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ABSTRACTS

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1. S-100 AND GLIAL FIBRILLARY ACIDIC (GFAP) PROTEINS: IMMUNOHISTOCHEMICAL MARKERS OF THE FOLLICULO-STELLATE CELLS OF THE *Lagostomus maximus maximus* PITUITARY

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Immunohistochemical techniques has enabling to extend the morphological study of the folliculo-stellate (FS) cells. The pituitary gland of viscacha (*Lagostomus maximus maximus*) exhibits follicular structures formed by FS cell. The aim of this work was to immunohistochemically identify FS cells of pituitary gland using anti-S-100 and anti-GFAP antisera. Moreover, lanthanum hydroxide was used as intercellular marker. Pituitary glands of adult male viscachas were processed for light and electronic microscopy. The immunolabeling pattern of the S-100 was nuclear and cytoplasmic and cytoplasmic for GFAP. FS cells were present in the pars intermedia and pars distalis of pituitary gland. They were spread around pituitary follicles and their cytoplasmatic processes were found between neighbouring endocrine cells. The tracer penetrated freely into the intercellular spaces, delineating the cellular borders of granulated and nongranulated cells. These results suggest the FS cells probably participate in a network of intrahypophyseal communication and may be involved in the modulation of the adeno-hypophyseal activity. The expression of S-100 and GFAP suggest of neuroectodermal origin of the FS cell of viscacha pituitary gland.

2. RAT AND BOVINE PARS TUBERALIS IMMUNOCYTOCHEMISTRY WITH FB12 ANTIBODY (LIGHT-DARKNESS CYCLE 12:12)

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The pars tuberalis is a well defined area of the vertebrates adeno-hypophysis. It consists of cells that can be classified in two groups: one of follicular cells, and the other one of secretory cells. Secretory cells are divided in two types: one type of cells that are present in other regions of the adeno-hypophysis, namely "pars distalis like cells" and the other group of specific PT cells, which secrete a substance of still unknown function.

The aim of this study was to immunolocalize the PT cells in bovine and rat by means of an antibody denominated FB12, which specifically recognizes PT cells. The antibody was obtained from formalin 5% fixed bovine PT extracts, fixed in 5% paraformaldehyde and homogenized in sodium bicarbonate. Rats were inoculated 4 times with this immunogen, with the highest titres in the third and the fourth bleeding.

In rats, the immunocytochemical study of the PT slides showed that the antiserum recognizes a PT cell population, with an immunoreaction in most of the cells, at a 1:4000 dilution. This reaction was circumscribed to small areas of the paranuclear region. In bovine, immunoreaction was observed at a dilution of 1:16.000 in cells located in the internal part of the pars tuberalis.

These results strongly suggest the specific nature of both bleedings to recognize a specific secretion of the PT cells from both rat and bovine.

3. ELECTRON MICROSCOPY OBSERVATION OF THE BOVINE PARS TUBERALIS (LIGHT-DARKNESS CYCLE 12:12)

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The pars tuberalis is a well defined area of the vertebrates adeno-hypophysis. It consists of cells that can be classified in two groups: one of follicular cells, and the other one of secretory cells. Secretory cells can be divided in two types: one kind of cells that are present in other regions of the adeno-hypophysis, namely "pars distalis like cells" and the other group, that differs ultrastructurally from the adeno-hypophysis called specific or inherent cells.

The objective of this work was to study the bovine PT by electron microscopy. Samples from the bovine median eminence-PT region were fixed by immersion in Zamboni (48hs). Subsequently were treated with osmium tetroxide in 0.1M phosphate buffer pH7.4 for 2 hours. After dehydration, they were included in metacrylate. Ultrafine slides were stained with uranyl acetate and lead citrate. Semifine slides were stained with toluidine blue.

Bovine PT showed cells follicularly organized around the median eminence, separated by a thin connective layer of tissue. This follicles are distributed among the vases of the portahypophysis primary system, with a central light, filled with a still unknown colloidal substance. Follicles are formed by different cell types, surrounded by a thin mesh of connective tissue.

4. HISTOLOGY OF THE CORONA OF SCENTED AND SCENTLESS FLOWERS OF *PASSIFLORA* SPP. (PASSIFLORACEAE)

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The histology of plant osmophores has been poorly studied. With the aim of knowing thoroughly the structure and function of the corona of *Passiflora*, *P. caerulea* (with odour perceivable by humans) and *P. suberosa* (without odour) were analysed. Histochemical tests and observations with TEM of the pieces in which odour is perceived in the scented flowers were performed in both type of flowers, in anthetic and in recently closed ones. The staining of the epidermis and subepidermal layers with sudan IV was positive, more homogeneous in *P. suberosa* but more intense in *P. caerulea*. In both species the epidermis was formed by papillae, shorter in *P. suberosa*. Ultrastructurally, the epidermal, subepidermal and parenchima cells showed dictyosomes, mitochondria, ERr, plastids and lipidic globules in different amounts. Plasmodesmata connected the epidermal, the subepidermal and both types of those cells. Vesicles in exocytosis in the epidermal cells outer tangential wall, and an osmiophilic substance between the plasmalemma and the wall and also in the cuticle were observed. The wall became more fibrillar near the thin cuticle and the microfibrils intruded inside it. The tissues of both species appeared more vacuolated in post-anthetic flowers.

Both species exhibit secretory tissue. *P. suberosa* emits some substance, although not detectable by our olfactory sense.

5. INDUCTION OF APOPTOSIS IN BOVINE GRANULOSE CELLS (CG) BY DISRUPTION OF THE CALCIUM DEPENDENT CELLULAR UNIONS

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Cellular adhesion is necessary for the organization of the CG. The Ca^{++} fulfills an important role stabilizing cellular unions, for example in the cadherin mediated cell contact. Alterations at this level generate cellular changes that may lead to apoptosis. The objective of the work was to establish "in vitro", the relation between the loss of the calcium dependent contacts with the integrity and the apoptosis of the CG. The model was a bovine CG cell line treated with EGTA, with the purpose of disrupting the Ca^{++} dependent homophilic unions mediated by E-cadherin. Cellular viability (CV) was determined by exclusion with Tripan blue stain and apoptosis index (IA) by staining chromatin with DAPI and TUNEL. Treatments were made with 100nM and 9nM EGTA, and without EGTA. Simultaneously, the role of the serum depletion was determined by repeating the same study in cultures with and without t5% bovine foetal serum (SFB). Obtained data were statistically analyzed by a 2x3 factorial design. Although the treatments generate evident apoptotic changes, in the different treatments with EGTA there was no statistically significant differences between the percentage average of VC and the IA determined by DAPI. The tests by TUNEL validate the apoptotic changes demonstrated with DAPI. In treatments with and without SFB significant differences ($p < 0.051$) between CV average were seen, as for the IA ($p < 0.054$). Since the serum factor must be considered in the study of apoptosis, also the effect of the EGTA to different concentrations from SFB has been analyzed.

6. MICROSCOPIC ORGANIZATION OF UTERUS IN THE SMALLNOSE FANSKATE SYMPTELYGIA BONAPARTII (MÜLLER & HENLE, 1841) (CHONDRICHTHYES, RAJIDAE)

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The smallnose fanskate is oviparous and use the Bahía Blanca estuary as an egg case laying zone during late spring and summer. The function of the uterus in this species is to host the eggs during the tanning process and until oviposition.

The aim of this work is to describe the histological organization of uterus of *S. bonapartii*. For the present study 8 adult females were caught in the Bahía Blanca estuary. Samples were processed according to routine techniques. The uterine wall was composed by three layers, with a well developed mucosa. It was lined with simple ciliated columnar epithelium that invaginated to form tubular glands. The subjacent lamina propia, of loose connective tissue was highly vascularized. The muscularis was composed of inner circular and outer longitudinal layers of smooth muscle bounded by a serosa. According to the features of these layers, two zones in the uterus can be distinguished. None of the examined uterus contained eggs, so the previous description corresponds to non pregnant females. Although previous studies about this species were not found, the general histology was coincident with the description for other species of north hemisphere skates.

This work was supported by a SGCyT-UNS PGI 4/B100. We thank to Prefectura Bahía Blanca for their invaluable help.

7. EPIDERMAL CHARACTERISTIC OF *Prosopis denudans* Benth IN DIFFERENT STADIUMS OF THE DEVELOPMENT

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Prosopis denudans Benth is the southern algarrobo but of the Argentina, used as forage, firewood and residents' food by their high nutritious value, ii vegetates in arid areas of Chubut, Sta. Cruz and Río Negro.

The epidermis is a key tissue in the water economy of the native species of the arid and semi-arid areas.

The objective of this contribution was to characterize the epidermis to foliate of seedling and mature plants of two origins of *P. denudans* of the province of Chubut.

Material of seeds and leaves were obtained from two places of the province of Chubut: Garayalde and Cerro Dragón. Cotyledons and true leaves of seedlings obtained in laboratory and field individuals' leaves were processed according to habitual fixation techniques and colored for the anatomical study with optic and electronic microscope of sweeping.

In folioles of the completely expanded compound leaf of mature and juvenile plant and in cotyledons it was determined the stomatal type predominantly anomocytic and unicellular trichomas, in epidermis adaxial and abaxial were different cells tapes in shape in the different development stadiums. We compared characters of two accessions.

8. ON THE PRESENCE OF TRYPTASE-AND CHIMASE MAST CELLS AND MICROVESSELS IN NON- SMALL CELL LUNG CANCER

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Two types of human mast cells are overexpressed in cancer tumors: tryptase-positive mast cells (MCt) and tryptase-chymase positive mast cells (MCtc), but their functional significance remains controversial. Their distribution in immunostained specimens from 29 lung cancer patients and its relation with local microvessel density (MVD) and other clinic-pathological parameters including global survival was investigated. MCt was the predominant phenotype in all tumor regions, and both MCt and MCtc, and MVD counts in the border of the tumor region were significantly higher than in the intratumoral zone ($p < 0.001$). Only a significant correlation between MCt and tumor stage was observed (Pearson, $p < 0.05$). The univariate analysis showed that patients with low MCt counts had the longest survival (Log-rank test, 5.1; $p = 0.02$). Nevertheless, Cox analysis indicated that MCt cannot be taken as an independent prognostic factor. In the intratumoral zone, a correlation between MCtc and MVD was observed ($r = -0.68$ $p < 0.05$) suggesting they are involved in tumor angiogenesis.

9.

IN SITU DETECTION OF REACTIVE OXYGEN SPECIES IN THE CENTRAL NERVOUS SYSTEM OF ADULT RATS EXPOSED TO VANADIUM(V)

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Neurotoxic effect of vanadium (V) through reactive oxygen species (ROS) generation in the central nervous system (CNS) of adult rats was previously reported. ROS are potentially toxic species that include NO and mediate several neurotoxicity mechanisms and neuropathological events such as Parkinson and Alzheimer. The aim of this work was to compare two methods, an indirect and a direct one, for the cellular localization of ROS and NO, on the central nervous system of vanadium exposed rats. 90-day-old male Wistar rats were divided into two groups: Treated rats: were intraperitoneally injected with 3 mg/kg body weight of sodium metavanadate for 5 consecutive days. Control rats: were injected with saline. Modified method of Vincent and Kimura (1992) in free floating sections of brain as an indirect method for NOS activity detection was used. In this technique, neurons with NADPH diaphorase activity were stained with Nitro Blue Tetrazolium (NBT). The DAB-Mn-Co method (Kerves *et al.*, 1997) in fresh frozen cryostat sections of brain for *in situ* localization of ROS was used as a direct method. NBT staining showed a higher NADPHd activity in cerebellum, hippocampus and paraventricular hypothalamic nuclei in vanadium exposed rats. DAB-Mn-Co staining also showed ROS production in the pyramidal cell layer neurons of the brain cortex of treated rats. This method improved the localization of ROS in the brain of vanadium treated rats

10.

MORPHOLOGY OF THE CAPITULA OF *PLUCHEA SAGITTALIS* (ASTERACEAE)

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The use of medicinal plants is an increasing practice in our country, being the treatment of digestive disorders the most spread use at present. *Pluchea sagittalis* (Lamb.) Cabrera ("lucera", "quitoc"), a native species, is used mainly as a digestive herb, existing numerous reports on its use. The aim is to study the morphology of the capitula in order to determine diagnostic characters to allow the identification of this species in commercial samples. Observations of fresh, dried and fixed material were made under stereoscopic and compound microscope. The capitula are flattened and the peduncles are pubescent. The receptacle is flat and glabrous, and the involucre is formed by external obovate and inner lanceolate bracts. Capitula are disciform and heterogamous. The marginal florets are functionally pistillate being their corollas white, gamopetalous, trilobulate and filiform. The ovary is inferior and the style exerted and deeply bifid. The fruit is a cypsela. Glandular hairs occur in bracts, corolla, ovary and fruit. A row of simple hairs forms the papus. The central florets, in lower number, are functionally staminate, having a white to purplish gamopetalous corolla, with tube and 5-lobed limb. They have five stamens with fused and exerted anthers; the style is bifid and exerted. The external face of the limb shows glandular and eglandular hairs. In all cases, the glandular and eglandular hairs observed in the inflorescences are similar to those described for the leaf of the same species.

11.

MORPHO-HISTOMETRIC COLONIC STUDY IN THREE DIABETIC LINES OF RATS

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The possible influence of dysmetabolopathies on colonic function was morpho-histometrically assessed in eSS (non obese diabetic rats), β (obese diabetic rats), eSMT (overweighed diabetic rats) and eumetabolic Wistar rats (W). Male animals of 4, 8 and 12 months of age (4/age) were sacrificed with ether overdose. Specimens of distal colon were dissected out, washed with PBS, measured and weighed, fixed in Carnoy's fluid, included in paraffin wax and stained with Giemsa and Hematoxylin-Eosin. Wall and mucous width and number of goblet cells/crypt were determined through a linear devise located in the eye-piece. Data are expressed as $x \pm sem$ Among them, we outlined at 12 months of age: Absolute body weight (BW): eSMT vs. W ($314.40 \pm 33.33 < 466.71 \pm 43.76$ g)($P < 0.05$); Absolute colonic weight β vs W (3.36 ± 0.12 g $> 2.71 \pm 9.26$ g)($P < 0.05$). Relative colonic weight: β vs. W ($0.79 \pm 0.03 > 0.59 \pm 0.04$ g/100g BW) ($P < 0.05$); Colonic length: β vs. W ($26.82 \pm 0.39 > 22.6 \pm 2.10$ cm.)($P < 0.05$); Wall width: eSMT vs. β ($675 \pm 120.04 > 365.24 \pm 22.85$ μ m)($P < 0.05$); Mucous width: (n.s.); Number of goblet cells/crypt: eSS and eSMT vs β and W (23.53 ± 4.07 and 23.00 ± 3.00) $> 9.95 \pm 1.49$ and W (10.58 ± 1.47)($P < 0.05$). The onset of diabetes in eSS (6 mo.), in eSMT (3 mo.) and in β (8 mo.) as well as the overweight (eSMT) and obesity (β) do not seem to offer a defined functional related morpho-histometric pattern in the colon of the studied line of rats.

12.

APOPTOTIC EFFECT OF AN ANALOG OF GnRH (ACETATE OF LEUPROLIDE) ON AN ESTABLISHED LINE OF BOVINE GRANULOSA CELLS

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It is known that the GnRH is also expressed in the ovarian tissue. GnRH receptors (GnRHr) in the granulosa (GC) and luteal cells have been identified. Analysis of the hormonal regulation with agonists of GnRH (GnRH-a) *in vivo* and *in vitro*, have demonstrated an increase of the atretic follicles in ovaries of rats. Also, have been determined that the *in vivo* administration of GnRH-a increases the cellular death by apoptosis in ovarian tissue and GC. The objective of work is to analyze *in vitro* if a GnRH-a, the acetate of Leuprolide (LA), has apoptotic effect on an established line of bovine granulosa cells (BGC). For it, three experiment of activation were made (6, 12 and 24 hs) and three doses of LA (1, 10 and 100 nM), under two experimental conditions (0 and 5% of bovine fetal serum). Each experiment was made by triplicate analyzing the results by ANOVA two way and a Bonferroni's test comparison averages. The apoptosis index was determined by the DAPI chromatin stain, validating the results by TUNNEL. Analyses and comparison of the results show activation of the apoptosis and significant differences respect to control (44% activation, $p < 0,001$) at the 24 hs of incubation in the presence of 5% SFB and LA 100 nM. This result it was validated by TUNNEL, being in the same conditions a 39% of activation. Similar results were obtained in bovine GC of primary culture of follicles of medium size (5 to 8 mm). Finally we concluded that LA active the apoptosis processes in the system of BGC and for these experimental conditions.

13. GLYCOCONJUGATES DETERMINATION IN THE INTESTINAL TRACT OF THE ATHERINE (*Odonthestes bonariensis*) OF TWO LACUSTRINE WATERSHED

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The atherine is a South American species from rivers very important for fishing and consequently of socioeconomical relevance. Numerous studies were done about its biology but literature related to the glycoconjugates involved within the alimentary process is limited. It is our objective to determine the glycoconjugates presence in the intestinal tract of the atherine of two lacustrine watershed. Specimen of Embalse Río Tercero (Córdoba) and of the Dique La Florida (San Luis) with different physical-chemical characteristic were used. Samples of the intestine were removed, processed by conventional histological techniques and for lectin histochemistry. The used lectins were: ConA and PEA (glucose/mannose); SBA and DBA (N-acetyl galactosamine) and PNA (D-galactose). The glucose/mannose presence was determined in brush border and in a lesser grade in the enterocytes of the middle region of the intestine. N-acetyl galactosamine prevailed in brush border and goblet cells of the middle intestine; while D-galactose was present, mainly, in the anterior intestine. It is concluded that the most reactive epithelial structures showed glucose/mannose and N-acetyl galactosamine in the region of the middle intestine. The presence of D-galactose/N-acetyl galactosamine was more important in the anterior intestine region. The amount of goblet cells reactive at used lectins, was more in the specimen of Embalse Río Tercero.

14. FIXATION MEDIA OF LLAMA (*Lama glama*) LIVER

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The objective of this work was to observe the particularities of the fixation liver media in this species. Ten llamas, at the age of 2 years old, with a body weight of 98 ± 14 kg, from the Veterinary Science School Unicen, were used. The animals were soothed and anesthetized. We proceeded to the dissection of the jugular vein and the carotid artery for bleeding. After the death the cadavers were hidrotomized and the jugular vein was occluded. A solution of 10% formaldehyde was injected to complete the 7% of corporal weight for fixation. In order to allow the best structures fixation a modified position II of Chaveau was used. The dorsal border of the lesser omentum is attached in the visceral surface of the liver; its right extremity shows a free margin between the liver and the pancreas body where the porta vein is found. The ventral border extends from the cardias to the duodenum. It attaches in the istms, lesser curvature of the caudal compartment of the stomach, right lobe of the pancreas and cranial duodenum. The coronary ligament passes between the diaphragm and the liver around the caudal vena cava. The falciform ligament is very thin. It extends obliquely over the parietal surface, from the coronary ligament to the ventral border of the liver but does not extend to the ventral abdominal wall. The right triangular ligament is strong. It attaches the right lobe to the diaphragm. The left triangular ligament, the round ligament and the caudate ligament are absent.

Key Words: liver, fixation media, ligaments, omentum, llama.

15. PRIMARY CULTURES FROM BOVINE VAGINAL AND ENDOMETRIAL CELLS: POSSIBILITIES OF APPLICATION

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Though there is a wide variety of cellular lines for application in the field of medicine, primary cultures offer an activity and functionality cellular system, which simulates original tissue conditions. Generally primary cultures are developed from fetal cells lacking characteristics of adult tissues. In order to find a study model for bovine reproductive pathogens, that can reproduce with high accuracy the natural biologic processes, primary cultures from adult bovines endometrial (EC) and vaginal (VC) cells were performed, considering that endometrial and vaginal cells are their host cells. Cells were obtained from vaginal and uterine biopsies from healthy adult bovines and were cultivated in Minimal Essential Medium, MEM, with antibiotic and bovine fetal serum, at 37°C and 5% CO₂ atmosphere. A strain of *Campylobacter fetus venerealis* isolated from vaginal mucus of a heifer was utilized as pathogen. Bacterial adhesion and citopathogenic effect, and citotoxicity were evaluated on monolayers cultivated in plates with an initial cellular concentration of 1×10^5 for CE and 2×10^6 for CV. Both, CE and CV cultures, were confluent in five days, and kept their multiplication capability for 40 days without suffering visible changes. Bacterial adhesion and citopathogenic effect, and citotoxicity were observed at 48 and 72 h. The results show the feasibility of producing primary cellular cultures of tissues from adult individuals' reproductive apparatus, and the possibilities of application for different bovine reproductive pathogens.

16. LECTINHISTOCHEMICAL PATTERN OF THE C5 SEGMENT OF THE SPINAL CORD OF YOUNG, OLD AND SENILE RATS

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Lectin histochemistry (LHC) determines the presence and distribution (pattern) of carbohydrates in different organs. LHC done in some regions of the encephalon have shown that said pattern varied with age. Since no works document the carbohydrates pattern present in the spinal cord of rats we decide to analyse the C5 segment and to observe the possible changes related with age. Young (5 mo.), old (22 mo.) and senile (28 mo.) female rats were used. Tissue samples were embedded in paraffin. Eleven biotinylated lectins were used: Con-A, SBA, DBA, RCA-I, PNA, PSA, JCA, VVA, UEA-I, LCA and WGA. Diaminobenzidine was used as chromogen, the complex ABC as the system amplifier and hematoxylin as a counterstain. Results were expressed in a qualitative system of values (0-3). DBA, WGA, PNA and UEA-I did not stain structures, being very light for LCA and JCA. PSA and VVA marked neurons in the 3 studied ages. RCA-I intensely marked the glycocalix of the ependyma and neuropil of laminae I and II. SBA marked only the neurons of the young animals, while it marked carbohydrates present in the ependyma and the cuneiform sulcus of the white matter in senile rats. Con-A intensely marked the paranuclear region of the glial cells present in the white matter. Although age variations observed in other nervous organs were not seen, the present study served as base of knowledge of the LHC pattern of the C5 segment of the rat.

17. HYPOTHALAMIC DOPAMINERGIC NEURONS OF ADULT RATS SUBACUTE OR CHRONICALLY EXPOSED TO 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

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2,4-D is an herbicide used worldwide. Dopaminergic system alteration in rats exposed to 2,4-D through lactancy has been reported in previous histological and biochemical studies in our laboratory. In substantia nigra and ventral tegmental area a decreased neurons' tyrosine hydroxylase (TH) immunostaining in 2,4-D-exposed pups was observed. The aim of this work was to perform an immunohistochemical quantitative study of the hypothalamic dopaminergic neurons on adult rats exposed to 2,4-D. Wistar rats were made pregnant and exposed to 2,4-D (50 mg/kg/day, through diet) from day 16th of gestation to weaning. 2,4-D was dissolved in alcohol solution, mixed with food and dried before starting to feed the diet. After weaning, pups were divided in two experimental subgroups: **T1:** pups were fed with untreated diet until sacrifice (postnatal day 90, PND90). **T2:** pups were maintained on the 2,4-D treated diet until PND90. Control animals received standard diet. Serial coronal sections -from plates 18 to 35 of the Paxinos and Watson atlas- were immunostained according to Sternberger's PAP technique using a monoclonal anti-TH primary antibody. Data showed a decrease in the number of dopaminergic neurons in the paraventricular hypothalamic nucleus (PVH), in T₁ rats. 2,4-D exposure during pregnancy and lactation produced a permanent neuronal decrease in PVH dopaminergic nucleus. However, with the chronic 2,4-D exposure an adaptable response was observed.

18. MORPHOMETRIC ANALYSIS OF THE LONG BONES OF THE FORELIMB OF CHAETOPHRACTUS VILLOSUS (XENARTHRA, DASYPODIDAE)

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A morphometric study of the long bones of the forelimb of *C. villosus* was performed with the aim of detecting possible intraspecific variations of size and shape. Females (n = 18) and males (n = 22) coming from the surroundings of Bahía Blanca were used. The total length of the humerus (LH), the radius (LR) and the ulna (LU); and the transverse diameter of the humerus (DH), the radius (DR) and the ulna (DU) were measured. Univariate and multivariate analysis techniques were applied; the structure of the correlation matrices of the female and male populations were estimated and analyzed separately with the aim of studying the relationship between characters. Significant differences were detected only in DR (p > 0,01), with higher values for the females. The discriminant function obtained (D = -17,5 + 5,1 DR) classified correctly the 72.5% of the individuals, which constitute a useful tool for the identification of sexes. The analysis of the correlation matrices of the considered morphometric characters showed that the lengths of the male bones were highly correlated, while for the females the highest correlations were observed between the lengths and diameters of the humerus and the ulna.

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19. COMPARATIVE STUDY OF THE LINGUAL DORSAL SURFACE IN EUPHRACTINI OF BUENOS AIRES PROVINCE (MAMMALIA, XENARTHRA)

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Different types of papillae are located on the dorsal mucosa of mammalian tongue: filiforms, fungiforms, foliated and circumvallated. They differ in shape, size and number between species, and their development degree could reflect food habits of certain specie. The aim of this study is to compare the characteristics of lingual dorsal surface of three armadillo species from Buenos Aires Province: *Zaedyus pichiy*, *Chaetophractus vellerosus* and *C. villosus*, by scanning electron microscopy. Tissue was fixed in diluted Karnovsky solution and then processed with routine histological techniques. Observations of tongue surface were made using an Evo 40 XVP (Cambridge, England) scanning electron microscopy at 7 kV. Prominent conical filiform papillae are mainly in the anterior third of the tongue followed by fungiform papillae in less proportion. Fungiform papillae have visible taste pores on surface. Filiform branched papillae are numerous in the middle third of the tongue. Their branches vary in number between 3 and 5 in *Z. pichiy* and *C. vellerosus*, and between 3 and 9 in *C. villosus*. In the posterior third there are two circumvallated papillae, placed at both sides of the midline of the tongue and they are surrounded by a groove. Posterior to circumvallated papillae, the filiform papillae are smaller and scarce. The presence and distribution of filiform, fungiform and circumvallated papillae are similar between the studied armadillo species.

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20. LIFE SPAN OF CHICKEN THROMBOCYTES IN THE BLOOD

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Thrombocytes (TBCs) are the avian and lower vertebrates counterparts of mammalian platelets. Structure and function of avian TBCs is rather well known, but very little is known about its kinetic. In this paper, we estimated the life span of chicken thrombocytes in blood by two different methods: 1) chicken TBCs were isolated by centrifugation followed by slow decantation. After being washed they were labelled with ¹¹¹Indium-oxinate and injected intravenously to 7, 1 month age, chicken. Then, the recovered radioactivity in blood after 1, 3, 24, 48 and 72 hs. Was measured 2) In view of the known ability of avian TBCs to phagocyte abiotic particles, indian ink (Pelikan Argentina, Bach 0206 T1) was injected intravenously to 10, 1 month age, chicken and the ratio of labelled TBCs in May Grunwald- Giemsa stained blood smears after 3, 24, 48 and 72 hours after injection was microscopically determined. A mean curve of time variation was made for the two methods. With both methods we detected an initial deep falling in labelled TBCs, perhaps due to activation and fast phagocytosis of a fraction of labelled platelets. After 24 hs. there was a gradual falling of labelled TBCs. in a linear relation with time. The estimated life span by extrapolation was near 6 days in both cases.

21. MORPHOLOGICAL AND MORPHOMETRICAL STUDY OF THE OVARY OF *Chaetophractus villosus*

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Ovarian morphology, ultrastructure of ovarian follicles and morphometry of oocyte and follicular cells in different stages of development in the armadillo *Chaetophractus villosus* are described. Material was processed according to standard methods. For morphometrical study, follicles and their oocytes were measured. Mean values and standard deviation of both diameters were calculated at each stage. Mean diameters were compared with Tukey-Cramer's test.

Ovaries are pair organs, cylindrical, with rounded extremes, with a ventral cortex, containing the ovarian follicles, and a dorsal medulla, of loose connective tissue, with numerous blood vessels. Relations with the oviduct, and structures associated with the ovary are described. Primordial, intermediary, early and late primary, secondary, tertiary and preovulatory or Graafian follicles were identified. Ultrastructural changes during follicular development in oocyte and follicular cells are detailed. By the morphometric study we demonstrated that the growth of oocyte and follicle conforms a biphasic pattern, reaching the oocyte the complete growth in the tertiary follicle stage, according with the typical pattern found in eutherian mammals and some marsupials.

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22. CONTRIBUTION TO THE MORPHOLOGY OF HARPACTORINAE: THE GENUS *PSELLIOPUS* BERGROTH (REDUVIIDAE: INSECTA)

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The Harpactorinae is the largest and most diverse subfamily of Reduviidae. In spite of its size, the subfamily has been poorly studied in recent years. The Harpactorinae are distributed worldwide, most dominantly in the tropics. The adults and nymphs are diurnal, and occur in a variety of habitats. The purpose of this contribution is to give new characters that are useful to separate the species of the genus *Pselliopus* (an Harpactorinae). This study has been based on material provided by national and international institutions. The morphological characters were described and illustrated with a Wild M-stereomicroscope. For this revision a total 23 measurements. The male and female genitalia gives important characters to separate the following species: *P. barberi* (Davis), *P. cinctus* (Fabricius), *P. dantei* (Brailovsky), *P. flaviceps* (Brailovsky), *P. inermis* (Champion), *P. infuscatus* (Champion), *P. ivanicus* (Brailovsky), *P. karlenae* (Hussey), *P. latifaciatus* (Barber), *P. latispina* (Hussey), *P. limai* (Pinto), *P. lineaticeps* (Champion), *P. majesticus* (Brailovsky), *P. mexicanus* (Champion), *P. nigropictus* (Champion), *P. ornaticeps* (Stål), *P. punctipes* (Amyot & Serville), *P. rufofasciatus* (Champion), *P. spinicollis* (Champion), *P. tuberculatus* (Champion), *P. ventus* (Brailovsky) and *P. zebra* (Stål).

23. IMMUNOHISTOCHEMICAL CUANTIFICATION OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IN CANINE MELANOCYTIC TUMORS: NUMBER OF RANDOMLY SELECTED FIELDS NEEDED TO HOMOGENIZE ITS VARIANCE

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PCNA is a nuclear protein that has been used as indicator of cellular proliferation in canine melanocytic tumors (MT). The number of random fields recommended for its quantification differs between authors. The aim of this study was to determine the number of random fields needed to homogenize the variance of quantification of PCNA in canine MT. Immunohistochemistry was performed in eight file cases, four benign (BMT) and four malignant MT (MMT), using a MoAb against PCNA (clon PC10, Dako) diluted 1:50. Detection was carried out by the LSAB2® System HRP (Dako Cytomation) and stained with diaminobencidine (DAB, SK-4800, Vector). The proliferation index (PI) was determined counting the number of positively stained cells from 100 tumor cells increasing the number of fields (Obj 40x), from n = 1 to n = 10, on digital images assisted by a guide reticle. This was repeated four times in each tumor to determine the value of coefficient of variation (CV). The CV showed a tendency to decrease in all tumors by increasing the number of fields. In all but one BMT, the CV showed no fluctuation counting from six fields onwards. In MMT the CV dropped to a level below than that observed in BMT. Thus, to determine de PI, counting of at least six random fields is recommended.

24. EXPRESSION OF NUCLEAR CELL PROLIFERATING ANTIGEN (PCNA) AS CRITERION FOR DIFFERENTIATION BETWEEN BENIGN AND MALIGNANT MELANOCYTIC TUMORS IN DOGS: A PRELIMINARY STUDY

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The differentiation between benign (MB) and malignant (MM) melanocytic tumors (MT) can be difficult if performed through conventional histopathology. Additional markers may be helpful. The expression of PCNA has been shown to correlate with biological behavior in canine MT. The aim of this study was to find a possible correlation between the expression of PCNA and histopathological diagnosis of benignancy and malignancy of MT. Immunohistochemistry was performed in file cases, 5 BM and 4 MM, using a MoAb against PCNA (clon PC10, Dako) diluted 1:50. Detection was carried out by the LSAB2® System HRP (Dako Cytomation) and stained with diaminobencidine (DAB, SK-4800, Vector). The proliferation index (PI) was determined counting the number of positively stained cells from 100 tumor cells in 10 random fields (Obj 40x) on digital images assisted by a guide reticle. The correlation between variables was evaluated by the Fisher exact test ($p < 0.01$). The PI in BM varied from 20 to 30%. In MM the PI varied from 60 to 65%. There was significant correlation between variables ($p < 0.01$). Thus, PI lower and higher than 30% could be related to benignancy and malignancy respectively. A greater number of tumors is needed to increase the sensibility of the study.

25.

ERYTHROPOIETIC MODULATION BY RENIN-ANGIOTENSIN-ALDOSTERONE*D'Anna MC, Veuthey TV, Gatti CJ, Giambelluca MS, Elía MA, López G, Roque ME.**Fisiología Humana. Biología, Bioquímica y Farmacia. UNS. Bahía Blanca. Argentina. E-mail: mroque@uns.edu.ar*

In previous studies we showed that the pharmacological inhibition of the Renin-Angiotensin-Aldosterone (RAA) system affects the time range of the splenic restorative response to hemolysis and that the kidney assumes a post-hemolytic erythropoietic function. The objective was to assess whether the kidney presents a delayed response analog to the spleen when the RAA axis is inactivated. Mice CF1 (43±3g) were grouped into: 1) **G1 CONTROL** (n=33): 300µl physiological solution (PS) intraperitoneal (ip) (10 days); 2) **G2+ENALAPRILATE (E)** (n=33): Enalaprilate ip (10mg/kg/300µL) (10 days); 3) **G1+PHZ; G2+E+PHZ**: phenylhydrazine (PHZ) ip (60mg/kg/300µL) (Days:10,12); 4) **G3(-E/-PHZ)** (n=33): ip PS (20 days). Retroorbital blood was obtained until day 20 (every/2 days) (n=3). Anemia evolution and recovery were assessed by means of Hb, HCT, and reticulocytes. The dissected kidney, fixed using Bouin solution and 10% formalin, was processed for H&E. Statistical significance was set at $P<0.05$. Mice received free food and water. Cellular islands that characterize active erythropoiesis were semi-quantified with a pre-established score (10xfields): a) 0-2 islands/10fields:++; b) 3-7 islands/10 fields:++; c) 8-10 islands/10fields:+++; d) >10 islands/10fields:++++. In **G2+E+PHZ**, Hb (13.7g/dL±1.2), HCT (49.5%±0.7), and reticulocytes (33%±5) showed a delayed recovery of anemia (day:20) with regard to **G1+PHZ** (Day:16): Hb: 14.2g/dL±1.3; HCT: 50.0%±0.8; reticulocytes 31%±4 ($P<0.05$). Renal cellularity confirmed the delayed erythropoietic recovery: 1) **G2+E** (Day0):+; 2) **G2+E+PHZ** (Day16):++; Day20:++++; 3) **G1CONTROL** (Day 0): +; 4) **G1+PHZ** (Day16):++++; Day20:+; 5) **G3(-E/-PHZ)**: (Day 0,16,20):+. The kidney behaves in the same way as the spleen with regard to erythron recovery when the erythropoiesis is modulated by inactivation of the RAA axis. We may conclude that inducers of cellular adhesion to the medullar stroma and/or stromal inhibitor production would affect the migration and nestling of erythropoietic precursors in these tissues.

26.

IMMUNOHISTOCHEMISTRY OF MOTILIN IN THE MUSCULAR TISSUE OF THE INTESTINAL TRACT OF HORSE FOETUS AT DIFFERENT AGES*Dauria PG, Castagnino RA, de la Cruz JP, Sona L, Perrotta F, Mac Loughlin VH, Bonino F.**Histología. Depto. de Anatomía. FAV-UNRC. Río Cuarto.*

The motilin is a gastrointestinal hormone secreted by the enteroendocrine cells of the digestive system that participates in the control of the contractions of the smooth muscle of stomach and intestine. It is objective of this work to identify motilin in the muscular tunic of the intestinal tract of foetus of horse at different ages. Samples of duodenum, jejunum-ileum and colon of foetus of 120, 240 and 300 days of gestation were used, fixed in formol buffered to 10%, included in paraffin and then subjected to immunohistochemistry techniques using the polyclonal antibody anti-motilin raised in rabbit. The results showed: in 120-day foetus, moderate to intense reaction in the longitudinal muscular of duodenum; intense reaction in the two muscular layers of jejunum-ileum, while in colon there was not reaction; in 240-day foetus, moderate reaction in mienteric plexuses of small intestine, while in colon there was intense reaction in the longitudinal muscular; in 300-day foetus, moderate to intense reaction in the two muscular layers of duodenum; moderate reaction in the circulate muscular of jejunum-ileum, moderate reaction in the two muscular layers of colon. Its concluded that motilin is present in muscular tissue of the intestinal tract of horse foetus at different ages, being more evident in colon of horses of more foetal development.

27.

GLYCOCONJUGATES IN THE INTESTINAL TRACT OF THE SNAIL*De la Cruz JP, Castagnino RA, Navarro OE, Daita JC, Dauria PG, Tissera JL, Cortegiano F, Alem PJ.**Cátedra de Histología/Zoología. Facultad de Agronomía y Veterinaria. U.N.R.C.*

The breeding of snails of the gender *Helix* is an activity in continuous expansion in the county of Cordoba likewise in the country, with the biggest hatchery in the world located in this county, and keeping in mind the quality of its meat very appreciated for exportation. The objective is to determine, through the lectin histochemistry technique the glycoconjugates present in the intestinal tract of the snail that are involved in the alimentary process. Mature snails were used, to which serial cuts were done, to analyze the intestinal tract. Samples were fixed in formol buffered to 10%. As markers the following lectins were used: Con A and LCA to determine glucose/mannose; DBA to identify N-acetilgalactosamine / D-galactose and UEA-I to show fucose; while for the detection and later development the complex avidine-biotine peroxidase (ABC) and diaminobencidine (DAB) were used respectively. The data analysis showed moderate to intense reactivity of brush border against the lectins Con A, LCA and UEA-I, while there was no reaction against the DBA lectin. We conclude that in the brush border of the snail intestine the presence of glycoconjugates mannose, glucose and fucose was determined.

28.

ULTRASTRUCTURAL REDESCRIPTION OF *CHORDODES MOUTONI* CAMERANO, 1895 (*GORDIIDA*, *NEMATOMORPHA*)*De Villalobos C, Zanca F, Achiorno C.**Facultad de Ciencias Naturales y Museo. La Plata, Argentina. E-mail: villalo@fcnym.unlp.edu.ar*

Gordiida is distributed throughout tropical and subtropical worldwide. Up to now about 300 species belonging to 21 genera, have been described. The genus *Chordodes* with 90 species is the widest genus within the *Gordiida* and has the greatest diversity of cuticular structures (areoles). The diagnostic feature of this genus is the presence of crowned areoles. At moment numerous species of *Chordodes* from different regions of the world, have been investigated. Only one species, *Chordodes moutoni* is known from China but the documentation of characters is not enough and its status appears to be uncertain. The aim of this study is to reinvestigate *C. moutoni* by scanning electron microscope (SEM). Holotype (male) and paratype (female) from Muséum National d'Histoire Naturelle were investigated. Fragments of mid-body were dehydrated in ethanol and critical point-dried. The body cuticle of the holotype, shows five different types of areoles; simple, tubercle, conic and high areoles, crowned areoles with apical short filaments surrounded by 10 areoles with bristles on top. The body cuticle of paratype includes all five types of areoles described, but along the ventral midline, one further type of crowned areoles that occur with very long filaments on top is present. This particular feature in females was considered as sexual dimorphism in cuticular pattern and has been mentioned for South American and Africa *Chordodes* species. This study widens the original description and allows to consider *C. moutoni* as valid species.

29. GLYCOCONJUGATES IN GILLS OF *Odontesthes argentinensis* (ACTINOPTERYGII, ATHERINOPSIDAE)
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The occurrence of mucous cells is a common feature of the teleosts fish. The glycoconjugates (GCs) present in the mucus are related to different functions such as lubrication, protection, inhibition of microorganisms growth and the osmotic function. Mar Chiquita Lagoon is the only Argentinean estuary of classic restricted type, that makes this lagoon a highly changing environment related to the salinity of the water. Among their ichthyofauna *Odontesthes argentinensis* stands out characterized as a marine dependent estuary. Its euryhalinity facilitates the adaptation to the different levels of salinity in the diverse sectors of the lagoon. The objective of this work is to analyze the composition of carbohydrates in the mucins of the mucous cells in the gills of the *Odontesthes argentinensis* of the Mar Chiquita Lagoon. Techniques were carried out to differentiate GCs: 1) GCs with oxidizable vicinal diols; 2) total sialic acids; 3) GCs with sialic acid residues; 4) sialic acid residues substituted; 5) neutral GCs; 6) GCs with carboxyl groups and/or with sulphate esters; 7) highly sulphated GCs. These techniques allowed the recognition of a single type of mucous cells in the filaments. The mucous cells showed neutral, sialylated and sulphated GCs. The different GCs elaborated and secreted by the mucous cells in the gills of *O. argentinensis* show a high-level of histochemical complexity, related with the multiple functions that the mucus plays in the euryhaline fish.

30. ACUTE CADMIUM INTOXICATION: ACTION ON PLACENTA AND FETUSES

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Cadmium is an environmental toxicant that produces adverse effects on the placenta and prenatal development. The aim of the present work was to study possible alterations on placental and fetal growth in Wistar rats intoxicated with cadmium at different moments of pregnancy. The cadmium chloride dose given was 0(G5, control group) and 10 mg Cd/kg body, at days 4(G1), 7(G2), 10(G3) and 15(G4) of gestation by subcutaneous injection. Pregnant rats were sacrificed at day 20 of gestation. Placental diameter and average weight were registered. The placentas of each rat were used for histological studies and to determine Cd concentration (ppm DM). Some placentas were fixed with buffered formol. Sections of 5 µm were stained with H/E and observed with an optic microscope. The following data were registered: weight of fetal vesicles, weight and different fetal measurements (fetal length: greatest fetal length, crown-rump fetal length, and head fetal length) and the number of fetuses per female. The results were analyzed by ANOVA or the Student t-test. The placental diameter of the G1 group was minor than the G5 (control group) (P<0.05). Although there were reabsorptions in the rats treated with Cd, the differences on the number of offspring per female were not significant among groups (P>0.05). Histopathological findings in the placentas of intoxicated rats were: labyrinthic congestion and coagulative necrosis. The highest placental Cd concentration was determined on G4 group. We concluded that in our working conditions, Cd is deposited on the placenta, altering its structure, without altering the size of fetuses.

31. VARIABILITY OF ENAMEL PRISM MORPHOLOGY

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Interpretations of human enamel prism morphology and organization is not univocal. Bhaskar (1986), Gomez de Ferraris (1999) and others stated that prism transverse morphology include a head and a tail. Ten Cate (1989) stands that prisms are round, surrounded by interprismatic substance. Martin and Boyde (1984) define "patterns" as prism arrangements in a transverse plane. We evaluated the prism morphology and arrangement in human enamel using S.E.M. Twelve healthy teeth were selected after their extraction by prescription. Two groups of teeth were analyzed: in one of them the samples were prepared by fracture and in the other one, samples were included in epoxy resin polished and etched with 10% hydrochloric acid for 4 seconds. Prisms were best observed within a range of 300X-1.200 X under the SEM. In all cases a variable quantity of interprismatic substance was found. Regarding their arrangement, prisms were observed both disperse or aligned in rows bordered by thick interprismatic matrix. Transverse cuts of individual prisms showed different "head and tail" morphology, much depending on the actual angle of the polished section. We conclude that the form of prism sections highly depends on the angle at which the (ideal) planes were made and the crystallites orientation. Patterns mentioned by Martin and Boyde are not appropriate for the study of human enamel microstructure; in fact, they are observational artifacts.

32. FOLLICULOGENESIS IN THE OVARY OF *MUSTELUS SCHMITTI*, SPRINGER 1939 (CHONDRICHTHYES, TRIAKIDAE)

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Mustelus schmitti is a common shark in the Bahía Blanca estuary. The aim of this work was to describe the folliculogenesis in the ovary of *Mustelus schmitti*. We worked with 5 mature females. The material was processed with routine histological techniques. This species has a unique left ovary, embedded in the epigonal organ. A simple cuboidal epithelium covers the ovary. Beneath this epithelium is the tunica albuginea, formed by connective tissue and smooth muscle fibers. Primordial follicles are present in groups under the tunica albuginea. They have a prominent nucleolus and are surrounded by a simple squamous layer of follicular cells. Externally, a theca with muscle fibers and connective tissue is found. This theca does not vary significantly in the different stages. During the development of the oocyte, follicular cells become cubic and finally, columnar. In any of the stages it is observed the granulosa formed by many layers of cells. Zona pellucida increases its width while the oocyte becomes larger. Our results differ from other viviparous sharks where there is a right ovary. Folliculogenesis was coincident with the description for other species of chondrichthyes. This work was supported by a SGCyT-UNS PGI 24/B100. We thank to Prefectura Bahía Blanca for their invaluable help.

33. MORPHOLOGY OF THE CLASPER GLAND OF SYMTERYGIA ACUTA. GARMAN, 1877 (CHONDRICHTHYES, RAJIDAE)

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Male skates of all species have copulatory organs denominated claspers, used to transfer the sperm to the female skates, associated with them there is the clasper gland. In this work we analyse the morphology of the clasper gland of *S. acuta*. For the present study 5 adult males were caught in the Bahía Blanca estuary. The clasper glands were dissected, fixed in Bouin in seawater and processed according to routine techniques. The clasper glands are paired, ovoid structures enclosed within muscle sacs, denominated clasper gland sacs. There are composed of simple tubules glands, that are radiated dorsally from the papilla which receives several secretion of many collector ducts in which the tubules exits the secretion, this ducts have high gland cells. All the gland is surrounding by transversal striated muscle tissue. Probably, the contraction of this muscle forces the secretion from the papilla to the sac and the contraction of the sac's striated muscle exits the secretion to the clasper groove. Results show that clasper glands of *S. acuta* are similar to other species of skates from the north hemisphere.

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34. ACTH CELLS OF PARS DISTALIS OF VISCACHA: IMMUNOHISTOCHEMICAL STUDY IN RELATION TO SEASON, SEX AND GROWTH

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Environmental winter conditions such as short photoperiod, low temperature, reduced hydric availability and variations in the composition of food are the stressors that provoke the activation of the hypothalamo-hypophysial-adrenal axis. The aim of this work was to immunohistochemically identify ACTH cells of pars distalis in adult male viscachas throughout the year and after melatonin administration, and in immature male and adult female animals. The distribution, shape, immunolabeling pattern, percentage immunopositive area and major cellular and nuclear diameters were analyzed. The percentage area and the major cellular diameter showed lower values during June-July and a decrease in the percentage immunopositive area was observed after the administration of melatonin in adult male. The distribution, morphological characteristics, immunolabeling pattern and percentage immunopositive area of ACTH cells in immature male showed differences in relation to what was observed in adult male. It was observed that ACTH cells of female were smaller in relation to adult male. Our results in adult male viscacha demonstrate that the morphology of the ACTH cells varies according to the different seasonal conditions, thus participating in the adaptation process of this rodent to the environment. The melatonin might inhibit the synthesis of ACTH, probably when affecting some secretagogue of this pituitary hormone. Moreover, the morphological variations observed between adult and immature male viscachas and between both sexes suggest that the steroid gonadal hormones might act on the development and activity of ACTH cells.

35. EPICUTICULAR WAXES AND SILICON DEPOSITS IN Festuca pallescens (St. Yves) Parodii LEAVES

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Festuca pallescens is a forage resource of the semiarid Patagonia. Epicuticular waxes and silicon content of the leaves affect the regulation of the water status of the plants through a reduction in transpiration and a greater leaf reflectivity to incoming solar radiation. The objective was to study the silicon content and distribution, and the quantity and morphology of the epicuticular waxes placed over the leaves of ecotypes of *Festuca pallescens* (Pampa del Castillo (PC), Media Luna (ML), Triana (T)) that are arranged along an increasing moisture deficit gradient. Plants were grown in a greenhouse. Treatments were: Control (soil kept at field capacity) and Stress (irrigation suspended until -3.5 MPa, and then irrigated to field capacity, three times). Epicuticular waxes were quantified gravimetrically after dilution with Cl_4C and the leaf surfaces were observed with a scanning electron microscope. Leaf silicon content was measured in liquid extracts with a plasma spectrophotometer. The depositional pattern of silicon in leaf tissues was observed by SEM and silicon dot maps were done with energy-dispersive X-ray analysis. The ecotype PC accumulated 2.7 times more wax in stressed plants. A uniform wax layer was observed in all ecotypes, but in PC wax tubes juxtapose into tufts on the surface. In PC and T silicon content was 30% higher in stress. Silicon accumulation pattern was different for adaxial and abaxial surfaces. Adaxial epidermis showed silicon in trichomes while in abaxial surfaces silicon occurs in specialized cells.

36. CHANGES IN THE QUANTITY AND STRUCTURE OF EPICUTICULAR WAXES IN ECOTYPES OF Trichloris crinita UNDER WATER STRESS

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The nature of changes of the epicuticular waxes depends on the intensity and duration of the stress so much as in the genetical ability to face up the harshness of the environment. The structure, layout and quantity of epicuticular waxes in ecotypes of *Trichloris crinita* (Ñacuñán (Ñ), La Jarilla (J), El Retamo (R), La María (M), Loventue (L), La Araña (A)) adapted to sites of contrasting moisture availability were studied to evaluate the variation of those parameters under a severe water stress. Plants were grown in the greenhouse. Treatments were: control (plants kept at field capacity) and stress (irrigation was suspended during 45 days). Waxes were quantified gravimetrically after dilution with Cl_4C . The adaxial and abaxial surfaces of the leaves were observed with a scanning electron microscope. In general, there was a large deposition of wax in the stress. The wax quantity of J and A under stress was greater than in the control (132 and 145%, respectively). In the stress treatment of Ñ leaf wax doubled the content of the control, but that quantity was lower than the control of other ecotypes. There were no major deposition of wax in R, M and L under water stress. The deposition of waxes was disposed as a reticulate thread with the exception of A that under stress showed atypical wax structures and M that presented differences in both leaf surfaces. Longer threads than the previous described were covering the stomata, perpendicularly to the stomatal pore.

37. MICROANATOMY OF THE THYMUS IN THE ADULT AMPHIBIAN *BUFO ARENARUM*

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The thymus is composed of elements derived from the ectoderm, endoderm and mesoderm. This multiple origin determines a very complex network of different phenotypic cell types that, together with the immune cells, create a unique microenvironment, essential for T-cell differentiation and immune homeostasis. The morphological and functional properties of the stromal cells implicated in this process are still a subject of discussion.

The cytoarchitecture of the thymus in the common toad *Bufo arenarum* was studied by means of histological, histochemical and electron microscopic methods.

Each thymus presents a variable number of interconnected lobules and a connective capsule. Cortex and medulla were clearly distinguishable. Four types of reticuloepithelial cells can be recognized in the stroma: 1) limiting cells; 2) cortical and medullar cells; 3) nurse cells and 4) Hassall's-like cells forming corpuscles.

Other cell types are observed: myoid cells, cystic cells, goblet cells, serous cells, macrophages, plasmocytes, granulocytes, erythrocytes, neuroendocrine like cells and unidentified cells. Concluding, the thymus in this species is similar to that found in other amphibians and vertebrates. It is worthwhile to mention the presence of multicellular "mammal-like" Hassall's corpuscles and follicular structures resembling thyroid follicles. Results of the investigation suggest that the thymus of the adult common toad *Bufo arenarum* may be as a useful model for studying the thymus cytoarchitecture, phylogeny and function.

38. LECTINHISTOQUÍMICA EN EL ÁREA PELUCIDA DE LA OVARIA DE LA VIZCACHA DE LA PLATA (*Lagostomus maximus maximus*)

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Studies carried out in several species of mammals have shown that the pellucid zone (PZ), present in the oocytes of the developing ovarian follicles is composed by complex and heterogeneous glycoproteins (GP). The PZ of the viscacha stained with PAS and Alcian blue at early stages of the follicle development was already described by us. Our present goal was to describe the carbohydrate pattern present in the PZ of the viscacha. Twelve wild, mature viscachas were used. The ovaries were embedded in paraffin and incubated with the following lectins: WGA, Con-A, PNA, SBA, RCA-I, UEA-I and DBA. Diaminobenzidine was the chromogen, the complex ABC the developer, haematoxylin the counterstain. The intensity of the reaction was expressed in terms of 0 to 3. The results demonstrate that the distribution of carbohydrates in the PZ varies according to the physiologic state of the female: in the pregnant viscachas (recent or advanced), the lectina WGA marked with a value of 2, while in non-pregnant females had a value of 3. The SBA lectin intensely marked the PZ of non-pregnant females, being negative (0) in the pregnant animals. The DBA and UEA-1 lectins reacted in a variable way in when exposing to pregnant and non pregnant tissues. Our results partially coincide with those observed in other species, indicating that the PZ of the viscacha present its own characteristic carbohydrates pattern.

39. EFFECTS OF EXPERIMENTALLY INTOXICATED BOVINES AND RABBITS WITH *Solanum glaucophyllum* ON THE THYMUS: A COMPARATIVE STUDY

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Solanum glaucophyllum (Sg) is responsible for the "Enzootic Calcinosi". Its active principle is the vitamin D that has been shown to regulate the immune response and the cellular differentiation. Our goal was to study the effects of Sg on the thymus of experimentally intoxicated rabbits and bovines. Rabbits and heifers were intoxicated and immediately sacrificed, during 15 and 30 days. Control groups were sacrificed in the day 0, 15 and 30 of the experiment. Lectin histochemistry on paraffin embedded material was done using PNA, WGA, DBA, RCA-I, UEA-I, SBA, Con-A. Immunohistochemistry for cytokeratins (CK) and S100 protein was also performed. CK identified, in both species, all the epithelial cells of the thymus. S100 marked medullar cells in bovine, reaching cortical and subcapsular areas in rabbits. PNA marked immature thymocytes in bovines but not in rabbits. DBA marked cells located in the cortical-medullar junction in bovines. WGA marked the cytotreticular net in both species while RCA-I only in the rabbit. In rabbits, SBA bound to medullar cells. In the intoxicated rabbits neither cortical atrophy nor relative and gradual increment of connective tissue or of non lymphoid cells as found in bovines were observed. Moreover, in the intoxicated rabbits the relationships cortex: medulla and lymphoid cells: non lymphoid cells do not change. It is suggested therefore that Sg would not affect in the same way the thymus of rabbits and bovines.

40. MICROANATOMY OF THE OVIDUCT AND THE OVIDUCAL GLAND OF *SYMPTELYGIA ACUTA* GARMAN 1877

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Rays show reproductive characteristics as internal fecundation and oviparity that makes them susceptible to fishery pressure. The bignose skate is commercially fished in the Bahía Blanca harbour and there is little information about biological features of this species. In this work, we studied the microanatomy of the oviducal gland and the oviduct of *S. acuta*. Eight adult animals were used. The fixation was made with 2.5% glutaraldehyde in 12% sucrose cacodylate buffer and then with 1% osmium tetroxide, immediately after the capture. The material for transmission electron and scanning microscopy was processed according to standard techniques. Results showed a folded tubular oviduct with tubular glands and mucous cells in the surface. The oviducal gland showed the typical zonation. The epithelium was simple columnar with ciliated and mucous cells, except in the spinnerets of the "baffle" zone where the epithelium lacks glandular cells. In all regions tubular simple or ramified glands produced secretion. The scanning images clarified about the distribution of epithelial plates. Our results depicted similitudes with other batoid fishes in the oviduct structure as in their specializations and give new information about an economically and conservationally important species.

This work was supported by a SECyT - UNS PGI 24/B100. We thank Prefectura Bahía Blanca for their invaluable help.

41. DISTRIBUTION PATTERN OF INTERSTITIAL CELLS OF CAJAL IN THE BOWEL OF THE CAT

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The interstitial cells of Cajal (ICC) are special class of cells dispersed in the smooth muscle and enteric neurons. They are established as pacemakers, in propagation of slow waves and as mediators of inputs from enteric motor neurons. ICC express c-kit receptor. This immunohistochemical study analyzed the distribution of c-kit positive cells using CD117/anti c-kit antibody. Segments of duodenum, jejunum, ileum, colon and rectum were removed from six adults males cats and processed for conventional histology. Sections (4±1 µm) were incubated with CD117. In duodenum, positive cells were observed around the myenteric plexus. In muscle layers, c-kit positive cells appeared with one or two processes running parallel to the musculature. These cells had elliptical shape with short cytoplasmic processes. In the colon, c-kit positive cells formed a continuous layer in relation to myenteric plexus. Others positive cells were observed at the submucosal border. In the rectum, the positive cells of fusiform aspect appeared isolated in the muscular tunica. These c-kit positive cells by their shape, location and relations with the enteric nervous system could be considered ICC. The distribution seems to be similar to those reported for other species.

42. EVALUATION OF ANGIOGENESIS WITH THE EXPRESSION OF VEGF AND CD34 IN HUMAN NON-SMALL CELLS LUNG CANCER

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Angiogenesis is an essential process in progression of malignant tumors, the most potent angiogenic factor is vascular endothelial growth factor (VEGF). The CD34 is an endothelial antigen that has been used to highlight the microvasculature vessel density (MVD) as a direct marker of degree of neoangiogenesis. In the present study we report the VEGF expression and its relationship with MVD, measured by CD34, in two lineage of non-small cell lung cancer (NSCL): adenocarcinomas and epidermoid carcinomas with low differentiation, in order to consider the possibility of using the correlation between both antibodies as prognosis factor. Tumor sections were stained by immunohistochemistry for CD34 and VEGF. The results showed that the mean value of VEGF for adenocarcinoma was significantly higher than the one for epidermoid carcinoma ($p < 0.001$). On the other hand, the mean of MVD, measured by Student-Newmann-Keuls Multiple Comparisons Test, didn't show significant differences between both types of tumors. The conventional factors taken into consideration (age more than 60, sex, and presence of lymph nodes) wasn't significantly related to the angiogenic factors examined. In conclusion, we have confirmed previous work claiming that VEGF and CD34 are excellent markers of neoangiogenesis in NSCLC. In prospective studies we will analyze the VEGF and CD34 expression in tumors with different degree of differentiation.

43. HUMAN THYMUS NEUROTROPHINS RECEPTORS (*Homo sapiens sapiens*)

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Neurotrophins (NGF, BDNF, NT-3, NT4/5) are growing factors implicated in the differentiation and maintenance of nervous system cell population. Neurotrophins mediate their effects by interacting with specific cell surface tyrosine kinase receptors, trkA, B and C. Briefly, NGF binds to and activates trkA, BDNF and NT-4/5 interacts with trkB and NT-3 mostly do it with trkC. This work goal was to determine the spatial distribution of these receptors in foetus human thymus by immunocytochemistry (ICC).

Foetus human thymus samples (n=10, 25-35 weeks) were donated by Asturias Central Hospital, Oviedo (Spain). Samples were fixed in 10% buffered formaldehyde and then processed with ICC technique, applying ABC method with rabbit polyclonal antibodies (Santa Cruz Biotechnology, CA, USA).

The preliminary results showed a positive immunoreactivity in medullar reticular epithelial cells around Hassall bodies to trkA. Cortex reticular epithelial cells were trkC (+) immunoreactives; however trkB receptor was negative observed throughout the thymus.

Both types of thymus reticular epithelial cells are related to support and perform positive-negative T cells selection, in addition, these cells provided growth factors for T lymphocytes survival during maturation process. Newly reports suggest that trk ligands of intra or/and extrathymic sources, could regulate the functions of thymic cells. The demonstration of trk receptors in human thymus stromal cells could be of importance, because of their role in providing an appropriate microenvironment to T cells.

44. *Sus scrofa domestica* THYMUS HIGH AFFINITY PROTEINS (TRKS) NEUROTROPHINS RECEPTORS LOCALIZATION. SECOND PART

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Neurotrophins are known as growing factors that participate in some neuronal populations differentiation, development and maintenance. On the other hand there are evidences that these molecules can work on other tissues that express mRNA and/ or their receptors, as rat and mouse lymphatic systems and man's palatine tonsils. This goal of this investigation is to determine Trk receptors localization by means of immunocytochemistry and western-blot in pig's thymus. Young pigs thymus (n=20) were immediately fixed in 10% buffered formaldehyde and processed by routine techniques (H-E) and blue metilene. Trks proteins were labeled with rabbit polyclonal antibodies, with ABC method. In order to detect Trk expression by Western blot, homogenates were electrophoresed in SDS-PAGE 12.5% and developed with Trk specific polyclonal antibodies. TrkA y Citokeratine is positive in medullar reticular epithelial cells. TrkB was visualized in medullar monocyte-macrophage cells series. S100 agrees with reticular cortical cells and TrkC with interdigital dendritic cell in cortex-medullar union. The three Trk proteins were identified in pig thymus by Western blot analysis. Pig's thymus Trks proteins develop an appropriate microclimate for tymphocytes maturation and differentiation and they also may act in a paracrine way.

45. MORPHOLOGICAL AND BIOCHEMICAL STUDY OF THE LUTEAL POPULATION OF VISCACHA (*Lagostomus maximus maximus*)

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The corpus luteum is a fundamental structure of pregnancy. Our laboratory researches the reproductive biology of viscacha, a rodent of our country. In this work, we present the results of the morphological and biochemical study of the luteal population of viscacha. Pregnant and non-pregnant viscachas were captured in its habitat, then anaesthetized and killed by decapitation. The ovaries were removed, fixed and processed for light and electron microscopy. The ovarian and serum progesterone (P) were determined by radioimmunoassay. In the viscacha ovary, we described accessory corpus luteum (ACL) and true corpus luteum (TCL). The ACL are formed from luteinized primary follicles containing the oocyte and the zona pellucida. The TCL are formed from ovulating follicles; they are big, abundant and positive to 3 β -HSD histochemical reaction. The predominant cell type derives from the granulosa cells (type I). These cells contain abundant mitochondria, dense bodies and dilated cisterns of endoplasmic reticulum. The following table resume the results of P:

	Pregnant	Non-pregnant	Student's t-test
Ovarian P (ng/ml)	0.80 \pm 0.06	0.080 \pm 0.034	p<0.001
Serum P (pg/ml)	40.5 \pm 4.3	18.3 \pm 2.5	p<0.001

(Values are average \pm SE)

In viscacha, the gestation length is approximately five months. The morphological and biochemical characteristic of TCL suggest that they are able to synthesize and secrete sufficient progesterone for to maintain a long pregnancy.

46. EMBRYOLOGICAL STUDIES ON TWO COMMERCIAL HYBRIDS OF SUNFLOWER (*HELIANTHUS ANNUUS*)

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The abortion of sunflower fruits during its development may be due to abnormalities in the reproductive process. This diminishes the potential yield of the cultivars and harvest. However, there are few investigations done on the subject. Microsporogenesis, microgametogenesis and the sporofytic structures related have been studied on hybrids CF17 and P30. Classic histological techniques were followed. The anther wall consists of epidermis, endothecium, one middle layer and a plasmodial tapetum. The latter integrates in the free microspore stage. Before losing its individuality, the cells of the tapetum present two nuclei. A peritapetal membrane encloses pollen grains, which are 3-colpate and shed at 3-cellular stage. The ovule is anatropous, tenuinucellate and unitegumentate. Megaspore tetrads are found in linear disposition and the chalazal one develops into a Polygonum type embryo-sac. The antipodes, usually two, present numerous nuclei, are separated between themselves and the middle cell by thick walls and begin to degenerate after fertilisation. Rests of them are still observed at the globular embryo stage. Both hybrids present nuclear endosperm. The endothelium becomes thicker during the first stages of embryogenesis. P30 presents different kinds of embryos. Results are confronted with the existing bibliography and differences between these and other genotypes are discussed.

47. MORPHOLOGICAL STUDY OF THE MOUTHPARTS OF THE POLICHAETE *EUNICE ARGENTINENSIS*

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The genera Eunice are wandering polichaete that inhabit shallow waters with rocky or muddy bottoms. *Eunice argentinensis* is one of the largest polichaete in Golfo Nuevo, which makes it a high energy prey for birds, marine mammals and fishes. The knowledge of prey size is usually important in diet studies of predators, because the size of the prey helps to estimate the energetic input in the predator. The only pieces of the polichaete that remain in the feces of the predator are the hard parts like the mouthparts. Therefore the study of the mouthparts of the polichaete could help to infer the size of the prey and its biomass. The aim of this study was to find which of the mouth parts are the better indicators of the size of an individual.

Thirty-three individuals were sampled in Golfo Nuevo. Organisms were weighed and measured. Jaws and maxillae were extracted from the individuals and then measured.

Regression analysis ($\alpha=0.05$) were used to determine which of the mouthparts were the best indicator of the body size.

The mouthpart AIII is the best indicator of the body size of *Eunice argentinensis*, because it is the best fit and it suffers the lesser degradation during the analysis.

48. EARLY PREGNANCY FACTOR LOCALIZATION IN PORCINE PLACENTA

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Early Pregnancy Factor (EPF) is a protein secreted during the first gestation stages, with growth regulatory and immunomodulatory properties. It is a seric marker of fertilization, essential for the initiation and maintenance of the pregnancy. The objective of this work was to study the localization of the EPF in swine placenta. Placenta from 30 days of gestation sows were used. EPF localization was determined by immunohistochemistry (Biotin-Streptavidin-Peroxidase) using polyclonal anti-swine EPF antibody developed in rabbit immunized with synthetic EPF. The results reveal differences in the staining pattern between maternal and fetal placental tissue. The maternal placental tissue did not present specific staining for EPF. On the other hand, embryonic placental tissue showed high intensity EPF expression in endoderm from allantois wall and in connective tissue from fetal corion. These results indicate that during the stage of gestation studied, part of EPF originates in fetal tissues, coinciding with the theory that the EPF is formed by two separated molecules that *in vivo* have different origin. EPF-A originates in oviduct during the estrus and pregnancy and EPF-B that is produced by ovary during the pre-implantation pregnancy and by embryo at post-implantation. The fetal corion is a tissue that suffers, at post-implantation period, an intensive process of cellular proliferation, so the detection of EPF in this tissue is coincident with its functionality as autocrine and paracrine growth factor of normal cells.

49.

 α -NAPHTHOFLAVONE INDUCES APOPTOSIS IN PORCINE GRANULOSA CELLS (PGC)Guaragna C¹, Lombardo DM¹, Pecci A^{2,3}, Barañao JL^{3,4}.¹Fac. Cs. Veterinarias, UBA; ²Dpto. Química Biológica, FCEyN-UBA; ³IFIByNE-CONICET; ⁴IByME-CONICET.

α -naphthoflavone (α -NF) is an antagonist at the aryl hydrocarbon receptor (AhR), the cellular receptor of polycyclic aromatic hydrocarbons and dioxins, that inhibits AhR activity via a classical competitive binding mechanism. The AhR is involved in the metabolism of a variety of environmental chemicals. AhR is part of an emerging family of ligand-activated transcriptional regulators that control a variety of target genes including the *Cyp1A1*. However, α -NF has been reported as an AhR agonist depending on their concentration and cellular context. Recent findings indicate that the AhR may have a central role in ovarian physiology and regulate follicular growth during pre- and postnatal life. In bovines, the AhR may be involved in the progression of oocytes maturation and in promoting the resumption of meiosis. This effect is blocked by α -NF. The aim of this work was to evaluate whether α -NF modulates the programmed cell death of porcine granulosa cells. Primary cultures were obtained by aspirating follicular fluid from 3-6 mm follicles. Cells were treated with α -NF (1 a 10 μ M). Apoptosis was evaluated by chromatin staining with DAPI that shows the morphological changes typical of apoptosis and a dose dependent induction. Detection of translocation of phosphatidylserine by annexin V binding indicated that cells died by apoptosis. The caspase 3 activity was assayed by the cleavage of a colorimetric substrate and confirm the results mentioned above.

50.

GAULTHERIA NUBIGENA (ERICACEAE): A SPECIES IN DANGER

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Gaultheria nubigena (Phil.) Burt & Sleumer occurs in the south of Argentina and Chile where it is very rare and herbarium specimens are extremely scarce. Its importance lies in its apparent decline and possible danger of extinction. All available herbarium material is being used to study its distribution, phenology, reproductive structures and to evaluate its status.

Dry specimens were observed directly under the stereoscopic microscope and boiled flowers or fruits were observed under water. Material has been requested from all Argentinean (38) and Chilean (11) Herbaria, plus a few European and American ones, but only six have a few specimens. The species distribution is highly restricted and occurs in the forest zone of the Cordillera. Specimens have been sporadically collected, and their location is poorly documented which makes it almost impossible to find them. It seems that in most cases it has been found by chance.

The new specimen found in 2004 (Parque Nacional Nahuel Huapi), 52 years after its last collection in this country, indicates the rareness of the species. This species (It) is apparently hermaphroditic and one of the only two southern Argentinean Ericaceae in which flowers occur in inflorescences. It is very important to cultivate it in order to prevent its extinction; so we will try to get seeds and/or cuttings for this purpose.

51.

COMPARATIVE STUDY OF GASTRIC MILLS IN LITHODES SANTOLLA, L. TURKAYI AND L. CONFUNDENSHuespe AV¹, Gómez Simes E², Vinuesa JH^{1,3}, Pastor de Ward CT¹.¹Centro Nacional Patagónico, CONICET, Puerto Madryn, Chubut, Argentina. E-mail: anahuespe@yahoo.com.ar; ²Universidad Nacional de la Patagonia "San Juan Bosco", Fac. de Ciencias Naturales, Puerto Madryn, Chubut, Argentina. ³Universidad Nacional de la Patagonia "San Juan Bosco", Fac. de Humanidades, Comodoro Rivadavia, Chubut, Argentina.

In Argentinean maritime littoral three species of the genus *Lithodes* (*L. santolla*, *L. turkayi* and *L. confundens*) (Crustacea, Decapoda, Anomura) are recorded. The family Lithodidae is very important because many species are subject to commercial fisheries and studies on growth, reproduction, embryonic and larval development, culture and parasitism were carried out in different species of the family to evaluate their potential as a fishery resource. The structure of gastric mills in crustaceans is also used as an approach to establish phylogenetic relationships between groups and food relationships. Only few papers were published related to anomuran decapods. It was considered relevant to undertake a descriptive and comparative morphological study of the gastric mill ossicles in the anomuran decapods genus *Lithodes* to know if there is any kind of interspecific or intraspecific variation within the species. The main aims of this study are: 1) to describe the gastric mill morphology in *L. santolla*, *L. turkayi* and *L. confundens* and 2) to compare those ossicles useful in taxonomy; by means of scanning electron microscope digital photographs and stereomicroscope observations. These gastric mill ossicles are useful to recognize and identify these three species when present in their predators gastric contents because these ossicles are very hard chitinous structures that resist the digestive process.

52.

TRIATOMA INFESTANS' OVARIUM POST-EMBRIONIC PEDICELLUM. A MORPHOLOGICAL AND ULTRASTRUCTURAL STUDY

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Triatoma infestans' ovaries are telotrophic as in other hemiptera heteroptera insects; each one is composed by seven ovarioles. Such elementary ovaries structures are already formed early in the embryogenesis. At the 9th or 10th day after oviposition they are distinguishable. After egg etching post-embryonic development takes place in five successive instar stages, each one with its molt, reaching the final winged adult insect. Throughout this cycle the ovarioles differentiate in peculiar regions. The present study is an attempt to analyse from a histological and ultra-structural point of view changes suffered by one of these regions: **the pedicel**; duct that joints each ovariole with the lateral oviduct. For this purpose the insects were narcotised, dissected "in situ" with Karnovsky fixative, each ovary pike-up, included in Polybed-Araldite and sliced in 1 μ m to 2 μ m sections for optical microscopy (phase contrast, dark field, DIC) or in ultrathin 50nm – 60nm sections, Reynhold contrasted, for TEM. It should be stressed that data referred to this subject is very little even for any other species of the insect order mentioned, it is important to note that our study allows us to observe the pedicel as a non well defined zone during early development. Their organogenesis was followed at a histological level, and the differentiation degree and functional stage of the cells and their interaction as shown by the cell-junction type founded at different nymphal instar was followed by TEM. A constant secretory activity is present at the third instars. The present study allows a better understanding of this ovariole zone as well as correlates its participation and functional activity during gametogenesis

53. CONTRIBUTION TO THE MORPHOLOGY OF THE SUBFAMILY HARPACTORINAE: THE GENUS *ARILUS* HAHN (REDUVIIDAE; INSECTA)

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The predatory assassin bugs, *Reduviidae*, are one of the largest and morphologically most diverse families of the true bugs. *Reduviids* range in size from relatively small and extremely delicate insects of only a few millimeters in length, such as members of the genus *Empicoris* Wolff (*Emesinae*), to very big, such as *Arilus*, which may attain a length of nearly 40 mm. The present revision was prompted by the lack of a critical taxonomic treatment of all the nominal species recognized in *Arilus*. Up to this moment the genus is constituted by: *A. cristatus* Linné (Guatemala, México, USA); *A. gallus* Stål (Colombia, Ecuador, Panama, Peru, Venezuela); *A. depressicollis* Stål (Mexico); *A. carinatus* Forster (Argentina, Bolivia, Brazil, Guatemala, Mexico, USA) and *A. nigriceps* Herrich-Schaeffer (South America). This study has been based on material provided by national and international institutions. Morphologic characters were described and illustrated with a Wild M- stereomicroscope. For this revision a total of 4 measurements and 11 ratios were selected. The methods of extraction, dissection, inflation and drawing of the male and female genitalia were detailed by Coscarón (1983, 1994). After this study we confirm that the genus *Arilus* is composed by the species mentioned before. Morphological characters allow an unambiguous species identification in most cases, and particular characters of the pronotum and genitalia indicate well delimited species groups. New geographical distribution are given for *A. carinatus* and *A. gallus*.

54. PERICARP DEVELOPMENT OF SUNFLOWER (*Helianthus annuus* L.) FRUITS UNDER TWO LIGHT STRESS PERIODS

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The structure of the pericarp (Pe) of sunflower consists of an epidermis with proper hairs; an hypodermis; the phytomelanin layer; a middle layer (ML) made up of 10-15 layers of axially orientated cells, with decreasing sclerification towards the center, interrupted by rays of parenchymatic cells at regular intervals and close to the inner epidermis, a parenchyma of thin walled and loosely packed cells, completely compressed by the seed at fruit maturity. The vascular bundles are located in the inner edge of the ML. Changes in the final anatomical structure of the Pe can be associated with environmental and cultural conditions during fruit development. In this work the shade effect on Pe development of fruits from the peripheral and mid positions on the sunflower capitula was analyzed. Two shade treatments were applied to sunflower crop: TS1, 10 days before anthesis of the peripheral flowers, and TS2, at early post-anthesis (10 days after full anthesis, FA). Fruits sampled at FA, 10 days after FA and at physiological maturity (PM) were processed and cross sectioned with conventional techniques for light microscopy. TS1 treatment affected the carpel cell division phase, limited to the preanthesis period, reducing the number of cell strata of the ML and increasing the cell walls sclerification. On the other hand, TS2 treatment affected the development of secondary walls of the cells of the ML reducing the number of sclerified layers and the thickness of their walls. Even the timing of light stress induced variations in the final anatomical structures of the pericarps, at PM both shade treatments produced a similar and significant reduction in their dry weight.

55. HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF THE CFM-27 TUMORAL LINE

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Tumors obtained by induction in Wistar rats with n' nitroso n' methyl urea (NMU), demonstrated an histopathological pattern corresponding to an alveolar mammary adenocarcinoma. Those originated in mice only treated with medroxiprogesterone (MPA) were ductal mammary carcinomas. Inductions with NMU in mice Balb/c did not reproduce the tumorigenic effect. The induction and later promotion with MPA in mice Balb/c generated an alveolar mammary adenocarcinoma. After several syngeneic passages of tumoral fragments obtained by induction and promotion a tumoral line with different histopathologic character was established. Mice of 5 weeks were induced with three doses of NMU (50 mg/kg) and three doses of MPA (40 mg). Two months after induction tumors were developed and detectables by palpation. These were transplanted in syngeneic form to female mice without immunosuppression. They were carried out 42 successive passages *in Vivo* and samples were frozen at -120°C to repeat passages starting from the same one. The transplants were in the inguinal pleat. With a latency of 7 days they were developed tumors locally, differing from the original pattern to a sarcoma of small cells. With the histological routine Trichomics were carried out and the cellular pattern was determined by immunohistochemistry, using membrane markers like Cadherin E, N, Cytoqueratin P8 and Pan cytoqueratine. The expression of receptor of hormon-dependence was evaluated with Progesterone monoclonals anti receptor of and anti protein P29. Up to now the CFM-27 line maintains its histopathologic pattern without changes in successive passages, being able to be a tumoral model repeatible for the study of different biological aspects of neoplasia.

56. HISTOCHEMICAL CHARACTERIZATION OF THE CLAW CLOSER MUSCLE OF *Chasmagnathus granulatus*

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Morphological, physiological and histochemical criteria divide crustacean muscle fibers into two main categories: fast and slow. Histochemically, these fibers can be differentiated according to the myosin ATPase and oxidative enzymes activity. The claw is a multifunctional organ, which is used by the crustacean during feeding, mating, agonistic interactions and burrowing. It's composed by two antagonistic muscles: the closer and the opener. The aim of the present study is to histochemically characterize the claw closer muscle of *Chasmagnathus granulatus*. Adult male crabs on intermolt (N=3) collected from the Mar Chiquita Lagoon (Bs. As., Argentina) were acclimated in aquarias for ten days. The specimens were cold anesthetized and their claws were removed. The closer muscle was fixed in liquid nitrogen. Cryosections were subjected to histochemical techniques: myosin ATPase (pH 9.4; room temperature and 4°C), SDH and PAS. The m-ATPase showed moderate contracting fibers. The SDH activity was subsarcolemmal and two types of fibers were found: S₁ and S₂, of weak and strong reaction, respectively. S₁ fibers were predominant, larger and PAS negative, whereas S₂ fibers were scarce, smaller and with weak reaction to PAS. Concluding, the claw closer muscle of *C. granulatus* presents two types of fibers: S₁ (moderate m-ATPase, weak SDH and negative PAS) would belong to slow "twitch" fibers and S₂ (moderate m-ATPase, strong SDH and feeble PAS) to slow "tonic" ones. The claw closer muscle composition of *C. granulatus* would be linked to its functional demands.

57. OBSERVATIONS ON THE OVARY HISTOLOGICAL STRUCTURE OF THE LLAMA (*LAMA GLAMA*)

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The llama is a south american camelid with international economic interest. The objective of this work was to characterize the ovary histological structure of the llama. Three ovaries were processed by routine histological methods for optical microscopy. They were stained with hematoxylin-eosin, Masson trichomic and periodic acid Schiff (P.A.S.) stainings. The ovaries showed spheroidal shape measuring $0,83 \pm 0,11$ cm in length and $0,85 \pm 0,003$ cm in width. The cover epithelium was high cuboidal ($10,18 \pm 1,83 \mu\text{m}$). The tunica albuginea was observed as a thick dense layer with cells and fibers in whirled disposition. In the cortex the connective tissue between the follicles showed cordonal disposition toward the medullary region. In the antral follicles, the granulosa layer was observed as a stratified epithelium with cuboidal cells toward the lumen and columnar cells toward the periphery. The theca interna showed a cellular composition while the theca externa showed a fiber composition. The medulla was formed by irregular dense connective tissue rich in blood vessels. At the hilum tubular ducts with low cubical epithelium were found; which are remnant of the fetal indifferent gonad, the rete ovarii.

58. DYNAMICS OF GROWTH OF TOMATO "PLATENSE" ROOT SYSTEM AFTER TRANSPLANTATION

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Given the current relevancy of Tomato "platense" which is more required than hybrids ones by its intensive flavor, in this work was outlined the study of the dynamics of growth of its root system during 20 days after transplantation.

Plants of 7cm approximately of height were transplanted to handles. They were accomplished four observations, one for week, with three repetitions. Following parameters were registered: plants height, number and length of adventitious roots and of ramifications of first, second and third order. Total length of radical system was estimated by Newman's method and graphic and adjustment curves were made. Length of the radical system increased linearly. The growth rate was 84 cm/week ($p < 0,01$) Adventitious roots length (8% to the total length of root system), was quadrupled at the end of the experience, tending to even to the first order roots. Second order ramifications provided of constant way during the trial. On the other hand, growth of third order ramifications began near the end of experience. Upon ending the experience the total length average was of 368.4 cm; composed by 33% of adventitious roots, 36% by ramifications of first order, 23% by those of second order and 8% by the third order ramifications. This growth dynamics would permit a better distribution of the tomato root system in soil profile and greater efficiency in the function of water and ions uptake.

59. HISTOLOGICAL AND MORPHOMETRICAL STUDIES OF SEXUAL ORGANS IN RATS EXPOSED TO 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

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Histological and morphometrical studies of sexual organs complement the determination of developmental and maturative parameters when reproductive toxicity must be analysed.

Virgin female 90 day-old Wistar rats were made pregnant, and the treated group was exposed to 2,4-D (70 mg/kg/day, sprayed on food) from gestation day 16 onwards. On postnatal day 23, pups were weaned and the treated group continued to be fed with 2,4-D until sacrifice at 45, 60 or 90 days of age. Histological studies were performed in ovaries and testes fixed, paraffin embedded and stained with hematoxyline-eosine. In ovary, the number of corpora lutea (CL) and primordial (prF), primary (PF) and secondary (SF) follicles were counted. In testes, mean tubular diameter (TD) and height of the seminiferous epithelium (HE) were measured.

Histological studies reveal no histopathological alterations in these organs. The differential ovarian follicles counts showed significant diminutions ($p < 0,05$) in number of prF, PF and CL in rats treated for 60 days and in the number of PF and SF in rats treated for 90 days. The TD was significantly increased ($p < 0,05$) in rats treated for 45 days, without alterations in HE.

These results, together with the others parameters already determined, suggest an indirect testicular toxicity and a direct ovarian toxicity of 2,4-D.

60. HISTAMINE AND SEROTONIN EXPRESSION IN THE OVIDUCTAL MAST CELL POPULATION DURING EARLY PREGNANCY IN THE RAT

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The oviduct is the site of fertilization and early embryo development. The aim of this study was to investigate the morphological changes of histamine- and serotonin-containing mast cells in the oviduct of the rat during early pregnancy. Oviducts were obtained from Wistar rats on days 3, 4, 5, 6 and 9 of pregnancy ($n=3$ per group). The oviductal tissues were fixed in Bouin's fixative and processed for embedding in paraffin wax. Immunohistochemistry for histamine and serotonin was performed on serial sections, using a sensitive peroxidase-labelled streptavidin-biotin detection system. The number and area of immunostained mast cells were measured by videomicroscopy and image analysis. Statistical analysis: ANOVA-Scheffé's test. On day 4, the histamine- and serotonin-containing mast cell number was significantly ($P < 0.01$) lower compared with the other days of pregnancy. These results suggest that histamine and serotonin are released by mast cells during the pre-implantational period.

61. SIGNIFICANT CARIES INDEX IN A RURAL CHILDREN POPULATION

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Prevalence caries indexes like DMFT (Disease, Missing, and Filled Teeth) and DMFS (Idem Surface) describe the amount of the individual prevalence of dental caries. To evaluate oral health population, you use position measures (media, mode, and median) and, probably, they are not enough to include extreme individual values. Significant Caries Index (SIC), in which you select one third of the population with the highest caries scores and calculate the mean DMFT for this subgroup, indicates if you have to continue with a Prevention Program. The aim of this study was to evaluate the dental oral health in a rural population from the Municipio of Florencio Varela. Sixty-three children were studied without evident pathologies, in-groups 0-6; 7-12 and 13-17 years old. Different Indexes were determined: For permanent teeth DMFT) and DMFS; For temporary teeth: dmft and dmfs; Plaque Index (Löe and Silness); Sugar Moments and SIC. Main caries activity was observed in 0-6 (dmft median 6 and dmfs median 15) and 13-17 years old (DMFT median 6 and DMFS median 16). SIC values were in a direct relation with them. For the first group SIC dmft 10; dmfs 26; second group SIC DMFT 9 and DMFS 34. As they are high values –high risk- we have to continue the Prevention Program. In 7-12 years old group, DMFT and DMFS median 0,5 we may infer that those values could be due to a very good maternal-infancy attendance before 2001 crisis. But SIC 4 and 7,5 showed that were some children in risk yet. Sugar Moments median 6 was not important as groups' differences.

62. IMMUNOHISTOCHEMICAL LOCALIZATION OF IGF-I IN PORCINE PLACENTA

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IGF-I is a protein molecule structurally related to insulin and it is the major growth factor in blood. IGF-I has important effects on pregnancy because it regulates fetal, placental and endometrial growth through autocrine and paracrine mechanisms. The purpose of this study was to investigate the localization of IGF-I in placental tissues of 30 and 60 days of gestation in mixed breed from a local slaughterhouse. After slaughter, the reproductive tracts were collected, samples of maternal-fetal interface were taken and the indirect immunoperoxidase technique was applied. We found a positive immunoreactivity in the apical membrane of the trophoblastic cells and in the lumen of some endometrial glands. This activity was stronger in the placentas of 60 days of gestation. We also observed this activity, though to a lesser degree, in the endothelial blood vessels and in the maternal epithelium. These results suggest the importance of IGF-I in the growth of both the conceptus and placenta. Besides, the presence of this factor in the endometrial glands could be related to fetal nutrition. Although IGF-I is crucial in the implantation period, our results show the presence of this factor is preferentially at 60 days of gestation. It is from this period onwards that the fetuses grow exponentially and increase their metabolic and nutritional demands which can be satisfied by IGF-I in an autocrine and paracrine manner.

63. PRESENCE OF CALLOSE IN THE MEGASPOROGENESIS OF APOMICTIC AND SEXUAL PLANTS OF WEEPING LOVEGRASS

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Apomixis is defined as a mode of asexual reproduction where seed is produced from the ovule tissue, avoiding meiosis and fertilization. This mode of reproduction is distributed among the tropical grasses, including weeping lovegrass (*Eragrostis curvula*), a forage grass native from Southern Africa and naturalized in Argentina. In sexual plants a layer of callose is deposited surrounding the arquesporeal cell and persists until the stage of embryo sac. According to the literature, in diplosporous apomixis there are not callose deposits, allowing to distinguish sexual from apomictic plants. The aim of this work was to differentiate sexual and apomictic pistils of weeping lovegrass using a technique that involve a differential staining for callose. In order to do that inflorescences were collected at the beginning of anthesis in sexual (dihaploid plants obtained by *in vitro* culture from the apomictic cv. Tanganyika) and apomictic (cv. Tanganyika) plants. The spikelets were fixed in FAA, included in paraffin and cut in slices of 10 µm. Slices were stained with aniline blue, a specific dye for callose, and observed with a fluorescence microscope. After that, slices were stained with safranin-fast green. In pistils of sexual plants it was possible to observe a fluorescent pattern typical from arquesporeal cell, tetrad and also in the cells surrounding the embryo sac. As an unexpected result it was possible to observe also callose in the stages of arquespore and functional megaspore in pistils of the apomictic plants. However, the observed patterns differed from those of sexual plants. This different patterns of callose deposits are a useful tool to distinguish between apomictic and sexual plants of weeping lovegrass.

This is the first report about the presence of callose deposits in the megasporogenesis of diplosporous apomictic plants.

64. STUDY OF THE HISTOLOGICAL STRUCTURAL ALTERATIONS IN THE MUSCULAR FIBERS OF THE EQUINE PRODUCED BY THE THERAPEUTIC ULTRASOUND

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The skeletal muscle lesions are frequent in the sporting equine. The therapeutic ultrasound is one of the mostly used electrophysical agents as physiotherapeutic for all those muscular, tendinous and articular affections, in which deep heat is beneficial. To detect their probable deleterious effects on the muscular fibers their morphology was studied with the optic and electronic microscope. The oxidative profile of the muscular fibers was studied by means of the activity and distribution of the Nicotinamide Adenine Dinucleotide tetrazolium reductase. (NADH-TR), using normal dosage and overdose. Ultrasound was applied to 6 clinically healthy horses in a pulsative form on the longissimus dorsi muscle, on the right side at an intensity of 3 watts / cm² and on the left side at 3,5 watts / cm², during 30 minutes for 10 days. Muscular biopsies were taken at the beginning and at the completion of the treatment. With the optic microscopy both, pretreatment samples and that underwent through treatment presented histological structure without alterations. With the reaction of NADH-TR the value of the metabolic activity in NADH with dose of 3 w/cm², was similar to the normal muscle while those tried with 3,5 w/cm² evidenced a weak blue coloration and a low value. With electronic microscopy, it was observed with dose of 3 w/cm² muscular ultrastructure was conserved, while with 3,5 w/cm² microedematization and increase of the interfibrillar distance occurred. The mitochondrias were visualized as broken into fragments and with loss of the crests. The therapeutic ultrasound is useful but the intensity should not exceed the 3 w/cm² to avoid injure of the ultrastructure which is as important and necessary as the mitochondrial crests where the cellular metabolism takes place.

65.

ORAL SQUAMOUS CELL CARCINOMAS: AMPLIFICATION OF PROTO-ONCOGENE *c-erbB-2*

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It was suggested that *c-erbB-2* amplification and/or over-expression in carcinomas is a bad prognosis indicator of different carcinomas because they have a more aggressive development in comparison with others of the same histological grade but without over-expression. The HPV 16 is associated with oral cancers. Co-expression of E6/E7 and *c-erbB-2* could induce metastasis. In order to analyze *c-erbB-2* proto oncogene amplification on oral squamous cell carcinomas HPV+, 30 HPV+ and 12 of normal oral mucosa samples were examined. Co-amplification technique was applied with reference gene using locus tyrosine kinase (one copy) as control gene located on the same chromosome. Electrophoretic reading was made on polyacrilamida gel (6%) and post stain with ethidium bromide. Relationship between intensity of bands was performed by digitalization of images (Kodak Program). The genetic amplification of *c-erbB-2* on squamous cell carcinomas was 10% and 0% on control examples. There was no statistically significant difference when we compared both groups: $p=0.035$. When we analyzed proto-oncogene amplification with viral infection, we found association with low risk viral types (26.3%) in comparison with HPV-samples. There was no significant difference with high risk viral types: $p=0.36$. We conclude that in the presented series, the activation of *c-erbB-2* was associated with the low risk viral types. We believe that it might be another co-factors and, that it would be possible that low risk HPV viral types could represent a role in tumor induction.

66.

TRITRICHOMONOSIS IN A MICE MODEL: CHANGES IN THE PATTERN OF CARBOHYDRATE IN THE GENITAL EPITHELIA IN PREGNANT FEMALESMonteavaro C¹, Gimeno E², Soto P¹, Echevarria H¹, Portiansky E², Catena M¹, Barbeito C^{2,3}.¹Microbiología, FCV UNCPBA. ²Instituto de Patología; ³Histología y Embriología, FCV UNLP. Buenos Aires. Argentina. E-mail: cmontav@vet.unicen.edu.ar

In previous papers we have demonstrated the existence of modifications in the lectins union pattern in different genital organs of female bovine and mice that were infected with *T. foetus*. Because of the difficulties of developing a long-term model with bovine, we worked in mice BALB/c females in the estrous phase. These animals were inoculated with *T. foetus* by intravaginal via with *T. foetus*. They were mated two weeks after infection. During the pregnancy, every day infected females and their controls were sacrificed. Vaginae and uteri from each individual were imbedded in parafin and sectioned for their incubation with the following lectins: SBA, Con-A, UEA-1, PNA, RCA-1, DBA and WGA. We have found, in the group of pregnant animals, modifications in the union pattern of lectins in the uterus and vagina. These changes are related to intensity and distribution of carbohydrates, which could be a consequence of the reaction against the protozoan. The modifications could be due to the enzymes effects produced by *T. foetus* to favour the penetration and adhesion.

67.

DETECTION OF ENDOMETRIAL GLANDULAR LYMPHOCYTES IN INFECTED BALB/c MICE WITH *Tritrichomonas foetus*Monteavaro C¹, Soto P¹, Gimeno E^{2,4}, Echevarria H¹, Catena M, Portiansky E^{2,4}, Barbeito C^{2,3,4}.¹Lab Microbiol. Clín. y Experimental, FCV, UNCPBA. ²Inst. Pat. and ³Cát. de Hist. y Embriología, FCV, U.N.L.P. Bs. As. Argentina. ⁴CONICET.

Tritrichomonas foetus is a flagellate protozoan that it produces embryonic death and abortions in bovine. A model was developed in BALB/c mouse to study this disease. In the gestation during the installation of the conceptus is formed the decidua. This contains cells, as fibroblasts, macrophages and lymphocytes citotóxicos. There is a change in the distribution of lymphocytes glandular endometriales (GMG) decidual tissue; this change response to hormonal signs. They also protect to the embryo of the maternal immunologic reaction. The GMG, they are cytotoxic cells that you/they appear in the basal decidua during the early periods of gestation; later on their number diminishes and they are located in the mesometrial triangle. The GMG is characterized by the accumulation of cytotoxic mediators like: perforinas, proteinasas, tumor necrosis factor and granzimas B. For the composition of their granules it can visualize them with PAS and with some lectinas, especially DBA. By means of these techniques these cells were studied in the mouse infected with *T. foetus*. Samples of uterus of infected mice pregnant BALB/c for via intravaginal with two dose of 9×10^5 *T. foetus* and controls that were sacrificed among the days 6 at 12 of pregnancy were taken. The cuts were colored with PAS or incubated with the lectina DBA. Both techniques allowed to identify the GMG, so much in the animals controls as in those infected. The morphology and the distribution of the cells was similar in both groups and coinciding with other authors. The present work is a previous study that will allow us to use these techniques in recounts of GMG, to determine possible changes in the number of cells in the infected animals.

68.

EFFECTS OF GESTATIONAL CHRONIC STRESS ON NPY NEURONS DURING SNC DEVELOPMENT IN RATS

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Due to the wide distribution of NPY-neurons on the SNC it is suggested that neuropeptide is an important molecule for the regulation of brain functions and also in different physiopathological processes. In hypothalamic regions and in the anterior brain they are closely associated to GnRH neurons and to some other neurons that would favor the neuroendocrine modulation. The aim of this work was to determine the chronic stress effects on the NPY neurons on the fetal development of the anterior, medium and posterior brain. Wistar rats kept under laboratory standard conditions were used. Sagittal cuts of fetal heads of 15, 17 and 19 days of gestational age were performed. Immune-marks for NPY neurons were done (Ac anti-NPY, Sigma, Co). Which were quantified by stereological analysis of images. Data were analyzed for intra and inter groups with the non parametric tests of Kruskal-Wallis. Significant differences were found in the anterior, medium and posterior brain ($p<0.01$) in the three ages of intra group control fetus (CF) and stressed fetus (SF). Highly significant differences were found in anterior and medium brain ($p<0.01$) when comparing CF vs. SF of 15 days of age while highly significant differences were found in the anterior brain ($p<0.01$) in those of 17 days. The differences were significant in anterior brain ($p<0.05$) and highly significant in medium and posterior brain ($p<0.01$) at 19 days. It is concluded that gestational chronic stress produces an increase of NPY positive neurons in the studied brain areas that might become in one of the emotionality alteration factors in postnatal stages facilitating behavioral alterations.

69. DISTRIBUTION OF RAMIFICATIONS IN PEPPER (*Capsicum annuum* L.) ROOT SYSTEM

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The efficiency of plants in water and ions uptake depends on the extension and spatial configuration of their root system. Efficiency is directly influenced by lateral roots development that increase the absorption area and the volume of explored soil. Frequency and position of lateral roots are conditioned by the genotype and influenced by the environment. In this work is described the distribution of root system ramifications of pepper for paprika, in the first 30 days of growth. They were visually analyzed, by nude eye and under magnifier binocular, the radical plant systems cultivated in handles, those which were extracted in different stages of growth and washed carefully in order to not to injure the roots. Pepper forms a typical radical system, with a principal axis that presents secondary growth. Ramifications of successive orders born acropetally. First order ramifications are located on the principal shaft gathered in two longitudinal, opposite bands, that coincide with the position of the cotyledons. Between them there are two bands that lack totally ramifications. However, the angle in which ramifications born permits radical system to explore all soil cylinder that surrounds to the principal shaft. When the plant has three or four leaves, a number of adventitious roots primordia can be observed on the hypocotyle. Those located in greater proportion in two longitudinal bands that coincide with cotyledons position and are separated by bands, and those that exist as smaller number of roots primordia.

Key words: pepper, root system, ramification

70. MORPHOLOGY AND SURVIVAL OF *GRINDELIA VENTANENSIS* (ASTERACEAE) IN RELATION WITH ITS POTENTIAL AS AN ORNAMENTAL PLANT

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Most plants used in Argentina as ornamentals are exotic, with the concomitant underestimation of native biodiversity and risks of invasion into wild lands. *Grindelia ventanensis* is an endemic shrub from Ventania Mountains (Buenos Aires) with potential as a garden plant and in resins industry. In this work, morphological features of the species and their relationship with seedling survival are evaluated in order to assess its cultivation potential. Characteristics of the cypsela and number of cypselas per capitulum were assessed for two cultivated populations and nine wild ones, a first description of the plantule was made and seedlings growth and survival were studied. The number of cypselas per capitulum ranges between 82 and 234, with important size differences depending on the population of origin. Cotyledons emerge at the second day of plantule life and differ in length and width among populations. A high mortality of plants originated in cultivated populations was observed. Cultivated plants start flowering before the wild ones, and among the last ones, those in the valleys flower before the ones in the hills. Differences in the size of the cypselas and in seedling growth could have a genetic origin and may be responsible for the variations observed in seedling mortality. Our results indicate that *Grindelia ventanensis* is a species with high cultivation perspectives but the origin of founding propagules has to be considered in order to establish populations with high reproductive potential.

Key words: native plants, conservation, sustainable use.

71. RADICAL SYSTEM OF CHERRY TOMATO POST TRANSPLANTATION

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In order to obtain healthy and vigorous plants from the beginning of the cultivation, it is important to know the plant's radical system. The peculiar feature of tomato is that its radical system (main axis and ramifications) is affected by the cultivation methods (transplantation). Therefore, our main objective was to characterize the radical system of the tomato plant in its post transplantation phase. For this reason, during the months of November and December, 2004 cherry tomatoes were cultivated in flowerpots and in natural environmental conditions. This experiment was carried out at the Faculty of Agronomy in Tucuman. The plants were transplanted 16 days after the sowing had taken place. The measurements were recorded every 7 days selecting 3 plants at random each time. The parameters taken into account were: the length of the air part (cm); length of the principal root (cm); total length of the radical system (cm); number of first order ramifications; total dry weight (g). The relationship between the total length of the radical system and the length of the air part was calculated. During the first 28 days of post transplantation, the air part was a 36% longer in comparison with the principal root. Later on, this situation was reverted, being the root a 18,5% longer than the air part. This was reflected in the relationship root/scion that increased from 0,92 to 9,78 during the five week period experiment. From the third week onwards and after overcoming the transplantation stress, the total length of the radical system increased a 118%. This remarkable increase was mainly due to the contribution of the first order ramifications and to a lesser extent, to the growth of the principal root, the one which was affected by the transplantation.

72. DOUBLE CUTICLE SCULPTURE IN ECHINISCIDAE (HETEROTARDIGRADA): THE CASE OF *ECHINISCUS RUFOVIRIDIS* DU BOIS- RAYMOND MARCUS 1944

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Descriptions of numerous species of echiniscids include references to a two-fold cuticle sculpture, *i.e.*, a thick heterogeneous sculpture and a fine homogeneous one. This sculpture can be recognized under light microscope with variable focusing; thicker sculpture can be observed with upper focus, while the fine one can be observed using the lower focus point. In this contribution we attempt an explanation of the light microscope images using ultrastructural studies in *Echiniscus rufoviridis*. Scanning electron images reveal that the thick granulations correspond to conical depressions of the surface, while the fine granulation can be attributed to the intracuticle structure. TEM sections perpendicular to the surface provide evidence that suggests that the fine granulation is due to the basal part of the internal epicuticle, which at this level is constituted by flat lacunae separated by low columnar structures. Sections parallel to the surface confirm that the localizations of such columnar structures correspond to the optical image of fine granulation. Available information on other echiniscids suggests that the postulated explanation for *E. rufoviridis* may be also applicable to other species with double cuticle sculpture. Fine granulation may have always the same origin, *i.e.*, basal supports of the internal epicuticle, perpendicular to the surface and more or less visible by transparency according to thickness and structure of the upper layers. Thick granulation would correspond to the true surface sculpture constituted by depressions or elevations, according to the species considered.

73.

EVOLUTIVE MORPHOLOGY OF THE RED GARLIC CLOVE PROTECTIVE LEAF (*Allium sativum* L.)

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Literature shows references on the anatomy of the garlic clove protective leaf but descriptions are based on recently harvested material. During storage, developing garlic bulbs loose water and some structures become differentiated until the physiological maturity is reached. This work shows the morphological changes that take place in the red garlic clove protective leaf from harvest (*inmatura* stage) to physiological maturity. Observations were made using light and electron microscopy.

The protective leaf consists of a single layer of straight elongated cells in the clove main axis, with sharp edges, primary cell walls and wide lumen with a visible nucleus. As maturation progresses it becomes dry and hard, the epidermal cells deposit lignified secondary walls so their lumen mostly reduces and pits can be noted. This process is delayed in the upper third of the leaf where stomata are under differentiation.

At early stages, the mesophyll consists of several layers of turgid parenchymatic cells and, closer to the outer epidermis, there is a single layer of square cells containing a homogeneous solution of anthocyanic pigment, responsible of the red colour of this leaf. As the leaf undergoes maturation, the mesophyll layers compress and obliterate, with the exception of the outer layer. Inside those uncollapsed cells, no reported before calcium oxalate crystals are formed while the pigment solution migrates towards the outer epidermal cells. Finally, it is found precipitated in the narrow lumen of those cells as dense clots. Those structures stand for the protective nature of this leaf.

74.

STOMATA AND WAXES PRESENT IN THE STORAGE LEAF EPIDERMIS OF THE GARLIC CLOVE (*Allium sativum* L.)

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The clove is considered the most important part of the garlic plant due to its double role of food and seed. It consists of three modified leaves: the protective leaf (thin and resistant) lining the storage one, that encloses the developing sprout. The storage leaf, the bulk of the clove, mainly contains reserve substances and water. Literature has descriptions about morphological and anatomical characteristics of the storage leaf, however nothing is said about stoma features and presence of epicuticular waxes here described. Studies were made using light and electron microscopy.

The outer epidermis of the protective leaf consists of a single layer of elongated cells with primary walls, arranged in longitudinal rows parallel to the clove main axis. In some cells, both terminal walls are perpendicular to the main axis while in others, one of them is oblique. The apical and basal thirds of the protective leaf show anomocytic stomata spread among the longitudinal rows of cells. Some stomata are elongated in the direction of the epidermal cells but others are equidimensional with an oblique main axis. The formation of different stomata could be related with the terminal wall (oblique or perpendicular) of the protodermic cell where the first asymmetric division takes place in the stomatic differentiation process.

The epidermis outer surface is covered by amorphous aggregates of epicuticular waxes irregularly distributed that block the stomatal pores so making them non functional. Those elements are compatible with a strategy developed by this leaf in order to reduce the water loss.

75.

THE ANATOMY OF *Nitrophila australis* Chodat & Wilczek var. *australis*, AN ENDEMIC HALOPHYTE FROM ARGENTINA

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The anatomy of *Nitrophila australis*, a rhizomatous species of the Chenopodiaceae, was studied since few such studies of representatives of this family have been made. It is found in the Salitral de la Vidriera (Buenos Aires). The leaves have large epidermal cells, there is abundant cuticle and the stomata are superficial and anomocytic. The chlorenchyma has no particular characteristics, consisting of smaller cells than the parenchyma which surrounds the vascular bundles; there are druses in both tissues. There is a large central vascular bundle and six smaller ones on each side. All of them have xylem, phloem and a cap of collenchyma cells next to the phloem. The stem epidermis is similar, with a layer of angular collenchyma below. The cortical parenchyma has large intercellular spaces, and druses in some cells. A layer of collenchyma surrounds the stele, forming caps in some of the bundles and sheaths in others. The stele is made up of four vascular bundles, two large and two small, placed in pairs. The phellogen in the rhizomes is superficial, producing middle-sized cork cells with slightly thickened cell walls. A large number of starch grains and a few druses are found in the cortical parenchyma. Small groups of fibres are sometimes present outside the primary phloem. The stele is formed by a variable number of vascular bundles. These characteristics are unlike those found in similar halophyte genera of the Chenopodiaceae.

76.

HISTOLOGICAL CHARACTERIZATION OF LIVER FROM ALUMINUM CHRONICALLY INTOXICATED RATS. EFFECT OF THE VITAMIN E.Pisani GB¹, González MA², Alvarez ML^{1,2}, Roma MG², Quintana AB¹, Lugano MC¹, Carrillo MC^{1,2}.¹Morfología. ²IFISE-CONICET. Fac. de Cs Bioq. y Farm. U.N.Rosario. ³Fisiología Humana. Fac. de Bioq. y Cs. Biol. U.N.L. Santa Fe. Argentina.

Human exposure to Aluminum (Al) has increased. In experimental animals, Al exposure has an impact on biliary secretion mediated by oxidative stress. Our aim was to characterize through a morphometric model, liver histology from Al-chronically intoxicated (Alci) rats, treated or not with Vitamin E (Vit E). Male Wistar rats were divided in groups: Controls (G1); Alci (G2); treated with Vit E (G3) and Alci + Vit E (G4). On liver sections we determinate: the number of bile ducts (NBD) (counted); volume density of portal areas (Vvpa) expressed as percentage (stereologically); hepatocellular necrosis (HN) and portal tract inflammation (PTI) (scoring system); fibrosis percent index (FI) (digital analysis) and apoptotic index (AI) (TUNEL).

	G1	G2	G3	G4
NBD	1,20±0,02	2,43±0,41*	1,26±0,07	1,31±0,10
Vvpa	1,30±0,04	4,93±0,72*	1,33±0,06	1,65±0,50
FI	0,60±0,06	1,18±0,21*	0,62±0,11	0,55±0,01
HN	1,0±0,0	2,1±0,4*	1,0±0,0	1,1±0,3
PTI	1,0±0,0	2,4±0,5*	1,1±0,1	1,2±0,4
AI	0,010±0,001	0,046±0,023*	0,010±0,001	0,015±0,005

Mean±SD. One-way ANOVA, following by Tukey-Kramer or Dunn's test, n=5, *p<0,05 vs G1, G3, G4. Morphometric model used let quantify histological alterations on Alci rats showing that these alterations were reduced when rats were treated with Vit E.

77. MORPHOLOGICAL CHANGES OF HEPATIC RAT ENDOTHELIAL CELLS DURING COLD PRESERVATION/ REPERFUSION

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Liver cold preservation/reperfusion induced injuries on hepatic endothelial cells (HEC). The aim of this work was to study the effect of the addition of a Nitric Oxide (NO) donor [S-nitrosoglutathione (GSNO)] to the University of Wisconsin solution (UW) on morphological changes produced during cold ischemia/reperfusion. Livers of Wistar male adult rats were preserved in UW solution (48hs-0°C) and reperfused in the Isolated Rat Liver Model (60min-37°C). Four GSNO concentrations were analyzed: 50, 100, 250 y 500 µM and normal controls (NC) and reperfusion controls (RC) were established. Liver samples were taken after reperfusion and fixed in 10% PBS-formaldehyde and embedded in paraffin. Sections of 7 µm thick were stained with H&E for light microscopy (LM) and phase contrast microscopy (PCM) analysis. Other samples were storage in liquid N₂ to perform immunohistochemistry for the antigen associated to factor VIII (FVIII-RA). The LM of H&E from NC showed fusiform HEC next to the sinusoidal pole of the hepatocytes. RC had both fusiform and rounded HEC. Reperfused groups evidence rounded HEC partially detached from the perisinusoidal extracellular matrix. 100 µM GSNO reduced the alterations. PCM observation shows rounded HEC with a refringent light perinuclear halo. FVIII-RA showed uniform granular marks in NC. Groups 48hs, 50, 250 y 500 µM GSNO has fewer positive HEC with polarized marks. In conclusion, 100 µM GSNO was the best concentration to avoid HEC injuries due to cold preservation/reperfusion.

78. FIBERS THAT CONTAIN COLLAGEN OF TYPE I and III IN MEAT OF BROILER CHICKENS

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The intramuscular connective tissue intervenes in the tenderness of the meat, dependence of amount to fibers rich in collagen of type I (collagen fibers), and those that possess collagen of type III (reticulin fibers). The objective of the present study was to characterize to determine and to compare the type and quantity of the fibers mentioned in the *pectoralis* muscles and *iliotibialis lateralis* from commercial broiler chickens versus samples obtained of broiler chickens brought up to homemade way. The experimental group was fed ad-libitum with 2/3 of balanced standard and 1/3 of vegetable shattered corn in growing quantities along the period of breeding, until reaching 50 g daily by animal; they were lodged in extensive environments during the night and in poultry coops with natural pasture during the day and they were killed between the 65 and 70 days of age. Samples of the muscles mentioned of chickens of both groups were taken immediately after the death and they fixed in formol al 10%, pH 7; then they were processed for the classical methods to obtain histological courts, to which applied them itself the method of picosirius-red-polarization for its coloring and observation to optic microscope. The fibers with an increase of 200X were counted by means of a *reticulum* of lines and the morphometric calculations were carried out. Statistically demonstrable differences in the quantities they were found and distribution of the collagen and reticular belonging to the muscles studied, as thus also among the groups of chickens studied.

79. A MORPHOLOGICAL AND BIOCHEMICAL STUDY OF THE ADRENAL RETICULAR ZONE IN THE LAGOSTOMUS MAXIMUS MAXIMUS. EFFECT OF THE PHOTOPERIOD

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The animal species that develop their vital cycles in close contact with nature are conditioned by the variations in the environmental signals, called zeitgeber. The natural photoperiod is the most important one since it constitutes the most predictable signal. Reproduction and adaptation to the environment are two characteristics synchronized by the light-darkness period, modulated by the pineal activity and regulated by the neuroendocrine system. The objective of the work was to study the effect of the photoperiod on the histophysiology of the adrenal reticular zone. We present the results of the morphological analysis of the cells in this zone and the androstenedione tissue levels. Summer: A great amount of big-nucleus cells with fine chromatin and evident nucleoli is observed. The clear cytoplasm exhibits activity characteristics. They are grouped in clusters. The blood capillarization is broad and evident. Tissue androstenedione: 693 ± 26.67 ng/g. Winter: The reticular zone shows a significant increase of the cells with hyperchromatic nuclei and scarce and dense cytoplasm. Tissue androstenedione: 347 ± 31.46 ng/g. The significant histoarchitectural difference between summer and winter together with the different tissue levels of androstenedione permits to postulate that during winter there is a marked decrease in the endocrine activity of this adrenal zone. We conclude that the morphofunctional variations found in the reticular zone are probably regulated by the environmental photoperiod and modulated by the pineal activity.

80. MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF ADRENAL MEDULLA IN AN WILD RODENT (Lagostomus maximus maximus)

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The sympathetic nervous system and the adrenal medulla constitute a functional unit in stressor response to the environmental aggression. The hydric and dietary restrictions, temperature, social interaction and natural photoperiod are exogenous synchronizers of the adrenomedullary function. In this work we present the morphological results obtained in adrenal medulla of the *Lagostomus maximus maximus*. We have used histological and immunohistochemical techniques. The glial cells were stained with anti-S-100 protein. These techniques have enabled the morphological study and distribution of the different cellular types. The adrenal medulla is irrigated by blood vessels of great size and thin walls. Chromaffin cells, sympathetic neurons and glial cells are present in adrenal medulla. The former produce catecholamine. They are forming glomerular and cordonal structures. The chromaffin cells are very polarized, with nucleus placed in the region basal. The apical cytoplasm is in contact with the blood vessel. The immunoreaction for S-100 protein shows that the glial cells are located in the basal region of the glomerular structures. On the other hand, the cytoplasmic processes of the glial cells are in contact with the basal membrane of the capillary. Our results suggest that probably the glial cells participate in the modulation of the stressor endocrine response.

81. PROBABLE EFFECT OF THE HYDRIC RESTRICTION ON THE SYNTHESIS OF CORTICOIDS IN A WILD LIFE RODENT (*Lagostomus maximus maximus*)

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The hormones synthesized by the adrenal gland are related to the processes involved in the retention of liquids and to the stress mechanisms, all of which have a fundamental function in the maintenance of the homeostatic equilibrium. In periods of scarce hydric availability, the plasmatic levels of aldosterone and corticosterone increase due to the decompensation produced by water deprivation, thus decreasing the sodium contribution to the organism. We present the results obtained from the morphometric analysis of glomerulose and fasciculate cells, and the levels of corticosterone in periods of high and low hydric availability, summer and winter respectively. Summer. glomerulose cells, nuclear volume (n.v.): $115 \pm 1.07 \mu\text{m}^3$. Fasciculate cells, n.v.: $286.4 \pm 14.72 \mu\text{m}^3$. Serum corticosterone $1.755 \pm 0.216 \text{ ng/ml}$. Winter. Glomerulose cells, of empty cytoplasm, n.n.: $88.24 \pm 9.9 \mu\text{m}^3$. Fasciculate cells, of empty cytoplasm, n.v.: $163.7 \pm 7.78 \mu\text{m}^3$. Serum corticosterone: $4.650 \pm 0.369 \text{ ng/ml}$. The results allows us to postulate that the winter hydric restriction induces the synthesis and liberation of mineralocorticoids stimulating the reabsorption of water with the subsequent decrease of diuresis and loss of salt. Besides, the significant increase of the glucocorticoids might reestablish the homeostatic equilibrium altered by stress and hydric deficiency, thus protecting the organism. This is, undoubtedly, a survival strategy that permits the wild life rodents living in dessert areas to survive during periods of marked drought.

82. COMPARATIVE EMBRYOLOGICAL STUDY BETWEEN *OXALIS NIEDERLEINII* KNUTHZ AND *OXALIS HISPIDULA* ZUCCARINI (OXALIDACEAE)

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Less than 1% of the 800 species of the genus *Oxalis* have been embryologically investigated. The taxonomic delimitation in many cases is not clear. According to what has been observed so far, embryological studies could provide important data to order the genus more naturally. The present work compares the embryology of two species that belong to different sections; *O. niederleinii* Sect. Corniculatae and *O. hispidula* Sect. Ionoxalis. Classic histological techniques were followed and observations were done with a L.M. in order to accomplish these results. The young anther wall presents four layers: epidermis, endothecium, one middle layer and a secretory tapetum which cells are 3- nucleate in *O. niederleinii* and 1 or 2 nucleate in *O. hispidula*. Microspore tetrads are tetrahedral in *O. niederleinii* while in *O. hispidula* there is a tendency to wards rhomboidal or isobilateral. Orbicules are observed in both species since in microspore stage. Pollen grains are shed at 3-cellular stage in *O. niederleinii* and at 2-cellular stage in *O. hispidula*. Both species present a Polygonum type embryo sac. During the early stage at development a nucellar epidermis is observed. In *O. hispidula* it gradually disintegrates in the micropylar end while in *O. niederleinii* it does so almost completely. Therefore the embryo- sac is direct contact with the inner integument.

83. SILICA BODIES IN SPECIES OF CAREX AND UNCINIA (CYPERACEAE)

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Silica bodies are common in the Cyperaceae family and their morphological characteristics are constant and peculiar to the different species, so they are of reliable taxonomic value. During the anatomical study of *Carex polysticha* Boeck., *C. brongniartii* Kunth., *C. catharinensis* Boeck. and *Uncinia phleoides* (Cav.) Pers. silica bodies were seen in the epidermal cells of stems and leaves. These organs were cleared and treated with phenol so that the form and arrangement of the silica phytoliths could be studied.

Silica deposits are found in the epidermal cells of the stems and leaves, above the sclerenchyma tissue which is attached to the vascular bundles; they are conical, with their bases on the internal periclinal walls and with their apexes towards the external periclinal walls. In some cases a cone may be surrounded by smaller cones found in a ring on the same platform (satellites). These bodies are found in both the adaxial and abaxial epidermis in the leaves of *Carex*, but in *U. phleoides* they are found only in the abaxial. Crib or bridge shaped silica bodies were found on the external periclinal walls in the leaves of *C. brongniartii* and *C. catharinensis*, and irregular formations of porous or nodular appearance were seen on any of the walls. This was observed in some of the epidermal cells on the edge of the leaves of both species. The conical bodies are considered typical of the family, whereas the cones of variable morphology are atypical. All these silica bodies are classified as micro-phytoliths and are of the Pileolita type.

84. MORPHOLOGY AND SEED ANATOMY IN REPRESENTATIVE VIOLA SPECIES

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The dispersal of seeds by ants is a widespread phenomenon involving many groups of plants. Majority of *Viola* species (Violaceae) are diplochores. Their seeds, small, with undeveloped elaiosomes are first ejected ballistically from the capsule, in order then to be located and removed by ants. Other seeds, large and with well developed elaiosomes depend on the presence of some medium-sized ground-foraging ant species for dispersal and they are confined to species of a single group, *Viola* subsect. *Rostellatae*.

The aim of this study is to describe and to compare the morphology and seed anatomy of representative *Viola* species belonging to each one of these groups - *V. roigii* Rossow/ *V. maculata* Cav. and *V. odorata* L.-, with particular emphasis in the elaiosome.

Seed samples for anatomical studies were fixed in glutaraldehyde in phosphate buffer, dehydrated and embedded in Spurr's resin. Stained cross sections were observed by light microscopy. Micro-morphological studies were carried out with scanning electronic microscopy.

The elaiosome is composed by small basal cells, and the outer ones, very elongated. Both types of cells present lipids but the outer ones, in greater quantity. Seed coat surfaces exhibit characteristic cellular patterns and the mechanical layer presents differences in relation with the number of elongated sclereid rows.

85. DIFFERENT MICROMORPHOLOGICAL PATTERNS IN SEEDS OF SELECTED *HYBANTHUS*'S SPECIES (VIOLACEAE)

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The genus *Hybanthus* Jacq. with around 100 species is widely distributed in tropical and subtropical regions. The seed is ovoid to ellipsoid, with linear raphe, abundant endosperm and without outstanding macromorphological characteristics that allow to differentiate the species.

The objective of this study was to describe the seed coat micromorphology in five previously selected South American species, in an attempt to establish references patterns for the genus *Hybanthus*. The selected species to study their mature seeds by means of scanning electronic microscopy were: *H. atropurpureus* (A. St.-Hil.) Taub., *H. bigibbosus* (A. St.-Hil.) Hassl., *H. leucopogon* Sparre, *H. nanus* (A. St.-Hil.) Paula-Souza, and *H. velutinus* Schulze-Menz.

The seed coats of these species displayed suites of characters of the primary and secondary sculpture, unique for each taxon. The patterns of seed coat micromorphology were mainly established considering presence of isodiametric or elongated in one direction cells, superficially polygonal or irregular cells, raised or depressed anticlinal cell walls, foveolate or striate - parallel or intercrossed-outer periclinal walls, stomata. This study is one of first attempts related to the micromorphology of seeds of *Hybanthus* and, taking into account the existing variability, we expect to define other different patterns in future analyses.

86. GENERATION OF ANTIPARASITIC RESISTANCE MECHANISMS IN THE TREMATODE *FASCIOLA HEPATICA*

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Helmints possess molecular and biochemical mechanisms to protect themselves from xenobiotic exposure, among them the antiparasitics used to their control. Its detoxification mechanisms include the flavin monooxygenase system (FMO) and the cytochrome P450 family (Cyt P450). *Fasciola hepatica* parasites the liver of herbivore animals, being also very important in tropical and subtropical zones its occurrence in human liver. When faced to a fasciolosis, triclabendazole is indicated (TCBZ), an antihelmintic benzimidazole that depolymerizes microtubules from the helminth, with the concomitant lack of function, loose of anchorage and death. TCBZ is trematocide in both immature stages and in the adult form, but its indiscriminate use has resulted in development of resistant phenomena. The objective of this work was addressed to investigate the manifestation of parasitic resistance comparing the detoxification capacity of a resistant (R) and sensible (S) strain of *Fasciola hepatica* to TCBZ, and to evaluate certain molecular characteristics of its tubulin molecule.

Our results showed that TCBZ is oxidized by both microsomal fractions to its metabolite TCBZ sulphoxide by the two metabolic routes, although the use of metabolic inhibitors demonstrated that strain R possesses a higher detoxification activity, due to an enhanced expression of the FMO route. When immunochemical techniques were used to analyze molecular characteristics of tubulin from both strains, immunoreactivity could be detected in the S strain, but not in the R. These results are straightly involved in the knowledge of the differential susceptibility of each strain to TCBZ and to the understanding of the manifestation of antiparasitic resistant phenomenon.

87. POLLEN MORPHOLOGY OF ENDEMIC PLANTS OF THE VENTANIA RANGE

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Higher plants have alternation of the gametophyte (n) and sporophyte (2n) generations during their life cycle. Male gametophytes are contained in the pollen grains, which exhibit great morphological variation. Most modern plants are adapted, according to the vehicle utilized for pollen transfer, either to wind (anemophilous) or to insect (entomophilous) pollination.

The main objective of this investigation was the morphological characterization of the pollen grains of plants endemic to the Ventania Range. Optical and scanning electron microscopes were used to study pollen samples of Asteraceae, Brassicaceae, Cactaceae, Fabaceae, Malvaceae, Plantaginaceae, Poaceae and Polygalaceae.

Fifteen out of the sixteen studied species have monads, and only one has polyads. In regard to apertures, porate, colpate and colpate grains were observed. According to the exine ornamentation grains could be classified as reticulate, echinate, psilate or verrucate.

All the studied species in this investigation are of restricted distribution. Their importance as allergens for humans has not been studied, however, being rare or scarce, the probabilities of any of them to become a problem seem remote. No previous information about these species has been found in the current palynological literature.

88. FSH AND LH IN VITRO RELEASE FROM GONADOTROPHS OF LAYING HENS, RELATED DOSES AND PULSE-FREQUENCIES OF cGnRH-I

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The effects of pulse-frequencies and doses of cGnRH-I on FSH-LH release and percentage of laying hens' gonadotropes were determined *in vitro*. Pituitary cells' cultures were stimulated with four 5 min-pulses of cGnRH-I 1 or 10 nM every 15, 30 or 60 min, including controls. The supernatants were collected and stored at -70°C until performing an indirect Enzyme Linked Immunosorbent Assay (ELISA) using chLH 1:1000 and chFSH 1:2000 antisera. The FSH-LH cells were identified by indirect immunocytochemical method (ABC-Vector Labs.) applying chFSH 1:6000 and chLH 1:8000 antisera, and quantification was carried out by stereological analysis (VIDA-K-system). Data were statistically processed (ANOVA, Kruskal-Wallis, Scheffé; p < 0.05). FSH was secreted with both doses of cGnRH-I every 15 min and with 10 nM every 30 min, without FSH-tropes percentages changes at any GnRH dose or pulse-frequency. LH was released with 10 nM every 60 min., and significant differences in LH-tropes percentages were seen with 1 nM, 15 vs 30 min (p=0.001), 30 vs 60 min (p=0.013) M±SE: 4.67±2.32%-16.69±1.63%; 16.69±1.63%-7.74±1.67%; with 10 nM, 30 vs 60 min (p=0.028): 14.74±1.28%-7.39±1.32%. High and moderate pulse-frequencies of cGnRH-I (15-30 min.) increased FSH secretion in a dose-independent and dependent manner respectively, without changes in FSH-tropes percentages, while lower pulse-frequencies (60 min) caused LH release in a dose-dependent manner, decreasing LH-tropes percentages.

89. COMPARATIVE MORPHOMETRIC STUDY OF THE MIDDLE EAR BONES OF THE EUPHRACTINI (XENARTHRA, DASYPODIDAE) FROM THE BUENOS AIRES PROVINCE

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A morphometric study of the middle ear bones of adults of *Chaetophractus villosus* (n=14), *C. vellerosus* (n=13) and *Zaedyus pichiy* (n=14), coming from the surroundings of Bahía Blanca, was performed. The bones were extracted by removing the ventral wall of the tympanic bulla. The total length, the head diameter and the manubrium length of the malleus; the total width and the total length of the incus; the total length and the base width of the stapes were measured. Parameters of centralization and dispersion of the measurements considered as well as of the percentages that represent these measurements with respect to the skull length were calculated. Mean values were compared with Least Significant Difference at 5% following simple ANOVA. The results showed that the studied structures are similar in the three species. Notwithstanding the skull of *C. villosus* is the biggest, the middle ear bones are relatively larger in *C. vellerosus*. The present study constitute a valuable contribution for the identification of adaptive features of these burrowing mammals in relation to the sound transmission in underground environments.

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90. SPERM MORPHOLOGY OF SAIMIRI BOLIVIENSIS (PRIMATE: PLATYRRHINI)

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The sperm morphology and morphometry have diagnostic value for the taxonomic determination of different species of mammals. In Eutheria several adaptive models (sexual selection, sperm competition and coevolution of the reproductive biology) have been postulated to enlighten the evolution of spermatozoa. However, these studies are scarce in Primates, as well as a model describing the possible evolution of these characters. In this work, we characterize the sperm morphology of the squirrel monkey *Saimiri boliviensis*. The spreads were performed from testicular biopsies and subjected to differential staining. The proportions measured were (Mean ± ES): total length: 71.39 ± 0.35 µm, head length 5.71 ± 0.02 µm, acrosome length: 3.70 ± 0.02 µm, midpiece length 12.20 ± 0.12 µm and tail length: 65.68 ± 0.12 µm. The midpiece was larger than the one observed by other authors in *A. caraya*, *C. apella* and *A. paniscus*. The total length is larger than *A. caraya*'s, and shorter than *A. paniscus*'s. No significant differences were observed in the length or width of the spermatid head. The tail insertion is central. Methodologies such as gamete cryopreservation, assisted fertilization techniques performed in zoological gardens and research centers, make this parameter a necessary variable for the implementation, in captivity and wild life, of biotechnologies applicable in Biodiversity Conservation Programs.

91. MORPHO-ANATOMICAL CHARACTERS IN PATAGONIAN LEGUMES RELATED TO ITS WATER ECONOMY

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Astragalus cruckshanksii (Hook. et Arn.) Griseb, *Glycyrrhiza astragalina* Gillies ex Hooker et Arnott. and *Adesmia lotoides* Hook. are native legumes of the extrandean Patagonia that vegetate in different environment of it. The extrandean Patagonia is characterized by a high water deficiency due to low precipitation and frequent winds. The permanence of the species in arid and semiarid ecosystems depends on their utilization of the available water. The objective of this work was to study the anatomical and morphological characters related to the water economy *A. cruckshanksii*, *G. astragalina* and *A. lotoides*.

There are many antecedents of the presence of xeromorphic characters in native plants of arid and semiarid regions.

All three species studied have small leaves, thick cuticle and stomata in both side of the leaf with a high density of stomata per unit area. There are glands hairs in all plant organs in *G. astragalina*.

A. cruckshanksii, have adpressed hairs in the abaxial epidermis of the folioles but not in the adaxial epidermis.

The bifolioles of the leaves of *A. lotoides* are covered by peltate hairs that give them a grayish color. The all trichomes studied have also cutinized walls in the basal cells.

The presence of starch and specialized cells with inclusions like crystals and tannin has been determined. We discuss the structure and function of this factors, that would favor the permanence and spread of those species in the region.

92. EQUINE LAMINITIS INDUCED BY SNAKE VENOM FROM ARGENTINA

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Many Elapidae and Viperidae inhabit northeastern Argentina, and the dangerously venomous ones are a serious threat to the wellbeing of both men and animals. *Bothrops* snakes (yará) are the most frequent cause of envenoming and, where horses play an important role in farm activities.

Bothrops venoms have components that induce systemic hemorrhage, coagulation disorders, cardiovascular shock and acute renal failure. Complex local effects, such as hemorrhage, edema and myonecrosis may also be present in the envenomed animal.

The horses involved in this study had this symptomatology and exhibited symptoms consistent with laminitis in the bitten and in the contralateral limb.

Limbs were disarticulated at the metacarpal-phalangeal joint and sectioned with a bandsaw, following the protocol of Pollitt (1996). Tissue blocks of the inner hoof wall were fixed in 10% formalin, dehydrated in alcohol and embedded in paraffin wax. Sections 5 µm thick were cut and stained with hematoxylin and eosin (H & E) and periodic acid Schiff (PAS) reagent and examined with a Leitz light microscope. Laminitis lesions were characterized by separation of the hoof lamellar basement membrane (BM) from basal cells of the epidermis. These results demonstrated that *Bothrops* snake venom can induce acute laminitis. We conclude that components of the venom, probably a metalloproteinase, produces severe lesions in the hoof early in the envenoming process. Antivenom therapy must be initiated as soon as possible in order to prevent complications, not only to save the life of an envenomed horse, but also to avoid the dysfunctional sequela of laminitis.

93. GINGIVAL BLEED INDEX IN PREGNANT DURING THE FIRST QUARTER AND THE THIRD QUARTER OF GESTATION

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Gingival inflammation and hiperplasia wich are frecuently observed in the puberal period, menstrual. Cycle and pregnancy, obey to the increase of circulation of female sexual hormones. Gingivitis increase seriously during pregnancy from the second mouth of pregnant and reach the high level in the eight mouth of pregnant. The increase of movility and depth of pouch. The inflammation appears in the second mouth coincident with the increase of the estrogen and progesterone and agravate in the eight mouth, when the level of both hormones increasses in blood. The gingival inflammation normally occurs with more or less prodigal bleed of gams. The qualitative and quantitative study of gingival bleed was determined by different index which evaluates the grade of seriousness of it. In the present work we proposed to determine the level of gingival bleed between the first and the eoght mouth of gestation to find the period of gratest incidence and gravity of it. Attendingof the objctive we worked with 60 pregnant, 30 of them were in the first quarter of pregnancy and 39 were in the thirt quarter of pregnancy. We used the bleed simplify index with the use of sounds and explorers of the periodontal pouch and gingival hendedura. The technical sounding from mesial and distal through vestibular and from distal and mesial through palatine and lingual. The O degreerrespond to absence of bleed and the 1 degree respond to gingival bleed. From the examined sample we found 66% O grade and 34% 1 grade for the first quarter of pregnancy.

15% O grade and 85% 1 grade for the third quarter of pregnancy. We concluded that the gingival bleed increasses in considerate levels from the first to the third quarter of pregnancy.

94. EPICUTICULAR WAX MORPHOLOGY OF SOYBEAN LEAVES UNDER WATER DEFICIENCY

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Our objective was to determine the morphological changes of epicuticular waxes in leaves of soybean plants grown under high and low irrigation conditions (HI and LI, respectively). The water status of the plant (leaf water potential, stomatal conductance and leaf relative water content) was significantly higher in plants grown in HI than LI-soils. LI treatment resulted in a significant decrease in the dry weight of the stems and leaves which was correlated with a significant decrease in leaf area in comparison with HI-plants. Scanning electron microscopy analysis showed that the epicuticular wax film consisted of an amorphous deposit with irregularly edged plates, arranged perpendicular to the leaf surface. These plates were uniformly distributed over the entire surface of the leaves. The wax film of the abaxial leaf plane consisted of deposits of a material of similar fine structure but much denser in LI than in HI plants. The total wax content per unit leaf area increased almost 3 times in LI compared with HI plants. The wax composition also changed, since all components augmented, the most hydrophobic constituents increased the most. Among these were the very long odd-chain alkanes (C25 to C31). The chemical alteration in the leaf wax content and composition may represent physiological-adaptive responses of soybean plants to water stress.

95. HISTOLOGICAL CHANGES TILL 18 MONTHS OF AGE IN TESTIS AND EPIDIDYDIMIS FROM OBESE, DIABETIC RATS

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We report epididymal and testicular histology in beta line rats (obese, late diabetes) at different ages.

Male, obese and late diabetic beta line rats were sacrificed with ether overdose. 4-5 rats from 2,4,8,12,18 month old rats: testes and epididymis were removed and weighed. Testes were fixed in Bouin's fluid and refixed in 4% formaldehyde in PBS. One epididymis were fixed in 4% formaldehyde in PBS, the other processed for spermatozoal count. Samples were histological processed. Until the 8th month, testicular and epididymal histology were quite similar to other "control" rats. After 12 months alterations of testicular germinal epithelia could be described. This findings were even worse at 18 months. Spermatozoal count and epididymal histology correlated with testicular changes. At 18 months spermatozoal count were separated in two groups. Epididymal histology could be described with several degrees of alterations, some of them included lost of tubular images, with atrophic epithelia without tubular content. Beta rats is a line of obese and diabetes onsets at 8 months of age. In spite of these facts, testicular alterations appear earlier than in other diabetic rats.

96. OXIDATIVE STRESS IN A MOUSE MODEL

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Mean erythrocyte life depends on processes affecting its cellular metabolism and functionality. Oxidants such as Phenylhydrazine (PHZ) induce phenomena characterized by lipid peroxidation, Hb denaturalization, and presence of phagocytosed Heinz bodies, mainly by splenic macrophages. The determination of the minimum effective dose of oxidative stress inducing PHZ in mice Strain CF1 was proposed in order to understand medullar and splenic function in hemolytic anemia. Mice CF1 (43±3g) were grouped into: **G1-CONTROL**: intraperitoneal (ip) physiological solution/300µL (Days:0,2 of the protocol) (n=9); **G2-EXPERIMENTAL**: PHZ ip between [0.5-24.8mM] /300µL (Days:0,2) (n=72). Samples was obtained from G1 and G2 (Days:0,4,6) (n=3). Anemia Evolution and Recovery: Hb and reticulocytes. Erythrocyte Oxidation degree: Heinz bodies by optical microscopy. Splenic Phagocytosis: Scanning electron microscopy. Medullar Erythropoiesis Magnitude: Optical microscopy. Statistical significance was set at $P<0.05$. Mice received free food and water. The [PHZ] dose-response curve vs. Hb/Reti/Heinz bodies showed: 1)[0.5-1.5mM] (Day:4): lack of anemia; 2)[2.5-24.8mM] (Day:4): the degree of anemia was directly correlated to [PHZ]. The hemolytic crisis took place on Day:4 [PHZ:24.8mM] (Hb:9.8g/dL±0.9). Doses [2.5-8.2mM] induced mild anemia (Hb:10.8-13.4g/dL) Reticulocytes showed erythropoietic recovery (Day:6):46.6%±3.0 [PHZ:24.8mM] and 18.62%±3.9 [PHZ:8.2mM], regarding the control group (1.5%±0.5) ($P<0.05$). The increase in Heinz bodies was parallel to [PHZ] (Day:4): 96.0%±2.8 [24.8mM] and 72.5%±2.38 [8.2mM] regarding non-hemolytic doses and G1. Medullar Quantification (Day:4): 1)Mild Anemia:16.5-20.5cel.x10⁶/g; 2)Marked Anemia:5.4-11.9cel.x10⁶/g; 3)CONTROL:8.5-11.5cel.x10⁶/g ($P<0.05$) The degree of anemia depends on PHZ, the bone marrow assumes active erythropoiesis in mild anemia but not in marked anemia, in which the spleen compensates volemia. Cellular indicators of oxidative damage efficiently valued the process with the presence of Heinz bodies and their splenic phagocytosis.

97.

ERYTHROPOIESIS IN A MAMMAL MODEL*Veuthey TV, Gatti CJ, D'Anna MC, García B, Pennacchiotti G, Roque ME.**Fisiología Humana. Biología, Bioquímica y Farmacia. Univ. Nac. del Sur. Bahía Blanca. Argentina. E-mail: mroque@uns.edu.ar*

The murine spleen restores volemia after hemolysis and there is evidence on the contribution of the liver. The erythropoietic activity of the kidney, the erythropoietin producing organ, remains unknown. The purpose of our work was to study the renal and hepatic erythropoietic involvement assessing the cellularity characterizing erythron recovery. Mice CF1 (43±3g) were grouped into: **G1-CONTROL** (n=9): 300µL intraperitoneal (ip) physiological solution (Days:0,2); **G2-EXPERIMENTAL** (n=9): Phenylhydrazine ip (60mg/kg/300µL) (Days:0,2). In G1 and G2 retroorbital blood was obtained (Days:0,4,7) (n=3). Anemia evolution and recovery were determined by Hb, HCT, and brilliant cresyl blue reticulocytes. The animals were euthanized by decapitation (days:0,4,7). Tissue dissection was performed by abdominal incision and then fixed using Bouin solution and 10% formalin and processed for H&E. Statistical significance was set at $P<0.05$. Mice received free food and water. Erythropoietic cellular islands were semi-quantified by a pre-established score (10xfields): a)0-2 islands/10fields:+; b)3-7 islands/10fields:++; c)8-10 islands/10fields:+++; d)>10 islands/10fields:++++. Renal and Hepatic Erythropoiesis: Transmission Electronic Microscopy (TEM). Mean values (±DE) for G1 and G2 (Day:0) were: Hb (15.5±0.7g/dL), HCT (48±2.5%), and reticulocytes (2.5±0.6%). In G2 (Day:4) Hb (9.8±0.9g/dL), HCT (31±2.0%), and reticulocytes (8.0±2.6%) showed acute hemolysis. While on Day 7, a clear compensation of anemia was observed: Hb (14.9±0.6g/dL), HCT (45.5±2.5%), and reticulocytes (46.6±3.0%) ($P<0.05$). G1 values remained unchanged during the experiment. The score showed: 1)G2-KIDNEY: Day0:+; Day4:+++; Day7:++++; 2)G2-LIVER: Day0:+; Day4:+++; Day7:++++; 3)G1-LIVER-KIDNEY (Days:0,4,7):+. TEM showed undifferentiated erythrocytic cells. The presence of islands with erythropoietic morphology indicates that in the acute hemolytic crisis in the kidney and liver they undertake a compensating erythropoietic role, with a different degree of involvement.

98.

ULTRASTRUCTURAL STUDY OF THE NERVOUS SYSTEM OF PSEUDOCORDODES BEDRIAGAE*Zanca F, de Villalobos C, Restelli M.**Facultad de Ciencias Naturales y Museo UNLP Paseo del Bosque S/N. La Plata, Argentina. E-mail: marestelli@speedy.com.ar*

The central nervous system of the Gordiida is formed by a ventral nervous cord and a ganglion in the posterior region of the body, the cloacal ganglion. The ventral nervous cord runs along the whole body and is located in a ventral cavity, the perintestinal cavity. It is connected to the epidermis through the neural lamella. As there are few previous studies on the microanatomy of this system, the aim of this paper is to describe the ultrastructure of the central nervous system of *Pseudochordodes bedriagae*. Six specimens of this species were collected in El Negro stream in Sierra de la Ventana. Transversal cuts 0.05 to 1 mm wide were made of the anterior, medial and posterior region of the body. They were fixed in 2% glutaraldehyde 0.1M cacodylate buffer, included in araldite; the ultrafine cuts were stained with uranyl acetate-lead and observed with transmission electronic microscope. The ventral nervous cord as well as the neural lamella are formed by "neuropile", that is to say exclusively by cellular projections. The caudal ganglion is formed by neuronal and neuroglial cells. The neurons are big with irregular nuclei, with lax chromatin and a clear cytoplasm, rich in vesicles and organules. The neuroglial cells present irregular nuclei with perinuclear chromatin and electron cytoplasm, dense and rich in microfilaments with scarce organoids.

99.

MORPHOLOGIC AND MORPHOMETRIC STUDIES OF PANETH CELLS IN *Solanum glaucophyllum* INTOXICATED RABBITS*Zanuzzi CN^{1,2,3}, Fontana PA¹, Portiansky EL^{1,3}, Barbeito CG^{1,2,3}, Gimeno EJ^{1,3}.**¹Institute of Pathology; ²Department of Histology and Embryology. School of Veterinary Sciences. UNLP. 60 y 118. (1900) La Plata. Buenos Aires. Argentina. ³Members of CONICET. E-mail: carozanuzzi@fcv.unlp.edu.ar*

Paneth cells (PC), located at the bottom of the small intestinal glands, secrete antimicrobial peptides. PC can react to different aggressors by altering their number, form, localisation, and its granule's composition and morphologic characteristics. The 1,25 dihydroxvitamin D₃ enhances antimicrobial peptide synthesis and the exocytosis from diverse secretory cellular types. The goal of this work was to determine PC's morphologic and morphometric changes in *Solanum glaucophyllum* (Sg) intoxicated animals, during 15 and 30 days of intoxication. Jejune samples were obtained from 5 *New Zealand* rabbits of each group (control and intoxicated), embedded in paraffin and stained with HE. Sixty PC per animal were morphometrically analysed. Cell area, aspect, major and minor axis, perimeter and roundness were evaluated. Statistical differences were observed in the three analysed groups. Control group PC have a mean area of 89.66 µm², pyramidal shape, not well-established cellular limits and a heterogeneous amount of eosinophilic granules. The PC area raised to 126,85 and 181,76 µm² in 15 and 30 days intoxicated rabbits, respectively. PC had a spherical shape, slightly stained cytoplasm, more eccentric nucleus and well defined cellular limits. PC changes of intoxicated rabbits could be attributed to the antimicrobial peptide synthesis, induced by 1,25 dihydroxvitamin D₃ contained in Sg.

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