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C.1.

VITAMIN A DEFICIENCY INDUCES PROOXIDANT ENVIRONMENT, INFLAMMATION CHANGES IN LIPIDS METABOLISM AND HISTOARCHITECTURE IN AORTA

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We evaluated whether nutritional vitamin A deficiency generates oxidative stress and inflammation in rat aorta. Rats fed the vitamin A- deficient diet were characterized by sub-clinical plasma retinol concentration and showed increased serum and aorta concentrations of TBARS Lower activities of CuZnSOD, GPx, and CAT were observed in aorta of the vitamin A-deficient group. The binding activity of NF- kB was higher in vitamin A- deficient animals. NO production evaluated as nitrite increased in aorta and serum, associated with a higher expression of iNOS, eNOS and COX-2 in aorta of vitamin A-deficient rats. When the lipid metabolism in the aorta of rats fed on a vitamin-A-deficient diet was evaluated, we observed a hypolipidemic effect (lower serum triglyceride and cholesterol levels) and a decreased serum paraoxonase 1/arylesterase activity. The triglycerides, fatty acid synthesis and mRNA expression of diacylglycerol acyltransferase 1, total cholesterol, free and esterified cholesterol, and phospholipids were increased in the aorta of vitamin-A-deficient rats. The phospholipid compositions showed an increase in phosphatidylcholine (PC), phosphatidylinositol plus phosphatidylserine and phosphatidylethanolamine, a decrease in sphingomyelin. In the aorta, the increase in triglycerides was associated with The increased PC content was attributed to an increased synthesis, as measured by [methyl-(14)C]choline incorporation into PC and high CTP:phosphocholine cytidylyltransferase-alpha mRNA expression. The lipoprotein lipase and lectin-like oxidized low-density lipoprotein receptor 1 mRNA expression levels increased in the aorta of vitamin-A-deficient animals. The incorporation of vitamin A into the diet of vitamin-A-deficient rats reverted all the changes observed. Simultaneously we observed modifications in aorta morphology. Vitamin A deficiency could be considered a risk factor for vascular disease.

C.2.

CHRONIC STRESS, MODULATION OF GENE EXPRESSION IN THE HIPPOCAMPUS AND THE RELATIONSHIP WITH SYNAPTIC PLASTICITY

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Depression in humans is the consequence of genetic (susceptibility genes) as well as environmental (chronic stress) factors and neuronal remodeling is considered an important event in the response to chronic stress. We analyzed gene expression in the hippocampus of *Tupaia belangeri* subjected to psychosocial stress and mice subjected to restraint stress. Several genes were found to be down-regulated in their expression. Among them is M6a (membrane glycoprotein 6a). Analysis of M6a transcript shows that it is present in neuronal bodies of granular and pyramidal cells. M6a protein is located on neuronal plasma membrane of granular and pyramidal cells and in dendritic extensions. When primary cultures of hippocampal neurons were transfected with a clone expressing M6a, they showed about two-fold increase in the number of neurites and a higher filopodium/spine density. These dendritic protrusions were in close contact with axons, presumably indicating synaptic sites. The increase in cell extension number was also detectable after transfection of neuronal (N2A and PC12) and non-neural (COS-7) cell lines. Further demonstration of M6a involvement in neuronal development was obtained by RNA interference (RNAi). RNAi of M6a transcripts resulted in a decrease of about 50% in the number of spine/filopodia and in the number of synaptophisin (presynaptic) clusters. In summary, these results suggest that M6a is involved in the synaptic plasticity/neuronal remodeling known to occur during chronic stress.

References: *Eur J Neurosci.* 19:659, 2004; *J. Neurosci. Res.* 78: 702, 2004; *Proc Natl Acad Sci.* 102: 17196, 2005; *Reviews in the Neuroscience* 16: 43, 2005; *Biol. Psychiatry* 59: 244, 2006.

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C.3.
MECHANISMS OF NON-VISUAL PHOTOTRANSDUCTION IN VERTEBRATES

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In mammals, a non-image forming circuitry conveys photic information from the retina to the brain regulating diverse non-visual functions. In birds, the retina, pineal gland and likely deep brain components are photoreceptive. We investigated light perception, in the absence of functional photoreceptor cells (PRCs), in GUCY1* chickens that suffer PRC degeneration and blindness at hatch, by assessing the pupillary light reflex (PLR) and the synchronization of feeding rhythms to various 12:12 h light-dark (LD) cycles. Blind birds displayed light responses in both the PLR and the entrainment of feeding rhythms. Feeding rhythms were entrained to different LD cycles even when blind chickens had the head covered. Also, we observed PLRs to monochromatic light of 430, 480 and 500 nm in GUCY1* chickens with maximal responses at 480, resembling a melanopsin-like photopigment. We previously found that retinal ganglion cells (RGCs) contain clocks synthesizing melatonin rhythmically (Garbarino *et al*., JBC 2004). Now, we found that embryonic RGC cultures express ancestral rhabdomeric photoreceptor markers such as *Pax6, Brn3*, melanopsin and the G-protein q. After synchronization to a 12:12 h LD cycle, dark-maintained RGC cultures exhibited a daily variation in ^{3}H-melatonin levels, which was significantly inhibited by light (Contin *et al*., FASEB J 2006). This effect was further increased by the chromophore all-trans retinal, and suppressed by specific inhibitors of the invertebrate photo-cascade involving phosphoinositide hydrolysis and Ca^{2+} mobilization. The non-visual photoreceptive capability of RGCs in the inner retina can be essential to temporally regulate development and physiology in response to ambient light even in the absence of formal vision.

C.4.
REPAIR OF OXIDATIVE DNA DAMAGE IN MAMMALIAN CELLS

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DNA can be damaged by either endogenous or exogenous compounds. This is underscored by the cancer-prone phenotype of human cells defective in DNA repair processes. Reactive oxygen species (ROS), generated either by the normal metabolism of the cell or by exogenous agents, is at the origin of either modified bases or strand breaks in DNA, having genotoxic or mutagenic consequences. Organisms have evolved multiple DNA repair mechanisms. Repair of modified bases in DNA is accomplished through base excision repair (BER). BER is initiated by a specific DNA glycosylase that recognizes and excises the altered base to yield an abasic (AP) site. After cleavage of the AP site by APE1, re-synthesis and ligation take place. In mammalian cells, the highly mutagenic 8-oxo-G is recognised by the DNA glycosylase hOGG1. We have cloned and characterised the enzymatic activities of this protein as well as the impact of mutations or polymorphisms in the *OGG1* gene from tumours. The study of protein-protein interactions that allow a coordinated BER process lead us to explore the function of XRCC1 in BER. Although XRCC1, essential for the maintenance of genomic stability, has not a known enzymatic activity, it participates in the first step of BER by interacting and stimulating the human DNA glycosylases hOGG1, NEIL2, MPG and NTH as well as with APE1. *In vivo* localisation studies show that after genotoxic treatments these DNA glycosylases can be found associated with XRCC1 foci. We present a BER model in which XRCC1 is recruited to the repair of modified bases by the enzyme recognizing the lesion. XRCC1 would then coordinate the subsequent enzymatic steps and modulate the activities of all the proteins involved through a series of sequential interactions. Such a mechanism would avoid the exposure of potentially genotoxic DNA repair intermediates to the cellular milieu. Recent findings on hOGG1 modifications induced by a cellular oxidative stress will be presented. Results from cellular and biochemical approaches also suggest that BER might be taking place in specific nuclear structures upon induction of oxidised bases in DNA by an oxidative stress.

C.5.

OOCYTE MATURATION AS A MODEL FOR THE STUDY OF PROGESTERONE NON GENOMIC MECHANISM

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The amphibian oocyte is a very usseful experimental model for the studies of steroid action at the plasma membrane. In amphibians, progesterone interacts with the oocyte surface to trigger a complex chain of morphological and biochemestry events that induces germinal vesicle breackdown. We have interest to study the inmediate, non genomic, effects of progesterone on metabolic pathways which generated second messengers and initiate the meiotic oocyte maturation. This interest was focussed in the comparative study of formation of second messengers (phospholids, purines and calcium) derived of the activation of G protein/s of the oocyte membrane that participate in the progesterone induced meiosis resumption. Theres is some evidence that the progesterone receptor is coupled to heterotrimeric G ptoteins, which inhibits adenylate cyclase. Consistently, the cAMP-dependent kinase (PKA) is a potent inhibitor of oocyte maturation.
In addition to cAMP others messengers, such those generated by the hydroliys of membrane-bound phosphatidylinositol-4,5-bisphosphate (PIP2) could be involved in the maturation process. Thus, inhibition of PIP_2 hydrolysis with neomicine blocks the spontaneous maturation, suggesting that products of this process, DAG and IP_3 participate in the maturation. Moreover, the activation of the PKC, by means of phorbol ester induces the germinal vesicle breakdown in oocytes treated. Other lipids as arachidonic acid could be involved en progesterone induced maturation. Althoug there is little information concerning the participation of the AA cascade and the role of prostaglandins (PGs) in this process.
In the amphibians Ca^{++} has been proposed as a second messenger for maturation. In *Bufo arenarum* we demonstrated that the reinitiation of meiosis is a process independent of extracellular calcium and that oocyte require adequate levels on intracellular calcium for germinal vesicle breackdown to occur.

C.6.

ENVIRONMENTAL ASSESSMENTS: PURPOSE, BENEFITS, APPLICATIONS

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Although scientific knowledge and understanding of environment is behind our needs, the last years have provided with some assessment tools to make a progress in our interaction with it. Environment is complex, covers a large variety of topics, ethical, chemical or biological, between other issues. The application of the scientific method to the natural world requires acceptance of certain assumptions like the existence of world patterns in natural events, and that those patterns could be known and recognized via analysis and observation; that specific observations could be generalized and that generalizations should be tested and verified. Observations and generalizations have a certain degree of uncertainty. Probability is a way of expressing uncertainty of our observations. The high complexity of environmental issues has lead to the elaboration of several approaches and methodologies of assessment, some of them well established within regulations and a few decades of enforcement. In general, environmental assessment is defined like a process to predict the environmental effects of proposed initiatives before they are carried out. An environmental assessment identifies possible environmental effects; proposes measures to mitigate adverse effects; predicts whether there will be significant adverse environmental effects, even after the mitigation is implemented. They should be conducted as early as possible in the planning and proposal stages of a project for the analysis to be valuable to decision makers and to incorporate measures into the proposed plans. Although many steps help to identify possible environmental effects and provide the means to introduce mitigative measures, some projects are strongly objected by the public or the administration.

C.7.

LONG SHELF LIFE TOMATOES WITH FRUIT QUALITY: THE GENETIC CONTRIBUTION OF *LYCOPERSICON* GENETIC RESOURCES

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Long shelf life (SL) tomatoes (*Lycopersicon esculentum*) were currently obtained by genetic engineering or by crosses among normal and mutant genotypes (N and M). Both breeding strategies had disadvantages, since genetically modified crops are not accepted by consumers and M provoke unfavorable pleiotropic effects on fruit taste, texture and color. Exotic genotypes (E) of *Lycopersicon* are a source of genetic variability. We evaluated a set of N and M together with E, finding that E had longer SL than N, but shorter than M. They also had adequate values of fruit quality traits, though low weight (W). Crosses were made among N, M and E. Hybrids among N and E did not significantly differ from those among N and M for SL, while hybrids among M and E had the longest SL. Reduction in fruit quality was more noticeable in the hybrid among N and M. A divergent–antagonistic selection for SL and W was begun in a F_2 population between a national N cultivar ('Caimanta') and accession LA722 of *L. pimpinellifolium*. After six cycles of selection, 17 recombinant inbred lines were obtained, which combined fruit attributes from both parents. We study the genetic basis of these long SL tomatoes by molecular and biochemical approaches. AFLP fragments associated to SL, W, and other quality traits were detected, which could be applied in marker assisted selection. A distinctive protein profiling was found among ripening stages and among genotypes, accounting for the phenotypic variation of N, M and E fruits. A differential expression of glutamine synthetase and glutamate dehydrogenase was observed among N, M and E in green and mature fruits, while lower levels of glutamate in mature fruits were found in the longest SL genotypes.

Young Researcher´s Symposium:

C.8.

REGULATION OF ALTERNATIVE SPLICING BY THE CELLULAR MICROENVIRONMENT

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Alternative splicing is considered the most important source of protein diversity in vertebrates and its regulation by extracellular cues is a key event in the control of gene expression. We use mouse mammary cell lines and two different genes, fibronectin and Rac1, to study the linkage between the cellular microenvironment and the splicing machinery.

We found that a laminin-rich basement membrane down-regulates the inclusion of two fibronectin alternative regions, EDA and IIICS, through a JNK-dependent pathway in epithelial cells. Using a cell culture system that simulates mammary epithelial-stromal communication we found that soluble factors from a mammary mesenchymal cell-conditioned medium as well as different growth factors up-regulate the inclusion of these two alternative regions via PI 3-kinase. The laminin-rich basement membrane blocks the effect of the mammary mesenchymal cell-conditioned medium, extending the already proposed antagonism between these two pathways to the field of alternative splicing. These results highlight the fact that the splicing pattern of a single transcript is the read out of an intricate network of different signaling cascades**,** strengthening the view that the control of alternative splicing is as complex and relevant as transcriptional control, together accounting for the spatiotemporal requirements of gene expression.

We also found that the signaling pathway triggered by soluble factors not only affect mRNA splicing but also alter translation of reporter mRNAs containing a fibronectin EDA exonic splicing enhancer. These effects on splicing and translation are dependent on SR proteins and can be duplicated by over-expressing a constitutively active Akt, a downstream target of PI 3-kinase. These results show how SR protein activity is modified in response to extracellular cues leading to a concerted regulation of splicing and translation.

Expression of Rac1b, a splicing isoform of Rac1, is induced by exposure of normal mouse mammary epithelial cells to the matrix metalloprotease MMP-3, and Rac1b activity is required for MMP-3-triggered epithelial to mesenchymal transition, a phenomenon characterized by the loss of intact E-cadherin, increased motility and invasiveness, down-modulation of epithelial markers and up-regulation of mesenchymal ones. Rac1b is generated by inclusion of a 57-nucleotide exon, accumulates in tumors and shows transforming properties when over-expressed in cultured cells. We are currently investigating the *cis*-acting elements and *trans*-acting factors involved in the regulation of Rac1 alternative splicing by MMP-3.

C.9.
DYNAMIC INTERACTIONS BETWEEN 14-3-3S AND CELLULAR PHOSPHOPROTEINS

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The 14-3-3 proteins were described as a family of acidic proteins within the mammalian brain, implicated in a variety of human diseases. In 1995, was demonstrated for the first time that 14-3-3 proteins could bind specifically to phosphoserine-containing peptides, became the first example of a specific phosphoserine/threonine-binding protein (1, 2).
In our Lab recently applied three different predictors of intrinsically disordered protein regions to investigate disorder in partners for the binding of 14-3-3 proteins. Our results support a general involvement of intrinsically unstructured proteins in the binding to 14-3-3 proteins. We propose a mechanistic model, where many proteins acting as partners of 14-3-3 proteins could undergo disorder-to-order transition upon the binding. Molecular recognition involving disordered proteins is possible only if the match between the interacting proteins is strong enough to provide enough longevity so that the induced fit takes place between partners. Also, protein interactions are critically dependent on just a few residues. Joining these two ideas, we could identify the specific amino acid side chains (structurally constrained = anchors) that help to stabilize a native-like bound intermediate and those that stabilized the final complexes. These findings have important implications in the studies of the regulation of the 14-3-3 protein complexes, because whereas the Eukariotic Linear Motifs are present in all 14-3-3 protein partners, anchors residues are specific of each complex. *In vivo* studies of 14-3-3 and its partners fused to sequences of YFP fragments enable us to visualize protein interactions *in vivo* with minimal perturbation of the normal cellular environment. After co-transfection with these constructs, HeLa cells presented fluorescence in the cytoplasm only, due to the NES sequence in 14-3-3. Anchor mutants analyses are correlated with our *in sicilic* predictions.

References:

1. Mackintosh C (2004) *Biochem J* 381: 329-342; 2. Muslin AJ *et al*. (1996) *Cell* 84: 889-897; 3. Bustos DM, Iglesias AA (2006) *Proteins* 63: 35-42.

C.10.
A WINDOW TO THE CRETACEOUS OF PATAGONIA

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"La Buitrera" is a locality that belongs to the Candeleros Formation (90-95 mya) located in northern Río Negro Province. The well-preserved fossils show evidences of aerial exposure and association to paleosols and ichnofossils. The vertebrates from "La Buitrera" lived a peculiar moment commonly known as the "Era of the Giants". At that time several lineages of long-necked sauropods lived at Patagonia. Among the carnivorous dinosaurs are the mega-predator *Giganotosaurus*, abelisauroids and southern dromaeosaurids, related to birds, becoming abundant. No ornithischian dinosaurs have been found at "La Buitrera". Crocodiles include the fox-like, terrestrial araripesuchids, carrying only two lines of scutes and long legs. Both juvenile and adult specimens have been found. Eilenodontine sphenodontids like *Priosphenodon* are the most abundant reptiles at "La Buitrera", exceeding 200 specimens. Snakes are peculiar. The finding of *Najash*, a fossil snake with a sacrum, pelvis and strong and functional hindlimbs positioned outside the ribcage, and the plesiomorphical features in the skull, place this species as the basalmost known snake, including the scolecophidian fossorial forms, and the marine pachyophid snakes, now confirmed as an early radiation of macrostomatan snakes. Additionally, as it comes from a clearly terrestrial environment, contributes to an old debate, supporting a terrestrial rather than marine origin for snakes. The "La Buitrera" fauna provide us the very rare opportunity to contemplate the medium size faunal components of the Late Cretaceous of Patagonia, better known by their giants. The continuity of the work in the area remains implicit in the new yearly discoveries, like the pterosaurs found at 2005, now in preparation.

1.
CYTOKINE EFFECT ON JEG-3 CELLS: CORRELATION BETWEEN LEPTIN EXPRESSION AND METALLOPROTEASES ACTIVITY AND EXPRESSION

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Previously, we reported that JEG-3 cells (model for cytotrophoblasts) express leptin and metalloproteases (MMP-9 and 2) and the regulation of these enzymes by leptin or the addition of an antisense oligonucleotide for leptin. We now present the regulation of MMP-9 and 2 by γ-interferon (IFN), IL-1β and leptin, all possibly involved in implantation. The objective was to study the role of these cytokines in the trophoblastic invasion process, using as an indicator the metalloprotease expression. JEG-3 cells were cultured in DMEM-1%FBS, in the presence of IL-1β [0-100 pg/ml], IFN [0-50.000UI/ml], leptin (100ng/ml) combined or alone. After 3 days, we evaluated MMP-9 and 2 activities and MMP-9 expression by zymograms and Western blot, respectively. Bands were densitometrically analyzed. We also analyzed the endogenous expression of leptin. The culture of JEG-3 cells in the presence of IL-1β [0-50 pg/ml] resulted in a 4-5 fold decrease in endogenous leptin. This correlates with a 2-fold increase in the expression and activity of secreted MMP-9. No changes were observed in the activity of MMP-2. When JEG-3 cells were cultured in the presence of IFN, no changes were observed in the expression of endogenous leptin, or in MMP-9 and 2. In conclusion, taking those cytokines as related to the implantatory process, we could observe an increase in the expression and activity of MMP-9 by IL-1β but not with IFN. IFN, a TH1 cytokine, with possible deleterious effects on the mother-embryo interaction, does not seem to be involved in the metalloprotease-mediated invasion. IL-1β does not have such a definitive role in this process, as IFN, and seems to contribute to MMP-9 mediated invasion.

2.
CAVEOLIN-1 FACILITATES PROGESTERONE RECEPTOR TRANSCRIPTIONAL ACTIVITY IN BREAST CANCER CELLS

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We have previously identified caveolin-1 (cav-1) as a gene whose expression is upregulated by the synthetic progestin medroxiprogesterone acetate (MPA) through a mechanism involving the classical progesterone receptor (PR). These findings were achieved in the progestin dependent murine mammary tumor C4HD, which expresses high levels of PR. We have demonstrated that MPA-induced C4HD epithelial cells proliferation was significantly reduced when cav-1 expression was suppressed. Here we evaluated whether the suppression of cav-1 expression interferes with PR transcriptional activity. For this purpose, transient transfections with a progesterone response element (PRE)-luciferase construction were carried out in C4HD cells, breast cancer T47D and LM3 cells, these last ones cotransfected with PR. MPA-induced PR transcriptional activity was significantly reduced upon suppression of cav-1 expression with antisense oligodeoxinucleotides in C4HD and LM3 cells. Cav-1 overexpression in T47D cells increased PR transcriptional activity. We additionally showed, using coimmunoprecipitation assays and confocal microscopy, that MPA treatment for 5 or 10 min induces cav-1 and PR association in C4HD cells. Finally, a recent mutational analysis performed in human breast carcinomas has identified in 16% of the cases a specific point mutation of cav-1 gene at codon 132. This led us to evaluate the possibility that cav-1 might be mutated in proline 132 in C4HD tumor, contributing to its transforming role. By PCR-RFLP mutational screening and sequencing analysis we showed that cav-1 gene is not mutated at codon 132. We can conclude that cav-1 facilitates PR transcriptional activity and that our previous results indicating that cav-1 expression is associated with C4HD cells proliferation cannot be attributed to codon 132 mutation of cav-1.

3.
BETA-ADRENERGIC RECEPTORS IN BREAST TUMOR CELL LINES

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Our group has previously described the presence of α_2-adrenergic receptors (α_2-AR) in different human and murine breast cancer cell lines. We have found the presence of different subtypes of α_2-AR in the human HS-578T cell line by RT- PCR and by immunofluorescence in the murine MC4-L5 tumor cell line. Specific α_2-adrenergic agonists enhanced cell proliferation in both cell lines. The presence of β-adrenergic receptors was observed by other groups in the human breast cancer cell lines MCF-7 and MDA- MB-231.
In order to further characterize adrenergic receptors in breast cancer cell lines, we have evaluated the presence of β-adrenergic receptors in the human HS-578T cell line and in the murine MC4-L5 one. We have seen positive staining for β_2-AR and α_2-AR by immunocytochimestry and immunofluorescence in both cell lines. Both essays were performed using specific antibodies against β_2-AR and α_2-AR in 1:50 final dilution.
In order to study the biological effect of these receptors, we have analyzed cell proliferation using the [^{3}H]-thymidine incorporation method in the presence of the natural adrenergic agonist (epinephrine, EPI) and a specific β–adrenergic specific agonist (isoproterenol, ISO). Cell proliferation was significantly enhanced after the treatment with EPI in both cell lines, the murine MC4-L5 showing an EC50=0.13 pM, whereas the human HS-578T cell line showing an EC=0.16 nM. On the other side, ISO induced a significant decline in [^{3}H]-thymidine incorporation in both cell lines with values of EC=1.4 pM and 17nM for MC4-L5 and HS-578T respectively. The results obtained agree with those previously observed in our laboratory using other breast tumor cell lines and suggest that epinephrine should be acting mainly trough α_2-ARs, which are associated with enhanced cell proliferation and not trough β-ARs associated with a decline in cell division.

4.
HEREGULIN (HRG) ACTIVATES SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) BY A MECHANISM DEPENDENT ON ERBB-2, SRC AND JANUS KINASES 1 AND 2 (JAK1 AND JAK2) IN BREAST CANCER CELLS

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We have already demostrated that HRG, ligand of the type I receptor tyrosine kinases, induced transcriptional activation of Stat3. We performed this study in an experimental model of hormonal carcinogenesis in which MPA induced mammary adenocarcinomas in female Balb/c mice and in the human breast cancer line T47D. Here, we found that HRG induced Stat3 phosphorylation on tyrosine 705 and this effect was inhibited by blocking ErbB-2 expression with antisense oligodeoxynucleotides (ASErbB-2), with the specific Src inhibitor PP2 or with the selective ErbB-2 inhibitor AG825. Transfection with Jaks inactive forms (JakDNs) had inhibited HRG capacity to induce Stat3 phosphorylation and its transcriptional activation in both C4HD and T47D cells. Assesment of the molecular mechanisms involved in HRG-induced Stat3 tyrosine phosphorylation showed that assembly of a functional protein complex between ErbB-2 and Src is required in order to recruit and phosphorylate Stat3 on tyrosine 705. ErbB-2 and Stat3 also interact in the nuclear compartment since HRG induced both ErbB-2 and Stat3 nuclear translocation and a striking nuclear co-localization of both proteins.
Our findings show, that HRG induces Stat3 transcriptional activation acting through a mechanism that requires the assembly of a functional protein complex among Stat3, ErbB-2 and Src in human and murine breast cancer cells.

5.

EXPRESSION AND INTEGRATION OF HPV16 AND HPV18 GENOMES IN CERVICAL CANCER AND HIGH GRADE LESIONS

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Introduction: Up-regulation of high grade HPV E6/E7 onco-protein expression is necessary for malignant phenotype support in cervical carcinoma, and its regulation would be altered by viral integration. The aim of this study is to evaluate HPV16 and HPV18 E7 expression and its integration status in cervical cancer and high grade lesions in patients from Buenos Aires. **Methods:** HPV16- and HPV18-E7 expression was analyzed by RT-PCR, and viral integration status by APOT method. **Results:** 19 out of 20 analyzed samples showed HPV DNA, of which 12 expressed HPV16-E7, 5 HPV18-E7, and 2 co-expressed both. Among HPV16-E7 positive samples 4 expressed both episomal and integrated derived transcripts, 3 only integrated forms, and 5 could not be analyzed. On the other hand, we found only integrated derived transcripts among the 5 HPV18-E7 positive samples. **Conclusions:** All the HPV16/18-E7 expressing samples showed integrated derived transcripts. In some cases these transcripts coexisted with episomal ones, whereas only integrated derived transcripts were observed among HPV18-E7 positive samples. These results suggest that HPV16 and HPV18 integration would be a common event in cervical cancer and high grade lesions. The co-existence of integrated and episomal HPV16 genome could be the result of tumoral heterogeneity, with cells expressing one or both forms. It remains to be evaluated if lesion evolution selects clones that harbor only HPV-integrated genomes.

6.

TUMOR NECROSIS FACTOR ALPHA (TNFα) INDUCES BREAST CANCER CELL PROLIFERATION THROUGH TNFα RECEPTOR TYPE 1-MEDIATED NF-κB ACTIVATION VIA JNK AND AKT

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We demonstrated that TNFα induces proliferation of murine breast cancer cells C4HD, from the medroxyprogesterone acetate-induced mammary tumor.
To explore the role of each receptor in cell growth, signaling pathways and NFκB activation, we used genetically engineered muteins of murine TNFα which bind selectively either TNFR1 (R1-TNF) or TNFR2 (R2-TNF), or both receptors (R1R2-TNF). Proliferation assays, by [^{3}H]-thymidine incorporation, showed that R1-TNF and R1R2-TNF induced proliferation to the same magnitude as wild type TNFα, but R2-TNF induced it modestly. Activation of signaling pathways, performed by western blots, demonstrated that TNFR1 induced p42/p44 MAPK, JNK, Akt activation to the same level as wild-type TNFα, while mutein to TNFR2 induced p42/p44 MAPK but had poor effect on JNK, Akt activation. To study NF-κB transcriptional activation, C4HD cells were transiently transfected with a κB-luc reporter construct. R1-TNF and R1R2-TNF induced a 3-fold activation of NFκB, while R2-TNF failed to stimulate luciferase activity. Blockage of JNK and PI-3K/Akt, with SP600125 and LY294002 respectively, abolished TNFα-induced NFκB transcripcional activity while blockage of p42/p44 MAPK with PD98059 had minimum effect.
Our results suggest that breast cancer cell proliferation induced by TNFα is mainly mediated through TNFR1 which, through the activation of JNK and Akt, stimulates NF-κB, a key transcription factor in this process. Despite the fact that p42/p44 MAPK is essential for TNFα's proliferative effects, its role is not related to TNFα's capacity of activating NF-κB.

7.

PLASTICITY OF MESENCHYMAL STEM CELL (MSC) FROM BONE MARROW (BM) OF PATIENTS WITH LUNG AND BREAST CANCER

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MSC are able to self-renewal and to differentiate. MSC form fibroblast colonies (CFU-F) when grown in FBS. Previously, we observed a decrease of the cloning efficiency of MSC to give CFU-F, and of the CFU-F size in BM of untreated advanced breast and lung cancer patients (BCP and LCP) vs. normal volunteers (NV). Proliferative activity of NV-MSC is directly proportional to their multipotentiality. In order to study the influence of tumor development on the relationship between self-renew and plasticity of MSC, we evaluated the osteochondroadipogenic differentiation of MSC on 7 BM chemonaive advanced BCP, 7 LCP and 7 NV. MSC derived from low density 3rd passage were cultured for 21 days in osteogenic, adipogenic, or chondrogenic medium. Cells were stained with Giemsa, Von-Kossa, Alizarin Red, OilRed-O and Toludine blue (TB). Immunocytochemistry for osteocalcin and type II collagen were done. We also characterized the cells into a pattern from grade 0 to 3; 0=fibroblast like, 1=in the way to osteoblast, 2=osteoblast, 3=osteocyte. ***Adipogenic*:** 100% NV cultures responded achieving morphologies with characteristics of this linage vs. 57% BCP and LCP. # adipocytes/petri dish (X±SE):**NV**=468±215**a**, **b**; **LCP**=98±66**a**; **BCP**=4± 3**b** (Mann Whitney: a=p< 0.03; b=p< 0.001). ***Chondrogenic*:** all cultures developed chondrogenic pellets. +TB staining with chondrocyte-like lacunae and aggrecan-rich extracellular matrix (EM) were evident in histological sections, and type II collagen-rich EM. An increase of chondrocytes/pellet was seen in patients versus NV (LCP and BCP vs. NV =p<0.026 and <0.002, Mann Whitney). ***Osteogenic*:** patient´s cultures showed a 15±7 (BCP) and 10±3% (LCP) of cells differentiated vs. 24±4% of NV. 43%NV cultures achieved grade 1 and 57% grade 2-3. In BCP, 57% of the cultures differentiate. From these ones the 75% reached grade 1 and 25% grade 2-3. Grade 1 was achieved by 6/7 LCP cultures and the other grade 3. Patients showed heterogeneous distribution of calcium as well as an inadequate mineralization of EM. All cultures showed a weak expression of osteocalcin.
Results showed that BCP and LCP-BM had a high % of small renewing MSC, which gave small CFU-F and had less osteoadipogenic potential.

8.

STUDY OF THE EFFECT OF LIPID INHIBITORY FACTORS, SECRETED BY MAMMARY EPITHELIAL CELLS, ON ADIPOCYTE DIFFERENTIATION AND OXIDATIVE METABOLISM

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Previously, we reported the inhibition of triglyceride accumulation (Tg) by factors secreted by mammary epithelial cells (NMuMG), on 3T3-L1 adipocytes. We now extend this study to other epithelial and non-epithelial cells and include parameters of oxidative stress. We used conditioned media from: NMuMG (CM), HeLa, HEP G2, JEG-3 and glyoma C6. The 3T3-L1 cells were differentiated in the presence or absence (PC, positive control) of the conditioned media. Total triglyceride content, superoxide dismutase and glutathione peroxidase activities were determined. CM inhibited Tg accumulation 65.7% (CM: 0.94 ± 0.07 μg Tg/μg prot vs. PC: 2.74 ± 0.49 μg Tg/μg prot, p<0.01). HEP G2, JEG-3 and glyoma C6 resulted in a lower reduction (p<0.01) compared to CM: 50%, 17% and 37%, respectively; while conditioned medium from HeLa cells resulted in a 56% inhibition, not significantly different from CM. Differentiation induced SOD activity (differentiated: 41.5 ± 2.4 U/g prot, control: 26.0 ± 0.4 U/g prot, p<0.01) and GPx (225 ± 12 U/g prot, control: 35 ± 13 U/g prot, p<0.01). CM and HeLa conditioned media did not inhibit enzyme activity. In order to discard a serum effect, NMuMG cells were cultured with different FBS concentrations (1-20%). All CMs inhibited Tg accumulation similar to 10% FBS. These results show that factors present in conditioned medium from NMuMG cells inhibit Tg accumulation in 3T3-L1 adipocytes, independent of serum content and to a greater extent compared to other epithelial or non-epithelial cell types. Differentiation of 3T3-L1 cells resulted in an induction of SOD and GPx enzymes and CM did not affect this induction, thus indicating that it did not alter the redox state in the cells.

9.

IMPORTANCE OF DICKKOPF-1 (DKK-1) ON MESENCHYMAL STEM CELL (MSC) EXPANSION FROM BONE MARROW (BM) OF PATIENTS WITH LUNG (LCP) AND BREAST CANCER (BCP)

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Although some of the *in-vitro* growth characteristics of human BM-MSC have been documented, the molecular mechanisms by which MSC regulate their own growth in culture are poorly understood. There is no explanation for the observation that when early passage MSC are replated at low density, they display a lag period of 3 days, followed by a phase of rapid exponential growth, and then enter a stationary phase. When BM mononuclear cells are cultured in α medium with 20% FBS, MSC and stromal progenitors give fibroblast colonies (CFU-F) after 14 days. MSC-self renewal and the induction of CFU-F occurred between days 3-7 while the increase in colony size occurred between days 7-14. Works suggest that Dkk-1 allow the MSC and the stromal progenitors from CFU-F reenter the cell cycle. At the end of lag phase and in the early log phase, MSC or CFU-F secretes Dkk-1. We have previously described that BM from untreated advanced LCP and BCP have significantly lower # of CFU-F and a significantly decreased of CFU-F size vs. the values normal volunteers (NV). We also found that these patients have no difference in the levels of most growth and inhibitor factors of CFU-F formation in conditioned mediums (CoM, 7 and 14 days) of CFU-F cultures vs. NV. Thus, we have now further evaluated the levels of Dkk-1 in CoM (*7 and 14 days*) of CFU-F cultures and of confluence primary cultures (*CoM from the last 7 days*) from BM of 12 NV, 13 LCP and 15 BCP by ELISA development kit. Dkk-1 levels were expressed as X±SE (pg/ml): **CoM of CFU-F(day7):** *LCP*=2,015±429 **a,** *BCP*=1,798±469 and *NV*=1,602±399 **b** and **14day:** *LCP*=4,276±691 **a,c**, *BCP*=2,665±611 **d** and *NV*=14,484± 4,034 **b,c,d**(a=p<0.01; b=p<0.0001; c=p<0.0008; d=p<0.0002; Mann Whitney). These patients also had a deficient cloning capacity of BM-MSC vs. NV as we previously published (#CFU-F from LCP and BCP vs. NV=p<0.001, Kruskal Wallis) and a significant decrease of the # of stromal cells/ field of CFU-F (LCP and BCP vs. NV= p<0.01 and 0.05, respectively, Dunn Multiple Comparison). No difference was found in Dkk-1 levels in CoM of confluence primary cultures from patients and NV. In conclusion, the low #of CFU-F and the decrease of CFU-F size in patient cultures could be consequence of a decreased secretion of Dkk-1 in 14 days CoM of CFU-F.

10.

ORIGIN OF TESTICULAR TUMORS IN TRANSGENIC MICE OBTAINED BY TARGETED ONCOGENESIS USING AN AMH PROMOTER/SIMIAN VIRUS 40 T ANTIGEN (SV40T) FUSION CONSTRUCT

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Transgenic mice bearing a construct in which the SV40T expression is directed by the Antimullerian Hormone (AMH) promoter (AT mice) develop testicular tumors in adult life. These tumors are preceded by interstitial hyperplasia and microtumors. Their phenotype indicates a mixed Leydig-Sertoli differentiation. Our aim was to study the cellular origin of these tumors by immunohistochemical localization of SV40T, AMH and 3β-hydroxysteroid dehydrogenase (3β-HSD) in fetal (12.5 and 14.5 dpc) and postnatal (3-15 day-old) mice. No tumors or hyperplasia were seen in control mice, and SV40T was always negative. 3β-HSD was positive in all Leydig cells of control and AT mice. AMH expression was intense in Sertoli cells until 7 days and progressively diminished to disappear at 15 days in control and AT mice. This very same pattern and chronology of expression was observed for SV40T in AT Sertoli cells, which is compatible with the normal postnatal downregulation of the AMH promoter. SV40T was also expressed in focal hyperplasias in the interstitium of all postnatal AT mice where it colocalized with 3β-HSD indicating their Leydig cell origin. The unexpected finding that an oncogen directed by a promoter specifically active in Sertoli cells has given rise to testicular tumors with Leydig differentiation is compatible with the origin of Leydig and Sertoli cells from a common precursor in the specific stroma of the gonadal ridge. These results would indicate that Leydig cells, transformed by the presence of the oncogen, give rise to tumors because they lack the mechanisms of AMH promoter downregulation that normally operates in postnatal Sertoli cells.

11.

NUCLEAR EXPRESSION OF HEME OXYGENASE-1 (HO-1) IN PROSTATE CANCER

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Several factors are involved in the development of prostate cancer (PCa): age, genetics, environment, diet, infectious agents and androgen exposition, inducing an imbalance in the redox state of the tissue. HO-1 is a microsomal key enzyme in the antioxidant defense. Association between HO-1 expression and malignancy is controversial. Nuclear translocation was associated with a decrease in biological activity, phosphorylation or a loss of the C-terminus. We studied the subcellular localization of HO-1 by immunohistochemistry and immunocytochemistry. We analyzed 89 patients with PCa (Gleason grade 4-9) and 39 cases of benign hyperplasia (BPH) and used PC3 and LNCaP cell lines. We found high nuclear staining of HO-1 in tumors (58/89, 65%), compared to peritumoral tissue and BHP (31/87, 35% and 9/39 23%, respectively, p< 0.05). Relative risk after nuclear staining of HO-1 was 1.8 in tumor *vs* non-tumoral parenchyma and 2.8 in tumors *vs.* BHP (Fisher's Test). In cell lines, there was an induction of HO-1 after treatment with the activator hemin. We isolated nuclear and cytoplasmic fractions and determined the expression of HO-1 by Western blot, and verified in both lines nuclear expression of the protein. Immunocytochemistry assays in cell lines confirmed the results, detecting nuclear expression in approximately 10% of the cells. We show, for the first time in an elevated number of patients with PCa, that HO-1 localized in the nucleus. Although the translocation mechanism of HO-1 towards the nucleus is still unknown, the modulation of its expression and localization could be a target for the development of new therapies.

12.

SIGNALLING PATHWAYS ASSOCIATED WITH THE BICARBONATE-DEPENDET RAT SPERM CAPACITATION PROCESS

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Results from our group demonstrated that bicarbonate is required to support rat sperm capacitation by regulating protein tyrosine phosphorylation, migration of epididymal protein DE/CRISP-1 to the equatorial segment and expression of sperm fusion ability. However, while phosphorylation occurred even when bicarbonate was replaced by a cAMP analogue, the other two parameters seem to require additional bicarbonate properties. To further study the pathway involve in the bicarbonate regulation of rat sperm capacitation, intracellular cAMP levels were measured by RIA in sperm incubated in the presence or absence of the anion. Results showed a time-dependent increase in cAMP levels only in sperm incubated in the presence of bicarbonate. To investigate whether this bicarbonate-dependent cAMP accumulation was induced by phorbol esters, sperm were incubated in the presence or absence of bicarbonate, with or without 10 μM PMA. Results showed that PMA produces a significant increase in sperm cAMP levels only in the presence of bicarbonate. In order to test whether the cAMP produced was required for the three capacitation parameters previously analyzed, the involvement of PKA was studied using the PKA inhibitor H89. Tyrosine phosphorylation was significant reduced by addition of 30 μM H89. However, migration of DE/CRISP-1 was not affected, and the expression of the fusion ability could only be partially inhibited by H89, even in the presence of the inhibitor during gamete co-incubation. Taken together, our results provide further information on the regulation of rat sperm capacitation by bicarbonate, confirming that some capacitation-associated events, although bicarbonate-dependent, would be cAMP/PKA-independent.

13.
NITRIC OXIDE AND ITS REGULATION IN MURINE ORGANOGENIC EMBRYOS AFTER PERIGESTATIONAL ALCOHOL CONSUMPTION

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Maternal alcohol consumption produces increased early pregnancy loss and teratogenic effects. The prenatal pathogenesis of CNS produced by perigestational alcohol ingestion has not been studied. Nitric oxide (NO) regulates cell survival, apoptosis and differentiation, and interacts with prostaglandin (PG) system, while altered NO levels may contibute to neurodegeneration.The aim was to study the NO in organogenic embryos and the alterations of PGE-NO interrelationship involved in defective neurogenesis after prenatal alcohol ingestion. Female mice were intoxicated with 10 % ethanol in drinking water for 15 days before and during pregnancy up to day 10 (T). Control females received maltose-dextrin in water (C). T had increased abnormal % of E.10 stage embryos (30% vs 10.7%, $p<0.001$), with defective neural tube (NT) closure (scanning microscopy), altered neuroepithelium, reduced mitotic cell Nr (HyE) and diminished body-cephalic proportion (C:0.5±0.02 vs. T:0.4± 0.01, $p<0.05$). Endogenous Nitrites (Ni) levels (Griess reaction, kit) were significantly reduced in E.10 embryos from T compared to those of C ($p<0.01$), which correlated with altered NT neuroepithelial pattern of immunostaining of nNOS. Embryo incubations with NO-donors (Nonoate), NOS inhibitors (L-NMMA) or COX-2 inhibitors (Meloxicam) demonstrated that interrelationship NO-PGE (RIA) and PGE-NO resulted deregulated in T-derived embryos while C-embryos had a positive modulation. In conclusion, deficient NO levels after maternal alcohol ingestion may lead to embryofetal microcephaly due to deregulation of PG-NO pathway that participate in neuronal proliferation and differentiation of early NT development.

14.
EVIDENCE FOR THE PARTICIPATION OF EPIDIDYMOSOMES IN THE ASSOCIATION OF "DE" WITH SPERMATOZOA

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Rat protein DE, synthesized by the epididymal epithelium, associates with the sperm surface during sperm maturation. Sequential protein extraction from epididymal sperm revealed the existence of two populations of DE on sperm: a loosely bound population released during capacitation, and a strongly bound population involved in gamete fusion. While the loosely-bound population is associated with sperm by electrostatic interactions, the mechanism that anchors the tightly-bound DE to sperm remains unknown. Recent evidence shows that membranous vesicles present in the epididymal fluid, named epididymosomes (Ep), are involved in the transfer of some epididymal proteins to sperm. To investigate the participation of Ep in the transfer of the strongly-bound DE to sperm, Ep were isolated by ultracentrifugation of rat epididymal fluid and examined by electron microscopy to confirm their correct isolation. Western blot (Wb) analysis using anti-tubulin as a sperm marker and a specific antibody against DE, revealed both the absence of sperm and the presence of DE in the vesicle preparation. To examine the anchoring of DE to the Ep, the vesicles were subjected to different extraction treatments and the presence of DE in the protein extracts was analyzed by Wb. As previously observed for DE on sperm, PBS, 2M NaCl, low pH(3) and 5U/ml PLC-PI (phospholipase C specific for inositol) were unable to remove DE from the Ep, while high pH(11), 250mM DTT and 1% Triton X-100 completely extracted the protein from the vesicles. These results indicate the existence of a population of DE strongly bound to Ep, supporting the involvement of these epididymal vesicles in the transferring of DE to sperm during epididymal maturation.

15.
TGF-β1 TESTICULAR EXPRESSION IN TRANSGENIC MICE OVER-EXPRESSING hCG. *IN VITRO* EFFECT OF hCG AND PROGESTERONE IN FVB/N MICE TESTES

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Transgenic male mice (TG) over-expressing α and β subunits of hCG are infertile and show enhanced steroidogenesis with high levels of testosterone and progesterone. The chronic hCG hyper-stimulation leads to Leydig cell hyperplasia and hypertrophy in prepubertal mice, being reduced at adulthood (Rulli *et al.,* Endocrinology, 144: 4980, 2003). Transforming growth factor-β1 (TGF-β1) is a growth factor that regulates cell cycle progression, cell differentiation, reproductive function and development. The aim of this study was to analyze by RT-PCR the expression of TGF-β1 and endoglin (a co-receptor which leads to proliferation) in the testes of TG at 3 and 8 weeks of age, as well as the "*in vitro*" effect of hCG and progesterone on TGF-β1 testicular expression in wild type mice (FVB/n) at both ages. TG showed an increase in the expression of TGF-β1 at 8 weeks of age ($p<0.05$). Endoglin showed an increment on its expression at 3 weeks of age ($p<0.05$). "*In vitro*" incubation (1 hour) with hCG (10UI/ml) induced the expression of TGF-β1 at both ages studied ($p<0.05$). Otherwise, "*in vitro*" incubation (1 hour) with progesterone (10^{-5} M) had no effect on TGF-β1 expression. These results indicate that high and chronic levels of hCG induced TGF-β1 testicular expression in TG at 8 weeks of age. In contrast, this factor is not regulated by progesterone, at least in these experimental conditions. The expression of endoglin in TG correlates with the hyperplasia and hypertrophy of Leydig cells in prepubertal animals. The temporal expression of TGF-β1 might influence the growth and development of the testes in this experimental model.

16.
EFFECT OF ALPHA-TOCOPHEROL ON CAPACITATION OF FROZEN-THAWED PORCINE SPERMATOZOA

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Sperm cryopreservation is associated with the production of reactive oxygen species (ROS), producing membrane changes similar to those occurring during capacitation. Capacitation is the physiological process that give spermatozoa fertilizing ability. Protein tyrosine phosphorylation is associated with sperm capacitation and acrosome reaction in several mammal species. Porcine spermatozoa from frozen-thawed semen are very sensitive to lipid peroxidation. Alpha-tocopherol (αT) protects sperm membrane against oxidative damage improving sperm motility. The aim of this work was to study the influence of αT, added to the semen extender, on capacitation of cryopreserved porcine spermatozoa. Sperm samples frozen with and without αT (200 μg/ml) were incubated with bicarbonate (40 mM), used as sperm capacitation inducer. Patterns of sperm capacitation were determined by chlortetracycline assay and by changes in protein tyrosine phosphorylation using an specific anti-phosphotyrosine monoclonal antibody. Sperm motility and viability were evaluated by optical microscopy and eosin-nigrosine technique, respectively The presence of αT did not modify the capacitation level respect to control samples. However, a decrease in the percentage of spermatozoa with capacitation-like changes was observed in samples frozen with αT. Protein tyrosine phosphorilation pattern did not show significant differences between treatments. Motility was higher ($p<0.05$) in samples frozen with αT. Viability did not differ ($p>0.05$) between treatments. The addition of αT to the semen extender would protect sperm membrane against ROS action generated during the cryopreservation process increasing sperm motility. Though, it would fail to improve the capacitation status of frozen-thawed porcine spermatozoa.

17.
INTRACELLULAR CALCIUM IS MODULATE BY SRC- TYROSINE KINASE ON CAPACITATED BOVINE SPERMATOZOA

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Calcium increase is a crucial event for capacitation. In bovine Heparin (H) and quercetin (Q) induce capacitation. The purpose was to determine in sperm capacitation intracellular calcium concentration (Ca_i) variation , voltage dependent calcium channels (VDCC) activation and src-tyrosine kinase (TK) participation. Shimatzu fluorescence spectrophotometer was used to measure (Ca_i) by Fura 2-AM and confocal microscopy was used to determine (Ca_i) distribution by Fluo3-AM. Capacitation was evaluated by chlortetracycline and the viability by trypan blue stain. In the presence of H or Q, 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±118.0 nM, respectively) vs control ($p<0.05$). 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. PP2 (specific inhibitor of C-src-TK) provoked a capacitated percentage and (Ca_i) decrease in spermatozoa capacitated by H or Q ($p<0.05$). In H capacitated spermatozoa with PP2, fluorescence significantly diminished compare to H treatment. Metoxyverapamil, specific inhibitor VDCC failed to block Q calcium increase and capacitation induction in contrast to H treatment ($p<0.05$). In bovine spermatozoa, H and Q induce capacitation with similar (Ca_i) increase and different intracellular distribution suggesting that different compartments are involved to induce calcium dependent signals regulated by src- TK, required for capacitation.

18.
HCG AND CAMP REGULATE LEPTIN EXPRESSION IN TROPHOBLASTIC CELLS

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Leptin is a 16kDa protein discovered in adipose tissue as the regulator of energy balance in the organism. Its expression, as well as its receptor's, were observed also in placenta, with a potential role in growth, angiogenesis and immunomodulation, thus affecting both maternal and fetal functions, via autocrine or paracrine signals. Previous results demonstrated that, in trophoblastic cells (JEG-3 and BeWo), leptin promoter is active with specific elements regulating its basal activity. We also demonstrated that estradiol treatment (0.1 μM) or hCG (10-100 IU/ml) presented an inducer effect on protein expression. The objective of the present work is to study the regulatory mechanisms involved in leptin expression by hCG, using BeWo cells as a model. We analyzed results from Western blots and reporter gene in experiments of transient transfection, using plasmids with serial deletions of the promoter for leptin gene. We observed up to 5-fold stimulation in the expression of leptin by hCG, with all the regions of the promoter analyzed. Analysis *in silico* of the promoter showed possible regulatory elements, related to the cAMP signaling pathway. Western blot analysis showed that 1 μM cAMP is capable of stimulating leptin expression, while 1 mM is inhibitory. Similar results were observed at transcriptional levels through the reporter gene expression. Moreover, we determined that 1 μM cAMP inhibits hCG (100 IU/ml) promoter activation. We finally analyzed the MAPK signaling pathway. Treatment with PD98059 (10 μM) (specific inhibitior for MEK) did not show any effect on the induction of leptin expression by hCG. The results obtained contribute to increase the understanding of the regulatory mechanisms on leptin expression in human trophoblasts by hCG and cAMP.

19.
CLONING OF GLYCOPROTEIN HORMONE ALFA SUBUNIT IN PEJERREY *Odontesthes bonariensis*. EXPRESSION PROFILE OF GONADOTROPIN SUBUNITS AND RELATIONSHIP WITH GnRHs EXPRESSION AND PLASMA SEX STEROID LEVELS IN MALES

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In the present work the cDNA encoding pejerrey glycoprotein hormone alfa subunit (pjGPH-α) was cloned and characterized. Also, pituitary expression of pejerrey gonadotropin subunits (pjFSH-β, pjLH-β and pjGPH-α) was analyzed by real time PCR and compared to brain expression of endogenous GnRHs (sGnRH, pjGnRH and cGnRH-II) and plasma sex steroid levels in adult pejerrey males. The nucleotide sequence of pjGPH-α consisted of 677 bp containing an open reading frame of 366 bp. The signal and the mature peptide were estimated to have 23 and 98 aa respectively, and as in other teleost species. This protein has 10 cysteine residues and 2 potential N-glycosilation sites at Asn 55 and Asn 80. The aminoacid sequence of pjGPH-α mature peptide was compared with other teleost fishes GPH-α sequences showing the highest homology (80.5%) with a perciform fishes. Expression results showed that maturing males had high levels of pjFSH-β and pjGPH-α subunits, and intermediate levels of pjLH-β when compared with running ripe and spent stages. These animals had the lowest plasma T and 11-KT values as well as low expression of sGnRH, pjGnRH and cGnRH-II. Running ripe males had the lowest expression of pjFSH-β and the highest expression of pjLH-β and pjGPH-α subunits, and of the three GnRHs genes. At this stage, it was observed the highest values of T and 11-KT. Spent males showed low expression of the three GtHs subunits, sGnRH, pjGnRH, and low levels of T. At this stage, 11-KT levels and cGnRH-II expression showed a tendency to decrease but the values were not statistically significant to running ripe stage. The present results would suggest that T and 11-KT modulate the expression of the pjFSH subunits. The expression of the anterior brain GnRH variants, sGnRH and pjGnRH is correlated with pjLH-β expression and reinforce the importance of the forebrain GnRH variants on the regulation of pituitary function.

20.
MECHANISM OF PARTICIPATION OF TESTICULAR PROTEIN TPX-1 IN GAMETE FUSION

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Epididymal protein DE and testicular protein Tpx-1 are two homologous members of the CRISP (Cysteine-RIch Secretory Protein) family. Evidence from our group indicates that both proteins participate in gamete fusion in rodents and human. While DE mediates gamete fusion through its binding to egg complementary sites, the mechanisms underlying the functional role of Tpx-1 remain unknown. To investigate the involvement of egg binding sites for Tpx-1, zona-free human and mouse eggs were incubated with bacterially-expressed Tpx-1 coupled to MBP (Maltose Binding Protein) (recTpx-1), and then subjected to indirect immunofluorescence (IIF) using anti-MBP. While fluorescent labeling was observed over the entire human egg surface, coincident with the fusion ability of the human oolema, mouse eggs exhibited labeling over the egg surface and a negative area as described for DE in mouse eggs. In view of recent results showing that the egg binding ability of DE resides in a region of 12 amino acids in which Tpx-1 exhibits only two substitutions when compared to DE, we investigated whether these two proteins were interacting with the same egg-complementary sites. Zona-free mouse eggs were incubated in medium containing a fixed concentration of recTpx-1 and increasing amounts of purified DE, and the binding of recTpx-1 to the egg analyzed by IIF. Results showed that exposure to DE resulted in a gradual decrease in the fluorescent staining for Tpx-1, indicating that both proteins share binding sites on the egg. These results support the participation of both DE and Tpx-1 in gamete fusion through their interaction with egg binding sites, suggesting the existence of a functional cooperation between homologue molecules as a mechanism to ensure the success of fertilization.

21.

INCREASED VEGF EXPRESSION IN MATERNAL-FETAL INTERFACE IN A MULTIPARITY MOUSE MODEL

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We previously reported that multiparous mouse placentae showed an increase in the number of macrophages and in the grade of trophoblast invasion. The aim of this study was to analyze VEGF and VEGF-sR1 in the same animal model. **Methods:** *CBA/JxCBA/J, CBA/JxBALB/c* and the abortion-prone *CBA/JxDBA/2* mouse combinations were divided into three groups: Primiparous Young (PY): 3.0±0.5 months old; Primiparous Old (PO): 8.5±0.5 months old and Multiparous Old (MO): 8.5±0.5 months old with 4 pregnancies. Non-pregnant (NP) *CBA/J* sera plus placentae and sera from term pregnancies were obtained. Placental VEGF expression was investigated by IHC, and serum VEGF and VEGF-sR1 levels by ELISA. **Results:** All groups showed similar quantity of VEGF+ cells in spongiotrophoblast and laberynthine. Among the PY groups, *CBA/JxDBA/2* presented a lower expression of VEGF in the area next to the decidua compared to that observed in the normal *CBA/JxBALB/c* combination. All MO groups showed a similar high expression of VEGF in this region. In contrast, at term of pregnancy, sera from the primiparous *CBA/JxBALB/c* group showed the highest concentration of VEGF-sR1 while both *CBA/JxBALB/c* and *CBA/JxDBA/2* multiparous females demonstrated similar and low circulating levels. All groups analyzed showed no differences in VEGF levels. **Conclusions:** Multiparity increased VEGF expression in maternal-fetal interface without modifying circulating levels. We suggest that multiparity enhances trophoblast invasion and that VEGF participates in this process.

22.

IMMUNODETECTION OF EPITHELIAL CADHERIN IN COW OVIDUCTAL EPITHELIAL CELLS AND *IN VITRO* MATURED CUMMULUS-OOCYTE COMPLEXES (COCs)

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After mating, few spermatozoa reach the cow oviduct to form a sperm reservoir; there, the plasma membrane overlaying the acrosome interacts with the cilliated surface of oviductal epithelial cells until signals associated to ovulation cause sperm release, possibly by capacitation-related events. At the site of fertilization, sperm initially make contact with the cumulus cells, and then interact with the oocyte *zona pellucida (ZP)* and the oolemma. Epithelial cadherin (Ecad) is a Ca^{+2} dependent cell-cell adhesion molecule present on the surface of non-capacitated, capacitated and acrosome reacted bull spermatozoa in cell regions proposed to participate in the interaction with oviductal cells and oocyte envelopes (1). The aim of the study was to evaluate localization of Ecad in oviductal tissue sections, as well as in cumulus cells and oocytes from *in vitro* matured COCs. Immunohystochemical analysis of sections from different regions of the oviduct during the periovulatory period, immunocytochemistry of *in vitro* matured oocytes and their surrounding cells, and Western immunoblotting (WIB) of denuded oocytes was done using a specific anti Ecad antibody (sc#7870; Sta Cruz Biotech). Presence of Ecad was revealed in oviductal epithelial cells and their secretions, in the surrounding cells accompanying the matured oocyte, in the ZP and in the oocyte plasma membrane; a 120 KDa form was immunodetected by WIB of whole oocyte protein extracts. Altogether, these results suggest the involvement of Ecad in the interaction between bull spermatozoa with the oviductal epithelial cells in the formation and release from the sperm reservoir, as well as during gamete interaction leading to fertilization.

(1)Caballero *et al*. J Androl (2006), Suppl. March April, pg. 72.

23.

ACTIVATION OF *BUFO ARENARUM* OOCYTE MATURATED *IN VITRO* BY MICROINJECTION OF HOMOLOGOUS SPERM EXTRACTS

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Several hypothesis have been proposed to explain the mechanism by which the sperm activate the oocyte. Some hypothesis assumes that after gamete fusion,the sperm may introduce, a soluble factor that triggers the activation process in the oocyte. Another hypothesis suggests the interaction between sperm and oocyte surface.
In this work, we investigated the effect of the microinjection of homologous sperm soluble fractions on the activation of *Bufo arenarum* oocytes maturated *in vitro*.
We used a swim-up procedure to select motile sperms. The good quality sperms were lysed by cycles of freezing (-70ºC) and thawing (25ºC). The lysate was centrifuged at 14.000 RPM and the supernatant was collected as a sperm extract. The supernatant was chromatografied by gel filtration (Bio-gel P-60). The different fractions from the sperm extract were microinjected using ICSI (Intracytoplasmic Sperm Injection) micropipets HUMAGEN™ FERTILITY DIAGNOSTICS. The injections were performed at 20°C in Tris saline "free calcium".
The activation parameters considered were the disappearence of the white spot, the elevation of the vitelline envelope and the cortical granules exocitosis. The results indicate that only the fraction XVII have biological activity when it is microinject into the egg cytoplasm. The fraction XVII was analyzed by SDS gel electrophoresis. The observations indicate that it is a protein of approximately 22 KDa.
We could conclude that the sperm of *Bufo arenarum* have a soluble factor able to induce the activation when is microinjected into the oocyte.

24.

LACTATE CONSUMPTION IN THE CAPACITATION OF CRYOPRESERVED BOVINE SPERMATOZOA

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Mammalian spermatozoa are highly differentiated cells that have a metabolic compartmentalization with a little cytoplasm and a reduce ability to translocate metabolic intermediates from one region to another. Heparin induces a respiratory burst and lactate dehydrogenase activity decrease in bovine spermatozoa. Pyruvate and lactate are mitochondrial sources of oxidative energy in frozen- thawed spermatozoa. The aim of the study was to evaluate lactate consumption during capacitation. Lactate and pyruvate were used as oxidative substrates. Capacitation was evaluated by chlorotetracycline and de viability by trypan blue stain. Data was analyses by ANOVA and Tukey test ($p<0.05$). Heparin capacitation with lactate alone induced a decrease of capacitation percentage (30%) compared to the percentage obtained in the presence of lactate and pyruvate ($p<0.05$). In the absence of oxidative substrates no heparin capacitation was observed ($p<0.05$). Acrosome integrity and viability were similar in all treatments except in the absence of substrates where was obtained significant decrease (52%) in the sperm viability ($p<0.05$). Lactate concentration in the medium was different in samples treated with lactate/ pyruvate ($0.39\pm0.06 \times 10^6$ spermatozoa) and lactate alone ($0.59\pm0.02 \times 10^6$ spermatozoa) ($p<0.05$). The absence of oxidative substrates inhibited the capacitation induction but only 40% of the sperm population was still alive. Heparin capacitation induction requires the presence of pyruvate and lactate in order to permit the translocation of substrates through the mitochondrial membrane for obtaining oxidative energy for the capacitation process.

25.

DEVELOPMENT OF IMMUNOREACTIVITY TOWARDS EPITHELIAL CADHERIN DURING EPIDIDYMAL SPERM MATURATION. STUDIES IN BOVINE AND MURINE MODELS

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Sperm acquire fertilizing ability during epididymal maturation. Epithelial cadherin (Ecad) is a Ca^{+2} dependent cell-cell adhesion molecule present in *cauda* epididymal mouse and ejaculated bull spermatozoa, in cell regions proposed to participate in fertilization (1,2). The aim of the study was to assess Ecad localization in epididymal spermatozoa recovered from *caput*, *corpus* and *cauda* epididymis, and compare it with that on testicular sperm cells. In addition, expression of Ecad in mouse and bovine epididymis was confirmed by immunohystochemistry, and immunodetection in epididymosomes was evaluated by immunoelectron microscopy (IEM) and Western immunoblotting (WIB). Evaluations were done using specific anti Ecad antibodies towards different protein regions.
Immunocytochemical studies revealed a specific signal for Ecad in the apical ridge (bull), acrosome (bull,mouse), and the post-acrosomal region (bull,mouse) of *caput*, *corpus* and *cauda* sperm; contrasting, negligible levels of Ecad were detected in cells recovered from the testis, showing a homogeneous localization along the cell (mouse) or only in the postacrosomal region (bovine). Expression of Ecad in the epididymis was confirmed in both species, and its presence in epididymosomes was revealed by IEM (mouse) and by WIB (bovine), showing Ecad forms of 120, 110, and 35 KDa. In conclusion, remarkable differences in Ecad immunodetection were found between testicular and epididymal sperm. Its expression in the epididymis and presence in epididymosomes suggests a role of these vesicles in Ecad transfer during epididymal maturation.
(1) Veiga *et al*. J Androl (2005), Suppl. March April, pg. 83. (2) Caballero *et al*. J Androl (2006), Suppl.March April, pg. 72.

26.

ACROSIN ACTIVATION IS REGULATED BY PROTEIN KINASE C AND TYROSINE KINASE IN ACROSOME REACTION INDUCED BY LYSOPHOSPHATIDYLCHOLINE

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Sperm acrosin is an important proteolytic enzyme, capable of hydrolyzing zona pellucida, with a vital role in the fertilization. Heparin (H) and quercetin (Q) induce capacitation in bovine spermatozoa. Lysophosphatidylcholine (LPC) induces acrosome reaction (AR) in capacitated bovine spermatozoa. The aim of the study was to determine the variation of the proacrosin-acrosin system in AR and its relation with intracellular signals system that depend on protein kinase C (PKC), tyrosine kinase (TK) and voltage dependent calcium channels (VDCC) activation. Enzyme activity was determined spectrophotometrically using BAPNA as specific substrate of acrosin. Capacitation and AR were evaluated by chlorotetracycline and the viability by trypan blue stain. Data was analysed by ANOVA and Tukey test ($p<0.05$). Genistein, specific inhibitor of TK blocked AR induction (5.4±2.9% AR) and provoked acrosin activity decrease to control level (185.51±92.73 $\mu U/10^6$ sp) ($p<0.05$). The total level of acrosin activity registered (895.1±130.91 $\mu U/10^6$ sp) indicated that 75% of acrosin exists as zymogen form. Activation of acrosin is not modified by the inhibition of VDCC (714.49±154.18 $\mu U/10^6$ spermatozoa) but blocked AR induction by LPC in heparin capacitated spermatozoa. GF-109203X, an specific inhibitor of PKC, decrease 50% the activation of acrosin induced by LPC. Proacrosin-acrosin system participates partially in the exocytotic process. The proacrosin-acrosin system is regulated by PKC and mainly through tyrosine kinase activity. Acrosin activation is not dependent on calcium influx through VDCC but acrosome reaction induced by LPC requires VDCC activation in cryopreserved bovine spermatozoa.

27.

TESTICULAR INTERLEUKIN 1β (IL1β): ITS ROLE ON THE REGULATION OF CYCLOOXYGENASE 2 (COX2) EXPRESSION AND POSSIBLE RELEVANCE TO MALE FERTILITY

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We have described: 1) an increased number of macrophages (MAC) containing IL1β and induction of interstitial COX2 expression (key enzyme in the biosynthesis of prostaglandins, PGs) in testicular biopsies from infertile men, 2) COX2 expression in testes of adult Syrian hamsters exposed to a long-day photoperiod (LD, 14h light: 10h dark) but not in those exposed to a short-day photoperiod (SD, 6h light: 18h dark), 3) PGJ2 induction of human testicular fibrosis, 4) PGF2α inhibition of hamster testicular testosterone production (Frungieri *et al*, Fertil Steril 78, 2002; PNAS USA 99, 2002; Endocrinology 147, 2006).
The aims of this study were to investigate the testicular expression of IL1β, its receptors (RI and RII), the antagonist receptor (Ra), as well as the effect of IL1β on COX2 expression.
Expression of IL1β and RI was detected by RT-PCR and immunohistochemistry in COX2-positive Leydig cells isolated by: 1) laser microdissection from human biopsies of patients with germ arrest and Sertoli cell only syndromes, 2) Percoll density gradient from LD-hamsters. In addition, IL1β significantly increased COX2 expression (RT-PCR, ´fold change´ units, basal: 1±0.2; IL1β 50 ng/ml: 8.1±0.7, $P<0.05$) in LD-hamster Leydig cells. COX2-negative Leydig cells from SD-hamsters did not expressed IL1β.
Thus, IL1β induces testicular COX2 expression in hamsters. Normal human testes with few IL1β-positive MAC do not express COX2, but pathological human testes with an increased number of MAC show expression of IL1β, RI and COX2 in Leydig cells suggesting a key role of IL1β on testicular COX2 expression and PGs production in male infertility.

28.

DETECTION OF ESTROUS CYCLE OF COATI NASUA NASUA (CARNIVORA: PROCYONIDAE) IN CAPTIVITY

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Estrous cycle is defined as the period of time when the female shows physiological signs of receptivity to the male and will stand for mating. The length of the period of estrous varies among species. Females of coati Nasua nasua have a monoestrous cycle with ovulation probably induced by coitus. The objective of the present investigation was to identify predictors of estrous cycle for improvement of the reproduction of this specie in captivity. In six females the following variables were analyzed three times at random during June, July, August and September: visual observations of vulva aspects, measurement of vulva dimensions (length and wide) and cytological analysis of samples of uterus tissue. Rectal temperature was also monitored. In August, vulva showed pink color and it became noticeably swollen (congested vulva), without secretion in comparison with other months. Length and wide of vulva were 9.4 ± 0.4 and 9.1 ± 0.5 mm in June-July, 23.5 ± 0.5 and 24.0 ± 1.0 mm in August and 12.1 ± 1.5 and 12.7 ± 2.5 mm in September, respectively. In August the superficial eosinophil cells were 100% whereas that in June, July and September, the percentage decreased to 10.3 ± 1.5%. Rectal temperature was 37.2 ± 0.2°C in June-July, 38 ± 1°C in August and 40.1 ± 0.9°C in September.
These data demonstrate that the estrous cycle of females of coati Nasua nasua take place during August, showing the reproductive apparatus favorable physiological signs for pairing.

29.

HEPARAN SULFATE AS PUTATIVE DECONDENSING AGENT OF HUMAN SPERMATOZOA *IN VIVO*: PRELIMINARY EVIDENCE OF ITS PRESENCE IN THE MAMMALIAN OOCYTE

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Human sperm decondense *in vitro* in the presence of heparin (Hep) and glutathione (GSH). Previous studies from our laboratory have shown that heparan sulfate (HS), but not other glycosaminoglycans (GAGs), has a decondensing ability similar to that of Hep *in vitro* and we have proposed HS as a putative protamine acceptor *in vivo*. The aim of this study was to investigate the presence of HS in the oocyte, via two different strategies. Semen specimens were provided by normospermic (OMS) donors. Oocytes were obtained from the oviducts of 8 weeks-old CF1 female mice, hormonally stimulated with PMSG (5 UI) and hCG (5 UI), and stripped of both cumulus cells (hyaluronidase 0,1% in PBS) and zona pellucida (acid Tyrodes, pH 2.5). 1) Methanol-fixed oocytes were stained with Rubipy (Tris (2,2´-bipyridine) Ruthenium (II) Chloride) 1 mg/ml in distilled water at pH 1.5 for preferential binding to sulfate groups. 2) Sperm were decondensed on microscope slides with Hep 46 μM + GSH 10 mM or mechanically crushed oocytes + GSH 10 mM, for 3.5 h. 1) Analysis by confocal microscopy suggested the presence of sulfated GAGs in the cytoplasm of all oocytes observed. 2) There were no significant differences in sperm decondensation in the presence of oocytes/GSH (38±8%) vs. Hep/GSH (41±6%) (n=7, Paired Student, p=0.25). Addition of heparinase to decondensation milieu significantly inhibited sperm decondensation (3±1% vs. 50±14%) (n=3, Paired Student, p<=0.05) whereas addition of either chondroitinase ABC or hyaluronidase did not (48±11% and 41±8% respectively). These results constitute the first evidence in the literature on the presence of HS in the ooplasm and support its role as protamine acceptor *in vivo*.

30.

REACTIVE OXYGEN SPECIES REQUIREMENTS FOR PORCINE SPERM CAPACITATION

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Mammalian spermatozoa must undergo a preparation process denominated capacitation to be able to fertilize mature oocytes. Bicarbonate induces capacitation by activating adenylyl cyclase to produce cyclic-AMP. At physiological concentrations, reactive oxygen species (ROS) are messengers that influence sperm function, activating enzymes or other mechanisms involved in the capacitation process. The aim of this work was to study the parcipation of ROS in the capacitation process of cryopreserved porcine spermatozoa. Samples of frozen-thawed spermatozoa were capacitated with bicarbonate in the presence of ROS scavengers (superoxide dismutase, catalase or hemoglobine). Sperm motility and viability were evaluated by optical microscopy and eosin-nigrosine technique, respectively. The percentage of capacitated spermatozoa was determined by chlortetracycline assay. The capacitation level was not affected by catalase (25 to 300 U/ml), hemoglobine (5 to 40 μg/ml) or superoxide dismutase (250 to 750 U/ml). However, a dose-dependent decrease ($p<0.05$) was observed in the percentage of capacitated spermatozoa starting from 1000 U/ml of superoxide dismutase. Motility and viability were not affected with any compounds used. According to our results, the lack of effect observed when catalase or hemoglobine were used, suggest that hydrogen peroxide or nitric oxide are not involved in the capacitation of cryopreserved boar spermatozoa. However, the ability of superoxide dismutase to inhibit sperm capacitation in the presence of bicarbonate, may indicate the generation of superoxide anion during such process. A membrane oxidase may produce the superoxide anion necessary for activating cellular mechanisms involved in boar sperm capacitation.

31.

ARTIFICIAL INSEMINATION USING COOLED SEMEN: PRELIMINARY RESULTS IN LLAMAS

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The objective of this study was to obtain a pregnancy in *Lama glama* by designing a protocol for artificial insemination (AI) using cooled llama semen. Twenty three females and six adult males were used. Semen was collected using electroejaculation or artificial vagina. The following ejaculate characteristics were evaluated in both raw and cooled samples: volume, concentration, motility, membrane function (HOS test), membrane integrity (using fluorochromes: CFDA/PI) and sperm morphology. Each raw ejaculate was extended 1:1 with 11% lactose-20% egg yolk and cooled to 5ºC in 2 h 30 min, remaining at that temperature during 24-30 h. Follicle dynamics were followed daily using transrectal ultrasound and rectal palpation. When a dominant follicle in the growth phase (≥ 7 mm) was detected, ovulation was induced administering 8 μg busereline (IV). Each female was transcervically inseminated, at the uterotubal junction ipsilateral to the ovary with the dominant follicle, with a complete ejaculate. Insemination dose was 51.52 ± 49.93 x 10^6 normal, live sperm (mean ± SD). Females were randomly divided into two groups: group A (n=10) were inseminated 23.4 ± 2.59 h post busereline injection and group B (n=13) were inseminated immediately post ovulation which was detected by ultrasonography (28.0 ± 1.3 h) (mean ± SD). No pregnancies were detected in group A, while for group B, pregnancy rate was 23% (3/13). These results indicate that it is possible to obtain pregnancies with AI using cooled semen and that the success of the technique would depend on proximity to ovulation.

32.

EFFECT OF EXOGENOUS AND ENDOGENOUS HYALURONIC ACID ON BOVINE *IN VITRO* FERTILIZATION AND SUBSEQUENT EMBRYO DEVELOPMENT

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Cumulus mucification occurs during cumulus-oocyte complex (COC) maturation as a consequence of the deposition of mucoelastic compounds, mainly hyaluronic acid (HA). This glycosaminoglycan (GAG) could be involved in providing an adequate environment for the fertilization process. The aim of this work was to study the effect of endogenous and exogenous HA added to the fertilization medium on cleavage (CR) and blastocyst rates (BR). COCs were matured in 199+5%FBS+FSH+LH in presence or absence of DON, an inhibitor of the HA synthesis. *In vitro* fertilization (IVF) was carried out in mSOF supplemented with BSA (negative control), heparine (He) (positive control) or HA. *In vitro* embryo development was performed in IVC-mSOF. COCs matured in presence of DON shown a lower CR and BR compared with COCs matured without DON (CR: 54% vs 70.9, BR: 14% vs 31.1%). These effects were reversed when the fertilization medium was additioned with HA (CR: 69.8%, BR: 32.1%), similar to the rates obtained with He ($p<0.05$). The addition of exogenous HA to IVF medium did not modify CR and BR of COCs matured without DON compared to the negative or positive control. Data show that the lack of endogenous HA synthesis inhibit events related to the fertilization process. The prevention of this effect produced by the addition of He and exogenous HA in IVF medium indicates that the presence of GAGs is necessary for fertilization, but developmental competence is not coupled directly with the process of endogenous HA synthesis.

33.
MINERAL PROFILE STUDY IN DIFFERENT PHYSIOLOGIC STATES IN DAIRY COWS

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Mineral profile study in dairy cows is important because allows to evaluate metabolic dysfunctions origin that would cause an adverse effect in the production. Minerals study in different physiologic states, of two dairy farm of Santa Fe center region was the objective. One worked with 15 cows of Cuenca del Salado and 15 of Pilar, during the months: August of 2005 to January of 2006. Mineral levels were determined by atomic absorption spectroscophy. For the treatment of the data , ANOVA statistical method was applied. Minerals concentrations were inside of normal range. However, copper values, were below the optimal value (1µg/ml) without being observed deficiency clinical answer.Later to the parturition, a decrease in the values of calcium was observed. Copper and calcium values in different physiologic states (gestation, pre-parturition, pos-parturition and lactation) were: Cu: 0,58± 0,13;0,63± 0,07;0,64± 0,08;0,72±0,09mg/dl; Ca: 9,22± 0,6; 9,16± 0,58; 8,99± 0,84;8,60± 0,60mg% for Pilar and Cu: 0,53± 0,12; 0,74± 0,10; 0,73± 0,09; 0,69± 0,08mg/dl; Ca:9,97±1,41; 10,36±1,5; 9,79± 1,53; 9,11±1,14mg% in Cuenca del Salado. Copper values in gestation, coincident with the winter, differ significantly ($p < 0,05$) of those in period of lactation corresponding to summery. The copper increased in that period. The above-mentioned, it could be explained through levels variation of Cu in the pasture according to variation seasonal. In the Cuenca del Salado, the concentration of calcium decreased significantly ($p<0,05$), between the periods of pre-parturition and lactation, fact attributable to mineral mobilization during for production, also to a limited contribution of the diet.

34.
PEROXYNITRITE INDUCES CAPACITATION IN CRYOPRESERVED BOVINE SPERMATOZOA

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Bovine spermatozoa require a preparatory process called capacitation, to fertilize mature oocytes, culminating in an exocytotic event, the acrosome reaction (AR). Superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide and nitric oxide (NO) are involved in capacitation and AR in bovine spermatozoa. NO and $O_2^{\bullet-}$ produce peroxynitrite ($ONOO^-$) in a diffusion-controlled reaction. Our aim was to study the participation of exogenous $ONOO^-$ in capacitation of cryopreserved bovine spermatozoa. The spermatozoa were incubated at 38ºC in TALP medium with Ca^{2+} and bovine serum albumin during 45 minutes with heparin (10 IU/ml) or in the presence of 3-morfolinosidnonimina (SIN-1, $ONOO^-$ donor) in concentrations ranging from 1 to 20 µM. Capacitation percentage was determined by chlortetracycline fluorescence assay (CTC) at 15, 30 and 45 minutes of incubation. Progressive motility and viability were determined by light microscopy and eosin/nigrosin technique, respectively. Spermatozoa previously capacitated (with 10 IU/ml heparin or 10 µM SIN-1) were incubated at 38ºC for 15 minutes in the presence of 30% follicular fluid, as physiological AR inducer. AR and true AR percentages were determined by CTC and Differential Interference Optical-Contrast microscopy (DIC), respectively. A SIN-1 concentration of 10 µM reached 23±2% of capacitation, which is significantly different from control group (4,6±1,62%). Capacitation was achieved in the first 15 minutes, showing no significant differences at 30 and 45 minutes. The percentage of acrosome reaction induced by follicular fluid in spermatozoa previously capacitated with SIN-1 was significantly different to the control group. Peroxynitrite induces capacitation, that leads to physiological AR, in cryopreserved bovine spermatozoa.

35.
TRANSPORT CONDITIONS OF PORCINE OVARIES AFFECTS BIOCHEMICAL PARAMETERS OF FOLLICULAR FLUID AND OOCYTE MATURATION COMPETENCE

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Percentages of oocyte *in vitro* maturation are very variable in the porcine species. Changes in the extracellular environment surrounding the oocytes could affect future oocyte performance. Biochemical parameters of follicular fluid could be influenced by different transport conditions of the ovaries. It was determined that glycolysis is one of the most important pathway present in follicular somatic cells. The aim of this work was to evaluate glucose and lactate concentrations and pH in follicular fluid according to different storage times and temperatures with two transport solutions, related to the oocyte maturation capacity. Biochemical parameters were determined at 2, 4, 6 hours and 15, 25, 35ºC with physiological saline or physiological saline + glucose 5.5 mM. Glucose and lactate concentration in follicular fluid was measured in a spectrophotometric assay and pH was determined with a pH meter. Cumulus-oocyte complexes were matured in medium 199, 10% FBS, FSH + LH for 48 h. Maturation was evaluated by the presence of metaphase II chromosome configuration. Glucose concentration and pH diminished and lactate concentration increased when storage time or temperature increased ($p<0.05$). No significant differences in the behavior of studied parameters were observed between the transport solutions. The higher meiotic maturation rates were observed with ovaries transported at short times and high temperatures ($p<0.05$). Both time and temperature storage seems to be important conditions to take into account for transportation of porcine ovaries. Metabolically more active follicles, evaluated through glycolytic activity, may contribute to maintain the maturation competence of porcine oocytes.

36.
***EX OVO* DEVELOPMENT OF CHICK EMBRYOS**

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In a previous experimental study, a standardized technique was developed to optimize the *ex evo* development of chick embryos. The aim of the present study was to determine the percent of embryonic survival to at least 14 days of total incubation. Fertilized eggs(*Gallus sp* Negra INTA) were used. These were incubated *in ovo* at 37ºC with 60% relative humidity for 72 hs. The *ex ovo* embryo development was carried out in a polystyrene container (4 x 7-cm-diameter, 0.2 mm-thick, 1/8kg, Huhtamaki Argentina) placed inside another high density polyethylene container with screw top (8.5 x 9-cm-diameter, model T 500 mL TE&T®) containing 50 mL of distilled water. All culture chambers were maintained at 37ºC at saturation humidity in a standard laboratory incubator. Each experiment included 24 embryos and was repeated five times. Embryos were monitored every 48 hs and staged (HH, 1951). The embryonic survival was estimated at E5, E7, E10, E12 and E14. The embryos were killed at -20ºC at E14 and were fixed in formalin. The embryo stage recorded at each time-points evaluated were consistent with an earlier study (HH, 1951). The embryonic survival was 82±10%, 62±9%, 48±8%, 38±3%, 24 ±10% at E5, E7, E10, E12 and E14 respectively.
The results described here indicate that the described technique allows the *ex ovo* chick embryo development and may constitute an appropriate model to use chick embryos for experimental embryology and pathology studies.

37.

HEMATOLOGIC PROFILE VARIATION OF DAIRY COWS IN GESTATION AND LACTATION

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Hematologic parameters constitute paraclinic search that allow to study disorders in the health and nutritional deficiencies. Normal values are influenced by the animal physiologic state among other variables. The objective of this work was to analyze the variations of hematologic profile, during the physiologic states of gestation and lactation in dairy cows of two dairy farm of Santa Fe center region. One worked with 15 cows of Cuenca del Salado and 15 of Pilar. Hematologic determinations performed in the anticoagulated blood (EDTA) from the jugular vein were: Hematocrito (%); Red globules (millon/mm^3), White globules (/mm^3), Hemoglobin concentration (g/dl), and percentage formula; using hematologic meter and May Grunwald-Giemsa´s method. For data treatment, the statistic method ANOVA was applied. Results obtained for the different hematologic parameters were inside bibliography normal ranges. Mean values of GR, Hto and Hb for Pilar's field were: GR:7.43± 0.39; 6.98± 0.60 millones/mm^3; Hto:33.33±1.80;31.27± 2.60%; Hb: 10.31± 0.62; 9.84± 0,86 g/dl; and for Cuenca del Salado: GR: 7,05± 0,33; 6,95± 0,46 millions / mm^3; Hto: 30.4± 2.38; 28.67± 2.55%; Hb: 9.7± 0.72; 9.17± 0.63 g/dl, in gestation and lactation respectively. Results corresponding to erythrocytary series were smaller in gestation than in lactation period for both fields. Eosinófils values increased in Pilar's field: 2.87 ±1.53% and 4.8 ±2.51% in gestation and lactation respectively; attributable to an allergic phenomenon of sensitization to own milk.

38.

NUCLEAR AND CYTOPLASMIC PORCINE OOCYTE *IN VITRO* MATURATION

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In vitro production of early porcine embryos requires an adequate maturation of oocytes. It has been observed that nuclear maturation rates are similar in porcine cumulus-oocytes complexes (COCs) with different characteristics of surrounding cumulus oophorus. The aim of this work was to evaluate oocyte meiotic and cytoplasmic maturation according to morphological classification of COCs. Ovaries from slaughtered gilts were kept warm during the journey to the laboratory and harvested COCs were classified according to cumulus characteristics in classes: A_1 complete-thick, A_2 complete, B_1 corona radiata and B_2 partially naked oocytes. COCs were matured in medium 199, 10% FBS, FSH+LH for 48h. After culture, oocytes were denuded and incubated in TBMm with fresh ejaculated spermatozoa during 18h. Meiotic maturation was evaluated by metaphase II and cytoplasmic maturation by sperm head decondensation and/or pronuclear formation. There were no differences in the percentages of meiotic maturation between classes (A_1 59.6%, A_2 58.5%, B_1 55.4%, B_2 51.9%). However, the percentage of cytoplasmic maturation was different between classes (A_1 55.0%, A_2 36.7%, B_1 27.3%, B_2 10.6%, $p<0.05$). A higher decondensated sperm head rates were observed in classes A ($p<0.05$), and the highest percentage of pronuclear formation was shown in class A_1 ($p<0.05$). Spontaneous partenogenic activation was observed only in classes B. Although the different classes of COCs are able to complete meiotic maturation, only oocytes surrounded with a complete-thick cumulus are competent for cytoplasmic maturation. Porcine oocyte nuclear maturation would be independent of the features of surrounding cumulus, whereas cytoplasmic maturation would be closely linked to cumulus characteristics.

39.

SYNCHRONIZATION OF THE FOLLICULAR WAVE USING INJECTABLE PROGESTERONE AND ESTRADIOL BENZOATE IN *Lama glama*

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The objective of this study was to evaluate the efficiency of two doses of progesterone (P_4) ® (Laboratorio Allignani) and one dose of estradiol benzoate (BE) ® (Laboratorio Allignani) for synchronizing the follicular wave in llamas in order to start superstimulatory treatments in absence of a dominant follicle. Two treatments were used on 40 adult females: A (n=20): 100 mg P_4 + 1 mg BE and B (n=20): 150 mg P_4 + 1 mg BE; in each case P_4 was applied during 5 days and BE only on the first day. The levels of plasma progesterone were measured using RIA. In group A, P_4 levels increased slowly after the first injection (day 0), reaching the highest level on day 1 (72.5 ± 35.6 nmol/l); in group B, the highest level was attained on day 1 (105.3 ± 60.0 nmol/l) (mean ± SD). Levels were lower in group A than in group B. No significant differences ($P>0.05$) were observed in the synchronization of the follicular wave between treatments. Considering the cost of the treatments, the treatment A would be the best for synchronizing the follicular wave.

40.

OXIDATIVE METABOLIC PATHWAYS INVOLVED IN BOVINE SPERM CAPACITATION

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Sperm catabolic processes produce energy for capacitation, required for fertilization. Heparin (H) glycosaminoglycan and quercetin (Q) a specific inhibitor of calcium-ATPase plasma membrane induce bovine sperm capacitation. The aim was to determine metabolic enzymes activities and their participation in the supply of energy and generation of the redox state to acquire fertilization capacity. Capacitation was induced with heparin and quercetin. Enzymatic activities (malato dehydrogenase -NAD (MDH), lactate dehydrogenase (LDH), isocitrate dehydrogenase-NAD (IDH) and creatine kinase (CPK)) were determined spectrophotometrically and oxidative stress (ROS) by spectrofluorometry with dichlorodihydrofluorescein diacetate. Chlortetracycline technique was used to evaluate capacitation. Samples were capacitated by H or Q. Capacitation percentage was increased by H or Q treatments respect to control ($P<0.05$) and no significant differences were observed in sperm viability. MDH activity diminished with both capacitation inducers (H $5.99\pm1.22.10^{-2}/10^8$spermatozoa, Q $4.6\pm0.5010^{-2}/10^8$ spermatozoa) while IDH- NAD remained constant with different inducers respect to the control ($1.00\pm .13.10^{-2}/10^8$sperm). In heparin or quercetin capacitated spermatozoa, CPK activity decreased respect to the control ($P<0.05$). ROS level remained as a control ($251.20\pm101.55 / 10^8$)($P<0.05$). In sperm samples, H decreased activity of LDH and MDH-NAD in contrast to Q treatment that maintained LDH activity as control level. Data suggest that H and Q induce capacitation through different metabolic pathway and maintain ROS level to modulate redox state and oxidative metabolism in cryopreserved bovine spermatozoa.

41.

MITOCHONDRIAL MORPHOTYPE´S DINAMIC ON EARLY DEVELOPMENT OF *IN VITRO* PRODUCED BOVINE EMBRYOS

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Objectives: Research on mitochondrial behaviour and it relation with vesicles presence during the first 4 days of *in vitro* produced bovine embryos development. **M&M:** Total of 20 embryos from 1 to 4 days (5 by stage) cultured on serum free media. The covered surface by hooded, orthodox and swollen mitochondrial types, and lisosomic/lipidic vesicles was quantified on ETM mycrographs at 8000X. Percent related on analysed surface were statistically probed with Kruskal-Wallis and Dunn methods. **Results:** Significant differences have not been found on vesicles between the 1st day and the 4th day. On all stages hooded mitochondrial forms prevails while the orthodox ones are scarce. In the second day the hooded forms decrease soon to be increased slightly towards day 4, whereas they swollen them are increased with a tip towards day 3.

DAY	1		2		3		4	
	%	σ	%	σ	%	σ	%	σ
Vesicles	29,59	7,89	31,66	7,68	31,59	10,10	39,70	13,67
Hooded mitochondria	4,86^{AB}	1,82	3,10^{A}	1,16	3,64^{B}	2,7	3,95	1,56
Orthodox mitochondria	0,20	0,25	0,064	0,164	0	-	0	-
Swollen mitochondria	0^{AC}	-	0,18^{BD}	0,2	1,35^{AB}	1,45	0,48^{CD}	0,87
Analized (μm²)	1783		1968		1779		2344	

* significant differences are indicate by identical superscripts (p<0,05).

Discussion: On serum free culture, mitochondrial dynamic would not have relation with the vesicle presence. Day 2 are critic for the mitochondrial changes; because the mitochondrial function depends on the coordinate expression between mitochondrial and nuclear DNA, these changes can be related with the switch between maternal and embryonic genome. On swollen types active mitochondrial internal surface notoriously decrease. The increasing of the covered surface of these types lead to the total embryo metabolism decrease.

42.

REACTIVE OXYGEN SPECIES ARE REQUIRED DURING BOVINE OOCYTE *IN VITRO* MATURATION

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Immature and matured bovine oocytes generate similar reactive oxygen species (ROS) levels. The addition of cysteine to the maturation medium increase reduced glutathione (GSH). Inhibition of respiratory chain increases ROS generation. The aim of this work was to determine ROS production in the presence of different treatments during 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 (control), 0.6 mM cysteine, 1 mM choro-dinitro benzene (CDNB, GSH removal) and 2 μM diphenyliodonium (IDP, NADPH oxidase inhibitor), at 39ºC, 5% CO_2 in humidified air for 22 h. Respiratory chain effectors, 1 mM potassium cyanide (KCN, cytochrome oxidase inhibitor) and 0,42 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP, mitochondrial uncoupler) were used. Meiotic maturation was determined by the presence of metaphase II. ROS production was determined in denuded oocytes at 0, 6, 10, 18 and 22 h by the ratio between 2´,7´-dichlorodihydrofluorescein diacetate (DCHFDA) and fluorescein diacetate (FDA) assays, analysing digital microphotographs. Maturation percentages of control, cysteine, IDP and CDNB were 78, 80, 75 and 32%, respectively. ROS levels expressed as DCHFDA/FDA ratio fluctuated throughout the 22 h of maturation, showing a similar pattern with different treatments. A significant decrease at 10 h was observed (P<0.05), recovering the initial value at the end of maturation. Potassium cyanide increased ROS levels at 10 h (P<0.05). The GSH removal, which does not increase ROS production, impaires normal maturation. ROS levels, generated mainly in mitochondrial respiratory chain, may be linked to key events associated with the maturation process.

43.

LOW DOSE DEEP INTRAUTERINE INSEMINATION IN MARES USING FROZEN-THAWED SEMEN. PRELIMINARY RESULTS

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Our aim was to improve the efficiency of deep intrauterine low dose insemination of frozen semen.
Semen from 5 stallions frozen by the protocol adapted by Miragaya *et al*. 2001 (treatment A), and semen from 4 stallions frozen by a commercial protocol (IMV Technologies, treatment B) were used. Twenty nine inseminations in 23 mares of age ranging from 4 to 14 years. When a follicle ≥ 35mm was observed, 2500 IU of hCG were administered IV. Ultrasonographic controls were made every 6 h. Once ovulation was detected, insemination was made at the uterotubal junction using a flexible catheter transrectally guided to the tip of the uterine horn ipsilateral to the ovary of the ovulated follicle. Insemination was always done within 6 h after ovulation. For treatment A inseminating dose was 64.66 ± 22.39 millions of progressive motile sperms and for treatment B was 44. 0 ± 11.34. Mares were evaluated 15 days later for pregnancy detection.
Statistical analysis were made using a Fisher´s test with a 95% of confidence.
Prime insemination pregnancy rate (PR) was 73.91% (17/23) and general PR was 68,96% (20/29). Prime insemination PR for treatment A was 83,33% (10/12) and for treatment B was 63.33% (7/11). No differences were observed between general PR and prime insemination PR, and between doses ≤ 50 million of progressive motile sperms and > 50 million of progressive motile sperms. Also, there were no significant differences between PR of mares inseminated with either treatment (A and B) (P>0.05).
Low dose deep intrauterine insemination with frozen semen at 6 h post ovulation allows to obtain similar results to those reported performing two inseminations (pre and post ovulation).

44.

STORAGE TIME AND TEMPERATURE OF PORCINE OVARIES INFLUENCE THE QUALITY OF IMMATURE OOCYTES AND SUBSEQUENT MATURATION CAPACITY

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The quality of immature oocytes is determinant in their *in vitro* maturation competence. Transport conditions of the porcine ovaries may affect the quality of immature oocytes and subsequent maturation capacity of female gametes. The aim of this work was to evaluate the viability and nuclear stage of immature oocytes according to different storage times and temperatures with two transport solutions, and its relationship with the oocyte maturation competence. Percentages of immature live oocytes and/or germinal vesicle stages were determinated at 2, 4, 6 hours and 15, 25, 35ºC with physiological saline or physiological saline + glucose 5.5 mM. Immature oocytes were denuded and stained in a solution of FDA and Hoechst 33342 to evaluate live/dead oocytes and nuclear status, respectively. Cumulus-oocyte complexes were matured in medium 199, 10% FBS, FSH + LH for 48 h. Maturation was evaluated by the presence of metaphase II chromosome configuration. Immature live oocyte rates diminished when time increased (p<0.05), but a protected effect was observed at 25ºC for longer storage times (p<0.05). Germinal vesicle rates decreased when time increased (p<0.05), but no differences were observed among storage temperatures. No significant difference in the immature oocyte quality was found regarding the two transport solutions. In coincidence, the higher rates of immature live oocytes with germinal vesicle and oocyte meiotic maturation were observed with ovaries transported at short times and high temperatures (p<0.05). These storage conditions would be more adequate to transport porcine ovaries. Immature live oocytes without precocious resumption of meiosis might be the most likely ones to mature properly.

45.

CUMULUS-OOCYTE COMPLEXES RECOVERY WITH DIFFERENT DOSES OF eCG IN LLAMA (*Lama glama*)

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The objective of the present study was to compare two superstimulatory treatments in relation to the number of follicles aspirated and COC's obtained. We used 20 non-pregnant, non-lactating females *Lama glama*. Follicular waves were synchronized using a single dose of 1 mg estradiol benzoate i.m. the first day plus an intravaginal device impregnated with 0.33 g of progesterone (CIDR®) inserted during five days; after the removal the groups received the corresponding superstimulatory treatment: (1) 1000 IU of eCG i.m. (n=10) and (2) 1500 IU of eCG i.m. (n=10). All females were monitored by ultrasonography and rectal palpation and when they presented more than two dominant follicles (≥ 0,7 cm) they received a single dose of 8 μg of buserelin i.v.. Twenty hours after, surgical approach was done by the flank. Ovaries were exteriorized and follicles were punctured using a 21 G needle and syringe containing PBS with heparin. COC's were found using a stereomicroscope. The 1000 IU and 1500 IU treatment groups did not differ with respect to the number of follicular aspirated (83 versus 137, respectively; $P >$ 0,05), the number of COC's collected (69 vs. 112; $P >$ 0,05) or the collection rate per follicle aspirated (83,1% vs. 81,7%, $P >$ 0,05). In summary, 1000 IU and 1500 IU of eCG were equally effective for ovarian superstimulation and oocyte collection but we observed a tendency towards a higher number of follicles with 1500 IU treatment. Cumulus-oocyte complexes were collected from more than 80% of follicles aspirated during laparotomy in llamas.

46.

***IN VIVO* RELATIONSHIPS BETWEEN HIF ALPHA, HSP 60, BAX AND CASPASE 3 IN MURINE ACUTE RENAL ISCHEMIA**

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Cell death post ischemic injury is involved in many diseases. Hypoxia inducible factor 1α (HIF-1α) is the oxygen sensitive subunit of this transcriptional master regulator of O_2 homeostasis.

By other hand, Hsps are chaperones involved in protein folding, refolding, transport, and translocation. Hsp 60 interacts with Bax, releasing it protein under injury during apoptosis. Moreover, there are evidences that elevated temperatures induced HIFα in murine kidneys.

The aim of this study was to assess the relationships operating between Hsp 60, Bax, caspase 3 and HIF α expressions in an experimental induced renal acute ischemia.

Left kidneys of male CF-1 mice were submitted to subtotal ischemia by ligation of the renal pedicle for 5, 15, 30 and 60 min. Right kidneys freely irrigated, were excised at the same scheduled times.

Nuclear and cytosolic proteins were fractionated onto 7.5 and 12% SDS-PAGE respectively and detected by westernblottings. HIF-1α was studied in nuclear fractions and the other proteins in cytosolic fractions. Positive controls for HIF became from post hypoxic renal extracts (6 h at 0,4 atm in hypoxic chamber). Ischemia triggers the up-regulation of HIF-1α, Hsp 60 and caspase 3 expressions in kidneys, while Bax showed progressive down regulation.

Correlations between HIF and caspase 3 were very significant (r=0.9601, P=0.095). The up-regulation of the chaperonine involves an inespecific attempt to deal with hypoxia, that finally concludes with Bax translocation to mitochondria and cell death.

In summary, the degree and timing of protein expression patterns suggest a linkage between the hypoxic stress signal and the apoptotic cascade induced by renal acute ischemia.

47.

EFFECT OF AROMATASE INHIBITORS ON PROLIFERATION AND APOPTOSIS OF ENDOMETRIOTIC LESIONS

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Recent studies have shown that patients with endometriosis (EDT) have increased aromatase P450 expression in the endometrial tissue. The objective of this study was to evaluate the "*in vivo*" effect of aromatase inhibitors (Anastrozole=Ana and Letrozole=Let) on the implantation, proliferation and apoptosis of ectopic endometrial tissue. For this purpose we induced endometriotic lesions in 51 two months old female Balb/c mice. Animals were divided in 3 groups: Control (n=19), Ana (n=16) and Let (n=16). Treatments consisted of saline solution (control) or a daily s.c. injection of Ana or Let (10 μg/day). After 28 days of treatment (beginning on day 1 post- induction of EDT), lesions were measured, proliferation was evaluated by immunohistochemistry using anti-PCNA antibody, and percentages of apoptotic cells was determined using TUNEL technique. We observed that although percentages of animals that developed EDT and the number of lesions per animal were similar in all groups, the size of the endometriotic lesions was significantly smaller in Ana group ($p< 0.05$ vs. control) and in Let group ($p<0.001$ vs. control). Additionally we found that both Ana and Let treatments produced a significant decrease in cell proliferation ($p<0.01$ and $p<0.001$ vs. control respectively) and a significant increase in apoptosis ($p<0.05$ and $p<0.01$ vs. control respectively). In conclusion, the treatment of EDT with aromatase inhibitors in this animal model did not prevent the establishment of the lesions but significantly diminished the size of the lesions. Also, aromatase inhibitors inhibited cell proliferation in the EDT lesions and increased the degree of apoptosis. This data favors further investigation of aromatase inhibitors as a treatment for EDT.

48.

ANTIAPOPTOTIC EFFECT OF VEGF AND IL-1β ON ENDOMETRIAL EPITHELIAL CELLS (EEC) CULTURES FROM PATIENTS WITH ENDOMETRIOSIS (EDT)

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In previous studies we observed that treatment with a GnRH agonist, Leuprolide Acetate (LA), increases the apoptosis in the EEC and decreases the production of VEGF and IL-1β. In addition it is known that VEGF and IL-1β are increased in the peritoneal environment of patients with EDT. The objective of this work was to evaluate the effect of the addition of VEGF and IL-1β on apoptosis induced by LA in the EEC. Primary cultures of the EEC were made from biopsies of endometrial tissue of patients with EDT and controls. Percentage of apoptotic cells (%ApC) was evaluated using the acridine orange - etidium bromide technique. We observed that treatment with LA 1000 ng/ml increased the % ApC from 25 ± 2% to 49 ± 3% in the EEC from control women and 20 ± 2% to 43 ± 5% in EEC from patients with EDT ($p<0.001$ vs. basal). The addition of VEGF at different concentrations (0.1, 1 and 10 ng/ml) 3 hours after the LA treatment, rescued apoptosis in EEC from controls: 29 ± 3%, 23 ± 2% and 22 ± 2% respectively ($p<0.001$ vs LA and $p>0.05$ vs basal); and from patients with EDT: 20 ± 3%, 18 ± 2% and 24 ± 5% respectively ($p<0.01$ vs LA, $p>0.05$ vs basal). In the same way treatment with IL-1β (0.1, 1 and 10 ng/ml) 3 hours after LA treatment, rescued apoptosis in EEC cultures from controls: 31 ± 3%, 25 ± 2% and 23 ± 36% respectively ($p<0.001$ vs LA, $p>0.05$ vs basal); and in EEC from patients with EDT: 21 ± 4%, 16 ± 2% and 19 ± 3% respectively ($p<0.001$ vs LA, $p>0.05$ vs basal). In summary, both VEGF and IL-1β showed antiapoptotic effects in EEC. Both factors could favor the survival and development of the ectopic endometrial tissue.

49.

PLACENTAL G-CSF CAN MODULATE *IN VITRO* THE SYNTHESIS OF FLT-1 BY PERITONEAL MACROPHAGES

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We have previously reported that multiparity status induces an increase of G-CSF expression in spongiotrophoblast, in the number of placental macrophages next to decidua and in the layers of invasive trophoblast tissue (ITT). Moreover, ITT cells showed an important expression of G-CSF, M-CSF, VEGF and its receptor Flt-1. Taking into account that M-CSF and G-CSF have proved to modulate macrophage activity, here we investigated whether placental culture supernantants (PS) were able to influence the secretion of VEGF and sFlt-1 by isolated peritoneal macrophages.
M&M: Term placentae were obtained from normal *CBA/J x BALB/c* and abortion-prone *CBA/J x DBA/2* crossbreedings, which were previously divided into 3 groups: primiparous young (PY: 3 months old), primiparous old (PO: 8.5 months old) and multiparous old (MO: 8.5 months old and four pregnancies). Placentae were minced and cultured (76 ± 4 mg/ml). The respective PS were incubated or not with anti G-CSF. Non-inflammatory peritoneal macrophages were obtained from BALB/c mice, cultured ($3x10^6$/ml) alone (CM) or in the presence of 10% of the different neutralized (ReN) and not neutralized (Re) PS. Controls of PS were also carried out (CPS).
VEGF and sFlt-1 were investigated in all the obtained culture supernatants by ELISA. The presence of G-CSF and M-CSF in the PS was checked by western-blot. Results: VEGF conc. (pg/ml): CM: 35±15; CPS: 80±20; Re and both ReN: not detected (nd). sFlt-1 conc. (ng/ml): CM: nd; CPS: between 36 and 47; Re: 140±30; ReN with anti G-CSF: diminishing of approx. 50%. Our results would indicate that G-CSF secreted by placentae induce the *in vitro* synthesis of sFlt-1 by peritoneal macrophages sequestrating the VEGF produced. We suggest that this mechanism may partially explain the increase of Flt-1 observed in multiparous placentae.

50.

THE ROLE OF THE CADERINS IN THE APOPTOSIS OF PORCINE GRANULOSA CELLS (CG)

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The normal physiology and the dynamics of the reproductive tissue depends in great measure of an appropriate contact cell to cell, which is mediated by proteins of the cadherins family, molecules Ca^{++} dependent such as the E and N Cadherin. In these circumstances, the atresia process for apoptosis of the CG, it could be related with the loss of this cellular contacts. In this work, the objective was to establish the relationship among the loss of the contacts Ca^{++} dependent with the integrity and the apoptosis of the CG. The study model was a cellular primary culture of porcine CG obtained by aspiration of antrals follicles, which were treated with EGTA, to obtain the disruptión of the homophilic unions. The treatments were made with EGTA 9 nM and 100 nM and without EGTA. Simultaneously, the role of the depletion of serum was determined making the same study in cultures with and without 5% SFB, both treatment at different times of induction 6, 12 and 24 hours.
In each case, the apoptosis index (AI) was determined by DAPI and TUNEL techniques. Also, was carried out a quantitative evaluation by analysis of images of the expression of Bax and Bcl-2 by inmuno cytochemical (ICQ). To different concentration of the inductor, the treatments with serum demonstrated a bigger AI by 9 nM (1,27; 2,58 and 1,38 to 6, 12 and 24 hs respectively). The activation was significant in the treatments of 12 hs with serum and in those of 6 hs without serum ($p \leq 0,05$). In the treatments with serum, activation was observed respect to the control. The AI in the treatments without serum was not significant. To concentrations of 100 nM the AI didn't demonstrate significant differences of activation. The quantitative determination of the expression of Bax and Bcl 2 for ICQ validates the previous results.

51.

CICLOOXYGENASE-2 (COX-2) INHIBITOR CELECOXIB INDUCES APOPTOSIS AND INHIBITS CELL GROWTH AND VEGF LEVELS IN ENDOMETRIAL EPITHELIAL CELLS (EEC) FROM PATIENTS WITH ENDOMETRIOSIS (EDT)

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There is room for improvement in the medical therapy of EDT. Celecoxib, strongly suppresses cell proliferation and induces apoptosis in different tumor cell lines and in oncogenic animal models. The purpose of this study was to evaluate the effect of celecoxib on cell proliferation, apoptosis and VEGF levels in EEC cultures from patients with EDT and controls. Primary cultures of the EEC were performed from biopsies of eutopic endometrial tissue of 14 patients with EDT and 10 controls. EEC were treated with celecoxib in different doses. Percentages of apoptotic cells (%Ap) were evaluated using the acridine orange-ethidium br. technique and cell proliferation, by ^{3}H-thymidine incorporation. VEGF levels were measured by ELISA in the supernatants of celecoxib treated EEC. Celecoxib 50, 75, and 100 μM induced apoptosis and inhibited cell proliferation in EEC from patients with EDT and controls (table I). Celecoxib 25, 50 and 100 μM decreased VEGF levels in EEC ($p<0.001$, 0.05 and 0.001 respect vs. basal).

Celecoxib (μM)	10	20	25	40	50	75	100
CONTROLS							
Cell prolifer. (% of basal)	86.6±3.7	80.2±4.0	67.7±5.6	64.2±4.1	58.0±5.8 *	43.7±11.0 **	36.20±8.3 ***
%Ap (fold increase)	1.6±0.22	1.6±0.19	1.4±0.2	2.2±0.45	2.5±0.44 *	2.7±0.3 ***	3.1±0.5 ***
EDT							
Cell prolifer. (% of basal)	84.3±4.0	79.0±3.2	64.9±6.8	68.3±3.0	55.0±4.3 *	37.6±4.8 **	34.0±3.5 **
%Ap (fold increase)	1.4±0.23	1.2±0.34	1.0±0.31	2.2±0.27	2.8±0.47 ***	3.3±0.6 ***	3.1±0.8 **

Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001 vs. basal

Conclusions: Celecoxib produced a significant and positive effect on apoptosis and cell proliferation on EEC and showed an antiangiogenic activity. These findings support the further investigation of COX-2 inhibitors as a treatment option in EDT.

52.

DOES MACROPROLACTIN AFFECT THE HUMORAL IMMUNE RESPONSE?

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Macroprolactin (MPRL) is a prolactin (PRL)-IgG complex, frequently found in hyperprolactinemic individuals and usually showing minimun clinical symptoms. On the other hand, PRL induces immunoglobulin production *in vitro*. It is known that asymmetrically glycosilated IgG molecules (AAb), which posses an oligosaccharide group in one of the Fab fragments, cannot bind large ligands and are unable to activate effector mechanisms. The AAb synthesis can be modulated by IL-6, estrogen, progesterone and glucocorticoids. We investigated the asymmetric/symmetric IgG ratio (ELISA test before and after Concanavalin A-Sepharose affinity chromatography) as well as the levels of IgG, IgA, IgM (IDR) and IL-6 (ELISA) in MPRL serum samples in comparison with hyperprolactinemic and normal sera as control. The results demonstrated: 1) The percentage of AAb was not modified in MPRL serum (control:26.8±2.4, n=10, MPRL:32.1±2.7, n=9). Similar results were obtained with high levels of PRL (PRL>50 ng/ml, 28.9±2.4, n=11). In contrast, this proportion increased when PRL was slightly elevated (PRL 24-50ng/ml:42.4±2.5, n=14, $p<0.05$ vs control). 2) Total IgG was significantly increased in MPRL and hyperprolactinemia (control: 1106±91mg/dl, n=10; MPRL: 1527.7±102.4, n=9, $p<0.05$; PRL>50 ng/ml:1488.9±104.2, n=9, $p<0.05$ vs control; PRL 24-50ng/m:1511.0±101.8, n=12, $p<0.05$ vs control). 3) No differences were observed in IgA, IgM and IL-6 serum levels between groups. These data suggest that in correlation with the absence of clinical endocrine sympthomatology the macroprolactinemia would not modify the quality of the immune response (AAb) in contrast to which observed when PRL is elevated, however, total IgG was increased as well as in hyperprolactinemia.

53.

APOPTOTIC VOLUME DECREASE: CAUSE OR EFFECT?

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A major hallmark of apoptosis is cell volume loss, which can be seen as a cellular shrinkage in a time-lapse recorded video. Studies in the last few years show how some apoptotic processes are early preceded by a significant volume decrease. In our work, we developed a time-lapse recording technique which allows us to follow the volume loss of BHK-21 and SHSY-5Y cells in real time. The setup was developed to keep cells under ideal conditions during long-term cultures (moisture, pH and temperature controlled). Furthermore, it allows the insertion of a micropipette which permits the focal release of substances in a way that can be controlled in time and space, then cells were monitored 24hs after treatment.
Staurosporine (STS), a bacterium alkaloid, is a well known PKC inhibitor and a broad-spectrum apoptotic inducer. In our work, cells were treated with 1uM of STS. Volume loss started immediately after treatment, achieving maximal shrinkage about 40 minutes later, and treatment is maintained for 20 minutes more. When at this point STS is retired from the medium, cells did not undergo apoptosis and a total volume reversion was observed after 2 hours. Moreover, the affected cells were able to divide, showing a completely normal behavior. In conclusion, we demonstrated that cell volume loss mediated by STS is not directly linked to the apoptotic event although probably the cellular stress induced by long term volume loss can activate an apoptotic pathway. In this context, we show how these innovative methods are useful to answer questions which, otherwise, can only be assessed in a very indirect way.

54.

FIRST DESCRIPTION AND IDENTIFICATION OF A POLYTENE CHROMOSOME OF *ANASTREPHA FRATERCULUS (WIED.) USING* MICRO- *FISH* ON MITOTIC CHROMOSOMES

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Anastrepha fraterculus is the South American fruit fly, a very genetically polymorphic species. It is a major pest which damages fruits causing important economic loses. We previously described 10 sexual and 9 autosomal mitotic chromosome variants. The analysis of polytene chromosomes is an important complement because they are the only chromosomes in perpetual interphase. The main metabolic activities occur in this stage of the cell cycle. Polytene chromosomes represent a lined up formation of hundreds of sets of the genome. Their nucleus-to-nucleus reproducible bands form a constant pattern, resulting valuable markers of changes in the genome that are caused by chromosomal rearrangements. However there is no information about its analysis in this species. The aim of the present work was to describe and identify a polytene chromosome in *A. fraterculus.* For this purpose, chromosome slides were obtained from salivary glands of third instar larvae from a particular laboratory strain. Slides were orcein stained and a polytene chromosome carrying a marker inversion was described.
Chromosome microdissection was used to isolate the inversion. A probe of 300pb was obtained and marked by Nick translation with Spectrum Orange-dUTP. The probe was used in fluorescence *in situ* hybridization (FISH) on mitotic chromosomes which were counterstained with DAPI. *In situ* hybridization showed that this DNA probe hybridized on the mitotic chromosome II in both females and males of this species. The results allowed for the first time to describe and to identify a polytene chromosome of *A. fraterculus*. These results enlarge the possibilities to locate and to study gene expression during the development of this endemic pest of Argentina.

55.

EVALUATION OF A MLVA METHOD TO CHARACTERIZE VEROCYTOTOXIGENIC *Escherichia coli* O157:H7 ISOLATES FROM ARGENTINA

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Verocytotoxigenic *Escherichia coli* (VTEC) can cause bloody diarrhea and hemolytic-uremic syndrome (HUS) in humans, being cattle the main reservoir. The most common serotype isolated from patients with SUH is O157:H7, although other ones are also detected. The standard method for typing O157:H7 is pulse field gel electrophoresis (PFGE). The complete genome sequenciation of two O157:H7 strains facilitated the search of alternative markers for molecular typing, like VNTRs (Variable Number Tandem Repeats). The analysis of multiple VNTR loci (MLVA- Multiple Locus VNTR Analysis), is a methodology with a high discriminative power, faster and simpler than the PFGE. In order to set up a MLVA method to characterize VTEC O157:H7 strains from Argentina, nine VNTR loci were amplified in 15 isolates from patients, food and cattle by PCR. The amplimers were visualized in denaturing polyacrilamide gels stained with silver nitrate. The results indicated an efficient amplification of the 9 loci. Six of them were amplified in two multiplex reactions and the other three, in monoplex . Thirty-nine different alleles were observed in the totality of the studied loci and isolates, distributed in 9 haplotypes. The genetic diversity index (D) showed values ranging from 0.49 to 0.72. These unpublished data, indicate the utility of MLVA method for the characterization of native VTEC O157:H7 strains, and reflect a high level of genetic diversity in the analyzed serotype. The characterization of further loci and other VTEC isolates will give greater information about the genetic variability of the native VTEC strains.

56.

ANALYSES OF MTHFR, CBS AND MTRR ALLELIC VARIANTS AS GENETIC RISK FACTORS FOR NEURAL TUBE DEFECTS (NTD) IN ARGENTINA USING THE TRASMISSION DESEQUILIBRIUM TEST (TDT)

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Neural Tube Defects (NTDs) are among the most common human congenital malformations though the etiological basis remain poorly understood. The aim of the study was to asses MTHFR 677 C>T, 1298C>A, CBS 68 bp insertion and MTRR 66A>G as genetic risk factor for NTD development in Argentina. We have included 100 non-syndromic NTD patients and 198 parents comprising 73 triads and 19 diads. The MTHFR alellic variants were analyzed by PCR followed by HinfI (677 C>T) or MboII enzymatic digestion (1298C>A). The 68 bp insertion was analyzed by PCR and subsequent BsrI digestion to verify linkage disequilibrium with 833T>C mutation. MTRR 66A>G was analyzed by mismatch PCR following AflIII digestion. Using the allele transmission disequilibrium test, we were unable to demonstrate a significant allele segregation bias of any of the genetic variants studied (p=0,529; p=0,655; p=0,532; p=0,529, respectively). Since several evidences demonstrate that MTHFR 677TT genotype is the major at risk genotype, we performed TDT analysis in families in which the parental genotypes would permit the offspring to have a TT genotype (Shields *et al*., 1999). Once again, no differences in the allele trasmission frequencies were achieved (p=0,330). Though preliminary results of a case–control study of our research group suggested that MTHFR 677TT genotype is a genetic risk factor, our present analyses may question those previous outcomes. Nevertheless, further investigations into a larger number of NTD families should be conducted, to better define the influence, if any, of MTHFR 677TT genotype in our population.

57.
MOLECULAR PHYLOGENY OF THE GENUS *PENNISETUM* AND *CENCHRUS* (POACEAE)

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The genus *Pennisetum* (Poaceae: Panicoideae: Paniceae) includes approximately 80 species distributed in tropical and subtropical regions worldwide, having its larger diversity in America and Africa. According to morphological characters, the genus is divided in seven sections: *Penicillaria, Brevivalvula, Gymnotrix, Heterostachya*, *Pennisetum, Dactylophora* and *Beckeropsis*. *Cenchrus* is close related to *Pennisetum*, and some species have been placed in both genera. They differ in the bristle shape, while in *Pennisetum* the bristles are free to the base, in *Cenchrus* are flattened and united below the base. Both genera belong to a monophyletic group together with *Setaria, Ixophorus, Paspalidium,* and *Stenotaphrum*, all characterized by having the inflorescence surrounded by an involucre of bristles ("Bristle clade").
The aims of this work were to analyze the monophyly of *Pennisetum*, *Cenchrus* and their relationship within the "Bristle clade". The monophyly of the sections of *Pennisetum* were also tested.
The study includes 33 species of *Pennisetum*, 7 species of *Cenchrus*, and 7 outgroups. We amplified and sequenced the chloroplast markers *ndh*F and *trn*L-F. The sequences were aligned by eye. The matrices were combined and analyzed using maximum parsimony, by the program TNT. The support for the clades was assessed using jackknife and GC analyses.
The strict consensus showed that *Pennisetum*, *Cenchrus* and *Odontelytrum* formed a monophyletic group, with high support values. Within this group, neither *Pennisetum*, nor *Cenchrus* were monophyletic, since both grouped together in a well-supported clade. Moreover, sections of *Pennisetum* were not monophyletic and need to be evaluated by a new morphological assessment.

58.
GENETIC BASIS FOR TASTE VARIABILITY IN HUMAN BITTER PERCEPTION

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There are individual differences on bitterness of 6-n-propyl-thiouracil (PROP) which is perceived very, medium or less bitter by supertasters (ST), taster (T) or non taster (NT), respectively. Previous results with a variety of psychophysical tasks showed that approximately 60% of the individuals received a reliable phenotypic tipification while the remaining exhibited much variability.
This study was undertaken to analyze this variability by means of bioinformatic resources (ClustalX and Phylip package). 36 gene sets obtained from GenBank and Expasy were analyzed. From these, 25 were intact bitter taste receptor genes (hTAS2R, G-protein-coupled receptors). Several possible subfamilies emerged from the phylogenetic tree obtained for this multigene family. By means of map view data visualization tool it was found a cluster of independent genes (hTAS2R43 to 50) located near hTAS2R13 and 14, giving a subfamily with high similarity in chromosome 12. Another clusters included hTAS2R38 in chromosome 7 and the hTAS2R1 in chromosome 5. SNPs taken from NCBI (databaseSNP) showed high SNP frequency/gene (mean value = 0.50%) from 0% (hTAS2R41, 43, 45, 47 without SNPs) to 1.94% (hTAS2R16). The display of several subfamilies and the high number of polymorphisms in hTAS2R receptors gene support the poor performance of the psychophysical methods to obtain the phenotypic tipification in ST, T and NT individuals. These results suggest the possibility of replacing the tests which associate phenotypes and PROP sensitivity for tests which associate phenotypes and the TAS2R genotype.

59.
CITOGENETICS STUDIES OF ADVANCED TRICEPIRO LINES BY MEAN OF FISH–GISH

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The tricepiros are synthetic hybrids with a trigeneric origin (wheat–rye–wheatgrass) and high forage value. They are obtained crossing triticales and trigopiros. The aim of this work is to study the chromosome number and genomic composition of two advanced lines, obtained at the Universidad Nacional de Río Cuarto, by crossing triticale LF42 (2n=6x=42) x trigopiro Don Noé (2n=8x=56), and triticale LF42 (2n=6x=42) x trigopiro "Horovitz" (2n=8x=56). Both trigopiros have different *Agropyron* (=*Thinopyrum*) ancestors. In Don Noé, the ancestor species is *Th. elongatum* or a related one with uncertain ploidy level, meanwhile in "Horovitz" is *Th. intermedium* (sin. *trichophorum*). *In situ* hybridization with the probe pSc119.2 and genomic DNA of rye (blocked with wheat) was performed on mitotic cells. In advanced generations, the two tricepiros, originally 7x, showed the same and stable chromosome number 2n=42. The *in situ* hybridization performed with genomic DNA showed the presence of the 14 chromosomes corresponding to the R genome. The probe pSc119.2 allowed the identification of most of the chromosomes, and made possible the characterization of 28 wheat chromosomes that would belong to the genomes A and B. The data obtained in the present work showed that the studied tricepiros are stabilized in 42 chromosomes and the rye R genome is retained. Furthermore, these tricepiros are different from each other because a pSc119.2 hybridizing region of the telomeric zone of the rye 7R chromosome pair is absent in one of them. Considering the fact that both tricepiros have the same triticale ancestor with the R genome, the difference found could be due to chromosome reorganizations stabilized by homocigosis.

60.
CYTOGENETIC CHARACTERIZATION OF THREE SPECIES OF HARPACTORINAE (REDUVIIDAE, HETEROPTERA, INSECTA)

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Members of the Reduviidae are of great importance in human health (since many Triatominae are vectors of Chagas' Disease) as well as agents of biological control. Cytogenetic studies in the subfamily Harpactorinae refer to only twelve species with diploid chromosome numbers between 12 and 28, showing simple and multiple sex chromosome systems. In this work we report for the first time preliminary cytogenetic results in *Apiomerus lanipes* (Fabricius) and we describe the karyotype and male meiosis of *Cosmoclopius nigroannulatus* (Stål) and *C. poecilus* (Herrich-Schaeffer) after conventional staining and C and DAPI banding. *Apiomerus lanipes* presents 2n= 22+XY, with a strikingly large autosomal pair; sex chromosomes are similar in size, and the second largest of the complement. Bivalents present as a rule only one terminal chiasma, but the largest one generally presents two chiasmata at terminal positions. Both *C. nigroannulatus* and *C. poecilus* have 2n= 28= $24+X_1X_2X_3Y$ with very small Xs chromosomes and a large heterochromatic Y that is C positive and DAPI bright. The three species have a long diffuse stage during which chromatin decondenses completely except for the sex chromosomes and heterochromatic blocks of the largest autosomal pair in *A. lanipes*. Our results are in agreement with previous reports on Harpactorinae and constitute the first studies in Argentinean species. This work will contribute to clarify the phylogenetic relationships within the Reduviidae.

61.

KARYOTYPE VARIABILITY IN A POPULATION OF THE HORN FLY *HAEMATOBIA IRRITANS* (L.) (DIPTERA: MUSCIDAE)

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The horn fly, *Haematobia irritans*, was introduced in Argentina in 1991 and it seriously affects cattle production. However, little is known about its biology and about its genetics. Only 2 previous cytological studies on the same laboratory colony from Kerrville, Texas, describe a single karyotype 2n=2x=10 without signs of heterogamety. The aim of our study is the genetic characterization of a mixed population representative of the North-Central region of Argentina. Wild females were allowed to oviposite in liquated bovine faecal matter under laboratory conditions (29ºC) to eliminate other immature muscids. Mitotic chromosome spreads from cerebral ganglia of third instar larvae (n=53) were Giemsa or Hoechst banded. Testes of 10 emerging adults were stained in lacto-propionic orcein to study meiosis.
This is the first time that karyotype variability in H. *irritans* is demonstrated. Chromosome complements of 2n=2x=10 were found in 37 specimens. The most frequent karyotype is characterized by 2 metacentric (1 and 2) and 3 submetacentric (3, 4, 5) chromosome pairs, one of the latter with a satellite. Other specimens showed rearrangements easily identified due to somatic pairing in the species. A polymorphism affecting chromosome number was observed in 26 specimens showing a 2n=2x=11 chromosome complement. The extra chromosome is a small acrocentric one and it is absent in testes preparations. Cytological samplings of emerging males as well as a screening to find female meiosis are required in order to determine if this small chromosome is a B-chromosome or could be associated to one sexual chromosome. Our results are relevant to develop control genetic strategies.

62.

PREMAURE OVARIAN FAILURE: STUDY OF POLYMORPHISMS 919A>G AND 2039A>G OF FSH RECEPTOR GENE AS PUTATIVE RISK FACTORS

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Two polymorphisms, 919A>G and 2039A>G, were described in exon 10 of FSH receptor (FSHR) gene. Although it was suggested that these polymorphisms may affect FSH levels and the ovarian response to this hormone, studies on patients with premature ovarian failure (POF) are limited and not conclusive. In order to analyze if these polymorphisms may be associated to POF, genotype frequencies were determined by PCR-RFLP in 65 POF patients and 72 controls above 40. No association was found between GG genotype and the risk of idiopathic POF (919A>G: $OR_{AA+AG\ vs.\ GG}$=1.01; $IC_{95\%}$=0.39-2.63; 2039A>G: $OR_{AA+AG\ vs.\ GG}$=1.03; $IC_{95\%}$=0.40-2.67). Polymorphism 919A>G was found to be in linkage disequilibrium with 2039A>G. Six POF patients and only one control presented the uncommon combinations 919G-2039A and 919A-2039G. The implication of both polymorphisms in serum hormone levels was analyzed in 44 normally menstruating women. Samples were divided into groups: 1) A-A/A-A genotype (n=8), 2) A-A/G-G genotype (n=27) and 3) G-G/G-G genotype (n=9). Hormone levels were measured by RIA or ELISA. No significant differences ($p>0.05$) were found between groups in mid-follicular phase FSH, E_2 and Inhibin B levels and mid-luteal phase Inhibin A levels. In conclusion, hormonal and genetic results suggest that polymorphisms 919A>G and 2039A>G of FSHR gene may not be involved in the etiology of POF. Nevertheless, differences in prevalence of the rare genotypes between POF and controls should not be overlook, and the biological implication of these rare genotypes should be further analyzed.

63.

DIARRHEA CAUSING BACTERIA IN A HOSPITAL OF ROSARIO CITY

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The ethiology of acute infectious diarrea differ from different countries and from different populations. The aim of this study was to know the incidence termophilic *Capylobacter* and other enterovirulent bacteria in our hospital. We study 304 ambulatory patients with acute diarrhea attended in Hospital Español de Rosario, Argentina in the period from 1/1/04 to 31/10/05. Fecal samples of 182 children less than 5 years old, and 122 patients ≥ 5 years were cultured acording to classical procedures and genetic probes looking for species of *Campylobacter, Salmonella, Shigella, Aeromonas, Vibrio, Yersinia, Plesiomonas* and diarrheogenic *Escherichia coli,* as was former described. Antimicrobial susceptibility of *Campylobacter* isolates was perform accordin with CLSI an ANLIS C Malbrán recommendations. RESULTS: *Campylobacter jejuni,* the most frequent pathogen, it was identified in 30 (9.9%) cases 8.8% in < 5 years old, and 11,5% in ≥ 5 years old, followed by *Salmonella enterica* 18 (5.9%), enteropathogenic *E coli* 7 (2.3%), enterotoxigenic *E coli* 6 (2%), *Aeromonas hydrophila* complex 3 (1%). Surprisngly there were not cases due to *Shigella* spp. In 87% of cases due to *C jejuni* the patients had feces with mucus, blood, and/or plymorphonuclear leucocytes. Among *C jejuni* isolates, 95% were susceptible to nitrofurantoin 89.5% to gentamycin, 89.5% to azitromycin, 88.9% to erythromycin, 88.9% to tetracycline, and 36.8% to the fluorquinolones. Most cases of patients with dysenteric diarrhea were due to *C jejuni*, and this species pesented high quinolone resistance.

64.

IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE PITUITARY FOLLICULO-STELLATE CELLS OF *Lagostomus maximus maximus*

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Pituitary folliculo-stellate (FS) cells have been investigated in several vertebrates to elucidate their function and origin. In our Laboratory we study the reproduction and adaptation mechanisms of viscacha (*Lagostomus maximus maximus*). It's a native rodent with nocturnal habits and seasonal reproduction. The purpose of the present work was to study the pituitary FS cells in the *Lagostomus.* The pituitaries of adult male viscachas were analyzed by immunohistochemistry techniques and transmission electronic microscopy. Lanthanum hydroxide was used as electrodense tracer. In the immunohistochemical study S-100 and glio-fibrillary acidic (GFAP) proteins were localized. The FS cells show cytoplasmic and nuclear immunolabeling for S-100 and cytoplasmic immunolabeling for GFAP. They are forming follicles with colloidal lumen, present cytoplasmic processes between endocrine cells. The ultrastructural study shows FS cells with irregular nucleus, moderate numbers of mitochondria, scarce rough endoplasmic reticulum and absence of secretory granules. The lateral membranes of the apical region are joined by prominent junctional complex and desmosomas. From the apical poles of the cells, microvilli protruded into the follicular lumen. The tracer penetrates freely into the intercellular spaces, delineating the cellular border of granulated and FS cells. Our results suggest that the FS cells of viscacha originate an extensive and permeate canalicular network of intercommunicated spaces freely between the granulated and FS cells. This cellular interaction probably is involved in the hormonal release control. Besides, the expression of S-100 and GFAP proteins might indicate a neuroectodermal origin of the FS cells in the *Lagostomus.*

65.

MELANIN CONCENTRATING HORMONE (MCH) AND GONADOTROPHIN RELEASING HORMONE (GnRH) AS REGULATORS OF SOMATOLACTIN IN THE TELEOST FISH *Cichlasoma dimerus*

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In most vertebrates, the pars intermedia (PI) presents only MSH cells but in teleost and lung fishes, somatolactin (SL) expressing cells can be observed too. The structure of SL is similar to vertebrate Growth Hormone and Prolactin. Moreover, a multitude of biological functions were described for this hormone. In *C. dimerus,* we found clear evidence about a possible involvement of SL together with αMSH and MCH in background adaptation (Cánepa *et al*., 2006). However, there is scarce information on the regulation of SL secretion.
This work analyzes the effect of GnRH and MCH on SL secretion and the morphological association between MCH and GnRH fibers with SL cells. We observed that MCH (10 μM, 24 hs) and GnRH (10μM, 24 hs) stimulate the SL secretion from organ-cultured pituitary. By double immunohistochemistry and confocal microscopy we demonstrate a close association between the MCH fibers proceeding from the nucleus lateralis tuberis (NLT) and SL cells. Furthermore, we found GnRH fibers from the ventral forebrain (in lower proportion than MCH) in association with SL cells. On the other hand, we observed that in the NLT, fibers of GnRH were associated to the soma and fibers of the magnocellular MCH neurons.
These results suggest that, in *C. dimerus,* SL is under hypothalamic control of MCH and GnRH. Morover the close association between GnRH fibers and MCH cells in the hypothalamus imply that GnRH also acts like a MCH neuromodulator.

66.

TEACHING LEARNING PROCESSES IN ANIMAL PHYSIOLOGY: BLOOD SUGAR QUANTIFICATION IN LABORATORY CONDITIONS

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Animal physiology is an experimental science that needs scientific laboratory practices and exercises in order to know its basic principles.
The aim of this study was to work on a laboratory practice performed by Biology students in order to manipulate, investigate and learn basic physiology techniques, use laboratory equipment and get accustomed with experimental animals. In laboratory conditions, the students measured blood sugar concentration working with three target groups of rats (Ratus.sp, Wistar) CG, AG, IG (control with physiology solution, adrenalin and insulin group). As a first stage, several animal behavioral patterns were registered, anesthesia (ether) was applied, and physiology sol. and hormone injections were done (adrenalin e insulin). Secondly, blood cardiac extraction was completed on each group, located in tubes and centrifuged. At last, tubes were incubated at 37ºC, readings by spectrometric absorbance were registered and the students measured and calculated blood sugar concentration. As a result of the experience, students were taught on basic lab knowledge, learned how to manipulate animals, worked with lab apparatus, and solved methodical analysis and calculus. We want to remark that during the four hours of activity, alumni were protagonist in the learning process dealing with common scientific situations in the Animal Physiology area.

67.

SOMATOTROPHS IN PITUITARY PARS DISTALIS OF VISCACHA. RELATION TO THE TESTICULAR ACTIVITY

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It has been reported that the gonadal steroids might regulate the synthesis and secretion of growth hormone (GH). We studied the regulation of the adaptative and reproductive processes in the viscacha (*Lagostomus maximus maximus*). This rodent presents a reproductive cycle with three gonadal periods: activity (Act.), regression (Reg.) and recovery (Rec.). The aim of this work was to study the somatotrophs in pituitary pars distalis (PD) in relation to the testicular activity. 1) Adult male viscachas during the reproductive cycle, 2) Immature and prepubertal male viscachas and 3) adult castrated animals were used. The somatotrophs were identified by immunohistochemistry. The percentage immunopositive area (%IA), cellular number (N) and cellular (CD) and nuclear (ND) diameters were carried out by image analysis. They were considered as a measure of the cellular activity. Somatotrophs were distributed throughout the parenchyma of PD. They were oval, pyramidal or round in shape with a voluminous nucleus that occupied most of the cytoplasm. These cells were usually found near to the follicular structures and along the sinusoidal surface. Morphometric analysis:

	Immature	Prepubertal	Adult-Act.	Adult-Reg.	Adult-Rec.	Control	Castrated
% IA	8.60±0.37 **	10.48±0.38	12.66±0.53	9.12±0.31 **	12.79±0.38	10.27±0.23	5.41±0.17 ***
N	35.20±1.56 **	48.06±2.33	45.84±1.62	28.19±1.61 **	41.08±2.53	53.87±1.91	26.56±1.12 ***
CD (μm)	9.32±0.14 *	9.18±0.12	10.49±0.14	9.37±0.10 *	10.62±0.15	9.43±0.14	8.43±0.12 *
ND (μm)	4.73±0.10	4.95±0.09	5.27±0.17	5.25±0.06	5.19±0.14	5.03±0.09	5.14±0.10

*P < 0.05; **P < 0.01; ***P < 0.001.

Our results suggest that the variations of the morphometric parameters of these cells are related to the testicular development and activity.

68.

SEASONAL AFFECTIVE DISORDER AND SMELL

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The AIM of this work has been to assess smell perception in subjects affected by seasonal depression (SD), on the basis that olfactory denervation unmasks photoperiodic responses in non-photocyclic animals. METHODS: 9 women with SD and 9 matched controls volunteered for olfactory determinations. Detection thresholds were determined using the n-butanol method. Identification was evaluated using the University of Pennsylvania Smell Identification Test (UPSIT). Assays were bilateral and unilateral (olfactory projections are predominantly ipsilateral). All subjects were euthymic and untreated at the time of the study. Beck's Depression Inventory was used for mood monitoring. RESULTS: 1). Detection thresholds revealed no differences between groups [$F(1,48)= 0.876$, N.S.]. 2). UPSIT performance was poorer in SD [$F(1,48)= 6.338$, $p< 0.015$ vs control subjects] only for the right nostril (all subjects were right-handed). DISCUSSION: our findings agree with previous evidence indicating lateralization of brain disfunction in SD and suggest that olfaction may be related to seasonal emotional rhythms in humans.

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69.
ARCHITECTURAL DESIGN OF THE SEMITENDINOSOUS MUSCLE IN PIGS

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Muscle subvolumes differ in architectural design, fibre types, fibre transversal sections, metabolic capacities, and number of capillaries. These differences between subvolumens are showed in oxidative and glicolitic capacities, colour, glycogen and lipids levels, all factors that have great influence in postmortem changes which conduct to transformation of muscles into meat.
Nevertheless, in many researchs were obtained contradictory results between muscles characteristics and meat quality parameters, because the muscles were considered as a whole. It has been our aim to carry out a study of the architectural design of the pig semitendinous muscle (PSTM), whose results will be applied to comparing the muscle characteristics and meat quality parameters in each subvolume.
The PSMT with the branch of the sciatic nerve of 5 pigs were immersed consecutively in a 10% formalin solution 30 days, a 25% acid nitric solution 10 days, rinsed, followed by blunt disection. The muscle belly was divided into a proximal medial (PM), a proximal lateral (PL), a distal medial (DM), and a distal lateral (DL) subvolumes, each one supplied by a primary branch of the sciatic nerve. PM and DM were besides indicated by a rudimentary intermediate septum. The length of the muscle fascicles were 35-40 mm in all subvolumes, with fibres arranged parallelly and sarcomeres ordered in series.
The PSTM has 4 subvolumes whose muscle fibrilar characteristics should be determined.
The correspondence with the white and red parts without details of structures documented by other authors should be established by histochemical techniques for its subsequent husbandry applications.

70.
SMELL CUES INVOLVE TASTE SENSITIVITY ALTERATION BY ETHANOL

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This article shows ethanol (Et) effects on taste thresholds as a function of gender and Et concentration (grade). Orosensory (taste, odor) influences were studied in relation to taste quality recognition' sensitivity.
Sixteen volunteers evaluated aqueous solutions of (mM): sucrose (7.31-61.4), citric acid (0.39-1.29), caffeine (0.25-2), NaCl (4.27-43.18) and their Et(12%, 6%)-mixtures. Taste thresholds were assessed by paired comparisons. Subjects compared tastant vs mixture and decided whether 'T=Mix' or 'T≠Mix' on a quali/quantitative basis. They should identify quality. 'Detection probability' results (DP= 'positive detection cases/ total cases' ratio) at each concentration and were submitted to multivariate analysis of variance (MANOVA), individual ANOVAs and LSD tests (SPSS 10.0™). Et reduced DP for sweetness (Sw) and bitterness (B) [F_{Sw} (1, 64)= 6.48, p< 0.01; F_{Bitt} (1, 64)= 4.39 p< 0.04]. Sw and B responses were related to gender [F_{Sw} (1, 64) = 4.02, p< 0.05; F_{Bitt} (1, 64)= 7.54 p < 0.008]. Sw and B responses were both related to alcoholic grade [i.e.: 0% (water), 6% Et, 12% Et] [F_{grade} (1, 64)= 4.02, p< 0.05; F_{Bitt} (1, 64)= 9.05 p< 0.004]. Deprivation of Et odor reduced the magnitude of Et effects on taste.
Et affected taste detection according to quality, gender and ethanol. Et affected perithreshold Sw and B. Since these are the same qualities perceived in pure Et dilutions (2, 3), present data might involve superimposed taste qualities. Perceptual confusion might enhance baseline signal so that taste increases become proportionally ineffective to make further differences.

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71.
BONE MARROW STROMAL CELLS MIGRATE TO DORSAL ROOT GANGLIA, INDUCE CHANGES IN NEUROPEPTIDE EXPRESSION AND REDUCE ALLODYNIA AFTER SCIATIC NERVE CONSTRICTION

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Peripheral nerve injury triggers neuropathic pain and induces changes in neuropeptide expression. Following brain injury, bone marrow stromal cells (MSCs) migrate to the lesioned hemisphere and mediate functional recovery. In this study we have analyzed the localization of MSCs administered to rats subjected to a sciatic nerve constriction. We have also assessed the expression of different molecules involved in pain transmission, as well as thermal and mechanical sensitivities. MSCs were isolated from adult rat tibiae and femurs, cultured in DMEM and labelled with Hoechst. 2 x 10^5 cells were injected into the right L4-dorsal root ganglia (DRGs) of rats subjected to a single ligature nerve constriction. Animals were tested for mechanical and thermal withdrawal thresholds after 1, 3 and 7 days. Lumbar DRGs were dissected out, cut, observed using a fluorescence microscope and processed for immunohistochemistry. MSCs acquired a perineuronal localization in the injected ganglia and migrated to the other ipsilateral lumbar DRGs. Nerve lesion induced a marked increase in the expression of the neuropeptides NPY and galanin and the neuronal isotype of nitric oxide synthase (nNOS) in primary afferent neurons. There was also a decrease in the number of cells expressing NPY-Y_1 receptor (Y_1r). These changes were prevented by the administration of MSCs. In parallel, both mechanical and thermal allodynias induced by nerve constriction were significantly decreased in MSC-transplanted animals. These results suggest that MSCs may modulate pain generation by modifying NPY, galanin, Y_1r and nNOS expression in primary afferent neurons after sciatic nerve lesion.

72.
BISPHENOL A AND THE PITUITARY: *IN VITRO* AND *IN VIVO* EFFECTS

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Bisphenol A (BPA), an endocrine disruptor, is a component of epoxy and polyestirene resins used in food packaging and dentistry. *In vitro* it has shown weak estrogenic actions, 10.000-fold less potent than estradiol (E_2), but it has pronounced *in vivo* effects as inducing irregular estrous cycles and cystic follicles in neonatally BPA-treated Sprague-Dawley (SD) rats.
First, we determined the *in vitro* effects of BPA on the pituitary response to GnRH (G). In primary pituitary cultures (PPC) from 13-day-old female SD rats, after 24 hours of BPA 1.10^{-7}M or E_2 1.10^{-7}M preincubation, an increase in basal FSH release was observed with regards to controls [FSH (ng/ml): Control=0.81±0.05; BPA=1.49±0.22; E_2=1.71±0.19, p<0.05], while only E_2 enhanced G-induced FSH release [G=3.39±0.01 vs. E_2+G=4.15±0.32, p<0.05]; neither BPA nor E_2 modified basal or G-stimulated LH release.
To further study a possible effect on LH, and on PRL, two new experimental models were used. The effects of chronic *in vivo* treatment with BPA on basal and stimulated LH and PRL release was determined in PPC, in addition to studying *in vivo* LH release after G administration. Females were treated daily, from postnatal days 1 to 10, with BPA dissolved in oil (500 μg/day, sc.) or vehicle and studied at postnatal day 13. *In vivo* pretreatment with BPA diminished LH release in PPC, but did not modify the response to G; an increase in basal PRL was determined (p<0.05). *In vivo,* rats pretreated with BPA had lower absolute serum LH levels, both basally and after G stimulation, than controls (p<0.05), although the G-induced percent of LH increase was similar.
We conclude that BPA has marked effects on pituitary secretion, modifying hormone release both after *in vitro* and *in vivo* treatments.
(CONICET, ANPCYT, UBA).

73.

EXPRESSION OF HIGH AFFINITY NEUROTROPHINS RECEPTORS (Trks PROTEINS) IN *Sus scrofa domesticus* SECONDARY LYMPHATIC ORGANS

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There are evidences that neurotrophins can work on other tissues that express mRNA and/or their receptors, as rats, mice and man lymphatic system. This investigation goal was to determine Trk receptors localization in pig's secondary lymphatic organs (spleen, lymphnodules, tonsils and Peyer´s patches). Samples from young pigs (n=20) were fixed in 10% buffered formaldehyde and processed by routine histological techniques. Trks proteins were labeled with rabbit polyclonal antibodies diluted 1:100 (Santa Cruz Biotechnology, CA, USA), employing the immunocytochemistry avidine-biotine-peroxidase method (ABC).
Spleen: TrkA and TrkB were localized in white pulp and in blood vessels inside lymphoid follicles. Trk C was identified in capsule, trabecules, and lymphoid tissue of red pulp.
Lymphnodules follicles: TrkC was found in cortical lymphoid tissue and capsule. TrkA and TrkB had negative immunoreactivity.
Palatine tonsils: TrkA and TrkC were identified in stroma follicles while TrkB was negative.
Peyer's patches: TrkA and TrkC were shown in lymph follicles center, TrkB showed negative immunoreactivity.
We conclude that TrkA and TrkC studied expression in pig's secondary lymphatic organs would indicate the probably action of NGF and NT-3 growing factors, while TrkB, was identified only in spleen, this would suggest a possible role of BDNF and NT-4/5 as growing factors in this organ, for maintenance of cellular populations.

74.

HUMAN BONE MARROW (BM) MESENCHYMAL STEM CELL (MSC) DIFFERENCIATION INTO CARDIAC PHENOTYPES ABLE TO EXPRESS CARDIAC PROTEINS

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Cardiac resident and non-cardiac stem cells with regenerative potential have been identified in human adult heart. The type of BM-cell source contributing more efficiently to cardiovascular regeneration (MSC, endothelial progenitor cells, or multipotent adult progenitor cells) is still under evaluation. Whether these cells become new cardiac lineage cells by phenomena of transdifferentiation or fusion is also being investigated, especially in human. The aim of the present work was the generation of an experimental homologous system of human BM-MSC able to differentiate into cardiomyocytes and to express cardiac proteins *in vitro*. Two differentiation protocols were selected: 5-azacytidine 10μM/24h or streptolysin O permeabilization 57ng/ml in the presence of primary culture of neonatal rat cardiomyocytes extract. Mononuclear cells from allogenic transplant donors were obtained by Ficoll-Hypaque gradient and subcultured at low cell density for MSC isolation. MSC was positive for prolil-4-hidroxilase, CD105, CD44 and exhibited differentiation capacity into osteoblasts/osteocytes, condrocytes and adipocytes. MSCs differentiated into cardiomyocytes were morphologically identified and phenotypically characterized by immunohistochemistry and PCR. One week after induction, some cells showed multinucleation and ball-like morphology; at week 2, cells assumed stick morphology. About 3 weeks after induction the cells formed myotube-like structures with adjacent cells. The treated cells were positive for desmin, sarcomeric α-actinin, MLC-2a, connexin-43, GATA-4, β-MyHC, Nkx 2.5, troponin I and SERCA-2, but showed a weak expression for β1-AR. Untreated cells expressed connexin-43, sarcomeric α-actinin and GATA-4. Until now, MLC-2a, troponin I y β1-AR were positively assayed by PCR. Our results provide a useful model for development of stem cell therapeutics and their potentiality in cardiac repairing under pathological conditions.

75.

TAXONOMY OF HARPACTICOIDA COPEPODS IN USHUAIA AND GOLONDRINA BAYS, BEAGLE CHANNEL, ARGENTINA

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Harpacticoid copepods are second in abundance and species diversity in benthic marine habitats. This group frequently occurs in planktonic samples as occasional forms, particularly in shallow and algae-covered coastal waters. Several shallow coastal zones in Ushuaia and Golondrina bays are characterized by the presence of dense macroalgae beds in which *Macrocystis pyrifera* is the dominating species. The latter serves as a substratum to a large number of organisms such as harpacticoid copepods, amphipods and isopods. The aim of the present study was to taxonomically identify the harpacticoid copepods found in mesozooplankton samples from both bays. Samples were collected in both bays (54º79', 54º85'S-68º22', 68º36'W) during August and December 2004. A Nansen net (0.30 m mouth; 200 μm mesh) and oblique tows were used for the collection. Copepods were identified at the lowest possible taxonomic level using a Leica DM LS 2 optical microscopy and an M Leica MZ 9.5 stereo microscope. Taxa belonging to13 harpacticoid families were identified in 24 samples. The species found in 11 of these families belonged to 15 genera. The families Tisbidae, Porcellidiidae and Thalestridae were dominant in the study area (with 75%, 62.5% and 58.33% frequency of occurrence, respectively). Ameiridae, Canthocamptidae and Idyanthidae played a minor role, not reaching 4.17% each one. The genus *Porcellidium* showed the highest frequency of occurrence (62.5%) while *Tisbe* and *Scutellidium* showed 50%. Three new species belonging to the genera *Mesochra*, *Idyanthe* and *Scutellidium* were found in both bays. This study is the first record of the taxonomy and occurrence of harpacticoid copepods in Ushuaia and Golondrina Bays.

76.

INVENTORY OF THE NABIDAE (HETEROPTERA, INSECTA) FROM ARGENTINA

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The Nabidae, or "damsel bugs", are generalist predators. Most members of the Nabinae appear to be predators on a wide variety of small arthropods including both pest and beneficial species. Their polyphagus habit, sometimes considered a detriment to their usefulness in control programs, may also allow them to persist in times of low target pest density contains. In contrast, the Prostemmatinae appear to prey exclusively on other Heteroptera. All of the Nabidae are generally herbicolous, arboricolous or ground- inhabiting. The family includes 21 genera and about 500 species distributed worlwide from about 70º N up to 56º S. Nabidae have been placed within the superfamily Cimicoidea in the group Cimicomorpha. Pennington (1921) cited 2 genera and 7 species for Argentina. With the accesss of bibliography and material from different institutions we mention 4 genera with 12 species. These taxa are distributed from the north of Argentina to Tierra del Fuego.

77.

VERTICAL DISTRIBUTION OF *EURYTEMORA AMERICANA* (CRUSTACEA, COPEPODA) DURING A TIDAL CYCLE IN THE BAHIA BLANCA ESTUARY

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Vertical distribution of zooplankton is a complex phenomenon involving differents behaviors that change according to species and individual. This distribution depend on factors suchs as age, sex and spawning and external factors such as tidal cycles, lunar phase, light intensity, temperature and salinity. This study analyze the influence of tide and physical and chemical variables on vertical distribution of developmental stages of *E. americana*. Sampling was conducted at Cuatreros Port in August 2005. Samples were taken every three hours at surface and bottom over a 14-hour period. Submersibles pumps discharging into 200 μm mesh nets were used. Salinity, temperature, chlorophyll a, particulate organic matter and suspended sediment were measured at each time. Temperature range was 9.8-11.6ºC and salinity 29.3-33.2. Chlorophyll a was higher at surface whereas the highest concentration of suspended sediment was registered during flood at bottom. Densities at surface ranged between 3.1-1725.7 ind m^{-3} and between 4.7-971.9 ind m^{-3} at bottom. Copepodids I-III dominated at surface during all tidal cycle and the highest value was observed during ebb. A similar pattern ocurred at bottom, although during flood copepodids IV-V were more abundant and the differences in abundance were not as marked as in surface. Adults males and females showed a similar pattern along the tidal cycle (surface and bottom) but males were always more abundant than females. Nauplius, copepodids I-III and IV-V were, in general, more abundant at surface while males and females at bottom. Differences in the abundances would indicate that the stages are distributed differentially in the water column and that the tide influences their position in the water column.

78.

OUABAIN-INSENSITIVE Na^+ATPase ACTIVITIES IN MUSCLE OF *Chasmagnatus granulatus* UPON HYPERGULATION

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Two Na^+-stimulated ATPases, an ouabain–sensitive Na^+K^+ATPase (NKA) and an ouabain–insensitive Na^+ATPase (NA), with distinct properties, appear to occur in several animal tissues. In invertebrates, NA is involved in intracellular homeostasis of Na^+. As part of our studies on regulatory mechanisms at the biochemical level of the euryhaline crab *C. granulatus* from Mar Chiquita coastal lagoon (Bs. As. Province), we previously showed the occurrence of salinity dependent NKA and alkaline phosphatase activities in chela muscle suggesting a role for this tissue in regulatory mechanisms evoked upon osmo-ionic stress. The aim of this work was to study the occurrence and biochemical characteristics of NA in chela muscle. Male adult crabs were acclimated for at least 10 days to 10‰ salinity. NA activity was determined in chela muscle homogenates (0.25M Sucrose/0.5mM EGTA-Tris pH 7.4) by measuring ATP hydrolysis (0.05-13.00 mM) in the presence of (mM): 20 imidazole (pH 6.2-7.8)/130 NaCl/0.5 EGTA/1 ouabain/1 NaN_3, in the presence (furosemide-insensitive, FI, NA activity) or the absence of 2 furosemide. The difference of both assays represents the furosemide-sensitive (FS) NA activity. Control: without NaCl. 3-6 independent experiments were carried out. Two NA activities were detected in chela muscle, a FI and a FS (88.2±11.9 and 117.5±23.2 nmoles $Pi x min^{-1} x mg\ prot^{-1}$ respectively). FI and FS activities showed a Michaelis-Menten kinetics ($K_{.m}$ = 0.021 and 0.224 mM, respectively). Maximal furosemide inhibition was at 2 mM (I_{50}=1.4 mM). Maximal activities occurred at pH 7.4 and NaCl 130 mM. The possible role of NA activities in mechanisms of adjustments secondary to hyperegulation in muscle of *C. granulatus* remains to be investigated.

79.

BIODIVERSITY OF REDUVIIDAE (HETEROPTERA, INSECTA): THE GENUS PSELLIOPUS

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The Reduviidae are predators of arthropods, polyphagus or specialized feeders. Blood-sucking Triatominae attack different Tetrapoda, and some Triatoma species are important as vectors of Chagas disease in South America. It includes 23 subfamilies 930 genera and 6.800 species. The distribution is about cosmopolitan though most taxa inhabit tropics and subtropics. Among the Reduviidae are the Harpactorinae, one of the largest and more diverse subfamily. They are distributed worldwide, most dominantly in the tropics. The genus *Pselliopus* comprises 22 species:
P.barberi (Davis), ***P.cinctus*** (Fabricius), ***P.dantei*** (Brailovsky), ***P.flaviceps*** (brailovsky), ***P.inermis*** (Champion), ***P.infuscatus*** (Champion), ***P. ivanivus*** (Brailovsky), ***P.karleanae*** (Hussey), ***P.latifasciatus*** (Barber), ***P.latispinia*** (Hussey), ***P.lima*** (Pinto), ***P.lineaticeps*** (Champion), ***P.majesticus*** (Brailovsky), ***P.mexicanus*** (Champion), ***P. nigropictus*** (Champion), ***P. ornaticeps*** (Stal), ***P. punctipes*** (Amyot & Serville), ***P.rufofasciatus*** (Champion), ***P. spinicollis*** (Champion), ***P. tuberculatus*** (Champion), ***P. ventus*** (Brailovsky), ***P. zebra*** (Stål), they are distributed from North to South America.The information is reduced and mainly regional: Fracker (1912) and Barber (1924) for the neartic Region and Champion (1899) and Brailovsky (2004) for Central America. The purpose of this contribution is to describe two news species. The material used for this study comes from Mexico, NL.

80.

SEASONALITY VARIATION OF OXIDATIVE METABOLISM IN GONADS OF *Loxechinus albus* FROM BEAGLE CHANNEL

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Changes in the production of reactive oxygen species (ROS) on aquatic organisms had been related to environmental or physiological variations. Carotenoids are often found among sea invertebrates and had influence over their coloration. In addition, these carotenoids protect their lipid constituents against oxidation. *Loxechinus albus*, is a edible sea urchin inhabiting the Beagle Channel. Their gonads have a high value in the gastronomic international market when the proper color and texture are achieved. The aim of this work was to study the oxidative metabolism variations during different stages of the reproductive cycle in the gonads of sea urchins. Ten sea urchins were collected monthly (August 2004 - June 2005) on Beagle Channel (Tierra del Fuego). The gonads were separated and frozen at -40ϒC. The gonadosomatic index (IG) was used as an indicator of the progression of the reproductive cycle. This value showed a minimum in November. The content of Tiobarbituric Acid Reactive Substances- (TBARS), as an index of lipid peroxidation, showed the highest value in October. The content of lipid antioxidant (α-tocopherol and β-carotene) was quantified by reverse-phase HPLC and showed non significant differences over the year. The TBARS content decreased during March and December in coincidence with the increase in IG values, the variation could be related to the reproductive cycle. Such decrease in TBARS content could be linked to the protection afforded by the activity of several antioxidant systems; however it seems that lipid soluble antioxidants are not responsible for this effect since their content was not affected over the tested period.

81.

SHEATH OF THE MUSCLE RECTUS ABDOMINIS OF THE LLAMA (LAMA GAMA)

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It is very important in Veterinary Anatomy to study the abdominal musculature as it surgery is very often (laparatomies) and laparoscopies, specially those which are made through the muscle rectus abdominis (transrectal).
In this investigation we are interested in the study of the sheath of the muscle rectus in abdominis of preumbilical, postumbilical and prepubic regions. We can describe each one as internal and external leaves. We add the detailed explanation of born, insertions, structure and vascular and nervous relations of this muscular group that forms the walls of the abdominis.
We used for this study ten animals, eight female-adult llamas and two male-adult llamas. The instruments and techniques we used were as always we use.
The sheath of the muscle rectus shows differences in different topographical regions, localized between the aponeuroses of obliquus externus abdominis and obliquus internus abdominis in the preumbilical region, then they go deeper. The origin, insertions and structure of the muscles in the llama are similar to domestic species, we found different bones inserted in this musculature and in order to the particular position of the ilium bone.

82.

COMPARATIVE ANALYSIS OF MITOCHONDRIAL FATTY ACIDS IN THE BRAIN OF AVIAN SPECIES: AN ALLOMETRIC STUDY

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The acyl composition of tissue lipids varies in a systematic manner among species. Previous research has shown that tissue phospholipids from small mammals were more polyunsaturated than those from large mammal species, whilst the phospholipids from large mammals were more monounsaturated than those from small mammals. This relationship was present in all tissues examined (heart, liver, kidney and skeletal muscle) except the brain. The objective of this investigation was to examine the relationship between body size and mitochondrial fatty acid composition in the brain of manon, quail, pigeon, duck and goose. The fatty acid composition was determined in isolated mitochondria by gas-chromatography. The percentage of saturated fatty acids (SFAs) was significantly higher ($r = 0.92$; $P< 0.05$), whereas the percentage of monounsaturated fatty acids (MUFAs), mainly oleic acid (C18:1n9), was significantly lower in the larger birds (≅ 15% in goose vs 30% in manon). The allometric exponents were 0.014 and 0.09 for SFAs and MUFAs, respectively. There were no statistically significant allometric trends for polyunsaturated fatty acid percentage ($r = 0.30$) and unsaturation index ($r = 0.52$). The brain of all birds studied had a high content of docosahexaenoic acid (22:6n3) (≅ 12%) and high level of double bonds (as indicated by the unsaturation index ≅ 150). These results show that: 1) brain mitochondria of small birds had higher content of monounsaturated and lower content of saturated fatty acids and 2) the brain of the avian species examined, similarly to other vertebrates, had consistently high levels of C22:6 n3, indicating a tissue specificity.

83.

APPLIED ANATOMY OF LUMBAR PLEXUS OF THE LLAMA (*Lama glama*)

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The lumbar plexus is formed by the ventral branches of lumbar spinal nerves. In the llama, whose lumbar column has seven vertebral segments, the first four pairs of nerves are: L1: cranial iliohypogastric nerve; L2 caudal iliohypogastric nerve, L3 ilioinguinal nerve and L4 genitofemoral nerve.
The present work has as the main objective not only the anatomic face but we are interested in the practical application of our descriptions so you will be able to use paravertebral anaesthesia, in lateral transverse process or near intervertebral foramen. It adds to necessary subcutaneous and intramuscular infiltration on lines of incision, so you must know the nerves way.
It were dissecated eight vertebral columns: six female llamas and two male llamas, all of them were adult animals over five years old. The vertebral canal was opened and were made dissections applying conventional techniques and using simple instrumental.
As the result of our dissections, we discovered that the most important nerves, as the way they go and in order to make their obstruction are the first up to the fourth lumbar nerves and the last toracic nerve (T12).

84.

LIPID SOLUBLE ANTIOXIDANTS IN THE TISSUES OF THE EQUINE EYES

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The oxidative stress is a possible risk factor for eye diseases such as equine uveitis. The free radicals elicited during the inflammatory process often result in pathologic conditions. Polyunsaturated fatty acids (PUFAs) of membranes are the major targets for free radicals which produce lipid peroxidation. Because of the ability to scavenge free radicals of the lipid soluble antioxidants, the purpose of this study was: to quantify values of these antioxidants in serum and different ocular tissues; to determine the fatty acid composition of ocular tissues with higher lipid content such as iris and retina. Equine serum and different tissues of health eyes were utilized. Lipid soluble antioxidants were quantified by HPLC and the fatty acids composition of lipids from retina and iris were analyzed by GLC. The levels of lipid soluble antioxidant were expressed in nmol/mg of lipids. Retina and iris show the higher concentrations of α-tocopherol (4,42 ± 0,83 and 5,80 ± 1,49 respectively). The retinoids were detected in appreciable concentration in the retina (0,37 ± 0,05) and the carotenoids content was similar in the retina and iris (0,64 ± 0,18 and 0,64 ± 0,31 respectively). The content of α-tocopherol and carotenoids were 135,86 ± 26,28 and 11,22 ±2,50 in aqueous humor and 150,52 ± 8,62 and 32,43 ± 1,17 in serum. In the tissues poor in lipids (cornea and crystalline), minor levels of lipid soluble antioxidants were detected. The fatty acids compositions of retina and iris showed a high percentage of PUFAs mainly 22:6n-3 and 20:4n-6n. The present experimental results show that the eye has an endogen lipid soluble antioxidant system, and that their distribution in each tissue correlates with their susceptibility to oxidative damage.

85.

ANALYZE COMPARATIVE THE FATTY ACID COMPOSITION AND NON-ENZYMATIC LIPID PEROXIDATION OF THE SKELETAL AND CARDIAC MUSCLES OF ADELIE PENGUIN (*PYGOSCELIS ADELIAE*)

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Birds –particularly long-lived species– have special adaptations for preventing tissue damage caused by reactive oxygen species. The objective of the present study was to compare the fatty acid composition and non-enzymatic lipid peroxidation from the skeletal and cardiac muscles of Adelie penguin (*Pygoscelis adeliae*). Fatty acids located in total lipids of penguin skeletal and cardiac muscles homogenated were determined using gas chromatography and lipid peroxidation was evaluated using a chemiluminescence assay. The saturated fatty acid content found in homogenated of both muscles examined was approximately 30%, with a prevalence of C16:0 (palmitic). The total unsaturated fatty acid content found in homogenated of both tissue examinated was approximately 70%, with the prevalence of C18:1 n9 (oleic) (50%). In both muscles content of polyunsaturated long chain fatty acid decrease in the order C18:2 n6 (linoleic) > C20:4 n6 (arachidonic) > C22:6 n3 (docoxahexaenoic). The homogenates obtained from skeletal and cardiac muscles were not susceptible to lipid peroxidation. Light emission originating from skeletal and cardiac muscles was not statistically significant and the polyunsaturated fatty acid profiles did not change. Therefore, the unsaturation index was similar in both tissues. These results indicate that a low degree of fatty acid unsaturation in the skeletal and cardiac muscles of Adelie penguin, may confer advantage by decreasing their sensitivity to lipid peroxidation process.

86.

THE IMMUNOCYTOCHEMISTRY LOCALIZATION OF CHROMOGRANIN A IN THE COYPU (*Myocastor coypus*) STOMACH

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Chromogranins are proteins of the granin family found in the secretory granules of endocrine and neuroendocrine cells. They have been useful as markers of endocrine cells in several species. Chromogranins were observed between epitellial cells of the stomach tunica mucosa and those of the small intestine. The objective of the present project was to identify chromogranin positive cells in the stomach of coypu with immunocytochemistry techniques using anti-chromogranin antibodies. Samples from eight zones of stomach were obtained, samples were fixed in Bouin solution and processed by routine histological techniques. Five micrometer thick cuts were done and made to react with a polyclonal antibody anti-chromogranin. The streptoavidin-biotin-peroxidase system and diaminobenzidin chromogen were used to develop the antibody labeling. In every analyzed stomach zone, except cardial region, isolated cells reacting to the polyclonal antibody were mainly observed; in some cases two or three cell grouped were seen. Immunoreacting cells were located in the lamina propria glands of stomach tunica mucouse; a great proportion of cells were found in the intermediate and basal zones. Cells immunoreacting to chromogranine were in a zone characterized by its cytoplasmatic and granulated aspect.

87.

TROPHIC SPECTRUM OF SOUTHERN RIGHT WHALE EUBALAENA AUSTRALIS BY MEANS OF FAECAL ANALYSIS

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Eubalaena australis lives in Nuevo Gulf (Chubut) from April to middle of December after a long migration from its feeding areas at high latitudes. It was thought that *E. australis* did not feed at this breeding zone at all. However, trophic behaviours of whales have been recently observed in both Nuevo and San José Gulfs, especially during the springtime. Also, faeces of whales have been detected from whale-watching ships in the northeastern coasts of Nuevo Gulf. Faeces collected in October 2004 were qualitatively analyzed as part of a large project aimed to evaluate the plankton availability related to whale's trophic behaviour. Faeces preserved in alcohol 70% were homogenized and three 5 ml-subsamples were taken to identify the strong remains of food items. They were treated with glycerine and methyl blue to improve the viewing of food remains under stereo and optical microscopes. Some of this material belonged to mandibles and coxae of *Calanus australis* and/or *Calanoides carinatus,* large Calanidae copepods that live in this zone. Pieces of crustacean segments and antennae were also observed, some of which would probably correspond to euphausiids. In terms of relative abundance, copepod mandibles were the most abundant remains (>70%), non identified tegument parts were abundant (30-70%) whereas copepod coxae and prosomes were scarce (<30%). These findings, obtained for the first time in Argentina, are a clear evidence of whale-foraging either in this breeding area or previously into the shelf. The foraging on large copepods agrees with that reported for the northern right whale *Eubalaena glacialis* on *Calanus finmarchicus* in the North West Atlantic feeding areas.

88.

6PP, A PRENYLATED FLAVONOID FROM *Dalea elegans*, INHIBITS MITOCHONDRIAL RESPIRATION AND INDUCES MITOCHONDRIAL-DEPENDENT CYTOTOXIC ACTIVITY

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The prenylated flavanone 2´-4´-dihidroxy-5´-(1´´´-dimethylallyl)-6-prenylpinocembrin (6PP), obtained from the roots of *Dalea elegans,* shows antimicrobial activity. Flavonoids depict multiple biological activities including antioxidant effects and/or impairment of mitochondrial function. The aim of our work was to evaluate mitochondrial toxicity of 6PP. When evaluating oxidative phosphorylation system in rat liver mitochondria, 6PP (50, 100 μM) with malate-glutamate as substrate, stimulated O_2 uptake in state 4 and inhibited it in state 3 ($p<0.05$). When succinate was the substrate, 6PP inhibited O_2 uptake in state 4 at 100 μM ($p<0.01$) and inhibited it in state 3 at concentrations 12.5-100 μM ($p<0.01$). As a result, in both conditions respiratory control index values decreased at 6PP concentrations 12.5-100 μM ($p<0.01$). 6PP inhibited F_0F_1 ATPase activity in coupled mitochondria (37.2±6.5%-50 μM; 51.0±3.3%-100 μM; both $p<0.01$) and in submitochondrial particles (46.2±6.0% 100 μM; $p<0.01$). To assess cytotoxicity, MTT assay was employed. HEp-2 cells were incubated 24h with 6PP in presence or absence of 0.5% albumin. In the albumin-free group, 6PP was cytotoxic in a dose-dependent manner between 10 μM and 400 μM ($p<0.01$). Albumin decreased 6PP effect (inhibition of MTT reduction only at 200-400 μM, $p<0.01$), likely suggesting a lowered free fraction. We conclude that this flavanone affects mitochondrial respiratory chain and ATPase activity, inducing cytotoxic effects via mitochondrial damage. Further studies are needed to complete this evaluation.

89.

MORPHOMETRIC STUDY OF PLACENTAS FROM CADMIUM INTOXICATED RATS

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Exposure to cadmium determines structural changes in the placenta. In the present work, the effect of Cd administered at different pregnancy times (4, 7, 10 and 15 days of gestation) on the placental structure of rat was study. Samples of placentas obtained from Wistar rats, 20 days of pregnancy, both control and administered with $CdCl_2$, 10 mg Cd / kg at 4, 7, 10 and 15 days of gestation. All the placentas were weighed and measured, then processed for paraffin embedding. Histological slices were stained with Hematoxilin/ Eosin, for morphometric analysis. Measures were performed on different fields of each slice determining: a) distance between fetal vessels, b) greater length of fetal vessels and c) maternal space between capillaries. Results were analyzed by ANOVA. Significant statistical differences (P<0.05) were only observed between placental diameters from animals intoxicated on day 4 and controls. In the analyzed placentas, a statistically significant (P<0.05) increase in the distance between blood vessels and thickness of maternal space were observed between treated and control animals. Also, a decrease in the length of blood vessels (p<0,05) in treated animals compared to controls was observed. These results show that Cd intoxication affects the placental capillary bed, and could modify the placental blood flow and angiogenesis, which could contribute to a decreased fetal development.

90.

HEAVY METALS IN *ACARTIA TONSA* AND *EURYTEMORA AMERICANA* (CRUSTACEA, COPEPODA) IN THE BAHIA BLANCA ESTUARY

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The Bahía Blanca Estuary is a coastal environment that receives important discharges from surrounded industries and the Bahía Blanca city. Heavy metals are common pollutants in this type of effluents and zooplankton play an important role in the biogeochemical cycling of these metals in marine systems. The goal of this study was to determine the presence of cadmium, lead and copper in *A. tonsa* and *E. americana* and suspended particulate matter (SPM). Sampling was carried out in 2005 in five stations on the months with the highest abundance of *A. tonsa* (April) and *E. americana* (September). Plankton was sampled with a Nansen net (0.2 mm mesh size), SPM with a Van Dorn bottle and metals were determined by atomic absorption spectrophotometry. Cd concentrations in *A. tonsa* were below the detection limit (<0.2 μg/g), Cu between 31.05 and 9.99 μg/g and Pb between 38.33 and 11.38 μg/g. Cd in *E. americana* was below the detection limit and 10.81 μg/g, Cu between 1.27 and 153.65 and Pb below the detection limit (<0.5 μg/g) and 842.68 μg/g. In SPM sampled in April, Cd was below the detection limit and 16.20 μg/g, Cu between 4.03 and 21.47 μg/g and Pb between 12.04 and 38.90 μg/g. In September, Cd were below the detection limit, Cu below the detection limit and 3.86 μg/g and Pb below the detection limit and 3.37 μg/g. *E. americana* always presented higher metal concentrations than *A. tonsa*, however in SPM the highest values were found in April, when *A. tonsa* was present. Possible explanations for these seasonal differences may be related to their life cycle and ecology, incorporation strategies or bioavailability changes of the metals. Finally, though several factors determine metals content in zooplankton, enhanced levels found would imply anthropogenic contamination.

91.

LIPID PEROXIDATION OF BOVINE ERYTHROCYTES EXPOSED TO T- BUTYL HYDROPEROXIDE

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The erythrocytes are exposed normally to high concentrations of oxygen, are rich in polyunsaturated fatty acids and iron, and are involved themselves in an environment in which they are exposed to sources intra and extracelulars of free radicals. Was studied the use of hydroperoxides organic such as: cumene hydroperoxide, t-butyl hydroperoxide and hydroperoxides of fatty acids trying to elucidate the mechanisms across which the free radical ones provoke injuries in membranes of erythrocytes.Twenty Holstein male calves 1 year old (with a mean of 197 kg ± 8.3) was randomy selected into two groups: A-fed on supplemented diet with copper and B-fed on deficient diet in copper. The lipid peroxidation of erythrocytes exposed to t-butyl hydroperoxide (t-BHT) was analyzed, using as parameters the chemiluminescence (Cl) and the composition of fatty acids. The erythrocytes were isolated for centrifugation (1000g for 10 minutes to 4ºC), the plasma was discarded and erythrocytes were washed in isotonic phosphate buffer (PBS 5mM pH 7,4, 150 mm NaCl). The peroxidation carried out in an *in vitro* system non enzymatic, the quantification was realized measuring Cl, during 90 minutes. Analyzing the Cl depending on the time could be observed that the erythrocytes were affected by the lipoperoxidation, the maximum values being observed to 5 minutes (means of cpm total 100 x 10^{-3} group B and 85 x 10^{-3} group A) .There were no significant changes in specific fatty acids of both groups. Nevertheless the principal difference in fatty acids composition was in the percentage of the whole of fatty acids not saturated (35,9 % in the group A vs 27,06% in the group B, p <0.05), whereas also the relation was significant saturated /unsaturated, p <0.05. Consistently the unsaturation index was higher in the group A than in the group B.

92.

STUDIES ON GASTROPROTECTIVE ACTIVITY OF MEDICINAL PLANTS

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The use of vegetable species for the treatment of gastrointestinal pathologies promotes the conservation and rational management of resources, economic development and improvement of population life quality. Gastric ulcer is a pathological process with high incidence, which leads to important social and economic repercussions. The aim of the present work was to evaluate the gastroprotective activities of *Gaillardia megapotamica*, *Solidago chilensis*, *Aloysia gratissima, Parthenium hysterophorus* (native species) and *Rumex obtusifolius*, *Solanum eleagnifolium*, *Portulaca oleracea, Spartium junceum, Artemisia annua, Maytenus ilicifolia, Calendula officinalis* (adventive species) for possible anti-ulcer effects. Aqueous extracts of these species were orally administered to male *Mus musculus* mice, using absolute ethanol as necrotizing agent. The results were expressed in terms of an ulcer index, which was established according to an arbitrary scale. Significant differences were found between treated and control groups (p<0.05). In all cases plants prevented acute gastric mucosa injury induced by ethanol. Preliminary phytochemical assays revealed the presence of flavonoids and polyphenols. It is known that flavonoids, among other compounds, are involved in the gastroprotection. As aqueous extracts contain these constituents, the partial anti-ulcer activity could be due to their effects. The mechanism underlying this action is unknown. However, as a first step, the extracts should be fractioned and further studied. Specific methods are required to elucidate the mode of action and better evaluation of the gastroprotective activities of these plants.

93.

ANTIOXIDANT AND SCAVENGER PROPERTIES OF 6PP, A PRENYLATED FLAVONOID ISOLATED FROM *Dalea elegans*

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The polyphenolic components of higher plants may act as free radical scavengers or through other mechanisms contributing to anticarcinogenic or cardioprotective action. The prenylated flavanone 2´-4´-dihidroxy-5´-(1´´´-dimethylallyl)-6-prenylpinocembrin (6PP) was isolated from the roots of the Argentinian plant *Dalea elegans.* Previous results showed activity against oxacillin-sensitive and resistant *Staphylococcus aureus*. The aim of our work was to evaluate 6PP antioxidant properties. Enzymatic lipid peroxidation in rat liver microsomes was determined by measuring the *in vitro* formation of thiobarbituric acid-reactive substances (TBARS). 6PP significantly inhibited malondialdehyde production in a concentration dependent-manner between 6.25 and 100 μM, (88.5±4.3-%-100 μM, p< 0.01). Microsomal oxygen uptake was determined by the polarographic method, employing an oxygraph with a Clark electrode. 6PP addition inhibited uptake oxygen between 25 and 100 μM, p<0.01. The ability of scavenging free radicals was measured by DPPH reduction spectrophotometric assay, at 517 nm. 6PP exhibited significant scavenging activity in a concentration-dependent manner between 10 and 100 μM, (63±9%-100 μM, p<0.001). The antioxidant capacity of 6PP was quantitated spectrophotometrically through the formation of a phosphomolibdenum complex at 695 nm. 6PP was able to significantly reduce Mo (VI) to Mo (V) at 50 and 100 μM, p<0.05. Our preliminary results demonstrate that 6PP exerts both antioxidant and antiradical activities. Further studies are needed to evaluate possible therapeutic applications.

94.

HYPOGLYCEMIC ACTIVITY OF *Opuntia salagria* CLADODES IN RATS

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An important increase in the medicinal use of plant natural products has been observed in the last few years. Cactus *Opuntia* has been traditionally used as a valuable health supporting nutrient and its different parts are currently used in folkloric medicine. The number of people with *Diabetes mellitus* and its associated complications rise on a daily basis. The purpose of this study was to analyze the oral hypoglycemic activity of cladodes of *Opuntia salagria*, an Argentine autochthonous species, in transiently hyperglycemic and streptozotocin (STZ)-induced diabetic rats. To this end, *O. salagria* cladodes were collected from the area surrounding Bahía Blanca city, Province of Buenos Aires. After removing the spines, cladodes were sliced and dried at 40ºC in a forced air circulation oven and milled. The flour thus obtained was stored at 4ºC until required. A slimy suspension of *O. salagria* powder cladode was prepared (2g/100ml of water). The animals used were male Wistar rats (250-300g), which were made diabetic by intraperitoneal injection of STZ (35mg/kg body weight). The animals exhibiting blood glucose levels >350mg/dl were considered diabetic. The main effects of the oral administration of the suspension in doses of 100 or 200 mg/kg body weight are summarized as follows: in transiently hyperglycemic rats (Glucose Tolerance Test) cladode suspension decreased blood glucose levels at both doses, and in STZ-induced diabetic rats, the administration of the suspension for 30 days, produced a significant hypoglycemic effect. These results are indicative of the potential use of *O. salagria* cladodes to regulate the plasma glucose levels.

95.

IMT504, THE PROTOTYPE OF THE IMMUNOSTIMULATORY OLIGONUCLEOTIDES OF PyNTTTTGT CLASS AUGMENTS THE NUMBER OF BONE MARROW DERIVED ADHERENT CELLS WITH MULTIPOTENT DIFFERENTIATION CAPACITY *IN VITRO* AND *IN VIVO*

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Bone marrow (BM)-derived adult Mesenchymal Stem Cells (MSCs) have the capacity to differentiate in-vitro into different cell lines. This capacity makes them a likely cellular source for clinical application in tissue repair therapies. Here we report evidence indicating that IMT504, the prototype of the PyNTTTTGT class of immunostimulatory oligonucleotides, significantly augment the # of adherent fibroblast colony forming cells (CFU-F) with multipotent differentiation capacity, which are the most commonly properties to define MSCs. When rat BM cells were cultured in the presence of IMT504, the X±SE # of CFU-Fs increased as compared with untreated controls (2.1±0.7 vs. 0.9±0.3 x10^2/femur, p=0.04). Furthermore, rats inoculated with IMT504 had a significantly higher # of CFU-F both in BM cells (CFU-F: 1.2±0.3 vs. 0.4±0.2 x10^2/femur, p=0.04) and peripheral blood cell cultures as compared with untreated animals (0.37±0.14 vs, 0.05±0.04 /ml, p=0.03). On the other hand, CFU-Fs augmented either in-vitro or in-vivo by IMT504 stimulation possess capacity to differentiate to the osteogenic and adipogenic cell lineages as regular MSCs. Finally, we found that repair of an experimental bone defect was accelerated in rats subcutaneously injected with IMT504 as compared with untreated control animals two weeks after (area with consolidated bone:92.6±10.3 vs. 70.3±6.8 %, p=0.003). Importantly, when human BM cells were cultured in the presence of IMT504, the mean # of CFU-F also significantly increased as compared with untreated controls (CFU-Fs: 2.6 ± 1.2 vs. 1.0± 0.6 x10^1/10^6 plated). These results suggest the possibility of clinical use of IMT504 in bone and presumably other tissue repair therapies. Regarding this, it should be pointed out that in preclinical trials IMT504 has demonstrated to be a very safe drug.

96.

STUDY OF THE RHEOLOGICAL PROFILE OF HONEY FROM VARIOUS SITES IN RELATION TO PHYSICO-CHEMICAL AND SENSATION PARAMETERS

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The physico-chemical, sensory and rheologic properties of honey depend on production factors, such as geographic localization, weather or vegetation available to hives. They are also influenced by the extraction techniques employed. The correlation between rheologic and physico-chemical and sensation parameters was studied so as to classify samples according to their quality as accepted by consumers. The systems employed were Argentine honey from different beekeeping sites and production year. System 1: Santa Fé (Casilda) year 2004, System 2: Santa Fé (Casilda) year 2006, System 3: Santa Fé (Cañada de Gomez) year 2006, System 4: Entre Ríos (Victoria) year 2006 and System 5: Mendoza (Santa Rosa) year 2006. Rheologic determinations were performed with a Brookfield DVII programmable viscometer, Searle type (small sample adapter). Sensation testing was carried out according to those descriptors suggested by IHC (International Honey Commission of Apimondia). The pH was measured with a HANNA pH meter, model HI9017. In these systems, pH varied between 3.45±0.01 and 3.95±0.01. They showed markedly different rheological profiles. Relative viscosity at rpm varied from 3400±10% cp to 183000±10% cp, 4 of the systems being plastic non-dependant on time, and one, plastic with thixotropy. Sensation characteristics were within acceptability parameters. Despite the great difference in rheologic profiles, all 5 systems correspond to products physico-chemically and sensationally accepted. Demand, however, varies depending on consumers.

97.

FATTY ACID PROFILE OF FEATHERS OF *COLUMBA LIVIA* AFTER PESTICIDE TREATMENT

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In the study reported here in the effect of lindane accumulation and it effect of fatty acid composition and lipid content of kind of feathers of the rock dove, *Columba livia*. Lindane is an organochlorine pesticide that does not acumulate in any specific tissue or organ, except in storage fat. Fatty acid located in total lipids and lindane content were determined using gas chromatography. The saturated long chain fatty acid present in different kinds of feathers of rock dove were mainly 16:0 and 18:0 in a percentage of approximately 50-60%.The unsaturated fatty acid content was approximately 13% with a prevalence of C18:1n9, C18:2n6 and C18:3n3.The insecticide was not present in the feathers of control birds; however, it was found in the lindane group; rectrices, coat feathers, powder down (0.74 ± 0.28, 3.67 ± 1.25, 0.17 ± 0.07 ug lindane/mg lipid respectively. The fatty acid composition of pigeon feathers was significantly changed by lindane treatment. Percentages of polyunsaturated fatty acid and the unsaturation index isolated from powder down and coat feathers increased significantly, whereas percentages of monounsaturated from rectrices decreased. In this study we demonstrated that the fatty acid profile of three kind of feathers were different. The changed observed, in the feathers may produced marked alteration in structure an function with corresponding perturbations in several biological activities.

98.

IMMUNOHISTOCHEMICAL STUDY OF THE EFFECT OF TRICLABENDAZOLE IN FASCIOLA HEPATICA

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Introduction: *Fasciola hepatica* is a ruminant parasite with hepatic localization. It is a major zoonosis in subtropical areas, with great impact in cattle production. Benzimidazoles (BZDs) are the anthelmintic usually used to prevent its dissemination, being triclabendazole (TCBZ) the most widely used. The action mechanism of anthelmintic BZDs is through despolimerization of the endoparasite microtubules, with the concomitant loss of function, loosening and death. In the particular case of the trematode *F. hepatica*, some authors suggest the existance of other unknown mechanisms. The aim of the present study is to evaluate the effects of triclabendazole on the microtubular system of *F. hepatica* exposed to the drug "*in vivo*". Methodology: histological and immunohistochemical study of *F. hepatica* recovered from ovines, subjected or not (control) to a unique therapeutic dose of the drug.
Results: In histological slides of *F. hepatica* of ovines non-treated, tegument (plasmatic sincitia) was intact. Its immunostain was homogeneous and intense. In spiculas, there was more immunolocalization in the base than in the apex. Tegumental cells were completely stained, while cells from the parenchima were only stained peripherally. In *F. hepatica* of ovines treated, there was a partial desintegration of the tegument, with a diffuse immunostain, with vesiculous aspect in some areas. Spiculas were loosely stained only in the basal region. Conclusion: The immunohistochemical study and the histopathological findings with routine techniques, confirmed that the action mechanism of the drug produces alterations in the microtubules distribution.

99.

DECOLORIZATION OF MALACHITE GREEN IN SOILS BY *TRAMETES TROGII* AND *CORIOLUS VERSICOLOR VAR. ANTARCTICUS*

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Bioremediation is the use of biological agents to eliminate or reduce the concentration of toxic substances from a site. Ligninolytic fungi are feasible as those agents due to their extracellular enzyme production, as ligninolytic enzymes are unspecific and highly oxidative. The aim of this research was the evaluation of two white rot fungi for the degradation of the toxic dye malachite green (MG) in soils artificially contaminated and their relationship with ligninolytic enzyme production.
Cultures of *T. trogii* (BAFC 463) and *C. versicolor var. antarcticus* (BAFC 266) were made in 100 ml erlemeyer flasks with 12 g of dry soil and 2.4 ml of MG 0, 2.5, 5.0, 7.5 and 10 mM, and were incubated 20 d at 28 ºC. Inoculum consisted of 5 g of wheat bran colonized by the mycelia. Residual MG in soils was extracted with ethanol and spectrophotometrically measured at 618 nm. Extracts for enzymatic determinations were made from inoculated soils with water. Laccase and manganese peroxidase (MnP) were measured in extracts and enzyme activities were expressed as U (μmol/min)/g dry weight. Uninoculated controls were made.
Both strains were capable to decolorize MG in soils. When a 10 mM solution of MG was used *C. versicolor var. antarcticus* decolorized 93.58% and *T. trogii* 78.84%. Maximal enzyme activities were found in cultures with the highest concentration of MG in both strains. Maximal laccase activity was produced by *T. trogii* (1.23 ± 0.138 U/g) and MnP by *C. versicolor var. antarcticus* (0.02 ± 0.003 U/g).
C. versicolor var. antarcticus was the most efficient for dye degradation and is a suitable organism for its use as a microbiological agent for soil bioremediation.

100.

MORPHOANATOMY OF *PLUCHEA MICROCEPHALA* R. K. GODFREY (ASTERACEAE)

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Pluchea microcephala ("cuatrocantos") is a perennial native species from northern Argentina, Bolivia and NW of Perú, used for digestive and hepatic disorders. It grows in humid and saline soils, bordering watercourses. Morphoanatomical characteristics of leaves, stems and capitula have been studied in order to determine the most important characters for identification of this species in commercial samples. Light microscope observations were carried out on herbarium specimens, dissociated material, and serial transverse sections of restored herbarium material. Leaves are decurrent, hipostomatic, with eglandulate septate-flagellate trichomes and glandular trichomes with a biseriate foot and a two-celled head. The mesophyll is isobilateral with lipidic globules in its cells. Stems are winged, epidermal tissue has stomata and trichomes similar to those present in the leaves; the cortex is composed of aerenchyma and the eustele has collateral vascular bundles and parenchymatous or hollow pith. Capitula are disciform and heterogamous, grouped in dense apical corymbs with flared involucres formed by few rows of phyllaries. Outer purplish florets are numerous and present a filiform 3-lobulate corolla. Central florets, which occur in low numbers, have a tubular 5-lobulate corolla. The cypsela has longitudinal ribs and pappus formed by a row of hairs. The main diagnostic characters of *P. microcephala* are glandular and eglandular trichomes, the presence of lipidic globules in the mesophyll and capitula morphology. These characters constitute an important quality control tool to identify this species in commercial products.

101.
HARMFUL ALGAL MONITORING IN BEAGLE CHANNEL, TIERRA DEL FUEGO

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To determine the processes that favor the development of events of harmful algal blooms and their influence in mussel cultures, is being carried out a weekly monitoring of biological and oceanographic variables in Bay Brown (inside and outside) and Punta Paraná, Beagle Channel. These sites are closed to harvesting, generally during summer months, representing a serious problem for the health of consumers and the economy of the area. Results from phytoplankton indicated that the potential toxic species present are *Alexandrium* sp., *Dinophysis* sp. and *Pseudo-nitzschia* sp. *Alexandrium* sp. reaching 6000 cells/L during December of 2005 in the outside area of the Bay Brown. The values of toxins reached the 580 ug STX / 100 g. The increase in the number of toxic dinoflagelate was coincident with the maximum values of wind speed. Qualitative results from zooplankton for the same date revealed the presence of *Drepanopus forcipatus* and cypris larvae in all stations. *Podon* sp. was present only in Bay Brown whereas the *Munida subrugosa* zoeae although appeared in the three stations they were more abundant in Bay Brown (inside).. *Oikopleura* sp. also appeared in all the stations, being more abundant in Punta Paraná. A threshold of 1000 cells/L of *Alexandrium* sp was observed to overcome the 80 ug STX / 100 g. of mussel tissue, allowed for the human consumption. Starting from this such threshold in the phytoplankton until the detection of toxin in mussels, there was a lag time of 1-2 week. Low values of nitrite and nitrate was observed associated to the presence of high dinoflagelates biomass. A high percentage of zooplankton species observed are herbivorous which suggests a probable high grazing pressure on algal blooms, even during red tides.

102.
LIGNINASE PRODUCTION IN RESPONSE TO STRESS IN *STEREUM HIRSUTUM*

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Lignin degradation in nature is accomplished by an enzyme complex produced by white rot fungi. Main enzymes involved are lignin peroxidase (Lip), manganese peroxidase (MnP) and laccase. *S. hirsutum* improves its enzyme production with high concentrations of Mn, Cu, Cr and Fe. The aim of this research was to investigate the influence of pH and temperature on laccase and MnP production.
S. hirsutum (BAFC 2234) was cultured in synthetic medium. Incubation was at 8, 16, 23, 28 and 37°C and different combinations. Medium pH was adjusted in the range 3-10. Growth was measured as dry weight. Laccase and MnP activities were measured in supernatants and expressed as U(µmol/min)/ml. Data presented had a standard error of less than 5%.
Cultures exposed to different temperatures showed similar growth. Similarity in growth does not coincide with laccase production, the highest activity of this enzyme was detected in cultures grown at 16ºC, where it was 2.23 higher than at 28°C. MnP production showed a different pattern with an optimal value at 23°C. Temperature alternation during the incubation period showed that: maximal dry weight, was obtained in cultures grown 7 d at 28°C and 8 d at 8°C, a condition in which MnP is not detectable and laccase activity is minimal: 1.25 U/ml. Maximal laccase production, 8.28 U/ml, was obtained with 7 d at 28°C and 8 d at 34 °C, in coincidence with minimal growth. Alkalinized cultures had maximal laccase activity, 7.13 U/ml, whereas acidified cultures showed maximal MnP activity, 0.22 U/ml. Final pH of the cultures tends to neutrality, showing a buffer capacity of mycelium.
This strain has the ability to grow and produce ligninolytic enzymes under stress conditions that are feasible during bioremediation processes. Buffer capacity and enzyme improvement under stress conditions make *S. hirsutum* a candidate for its use in bioremediation.

103.
NUTRITIONAL ANALYSIS AND FLAVOR COMPOSITION OF *POLYPORUS TENUICULUS* (AGARICALES) CULTIVATED ON SAWDUST

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Mushrooms has become as an important source of food because they have high protein, carbohydrates, fibers, minerals, amino acid, vitamin contents and lower in fatty. In fact, they represent an important group for the industrial production of natural aromas and flavours "de novo" or by biotransformation of substrate with high commercial value. The aim of this work was to make nutritional- and flavour-composition analysis of *Polyporus tenuiculus* fruitbodies cultivated on *Salix* sawdust with soy flour and wheat bran as supplements. The volatile compounds were analyzed by GC-MS and identified on basis of comparison of their mass spectra with authentic standards when possible or with spectra from the Willey and NIST 98 libraries. Protein was determined by Kjeldahl; total carbohydrates by Feling Causse Bonans, previous HCL hydrolysis; Fatty by Soxhlet; ash in muffle at 550 ºC and fibers by 7069 methods (AOAC Methods 1980). The results of the volatile composition showed that 1-octen-3-ol (43%) was the majority component, followed by (Z,Z)-9,12-octadecadienoic acid methyl ester (12%), (Z,Z)-9,12-octadecadienoic acid (8.7%), 3-octanone (8.2%), 3-octanol (5.5%), hexadecanoic acid (5.3%), between the most important. When the nutritional composition was analyzed, it was found proteins 22%, carbohydrates 35%, fatty 8%, ash 6% and fibers 28%. In conclusion, 1-octen-3-ol, an alcohol, was the majority volatile component found in *P. tenuiculus* fruitbodies, which gives mushrooms its characteristic odor. This compound is important in food industry to reproduce mushrooms flavour. Moreover, the nutritional composition showed high fiber and protein content, making *P.tenuiculus* a very healthy food.

104.
PHOTOCHEMICAL FORMATION OF H_2O_2 AND ITS EFFECTS ON PHYTOPLANKTON COMMUNITIES OF THE BEAGLE CHANNEL, SOUTHERN ARGENTINA

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The most important source for H_20_2 formation are photochemical reactions between dissolved organic carbon (DOC) and ultraviolet radiation (RUV, 280-400 nm), particularly RUVB (280-320 nm).
High H_2O_2 concentrations produce deleterious effects to organisms via oxidative damage, with cellular membranes and photosynthetic pigments being key targets. The objective of this study was to evaluate how the effect of the increases in RUVB, resulting from the decrease of the stratospheric ozone on the Beagle Channel, modify DOC photochemistry. The oxidative stress on phytoplankton was determined by exposure of cells to different H_2O_2 concentrations and natural solar radiation. All the experiments were carried out in the Beagle Channel (Ushuaia, 54º 52'S, 68º 18'W). Water samples were filtered through 0.4 um filters to eliminate phytoplankton and bacterioplankton cells. Two treatments were analyzed using four-replicate samples for each one: water exposed to RUV > 280 nm and to RUV> 320 nm as control. To evaluate the possible oxidative stress on phytoplankton the oxidation of 2´7´-diclorofluorescein diacetate was measured. Three phytoplankton communities were exposed to natural solar radiation at 0 (control), 20, 30, 40 and 60 uM H_2O_2. A positive regression was observed ($P < 0.05$, $r^2 = 0.89$) in the production of H_2O_2 when exposing the seawater to RUV > 280 nm. In contrast, no significant regression ($P > 0.05$) was observed after exposure of the water to RUV > 320 nm. The different phytoplankton communities did not show significant oxidative damage due to H_2O_2 exposure at any of the concentrations used. **Conclusions:** DOC concentration in Beagle was of between 1000 and 2000 ug/l. The threshold RUVB dose producing H_2O_2 was 20 kJ/m^2. The H_2O_2 concentrations generated by RUVB on DOC have not produced an oxidative stress in any of the phytoplankton communities studied.

105.
COMPARISON OF EPIDERMAL CHARACTERS BETWEEN *ALLENROLFEA PATAGONICA* (MOQ.) KUNTZE AND *NITROPHILA AUSTRALIS* CHODAT & WILCZEK VAR. *AUSTRALIS*

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A. patagonica and *N. australis* have different leaf arrangements on the stem: they are appressed in the former, exposing its abaxial surface to the environment, whereas in the latter, both surfaces are equally exposed. It is intended to determine whether foliar arrangement produces any effect on the epidermal morphology.
Leaves were gathered at the Salitral de la Vidriera (Prov. Bs. As.), fixed in FAA and sectioned manually. Later, they were subdued to a weak dissociation.
In *A. patagonica* the abaxial epidermis is composed of somewhat isodiametric cells and the subsidiary cells form rings that delimit pores; the guard cells are sunken within these pores. The adaxial epidermis consists of variously shaped cells and the rings delimited by the subsidiary cells are not well defined. In both surfaces the epidermal cells show various kinds of papillae.
In *N. australis* the abaxial epidermis contains elongated cells and the stomata tend to be distributed in rows, while the adaxial epidermis shows polygonal cells and the stomata occur at random.
The stomatal index of each epidermal surface was compared with an ANOVA test in both species; this allows to affirm, with $p<0,05$, that there are differences between the mean values.
The epidermal arrangements show differences not only between species but between abaxial and adaxial epidermis. Independently of the degree of exposition, the abaxial surface always presents a larger number of stomata which agrees with the tendency observed in other species.