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C. 1.

INFLUENCE OF MATURATION IN THE BLOCK TO POLYSPERMY IN AMPHIBIANS

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In the fertilization process, the response of the oocyte to contact with sperm is known as activation and includes a series of events such as block to polyspermy, termination of meiosis II, extrusion of the second polar body, pronuclei formation, DNA replication and reorganization of the cytoskeleton. The process of activation starts a development program that will lead to the formation of a new individual.
In amphibians, the female gamete acquires competence to activate and start development during maturation, the period in which the nucleus reaches metaphase II of meiosis and the cytoplasm undergoes important morphological and biochemical changes.
The physiological maturation inducer in amphibians is progesterone, which acts at the level of the plasma membrane and starts a cascade of signaling events that lead to the activation of a cytoplasmic factor, the maturation promoting factor (MPF). The MPF is common to many species, although the way in which it originates and its mechanisms of activation differ considerably among them. The MPF is made up of a catalytic subunit produced by cdc2 and a regulatory unit, cyclin B. In some species such as *Xenopus* and *Bufo arenarum*, the MPF is present in immature oocytes in an inactive or pre-MPF form. The MPF is responsible for nucleus or germinal vesicle breakdown, chromatin condensation, and spindle formation.
In *Bufo arenarum*, the gradual increase infollicle stimulating hormone (FSH) that occurs during the reproductive period induces a change in the oocyte oxidative metabolism, decreasing oxygen consumption and increasing the synthesis of nucleic and fatty acids precursors. In this species we can find fully grown oocytes the whole year round. However, these oocytes differ in their biochemical behavior, and only oocytes with a biochemically mature cytoplasm are able to undergo spontaneous nuclear maturation when deprived of their follicles.
Most anuran amphibians present monospermic fertilization, so that the oocyte develops polyspermy prevention mechanisms with two phases or stages, a fast transitory one in which the oocyte plasma membrane is involved, and another slow and permanent that involves cortical granules and vitelline envelope. The ability to establish a block to polyspermy has been directly related to the acquisition of nuclear maturation; however, the denuded oocytes of *Bufo arenarum* matured *in vitro* present a high index of polyspermy when they are inseminated. This indicates that in this species nuclear maturation is a necessary but not sufficient condition for the block to polyspermy to be effective.The analysis of the structures related to the slow block to polyspermy in denuded *Bufo arenarum* oocytes matured *in vitro* indicates that cortical granules exocytosis occurs in a way similar to that of oocytes matured *in vivo*; the vitelline envelope, however, only becomes a fertilization envelope when pretreated with trypsin. Interestingly, oocytes matured *in vitro* inside the follicles acquire the capacity to block supernumerary sperm entry. However, the nature of the influence of the follicle in the process is still unknown.

C. 2.

TWO FACES OF THE MAST CELL

Conference Sociedad de Biología de Cuyo: Dra Alicia B. Penissi

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Mast cells are multifunctional and specialized secretory cells found widely distributed in the body, particularly associated with connective tissues. They originate from precursors in the bone marrow and migrate to peripheral tissues where they mature. These cells release, in response to activation by external stimuli, a variety of biologically active mediators. Mast cells have long been recognized as key cells of type I hypersensitivity reactions. Several lines of evidence, however, indicate that they not only express critical effector functions in classic IgE-associated allergic disorders, but also play important roles in host defense. Moreover, there is growing evidence that mast cells exert distinct non-immunological functions, playing a relevant role in tissue homeostasis, remodeling and fibrosis as well as in the processes of tissue angiogenesis. The identification of therapeutic agents for modulating mast cell function has been the focus of many laboratories for more than two decades. Selective stabilization of mast cells may represent a new approach in the treatment of mast cell-mediated diseases.

C. 3.

GENE EXPRESSION CONTROL AND ONCOGENESIS: FUNCTION OF THE KRUPPEL-LIKE TRANSCRIPTION FACTOR KLF6

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The transcription factors called "Krüppel-like factors" (KLFs) represent one of the most diverse set of regulators in vertebrate organisms. These molecules belongs to a family of GC-rich binding proteins characterized by the presence of three Cys2/His2 zinc fingers DNA-binding domains, which is the most abundant motif seen in transcription factors encoded by the human genome. Recent data indicate that an increasing number of Krüppel-like family members are involved in the control of cell proliferation and differentiation.

A specific protein of this family, KLF6, was identified in our laboratory on the basis of its properties to regulate transcription of several genes such as those encoding the Pregnancy-Specific Glycoproteins (PSG). Several independent studies reported that mutations in the KLF6 gene are associated with the development of different human malignancies like prostate carcinoma, glioblastoma and colorectal cancer, suggesting that KLF6 is the product of a tumor suppressor gene. A proposed function for the wild type form of KLF6 is the direct binding and activation of the $p21^{(CIP1/WAF1)}$ gene promoter in a p53-independent manner, leading to inhibition of the cell cycle.

Results obtained in our laboratory are consistent with a novel mechanism of KLF6 as an inhibitor of cellular proliferation counteracting the function of the c-Jun proto-oncogene product. The mechanism by which KLF6 produces these effects correlates with induction of the c-Jun protein degradation by the ubiquitin-proteasome pathway. All the effects of KLF6 on c-Jun were largely dependent of external cell stimulation mediated by phorbol esters tumor promoter. These stimuli modify KLF6 phosphorylation, promote its translocation from the cytoplasm to the nucleus and enhance KLF6 transcriptional activity.

In addition to the interaction with cJun, which is a cellular proto-oncoprotein, KLF6 is also targeted by a viral oncoprotein such as E1A encoded by *Adenovirus*, further supporting a KLF6 function in cell proliferation control.

Endogenous KLF6 protein level showed changes during the course of infection of human lung epithelial cells and was recruited *in vivo* for DNA-binding to the early E2A *Adenovirus* promoter according to Chromatin Immunoprecipitation and Real-time PCR approaches. Interestingly, we determined that KLF6 interacts physically with ATF7 whose role in the E2A transcriptional activation has been demonstrated.

The interactions of KLF6 with both c-Jun and E1A oncoproteins in the modulation of cell proliferation and its eventual impact in oncogenic transformation are relevant biological features that highlight its potential function as a tumor suppressor gene product.

1.

PARTICIPATION AND REGULATION OF CAVEOLIN-1 EXPRESSION IN THE PROGESTIN-DEPENDENT MURINE MAMMARY TUMOR C4HD

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We have previously demonstrated using the murine mammary tumor line C4HD that caveolin-1 (cav-1) expression is induced both *in vivo* and *in vitro* by the synthetic progestin medroxiprogesterone acetate (MPA) and that this regulation involves the classical progesterone receptor (PR). C4HD tumor is a progestin dependent mammary adenocarcinoma which expresses high levels of PR. At the present study we evaluated whether MPA was able to positively regulate cav-1 expression at transcripttional level in C4HD cells as well as the signaling pathway involved in the regulation of cav-1 at protein level. We further evaluated the capacity of MPA to induce cav-1 phosphorylation and determined the protein kinase responsible for this phosphorylation. Finally, we analyzed if cav-1 is involved in the proliferating activity induced by MPA on C4HD cells. Firstly, we transfected C4HD cells and human breast cancer T47D cells with a luciferase reporter vector under the transcriptional control of the mouse cav-1 promoter and we showed that MPA promoted strong cav-1 transcriptional activation (C4HD: 2,5 fold, p<0,001, T47D: 5 fold, p<0,001). In both cases the effect was prevented by the PR antagonist RU486. We additionally showed that MPA regulation of cav-1 expression involved the activation at least of two signaling pathways: MAPK and PI-3K. Short term MPA incubation of C4HD cells led to tyrosine phosphorylation of cav-1 protein, being Src the kinase responsible for this phosphorylation. Finally we showed, using antisense oligodeoxinucleotides to cav-1, that MPA-induced C4HD cells proliferation was significantly reduced as a conesquence of the suppression of cav-1 expression, demonstrating that cav-1 is a downstream effector of MPA that is responsible in part for the growth stimulation of C4HD breast cancer cells.

2.

BCL-X_L AND EPO-R INDUCTION DURING ERYTHROID DIFFERENTIATION ON BONE MARROW CELLS INJURED BY TAXOL CORRELATES WITH DELAYED APOPTOSIS

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The long form of B-lymphoma-x (Bcl-x_L), an outer mitochondrial protein, has been proposed to mediate the antiapoptotic action of erythropoietin (Epo) on erythroid progenitor cells.
We investigated in a time course study (1-10 days) Bcl-x_L and EPO-R induction on murine bone marrow (BM) cells after a single dose of Taxol (Tx) treatment (29 mg/Kg i.p). These expressions were compared to those evaluated after *"ex vivo"* Epo stimulation (BM cultures with 2 UI/ml Epo for 2 h, 37ºC, 5%CO_2). Bcl-x_L and EPO-R expressions (westernblotting) were correlated with total erythroid cells (x10^6/femur) and hemoglobin-synthesizing erythroblasts (%Fe^{59} uptake). On the 1st day post-Tx, BM erythroid cells fell 4 times compared to control (p<0.001), remained decreased until the 7th day (p<0.05) and returned near to normality by day 10 post-Tx. ^{59}Fe incorporation on hemoglobin-synthesizing erythroblasts post-Tx treatment revealed less isotopic uptake than control between 1 to 5 days (p<0.01). However, % ^{59}Fe uptake returned to normality from the 7th day until the end of the experience. Epo rh *"ex vivo"* treatment of BM cells caused overexpression of the EPO-R and the apoptotic supressor protein, Bcl-x_L between 7 to 10 days (p < 0.01) whereas it remained under control values from 1 to 3 days.
These results suggest that Bcl-x_L and EPO-R does not mediate the antiapoptotic effect of Epo, but it prevents ineffective erythropoiesis due to apoptosis in late-stage, hemoglobin synthesizing erythroblasts.
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3.

EXPRESSION OF FAS (CD95/APO-1) RECEPTOR SYSTEM ON HEMATOPOIETIC APOPTOSIS INDUCED BY TAXOL

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CD 95 (Fas/APO-1) system regulates several physiological and pathological processes of cell death. The aim of this work was to evaluate CD95 expression (immunoblotting) in a time-course study on hematopoietic recovery (0–10 days) using a murine model following a single dose of Taxol (Tx, 29 mg/kg i.p) in bone marrow (BM) cells with or without "ex vivo" human recombinant erythropoietin stimulation (Epo rh 1 UI/ml). Variations of CD95 expression were correlated with BM cellularities and apoptotic indexes (TUNEL assay). We noticed, on the 1st day post Tx, the maximal apoptotic index (24 ± 0.81 % p<0.01) and the minimal BM cellularity (28 ± 4.2 % under control p<0.001). Apoptosis returned to normal values (3.08 ± 0.61%) by the 3rd day, while BM cellularities decreased until the 4th day and started to recovery from day 5 post Tx.
Up regulation of the cell death receptor expression (CD95/Fas) was significantly noticed between the 1st and 2nd days (p<0.01 over control values). However, this expression decreased from the 3rd until the end of the experience. Evenmore, CD95/Fas patterns did not change with Epo rh *"ex vivo"* stimulation.
These results suggest that Tx changed CD95 receptor expression during hematopoietic recovery. These variations are directly correlated to hematopoietic cells prolixferation. Moreover, Epo rh failed to cause changes in the pattern of CD 95 expression, suggesting that once apoptosis has been triggered, the addition of this hormone did not modify the course of apoptotic hematopoietic recovery.
This work was supported with CONICET and SEGCyT-UNNE Grants.

4.

PROTEIN-TYROSINE KINASE (TK) PARTICIPATION ON INTRACELLULAR CALCIUM MOBILIZATION IN PITUITARY GH3 CELLS

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The GH3 clonal cell line, which originated from a rat pituitary tumour, secretes GH and PRL. It was shown to contain spontaneous Ca^{2+}-dependent action potentials due to the presence of voltage-sensitive Ca^{2+} channels (preferably L-type) in plasma membrane. The activity of these channels is critical in secretory events of endocrine cells. There are several studies in different tissues, on L-type channel regulation by the ser-threo kinases PKC and PKA. Less is known about such regulation by tyrosine kinases (TKs). The aim of our study was to evaluate the participation of kinases and specifically TKs on the activity of Ca^{2+} channels in GH3 cells. Intracellular calcium levels ($[Ca^{2+}]_i$) were measured second by second using a spectrofluorometric method with Fura-2/AM as a fluorescent indicator and KCl 12.5mM as a depolarization stimulus which enhances $[Ca^{2+}]_i$.
Chelerytrine (1uM, a PKC inhibitor) prestimulus produced a potentiation in $[Ca^{2+}]_i$ rise induced by KCl. By contrast 10uM H89 (a PKA inhibitor) produced the opposite effect.
25uM Genistein (a TK inhibitor) decreased and 50 nM okadaic acid (a phosphatase inhibitor) raised the $[Ca^{2+}]_i$ evoked by K+ suggesting a positive TK participation on Ca^{2+} channel activity (average increase over basal levels (%) achieved during 1 min after K+ (area):genist: 4192+166 , buffer: 5121+308, p<0.02; OK: 5930+235 , buff 5317+126 , p< 0.02). The activation of receptor TKs with EGF and VEGF potentiated the Ca^{2+} influx induced by K+, and AG1478 (a REGF inhibitor) decreased it.
The present results suggest that in GH3 cells PKA and TKs activities cause an increase of L-type Ca^{2+} channel activation. Apparently REGF may be involved in this effect.

5.

EXPRESSION OF PLACENTAL GROWTH FACTOR AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN OVARIAN TERATOMAS OF TRANSGENIC MICE HYPERSECRETING HUMAN CHORIONIC GONADOTROPIN

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Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally synthesized by the human placenta. It is structurally related to the luteinizing hormone, and acts through the same receptor. We have demonstrated that hCG overexpression induces the development of ovarian teratomas in transgenic mice (TG). These tumors are composed of disorganized tissues derived from embryonic and extraembryonic cells. Angiogenic factors are known to promote the formation of blood vessels, necessary to feed and maintain tumor growth. The placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family that has been shown to play an important role in promoting angiogenesis. Besides inducing its own signaling in endothelial cells, PlGF exerts its angiogenic action by synergising with VEGF. The objective of the present study was to analyze gene expression and immunolocalization of VEGF and PlGF, by RT-PCR and immunohistochemistry in wild-type (wt) ovaries and TG ovarian tumors. The relative gene expressions of VEGF and PlGF were significantly increased in TG tumors compared with wt ($p<0.05$). Immunohistochemical analyses demonstrated that these factors were localized in the remnant ovarian tissue, and in the trophoblast giant cells present in the teratomas, which are typically found in the mouse placenta. In conclusion, the present results suggest that the trophoblast giant cells of the teratomas would contribute in promoting the angiogenic process in these tumors, through the expression of PlGF and VEGF.

6.

THE EXPRESSION OF iNOS AND COX-2 ARE MODIFIED BY VITAMIN A DEFICIENCY IN RAT HEART

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We have previously reported that vitamin A deficiency induces alterations of lipid metabolism and antioxidant enzyme activities in rat heart. The aim of the present *in vivo* study was the evaluation of oxidative stress and inflammation parameters in heart. Wistar male 21 days old rats were fed during three months with free vitamin A diet (-A) and the same diet plus 8 mg of retinol palmitate/kg of diet (+A), respectively. Also, a group of eight deficient rats was fed with the control diet 15 days before sacrifice. Vitamin A deficiency was confirmed in plasma and liver by HPLC. Serum nitrites were determined by the Griess reagents. The nitric oxide synthase (iNOS) and ciclooxygenase-2 (COX-2) expressions were determined by Western Blot Analysis using polyclonal antibody solutions (Santa Cruz Biotechnology). Rabbit anti- β-actin polyclonal antibody was used as a control for protein loading. A Vectastain ABC – detection system was used for color development. Results were analyzed by one-way ANOVA. In –A rats the NO production increased in serum (8.1 ± 0.3 vs 16.9 ± 1.2 μmol $NaNO_2$/mg protein, $p< 0.001$) compared with +A rats. In heart of –A rats, the expression of iNOS and COX-2 increased ($p< 0.05$) in relation to +A. Vitamin A refeeding in –A rats normalized the serum NO levels, and iNOS and COX-2 expression to control values. The coordinated induction of both enzymes, iNOS and COX-2, can be a cellular effect resulting from redox changes induced by the vitamin A deficiency under the oxidative stress conditions. The incorporation of vitamin A to the diet of -A rats considerably improves the redox and inflammatory changes. Vitamin A has potentially beneficial effects on heart, which are related to its antioxidant protection.

7.

GROWTH OF THE HUMAN BREAST CANCER CELL LINES IBH-4, IBH-6 AND IBH-7 IN NUDE MICE

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We have developed in our Lab three human breast cancer cell lines which were sensitive to estrogen, progesterone and EGF. The aim of this work was to study their growth in nude mice. 1-2x10^7 cancer IBH-4, IBH-6 or IBH-7 cells were injected sc into female athymic nude female mice (N:NIH(S)-nu). Mice were treated with estradiol (0.5 mg) pellets, MPA (20 mg) depot or both. Tumors were measured with a Vernier caliper and tumor volume was calculated using the formula: $4/3\pi$*minor radius^2*main radius. The three cell lines proved to be tumorigenic. IBH-4 tumors formed poorly differentiated solid carcinomas, with fusocellular components and grew similarly in hormone-treated or untreated mice. They were highly vascularized and had a high mitotic index. The volume reached after 30 days, in absence of hormones, increased with the successive passages (p.) 2668 ± 290 (1º p.), 9056 ± 1035 (2° p.) and 7524 ± 1637 (3° p.) mm^3, $p<0.001$. Multiple lung metastases were observed in some mice.
IBH-6 tumors were poorly differentiated sarcoma-like tumors with a high mitotic index. Tumor size was greater in estradiol-treated mice. The volume reached after 30 days was higher after they were passed *in vivo* than when inoculated from cell cultures. The tumors frequently invaded the peritoneum and neighboring tissues. Lung, pancreas, lymph nodes and diaphragm metastases were observed.
IBH-7 carcinomas only grew in E2-treated mice. Tumors were ductal carcinomas showing slow growth. Metastases were observed in lung and uterus.
All tumors were cytokeratin positive and vimentin negative.
We conclude that the malignant IBH-4 and IBH-6 cell lines have a different sensitivity to hormones when growing in nude mice and when tested *in vitro*, whereas the IBH-7 cell line conserved *in vivo* the estrogen dependence.

8.

MAPK ACTIVATED BY HEREGULIN (HRG) AND BY THE SYNTHETIC PROGESTIN MEDROXYPROGESTERONE ACETATE (MPA) ARE ABLE TO PHOSPHORILATE STAT3 ON SER-727 IN BREAST CANCER CELLS

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We have recently demostrated that HRG, ligand of the type I receptor tyrosine kinases (RTKs), activates p42/p44 MAPK. We performed this study in an experimental model of hormonal carcinogenesis in which MPA induced mammary adenocarcinomas in female Balb/c mice. It is also well kown that HRG activates MAPK in the human breast cancer line T47D. Here we explored the capacity of HRG- and MPA-activated MAPK to modulate Stat3 serine 727 phosphorylation in C4HD and T47D cells. Although it is well kown that Stat3 phosphorylation on serine 727 is essential for its transcriptional activity, neither the mechanisms nor the kinases involved are known. We found that treatment with 20 ng/ml HRG or 10 nM MPA for 5 to 10 min induced serine 727 Stat3 phosphorylation, by performing western blots with the specific anti-phospho serine-727 Stat3 antibody. Blockage of MAPK activity by preincubation with the specific MAPK inhibitor U0126 (10 μM), inhibited not only the serine 727 Stat3 phosphorylation but also Stat3 transcriptional activation. We demonstrated that MAPK immunoprecipitated from HRG- or MPA-treated cells, induced serine 727 Stat3 phosphorylation in breast cancer cells, using an *in vitro* unradioactive phosphorylation assay.
Our findings show, for the first time, that HRG- and progestins-activated MAPK are able to phosphorylate Stat3 on residue 727 *in vivo* and *in vitro* in breast cancer cells. In addition, we found that MAPK-induced serine 727 phosphorylation of Stat3 is a requisit for Stat 3 transcriptional activity in breast cancer cells.

9.

IN VIVO ACTION OF AN α_2-ADRENERGIC AGONISTS AND ANTAGONISTS IN EXPERIMENTAL BREAST CANCER

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We have already described that α_2-adrenergic agonists enhance the proliferation of human and murine breast cancer cells *in vitro* at very low concentrations. They also stimulate the growth of several murine progestin-dependent tumors. The aim of this study was to evaluate the effect of α_2-antagonists *in vivo* using a murine mammary carcinoma and a human breast cancer cell line growing in nude mice.
CC4-3-HI tumors (progestin-independent mammary carcinomas) were inoculated s.c. in BALB/c female mice. After 24 hours they were separated randomly in 4 groups (n=x/gropu) and treated with clonidine (0.1 mg/kg daily), or rauwolscine (0.5 mg/Kg daily) or clonidine plus rawolscine or with vehicle. Tumors were measured daily. The specific α_2-adrenergic agonist clonidine significantly stimulated CC4-3-HI tumor growth, as compared with control group; on day 28: 2339,4 ± 293,7 mm^3 *vs.* 1299 ± 93,1 $p<0.05$. Rauwolscine, a specific antagonist, alone, reduced tumor growth (800,5 ± 218,8 mm^3, $p<0.05$), and combined with the agonist reverted its stimulatory effect (1011,1 ± 206,8 mm^3).
The IBH-4 human breast cancer cell line was inoculated in sixteen nude mice, and after 24 hours the animals were treated with clonidine or vehicle. Clonidine showed a stimulatory effect in (on day 23: 13419,6 ± 909,4 vs 6868,7 ±1369,3 mm^3, $p<0.05$). These results suggest that the α_2-adrenergic antagonist rauwolscine could be used to inhibit breast cancer growth.

10.

APPLICATION OF BORON NEUTRON CAPTURE THERAPY (BNCT) FOR THE TREATMENT OF UNDIFFERENTIATED THYROID CANCER (UTC) USING A COMBINATION OF TWO BORON COMPOUNDS

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BNCT combines the selective uptake of certain boron compounds with the irradiation of the tumor with a neutron beam. The ^{10}B is converted into ^{11}B, which releases an α particle and a nuclei of ^{7}Li, with a high linear energy transfer (LET), killing the cells located within a 10 nm radium. UTC is a very aggressive tumor which does not respond to standards therapies. Previous studies have shown that a human UTC cell line (ARO) has a selective uptake of p-borophenylalanine (BPA) and that mice transplanted with ARO cells had a 50% cure rate when they were treated by BNCT. When BPA is combined with a boronated porphyrin (BOPP) the amount of boron in the tumor increases (45 ppm vs 24 ppm). ***Aim:*** to explore the response to BNCT when the two boron compounds are administered. ***Materials and Methods:*** Nude mice were transplanted with the ARO cells and after 14 days the animals were distributed into the following groups: 1) untreated controls; 2) NCT: treated with the neutron beam only; 3) BPA (350 mg/kg bw) + irradiation; 4) BPA+BOPP (60 mg/kg bw) + irradiation. The mice were irradiated (RA6, Bariloche) with the neutron beam 83 min, corresponding to a fluence of 1.6×10^{12} n/cm^2. Tumor volume was measured during one month after irradiation. ***Results:*** Control group showed continuous growth of the tumor, while NCT had a partial delay; BPA and BPA+BOPP showed a complete stop of growth in 100% animals. When initial tumor volume was <50 mm^3 complete regression was observed in 1/4 mice from group BPA and 7/7 from group BPA+BOPP. ***Conclusions:*** the simultaneous use of the two boron compounds improves the efficacy, opening a new possibility for the treatment of UTC.

11.

PROLACTIN CONCENTRATION IN CONDITIONED MEDIA FROM HUMAN BREAST CANCER CELL LINES

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In rodents, prolactin (PRL) is involved in mammary gland carcinogenesis. In humans, recent work suggests a potential autocrine role of PRL because of its mitogenic effect, and its local synthesis. Although PRL release by the T47D human breast cell line has been reported, the very low amounts of this hormone released by other human breast cell lines was precluded by sensitivity of the assays. We used the Nb2 bioassay because it shows a high sensibility for PRL allowing measurement of tiny amounts of the protein in the conditioned media of other human breast cell lines.
We analyzed the PRL released to the medium by the human breast cancer lines T47D, MCF-7, IBH-4, IBH-6 and IBH-7. Cells were maintained in culture with DMEM-F12 supplemented with FCS 10%. The media was replaced by media without FCS with or without 10 nM E_2 at near confluence. The conditioned media were measured after 48 hs treatment. Nb2 bioassay was performed with the conditioned media using ovine PRL as standard. After two day of incubation, the cells were treated with [^{3}H]-thymidine and harvested after 24 hs.
All the cells lines used for the bioassay showed capability for the synthesis and release of PRL to the conditioned media. The E_2 upregulated the PRL release in T47D (5.574 ± 2.39 vs. 0.496 ± 0.171 ng, $p<0.0479$) and MCF-7 (33.91 ± 7.60 vs. 12.7 ± 0.967 pg, $p<0.0201$) cell lines. The opposite effect was found in IBH-4 (0.127 ± 0.0253 vs. 0.368 ± 0.0524 ng, $p<0.0004$) and with no change in IBH-6 cell line (2.421 ± 0.341 vs 1.984 ± 0.402 ng, NS).
It can be concluded that PRL is released in several human breast cancer lines, but the regulation by estradiol does not follow a common pattern.

12.

DISTINCT EPITHELIAL CADHERIN PROTEIN EXPRESSION PATTERN IN HUMAN BREAST CANCER CELL LINES IBH-4 AND IBH-6

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Epithelial cadherin (E-cad) is a 120 KDa membrane glycoprotein that participates in Ca^{2+}-dependent cell-cell adhesion (1). Aberrations in E-cad (i.e. decreased gene expression, presence of mutations, protein processing) have been associated with increased tumor malignancy and invasiveness in cancer cell lines *in vitro*. This study was aimed at evaluating E-cad protein expression analysis in IBH-4 and IBH-6 human breast cancer estrogen and progesterone-sensitive cell lines developed from patient's primary tumors (2), and compare it with that obtained with the MCF-7 cell line. **Materials & Methods:** Total protein extracts were prepared in Laemmli sample buffer from IBH-4, IBH-6 and MCF-7 cell cultures and from 2 days-Serum free (SF)-conditioned medium. E-cad protein forms were identified by Western immunoblotting using antibodies towards three different E-cad domains. **Results:** As previously reported, the whole protein and the soluble E-cad ectodomain were identified in MCF-7 cell extracts and SF-conditioned medium, respectively. Contrasting, very low levels of full-length E-cad were detected in protein extracts from IBH-4 and IBH-6; moreover, two truncated isoforms of 89 and 82 KDa were identified, both lacking the intracellular epitope, suggesting a different C-terminal end. In IBH-4 and IBH-6 SF-conditioned media, soluble E-cad could not be detected even after 20 times medium concentration. **Conclusion:** A distinct E-cad expression pattern was identified in IBH-4 and IBH-6 human breast cancer cell lines compared to MCF-7. Changes could result from alterations in post-transcriptional and/or post-translational processing, and may be related to the invasive behavior reported for these cell lines.

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13.

TUMOR NECROSIS FACTOR ALPHA (TNF) INDUCES PROLIFERATION OF A MOUSE MAMMARY ADENOCARCINOMA THROUGH A MECHANISM THAT REQUIRES P42/P44 MAPK, JNK AND AKT AND PARTICIPATION OF BOTH TNF RECEPTORS: TNFR1 AND TNFR2

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TNF exerts its biological effects through two distinct receptors: TNFR1 and TNFR2. In several breast cancers, TNF induces cell cycle progression but the signaling pathways and the roles of each receptor in this effect remain unknown. In a murine progestin-dependent tumor, C4HD, we have demonstrated that TNF induces its proliferation. The aims of the present work were to characterize the presence of TNFRs in C4HD cells, and to establish their participation in TNF-induced proliferation and signaling. To determine the signaling pathways induced by TNF, cells were stimulated with TNF 20 ng/ml and the phosphorylated form of p42/p44 MAPK, p38 MAPK, JNK or Akt, was detected by Western blot (WB). TNF induces activation of p42/p44 MAPK (3.14 ± 0.3-fold vs. control), JNK (2.5 ± 0.45-fold) and Akt (2.2 ± 0.4-fold), but not p38 MAPK. In proliferation assays, by [^{3}H]-thymidine incorporation, using specific inhibitors of each pathway (PD98059, SP600125 and LY294002 respectively), we observed that all pathways were essential for C4HD cell proliferation. We assessed TNFRs expression in C4HD cells, and we observed that they express TNFR1 and TNFR2, by using through flow cytometry and WB analysis. Using blocking and stimulating antibodies directed to TNFR1 or TNFR2, we observed that both receptors should be functional in order to increase signaling pathways and C4HD proliferation. In this work we have demonstrated that TNF induces C4HD cells proliferation through p42/p44 MAPK, JNK and Akt activation. In addition, we have determined that TNFRs are involved in TNF-induced C4HD proliferation and signaling.

14.

CADMIUM TOXICITY ON THE RAT PLACENTA

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Cadmium is a heavy metal, toxic for animals and humans. It can produce toxicity entering into the organism through the following vias: a) digestive (ingestion of contaminated water and feed) b) respiratory (inhalation of cadmium compounds) and c) through the skin. It can be deposited in the placenta, affecting its structure and producing prenatal development defects. The aim of the present work was to study the possible placental alterations in rats intoxicated with cadmium at different times of gestation. Twenty virgin female Wistar rats under standard laboratory conditions were matted with males. Pregnant rats were divided in 5 groups of 4 animals each, and were injected subcutaneously with 10 mg Cd^{+2}/kg b.w. at days 4 (G1), 7 (G2), 10 (G3) and 15 (G4) of gestation. The control group (G5), received similar volume of saline solution. On day 20 post conception, rats were sacrificed. Placental diameter and average weight were measured and registered for their study. The placentas of each rat were used for histological studies and to determine Cd concentration (ppm DM). Some placentas were fixed with buffered formol, dehydrated with alcohol and embedded in paraffin; sections of 5 µm were stained with H/E and observed with an optic microscope. Others were used to determine Cd concentrations by atomic absorption spectrophotometry. Histopatological findings in the placentas of intoxicated rats were: congestion of labyrinthic vessels and coagulative necrosis, being more prominent in G2 animals. Mean (± SD) Cd concentrations (ppm DM) determined in the different groups were: G1 (1.13 ± 0,322); G2 (7.54 ± 0,947); G3 (19.95 ± 1,220); G4 (58.49 ± 7,477) and G5 (0.59 ± 0,161). We concluded that Cd was deposited in the placentas studied, producing alterations observable with an optic microscope.

15.

EFFECT OF ALPHA-TOCOPHEROL ON THE PRECAPACITATION STATUS OF FROZEN-THAWED BOAR SPERMATOZOA

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Cryopreservation is associated with membrane destabilization and production of reactive oxygen species which leads to decrease cellular functionality. Natural antioxidants exert a protective effect on the plasma membrane in cryopreserved porcine spermatozoa preserving both metabolic activity and cellular viability. Freezing and thawing processes induce alterations similar to those occurring during capacitation denominated precapacitation state. The objective of this work was to analyse the influence of α-tocopherol on the precapacitation status in cryopreserved boar semen. Boar spermatozoa frozen with or without 0.2 mg/ml α-tocopherol were thawed in BTS extender at 37ºC. Routine parameters of semen quality were evaluated and capacitation status was determined by the epifluorescence chlortetracycline technique. Motility and acrosome integrity were higher ($p<0.05$) in samples treated with α-tocopherol. Viability did not differ ($p>0.05$) between treatments. A significative decrease in the precapacitation level was observed in samples frozen with α-tocopherol.

Parameter (%)	Control samples	α-tocopherol samples
Motility	39 ± 4[a]	46 ± 2[b]
Viability	49 ± 6[a]	52 ± 1[a]
Acrosome integrity	44 ± 1[a]	51 ± 3[b]
Non capacitated spermatozoa	45 ± 1[a]	55 ± 4[b]
Capacitated spermatozoa	11 ± 1[a]	8 ± 1[b]

Within a row values with different letters differ significantly ($p<0.05$, T student test). Five replicates per sample.
Antioxidant effect of α–tocopherol would preserve plasma and mitochondrial sperm membrane integrity retaining the ability to generate oxidative energy that is required for motility. Alpha–tocopherol protects sperm membrane from non-regulated capacitation-like modifications produced by the cryopreservation process.

16.

DIFFERENTIAL INTRACELLULAR CALCIUM DISTRIBUTION IN THE CAPACITATION OF CRYOPRESERVED BOVINE SPERMATOZOA

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Capacitation is a biochemical event that allows oocyte fertilization. Heparin (H) and quercetin (Q) induce capacitation through a binding to membrane receptor and an inhibition of Ca-ATPase of plasma membrane, respectively. Intracellular calcium (Ca_i) increase is one of the first signals of capacitation. The aim of the study was to determine the variation and distribution of (Ca_i), and tyrosine kinase participation in capacitation induction. Shimatzu fluorescence spectrophotometer was used to measure Ca_i in samples loaded with Fura 2-AM and confocal microscopy was used to determine intracellular calcium distribution using Fluo3-AM as calcium indicator. Capacitation was evaluated by chlortetracycline and the viability by trypan blue stain. Data were analyzed by ANOVA and Tukey test ($p<0.05$). In the presence of heparin or quercetin, capacitation percentage differs vs. control (10.0±0.5%) and not significant differences were observed in the viability pattern. Ca_i increased in H and Q treated samples (400.0±45.2 nM and 447.0±118.0nM, respectively) vs control ($p<0.05$). Sperm samples loaded with Fluo3-AM and capacitated with H or Q, showed different fluorescent patterns. Sperm fluorescence of H-treated samples increased in the acrosomal and mitochondrial areas and disappeared in postacrosomal region, in contrast to Q-treated samples in which the fluorescence was homogeneous in the whole sperm head maintaining the intensity in mitochondria. Genistein (150µM), specific inhibitor of tyrosine kinase provoked Ca_i (226.0±74.0nM) decrease and a capacitated percentage diminish in H-physiological induction, the same was observed with PP2 (specific inhibitor of C-src-tyrosine kinase) ($p<0.05$). Heparin and Quercetin induce capacitation with similar increase in calcium concentration with different intracellular distribution suggesting that different intracellular compartments are involved to induce calcium dependent signals required for capacitation. The physiological induction of heparin was regulated by tyrosine kinase including C-src in the capacitation of cryopreserved bovine sperm.

17.
GENERATION OF MICE LACKING THE EPIDIDYMAL PROTEIN "DE/CRISP-1" THAT PARTICIPATES IN THE FERTILIZATION PROCESS

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Epididymal protein DE/CRISP-1 mediates gamete fusion through complementary sites localized on the egg surface. To investigate its relevance for fertility, crisp-1 knock out mice were generated. The targeting vector was constructed using two fragment of the crisp-1 gene obtained from a genomic library from 129/Sv strains of mice, and two markers for selection. The positive selection marker was introduced between both targeting sequences, disrupting the exon 2. This construct was electroporated in embryonic stem cells derived from the 129/Sv mice and the G418 and ganciclovir resistant cells were selected. The homologous recombination between the vector and the crisp-1 gene, i.e. the disruption of this gene, was confirmed by Southern Blot and PCR on genomic DNA from those cells. Two independent clones were isolated and microinjected in mouse C57BL/6 blastocysts, which were then transferred to the uterus of pseudopregnant mothers. Twelve chimeras were born, from which three males bred to C57BL/6 females sired heterozygous animals for the deletion on the crisp-1 gene. Breeding of these mice produced knock out animals, in which the lacking of DE/CRSIP-1 protein in the epididymis was verified by Western Blot. The generation of these animals represents the first knock out mice produced for a protein identified in our country. In addition, these animals not only will address the relevance of DE/CRISP-1 for fertility, but will also contribute to the future development of methods for regulation of fertility and diagnosis and treatment of infertility.

18.
GONADAL STAGES IN *Nacella concinna* MALES DURING THE ESTIVAL PERIOD

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There is a lack of histological studies of the reproductive cycle of the mollusk *Nacella concinna* found in subantarctic waters. The aim of the present study was to describe the histological changes observed in different stages of the male gonad of *N. concinna* and the change percentage of each stage found during the sample period.
Sampling was carried out in December (1993), January and February (1994) on the 25 de Mayo Island, Antarctica. In each monthly sampling, fifteen (15) males with a valve length from 3 to 4 cm were collected. Gonads were fixed in Bouin and embedded in paraffin for histological analysis. The gonadal index (GI) was estimated as: gonad weight/total weight)*100. Gonadal stages were established by histological characteristics in each sample period, estimating the relationship with the GI. During the estival time, five phases of the sexual cycle were observed: Maturation (M), Advanced Maturation (AM), Full Maturation (FM), Partial Post-Spawned (PPO), PPO/R (Partial Post-Spawned with recovery).
In December, individuals in M, PPO and PPO/R coexisted (53%, 40% and 6.67%). In January, M, AM and FM phases were observed (21.4%, 14.29% and 14.29%) as well as a high number of PPO individuals (50%). In February, M and AM phases (33%, 60%) were observed again as well as a low number of PPO (6.67%). During the first two month, a low GI (16.18% y 12.67%) was observed, while in February (20.40%) a recovering that exceeded the initial value of December was observed. The great dispersion of the GI values is probably due to the existence of different gonadal stages in each month (asynchronic cycle) with a high percentage of mature individuals and a low percentage of them in a gonadal recovering stage. The decrease in the mean value of the GI would indicates that males spawning in December and January.

19.
IMMUNOLOCALIZATION OF EPITHELIAL CADHERIN IN MOUSE SPERM

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Epithelial cadherin (E-cad) is a membrane glycoprotein involved in cell-to-cell adhesion and signaling events. Studies from our group described its expression in human sperm and suggested its involvement in sperm-egg interaction (Marin-Briggiler *et al*, 2004). In the murine model, sperm preincubation with anti E-cad antibodies inhibited *in vitro* fertilization (Veiga *et al*, 2005). The aim of this study was to evaluate E-cad localization in mouse sperm recovered from the testis and different regions of the epididymis, and in cells incubated under conditions that resemble changes associated with fertilization. ***Methods***: Expression of E-cad was evaluated by immunocytochemistry in testicular and epididymal sperm, as well as in capacitated and calcium ionophore acrosome reacted-cells. Studies were carried out with an anti E-cad antibody (Santa Cruz Biotech.) that proved to inhibit mouse blastocyst development (embryonic arrest at 4-8 cell embryo stage; n=2). The sperm acrosomal status was assessed by staining with FITC-*Pisum sativum* agglutinin (FITC-PSA). Subcellular localization analysis of E-cad was done by immuno-electron microscopy (IEM). ***Results****:* E-cad was immunodetected in the acrosomal cap of testicular (13-57%) and epididymal sperm (*caput*=88-97%, *corpus*=81-96%, *cauda*=85-95%; n=3). IEM studies revealed E-cad localization in the plasma membrane of live sperm from *cauda* epididymis. All intact sperm (FITC-PSA acrosome positive) incubated under capacitating conditions were reactive for E-cad in the acrosomal cap; contrasting, cells that had undergone acrosomal exocytosis showed no signal in this region. In addition, a staining for E-cad was observed in the postacrosomal region in a high proportion of epididymal sperm (>60%), which remained after acrosomal exocytosis (n=4). ***Conclusion****:* These results show E-cad presence in testicular and epididymal sperm, and suggest its unmasking/addition during maturation. E-cad localization in the acrosomal and postacrosomal regions supports its participation in gamete interaction.

20.
HISTOLOGY OF 60-DAY OF GESTATION *MYOCASTOR COYPUS* (COYPU) FETUSES

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The aim of this work was to histologically describe coypu (Myocastor coypus) fetuses of 60 days of gestation. This was done using 14 fetuses processed with standard histological techniques. First, we focused the developmental stage of the different systems; for instance, the skin all over the body showed a germinative stratum 4.92-2.38 µm thick. Snout, forehead and eyelids were constituted by basal stratus and periderm. Organs of the digestive system presented stratified epithelium and two mesenchimal layers. Lungs had principal, segmentary and primitive subsegmentary bronchi. Gonads just started to differenciate into ovaries and testis in each sex. Pars distalis and Pars nervosa of the pituitary gland were already differenciated leaving small spaces of the Rathke´s pouch. The optical vesicle was constituted by the sensorial and pigmentary layers of retina, the coroidal vascular plexus, the vitreous body, the crystalline lens and peripheral mesenchimatic cells in a circular array. The Corti's organ showed vestibular and basilar membranes and it was surrounded by a net of mesenchimal cells and by a cartilaginous otic capsule. Hind and forelimb bones were identify as cartilaginous sprouts, also, in the same way, the vertebral body and lateral and transversal apophysis, the base of the craneus, jaw, ribs and sternum. Vertebral neural arcs projected until the covered two third of spinal cord. In the limbs, neck area and lateral body walls could be observed fascicles of myoidal cells. In every fetus observed we found intestinal bends inside the umbilical cord. Results indicate that the stage of prenatal development of coypu (Myocastor coypus) 60 pc days is the time point in which the slope of growth of the embryo makes a crucial change. Comparative study with equivalent stages of other mammals showed that coypu has a precocious anatomical and hystological differenciation.

21.

APOPTOSIS OF PORCINE GRANULOSA CELLS IS MEDIATED BY ARYL HYDROCARBON RECEPTOR

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The arylhydrocarbon receptor (AhR) is known to mediate toxic response to dioxin like TCDD and polycyclic aromatic compounds d has been extensively characterized from a toxicological viewpoint. However, it has recently been reported that the AhR may have a central role in ovarian physiology. The expression of AhR has been described in the ovary of rat, mouse, rabbit, cow and human. Recent findings indicate that the AhR may have a central role in regulating follicular growth during pre- and postnatal life. In bovine, the AhR may be involved in the oocytes maturation promoting the resumption of meiosis. On the other hand, it is known that the follicular atresia begins with the programmed cell death of granulosa cells. The purpose of the current study was to explore the role of the AhR in the apoptosis of porcine granulosa cells (PGC). Granulosa cells obtained by aspiration of 3 to 6 mm porcine follicles were culture and incubated with the AhR agonist β-naphthoflavone (βNF) and the antagonist α-naphthoflavone (αNF). The apoptosis was examined by annexin V-FITC staining and activity of caspase 3. The results showed that αNF 10 μM induces PGC apoptosis while βNF 10-20 μM showed no significant changes respect to the control. The αNF induction was significatively reverted by βNF when PGC were coincubated with the agonist and antagonist simultaneously. The results indicate that AhR may be involved in the apoptosis regulation of porcine granulosa cells.

22.

HORMONAL MODULATION OF ASYMMETRICALLY GLYCOSILATED IGG IN ENDOCRINE-GYNECOLOGY FAILURE WOMEN

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Asymmetrically glycosilated IgG (Asym IgG) molecules posses an oligosaccharide moiety which interferes with the antigen combination, so, these antibodies cannot trigger the effector mechanisms of the immune response. Previous works demonstrated that during pregnancy there is an increase in serum Asym IgG. Furthermore, estrogen, progesterone and glucocorticoids modulate asymmetric antibodies synthesis in murine hybridoma. Our aim was to evaluate the asymmetric/simmetric IgG proportion in endocrine-gynecology failure patients. We used sera from women with clinical symptoms and laboratory diagnose of hyperprolactinemia, hypotiroidism and polycystic ovarian syndrome (PCO) as well as sera from normal women as controls (ages: 20-40 years old). The percentage of Asym IgG was determined by ELISA test before and after Concanavalin A-Sepharose affinity chromatography. The results demonstrated: 1) When prolactin serum levels (PRL) were slightly elevated a significant increased in asymmetric/symmetric proportion was observed. However, the relation was not altered by higher prolactin levels (% Asym IgG: control: 27.1±1.8, n=7; PRL:24-50 ng/ml: 42.6±5.4, n=4, $p<0.05$; PRL >50 ng/ml: 31.0±5.0, n= 5, ns vs control). 2) In hypotiroid patients an increase in Asym IgG percentage was detected (49.0±5.8 %, n=3, $p<0.01$). 3) In PCO women also a higher proportion of asymmetric antibodies was found (39.4±3.2 %, n=6, $p<0.05$). These data suggest that certain endocrine pathologies, for example, hyperprolactinemia, hypotiroidism and polycystic ovarian syndrome could modulate the percentage of asymmetric glycosilated IgG synthesis and, as a consequence, alter the immune response quality.

23.

REGULATION OF CYCLOOXYGENASE 2 (COX2) EXPRESSION IN THE TESTIS

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Previously, we described the expression of cyclooxygenase 2 (COX2), key enzyme in the biosynthesis of prostaglandins (PGs), in testicular biopsies from patients with Sertoli cell only (SCO) and with germ arrest (GA) syndrome. Nevertheless, COX2 was not expressed in testicular biopsies from Idiopathic infertile patients revealing normal spermatogenesis (Frungieri *et al.*, *Proc. Natl. Acad. Sci USA* 2002; 99:15072). In this study, we analyzed by immunohistochemistry and RT-PCR, COX2 expression in testes of different species. COX2 was not expressed in mouse, rat, pig or monkey testes. We found COX2 in Leydig cells of 36, 45, 60 and 100 days-old Syrian hamsters kept in a long-day photoperiod (LD, 14/10 h light/dark), whereas COX2 expression was not detected neither in testes of prepubertal hamsters nor in testes of adult hamsters exposed to a short-day photoperiod (SD, 8/16 h light/dark) for 16 weeks in order to achieve the maximal sexual regression. *In vitro* studies performed in hamster Leydig cells followed by RT-PCR demonstrated that COX2 is induced by hCG, testosterone (T) and 3α-Diol, whereas melatonin reverted the hCG -stimulatory effect on testicular COX2 expression. Similar results were found when PGF2α production was determined in hamster Leydig cells (fmol/million cells, control: 102.69 ± 5.01, 100 mIU/ml hCG: 144.17 ± 3.55, 1μM T: 156.94 ± 5.87, 1μM 3α-Diol: 148.32 ± 5.71, $P<0.05$, 100 mIU/ml hCG + 1nM melatonin: 99.36 ± 6.00). In addition, we detected androgen receptor immunoreactivity in hamster Sertoli cells, myoid cells and Leydig cells. In summary, COX2 is expressed in hamster Leydig cells and regulated by hCG, T, 3α-Diol and melatonin during sexual development and adulthood.

24.

INFLUENCE OF GONADOTROPHINS ON GLYCOLYTIC ACTIVITY AND AMINO ACID METABOLISM IN PORCINE OOCYTE *IN VITRO* MATURATION

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Porcine embryo *in vitro* production technique is not satisfactory, the inadequate oocyte maturation is one of the main reasons. The lack of knowledge about factors involved in control of maturation includes the metabolic activity of cumulus-oocyte complexes (COCs). The effect of gonadotrophins on glycolysis, ammonia and protein production during maturation of different classes of COCs was studied. COCs from slaughtered gilts were classified according to cumulus characteristics in classes: A_1 complete-thick, A_2 complete, B_1 corona radiata and B_2 partially naked oocyte. COCs were matured in medium 199 + 10% FBS (control) + FSH + LH for 48h. Glucose (G), lactate (L) and ammonia concentrations in spent media were determined spectrophotometrically. Total protein of COCs was measured by Lowry's method. Maturation was evaluated by metaphase II. A higher G uptake and L production was observed in classes A respect to B (G: 27.6±2.0 vs 6.2±1.3, L: 57.0±6.3 vs 9.2±3.5 nmol/COC, $P<0.05$). Gonadotrophins increased glycolytic activity only in classes A (G: 13.3±0.6 vs 27.6±2.0, L: 33.5±4.5 vs 57.0±6.3/COC, $P<0.05$). A high correlation between G uptake and L production was observed (r=0,7), being 1/2 the G/L ratio in classes A. Higher protein content was observed only in matured classes A in the presence of hormones (3.1±0.3 vs 1.5±0.1 μg/COC, $P<0.05$). Ammonia production was higher in classes A respect to B (0.9±0.2 vs 0.4±0.1 nmol/COC), and the tendency was to increase with gonadotrophins ($P<0.1$). Glycolysis is the main fate of G uptake in COCs with complete-thick cumuli, which is stimulated by FSH + LH. Gonadotrophins probably increase uptake of amino acids for protein synthesis as well as their catabolism.

25.

EXPRESSION OF RAT CALTRIN I IN *E. COLI*

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Caltrin (calcium transport inhibitor) I protein from rat seminal vesicle secretion binds to the acrosomal region of spermatozoa during ejaculation, and inhibits extracellular Ca^{2+} uptake. It also inhibits the activity of trypsin like acrosomal proteases. New studies have indicated that caltrin I prevents spontaneous acrosome reaction without affecting the capacitation process, and it also participates in the mechanisms of sperm-oocyte recognition and interaction. Since the activity of this protein is dependent on its structural arrangement, we decided to generate recombinant caltrin to study the structure/function relationship using modified variants. The cDNA of rat caltrin I was obtained by RT-PCR of mRNA from SV. The amino acids sequence predicted from the codifying region completely agree with that determined by peptide sequencing of the secreted form of caltrin I consisting of 56 residues of amino acids and a molecular size of 6.2 kDa. The cDNA was amplified by PCR using primers designed to introduce the BamHI and NotI sites in the 5' and 3' ends, and then it was cloned in pGEX-4T-3 vector in frame with GST coding sequence. Recombinant plasmids purified from positive Escherichia coli DH5α colonies were subcloned in E. coli BL21 cells. Cells were grown at 37 °C for 4 h and the expression was induced by addition of 0.1 mM IPTG. The mayor expression product with an apparent molecular size similar to that expected for the fusion protein was recognized in the insoluble fraction of cellular lysates by SDS-PAGE and Western blotting using anti-caltrin and anti-GST antibodies.

26.

ABNORMAL SPERM NUCLEI IN BOAR EJACULATES

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Changes in nuclear material could originate fertility problems, thus, morphology of sperm nuclei is an important parameter of semen evaluation. Our aim was to determine the percentage of different sperm nuclear abnormalities, and defects on chromatin maturation and condensation from fertile boar samples. Fresh ejaculates from 18 fertile male were used. Feulgen Reaction was developed to observe nuclear morphology. Chromatin maturation and condensation were studied with Aniline Blue and Toluidine Blue. Aniline Blue stained immature sperm nuclei, where histones are present, and Toluidine Blue stained decondensed nuclei, where disulphure bridges are not completely formed. Thus, positive reaction to this stains indicate maturation and/or condensation problems. Nuclear morphologic abnormalities were consistent with those founded in other species. Mean of the eighteen male percentages standard error were: normal 97,74 ± 0,26 %; piriform 0,24 ± 0,10 %; small 0,74 ± 0,16 %; large 0,44 ± 0,14 %; rolled 0,37 ± 0,12 %; tapering 0,22 ± 0,10 %; vacuolated 0,21 ± 0,15 %; diploid 0,07 ± 0,08 %; teratoid 0,19 ± 0,08 %. Normal nuclei varied between 99,2% and 96,2 %. Mean percentage of non colored nuclei were 99,35 ± 0,31 % (100% to 97,2 %) for Aniline Blue, and 99,62 ± 0,12 % (100 % to 97,4 %) for Toluidine Blue. Feulgen Reaction stained properly boar sperm nuclei, revealing chromatin qualitative and quantitative defects. Vacuolated and abnormal forms revealed damage on chromatin integrity, and diploid nuclei showed changes on DNA content (dark stained, no morphologic changes). The three techniques showed high percentages of normal sperm nuclei in the studied population.

27.

α METRONIDAZOLE EFFECT ON SEMINIFEROUS EPITHELIAL CYCLE AND SPERM MORPHOLOGY IN MICE

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The aim of this work was to assess the effect of metronidazole (MTZ) on the synchronization of the stages of the seminiferous epithelial cycle and sperm morphology when the drug is administered in therapeutic doses to CFW male mice.
Sixty-day-old mice (n=10) kept in standard captivity conditions were used. The relative frequency of the cycle stages was established by counting spermatocytes in pachytene, spermatids and Sertoli cells (stage XII, 100x). For the morphological analysis, the following types of abnormalities were considered: 1) abnormalities in the flagellum, 2) abnormalities of the head, 3) lack of maturity and 4) multiple malformations. The variability observed in the strain in its natural state (control) was compared with that of the MTZ-treated group (v.ip. 130 mg/kg/bw) (exposed) according to the seven-day treatment protocol used in humans.
The results indicate that: a) there were no differences in number of stages in the seminiferous epithelium tubules between exposed and control groups; b) cells in stages I, V and XII showed a significant increase in number in treated animals ($p<0.05$); and c) the sperm morphology was severely altered in the exposed group when compared to controls ($p=0.019$). These findings present evidences that the observed alterations could constitute an important prezygotic barrier, due to morphologically anomalous spermatozoa, with the consequent decrease in the fertilization rate. Further studies are necessary in order to analyze the effect in the offspring.

28.

LOCALIZATION OF EPITHELIAL CADHERIN IN FROZEN-THAWED BULL SPERM IN DIFFERENT FUNCTIONAL STATES

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Epithelial cadherin (E-cad) is a Ca^{+2} dependent cell-cell adhesion glycoprotein. Its presence has been reported in human and murine sperm, and data suggest its participation in fertilization. The aim of the study was to assess E-cad localization in frozen-thawed bull sperm under experimental conditions that resemble sperm functional changes during fertilization. Evaluations (n=3/animal) were done in sperm from semen of 5 fertile Holstein bulls, as follows: 1) Frozen-thawed cells (semen extender removal with Sp-TALP), 2) Motile sperm selected by glass wool column filtration, 3) Motile sperm incubated in media with 60βg/ml heparin (control: same+5mM glucose) to promote capacitation, and 4) Capacitated (cap) sperm incubated with 100ig/ml Lysophosphatidylcholine (LPC) to induce acrosomal exocytosis. E-cad localization was done by immunocytochemistry with a specific anti E-cad antibody (Santa Cruz Biotech). In all cases, % live sperm, % progressive motile sperm and vigor (0–5 scale), capacitating status (chlortetracycline staining), and acrosome integrity (FITC- *Pisum Sativum Agglutinin*; FITC-PSA) was measured. In frozen-thawed and selected motile sperm (21±2% cap-sperm, 98±1% with intact acrosome; average ± SEM), a strong signal for E-cad was observed in the apical ridge, accompanied with a weak staining in the acrosome and post-acrosomal regions (63±3%; 77±2%; respectively). Under capacitating conditions (57±1% cap-sperm, 87±1% with intact acrosome), sperm were immunoreactive to E-cad in the apical ridge (57±6%) although no staining was observed in the acrosome. LPC-acrosome reacted (44±2% acrosome reaction) sperm lost the E-cad signal over the apical ridge in all cases, and showed an immunoreaction in the sperm head, suggesting E-cad localization in the inner acrosomal/nuclear membrane (54±4%) and in the equatorial segment (21±5%).
In conclusion, E-cad is present in non capacitated, capacitated and acrosome reacted bull sperm, localized in cell regions proposed to participate in the interaction with oviductal cells and oocyte envelopes.

29.

11-KET0TESTOSTERONE IN PEJERREY (*Odontesthes bonariensis*)

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11-oxo-androgens are well recognized as one of the main effectors of male differentiation in teleost fish and their synthesis involves the 11-β-hydroxylase (P45011B) enzymatic activity. The effect of 11-oxo-androgens in the induction of male phenotype in monosex genetic females is well known. In general, studies on the role of androgens during male sex differentiation are focused on the expression P45011B around the differentiating period. However, there are scarce available data on the capacity of young testes to produce androgens before, during or just after male gonad differentiation. We hypothesized that in pejerrey (a species with a strong temperature sex determination/differentiation, TSD) androgens play a major role during the critical period of determination/differentiation. Two complementary methodologies were developed in order to determine whether the P45011B is expressed and is active in immature pejerrey testes, and to identify the major androgens produced at early stages of gametogenesis. First we cloned and sequenced a partial fragment of mRNA of P45011B (360 bp) and then, by using biochemical techniques we found that 11-hydroxylase is yet active in immature (only spermatogonias) fish. In fact, immature testes were able to convert androstenedione and 17α-hydroxyprogesterone into 11-oxygenated derivatives. The main androgen synthesized at this stage was the 11-ketotestosterone (identified by TLC, HPLC and acetylation). The next step will include the analysis of changes in the enzyme expression and in androgen synthesis by gonads of a cohort of all male pejerrey during TSD.
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30.

SUPEROXIDE ANION INDUCES CAPACITATION IN CRYOPRESERVED BOAR SPERMATOZOA

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Mammalian spermatozoa require a preparation process denominated capacitation to be able to fertilize mature oocytes. At physiological concentrations superoxide anion, hydrogen peroxide and nitric oxide are considered the major reactive species that participate in redox reactions activating enzymes or other mechanisms involved in the capacitation process. The objective of this work was to determine the participation of exogenous superoxide anion in the capacitation of cryopreserved boar spermatozoa. Samples of frozen-thawed spermatozoa were capacitated with bicarbonate or different concentrations of xantine-xantine oxide-catalase system (X-XO-C; superoxide anion donor). Motility, viability were determined by optical microscopy and percentages of capacitated spermatozoa by the epifluorescence chlortetracycline technique. Motility was not affected by X-XO-C with xantine ≤ 0.05 mM + xantine-oxidase ≤ 5 mUI/ml + catalase 50 μg/ml, however a dose-dependent decrease was observed at concentrations of xantine ≥ 0.1 mM + xantine oxidase ≥ 10 mUI/ml + catalase 50 μg/ml. Capacitation was induced by X-XO-C with concentrations among 0.01 to 0.05 of xantine + 1 to 5 mUI/ml xantine oxidase + 50 μg/ml catalase at the same level ($p>0.05$) of bicarbonate (a well known capacitation inducer) preserving sperm viability. Low concentrations of exogenous superoxide anion are capable to induce capacitation in cryopreserved boar spermatozoa.

31.

EFFECT OF VEGF INHIBITOR TREATMENT (R1/FC CHIMERA) ON FOLLICULOGENESIS AND OVARIAN APOPTOSIS

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In the adult, angiogenesis occurs infrequently with the exception of the female reproductive system. VEGF is thought to play a key role in the regulation of angiogenesis in the ovary. This protein and its receptor Flk-1 are expressed in granulosa and theca cells. The objective of this study was to determine the rol of VEGF on the folliculogenesis and ovarian apoptosis. Female immature rats superovulated with eCG were injected with a VEGF inhibitor (Trap: R1/Fc chimera 0.1 or 0.5 μg/ovary) under the bursa of one ovary. The contralateral ovary was injected with vehicle (C). The ovaries were removed at different times for histological studies. Time course on follicle growth was made 12, 24 and 48 hours after surgery. The number of atretic follicles significantly increased after 48 h (0.5μg) (C: 10,46±0,54; Trap: 16,38±1,27, $p<0,05$) whereas the number of periovulatory follicles significantly decreased (C: 14,08±1,49; Trap: 8,54±1,72, $p<0,05$). No significant changes were observed when 0.1 μg was injected. Trap treatment (0.5μg) significantly increased the number of apoptotic cells detected by the TUNEL technique, 48 h after surgery (C: 3,17±0,82; Trap: 6,81±1,02 apoptotic cells/field, $p<0,05$). Preovulatory follicles (>400 μm) were dissected under a stereoscopic microscope and used for western blots. Trap injection reduced the ratio Bcl-xL/Bcl-xS and Bcl-2/Bax whereas the levels of Fas and Fas-L proteins did not change.
Conclusions: The inhibition of VEGF activity would produce an increase in ovarian apoptosis, leading to a larger number of follicles to atresia. The mechanism of this cytoprotection could be through a larger vascularization or through a direct effect mediated by its receptor in granulosa cells.

32.

SEROTONIN REGULATES THE EXPRESSION OF TGF-β1 VIA MAPK ACTIVATION IN LEYDIG CELLS OF GOLDEN HAMSTER

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In previous studies we described the role of serotonin (5-HT) as a local modulator of testicular steroidogenesis in Goden hamster (Neuroendocrinology, 76:53, 2002). In this report we analyze the involvement of 5-HT in the regulation of TGF-β1 expression, which participates in development and differentiation of Leydig cells. Testes of adult Golden hamster maintained in normal photoperiod (FN: 14h light: 10h dark.) were collagenized and the interstitial cells were purified using a Percoll gradient (90-40%). Total mRNA of purified Leydig cells fraction was used as template for a RT-PCR with specific primers for serotoninergics receptors type 1A and 2A, and the sequences were obtained from cDNA of each receptor. Then, the homology with other species was established. The TGF-β1 expression of Leydig cells incubated in basal conditions (Control) and stimulated with hCG (100UI/ml) (20 min) in presence or absence of 5-HT (1 uM) was analyzed by RT-PCR. The results show that 5-HT induces TGFb1 expression in basal conditions and stimulated with hCG (semi quantitative determination with the software Scion). There was also an increment in p42/p44 (erk1/2) phosphorilation with 5-HT treatment in basal conditions, evaluated by Western blot.
The stimulatory effect of 5-HT on TGF-β1 expression was abolished by P98059, an erk phosphorilation inhibitor.
In conclusion, the present results show that, in a photosensitive species like hamster, 5-HT modifies TGF-β1 expression through MAPK activation.

33.
REACTIVE OXYGEN SPECIES IN BOVINE OOCYTE *IN VITRO* MATURATION

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Oxidative modifications of cell components due to the action of reactive oxygen species (ROS) is one of the most potentially damaging processes for proper cell function; however, in the last few years it has been observed that ROS participate in physiological events. The aim of this work was to determine the possible variation of ROS production throughout bovine oocyte *in vitro* maturation. Cumulus-oocyte complexes were recovered by aspiration of antral follicles from ovaries obtained from slaughtered cows and cultured in medium 199 with or without cysteine 0.6 mM at 39ºC, 5% CO_2 in humidified air for 22 h. ROS production was determined in denuded oocytes each 2-h interval (0-22 h) calculating the ratio between the 2´,7´-dichlorodihydrofluorescein diacetate (DCHFDA) and fluorescein diacetate (FDA) fluorescent assays by the analysis of digital microphotographs. Meiotic maturation determined by the presence of the metaphase II chromosome configuration was 87%. At 18 h of maturation, 80% of the analyzed oocytes showed first polar body. ROS levels expressed as DCHFDA/FDA ratio fluctuated throughout the 22 h of bovine oocyte *in vitro* maturation, showing a significant decrease at 6 h and 18 h from the start of culture period ($P<0.05$). Oocytes matured in the presence of cysteine showed a decrease in ROS production ($P<0.05$). No significant differences were observed in esterase activity during *in vitro* maturation (FDA). In this study, the variation in ROS level during the complete process of oocyte *in vitro* maturation was determined for the first time in a mammalian species. During bovine oocyte *in vitro* maturation an important variation in ROS production takes place, decreasing in coincidence with the germinal vesicle breakdown and the polar body emission.

34.
IDENTIFICATION OF THE EGG-BINDING DOMAIN OF SPERM PROTEIN DE

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Rat epididymal protein DE is a member of the CRISP (cysteine-rich secretory proteins) family involved in sperm-egg fusion through its binding to complementary sites on the egg surface. The evaluation of the egg-binding ability of different recombinant fragments of DE suggested that this ability would reside in the region spanning from aa 114 to 158. In order to identify the active domain of DE, in this study we first evaluated the ability of that region (F7) to inhibit gamete fusion. The incubation of eggs with F7, but not F6 (aa 62-112), produced a significant and dose-dependant inhibition in the percentage of fused eggs (C:86±6%, F6:100%, F7 30 µM:42±4%), confirming that the active domain is located within F7. Interestingly, this region contains two characteristic motifs of the CRISP family, Signatures 1 and 2. To investigate whether these motifs were responsible for the binding of DE to the egg, 2 peptides, P1 (GHYTQVVWNST) and P2 (FYVCHYCPGGNY), corresponding to each motif, were synthetized and evaluated for their ability to bind to rat eggs. Indirect immunofluorescence results indicated that while eggs incubated with P1 showed a negative labeling, those incubated with P2 presented a bright fluorescent labeling similar to that previously observed with F7. A scramble P2 peptide, used as control, was not able to bind to the egg. The analysis of different CRISPs available indicated that those able to bind to the rat egg presented a Signature 2 very similar to that of DE, while those proteins that did not bind presented a lower homology. In summary, these results indicate that the egg-binding domain of DE corresponds to a 12 aa region highly conserved within the CRISPs, suggesting its relevance for the molecular mechanisms of other members of this family.

35.
CHROMATIN DISTRIBUTION IN DIPLOID AND HAPLOID BOVINE SPERM

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Spermatozoa with double DNA content, putative "diploids", are infrequent in different species, but they have an important role in the origin of triploidy. The aim of this work was to study if the increase in DNA content follows an isotropic or anisotropic growth model in bovine "diploid" sperm. Individual Feulgen-reacted nuclei were microspectrophotometrically studied and the major plane area, maximal absorbance, mean absorbance and mean absorbance per area were determined. The last measure corresponds to DNA content expressed in arbitrary units. "Diploid" nuclei were present in 0.15% of cells, showing a broader nuclear basal zone and a deeper Feulgen coloration than haploid sperm. Data rejection method was used to determine the DNA content and major plane area in haploid and "diploid" spermatozoa. Values (mean $\pm$ SD) obtained for normal and "diploid" sperm were respectively: a) major plane area 47.83 ± 2.51 µm^2 and 52.34 ± 1.41µm^2; b) mean absorbance $0.09 \pm 0.01$and 0.17 ± 0.02; c) maximal absorbance 0.30 ± 0.07 and 0.60 ± 0.14; d) DNA content 4.39 ± 0.39 UA and 8.69 ± 0.75 UA. A simple mathematical model was made in order to relate the sperm nucleus with an ellipsoid of revolution, in which the nucleus major plane area would correspond to the ellipsoid principal section (*x; y*) and the DNA content to the semi axis *y*. The volume increase of a solid is isotropic when *x*, *y* and *z* axis grow in the same way. In "diploid" nuclei the increase of DNA content would be anisotropic since the growth factor is 1,8 in the *z* semiaxis and 1,04 in *x* and *y* axes. Therefore, DNA increase in "diploid" bovine sperm follows an anisotropic growth that is maximum in the perpendicular direction of the major plane area.

36.
EFFECT OF ERYTHROPOIETIN ON BOVINE *IN VITRO* EMBRYO PRODUCTION

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Erythropoietin (EPO) is known to regulate the number of circulating red blood cells. Recently, EPO mRNA and EPOr mRNA were found in organs non related to erythropoiesis such as testicle, endometrium, oviduct and ovary. The aim of our work is to evaluate the effect of adding EPO to the *in vitro* maturation medium (IVMm) of bovine oocytes on the percentage of MII oocytes, cleavage and blastocyst rates. Bovine oocytes were obtained from slaughterhouse ovaries and matured *in vitro* for 22h in groups of 5 to 10 in 50µl droplets of IVMm (De Matos *et al*, Mol Reprod Dev. 62:203, 2002) with 0, 7, 14, 20 or 80mU/ml EPO. Then, they were denuded from their cumulus cells or incubated for 24h with $2x10^6$ bull sperm cells. Presumptive zygotes were denuded and cultured for 24h in synthetic oviduct fluid medium (SOFm) in co culture with granulosa cells and then the medium was replaced for SOFm with 1,5mM glucose. The embryos remained in this medium for 7 additional days. The MII oocytes, cleavage and blastocysts rates were compared between the different experimental groups using Fisher's test. The supplementation with EPO during IVM demonstrated a positive effect only on the 20mU/mL group by a significant increment on the blastocyst rate (52% vs 24,6% of the control group $p<0,05$). These results demonstrate an improvement on the efficiency of bovine *in vitro* embryo production (IVP). To our knowledge, this is the first report on the effect of EPO on *in vitro* embryo development. Further investigations will be necessary to determine the effect of adding EPO during other stages of IVP such as fertilization and embryo culture as well as its regulation in reproductive organs.

37.
EXPRESSION OF PLACENTAL GROWTH FACTOR AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN OVARIAN TERATOMAS OF TRANSGENIC MICE HYPERSECRETING HUMAN CHORIONIC GONADOTROPIN

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Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally synthesized by the human placenta. It is structurally related to the luteinizing hormone, and acts through the same receptor. We have demonstrated that hCG overexpression induces the development of ovarian teratomas in transgenic mice (TG). These tumors are composed of disorganized tissues derived from embryonic and extraembryonic cells. Angiogenic factors are known to promote the formation of blood vessels, necessary to feed and maintain tumor growth. The placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family that has been shown to play an important role in promoting angiogenesis. Besides inducing its own signaling in endothelial cells, PlGF exerts its angiogenic action by synergising with VEGF. The objective of the present study was to analyze gene expression and immunolocalization of VEGF and PlGF, by RT-PCR and immunohistochemistry in wild-type (wt) ovaries and TG ovarian tumors. The relative gene expressions of VEGF and PlGF were significantly increased in TG tumors compared with wt ($p < 0.05$). Immunohistochemical analyses demonstrated that these factors were localized in the remnant ovarian tissue, and in the trophoblast giant cells present in the teratomas, which are typically found in the mouse placenta. In conclusion, the present results suggest that the trophoblast giant cells of the teratomas would contribute in promoting the angiogenic process in these tumors, through the expression of PlGF and VEGF.

38.
EXPRESSION OF MÜLLERIAN-INHIBITING SUBSTANCE (MIS) DURING TEMPERATURE SEXUAL DETERMINATION IN THE PEJERREY FISH, *Odontesthes bonariensis*

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The process of sex determination/differentiation in bony fish species can by controlled either by genetic, environmental or combination of both factors. In the pejerrey fish, a fish with marked temperature-dependent sex determination (TSD), the proportion of males changes gradually from 0% at 19°C to 100% at 29°C, whereas at 23–25°C there is almost a 1:1 sex ratio. The anti-Müllerian hormone or Müllerian-inhibiting substance (AMH or MIS) is a glycoprotein belonging to the transforming growth factor b (TGF-b) superfamily. In mammals, MIS is responsible for the regression of the Müllerian ducts in the male fetus. For example, in mice, MIS starts to be expressed in Sertoli cells at the onset of testicular differentiation and its expression persists even after regression of the Müllerian ducts. However, the role of MIS on gonadal sex differentiation in teleost fishes, which do not form the Müllerian ducts, has not been cleared out yet. The first demonstration of sexually dimorphic expression of MIS mRNA during sex differentiation in a teleost fish species was demonstrated in the Japanese flounder and was then observed in zebrafish. In the present work were pejerrey larvae were incubated at 17°C, 25°C and 29°C and the expression of MIS was followed from hatching to 9 weeks old by relative quantitation real time PCR. MIS showed a statistically significant expression in male promoting temperatures starting at 5 weeks. The current results suggest that the up-regulation of MIS in pejerrey may play a role in male sex differentiation.
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39.
MITOCHONDRIAL LIPIDS INVOLVEMENT IN AMPHIBIAN MEIOTIC MATURATION

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Progesterone induces the resumption of meiosis in amphibian full-grown arrested oocytes through a nongenomic mechanism called meiotic maturation. Growing evidence indicates that lipids are involved in this process. The effect of progesterone-induced maturation on the content and composition of polar and neutral mitochondrial lipids was analyzed in *Bufo arenarum* full-grown oocytes. Ovarian oocytes, manually obtained, were incubated with progesterone to induce maturation. Mitochondrial fraction was isolated by centrifugation, lipids were extracted, separated by thin-layer chromatography and derivatized by methanolysis. Methyl esters were identified in a gas-liquid chromatograph. Phospholipid phosphorous was colorimetrically measured.
A significant fall in total phospholipids, due to phosphatidylcholine (PC) and sphingomyelin (SM) decreases was observed. With respect to the fatty acid composition, a decrease in PC polyunsaturated fatty acids, mainly in linoleic and arachidonic acids was determined. In phosphatidylethanolamine (PE), arachidonic acid showed a net increase. As a consequence of progesterone stimulation, decreases in linoleic and palmitic acids were observed in diphosphatidylglycerol and SM, respectively. Nervonic acid, a characteristic component of SM, underwent a net decrease. In addition, an increase in total neutral lipid amount and significative changes in their fatty acid composition were evidenced. Altogether, results suggest an active role of mitochondrial lipids during meiosis reinitiation.

40.
TREATMENT WITH AROMATASE INHIBITORS REDUCE THE ENDOMETRIOTIC LESIONS SIZE IN A MURINE MODEL OF ENDOMETRIOSIS

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Endometriosis (EDT) is considered to be an estrogen-dependent disease and recent studies shown that patients with EDT have high levels of aromatase P450 expression in the eutopic and ectopic endometrial tissue. Aromatase inhibitors, such as Letrozole (*Let*) and Anastrozole (*Anas*), are currently used in postmenopausal women to treat breast cancer, then the objective of this study was to evaluate the effect of *Let* and *Anas* as a possible treatment to reduce the endometriotic lesions (EDT-L). For this purpose we employed an experimental model of EDT in which we induced EDT-L by implanting autologous endometrial fragments in the mesothelium of Balb/c mice. A total of 48 female mice with inducing EDT were used, 18 of these that did not received treatment were used as controls (C), 14 were treated with a 10 μg/Kg daily dose of *Let* and 16 were treated with a 10 μg/Kg daily dose of *Anas* for 28 days beginning on day 1 post-induction of EDT. After treatment, animals were sacrificed and EDT-L were counted and measured (major diameter and thickness). Results were analyzed using One-way ANOVA (non-parametric) statistic test. Percentages of mice that develop EDT and the number of lesions founded per mouse were similar in all groups. However the size of EDT-L expressed as X ± S.E. was 43±25 mm^3 in C, while in *Let* was 3.3±0.6 mm^3 ($p<0.001$ vs C) and in *Anas* 8.3±1.4 mm^3 ($p<0.05$ vs C). In conclusion, the treatment with aromatase inhibitors at the beginning of the disease did not inhibit the development of the lesions but produced a significant reduction in the size of EDT-L. This treatment for endometriosis could be a promising new modality that requires further investigation.

41.

LACTATE DEHYDROGENASE ACTIVITY AND AMINOACID METABOLISM ARE INVOLVED IN THE CAPACITATION OF CRYOPRESERVED BOVINE SPERMATOZOA

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Capacitation is a sperm process required to fertilize the oocyte. Heparin (H) and quercetin (Q) induce capacitation through a membrane receptor-binding and the inhibition of Ca-ATPase of plasma membrane, respectively. Intracellular calcium increase (Ca_i) is the first signal of capacitation reaching to the same Ca_i level with either inductor. In spermatozoa, ammonia accumulation is attributible to the protein degradation, inhibiting the respiration and the Krebs cycle. The aim was to determine enzymes activities that contribute to the redox state and to supply the energy for capacitation. Malate dehydrogenase (MDH-NAD), alanine and aspartate aminotransferases (ALT and AST) and lactate dehydrogenase (LDH) were registered spectrophotometrically at 340nm (NADH-oxidation). Capacitation was evaluated by chlorotetracycline and the viability by trypan blue stain. Data were analyzed by ANOVA and Tukey test ($p<0.05$). Capacitation percentage (H o Q) differs vs. control, not significant differences were observed in the viability. H or Q produced a decrease of ALT and MDH-NAD activities vs controls ($0.45\pm0.5x10^{-2}$ U/10^8esp; $7.08\pm1.8x10^{-2}$ U/10^8esp respectivity). AST activity decreased with H ($0.49\pm0.11x10^{-2}$U/10^8esp) vs Q ($0.69\pm0.10x10^{-2}$U/10^8esp) and control ($0.77\pm0.14x10^{-2}$ U/10^8esp) ($p<0.05$). LDH dismishes 50% by H vs control (2.83 ± 0.66U/10^8esp). Oxygen uptake was increased with H ($17.03\pm3.21\mu LO_2$/h/10^8 esp) vs control and Q ($p<0.05$). Aminotransferases, MDH-NAD and LDH activities diminished and aerobic metabolism was increased in the H capacitation probably due to the aminoacid catabolism reduction. Q capacitation dismishes ALT and MDH-NAD to the control levels maintaining LDH activity to supply piruvate for cellular metabolism as a control. H and Q modulate the redox state and the metabolism inducing a typical metabolic capacitation pattern suggesting different intracellular signals activation.

42.

STUDY ON THE IDENTITY OF THE BINDING SITES FOR THE SPERM PROTEIN DE ON THE EGG

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Evidence on the molecules involved in mammalian gamete fusion is very scarce. Results of our group indicate that sperm protein DE participates in the fusion process through complementary sites on the egg surface. In an attempt to identify these sites, zona-free mouse eggs were treated with proteinases, glycosidases and lipases under different experimental conditions, and their effect on the association of DE to the egg was analyzed by indirect immunofluorescence (IIF). While the different treatments affected the oocyte´s fusogenicity, differences in the binding of DE were not observed. The next step was to carry out biochemical assays to isolate a possible DE–protein receptor (R) complex. Neither the immunoprecipitation of egg proteins (EP) incubated with DE, nor the incubation with DE of EP attached to nitrocellulose (ligand blot) allowed the isolation of the R, suggesting the dissociation of the DE-R complex due to a low affinity. The exposure to disuccinildisuberate (DSS), a crosslinker that covalently attaches proteins associated by ionic strength, allowed us to identify a high molecular weight band, which was not detected in control samples (without oocytes, protein or crosslinker), that might correspond to a DE-R complex. Considering that tetraspanine CD9 is the only molecule of the mammalian oocyte essential for gamete fusion, we investigated whether it had the ability to interact with DE. IIF studies in which competence between anti-CD9 antibodies and DE was carried out, indicated that there might not be a direct interaction between CD9 and DE. Together, these observations indicate that the binding sites for protein DE on the egg might correspond to a protein or protein complex of high molecular weight, which is in accordance with the evidences found in mammals and invertebrates.

43.

EFFECT OF ZINC DEFICIENCY ON RAT EPIDIDYMAL OXIDATIVE STRESS

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Little is known about the regulation of post-testicular factors, especially epididymal factors, in the sperm viability. On the other hand, the expression of the inducible nitric oxide synthase (iNOS) is presented like an answer in several models of oxidative stress. Previous studies of our laboratory showed an elevated level of lipoperoxidation and alterations of the lipid pattern in epididymis of zinc deficient rat. To evaluate the effect of nutritional Zinc deficiency on the iNOS expression and non-enzymatic antioxidants in rat epididymis, Wistar male rats (200 ± 20 g) were fed with AIN-93 diet (zinc deficient, ZD) or with AIN-93 + 30 mg Zn/ kg (Control, Co) during 2 months. The epididymis was homogenized in buffer containing inhibitors of proteases and triton X-100. Forty µg of proteins were loaded on 8% SDS-PAGE gel. iNOS was identified by Western blot with specific antibodies and bands were revealed using Vectastain ABC detection kit. Other determinations carried out in epididymis head and cauda were: total glutathione (GT), glutathione disulfide (GSSG), non-proteic thiols (TNP) and metallothionein (MT). In epididymal head: GT, TNP and MT did not change but GSSG decreased with a ($p< 0.005$). In epididymal cauda: GT increased ($p< 0.05$), GSSG ($p< 0.0001$) and MT ($p< 0.01$) decreased and only TNP did not change. The inmunobloting showed iNOS expression without significant differences between Co and ZD in head and cauda. We can conclude that the zinc deficiency would not induce the expression of iNOS and that the cauda of the epididymis is more affected by oxidative damage than its head. It might be conceivably that these alterations lead to modifications in the spermatic maturation.

44.

IDENTIFICATION OF EPITHELIAL CADHERIN IN MEMBRANOUS VESICLES ISOLATED FROM HUMAN SEMINAL PLASMA

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Mammalian seminal plasma contains small membranous vesicles, called prostasomes (prostate derived vesicles) and epididymosomes (epididymis derived vesicles), that transfer proteins to the sperm plasma membrane. Our group previously described the presence of the cell-cell adhesion glycoprotein Epithelial Cadherin (E-cad) in human seminal plasma (hSP) and in ejaculated sperm, and confirmed previous evidence of its expression in the epididymis (Vazquez-Levin *et al*, 2003). The aim of this study was to evaluate the presence of E-cad protein forms in both soluble and membranous vesicle fractions of the hSP. **Materials and Methods:** hSP was obtained from normozoospermic donors (WHO, 1999). Membranous vesicles were isolated by ultracentrifugation of hSP at 100,000xg and further purification by gel filtration on Sephadex G-200. Protein extracts from total and ultracentrifugated hSP, from membranous vesicles recovered before and after gel filtration analysis, and from human sperm were prepared in Laemmli sample buffer and analyzed by SDS-PAGE and Western Immunoblotting using antibodies towards three different E-cad domains. **Results:** Four high molecular weight forms of E-Cad were detected in protein extracts from crude hSP and from purified membranous vesicles: the full length 122 KDa mature protein, a N-*terminus* truncated 105KDa form, and two protein forms of 97 KDa and 86 KDa lacking the cytplasmic region; similar E-cad protein patterns were previously described in human sperm (Lentz *et al*, 2003). The 86 KDa form was also immunodetected in the ultracentrifuged hSP and may correspond to the E-cad ectodomain previouslydescribed in other cell systems. **Conclusion:** E-cad protein forms are present in the soluble fraction as well as in the membranous vesicles purified from hSP. Immunoelectron microscopy analysis of the purified vesicles will further characterize E-cad localization.

45.

SOME OBSERVATIONS ON THE MORPHOLOGY OF GESTATIONAL SACS IN *MYOCASTOR COYPUS* (COYPU), ORDER *RODENTIA*, FAMILY *MYOCASTORIDAE*

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Myocastor coypus (coypu) is a neotropical rodent. Its embryonic development has not been study. This work was undertaken to do a morphological characterization of gestational sacs corresponding to the first, second and third period in which gestation could arbitrarily be divided. The experimental included 41 fetuses of 60, 90, and 120 postcoitum (pc) days. In all cases, gestational sacs adopted an elliptical shape with the larger axis follows the line of the uterus. The space between gestational sacs had incomplete uterus ridges. Between 60 and 120 pc days it was observed a decrease inside the gestational saculi. In all fetuses ages, placentas were of discoidal type and wine-red colored with an under-placenta irregularly conic-shapped. The placental external apearance in the 60 pc days fetuses was unilobular, and in 90 and 120 fetuses had lobulations separated by shallows furrows. Placental longitudinal cuts showed a complex system of partitions and lobulations. The umbilical cord rolled up around the fetal body, had a proximal dilatation in 60 pc days; in the other two groups was more uniform alongside and no so spiral. It was observed a notorious increase in the weigth of the gestational saculus in the 90 and 120 pc days specimens, which corresponded with the fetus weigth increase. In all groups studied the fetal envelopes had the apeareance of thin and translucid membranes. The liquid inside these envelopes showed a transparent look and light ambar colored. These results allow to conclude that coypu (*Myocastor coypus*) is a rodent species with a comparatively most precocius fetal development.

46.

LYSOPHOSPHATIDILCHOLINE INCREASES ACROSIN ACTIVITY IN BOVINE HEPARIN-CAPACITATED SPERMATOZOA

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A high acrosin activity is associated with the normal fertility in human and bovine spermatozoa. Heparin (H) and quercetin (Q) induce capacitation through a membrane receptor binding and the inhibition of Ca-ATPase of plasma membrane, respectively. Lysophosphatidylcholine (LPC) induces acrosome reaction (AR) in capacitated bovine spermatozoa. The aim of the study was to determine the variation of proacrosin-acrosin system activity during capacitation, AR and the tyrosine kinase participation in the activation of acrosin, in cryopreserved bovine sperm. Enzyme activity was determinated spectrophotometrically using BAPNA as acrosin specific substrate. Capacitation and AR were evaluated by chlorotetracycline and the viability by trypan blue stain. Data were analyzed by ANOVA and Tukey test ($p<0.05$). Capacitation percentages in the presence of H or Q differ respect to the control, significant differences were not observed in the viability. Acrosin activity of H, Q and Q-LPC treated samples did not differ vs control (181,05± 145,60μU/10^6 esp). LPC increased the acrosin activity (686±149.86μU/10^6 esp) and only induced true AR (29.0±4.20%) in spermatozoa capacitated with H ($p<0.05$). Genistein, specific inhibitor of tyrosine kinase blocked AR induction (5.4±2.9%) and provoked acrosin activity decrease (179,09± 33,70μU/10^6 esp) to control level ($p<0.05$). The total level of acrosin activity registered (895,10±130,91μU/10^6 esp) indicates that 75% of acrosin from capacitated sperm and control samples, exists as zymogen form. LPC is capable of increasing 77 % the level of active enzyme only in H capacitated spermatozoa. H or Q- capacitation involves different intracellular processes, which don't allow that LPC can induce AR in Q- capacitated spermatozoa. The activation of proacrosin-acrosin system is regulated by tyrosine kinase activity and changes of acrosin activity are related to AR rather than capacitation, and only a proportion of proacrosin–acrosin system participates in exocytotic process in bovine sperm.

47.

VESICLE- AND MITOCHONDRIA-COVERED SURFACE IN IVP BOVINE EMBRYOS CULTURED IN SERUM-FREE AND SERUM-SUPPLEMENTED MEDIA

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Objetives: Investigating embryos' subcellular alterations, caused by serum-free (SFM) and serum-supplemented (SSM) culture media in *in vitro* production (IVP), and culture conditions that minimize those alterations. **M&M:** Total of eight embryos: from days 1, 2, and 3 (all SFM) plus day 3 embryos cultured (last 24 hs) with 5% oestrus-cow serum (3D-SSM). The surface covered by 333 mitochondria and 1207 lisosomic/lipidic-vesicles' sections was quantified on ETM micrographs at 8000X. **Results:** Vesicle decreases with SFM development. Mitochondria increases until the last stage, D 3. Vesicles' increases in SSM and mitochondria fell vis-a-vis the corresponding SFM stages. Ultrastructurally, some mitochondria of D3 embryos -SFM and SSM- were vacuolated. On SSM D3 embryos, some mitochondria show granulations observed on morulae by Brackett *et al.* (Biol Reprod 23: 189-205, 1980) and giants finger-like morphs (Crocco *et al*., Biocell 28: 359, 2004). **Conclusions:** Research continues on larger areas, stages, and treatments; hitherto shows (1) On SSM the responses of both organelles are tied. This culture modifies active mitochondrial surface. (2) Oft associated with SSM and degenerative stages, vacuolated mitochondria on SFM show that this metabolic response cannot be originated in serum.

Embryo stage (days) - Treatment	Analized (μm^2)	Versicle			Mitochondria		
		Surface (μm^2 +/- 0,01)	σ	Percentile	Surface (μm^2 +/- 0,01)	σ	Percentile
D 1 SFM	552	196,49	1,03	35,6%	26,9	0,14	4,9%
D 2 SFM	559	175,25	0,51	31,4%	20,51	0,13	3.7%
D 3 SFM	520	141,41	0,50	27,2%	30,65	0,28	5.9%
D 3 SSM	460	157,43	0,08	34,3%	24,05	0,20	5,2%

Vesicle and mitochondria surface on IVP embryo´ cells

48.

EFFECT OF VITAMIN A DEFICIENCY ON HEPATIC TRIGLYCERIDES IN RAT. EXPRESSION OF DIACYLGLYCEROL-ACYLTRANSFERASE 1 AND 2

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We have previously demonstrated that vitamin A deficiency alters liver phospholipid metabolism by decreasing its synthesis and content. This fact was associated to a reduced Fatty Acid Synthetase activity (FAS). Since this enzyme participates in the fatty acid synthesis, in the present study we examine the triglyceride content and mRNA expression of the regulatory enzymes involved in its synthesis in the liver of vitamin A deficient rats. Female Wistar rats at 21 days old were randomly weaned onto either a vitamin A deficient diet (-A group) or the same diet added with vitamin A 4000 IU/kg diet (control group) and fed for three months before sacrifice. Vitamin A deficiency was confirmed by retinol levels in plasma (0.55 ± 0.01 *vs* 1.89 ± 0.01 μmol/L, $p< 0.005$) and liver (0.06 ± 0.002 vs 1.80 ± 0.11 μmol/g, $p>0.001$) using HPLC. Liver lipids were extracted with hexane / isopropanol mixture (3:2, by vol), containing butylated hydroxytoluene as antioxidant. The TG were quantified after separation by TLC plates coated with silica gel G using hexane /diethyl ether /acetic acid (80:20:1, by vol) as solvent. The mRNA levels of hepatic Diacylglycerol acyltransferases (DAGAT 1 and 2) were measured by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). The bands were quantified using the software program Scion Image from NIH and normalized by the values obtained for β-actin. The statistical analysis was performed using Student´s t test. The vitamin A deficiency did not affect the triglycerides content in relation to control (0.77 ± 0.01 vs 0.73 ± 0.02 μmol/g liver). The mRNA levels of DAGAT-1 was increased ($p< 0.01$), while DAGAT-2 did not, in the liver of vitamin A deficient rats, compared with control. These results suggest that the regulation of liver triglycerides synthesis in the vitamin A deficient rats could depend of enzymes or factors other than mRNA DAGAT and FAS, which could be regulated by retinoic acid.

49.

COLECTOR VESSELS OF LYMPH IN THE ABDOMEN OF LLAMA *(Lama Glama)*

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The main objective of this work was to study the macroscopic characteristics of the collector vessels which draw in the lymphatic drainage from the different portions of the digestive system in the abdomen of the llama. 10 llamas, of 2 years old, from the FCV-UNICEN were analized, by lymphography technique, through the intranodular boarding of the jejunal lymphnodes and injection techniques with Chinese ink, toluidine blue and the modified Gerota technique. In this way the lymphatic drainage of the stomach and intestine was visualized, and complementary studies, as the radiological diagnostic and statistical analysis were performed. Starting from this information, the anatomical descriptions were carried out. The cistern chyli lenght is about 20 cm, with a diameter of about 5 mm, originated in ventral of the fifth lumbar vertebra. Its roots are double and the intermediate portion is an unique conduit, related to the fourth lumbar vertebra. The cranial portion ends in the aortic hiatus of the diaphragm. The gastrointestinal lymphatic trunk presents a straight itinerary of 5 cm and a diameter of 3mm, joins to the celiac lymphatic trunk and end together in the cistern chyli. The celiac lymphatic trunk is located to the right of the celiac artery. It is 3 cm of lenght and 4 mm of diameter. The efferent vessels come from the celiac, hepatic and spleen lymphnodes. The colic lymphatic trunk is formed by two lymphatic conduits of 10 cm lenght and 3 mm of diameter. They are located in the dorsal mesocolon. In conclusion, the big collector vessels follow the morphological model of domestical mammalians and distinguish for the lymphatic trunk.

50.

EARLY CHANGES IN IGG IMMUNOGLOBULIN LEVELS IN A MODEL OF PERINATAL ASPHYXIA

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Acute stress caused by hypoxia or poly-traumatisms alters the immune system. One of the most important complications in the clinic is then the increasing risk of infection. The immune system may be altered among other factors by changes in the levels of white cells, immunoglobulins or lactate, that have shown variations during the pursuit from the hospitalization up to two first weeks. In the present work, we have evaluated the effect of a perinatal asphyxia (PA) on one particular isotype of immunoglobuline (IgG) in Sprague-Dawley rats submitted to AP at normothermia (37ºC) or hypothermia (15ºC) at different age. (1, 7, 15 and 21 days-old; n=5-8/grupo). Immunoglobulins levels were determined by radial immuno-diffusion using specific monoclonal antibodies quantifying the diameters of the precipitation hale. The mean values from 15 days-old animals showed significant differences in AP respect to control animals (AP=6.00 ± 0.10 mm; C=7.7± 0.62 mm; $p<0.01$) but hypothermia avoid IgG alterations (H=7.23 ± 0.25 mm, p=0.3 when compared to control by Scheffé test after ANOVA). Similar differences were observed at 7 and 21 day-old animals. In animals exposed to AP, there is a decrease of IgG similar to it was described in humans after hypoxia or poly-traumatisms, the IgG change appears 7 days after hypoxia. Then this model could be used to explore other specific changes in the immune system. On the other hand hypothermia during hypoxia avoids the decrease on the isotypes of immunoglobulins like the IgG, probably acting by reducing the protein catabolism or avoiding suppression in the cooperation and production of antibodies, making this procedure after some modifications a possible therapeutic strategy.

(UBACYT-M020).

51.

DISTRIBUTION OF THE COLLAGEN SYSTEM FIBERS IN THE JEJUNUM -ILEUM IN LLAMA *(Lama glama)*

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This work was carried on by the "Biology Investigation group" of the Veterinary school-Unicen, framed in the investigation project: "Morphology of the digestive organs and mesenteric lymph nodes of the abdominal cavity in south american camelids". The objective was to study the different types of collagen fibers of jejunum-ileum of the llama *(Lama glama*). Samples were taken from three male llama, fixed in Bouin liquid and processed for light microscopy. The slices were stainned with Masson's stain, picrosirius-red and PAS-hematoxilyn. The different portions of jejunum-ileum were studied. A lot of type I collagen fibers was observed in the connective tissue of submucosa in the different portions, with picrosirius-red stain. Less proportion of type III collagen fibers was observed. Also type I collagen fibers were observed between both muscle layers and in the serosa with different distribution pattern according to the zone. In conclusion, according to the distribution of collagen fibers in the anatomical portions of jejunum-ileum were inconstant.

52.

LIPID PEROXIDATION OF ERYTHROCYTES OF EQUINE EXPOSED TO T-BUTYL HIDROPEROXIDE

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The erythrocytes cells are exposed normally to high oxygen concentrations, there are rich in polyunsaturated lipids and iron. In addition are exposed to sources of free radicals intra and extracellulars. Many studies were realized with organics hydroperoxides of fatty acids in order to understand the mechanism used by free radical to damage erythrocyte membranes. In this work was studied the non enzymatic lipid peroxidation, induced by ascorbate-Fe^{++} system of equine erythrocyte membranes exposed to tert-butyl-hydroperoxide (t-BHP), using as parameter chemiluminescence and fatty acids composition. The erythrocytes were isolated by centrifugation (1000g for 10 minutes at 4 ºC). The (buffy coat and the) plasma were discarded and erythrocytes were washed three times in isotonic phosphate buffer (PBS 5mM pH 7.4, 150 mM NaCl). Chemiluminescence was determined over 90 minutes and recorded as count per minute (cpm) every 10 minutes. The fatty acids (methyl esters) were analysed with GC-14A gas chromatograph. The equine erythrocytes were affected by lipid peroxidation, there were statistically differences between control total cpm (117 ± 17.6) and peroxidized (349.8 ± 96), the increasing of chemiluminescecne in peroxidized (10 mM t-BHP) respect to controls was of 198%. When was analysed the fatty acids composition was observed that the content of polyunsaturated C18:2n6, C20:4n3 and C22:6n3, decrease after peroxidation and the unsaturation index was significantly lower when was compared with control group. These results show that equine erythrocytes are sensible to oxidative damage and that the fatty acid composition of these membranes were modified by t-BHP prooxidant system.

53.

FATTY ACID PROFILES AND LIPID PEROXIDATION IN THE PENGUINS ADELIA (*PYGOSCELIS ADELIA*) AND PAPUA *(P. PAPUA*)

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The amount of fatty acids found in biological membranes is different between species and tissues of the same species. Birds are characterized by low free radical production than mammals of similar body size and metabolic rates.
The aim of the present study was examine fatty acid profiles and the susceptibility to lipid peroxidation in mitochondria obtained from liver and heart of two penguin species (Pygoscelis adelia) and (Pygoscelis papua). Chemiluminescence and fatty acid composition were used as an index of the lipoperoxidation process.
When we compared both species of penguin we observed that the amount of saturated and unsaturated fatty acids obtained from liver mitochondria was 54% and 46%, whereas in heart mitochondria of both species was 40% and 60% respectively.
In liver as in heart the predominant monounsaturated fatty acid was C18:1 n9. The polyunsaturated fatty acid percentages of mitochondria obtained from liver was 16% approximately whereas in heart was 18%. The relationship between C20:4 n6 / C18:2 n6 in liver and heart mitochondria was similar. The mitochondria obtained from liver and heart is not susceptible to lipid peroxidation. Light emission originating from liver and heart mitochondria was not statistically significant and the polyunsaturated fatty acid profiles did not change. Therefore, the unsaturation index was similar in both organs. The results suggest that the fatty acid composition and other factor may be involved in the protection to oxidative stress observed in these organelles.

54.

DEVELOPMENT OF A TECHNIQUE FOR ANATOMICAL STUDY OF LATERAL ABDOMINAL WALL IN THE LLAMA *(Lama glama)*

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Our main objective was to study the different layers of the lateral abdominal wall in the llama. Ten 2-years-old llamas with a corporal weight of 98.73 ± 14 kg, from the Veterinary Science School of UNICEN, were used. The animals were tranquilized and anesthetized. We proceeded to the dissection of the yugular vein and the carotid artery for bleeding. Immediately after the death the cadavers were hidrotomized, then the jugular vein was occluded. A 10% formol solution was injected to complete 7% of corporal weight, for fixation. In order to allow the best structures fixation a modified position II of Chaveau was used. The skin and the subcutaneous cover the abdominal muscles, since the cutaneous muscle is abscent. The abdominal muscles are plane and extensive with a muscular part and an aponeurotic part, they are in paired in superposed layers forming the lateral and ventral abdominal walls. The abdominal tunic (tunic flava) is thick and elastic, and is extended from the caudal edge of the costal arch until the tuber coxae. It is more developped in the ventral region of the abdomen. Their fibers are also attached to the edges of the cranial pubic tendon and the linea alba. The external and internal abdominal oblique muscles and the transversus abdominis form the muscular layer of this region. The transversus fascia is a fibrous expansion that covers the deep surface of the transversus muscle in the abdomen and is extended until the abdominal face of the diaphragm. This technique permits us to observe the abdominal organs in the original anatomical situation and without displacement.

55.

EFFECT OF DIFFERENT ISOMERS OF CONJUGATED LINOLEIC ACID ON NON-ENZYMATIC LIPID PEROXIDATION OF HEPATIC MITOCHONDRIA

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The polyunsaturated fatty acid composition, chemiluminescence and peroxidizability index of mitochondria obtained from rat liver, were studied after oral way administration of 9c-11t and 10t-12c isomers of conjugated linoleic acid. In the present study was found that the effect of both kinds of isomers was similar under our experimental conditions. After incubation of mitochondria in an ascorbate Fe^{++} system (120 min at 37°C) it was observed that the total cpm/mg protein originated from light emission: chemiluminescence was lower in liver mitochondria in the CLA group than in the control group. In mitochondria obtained from control rats, the most sensitive fatty acids for peroxidation were linoleic acid C18:2 n-6, arachidonic acid C20:4 n-6 and docosahexaenoic acid C22:6 n-3. In CLA group the statistically significant differences appear in linoleic acid C18:2 n-6, arachidonic acid C20:4 n-6 and docosahexaenoic acid C22:6 n-3 in liver mitochondria. As a consequence the peroxidizability index, a parameter based on the maximal rate of oxidation of fatty acids showed significant changes in liver mitochondria. These changes were less pronounced in membranes derived from rats receiving CLA *per os*. Our results confirm and extend previous observations that indicated that CLA may act as an antioxidant protecting membranes from deleterious effects.

56.

INFLUENCE OF DIET ON THE FATTY ACID COMPOSITION AND SENSITIVITY TO LIPID PEROXIDATION OF LIVER, HEART AND BRAIN MITOCHONDRIA FROM *Lonchura striata* (MANON)

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Comparative studies have demonstrated that the diet did not affect the fatty acid composition of membranes of different mammal species. The objective of this study was to assess the influence of the diet on fatty acid composition and sensitivity to lipid peroxidation of mitochondria from liver, heart and brain of manon. The mitochondria were obtained by differential centrifugation and incubated in an ascorbate- Fe^{+2} system, measuring chemiluminescence and fatty acid composition. The total unsaturated fatty acid percent of brain mitochondria was similar (60%) and those of liver and heart mitochondria were two times lower compared with the diet. The content of 18:1n9 and 18:2n6 in mitochondria from all the organs analysed was 1,4 times and 3 times lower compared with the diet, respectively. The content of 18:3n3 in brain mitochondria was similar and in liver and heart mitochondria was 2 times lower compared with the diet. The brain mitochondria have the highest content of 22:6n3, which was absent in the diet. Unsaturation index of liver and heart mitochondria was lower, whereas in brain mitochondria, it was higher compared with the diet. The analysis of chemiluminescence demonstrated significant lipid peroxidation in brain mitochondria whereas in heart and liver mitochondria was not affected by this process. Our results demonstrate that: 1) the distribution of unsaturated fatty acids in mitochondria from liver, heart and brain of manon was different and is not correlated with the diet composition. 2) The low unsaturation index of liver and heart mitochondria decreased the sensitivity to lipid peroxidation.

57.

BIODIVERSITY OF TRUE BUGS (HETEROPTERA) FROM RINCÓN SANTA MARÍA RESERVE (IBERA MACROSYSTEM, CORRIENTES, ARGENTINA)

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The Hemiptera currently ranks as the fifth most specious order of insects, after beetles, flies, wasps, and moths (Wilson 1992). There may be some 37.000 described species of Heteroptera, and perhaps 25.000 species remaining to be described (Schaefer & Panizzi 2000). The knowledge of biodiversity gives the basic information about richness, abundance and distribution of taxa; this data is necessary to take decisions about conservation and studies on ecosystems, ecology and cladistic biogeography. With the purpose to make a quali and quantitative inventory of terrestrial Heteroptera from Rincón Santa María Reserve, Ituzaingo (27º28' S- 56º34' W) (Ibera macrosystem, one of the most developed wetlands of the warm climate biosphere), Corrientes, Argentina; two entomological expeditions were done. We used four collecting methods: sweeping nets, beating nets, Malaise trap, mercury vapor light trap. We collected 349 spp. belonging to 24 families: spp. of 6 Alydidae, 4 Anthocoridae, 1 Aradidae, 2 Berytidae, 4 Blissidae, 24 Cydnidae, 2 Cymidae, 1 Colobathristidae, 15 Coreidae, 4 Geocoridae, 4 Largidae, 12 Lygaeidae, 74 Miridae, 5 Nabidae, 1 Oxycarenidae, 5 Pachygrontidae, 48 Pentatomidae, 3 Pyrrhocoridae, 51 Reduviidae, 13 Rhopalidae, 48 Rhyparochromidae, 8 Scutelleridae, 19 Schyzopteridae and 13 Tingidae. These results are biased by the collecting methods used, there are groups with very specific habits or behaviours that cannot be contempled by them, such us hematophagous, geophilous and laminophilous Heteroptera that were not found. Anyway, comparing these amounts with the recorded 110 gen. and 146 species from Corrientes (Coscaron 2003), we assume that the actual biodiversity is higher.

58.

GH AND PRL ALTERATIONS IN THE ADULT DOPAMINERGIC D2 RECEPTOR KNOCKOUT MOUSE MAY IMPAIR GLUCOSE METABOLISM

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In previous studies we have shown that the dopaminergic D2 receptor male knockout mouse (KO) has a lower GH secretion than their wild type (WT) counterpart during the first month of life (Endocrinology 143:1270, 2002). These mice are growth restricted with lower serum IGF-I and have chronic hyperprolactinemia. As GH and PRL may influence pancreatic beta cell function, we sought to establish if the alteration in these hormones had any effect on glucose metabolism in adult KO male mice. Methods: By immunohistochemistry the pancreata were analyzed for islet abundance and morphometry. *In vivo*, GTT was performed to study beta cell response. Pancreatic IGF-I and Pdx-1 mRNA expression were studied by real-time PCR. Results: During GTT, glucose levels in the KO were lower than in WT at 60 and 120 min. Furthermore, insulin secretion was higher in KO mice at 30, 60 and 120 min. When HOMA IR values were calculated, at 30 min, units were higher in KO mice indicating impaired insulin sensitivity. Preliminary data on the morphometry of the pancreas (n=3) showed that the adult KO mice had a similar percentage of islet area compared with the WT. Pancreatic mRNA expression of IGF-I and Pdx-1 was lower in the KO mice, while glucagon was increased. These results show that glucose metabolism is altered in D2R KO mice. There is an increased insulin resistance, and a decrease in the expression of genes related to beta cell function in the pancreas, also revealed in a reduction in HOMA beta cell index. These findings may be related to a faulty imprinting of the pancreas due to neonatal GH deficiency, or to the effects of chronic hyperprolactinemia observed in our KO mice.

59.

BROILERS´ THYMUS NEUROTROPHINS RECEPTORS EXPRESSION (*Gallus domesticus)*

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Neurotrophins are growing factors that take part in some neuronal populations' differentiation, development and maintenance .Some neurotrophins receptors´ variants with no defined function had been detected in other tissues (immune system, ovary, liver, kidney, etc). The main goal of this work is to identify high affinity neurotrophins receptors (Trk) in broilers´ thymus. Samples from twenty thirty days old chicken thymus were fixed in Bouin and buffered formaldehyde and processed with histological routine technique .Then, colored with H/E and inmunocitochemestry (LSBA) was applied to detect Trk proteins using rabbit polyclonal antibodies (Santa Cruz Biotecnnology, CA, USA). Samples were separated by SDS-PAGE and analyzed by WB SDS-PAGE using same polyclonal antibodies, in samples processed for Trak A Western blot receptors were located in medullar area, around Hassal corpuscles and capsule zone. TrkB was observed only in medullary dendritic cells. Trk C was not identified with ICQ, besides with Western blot, protein is found in its complete 140 and 90 kDal form as well as for TrkA and TrkB. TrkC molecular weight was about 50 kDal. Analysis results show that neurotrophins thymus Trk A and Trk B receptors were located in stromal cells while Trk C was only detected by Western blot. Thymus epithelial cells with Trk A receptors would modulate growing factor that is essential for its development. Also, neurotrophins would develop an adequate environment for tymocytes maturation. Researches continue to establish the neurotrophins role and their receptors in broilers´ immune system.

60.

EARLY CHANGES IN NITRIC OXIDE EXPRESSION IN ASPHYCTIC RETINA

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Perinatal asphyxia (PA) induces retinal alterations that are compatible with the pathological changes observed in the phase II of the retinopathy of the prematurity (ROP). In previous work we have shown that nitric oxide (NO) is an important neurotoxic agent in neurons of the central nervous system after long-term PA. Little is known about the short-term effects of PA, then in this work we evaluated the changes in retinal NO in 21 days-old-rats submitted to PA at 37°C or 15°C (hypothermia treatment). Immunohistochemistry for neuronal nitric oxide synthase (nNOS) showed strong staining in amacrine and ganglionar neurons in both, control and PA group. A significant increase (30%; $p < 0.01$) of the relative optic density (ROD) was observed for nNOS immunoreactivity in PA respect the control group. We also had observed a significant increment in the ROD for nitrotyrosin immunoreactivity after PA in ganglionar neurons (25% more than in control $p < 0.01$). Since nitrosylation is an indicator of peroxinitrite production this data is consistent with the ganglionar neuron death observed at long term PA by electron microscopy. Finally hypothermia avoids the neurotoxic effects of NO. Therefore, this data suggests that the neurotoxic NO effect in retina may starts at early moments after PA. Furthermore hypothermia treatment emerges as good therapeutic tools for ophthalmologic pathologies.

(UBACYT- M020 - CONICET PID 5782).

61.

PROGESTERONE (PROG) PREVENTS CELL DEATH AND MOTONEURON LOSS IN ORGANOTYPIC SPINAL CORD CULTURES AFTER TRAUMATIC DAMAGE

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PROG exerts protective and trophics effects throughout the central nervous system after injury. We developed a model of trauma in organotypic spinal cord cultures (OSCC) to study the mechanism of PROG neuroprotection. Spinal slices of three week-old mice were incubated at 35ºC for one week. PROG(10μM) was added to the medium one hour before the injury. The mechanical trauma was elicited using a weight drop model of injury. Following the spinal cord injury(SCI), we studied the effects of the injury and PROG on cell death, and the number of motoneurons. Assessement of injury-induced cell death was performed using propidium iodide(PI) and lactate dehydrogenase(LDH) activity in the culture medium. The identification of neurons was done by immunofluorescence using a monoclonal antibody anti neuron-specific transcriptional factor(NeuN). The number of PI positive cells and LDH activity after injury was higher in SCI group than in control slices, showing that cell death was increased in injured groups. PROG added to injured slices decreased the number of dead cells. SCI also induced a large loss of ventral motor neurons. However, the incubation of SCI slices with PROG increased the number of motoneurons showing a clear neuroprotective effect. To confirm the role of the intracellular PROG receptor (PR) in mediating the effect of PROG, we measured the number of NeuN and PI positive cells after PROG treatment in injury slices of PRKO. PROG restored the evaluated parameters only in 50% of the PRKO mice. Therefore, PR is not completely necessary for the effect of PROG on neuroprotecion. This observation, focused our attention to study alternative mechanisms of PROG action. Using RT-PCR three isoforms of membrane PR (α,β,γ) were detected in injured slices exposed to PROG. Our results demonstrated that the effect of PROG on the number of motoneurons and the number of cell death in spinal cord slices, could be mediated by PR and by alternative mechanisms such as mPR.

62.

ABNORMALITIES OF THE HIPPOCAMPUS ARE SIMILAR IN DOCA-SALT HYPERTENSIVE RATS AND SPONTANEOUSLY HYPERTENSIVE RATS

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Hippocampal neuropathology is a recognized feature of the brain of spontaneously hypertensive rats (SHR), but similar studies are lacking in another model of hypertension, the mineralocorticoid-salt treated rats. Our objective was to compare changes in hippocampal parameters in 16 week–old male SHR (BP≈ 190 mm Hg) and their normotensive Wistar Kyoto controls, with those of male Sprague-Dawley rats receiving (a) 10 mg DOCA every other day during 3 weeks and drinking 1% NaCl solution (BP≈ 160 mm Hg) and normotensive controls treated with (b) DOCA and drinking water, (c) drinking water only or (d) 1% NaCl only. In these experimental groups we determined: I) cell proliferation in dentate gyrus (DG) using the BrdU labelling technique; II) the number of glial fibrillary acidic protein (GFAP) positive astrocytes under the CA1, CA3 and DG; III) the number of apolipoprotein E (ApoE) positive astrocytes as a marker of potential neuronal damage, and IV) the number of neurons in the hilus of the DG. Changes were remarkably similar in both models: decreased cell proliferation in DG (WKY:55.6±5.55, SHR:31.31±3.28 $p<0.01$; CTL-H2O: 26.5±2.29, DOCA+SALT: 11.62±1.88 $p<0.01$ + cells/hemiDG), an increased number of astrocytes immunopositive for GFAP and ApoE (GFAP: CA1: WKY: 142.85±12.40,SHR: 279.54±16.07 $p<0.001$; CTL-H2O: 120.6±9.29, DOCA+SALT: 201.81±14.80 $p<0.001$ +astrocytes/mm2). The number of hilar neurons was 37% lower in SHR and DOCA+SALT compared to their normotensive controls. While hypertension may be a leading factor for these abnormalities, endocrine mechanisms may contribute, since hypothalamic-pituitary function, mineralocorticoid receptors and sensitivity to mineralocorticoid treatment are stimulated in SHR, whereas high exogenous mineralocorticoid levels circulate in DOCA-treated rats. Thus, in addition to the deleterious effects of hypertension, endocrine factors may contribute to the abnormalities of hippocampus in SHR and DOCA-treated rats.

63.

IMMUNOCYTOCHEMICAL IDENTIFICATION OF BOVINE PARS DISTALIS TYPE CELLS AND PARS TUBERALIS SPECIFIC CELLS (INTO A CYCLE LIGHT-DARKNESS 12:12)

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Pars tuberalis (PT) of adenohypophysis contains two types of cells, follicular and secretory. Secretory cells, in turn, has been reported to have two groups of cells; one is composed by cells present in Pars distalis (PD) and called "PD type" cells, the other is constituted by the specific cells of PT. The main objective of the present work was to identify the cell types present in the PT portion of the bovine pituitary gland. Neural stems of bovine brain were fixed by immersion in Zamboni solution and processed for inclusion in metacrylate. Peroxydase-antiperoxydase method (Sternberger *et al*. 1970) was used for immunocytochemical detection of different hormones of PD using monoclonal antibodies (TSH, LH, FSH, GH, ACTH, PRL, β endorfine) and a polyclonal antibody against a PT specific cells secretion product which we named as FB 12. Results show a great number of cells that reacts against FB 12 antibody which is located close to medium eminence (EM); however, that did not react against hypophyseal hormones antibodies. The antiserum FB 12 was used at 1:8000 and 1:16000 dilution over bovine hypophysis, no cellular type reacted under those conditions. Immunocytochemical study results, in which adenohypophyseal hormone antibodies were used, show that in bovine PT there is a few cellular groups reacting against LH, FSH, LTH and TSH; whereas, cells reacting against ACTH, GH and β endorfine were almost undetected. Altogether, these results suggest that PT cell population is mostly specific cell type.

64.

EVIDENCES OF GNRH-II PRESENCE IN RAT BRAIN AND PITUITARY

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The GnRH isoform known as GnRH-II is the most ubiquitous peptide of this family, being present from jawed fish to human beings. However, the presence of GnRH-II in such an important model as the rat is still object of discussion. Here we present chromatographic, immunologic and activity evidences supporting the expression of GnRH-II in the rat. Olfactory bulb, hypothalamus, remnant brain and anterior pituitary from a pool of 50 female adult rats were extracted and subjected to RP-HPLC on a Supelcosil LC-18 column. Fractions were collected and evaluated by two different RIA systems, specific for GnRH-I (antibody HU60, Dr. Urbanski) and for GnRH-II (antibody acII6, Dr. Okuzawa). Under these conditions the GnRH-I standard eluted in fraction 21 (f21) and was only detected with the GnRH-I RIA, whereas GnRH-II standard was only detected in the fraction 27 (f27) by GnRH-II RIA. In the olfactory bulb extract, the GnRH-I RIA system showed a peak in f21, whereas the GnRH-II RIA showed a single peak at f27. In the hypothalamus GnRH-I was detected in f21 whereas GnRH-II could not be detected. When the remnant brain and pituitary were analyzed, both GnRHs forms were detected. The immunological properties of f27 were tested in displacement experiments comparing to GnRH-II, and no differences were found. Previously, we reported that GnRH-II is able to release LH and FSH from rat pituitary cells, being this effect mediated by the classical GnRH type-I receptor. Here, we demonstrated that after 60min stimulation, both f27 and GnRH-II posses LH and FSH releasing activity in 12 day-old rat pituitary cell cultures (FSH ng/ml, C: 3.6±0.5, GnRH-II 10^{-9}M: 19.9±1.4, f27 10^{-9}M: 13.9±1.1, $p<0.01$ vs C; LH ng/ml, C: 12.2±0.8, GnRH-II 10^{-9}M: 45.0±6.5, f27 10^{-9}M: 27.8±2.4, $p<0.05$ vs C).
These results provide strong evidence for the expression of GnRH-II in rat with some regional distribution.
UBA, CONICET, ANPCYT.

65.

LIGHT INDUCED RETINAL DEGENERATION AND CORTICOSTERONE TREATMENT

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Continuous illumination (CI) induces retinal degeneration (RD) and increases glucocorticoid (GC) levels because it is a stressful condition. Our previous results have shown that adrenalectomy delayed RD induced by CI. The aim of the present work was to evaluate the relationship between different doses of CORT and its effect on the retina of adrenalectomized (ADX) rats. Adult male Sprague Dawley rats were ADX and submitted to CI (12000 lux) during 5 days. Along the illumination, animals were daily subcutaneously injected with either a high dose of CORT (HD-CORT= 4 mg/kg), a low dose of CORT (LD-CORT= 0.4 mg/kg), or vehicle (ADX-VH). Rats were sacrificed under anesthesia; eyes were fixed with 4% paraformaldehyde and embedded in paraplast. Sections were stained with hematoxylin-eosin and retinal thickness was determined using a Kontron-Vidas image analyzer. Data were statistically analyzed using the Student Newman-Keuls Multiple comparison test. After 5 days of illumination, retinal thickness of HD-CORT rats (167.3 ± 9.46 µm) was significantly thinner than in ADX-VH rats (193.21±9.06 µm $p<0.001$) or than in LD-CORT rats (192.06 ± 8.83 µm; $p<0.001$). No differences were found in the retinal thickness between LD-CORT rats and ADX-VH rats ($p > 0.05$). In summary, high doses of CORT contribute to RD while low doses seem to be innocuous. The present results support the idea that a stressful situation may contribute to the deleterious effect of CI due to high circulating levels of steroids.

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66.

PITUITARY PROLACTIN ISOFORMS IN TWO EXPERIMENTAL MODELS

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The oligosaccharide structure of glycoprotein hormones has been shown to play a key role in the secretion, metabolic fate and bioactivity of the hormone. Biologic versatility of Prolactin (PRL) hormone has been attributed to its microheterogeneity.

The aim of this study was to characterize the charge isoforms of PRL in:

Pituitary of adult males Golden hamsters maintained under long photoperiod (LP, 14hsL:10hsD) and adult male under short photoperiod (SP, 6hsL:18hsD) for 14 weeks.

Pituitary of 12m-old female mice intact (fbb, WT) and pituitary Prolactinoma of 12m-old female Transgenic mice overexpressing the β-hCG gene (TG). (Carino M. *et al*. SAIC, 2003).

Isoform mixes were fractionized from homogenized tissue by isoelectric focusing (pH: 3-10) and PRL concentration was measured in each fraction using RIA.

Results (relative % PRL in each pI range):

Hamsters: LP: 21.24±1.83% (pI 5.15-5.17); 17.35±0.58% (pI 4.50-4.72); 6.88±1.30% (pI 4.33-4.35); SP: 45.29±1.02% (pI 5.42-5.47).

Mice: WT: 47.52±1.05% (pI 4.33-5.50); 16.35±0.92% (pI 6.00-7.20); 25.30±1.20% (pI 7.80-8.30); RT: 72.20±1.11% (pI 4.33-5.50); 12.70±1.80% (pI 6.00-7.20)

The present results suggest that:

Charge isoforms variations in pituitary hamster PRL are related to the photoperiod. Three isoforms (more acidic) prevail in LP and only one in SP.

Pituitary Prolactinomas show less isoform heterogeneity with predominance of more acidic in relation to control.

67.

FGF-2 MEDIATED PROLIFERATION AND ANGIOGENESIS IN THE PITUITARY OF DOPAMINERGIC D2 RECEPTOR (D2R) KNOCKOUT FEMALE MICE

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D2R knockout (ko) female mice develop chronic hyperprolactinemia and pituitary hyperplasia. Recently, we have shown that female ko mice have an overexpression of vascular endothelial growth factor (VEGF) in folliculoestellate cells, suggesting a paracrine action of VEGF on pituitary endothelial cells (Endocrinology 146:2952:2005). Numerous cytokines and growth factors modulate angiogenesis in different conditions. Among them, the type-2 Fibroblast Growth Factor (FGF2) exerts its mitogenic action on a wide variety of cells, including endothelial and fibroblasts. We therefore wanted to study the participation of FGF2 in our experimental model. We observed that the conditioned media (CM) from *in vitro* pituitary cells of both genotypes enhanced the HUVECs growth, and an antibody against FGF2 blocked the action of the CM from ko mice (48% reduction) while in the wt a 30% reduction was observed, this was consistent with an increased FGF receptor expression in kos as determined by Western Blot. By immunohistochemistry we found FGF2 in the pituitaries, with a preferential localization in the basal membrane but also in endocrine cells. In ^{3}H-Thymidine captation assay 10 ng/ml FGF2 induced the proliferation of cells from both genotypes (cpm for ko: 494 $\pm$ 29 and 632 $\pm$ 33, wt: 985 $\pm$ 52 and 1278 $\pm$ 106, control and FGF2 respectively $p< 0.05$) and also increased prolactin secretion. When we studied the mechanism of signal transduction involved in the proliferative action of FGF2, ko cells showed a higher activation of MAPKs than wt cells (at 5 min. 236$\pm$ 30% and 158$\pm$ 8%, respectively, $p< 0.05$).

These results suggest the FGF2 may be involved in pituitary cell proliferation and secretion, as well as in angiogenesis in D2R ko female mice.

68.

SYNTHETIC STEROIDS WITH A SULFUR ATOM BIND TO GABA RECEPTOR FROM RAT CEREBELLUM

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Neuroactive steroids like alfa-hidroxi-alfa-pregnano-20-ona (ALLO) and its isomer 5ß modulate GABA action. The administration of these compounds is not useful for long term treatment because the rapid bio-transformation they suffer. The development of stable synthetic analogs with similar effect on the $GABA_A$ receptor would be useful to solve this problem. The interactions of three synthetic steroids containing a sulfur atom in position 6 with the GABA receptor have been evaluated. Studies were developed by competition assays using purified synaptosomes from male rat cerebellum. Changes in the binding of ^{35}S-tert-butylbicyclo-phosporothionate (TBPS, 10nM) and ^{3}H-flunitrazepam (FLU, 3nM) were evaluated in presence and absence of 5µM of GABA with the aggregate of increasing amounts (50-600nM) of ALLO, 6-tio-steroid (Ns1), its 6-sulfoxide (Ns2) or its 6-sulfone (Ns3). Incubations were carried on at 22ºC for 2h or at 4ºC for 90 min. and the nonspecific binding was determined using 2mM picrotoxin or 1mM diazepam respectively. The bound fraction was separated by rapid filtration through glass fiber filter. All steroids displaced TBPS binding in presence of 5µM of GABA, ALLO and Ns1 showed a similar behavior, whereas the other two steroids were less powerful ($p<0.05$). The IC_{50} obtained were: ALLO = 111.2±13nM; Ns1 = 179.8±19nM; Ns2 = 328.7±12nM; Ns3 = 360.1±16nM. All steroid also increase the FLU binding in the absence of GABA showing the following pattern Ns3>Ns2=Ns1=ALLO. The greater capacity of Ns1 to compete for TBPS binding site respect to the other two derivatives could be related to the absence of oxygen associated to the sulfur atom reducing its capacity to generate hydrogen bounds. Further '*in vitro*' and '*in vivo*' studies would be necessary to validate any of these synthetic steroids as a possible therapeutic drug.

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69.

PROGESTERONE (PROG) STIMULATES MYELINIZATION AND PROLONG THE SURVIVAL OF MICE WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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EAE is a demyelinating autoimmune disease of the Central Nervous System and a model of Multiple Sclerosis. As PROG was found to be neuroprotective in models of neurodegeneration and spinal cord injury, the main goal of this work was to analyze PROG possible effects in mice with primary demyelination. Female C57/BL6 mice were immunized with a myelin oligodendrocyte glycoprotein peptide (MOG40-54) emulsified in complete Freund´s adjuvant. A group of animals received a 20-mg pellet of PROG s.c seven days before the induction of the disease while another group of animals remained untreated. The following parameters were studied at the spinal cord level, using a computer-assisted image analysis system: 1-% of cellular infiltration in the white matter by Haematoxylin-eosin staining, 2-extent of demyelination by luxol fast blue staining and 3-astroglial reactivity by immunohistochemistry for glial fibrillary acidic protein (GFAP). Animals were clinically assessed daily and also evaluated in order to determinate if PROG prolonged the survival time. Results: We observed in treated (EAE+PROG) vs. untreated (EAE) animals: a- 40% reduction in the inflammatory cells infiltrated area (EAE+PROG:1.81±0.21% vs. EAE:4.56±1.46%; p=0.018). b- smaller extent of demyelination in white matter (EAE+PROG:8.18±0.93% vs. EAE:13.10±1.61%; p=0.021). c- the number of GFAP+ cells was not significantly modified by PROG treatment. Clinically, we observed a 45% significant increase of survival time in animals receiving PROG in comparison to the untreated group. (EAE:55% vs. EAE+PROG:100%;p=0.028). Conclusions: We found that PROG diminished the neuropathologic alterations evaluated in EAE. Whereas PROG could downregulate the immune response, increase remyelination by direct action on myelinating cells constitute an alternative and novel mechanism.

70.

EXPRESSION OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) IS DIMINISHED IN THE DENTATE GYRUS OF STREPTOZOTOCIN (STZ) DIABETIC MICE: REVERSION BY FLUOXETINE TREATMENT

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Type 1 diabetes correlates with several disturbances of the CNS in humans and in experimental animal models. The hippocampus is one of the most vulnerable structures. Previously we reported that STZ diabetic mice showed less dentate gyrus neurogenesis than controls and that a 10-day treatment with fluoxetine (FXT, 10 mg/kg BW) reversed this situation. In the present work our objective was to determine BDNF mRNA in the hippocampus of diabetic mice and the effect of FXT on this factor, which is closely related to neuronal plasticity and functionality. Male adult C57BL/6 mice were given STZ (195 mg/kg BW) and, after 10 days, FXT or vehicle was given for 10 more days. We performed in situ hybridization (ISH) with a S35 labeled probe to measure BDNF mRNA and quantified the optical density of the dentate gyrus in the autoradiographic films. In diabetic mice, BDNF mRNA level was significantly lower than in controls (CTL:129±4.3, STZ:110±2.75, $p<0.01$) and FXT reversed completely the effect of diabetes, leading to levels similar than in controls (STZ+FXT:125.4±3.0, $p<0.05$ vs. STZ; CTL+FXT:123.4±2.0). In another group of mice we performed non isotopic ISH for BDNF mRNA and BrdU detection of proliferating cells in the dentate gyrus and we found that recently divided cells are positive for BDNF mRNA. These results suggest that in the diabetic brain the expression of trophic factors is altered and that BDNF plays and a role in the action of FXT in the neurogenic process and over adult granule cells.

71.

EFFECTS OF cGnRH-I PULSE-FREQUENCIES ON FSH AND LH RELEASE FROM GONADOTROPES OF LAYING HEN *in vitro,* DETERMINED BY ELISAs

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cGnRH-I is the predominant gonadotropin-releasing hormone in adult birds which contributes to the differential regulation of LH and FSH biosynthesis and secretion. The aim of this work was to determine FSH and LH release on cultured laying-hen pituitary cells after administration of 1 or 10 nM cGnRH-I by four 5 minute-pulses every 15, 30 or 60 minutes. Pulse-frequency and dose-dependent effects were determined in six experiments including controls. After the last interpulse time the supernatants were collected and stored at –70ºC until the performance of an indirect Enzyme Linked Immunosorbent Assay (ELISA) using cLH and cFSH antisera in 1:1000 and 1:2000 dilutions, respectively. Supernatants were coated in duplicate on the inner surface of Immulon 2 plates and later blocked with the optimal solutions. They were incubated with each antiserum and subsequently with isotype-specific peroxidase-labelled anti-rabbit antibodies. H_2O_2/ *o*-phenylenediamine was added as substrate/chromogen and the optical density (OD) was determined at 492 nm. The ODs obtained for each anti-hormone were compared with the control groups and between each other. cGnRH-I administered in pulses every 15 and 30 minutes caused release of FSH with both doses every 15 minutes, while the 10 nM dose caused the same effect with pulses every 30 minutes. On the other hand, LH was released only with 10 nM dose of cGnRH-I administered every 60 minutes. In conclusion, high pulse-frequencies (15-30 minutes) of cGnRH-I increased FSH release in a dose-independent and dose-dependent manner respectively, while the lower pulse-frequency (60 minutes) increased LH release dose-dependently.

72.

HYPOTHALAMIC-PITUITARY-GONADAL AXIS EVALUATION IN $GABA_{B1}$ KO FEMALE MICE: CYCLICITY AND REPRODUCTIVE FUNCTION

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GABA, main inhibitory neurotransmitter, participates in endocrine regulation through receptors A, B or C. Recently, BALB/C mice lacking $GABA_{B1}$ subunit expression (KO) was developed (Neuron 2001, 31:47-58), exhibiting profound neurological alterations. We have demonstrated basal hyperprolactinemia in males and advanced LH post-castration rise in females (Medicina 64, II, p 167, 2004). Here we evaluated cyclicity and reproductive function in female KO mice and wild type controls (WT) from the same colony.
Estrous cyclicity: vaginal lavages were obtained daily from adult KO and WT mice for a fifty-day period (n: 18-22). *Reproductive function:* female adult mice of both genotypes were exposed to males of known fertility for a two-month period. Days to first delivery, the percentage of female mice pregnant after 30 days of male exposure and the number of pups/litter were recorded (n: 9-11). Female KO mice exhibited an extended estrus ($p<0.001$) and reduced proestrus period ($p<0.001$) as compared to WT controls. The number of mice becoming pregnant in the first 30 days of male exposure was significantly reduced in KO animals (% of pregnancies: WT: 87.5%, n=8 vs. KO: 37.5%, n=8, χ^2 test: $p<0.05$). The interval between exposing female KO mice to a male and delivery of the first litter was lengthened, though not attaining statistical significance ($p<0.11$). Only 1/8 WT vs. 3/8 KO females failed to get pregnant in the evaluated period. No difference in the number of pups/litter between genotypes was detected.
Conclusions: Female KO mice exhibited altered cyclicity and compromised reproductive function.
UBA-CONICET-ANPCyT.

73.

EXPRESSION OF THREE GnRH FORMS, FSH-β AND LH-α SUBUNITS DURING SEX DIFFERENTIATION AND AT DIFFERENT GONADAL STAGES IN THE PEJERREY FISH, *Odontesthes bonariensis*

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GnRH plays a pivotal role in the reproduction of all vertebrates. The primary function is to stimulate the synthesis and release of LH and FSH.
The presence of three GnRH variants is a common pattern in teleosts; however, the role of each form is not known. In this context, the objective of this work was to follow GnRHs and GtHs expression at different gonadal stages in pejerrey by semiquantitative-PCR. Also, GnRH gonadal expression was determined. The pejerrey possesses marked thermolabile sex determination and it was showed that the number of GnRH neurons in the preoptic area peaked during the temperature sensitive window. GnRH expression was also measured during the sex differentiation period. In adult fish, at the brain-pitutary level, pejerrey females showed a tendency to increase the mRNA levels of the three GnRH forms during mid vitellogenesis compared to previtelogenic and post-spawning stages. Males did not show a clear pattern maybe due to the fact that they showed an expanded spermiating period. At the pituitary level only the hypothalamic GnRH (pjGnRH) form was shown to be expressed at all stages. With respect to both gonadotropins a tendency to show a greater expression during early stages of gonadogenesis was observed in both sexes as already demonstrated in other multiple spawner teleost species. GnRH variants were also demonstrated to be expressed either at the ovaries and testis. The expression pattern of GnRHs in the gonads also showed seasonal and stage-dependent variations.These results represent the first step to understand the function of different GnRH forms in the teleost brain and gonads.
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74.

LIPOFUCSIN DEPOSITION IN THE DIGESTIVE GLAND OF LIMPET (*Nacella concinna)* EXPOSED TO ANTARCTIC GASOIL

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Lipofucsin deposition is a cytochemical parameter used to evaluate the effects of exposition to metallic and organic contamitants. There is information about the increment of lipofucsin in *N. concinna* as a response to the exposition to high temperatures, hydrogen peroxide and Cd, but no data is available to the exposition to antarctic gasoil (GOA). The objective of the present work was to evaluate variations in lipofucsin deposition in limpet exposed to GOA at two different concentrations and exposition times. Limpet were collected on 25 de Mayo Island (Antarctica) in 2002, and exposed to 0,05 y 0.1% GOA for 2 and 7days. The digestive glands were fixed in Bouin and embedded in paraffin. For each individual, the area of lipofucsin granules was estimated as web as the optical density in six areas of the glandular epithelium. The Schmorl reaction was used for the lipofucsin detection. Estimations were performed on digitalized images by using Image Pro Plus. Results are expressed as percentage of lipofucsin area in relation to the epithelial area epithelial and analyzed by ANOVA. Results indicate a marked increment of lipofucsins in time (0,92 a 3%) in limpet exposed to 0.05 GOA. After 7 days, the lipofucsin area in individuals exposed to 0.05 and 0.1, was 4 and 2 times greater than controls, respectively (3 and 1,21 vs 0,68). After 2 days, there were no significant differences between control and treated individuals.The correlation coefficient between lipofucsin areas and optical density was 0.97. The increment of lipofucsin in the digestive gland of *N. concinna* would be due to peroxidation reactions of lipoproteins as a consequence of oxidative stress induced by GOA.

75.

CHEMICAL COMPONENTS AND ANTIULCEROGENIC ACTIVITY OF *BACCHARIS POLIFOLIA* GRISEB

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The interest in the phytochemical study of the species belonging to the genus *Baccharis* derives from the fact that several of them, particularly those widely used in popular medicine, have been reported to have significant pharmacological and therapeutics properties. *Baccharis polifolia* Griseb, placed in the Cuyo region, have been used in folk medicine for gastrointestinal disorders. The aim of this study was to identify and characterize secondary metabolites present in *B. polifolia.* The methodology employed was the usual one in chemical-pharmacological investigations of natural product studies. From the chromatographic resolution of the ethanolic soluble fraction of the methanol extract, previously deffated with hexane, were isolated nevadensina, gardenina B, xanthomicrol and 7-methylsudachitine. Identification was performed by uni-and bidimensional spectroscopic techniques ^{1}H-NMR and ^{13}C-NMR and GC-ME combined techniques. The antiulcerogenic activity of *B. polifolia* was assessed in according to the method of Robert *et al.* Absolute ethanol administered orally was employed as necrotizing agent in Wistar rats. The degree of erosion was assessed from a scoring from 0 to 5 (maximal damage). Previously we have demonstrated that *B. polifolia* ethanolic extract prevents the formation of gastric lesions ($p<0.001$ vs. damage control). In this experiment, gardenina B and xanthomicrol decreased the ulceration size ($p<0.01$ vs. control). The higher activity of the ethanolic extract of *B. polifolia* can be accounted for the higher concentration of active compound in extract. These facts support the use in traditional medicine of *Baccharis polifolia* to treat digestive disorders.

76.

INDUCTION OF DNA AND PROLIFERATIVE KINECTIS DAMAGE IN CHINESE HAMSTER OVARY (CHO) CELLS BY INSECTICIDE PIRIMICARB

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Although various experimental data have provided evidence that pesticides can possess toxic properties in animals and in *in vitro* test systems after acute and chronic exposure, the information on the genotoxic effects of some of pesticides is limited and inconsistent. In the present study, the genotoxic potential of commonly used insecticide pirimicarb (2-dimethyl-amino-5,6-dimethyl-4-pirimidyl-dimethyl-carbamate) and one of its derivatives currently used in Argentina the Aficida® (Syngenta Agro S.A.) were studied in Chinese Hamster Ovary (CHO) cells by the analysis of the sister chromatid exchange (SCE), cell-cycle progression and single cell gel electrophoresis (SCGE) assays. The cells were incubated with the range of 5.0-200.0 μg/ml concentrations of the test substances for 24 at 37°C in 5% CO_2 by SCE and cell-cycle progression. In SCGE assay, concentrations of 50.0, 100.00 and 200.00 μg/ml of both compounds were used for 0.5 h at 37°C in 5% CO_2. Pirimicarb induced a significant increase in SCE frequency, a delay in the cell-cycle progression and single strand breaks over control values ($P<0.01$) when doses of 100.0 and 200.0 μg/ml were employed. Commercial formulation induced SCE and altered the rate of cell proliferation ($P<0.05$) only when 200.0 μg/ml was used. Based on these results, we demonstrate that the carbamate insecticide pirimicarb induces large DNA alterations and cytotoxic effects in CHO cells showing the need for additional studies on the genotoxicity of these substances and their adverse effects on human health.

77.
SAFETY EVALUATION OF *ARISTOLOCHIA ARGENTINA*: 14 DAYS STUDY IN RATS

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Aristolochia argentina (*Aa)* (family Aristolochiaceae), popularly known as "charrúa", is used in folk medicine as: antidiarrheic, astringent and antihemorroidal. The acute toxicity test after oral administration of 6000 mg/kg of *Aa* revealed non-toxicity at this dose. The aim of this study was to assess the toxicity at 14 days in rats. Infusion (10%) of the roots was prepared, 200 ml boiling water was poured onto 10 g *A.a.* and left to infuse for 20 min, then filtered and the moisture squeezed out. Wistar male and female rats were used. *Aa* was administered, p.o., at concentrations: 0 (control group), 0.25 (low-dose group), 0.5 (middle-dose group) and 1g/kg (high-dose group), respectively. Routine clinical observations, body weight and food consumption were measured. Peripheral blood was collected, hematology (RBC, WBC and leukocyte differential counts) and clinical chemical (ALT, AST, glucose, total protein, albumin) values were evaluated. The organs of each rat were observed grossly, and the following organs were weighted and organ/body weight ratios were calculed: lungs, heart, liver, kidneys, spleen, testes and ovaries. Parametric ANOVA method was used. No abnormal symptoms and clinical signs or deaths had been found in rats in each group during the test. No significant difference had been found in body weight and food consumption of rats in each test group ($p>0.05$). In addition, no significant differences were found in each hematology value, clinical chemistry value and organ/body weight ratio, either ($p>0.05$). The highest dose did not induce noticeable signs of toxicity. These results suggest that the infusion of *Aa* is of low toxicity and has the potential for application.

78.
EVALUATION OF THE GASTROINTESTINAL ACTIVITY OF *HEDEOMA MULTIFLORUM* IN RATS

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Hedeoma multiflorum Benth (Labiadas), is commonly known as "peperina de las lomas", is widely spread in Argentine and used in popular medicine as stimulating, digestive, carminative and vulnerary. The objetive of the present work was to assess the biological activity in the gastrointestinal tract of extract and infusion of *Hedeoma multiflorum* in rats.
Determination of gastric cytoprotective activity of dichloromethane extract and infusions of *H. multiflorum* in rats: The ulcer experimental model of gastric lesions were produced in according to the method of Robert *et al*. Absolute ethanol administered orally was employed as the necrotizing agent. The degree of erosion was assessed from a scoring system designed by Marazzi-Uberti and Turba. The results were expressed in terms of an ulcer index. Determination of small intestinal transit in mice: The effect of 10% infusion of *H. multiflorum* on small intestinal transit was tested using Ueda *et al*. method. The length traversed by the charcoal marker was calculated as a percentage of the intestine length. The statistical significance of difference among means was assessed by Student's t-test or analysis of variance (ANOVA) with multiple comparison method by Tukey-Kramer. The experimental results demonstrate that 10% infusion of *H. multiflorum* decreased small intestinal transit in mice ($p<0.05$). Moreover, 10% and 20% infusions of *H. multiflorum* do not prevent gastric mucosal damage induced by absolute ethanol in rats. The extract of *H. multiflorum* (250 and 500 mg/kg, p. o.) protects the gastric mucosa against the lesions induced by ethanol ($p<0.01$ vs. control). These facts support the use in traditional medicine of *Hedeoma multiflorum* to treat digestive disorders.

79.
DICAMBA EFFECT PRELIMINARY RESULTS ON MAMMALIAN CELL CULTURES

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Dicamba (2-methoxy-3,6-dicholorobenzoic acid) is an auxin herbicide widely employed to control broadleaf weeds in Argentina. Actually it is considered a class III product for its limited evidence for carcinogenicity according to IARC. It exerts its toxic effect by inhalation, contact or contaminated food ingestion. The aim of the present work was to assess the genotoxic effect of dicamba and its commercial formulation Banvel (dicamba 57.71%) on mammalian cells cultures. Induced damaged was quantified by the analysis of the sister chromatid exchange (SCE), cell-cycle proliferation (CCP), mitotic index (MI), cell viability (CV) and single cell gel electrophoresis (SCGE) assays. First four mentioned end-points were performed on human lymphocytes (HL) treated with 10, 50, 100, 200 and 500 µg/ml dicamba or Bavel doses and kept at 37°C and CO_2 5% till fixation. CHO (chinese hamster ovary) cells were exposed for 90 min with either compounds in the range of 50-200 µg/ml for SCGE. Results revealed that: 1) the 200 µg/ml dicamba dose induced a SCE significant increase ($p<0.01$) 2) Banvel induced a slight SCH augment not statistically significant; 3) HL treated by dicamba (100 µg/ml) and Banvel (200 µg/ml) presented a cell cycle delay; 4) the MI showed a significant decrease ($p<0.05$) in a dose-dependent manner; 5) VC registered a high diminution from the 50 µg/ml for both compounds; 6) the 500 µg/ml dicamba dose resulted cytotoxic; 7) only dicamba induced single strand breaks when 100 µg/ml were used. Results found suggest that dicamba is a DNA damage inducer and alters proliferative kynetics. More detailed studies are required to better characterize the mechanisms underlying dicamba genotoxic effects.

80.
CELLULAR ALTERATIONS IN DIGESTIVE GLAND OF LIMPET (*Nacella concinna*) EXPOSED TO CADMIUM

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In mollusk, pollution mainly affects the digestive gland and alters differently the behavior of basophilic (secretory) and acidophilic (digestive) cells. The objective of the present work is to evaluate numeric variations, level of vacuolation of these cellular types and height of basophilic cells in *N. concinna* exposed to two cadmium (Cd) concentrations for different times.
Animals were sampled in Antarctica (March, 2000). Collected limpet (40) were exposed to 0,25 and 0,5 $mg.l^{-1}$ Cl_2Cd in two fishbowls with filtered sea water . For each treatment, 4 limpet were removed at 0, 3, 7, 11, 17 and 25 days. Digestive glands were fixed in Bouin and embedded in paraffin. In 40 acini per animal the percentage of digestive and basophilic cells was estimated. The basophilic cells height was measured with a micrometric ocular. To evaluate the vacuolation, 3 degrees were determined, grade 3 indicating the presence of vacuoles in all the cell. Variables were analyzed by ANOVA. Results showed that at the highest concentration and exposition time, the percentage of acidophilics decreases while the percentage and height of basophilics increase. Vacuolation increased in both cell types. The decrease of acidophilics and their high vacuolation degree may be to lysosomal alterations driving to a progresive cellular destruction. The increment of basophilics and their height, along with their high vacuolation could be associated with an increased secretion of digestive enzymes and metal-bounding proteins. Basophilic cells would replace in time the lost acidophilic cells.

81.

EFFECT OF CADMIUM ON NADPH GENERATING ENZYMES IN DM 4800 SOYBEAN (*Glycine max.L*)

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The role of NADPH generating enzymes: isocitrate dehydrogenase (ICD), malic dehydrogenase (MD) and glucose 6 phosphate dehydrogenase (G6PD) in the antioxidative response of soybean roots to cadmium contamination was studied. Grown plants, were exposed on the 10th day of adaptation to hydroponic conditions in Hoagland's solution to intoxication with cadmium (40uM) during 0, 4, 6 and 24 hours. The enzymatic activity of ICD, MD and G6PD was determined in soybean roots. Results: ICD increased its specific activity in relation to the time of treatment with CD ($p<0.05$ time 4h, $p<0.01$ time 24h). The activity of G6PD decreased at 4 and 24 h ($p<0.01$). MD did not show any significant differences. We can suggest that cadmium alters the NADPH production, demonstrating an essential role of this cofactor to maintain the reduction equivalents of the GSH- GSSG system as part of the antioxidant defense responses, depending on the enzyme and time of treatment.

82.

DIURETIC ACTIVITY OF *Tetraglochin alatum* (ROSACEAE) IN RATS

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Tetraglochin alatum is a plant from the Rosaceae family commonly known as "espina de pescado", "horizonte" or "yerba de la perdiz" that grows in the central precordillera region of Argentina. The infusion of this specie is widely used in folk medicine as a diuretic agent. The purpose of this study is to determine the diuretic activity of *T. alatum* infusion in rats.

Adult Wistar rats, both sexes, were used and an infusion of the aerial parts of the plant at 10% was prepared according to Pharmacopea Argentina VI ed., also phytochemical assays were carried out. Control (saline solution) and Furosemide (reference drug) groups were established, all animal received orally the solutions according to Lipschitz *et al.*

The preliminary phytochemical assays showed the occurrence of flavonoids, genines, and saponins among others compounds, while rats treated with *T. alatum* infusion showed a significative diuretic effect respect the control one, $p< 0.001$. Flavonoids are responsible for diuretic effect in other vegetable species according to literature, therefore all compounds isolated from this plant will be submitted for chemical identification and diuretic assays each one.

83.

PROSTAGLANDINS AND NITRIC OXIDE AS MEDIATORS OF CHALCONES FROM *ZUCCAGNIA PUNCTATA* GASTROPROTECTION IN RATS

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Zuccagnia punctata Cav. is a monotypic species of the Fabaceae, known under the common name of "jarilla macho". Pederiva *et al.* reported the isolation of 2',4'-dihidroxychalcone (1) and 2',4'-dihidroxy-3'-methoxychalcone (2) as major constituents from the leaf resin of *Zuccagnia punctata*. In this study, role of prostaglandins (PG) and nitric oxide (NO) in the gastroprotection induced by 2',4'-dihidroxychalcone and 2',4'-dihidroxy-3'-methoxychalcone were evaluated. Gastric lesions, in Wistar rats, were produced according to the method of Robert *et al*. Absolute ethanol administered orally was employed as necrotizing agent. The degree of erosion was assessed from a scoring from 0 to 5 (maximal damage).The compounds studied, (1) (100 mg/kg, 1 ml p.o.) and (2) (100 mg/kg, 1 ml p.o.), prevent damage induced by absolute ethanol in rats. Both indometacin (10 mg/kg, i.p.), a PG synthesis inhibitor, and L-NNA (40 mg/kg, i. v.), NO synthase inhibitor, antagonised gastroprotective activity of (1) and (2). The last effect was reversed by L-Arg (400 mg/kg, i. v.). Statistical evaluation was performed using analysis of variance (ANOVA) with the multiple comparison method of Tukey-Kramer. Conclusion: Both compounds isolated from *Zuccagnia punctata*, 2',4'-dihidroxychalcone and 2',4'-dihidroxy-3'-methoxychalcone, prevent the formation of gastric lesions induced by absolute ethanol as necrotising agent. These findings suggest that prostaglandins and nitric oxide participate in gastroprotective mechanism of 2',4'-dihidroxychalcone and 2',4'-dihidroxy-3'-methoxychalcone, probably with effects in microcirculation and mucus production.

84.

SEASONAL VARIATIONS IN THE EXPRESIÓN OF THE mRNA ENCODING β_1-ADRENOCEPTOR AND AA-NAT ENZYME, AND IN THE AA-NAT ACTIVITY IN PINEAL GLAND OF VISCACHA - CORRELATION WITH SERUM MELATONIN

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Melatonin is a key molecule in the regulation of multiple functions of circadian and seasonal physiology such as reproduction. In photoperiodic mammals, melatonin plays an important role in adjusting seasonal reproductive capability. Viscacha (*Lagostomus maximus maximus*) is a photoperiodic and nocturnal rodent that exhibit an annual reproductive cycle. The aim of this study was to investigate whether biochemical parameters involved in melatonin synthesis in viscacha pineal gland exhibited an annual rhythm in parallel with the annual reproductive cycle.

The mRNA encoding β_1-adrenoceptor and mRNA encoding AA-NAT (arylalkylamine N-acetyltransferase) enzyme were determined by use of *in situ* hybridization. Activity of AA-NAT enzyme and serum melatonin were determined by radiometric method and radioimmunoassay, respectively.

An annual variation of mRNA encoding β_1-adrenoceptor was shown, with a maximum during autumn and winter. The mRNA encoding AA-NAT enzyme also exhibited an annual rhythm with the lowest and highest levels in May and August, respectively. The activity of AA-NAT enzyme, also reached a maximum in August. Serum concentrations of melatonin showed an increase during winter.

Our results are in concordance with several biochemical and morphological parameters of the reproductive axis of the male viscacha, which support the reproductive rhythmicity of this rodent. Thus, our data suggest that the pineal gland and melatonin, which is activated via the sympathetic system, could be involved in the photoperiodically dependent annual reproductive behavior of viscacha.

85.
MECHANISM OF GASTROPROTECTION OF THE NEW ANTIULCER AGENT XANTHATIN

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Xanthatin was isolated from *Xanthium cavanillesii* Schouw, known as "abrojo". Previously we reported that X prevents damage induced by several ulcerogenic agents (J. Ethnopharmacol., 2005). In this investigation, new results are presented in order to elucidate the action mechanism of this molecule. In the present study we discuss the roles of nitric oxide (NO), sulfhydryls (SH) and prostaglandins (PG) in the gastroprotection by X in rats.Gastric lesions were produced according to the method of Robert *et al*. Pretreatment with *N*-ethylmaleimide (NEM), a sulfhydryl-blocker, reduced gastroprotection afforded by X. Previous treatment with indomethacin, a cyclooxygenase inhibitor, attenuated the protection produced by xanthatin against ethanol-induced gastric mucosal lesions indicating that prostaglandins participate in its gastroprotective mechanism. Moreover we demonstrated that the gastric cytoprotective effect is antagonized by the NO synthase inhibitor, N^G -nitro-L-arginine. The inhibitory action of N^G -nitro-L-arginine is reversed by L-arginine, but not D-arginine. The findings suggest that NO is involved in the gastroprotection induced by X. NO participates in the gastric defense mechanisms by regulating the gastric mucosal blood flow and gastric mucus secretion. There is evidence to suggest that NO exerts some regulation over the synthesis of prostaglandins. Sulfhydryl compounds may be important in maintaining gastric mucosal integrity. These results suggest that endogenous nitric oxide plays an important role in the gastroprotection of xanthatin and there is partial participation by prostaglandins and endogenous sulfhydryls.

86.
COMPARISON OF THE CYTOTOXICITY OF COPPER BETWEEN OVARY CELL LINES AND OSTEOBLASTS

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Copper is frequently used as biomaterial for dental and intrauterine devices, either in its pure form or as alloy component. In order to evaluate the cytotoxic effect of this metal, we studied the behaviour of an osteoblastic cell line (UMR 106) and one derived from Chinese Hamster ovary cells (CHO K1), in presence of copper. Cells were cultured in 35 mm Petry plates containing a central copper disk of 0.314 cm^2. Previous experiments have shown that copper gradually releases ions to the culture medium, increasing its concentration as exposure time is extended. Cell viability was analysed by means of the differential staining technique with ethidium bromide and acrydine orange which allowed the calculation of dead and live cells proportions for different exposure times (3 to 72 h). Results revealed the existence of a toxicity gradient related to the radial distance, which varied according to longer exposure times but maintained a distance/ death relationship. The same trend was observed for the cell mitotic index. These findings suggest that the cell monolayer allows radial diffusion, which is similar for both cell lines, independently from the level of copper ion concentration in the culture medium.

87.
TREATMENT OF INFLUENZA IN A HOSPITAL OF SAN LUIS

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The diseases of the respiratory system represented the prevalent diagnosis (40.24%) in the Hospital del Sur of San Luis' city. Inside them, pharyngitis, (18.42%), bronchitis (18.29%) and influenza (17.14%) were more frequent. Having in mind the transcendence of the influenza, our aim was to determine its distribution for age and sex and to analyze the realized prescriptions for this pathology. During June of 2004, a observational, cross-sectional and retrospective study was carried out. Age, sex, diagnoses and recipes of 1583 patients were registered. The prescriptions were analyzed according to ATC and the diagnoses according to the ICD-10 classification.
Distribution (%): for age: <15 years old 24.16; ≥15 years old 75.84. For sex: F 48.33; M 51.67. Prescriptions: dipyrone 40.83; ibuprofen 21.66; acetaminophen 15.83; dexamethasone 14.16; diphenhydramine 13.33; amoxicillin 12.15; methylprednisone 5.83; ketorolac 2.5 and diclofenac 2.5. These drugs were prescribed alone or in different combinations, being the principal dipyrone with corticosteroids 12.5.
The distribution of the influenza cases was major in adults that in children, there was not significant difference with respect to the sex. The nonsteroidal anti-inflammatory drugs (NSAIDs) were the drugs more prescribed, dipyrone, followed by ibuprofen and acetaminophen. Corticosteroids, antibiotics and antihistamines continue in importance. Some therapeutic irrationalities were observed, such as the use of antibiotics in this pathology of viral origin without report of associated pathologies; the overuse of dipyrone respect to ibuprofen and acetaminophen, whose efficacy and safety are recognized; and the utilization of combinations of 2 NSAIDs or 2 corticosteroids.