different concentrations of glucose to model *in vitro* the glycation that occurs by hyperglycemia in diabetic patients. The protocol with these images includes the determination of the isolated cell percentages, the S aggregation parameters, and the coefficient of isolated cells (C_{CA}). A GUI (Graphic User Interface) was developed in Python to systematize the image analysis. The usability criteria of the GUI based on the Tkinter library are aimed at non-expert users. The image processing algorithms are contained in the OpenCV2 library, which uses the previously trained neural networks. The images were obtained with a digital camera attached to an inverted microscope and objective 40×. In this work, the process of parameter finding was optimized. The developed interface allows to use digitized images in a practical and even remote way. The interface gives the count and relations of total cells with the isolated cells through a histogram, including those grouped in *rouleaux* or clusters. Moreover, the code optimizes the images in the case of clusters employing a white balance. The process ensures that the cell-background contrast is such as to guarantee a meaningful interpretation of the phenomenon of erythrocyte aggregation. This developed code will be helpful for the analysis of images obtained by microscopy of red blood cells treated *in vitro* with different chemical agents and samples from patients.

A24
SANITARY MANAGEMENT IN BULBS OF *Zephyranthes candida* (LINDL.) CULTIVATED *IN VITRO*

Bueno M[1,3], Incremona, M[2]
[1]Biology, [2]Phytopathology. Facultad de Ciencias Agrarias, Universidad Nacional de Rosario.CC14 (S2125ZAA) Zavalla. [3]CIUNR. E-mail: miriam.incremona@gmail.com

The genus *Zephyranthes* Herb. It is represented by herbaceous, bulbous, and perennial plants, with great ornamental value. The propagation of *Zephyranthes candida* is done by seeds or bulbs. Plant tissue culture techniques decrease the time required for the vegetative cycle since the bulb reaches the minimum size to complete its reproductive cycle in a shorter time, and can also increase the production of seedlings by dividing the bulbs. In previous works, it was determined that the high percentage of contamination, which hinders *in vitro* multiplication, is due to the presence of fungal contaminants from the species *Aspergillus niger*, *Aspergillus flavus*, *Fusarium sp*., *Penicillium* sp., and *Saccharomyces* sp. In order to evaluate the presence of pathogens in the bulbs used as explants for the multiplication of *Z. candida*, two disinfection methods were tested: S: immersion for 10 min in 3% sodium hypochlorite with the addition of 2 drops of Tween 20 and C: immersion for 10 min in fungicide (Carbendazin: 2-methoxy carbamoylamino) and later 10 min in 3% sodium hypochlorite with the addition of 2 drops of Tween 20. Prior to disinfection, the bulbs were washed with running water under a tap, the first cataphylls were extracted, working with whole bulbs (BE) and bulbs cut transversely in half (BM). After disinfection, all the bulbs were rinsed with sterilized distilled water. 25 whole bulbs without fungicide (BES), 23 whole bulbs with fungicide (BEC), 19 bulbs cut transversely in half without fungicide (BMS), and 22 bulbs cut transversely in half with fungicide (BMC) were planted. The culture medium used was MS with 30 g/L of sucrose solidified with 8 g/L of agar, pH 5.8. The explants were grown in a growth chamber at 26 ± 2°C with a photoperiod of 16 h of light. The following were evaluated: (1) the percentage of contamination at sowing, (2) the percentage of bulbs with stems, and (3) the type of fungal pathogens present. The contamination percentages were 76%, 21%, 89%, and 31.8% for BES, BEC, BMS, and BMC, respectively. Shoot regeneration for whole bulbs was 92.5% for BES and 100% for BEC, 26% for BMS, and 68.13% for BMC. The fungal pathogens with the greatest presence were *Fusarium verticillioides*, *F. proliferatum*, and *Aspergillus* sp. The presence of bacteria was also detected, although with a lower incidence. The presence of fungal pathogens did not affect the regeneration of the bulbs, although it prevents the multiplication *in vitro* as it was demonstrated in previous works. The bulbs without the application of fungicides, both whole and half, presented a higher percentage of contamination. Regarding regeneration,

there were no differences between the treatments in whole bulbs. In the medium bulbs, there were significant differences between treatments. The best result for subsequent *in vitro* work was observed in the combination of BEC, pending the evaluation of the development of contaminated and uncontaminated shoots when brought to land.

A25
SEX HORMONES INFLUENCE THE RESPONSE TO ISCHEMIA–REPERFUSION ACUTE KIDNEY INJURY

Fussi MF[1,2], Rivabella-Maknis T[3], Bulacio RP[1,2], Hazelhoff MH[1], Marquez SB[4], Campagno R[1,2], De Vuono D[5], Massoni C[5], Riera JM[5], Lioi S[5], Molinas JL[6], Brandoni A[1,2], Monasterolo LA[1,2], Larocca MC[3], Molinas SM[1,2]
[1]Farmacología. Fac. Cs. Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario (UNR). [2]CONICET. [3]Instituto de Fisiología Experimental (IFISE-CONICET). [4]Anatomía y Fisiología Patológica. Fac. Cs. Médicas. UNR. [5]Laboratorio Central del Hospital Centenario de Rosario [6]Fisiología Humana. Fac. Cs. Médicas. UNR.
E-mail: ceciliapaparella@yahoo.com, arfernandafussi@gmail.com

Ischemia–reperfusion (IR) is one of the main causes of acute kidney injury (AKI). Several studies *in vivo* have demonstrated that 17β-estradiol administration protects against IR-induced AKI. It is not yet clear if endogen levels of sex hormones could condition the damage response to IR. Our aims were to characterize the renal response to IR damage in male and female rats and evaluate if there is a correlation between serum sex hormones levels and renal damage degree. Wistar male (MIR) and female rats (HIR) (N = 6 per group) were submitted to 40 min of unilateral renal ischemia followed by 24 h of reperfusion. Controls underwent sham operations (MC and HC). Functional and histological studies were performed. Plasma estradiol, progesterone, and testosterone were determined using the electrochemiluminescent immunoassay (ECLIA) technique. α-SMA, an epithelial–mesenchymal transition marker, was evaluated in renal tissue by Western blot. IR damage produced a decrease in glomerular filtration rate (GFR), estimated by creatinine clearance, which was greater in MIR (−38% $P < 0.05$ *vs.* MC) than in HIR (no difference *vs.* HC). IR damage cause an increase in urea serum levels that was greater in MIR (+65% $P < 0.05$ *vs*. MC) than in HIR (+36 $P < 0.05$ *vs.* HC). Histopathological evaluation of cortical renal tissue revealed acute tubular necrosis in MIR, while kidney damage in HIR was moderate. The renal abundance of α-SMA was increased in MIR (+450% $P < 0.05$ *vs.* MC) and did not change in HIR *vs.* HC. A positive correlation between GFR, and plasma estradiol was found in HIR ($\rho = 0.881$). Plasma progesterone did not show a correlation with GFR. IR damage caused a decrease in plasma progesterone and testosterone in MIR (−46% and −27% $P < 0.05$ *vs.* MC, respectively). In conclusion, consistent with the scientific literature, we found that IR damage was less severe in female than male rats. In this group, IR damage also altered normal levels of progesterone and testosterone. Our main finding was that female renoprotection could be mediated by high concentrations of endogenous estradiol. These data provide evidence in favor of sexual dimorphism in the pathophysiology of ischemic AKI. Further understanding of this sex-specific molecular mechanism could lead to the development of novel therapeutic strategies.

A26
EFFECTS OF MARIJUANA AND COCAINE CONSUMPTION ON SEMINAL PARAMETERS OF MEN WITH IDIOPATHIC INFERTILITY

Paparella C[1,2], Garnero I[1,3], Perfumo P[1,3]
[1]Unidad de Reproducción Humana Médicamente Asistida (URHMA) - Hospital Provincial del Centenario, Rosario. [2]Facultad de Cs. Bioquímicas y Farmacéuticas. [3]Facultad de Cs. Médicas. Universidad Nacional de Rosario.
E-mail: ceciliapaparella@yahoo.com.ar

Illicit drugs such as marijuana and cocaine are psychoactive substances that inside a living organism can modify its perception, mood, cognition, and motor functions. Marijuana from the plant species *Cannabis sativa* causes a progressive deterioration in the function of the hypothalamic-pituitary-gonadal axis, generating a decrease in the secretion of pituitary gonadotropins that alters the spermatogenic process and the synthesis of androgens. Cocaine is a stimulant drug whose consumption decreases sexual desire and causes erectile and/or ejaculatory dysfunction in men. Our objective was to evaluate the effects of marijuana and cocaine consumption on seminal parameters of men with idiopathic infertility. A retrospective study of cases and controls was carried out. 214 seminal samples were selected from patients who attended the URHMA between 2017 and 2021. Male smokers, exposed to extreme temperatures, toxic products, and with antecedents of andrological alterations were excluded. Three groups were formed: G1 (N = 117) non-consumers of illicit drugs, G2 (N = 54) chronic consumers of marijuana or cocaine, and G3 (N = 43) simultaneous consumers of both drugs combined. Sperm parameters of progressive motility, concentration, and morphology were evaluated. The Student's *t*-test was applied to compare the averages of the analyzed variables between the groups. MP (% progressive mobile spermatozoids) G1: 60.58 ± 25.98 *vs*. G2: 57.66 ± 26.10 ($P = 0.210$); G1: 60.58 ± 25.98 *vs.* G3: 51.67 ± 26.14 ($P = 0.022$); G2: 57.66 ± 26.10 *vs.* G3: 51.67 ± 26.14 ($P = 0.102$). C (10^6 spermatozoids/mL semen) G1: 54.31 ± 14.26 *vs.* G2: 57.87 ± 18.47 ($P = 0.186$); G1: 54.31 ± 14.26 *vs.* G3: 46.98 ± 19.42 ($P = 0.257$); G2: 57.87 ± 18.47 *vs.* G3: 46.98 ± 19.42 ($P = 0.063$). M (% gametes with normal morphology) G1:

6.37 ± 1.65 *vs*. G3: 3.56 ± 1.80 (P = 0.0006); G2: 5.82 ± 1.13 *vs*. G3: 3.56 ± 1.80 (P = 0.004). The group consuming both drugs simultaneously presented statistically significant differences in the seminal variables of MP and M (P < 0.05). The toxic effects of marijuana and cocaine consumption affect male reproductive function by altering sperm morphology and motility. The simultaneous combination of both drugs increases the negative consequences on sperm quality, being an important factor to consider in the study of the male within the couple with reproductive problems.

A27
EFFECT OF EGG CONSUMPTION ON THE LIVER OF OBESE AND DIABETIC RATS

Crespo LM[1], Ruiz MF[1], Serena R[1], Montenegro SM[3], Revelant GC[2], Marinozzi D[2], Elias D[2], Posadas MD[1]
[1]Chair of Biology. Faculty of Medical Sciences. [2]Bromatology and Nutrition Area. Faculty of Biochemical and Pharmaceutical Sciences. [3]CIUNR. National University of Rosario. E-mail: martaposadas@hotmail.com

Dietary recommendations that restrict egg consumption have been maintained for decades and the belief that egg consumption increased plasma cholesterol and therefore the risk of cardiovascular disease still persists in society. Based on the available scientific evidence, it can now be stated that, in the context of a balanced diet, moderate consumption of eggs (up to seven per week) would be safe for the healthy population. However, this would not be the case for diabetics, for whom greater caution would be appropriate. The controversy in diabetic patients regarding egg consumption is related to the increased risk of developing non-alcoholic fatty liver disease (NAFLD). We set out to evaluate the effect of egg consumption on the liver of obese and diabetic rats. 60-day-old male rats of the IIMB/Beta line, randomly separated into 3 groups (N = 6 each) received for a period of 8 weeks: Diet 0 (control): commercial balanced food for rats; Diet 300: Diet 0 + cholesterol 0.05 g /100 g of food provided by whole egg powder (comparable to 300 mg/day in humans) or Diet 600: Diet 0 + cholesterol 0.1 g/100 g of food provided by whole egg powder (comparable to 600 mg/day in humans). At the end of the experiment, blood was taken from the animals by venipuncture of the tail and liver enzymes aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and alkaline phosphatase (FOH) were determined in plasma. After sacrificing the animals, the liver was removed and weighed. For histomorphological evaluation, liver tissue samples were fixed in 40% buffered formalin, processed histologically, and included in paraffin. 5-µm sections were stained with hematoxylin–eosin to evaluate steatosis (percentage, grade, type, and location), inflammation (grade and location), presence of glycogen, and cholestasis. The degree of steatosis was established according to the Turlin score (percentage of hepatocytes containing lipid vacuoles) as "grade 0" less than 5%; "grade 1" between 5–25%; "grade 2" between 25–50%; grade 3 between 50–75%; and "grade 4" greater than 75%. The results (mean ± standard deviation) were analyzed with the ANOVA test and Tuckey's post-test using the statistical package Prism 3.0; significant differences were considered at a level of P < 0.05. The relative weight of the liver in g/100 g of biomass (0: 3.79 ± 0.27; 300: 3.79 ± 0.19; 600: 3.66 ± 0.18; P > 0.05), as well as the enzymes in U/L (ASAT: 0: 107.5 ± 21.7; 300: 113.4 ± 23.2, 600: 107.2 ± 22.0; P = 0.88 – ALAT: 0: 40.2 ± 8.7, 300: 40.0 ± 3.2, 600: 33.2 ± 4.1; P = 0.09 – FOH: 0: 214.0 ± 42.0, 300: 218.0 ± 26.0; 600: 185.0 ± 12.0; P = 0.14) did not differ significantly. The morphological study did not show differences either, in the three groups the same was observed: steatosis between grade 0 and grade 1, with microvacuoles, and to a lesser extent macrovacuoles, of perivenular and centroacinar location; zonally hepatocyte ballooning and sinusoidal congestion. In obese and diabetic rats of the Beta line, egg consumption did not affect liver function or morphological structure.

A28
TIME OF SYNTHESIS AND ACTION OF SOYBEAN EMBRYONIC AXIS GERMINATION PROTEINS

Yordán E[1], Gosparini CO[1,2], Perotti VE[3], Montechiarini NH[1,2]
[1]Lab. Fisiología Vegetal. [2]IICAR–CONICET. [3]Plataforma AGROBIOTEC-FCA. Facultad de Ciencias Agrarias. UNR. E-mail: nidia.montechiarini@unr.edu.ar

Seed germination is an expansion process that does not imply cell division and visualizes as the radicle protrusion through the seed covering layers. That cell expansion is driven by the water uptake during incubation under suitable conditions, specifically by cells in the elongation zone (EZ) of the embryonic axes (Ax), describing a typical triphasic water uptake dynamic in which the onset of phase III defines germination *sensu stricto*. Additionally, germination experiments showed that the germination of soybean Ax is possible from the mRNA synthesized in the seeds during development and accumulated in the Ax at maturity while requiring the *de novo synthesis* of the proteins for that process to occur. In the present work, the time of the synthesis of the minimum proteins to complete soybean Ax germination was evaluated. A total of 120 Ax were firstly incubated in distilled water and thereafter, samples of 10 Ax each were transferred consecutively every hour until 12 h to a 100 µM solution of cycloheximide (translation inhibitor), continuing the incubation in this last medium for the following 16 h. The dynamics of water uptake from the transference to cycloheximide was recorded, at hourly intervals up to 12 h, ending the experiment with the last weighting at 28 total h of incubation (water + cycloheximide). Also, the imbibition dynamics were evaluated for 10 Ax incubated in 100 µM

cycloheximide (negative germination control) and 10 Ax in distilled water (positive germination control). The Ax corresponding to the negative control experienced an increase in water uptake during the first 2 h that phase I lasted, then remained in phase II, without starting phase III of germination until the end of the experiment. Similar results were obtained for Ax incubated in water up to 6 h inclusive, which gained weight until reaching phase II at the time or after transference to cycloheximide, and thereafter stopped water uptake without entering phase III (germination) during the entire period of cycloheximide incubation. By contrast, the Ax incubated in water for 7 and up to 12 h showed a sustained volume increase, even after the transference to cycloheximide, with the greater initial and final water uptake the longer the time in the water was. These results showed that the previous incubation for 7 h in distilled water was sufficient to allow the initiation and completion of Ax germination even after the transference and incubation in cycloheximide. It is concluded the existence of a time window of, at least 6 h of incubation, for the synthesis and action of the minimum necessary proteins responsible for the soybean Ax germination.

A29
IN VITRO STUDY OF THE HEMORHEOLOGICAL ACTIVITY OF AQUEOUS EXTRACTS OF *Phyllanthus sellowianus*

Mascaro Grosso H[1], Buszniez P[1], Castellini H[2], Riquelme BD[1,3,4]
[1]Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). [2]Facultad de Cs. Exactas, Ingeniería y Agrimensura (UNR). [3]Grupo de Física Biomédica, Instituto de Física Rosario (CONICET-UNR). [4]Consejo de Investigaciones de la UNR. E-mail: hermanomascaro@gmail.com

Diabetes mellitus is currently one of the main threats to human health. Extracts of plant species have been used since ancient times to treat the symptoms of diabetes, being an alternative to the high cost of synthetic drugs. Among the numerous species of plants of medicinal interest for the treatment of diabetes is the *Phyllanthus sellowianus* Müll (Klotzch) Arg., native to Argentina and Brazil. This work aims to evaluate the effects of the *in vitro* hemorheological action of different aqueous extracts obtained from *P. sellowianus* on human red blood cells. The infusion, maceration, digestion, and cooking techniques were used to prepare aqueous extracts with leaves and bark of *P. sellowianus* and saline as solvent. The extracts presented acid characteristics and osmolarity outside the range of the necessary physiological conditions, and they were corrected at pH 7.4 and 300 mOsmol/L to confront it with human red blood cells. Red blood cells from healthy donors (N = 3) and the same incubated with glucose solutions (0.4 g/dL in PBS) were used to simulate *in vitro* the glycation that occurs due to hyperglycemia in diabetes. Erythrocyte Rheometer was used to determine the hemorheological parameter of erythrocytes treated with the extracts. The *in vitro* tests with the different extracts of *P. sellowianus* showed diverse effects on the viscoelasticity and erythrocyte aggregation of the red blood cells of healthy donors. When evaluating the effect on glycated red blood cells, it was observed that the extracts reversed the glycation effect on the surface membrane viscosity, bringing their values closer to those of the controls. The results obtained are of great importance regarding the hemorheological activity of the different aqueous extracts of *P. sellowianus*. They also provide relevant information for understanding the mechanisms of action by which these extracts or their components can be used as antidiabetics in phytomedicine.

A30
SEROLOGICAL DETECTION OF SEROREACTIVE CANINES TO PATHOGENIC LEPTOSPIRES IN THE SOUTHEAST REGION OF CORDOBA AND SOUTHWEST SANTA FE

Cane V[1-4], Cane J[2-4], Poli G[1], Anthony L[1], Giacomelli P[2], Muchut M[3], Ascaíni V[1], Francois S[1]
[1]Microbiología, Facultad de Cs. Veterinarias (FCV), Universidad Nacional de Rosario (UNR), Ruta 33 y Bv. Spangemberg S/N, (2170), Casilda, Santa Fe. [2]Cátedra de Dermatología Veterinaria, FCV-UNR. [3]Cátedra de Animales de Compañía, FCV-UNR. [4]Medax, Chañar Ladeado, Santa Fe. E-mail: valentinacane@fcv.unr.edu.ar

Leptospirosis is an infectious disease caused by pathogenic strains of Leptospira spp. that affects most animal species. In Argentina, it is one of the most important zoonoses. Dogs, due to close contact with humans, play an important role in the epidemiology of the disease, mainly in areas of high population density. The objectives of the work were: to detect seroreactivity to *Leptospira* spp. in dogs with and without clinical signs compatible with leptospirosis using the microscopic agglutination technique (MAT) and to identify the main serogroups of *Leptospira* spp. that affect canines that inhabit the southwest (SO) region of Santa Fe and southeast (SE) of Cordoba. Fifty-six samples of blood serum from dogs (*Canis lupus familiaris*), 23 females and 33 males of different ages and breeds, from the south of Santa Fe and Cordoba, were analyzed. The canines from urban environments had owners and had indoor/outdoor habits. Some presented clinical signs compatible with leptospirosis. Blood samples were obtained by venipuncture and sera were stored at −20°C. Reference strains of *Leptospira* spp. were used for the MAT: *L. interrogans*: Pomona Pomona; Icterohaemorrhagiae Copenhageni M 20, Canicola Canicola Hond Utrech IV, Australis Bratislava Jez bratislava, Pyrogenes Salinem, Sejroe Wolffi 3705, Autumnalis Autumnalis Akiyami A, Bataviae Bataviae Swart; *L. kirschneri*: Grippotyphosa Moskva V, Cynopteri Cynopteri 3522 C; and *L. borgpetersenii*: Ballum Castellonis Castellon 3. The

cut-off point was 1:50, considering a titer ≥ 1:400 a positive case. Thirty-nine seroreactive canines (69.64%) to *Leptospira* spp. were found: 15 females (38.46%) and 24 males (61.53%). It was observed that 5 sera (12.82%) reacted to a single serovar: 2 to Bratislava, with titers of 1:50 and 1:100, 2 to Cynopteri, and 1 to Canicola, each with a titer of 1:100. In the remaining 34 (87.17%), different cross-reactions were detected, within which the diagnosis of leptospirosis was established using MAT in dogs with clinical signs: 7 adult males (20.58%) and 1 young female (2 .94%). Five of the eight patients were from the SE of Cordoba in which the highest titers detected were Bratislava (1:1600), Canicola (1:800), Copenhageni (1:800), and Autumnalis (1:400). One of these individuals was considered positive in the second sample, still with a titer of 1:200 to Pomona, Bratislava, Autumnalis, and Cynopteri, since seroconversion to those serovars was observed (from negative to 1:200). In SW Santa Fe, 3 positive animals were found: 2 in which the highest titer found was for Canicola (1:51200 and 1:400) and 1 for Cynopteri (1:800). The results of the MAT allowed us to observe a high seroreactivity rate in the analyzed population, as well as a higher proportion of clinical cases of leptospirosis in adult male canines. The most frequently detected *Leptospira* spp serogroups were Canicola, Australis, and Cynopteri, the last two being detected for the first time in the studied region.

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FIRST STEPS FOR THE IMPLEMENTATION OF A NON-INVASIVE SCREENING PLATFORM FOR COLORECTAL CANCER USING DIGITAL PCR

Bogado J[1,3], Heckel SB[2,3,4], Girardini J[2,4], Sesma J[1,3,4]
[1]Facultad de Ciencias Médicas, Universidad Nacional de Rosario (FCM-UNR). [2]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (FbioyF-UNR). [3]Biología Molecular, Hospital Provincial de Rosario, Argentina. [4]Instituto de Inmunología Clínica y Experimental de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (IDICER-CONICET). E-mail: sofiaheckel@hotmail.com

Colorectal cancer (CRC) is the second cause of cancer-related death in Argentina and the world. Early diagnosis is critical as it increases up to 90% the chances of cure. Currently, colonoscopy is the gold standard method for CRC diagnosis. However, due to its invasive nature and technical complexity, it has a low adhesion rate, reducing the chances of survival. These difficulties, added to the cost of the intervention, limit its use in mass monitoring programs. Consequently, it is a priority to develop non-invasive methodologies for CRC diagnosis. In Argentina, the non-invasive monitoring method used is the fecal occult blood test, which presents insufficient sensitivity levels to provide an accurate diagnosis. The presence of DNA derived from tumor cells in feces or fluids has revolutionized the concept of diagnosis. The identification of genetic and epigenetic alterations in DNA derived from the primary tumor present in fluids and feces constitutes a target of great clinical potential. Mutations in KRAS and TP53 genes are among the most common in CRC. On the other hand, alterations in the methylation patterns of the promoters of specific genes are related to the development of the pathology. So far, there are no non-invasive methods with sufficient sensitivity to give an accurate diagnosis. For this reason, we propose to develop a non-invasive quantitative diagnostic method for CRC using an ultrasensitive and quantitative technology: droplet digital PCR (ddPCR). We started with biomarkers that have demonstrated their diagnostic potential in other non-invasive methods, with the aim of looking for the best combination of them. We have achieved a first set-up for the selective detection of one of the most frequent KRAS mutations, G13D, working with the HCT-116 cell line that presents an allele with the G13D mutation and a wild-type (WT) allele for KRAS. DNA extraction was performed by extraction columns and two methods of DNA disruption (sonication and use of restriction enzymes) we used to improve detection by Real Time-PCR. Once the purification of the DNA was set up, we carried out the assays by ddPCR. We used different concentrations of DNA from the HCT-116 cell line and in all cases, both alleles could be detected in a 1:1 ratio. The next tests to be carried out are to reduce the G13D/WT ratio, forcing the method to know its detection limit and validate it. In this pilot test, we fine-tuned the first determinations to detect a KRAS mutant allele in the presence of KRAS WT by ddPCR.

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COMPARISON OF BREAST CANCER MORTALITY DURING THE FIVE YEAR PERIOD (1988–92 AND 2013–17) IN THE CITY OF ROSARIO

Ferreira da Silva W[1], Pezzotto SM[1,2], Dagatti MS[1]
[1]Metodología de la Investigación Científica, Facultad Ciencias Médicas, Universidad Nacional de Rosario. [2]CIUNR. Santa Fe 3100 (2000) Rosario. E-mail: sgtwesllen@gmail.com

Breast Cancer (BC) is the leading cause of death from neoplasia in women in most occidental countries, including Argentina. The objective of this study was to know the current mortality rates (MR) from this disease in Rosario, to compare with those obtained for an earlier five-year period. The database of deaths by MC occurred among all women residing in Rosario during 2013–17 provided by the "Dirección General de Estadística, Municipalidad de Rosario" was analyzed. Age group-specific mortality rates, during the five-year period from 2013–17, were calculated and compared with the obtained data in a previous investigation in which the period analyzed was 1988–92. The Age-Adjusted Mortality Rates (AAMR) were also calculated, using the direct method of standardization to compare the rate

corresponding to 2013–17. The obtained rates are shown in the following data: 25–29 (1988–92: 2.6; 2013–17: 1.8); 30–34 (1988–92: 8.5; 2013–17: 6.3); 35–39 (1988–92: 15.5; 2013–17: 14.7); 40–44 (1988–92: 24.7; 2013–17: 22.5); 45–49 (1988–92: 32.6; 2013–17: 39.0); 50–54 (1988–92: 35.6; 2013–17: 64.9); 55–59 (1988–92: 76.9; 2013–17: 66); 60–64 (1988–92: 114.6; 2013–17: 102.8); 65–69 (1988–92: 119.1; 2013–17: 142.6); 70–74 (1988–92: 177.3; 2013–17: 129.6) and ≥ 75 (1988–92: 305.9; 2013–17: 212.4). The AAMR were 34.7 (1988–92) and 30.2 (2013–17), being lower than the one obtained in 1988–92. Disparities in the age group-specific mortality rates were also found, lowering in some age groups, and raising in others. In conclusion, in the two decades that have elapsed between the five-year periods analyzed, a tendency to decrease in AAMR is observed in Rosario. Analytical epidemiological studies would be necessary to detect possible factors associated with these changes in age-specific mortality rates.

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