

II LUSO-BRAZILIAN CONGRESS OF EXPERIMENTAL PATHOLOGY

XII International Symposium on Experimental Techniques

Faculty of Medicine, University of Coimbra

Section A - Hot spot: Monday 10th December 17 hours

Morphology and Physiology
General Pathology
Biomaterials and Biocompatibility;
Biology of Development
Regeneration and Differentiation
Regenerative Medicine.

A01-013 - Inflammatory reaction evaluation after intramuscular implantation of biomaterials for bone regeneration – connection with the material's physico-chemical characteristics
Figueiredo, A. 1,3,4; Figueiredo, C.4, Silva, O., Silva, L. 1, Coimbra, P. 2, Figueiredo, M. 2, Guerra F.3, Cabrita A1.

1 Experimental Pathology Service, Faculty of Medicine, University of Coimbra, Portugal
2 Chemical Engineering Department, University of Coimbra, Portugal
3 Dentistry Department, Faculty of Medicine, University of Coimbra, Portugal
4 Portuguese Catholic University, Viseu, Portugal

Introduction: Physico-chemical properties of biomaterials are the most important factors regarding their in vivo behavior, being responsible for the promotion of different biological responses. These properties may alter the behavior of immune system cells such as the macrophages. Features such as the chemical composition, crystallinity, granulometry, porosity, surface area and density are important factors for predicting different clinical responses. **Objectives:** The objective of this study was to relate the inflammatory response after intramuscular implantation of two biomaterials with the physical and chemical characteristics of these biomaterials, obtained a priori. **Materials and Methods:** Samples of two biomaterials (Osteobiol™ and Bonelike™) were tested regarding morphostructural properties: morphology was evaluated by SEM, particle size distribution by laser diffraction spectrometry, the porosity by mercury intrusion and density by gas pycnometry. The inflammatory response was studied in a sample of 15 rats, spread across 3 groups: G1 (Osteobiol™), G2 (Bonelike™) and G3 (control group injected with saline), in which intramuscular injection was made in the dorsal muscles. Immediately after death of the animal, the biomaterials were removed along with the peri-implant tissues. The cuts were stained according to the hematoxylin and eosin (HE) and Masson's trichrome (TM) techniques. **Results:** The materials tested proved to be different in all traits studied, a fact we attribute to their origin: Osteobiol™ is a xenogenous material and Bonelike™ is a synthetic one. Inflammatory

reactions were different, particularly the production of collagen and thickness of the capsule formed around the implanted bone particles, with Bone-like™ inducing a more intense inflammatory response. **Conclusions:** It is difficult to establish a direct relationship between the observed differences in the in vivo response and their physico-chemical composition, since the materials that were tested proved to be very different in all morphostructural characteristics. There are multiple factors that may account for the disparity of the inflammatory response, being impossible to identify one single factor to held accountable for these differences.

Keywords: inflammatory reaction, biomaterials, physico-chemical characterization.

A02-124 - Morphological and morphometric study of the right subclavian vein, directed to central venous access. Oliveira, T.H.A.1; Costa Sobrinho, A. V.1; Villarouco, F.M.O.1; Lins, C.C.S.A.1; Lima, V.J.M.1; Silveira, M.F.G.1; Gouveia, A.S.2

1 Universidade Federal de Pernambuco, Brazil
2 College Santa Maria - Pernambuco, Brazil

Background: The subclavian veins are continuations of the axillary veins and terminate at external extremity of the clavicle. This vessel joins the internal jugular vein to form the brachiocephalic vein behind the sternal extremity of the clavicle. It relates ventrally with the clavicle and dorso-cranially with the subclavian artery, which is separated medially by the anterior scalene muscle and phrenic nerve. Inferiorly, rests on the upper surface of the first rib. The right subclavian vein is frequently used to puncture venous blood, administration of drugs, nutrition support in debilitated patients and for measurement of venous pressure. **Objective:** Make a topographical study of the subclavian vein observing anatomical relations and morphometric data, for providing better guidance in the puncture. Thereby reduce the risk of possible complications. **Methodology:** Males adults cadavers (n=10), belonging to the Anatomy Department of the Universidade Federal de Pernambuco – Brazil, were dissected showing the right subclavian vein and its communications. Anatomical variations were analyzed and the length of the subclavian vein was measured with a caliper rule (accuracy of 0.05 mm). **Results:** It was observed that the junction between the right subclavian vein and the internal jugular vein occurs in 50% of the cases, behind the medial third of the clavicle, at the level of the clavicular tendon of the sternocleidomastoid muscle. The other half of cadavers presented the junction behind the medial third of the clavicle next to the sternoclavicular

articulation. The length (cm) of the subclavian vein varies from 2.13 to 3.42 with a mean of 2.78. Conclusion: The junction between the subclavian and internal jugular veins does not seem to be always positioned at the sternal extremity of the clavicle. The results do not permit to be established a single orientation method for the right subclavian vein puncture, requiring more reviews.

Keywords: Subclavian vein; puncture; GRAY, H.

A03-099 – Adipose tissue in DMBA administration - morphometric analysis. João Dias^{1,2}, Felipe Pereira^{1,2}, Eduardo Costa¹, Lucia Costa^{1,2}, Paula Pereira³, Silvério Cabrita¹.

1 Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

2 Faculty of Pharmacy, University of Coimbra.

3 Laboratory of Biochemistry, CiiEM, Institute of Health Sciences Egas Moniz, University Campus, Caparica, Portugal.

Dimetilbenzantracene is a carcinogenic compound present in several products with identified toxic action in some human tissues. With this study we tried to connect the presence of DMBA experimental intoxication with morphologic changes in the adipose tissue and if this condition can be modulated by the diet.

168 Sprague Dawley virgin female rats with 42 days were randomly distributed for seven groups of 24 animals each. Besides the control group, all the treatment groups were submitted to the carcinogen 1,12-dimetilbenzantracene (DMBA) at the dose of 20mg diluted on 1ml of olive oil orally administered by gavage plus dietary variations. The group I was fed with a standard diet of 300Kcal/100g and no carcinogen. Group II was submitted to the carcinogen and standard diet. Another group, group III, was submitted to the carcinogen and standard diet supplemented with beans 40g/Kg of diet (341Kcal/100g). The Group IV was submitted to the carcinogen and standard diet supplemented with olive oil (50ml/Kg of diet). The group V was submitted to the carcinogen and fiber supplement four times higher than the standard diet for a total 250Kcal/100g. Group VI was submitted to the carcinogen and a diet supplemented with fiber four times higher than the standard diet plus 19.95g of beans and 25ml of olive oil on 1Kg of standard diet, and the group VII was submitted to the carcinogen and standard diet (300Kcal/100g) supplemented with 41Kcal of a Mediterranean diet with sardine, tomato, pepper, onion and olive oil. The experience was 150 days long.

All animals were sacrificed at the end of a twenty-eight weeks period and a complete necropsy was performed. The slides of perirenal adipose tissue, stained by HE, were evaluated for morphometric changes, using the software ImageJ.

The adipocytes were classified in adipocytes with integrate membrane and adipocytes with ruptured membrane, and were distributed into five classes (very small - VS, small - S, medium - M, big - B and very big - VB) using the average and standard deviation, for the control group. The test group were compared with the control one.

The adipocytes area were classified according to

the following criteria: medium adipocytes (M), when the value varies between the range $[\mu-\delta; \mu+\delta]$, big adipocytes (B), when the value varies between the range $[\mu+\delta; \mu+2\delta]$, small adipocytes (S), when the value varies between the range $[\mu-\delta; \mu-2\delta]$, very big adipocytes (VB), when the value is more than $\mu+2\delta$ e very small adipocytes (VS), when the value is less than $\mu-2\delta$;

In the control group the percentage of each class was: 0 VS; 15.23 S; 68.46 M; 12.60 B; 3.71 VB. In the group II the percentage of each class was: 0 VS; 0 S; 55.06 M; 27.53 B; 17.41 VB. In the group III the percentage of each class was: 0 VS; 52.91 S; 45.49 M; 1.40 B; 0.20 VB. In the group IV the percentage of each class was: 0 VS; 1.04 S; 49.59 M; 31.54 B; 17.84 VB. In the group V the percentage of each class was: 0 VS; 7.05 S; 39.73 M; 28.77 B; 24.46 VB. In the group VI the percentage of each class was: 0 VS; 9.48 S; 79.79 M; 8.45 B; 2.27 VB. In the group VII the percentage of each class was: 0 VS; 9.26 S; 65.26 M; 14.74 B; 10.74 VB.

We found significant changes among the groups what suggest that the diet may interfere with this morphologic changes.

A04-007 - Morphometric analysis of the adipose tissue in a model of experimental administration of ocimum extract. Karen Cavalcanti¹, Eduardo Costa², João Dias^{2,3}, Rodrigo Farinha², Ivone Souza³, Silvério Cabrita².

1University Federal of Pernambuco, Recife, Brazil.

2Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

3Faculty of Pharmacy, University of Coimbra.

Ocimum campechianum Mill, a native plant of central and south America is known in northeastern Brazil as alfavaca of snake. It's an importance source of essential oils in the leaves, flowers and seeds of adult plants whose medicinal properties and aromatic condiments are recognized and used in natural medicine and regional cuisine (Silva, 2007). In traditional medicine is widely used as an analgesic, antipyretic, diuretic, and flu. Recent studies have demonstrated anti-inflammatory activity and healing (Sousa, 2004; Silva Junior, 2006). The objective of this study was to evaluate the perirenal adipose tissue morphometric changes associated with the administration of Ocimum campechianum Mill hydroalcoholic extract.

One group of ten male Wistar rats, 8 weeks old maintained during 8 weeks with no manipulation was used as a control group. Another group of ten male Wistar rats, 8 weeks old, was submitted to the oral administration, by gavage, of 75mg/kg of Ocimum campechianum Mill hydroalcoholic extract, five times a week, during 8 weeks.

All animals were sacrificed at the end of the twenty-eight weeks after and a complete necropsy was performed. The slides of perirenal adipose tissue, stained by HE, were evaluated for morphometric changes, using the software ImageJ.

The adipocytes were classified in adipocytes with intact cytoplasm membrane and adipocytes with ruptured cytoplasm membrane, and were distributed into five classes (very small - VS, small - S, medium - M, big - B and very big - VB) using the av-

erage and standard deviation, for the control group. The test group were compared with the control one. The adipocytes area were classified according to the following criteria: Medium adipocytes (M), when the value varies between the range $[\mu-\delta; \mu+\delta]$, big adipocytes (B), when the value varies between the range $[\mu+\delta; \mu+2\delta]$, small adipocytes (S), when the value varies between the range $[\mu-\delta; \mu-2\delta]$, very big adipocytes (VB), when the value is more than $\mu+2\delta$ e very small adipocytes (VS), when the value is less than $\mu-2\delta$;

In the control group the percentage of each class was: 0 VS; 15.23 S; 68.46 M; 12.60 B; 3.71 VB. In the group test the percentage of each class was: 0 VS; 0 S; 32.09 M; 23.13 B; 44.68 VB.

We found significant changes between the control and test group, with an increase of the adipocytes size and more rupture of cytoplasm membrane in the animals submitted to the administration of *Ocimum campechianum* Mill hydroalcoholic extract.

A05-217 - Peri-implantitis treatment: case series with medium and long-term follow-up. Martins, O1; Ramos, J.C. 1; Matos, S1.; Baptista, I.P1. 1Dentistry School – Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Introduction: Biofilm formation on implants can endanger long-term success of osseointegrated implants. Periimplantitis has a high prevalence and was identified in 28% to $\geq 56\%$ of subjects. Therapy of peri-implantitis can compromise non-surgical and surgical phase. The restitutum ad integrum with regeneration of lost tissue should be the ultimate goal, however with x-Ray we can only confirm the bone fill of the peri-implant defect.

Objective: The aim of this case series was to describe the clinical and radiographic follow-up of four patients with treated peri-implantitis.

Material and Methods: Four patients treated for peri-implantitis involving a total of 6 implants, were followed-up for a 46 months to 6,5 years period. All cases presented suppuration, bleeding-on-probing and peri-implant bone loss. After diagnostic, the first non-surgical treatment phase comprised hygiene individual procedures, mechanical debridement and local application of antiseptics and antibiotics. In case of persistence of clinical signs and/or pocket depth ≥ 5 mm a second phase, surgical, with mechanical debridement, surface decontamination with antiseptics and antibiotics and xenograft bone regeneration was performed. In the subsequent clinical and radiographic follow-up period, all cases showed improvement in the clinical evaluation parameters and none implant was lost.

Results: There is little reliable evidence about the most effective intervention for peri-implantitis therapy. According to some authors, the adjunctive use of local antiseptics,

antibiotics and bone-substitutes can improve clinical parameters. Our results, based on chlorhexidine/minocycline applications and, when needed, on bone regeneration with xenografts are in agreement with these conclusions.

Conclusion: Within the limitations of this limited case series, we conclude that it seems possible to successfully treat periimplantitis disease on a me-

dium and long term basis.

Keywords: peri-implantitis, treatment, follow-up

A06-109 - Morfometric evaluation of hepatic proliferation in a murine implant model of bio-material

Ribeiro, G.B2; Batista, J.J1 ; Cenedezi, J.M.1; Souza, J.C.1; Carvalho, L.A.1; Lavall, L.1; Andrade,S. P3; Mendes, J,B3; Campos,P.P1.

1 Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brasil

2 Universidade José do Rosário Vellano – UNIF-ENAS BH, Brasil

3 Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brasil

Introduction: Liver regeneration is a phenomenon known since the times of ancient Greece and is currently being studied associated with biomaterials due to the prospects that it can bring to the therapy of fulminant liver disease. Objective: To evaluate histological and morphometric parameters of liver tissue that proliferated in implants of polyester-polyurethane sponges inserted in the abdominal cavity of mice. Methods: 36 mice were divided into experimental and control group. The control group did not undergo any surgical procedure, while the experimental group received the implant sponges above the liver capsule that were removed at predetermined intervals of times: 04, 08, 12 and 25 days post-implantation. Both control and animals in the experimental group were euthanized for removal of liver sample (control group) and the biomaterial (other groups). The implants collected were processed and stained by AgNOR techniques, hematoxylin and eosin, picrossíruis and Schorr for histological analysis. Results: We observed an increase in the area of liver tissue in the matrix of the sponge implant and in the number of nucleolar organizer regions (NORs) present in the nuclei of these hepatocytes during the study period, however the amount of collagen and veins did not differ from the control liver, indicating that there were no fibrosis in the proliferating tissue. There were no histopathological changes in the new tissue that proliferated in the biomaterial. The analysis of fibrovascular tissue rich in vessels that infiltrates the polyester sponge implant showed maintenance of the number of vessels and an increase in the amount of collagen during the evaluation period, this is probably due the fact that this tissue functioned as a support for the migration and organization of liver cells. Conclusions: This work proposes an innovative and well-defined model for study cells and molecules involved in liver proliferation.

Key words: Liver, proliferation, biomaterial

A07-250 - Liver morphologic changes during the first week of live. Renato Travasos1, Eduardo Costa1, Rute Duarte1, Ana Fernandes1, Rodrigo Farinha1, Silvério Cabrita1.

1Experimental Pathology Service, Faculty of Medi-

cine, University of Coimbra.

During the first week of life there are an physiologic inflammatory infiltrate in the rat liver, that progressively vanish. The aim of this study is to evaluate, using the morphometric analysis, the presence of the lymphocytes in the liver during the first week of life.

Five animals of each chosen age, one day, three day, five days and seven days were studied using HE liver sections. From each animal five liver sections were submitted to the microscopic evaluation using the ImageJ Software to count the lymphocytes present in this tissue. From each section was chosen the five areas with more lymphocytes, at the area was centered in this cells.

For the animals one day old, we found 0.01098 ± 0.00454 cells/ μm^2 , ranging between 0.0075 and 0.0144, for a confidence interval of 95%. For the animals three days old, we found 0.00695 ± 0.00210 cells/ μm^2 , ranging between 0.00802 and 0.00987, for a confidence interval of 95%. For the animals five days old, we found 0.0038 ± 0.0027 cells/ μm^2 , ranging between 0.00214 and 0.00547, for a confidence interval of 95%. For the animals seven days old, we found 0.0018 ± 0.0008 cells/ μm^2 , ranging between 0.0013 and 0.0022, for a confidence interval of 95%.

These results suggest that almost complete disappearance of live phymphocytes during the first week of life, and this period should not be used for models with inflammatory changes of this organ.

A08-253 - Immunohistochemical Analysis of Human Abdominal Aortic Aneurysms.

Marcos André Araújo Duque¹, Pedro Pereira Tenório¹, Jobson Ferraz do Nascimento¹, Joyce Golveia de Andrade¹, Daysiane Cristiny de Andrade e Silva¹, Flavio Oliveira da Silva¹, Rosangela Vidal de Araújo^{1,2}, Nicodemus Telles Pontes-Filho^{1,3}, Mario Ribeiro Melo-Junior^{1,3}

¹Laboratório de Imunopatologia Keiso Asami – LIKA1

²Associação Caruaruense de Ensino Superior – ASCES2

³Departamento de Patologia – CCS/UFPE3

INTRODUCTION: Abdominal aortic aneurysm (AAA) is leading causes of mortality in the elderly population due to increased breakage risk. AAA is a localized dilatation of the abdominal aorta (from the diaphragm and extending into forking left and right common iliac arteries) that exceeds the normal diameter 50% or >3 cm. The matrix Metalloproteinases (MMPs) are a group of zinc dependent proteins (enzymes) produced by a wide variety of cell types including smooth muscle cells, fibroblasts, inflammatory cells found into aneurysms. There is evidence that MMPs concentrations, particularly gelatinase (also known as MMP-2 and 72 KDa, gelatinase) and gelatinase B (also known as MMP-9 and 92 KDa, gelatinase) is high on the wall from non-dissectantes aneurysms. **OBJECTIVE:** the aim of this study was investigate the tissue expression of metalloproteinase-2, and -9 into 10 dissecting aneurysm cases in the cadavers from

Recife, Brazil. **METHODS:** The staining pattern of monoclonal antibody anti-Metalloproteinase-2 and anti-Metalloproteinase-9 was studied. All tissue samples were incubated with primary antibodies (Ab clones-4 anti-2, MMP -2: 800; Ab-3 anti MMP-9, 1: 2000; Ab-2 anti TIMP-1, 1: 600, and Ab-2 anti-2, TIMP 1: 500. The amplification reaction is obtained with streptoavidin system-biotin peroxidase (SABP), according to the manufacturer guidelines (Dako, USA). **RESULTS:** Different staining patterns in the AAA tissues were observed, evidencing that the MMP-2 and -9 have different roles in the pathogenesis of dissecantes aneurysms when compared to the non-dissectantes aortic aneurysms in humans. **Key words:** Aortic, aneurysm, Immunohistochemistry.

A09-253 - Epidemiological profile of the Human Aortic Aneurysms and Dissection.

Marcos André Araújo Duque¹, Pedro Pereira Tenório¹, Jobson Ferraz do Nascimento¹, Joyce Golveia de Andrade¹, Mônica Modesto Araújo¹, Daysiane Cristiny de Andrade e Silva¹, Nicodemus Telles Pontes-Filho^{1,2}, Mario Ribeiro Melo-Junior^{1,2}

¹Laboratório de Imunopatologia Keiso Asami – LIKA1

²Departamento de Patologia – CCS/UFPE2

INTRODUCTION: Aneurysm is an abnormal blood vessel location expansion, commonly applied to arterial swelling, although it can occur in any part of vascular system. Aortic aneurysms are characterized by progressive dilation of the vessel wall, involving the three tunics into a break. There are two forms: the saccular aneurysms (with narrow mouth) or fusiform (dilation into entire segment circumference). Abdominal aortic aneurysm (AAA) is leading causes of mortality in the elderly population due to increased breakage risk. Nowadays, studies indicated genre differences in the incidence of abdominal aortic aneurysms with prevalence in men, four times higher than in women. The AAA is found in approximately 6 to 8 men over 60 years old and its prevalence tends to increase with population aging. The decreased risk of developing AAA is lost in menopausal women, suggesting that reproductive events, including circulating estrogens, may play a protective role. **OBJECTIVE:** the aim of this study was investigate the incidence and drawing epidemiological profile of 47 aortic aneurysms cases in the cadavers from Recife, Brazil. **METHODS:** the deaths statements and statistical survey of necropsied corpses were analyzed. **RESULTS:** there was a greater prevalence of female 28 cases, and also observed higher incidence in the individuals between 60 and 79 years old. The main risk factors observed in our study: hypertension (n=30 cases), smoking (n=25), atherosclerosis (n=25), and alcoholism (n=16). The thoracic aortic aneurysm showed higher prevalence. Others lesions (abdominal aortic aneurysm, dissecting aneurysms or aortic dissecting hematoma or Pseudoaneurysm) were found too. Hemopericardium is a direct death cause in 25 cases, followed by Hemoperitônio (n=9) and Hemothorax (n=3). **CONCLUSION:** Several stud-

ies are carried out for further clarification regarding the epidemiological factors of aneurysms. Was observing an increase in the aortic aneurysms and dissection in Brazil. More studies are important for understanding the aneurysms, in order to elucidate further mechanisms related to pathogenesis.

Key words: Aorta, Aneurysms, Epidemiology

A10-007 - Embryonic development in rats subjected to treatment with *Mentha crispa* (Lamiaceae).

Cavalcanti, K.P.S. 1; Souza, I.A.2.; Anjos, F.B.R.3; Silva, E.V.4; Silva, E.C.B.4; Caribé, R.A. 4; Souza, V.M.S. 4; Lima, J.4 ; Silva Neto5, J.F.; Jimenez, J.5

1. Hospital das Clínicas-Lika-UFPE-University of Coimbra

2. Department of Antibiotics-UFPE

3. Department of Histology and Embryology-UFPE

4. Department of Pharmaceutical Sciences-UFPE

5. Department of Morphology and Physiology-UFPE

Background: The *Mentha crispa* (Lamiaceae), gathers properties tonic, stimulant and anti-spasmodic of Labiatae aromatic. Widely used in folk medicine in northern and northeastern Brazil, has proven action as amoebicide and giardicidal. Objectives: This study aims to evaluate the effect of aqueous extract of *Mentha crispa* orally in rats throughout pregnancy. Methodology: Were selected for the study, Wistar rats (aged 60 days) with regular estrous cycle. Females with confirmed pregnancy were administered orally with aqueous extract (leaves and stems) of *M. crispa* 160mg/kg and 220mg/kg doses. The females were divided into 6 groups treated and 6 controls (6 animals each) and administered during the three stages of pregnancy: conception phase (from day 1 to day 6), embryonic (from 7 to 13 days) and fetal development (from the 14st to 21th day). The control group was given only the vehicle. At the end of each phase of the cycle, the rats were sacrificed and then laparectomizadas being counted cycles pregnancy. Results: The *M. crispa* administered from day 1 to day 6 had no change in the deployment, the 7th to the 13th day all embryos remained normal development without visible changes occurred and no fetal malformation and 14[°] to 21 days gestation was completed as term pregnancy with complete absence of embryotoxicity and fetal malformation. In this investigation we found no embryotoxic and teratogenic effects such as changes in the implant (gestational interruption of the cycle), exophthalmos, harelip, evisceration, polydactyly, syndactyly, and reduced weight and hydrocephalus / anafacelia treated animals. The pregnancy was processed under normal conditions until its final phase. Conclusions: We can conclude that the aqueous extract of *M. crispa* showed no abortive effect, embryotoxic and teratogenic in laboratory animals.

Keywords: *Mentha crispa*, embryotoxicity, teratogenicity

A27-008 - Acid phosphatase ratio as a marker of the osteoclastic function in the ovariectomized rat model

Colaço, B1; Pires MJ1; Oliveira P1; Ferreira C1; Cerdeira T1, Silva, JC2; Fernandes MH2; Gomes, PS2

1. Centro de Ciência Animal e Veterinária (CE-CAV) - Escola de Ciências Agrárias e Veterinárias, UTAD, Portugal

2. Laboratory for Bone Metabolism and Regeneration – Faculdade de Medicina Dentária, U. Porto, Portugal.

Introduction: The ovariectomized rat model mimics the human condition of post-menopausal osteoporosis, being one of the most used models in translational osteoporosis-related research. In this model, the assessment of the bone tissue broadly relies on the determination of morphometric histological and radiographic indexes, or biomechanical assays. Thus, alternative procedures, based on minimal invasive techniques aiming to address the metabolic function of the bone tissue through the quantification of relevant biomolecules in body fluids, have been developed.

Objectives: This work aims to demonstrate the relevance of the serum acid phosphatase ratio (rACP), determined by the reason between the tartarate-resistant acid phosphatase (TRACP) and total acid phosphatase (tACP), as a simple and low cost methodology to monitor the osteoclast function in the ovariectomized rat model.

Methods: 32 female Wistar rats, 6 week old, were submitted to a bilateral ovariectomy (OVX) or simulated surgery (SHAM). Animals were euthanized one or three months following the surgical procedure (n=8) and the TRACP and tACP values were determined using a commercial kit. rACP was calculated and results were compared between groups and time points.

Results: No significant differences between the experimental groups were found, regarding the tACP and TRACP levels, for both one and three months periods. However, rACP was significantly reduced in OVX groups, comparing to SHAM groups, at both time points ($p < 0.05$).

Conclusion: The determination of the rACP may be considered a relevant parameter in the diagnosis and monitorization of metabolic conditions with altered osteoclastic function, such as that of osteoporosis. The rACP seems to be a sensitive parameter, taking into account the TRACP activity normalized by the tACP, outputting a standardized and individual ratio for each assayed subject.

Keywords: Osteoporosis; Acid phosphatase; Ovariectomized rat.

Section B - Hot spot - Monday 10th December 17 h

Nutrition and Dietetic
Food confection
Pharmacology and Therapeutic
Phytotherapy
Iatrogenesis
Pharmaceutical Sciences.

B01-042 - Chronic exercise influence on the amphetamine-dependent behaviour and dopamine in striatum, frontal cortex and hippocampus in the rat. Fontes-Ribeiro, C. A.1, Pereira, F. C.1, Silva, A. P1.

1Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, University of Coimbra, Portugal

Background: The dopaminergic mesocorticolimbic and nigrostriatal systems are involved in drug addiction. However, there is little information about the effect of exercise on addiction and on the synthesis, metabolism and action of dopamine (DA) in the presence of amphetamines. Objectives: This work aimed to verify the influence of exercise on addiction using an amphetamine (AMPH)-induced conditioned place-preference (CPP) in rats, and to determine DA and respective metabolites in striatum, frontal cortex and hippocampus. Methodology: Adult male Sprague-Dawley rats were randomly separated in two groups: one with and another without exercise (8 weeks running program in a treadmill, with increasing intensity). The CPP test was performed in both groups. There were three phases: 1st- Pre-conditioning: free access to both compartments, 20 min sessions with registration of the time spent in each compartment; 2nd- Conditioning: 8 consecutive days (45 min sessions) in the alternated compartments (4 days of 2 mg/kg AMPH alternated with saline injections); 3rd- Test day: free access to both compartments, 20 min sessions with registration of the time spent in each compartment. Animals were sacrificed 24 h after the last AMPH or saline, and striatum, hippocampus and frontal cortex were dissected for DA and metabolites measurement by HPLC-ECD. Results: Since in the pre-conditioning phase none of the animals presented preference for a compartment, all the animals were used. Rats without exercise showed preference for the compartment associated with AMPH, an effect which did not occur with the animals with training. Amphetamine decreased striatal DA content and turnover from trained and untrained rats. Training reduced and increased DA content in frontal cortex and hippocampus, respectively. Conclusions: Exercise may prevent the AMPH-seeking behaviour but not changes the AMPH impact on striatal DA. Exercise may alter DA dynamics in the frontal cortex and hippocampus which may be correlated with addiction.

B02-133 - Can perinatal undernutrition modulate the antioxidant enzymes activities in the heart? 1Barreto, M.D.P.; Nascimento,L.P.2;Silva Filho, R.C.3;Fernandes, M.P.3;Silva, W.T.2;Lagranha,C.J.3

1 Department of Pathology – University Federal of Pernambuco, Brazil
 2 Department of Nutrition, Academic Center of Victory – University Federal of Pernambuco, Brazil
 3 Department of Physical Education and Sport Sciences, Academic Center of Victory – University Federal of Pernambuco, Brazil

Background: In the critical period of development external factors, as the undernutrition, can induce the organism to undergo biochemical modification in the heart tissue. Objectives: Evaluate the effects of perinatal undernutrition in the heart tissue by activities of antioxidant enzymes: superoxide dismutase, catalase and glutathione-S-transferase. Methodology: Male Wistar rats were divided according to the diet offered to the mother during pregnancy and lactation in two groups: control (C, casein 17%,n=6) and low-protein diet (M, casein 8%, n=6). After lactation, the puppies began receive standard diet (LABINA). At the 100 days of age the animals were sacrificed and the heart was removed. Then, the analyses of the lipid peroxidation and the superoxide dismutase, catalase and glutathione-S-transferase were conducted. The values were expressed in mean and standard error of mean (X±SEM). The significance level was maintained in 5% (p<0.05). Results: There was increased lipid peroxidation in the undernutrition group (C=30.1±2.0; M=54.4±3,2 nmol/mg of prot). Related to the analyzes of the antioxidant enzyme, all enzymes of the undernutrition group showed lower values than control group: superoxide dismutase (C=3.36 ± 0.39 U/mg prot; M= 2.09 ± 0.39 U/mg prot), catalase (C= 62.99 ± 3.89 nmol/min/mg prot; M=39.5 ± 2.05 nmol/min/mg of prot) and glutathione-S-transferase (c=8.85±1.43 nmol/min/mg prot; M=3.6±0.5843 nmol/min/mg prot).Conclusions: This study suggests that undernutrition induced a deficiency in the antioxidant capacity in heart. This effect can favor, in the heart, an oxidative imbalance resulting in pathogenesis heart in adulthood because of negative stimulus during the critical period of development.

Keywords: undernutrition; antioxidant enzymes; heart.

B03-159 - Sitagliptin prevents blood-retinal barrier breakdown, inflammation and neuronal cell death in the retina of type 1 diabetic animals.

Gonçalves A.1; Leal E.2; Paiva A.3; Fontes-Ribeiro C.1; Reis F.1; Ambrósio A.F.2,4; Fernandes R.1
 1Laboratory of Pharmacology and Experimental Therapeutics, IBILI-Faculty of Medicine, University of Coimbra, Portugal
 2Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal
 3Histocompatibility Centre of Coimbra, Coimbra University Hospital, Portugal
 4Centre of Ophthalmology and Vision Sciences, IBILI-Faculty of Medicine, University of Coimbra, Portugal.

Background: Diabetic retinopathy, the main microvascular complication of diabetes, is a leading cause of vision loss and blindness. A novel class of oral antidiabetic agents, the dipeptidyl peptidase-IV

(DPP-IV) inhibitors, has shown to improve glycemic control by enhancing the levels of active incretin hormones, which increase insulin secretion in type 2 diabetics. Objectives: Evaluate whether sitagliptin can exert protective effects in the retina by a mechanism independent of insulin secretion in diabetic animals. Methods: Diabetes was induced in male Wistar rats at 8 weeks of age with streptozotocin. Experiments were performed 1 month after diabetes induction. Oral treatment with sitagliptin was initiated at 10 weeks of age, for two weeks, with a daily dosage of 5 mg/kg. Glucose, HbA1c and insulin levels were evaluated in serum or total blood. The blood-retinal barrier (BRB) breakdown was evaluated using Evans blue. The activity of DPP-IV was assessed using a fluorogenic substrate. The content and/or distribution of tight junction (TJ) proteins, CD26, IL-1 β , TNF- α and Bax was evaluated by western blotting and/or immunohistochemistry. Retinal cell apoptosis was assessed by the TUNEL assay. The number of CD34+ cells present in the circulation was assessed by flow cytometry. Results: Treatment with sitagliptin was able to prevent the increase in DPP-IV levels and activity induced by diabetes, having no effect on glucose, HbA1c or insulin levels in diabetic animals. This compound prevented the increase in BRB permeability and the alterations in the subcellular distribution of TJ proteins caused by diabetes. Furthermore, sitagliptin was able to prevent the increase in IL-1 β , TNF- α , Bax and TUNEL-positive cells in the retinas of diabetic animals. The number of CD34+ cells in the peripheral circulation of diabetic animals was significantly increased by sitagliptin treatment. Conclusion: Sitagliptin prevented retinal cell apoptosis and inflammation, and exerted beneficial effects on the BRB integrity maintenance in diabetic rat retinas. Sitagliptin may directly protect endothelial progenitor cells/endothelial cells, through the inhibition of soluble and membrane-bound DPP-IV. Keywords: Diabetic retinopathy; sitagliptin; blood-retinal barrier

B04-084 - Activity of crude extracts of two species of Asteraceae family in wound healing of mice.

Alves, Natália Ribeiro¹; Longatti, Tamara Ribeiro¹; Ferreira, Andréia Silva¹; de Sequeira, João Máximo¹; Oliveira, Claudia Di Lorenzo¹; Pereira, Luciana Xavier²; Parreira, Gleydes Gambogi²; Carneiro-Proietti, Anna Bárbara; Thomé, Ralph Gruppi⁴; Santos, Hélio Batista¹; Ribeiro, Rosy lara Maciel de Azambuja¹.

¹Universidade Federal de São João Del Rei, Campus Centro Oeste D. Lindu, Divinópolis, MG, Brazil.

²Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³Fundação Hemominas & Faculdade de Saúde e Ecologia Humanas (FASEH), Minas Gerais, Brazil.

⁴Universidade Federal de Juiz de Fora, Governador Valadares, MG, Brazil.

Introduction: Despite the wide application of plant extracts in traditional medicine, there are few studies that demonstrate their effectiveness on the process of wound healing.

Aim of the study: This study reports the effects of

extracts from two species of plants of the family Asteraceae (9IIE and 10IIE) and lyophilized platelets in wound healing through macroscopic and microscopic analysis.

Materials and Methods: The injury was caused by punching the cervical dorsal of mice (n = 10/group), which regions were treated by the following compounds: G1 (water-based gel); G2 (water-based gel + 9IIE); G3 (based gel water + 9IIE + lyophilized platelets); G4 (water-based gel + 10IIE); G5 (water-based gel + 10IIE+ lyophilized platelets) and G6 (water-based gel + lyophilized platelets). The wound healing rates were calculated in periods of 4, 9, 15 and 17 days post wounding. Day 9 was considered for histological analyzes.

Results: The reduction of the wound area with the complete closure occurred earlier in the group G4 on the tenth day. Time of wound closure was longer for G5, around 14 days, as it was G1. The closure of the wound treated animals of G2 and G3 was completed at the end of 13 days, while the G6 wound closure happened on the sixteenth day. Wounds of the groups G2, G3, G4 and G6 were almost completely resurfaced with neoeplithelium and skin appendages.

Conclusion: This study revealed that the treatment with the compound G4 was more efficient and might be inferred as a strong candidate for the treatment of wound.

Keywords: healing, plant extracts, Support from FAPEMIG, CNPq, CAPES

Presenting author: Rosy lara Maciel de A. Ribeiro

B05-113 – Effects of non-alcoholic beer on blood lipid profile and fat deposition in ovariectomized rats. Nery, M. S.1; Dias, A.L.A.1; Moriyama, D.F.1; Marincek, F.1; Duarte, F.O.2; Sene-Fiores, M.3; Pinto, F.G.1

¹Departamento de Morfologia e Patologia;

²Departamento de Ciências Fisiológicas – Univ. Federal de São Carlos (São Carlos – SP - Brasil); ³Grupo de Ótica do Instituto de Física de São Carlos - USP

Background: Dyslipidaemia and increase of fat deposition are common in women after menopause. It has been suggested that the consumption of non-alcoholic beer could provide several metabolic and cardiovascular benefits without negative alcohol effects. Objectives: To determine the non-alcoholic beer effect consumption on blood lipid profile and fat deposition in ovariectomized rats. Methodology: 27 female Wistar rats, aged 76 days, were distributed into 3 groups: untreated not-ovariectomized (UT-NO), untreated ovariectomized (UT-O) and treated ovariectomized (T-O). The rats were submitted to ovariectomy two weeks before initiating treatment with non-alcoholic beer, two times a week, in substitution of drinking water. The blood samples were withdrawn 105 days after initial treatment to determine the serum concentration of Triglycerides, Total Cholesterol, HDL-c, non-HDL-c, TGO, TGP and Creatinine. The liver and the white and interscapular brown adipose tissues were weighed and the lipid content percentage was determined by the gravimetric method. Results: It was not observed significant difference between

the groups in the blood components and the percentage of total weight gain. Ovariectomized rats showed an increase in both relative weight (g/100g of body mass) of the subcutaneous adipose tissue (UT-O=0.98; UT-NO=0.57; $p<0.05$) and in its lipid content (UT-O=56%; UT-NO=40.56%; $p<0.05$); reduction of the liver relative weight (UT-O=2.41; UT-NO=3,10; $p<0,001$) and an increase of liver lipid content (UT-O=1.9%; UT-NO=1.07%; $p<0.001$). The treatment induced a significant increase of relative weight of the interscapular brown adipose tissue (T-O=0.14; UT-O=0.07; $p<0.001$) and liver (T-O=3.00; UT-O=2.41; $p<0.01$), with reduction of liver lipid content (T-O=1.21%; UT-O=1.90%; $p<0.01$). Conclusions: regular intake of non-alcoholic beer does not affect significantly the blood lipid profile, weight gain or white adipose tissue depots, but induce an increase in both liver and interscapular brown adipose tissue relative weight; and prevent the increase of liver lipid content in ovariectomized rats.

Keywords: non-alcoholic beer; ovariectomized; adipose tissue

B06-114 - Effect of injectable combined hormonal contraceptive on feeding parameters of Wistar rats. Guzmán-Silva, MA1; Henriques, HN1; Couto, BB1; Boaventura, GT1, Pantaleão, JAS1. 1Fluminense Federal University, Brazil

Hormonal contraceptives used by women during the fertile period usually affect and increase the body weight. The aim of this study is to determine the effect of injectable combined hormonal contraceptive (HC) on body weight, food intake and feed efficiency in female Wistar rats.

The experimental protocol was approved by the Ethics Committee on Animal Research PROPPi/UFF (0010/2010). Twenty rats weighing 159.0 ± 1.7 g were randomly divided into two groups ($n=10$). Rats were s.c. injected once a week with 0.02ml of Mestigyna® (norethisterone enanthate and estradiol valerate) in the HC group and with distilled water in the control group. The treatments were performed until day 21. Throughout the experiment the body weight was measured every week, the food intake was registered every three or four days until day 28, and feed efficiency was calculated. Data were compared using the Student t test or Mann-Whitney test with significance level set at $P<0.05$.

Body weight gain was registered in both groups, with significant ($P<0.01$) lower increase in the HC group compared to control on days 14, 21 and 28. The average body weight in HC group was 184.9 ± 7.9 g, while in the control group was 198.5 ± 11.7 g. The food intake was significant ($P<0.05$) lower in the HC group compared to the control group on days 3, 9, 24 and 28. The average food intake in HC group was 17.1 ± 0.3 g and in the control group was 19.1 ± 0.1 g. The feed efficiency was lower in HC than in the control group ($2.45\pm 1.01\%$ vs. 3.35 ± 1.00 respectively), but no significant difference was observed.

Weekly HC treatment in female rats causes lower food intake and less body weight gain, but does not modify the feed efficiency.

Key words: hormonal contraceptive, feed efficien-

cy, rats.

B07-117 – Bone resistance and mineral chemical composition in ovariectomized rats treated with infusion of Morus nigra leaves. Marincek, F.1; Moriyama, D.F.1; Dias, A.L.A.1; Nery, M.S.1; Gonçalves, F. A. M.2; Ruffoni, L.D. G.3; Pinto, F.G.1 1Departamento de Morfologia e Patologia, Universidade Federal de São Carlos, Brasil 2Centro de Estudos Ambientais, Universidade Estadual Paulista, Brasil 3Departamento de Ciências Fisiológicas, Universidade Federal de São Carlos, Brasil

Background: Several studies have aimed to identify alternatives pharmacologically safer and able to inhibit or revert the process of postmenopausal bone fragility. In Brazil, infusions from *Morus nigra* leaves are empirically used to reduce climacteric symptoms. Objectives: This study was designed to determine the effect of treatment with infusion of *Morus nigra* leaves on bone resistance and mineral chemical composition in ovariectomized rats. Methodology: 27 Wistar rats aged 76 days were distributed into 3 groups: untreated not ovariectomized (UT-NO), untreated ovariectomized (UT-O) and treated ovariectomized (T-O). Two weeks after ovariectomy, continuous treatment was initiated with infusion of *Morus nigra* leaves, in substitution of drinking water. One hundred and five days after treatment the serum levels of calcium, phosphorus and alkaline phosphatase were measured; the mineral chemical composition of the lumbar vertebra (L4) was obtained by ICP-OES and resistance of the lumbar vertebra (L5) was assessed by biomechanical tests. Data were submitted to the One-way ANOVA and the Tukey-Kramer multiple comparisons test using the GraphPad InStat software (version 3.06). Results were considered statistically significant at $p<0.05$. Results: As compared to the control groups (UT-O/UT-NO), the ovariectomized animals presented lower serum levels (mg/dl) of Phosphorus ($3.37/4.81$; $p<0.001$). As effect of treatment (T-O/UT-O), there was increase in serum Phosphorus ($4.67/3.37$; $p<0.01$). The vertebral mineral composition and the biomechanical resistance were not affected by ovariectomy or by treatment. Conclusions: treatment with infusion of *Morus nigra* leaves may modify the blood Phosphorus levels, but it cannot change the resistance or the vertebral mineral composition in ovariectomized rats.

Keywords: *Morus nigra*; ovariectomized rats; bone

B08-115 – Blood lipid profile and fat deposition in adult ovariectomized rats treated with infusion of Morus nigra leaves. Dias, A.L.A.1; Nery, M.S.1; Moriyama, D.F.1; Marincek, F.1; Duarte, F.O.2; Sene-Fiorense, M.3; Pinto, F.G.1 1Departamento de Morfologia e Patologia; 2Departamento de Ciências Fisiológicas – Univ. Federal de São Carlos (São Carlos – SP - Brasil); 3Grupo de Ótica do Instituto de Física de São Carlos - USP

Background: Dyslipidaemia and increase of fat de-

position are common in women after menopause. In Brazil, infusions of *Morus nigra* leaves are empirically used to reduce climacteric symptoms. Objectives: To determine the effect of infusion of *Morus nigra* leaves on blood lipid profile and fat deposition in ovariectomized rats. Methodology: 27 female Wistar rats, aged 76 days were distributed into 3 groups: untreated not ovariectomized (UT-NO), untreated ovariectomized (UT-O) and treated ovariectomized (T-O). The rats were submitted to ovariectomy two weeks before initiating continuous treatment with infusion of *Morus nigra* leaves (10g/L) in substitution of drinking water. The blood samples were withdrawn 105 days after initial treatment to determine the serum concentration of Triglycerides, Total Cholesterol, HDL-c, non-HDL-c, TGO, TGP and creatinine. The liver and the white and interscapular brown adipose tissues were weighed and the lipid content percentage was determined by the gravimetric method. Results: It was not observed significant difference between the groups in the blood components and the percentage of total weight gain. Ovariectomized rats showed an increase in both relative weight (g/100g of body mass) of the subcutaneous adipose tissue (UT-O=0.98; UT-NO=0.57; $p<0.05$) and in its lipid content (UT-O=56%; UT-NO=40.56%; $p<0.05$); reduction of the liver relative weight (UT-O=2.41; UT-NO=3.10; $p<0.001$) and an increase of liver lipid content (UT-O=1.9%; UT-NO=1.07%; $p<0.001$). The treatment induced a significant increase of relative weight of the interscapular brown adipose tissue (T-O=0.12; UT-O=0.07; $p<0.01$) and liver (O/T=2.92; UT/O=2.41; $p<0.005$), with reduction of liver lipid content (T-O=1.23%; UT-O=1.90%; $p<0.01$). Conclusions: Treatment with infusion of *Morus nigra* leaves does not affect significantly the blood lipid profile, weight gain or white adipose tissue depots, but induces an increases in both liver and interscapular brown adipose tissue relative weight; and prevents the increase of liver lipid content in ovariectomized rats.

Keywords: *Morus nigra*; ovariectomized; adipose tissue depots

B09-112 – Influence of non-alcoholic beer supplementation on bone resistance and mineral chemical composition in ovariectomized rats.

Moriyama, D.F.1; Marincek, F.1; Dias, A.L.A.1; Nery, M.S.1; Gonçalves, F. A. M.2; Ruffoni, L.D. G.3; Pinto, F.G.1

1Departamento de Morfologia e Patologia, Universidade Federal de São Carlos, Brasil

2Centro de Estudos Ambientais, Universidade Estadual Paulista, Brasil

3Departamento de Ciências Fisiológicas, Universidade Federal de São Carlos, Brasil

Background: Several studies have aimed to identify alternatives pharmacologically safer and able to inhibit or revert the process of postmenopausal bone fragility. It has been suggested that the consumption of non-alcoholic beer could provide benefits on bone mineral density without the negative effects of alcohol. Objectives: This study was designed to determine the effect of non-alcoholic beer supplementation on bone resistance and mineral chemi-

cal composition in ovariectomized rats. Methodology: 27 Wistar rats aged 76 days were distributed into 3 groups: untreated not ovariectomized (UT-NO), untreated ovariectomized (UT-O) and treated ovariectomized (T-O). Two weeks after ovariectomy, treatment was initiated with non-alcoholic beer, two times a week, in substitution of drinking water. One hundred and five days after treatment, the serum levels of calcium, phosphorus and alkaline phosphatase were measured; the mineral chemical composition of the lumbar vertebra (L4) was obtained by ICP-OES and resistance of the lumbar vertebra (L5) was assessed by biomechanical tests. Data were submitted to the One-way ANOVA and the Tukey-Kramer multiple comparisons test using the GraphPad InStat software (version 3.06). Results were considered statistically significant at $p<0.05$. Results: As compared to the control groups (UT-O/UT-NO), the ovariectomized animals presented lower serum levels (mg/dl) of Phosphorus (3.37/4.81; $p<0.001$). As effect of treatment (T-O/UT-O), there was an increase in serum Alkaline Phosphatase (32,33/25,92; $p<0.05$) and in the vertebral concentration (mg/g) of Potassium (2.22/1.75; $p<0.01$). The biomechanical resistance was not affected by ovariectomy or by treatment. Conclusions: non-alcoholic beer supplementation may modify the blood Alkaline Phosphatase levels, as well as potassium levels in the lumbar vertebrae, but it cannot change the resistance or the major vertebral mineral components in ovariectomized rats.

Keywords: non-alcoholic beer; ovariectomized rats; bone

B10-114 - Changes induced by estradiol valerate and norethisterone enanthate in mammary glands and uterus of fertile rats. Guzmán-Silva MA1; Henriques, HN1; Couto, BB1; Boaventura, GT1; Pantaleão, JAS1.

1 Fluminense Federal University, RJ, Brazil

Combined progestogen-estrogen preparations have shown marked differences between species. The aim of this study is to evaluate the effect of a combined injectable hormonal preparation on mammary glands and uterus of fertile rats.

The experimental protocol was approved by the Ethics Committee on Animal Research PROPPi/UFF (0010/2010). Ten rats weighing 162.8 ± 1.5 g were randomly divided into two groups (n=5). Rats were s.c. injected once a week with 0.02ml of hormonal contraceptive (HC) Mesigyna® (norethisterone enanthate and estradiol valerate) in the HC group and with distilled water in the control group. Treatments were performed until day 21 and all rats were euthanized on day 28. Periodic vaginal cytology was performed to assess rats' hormonal status. Thoracic mammary glands and uterus were removed, fixed in Carson's modified buffered formalin and processed for paraffin embedding. Data were compared using the Mann-Whitney test with significance level set at $P<0.05$.

The hormonal stimulus (HS) of HC was characterized in vaginal smears by areas similar to metestrus and proestrus-like cell clusters. The HS was seen in all rats seven days after first injection,

lasting until the end of the experiment. The uterine weight was significantly ($P < 0.05$) higher in the HC group compared to control, both with respect to relative weight (1.15 ± 0.38 vs. 0.20 ± 0.03 , respectively) and absolute weight (2.30 ± 0.75 vs. 0.47 ± 0.07 , respectively). Mammary glands in the control group showed ducts and acini with one to two cell layers and slightly distended lumen. The HC group showed similar ducts and acini with slightly increased cell volume and greatly distended lumen with secretion containing fat droplets.

Weekly HC treatment in fertile rats modifies the cytology pattern and increases uterine weight by uterine fluid accumulation. Changes in mammary gland morphology were mammary lobe complete differentiation, similar to what occurs during pregnancy/lactation.

Key words: hormonal contraceptive, mammary gland, uterus.

B11-119 - Effect of different hormonal therapies on feeding parameters in Wistar rats

Henriques, HN1; Couto, BB1; Alves, RC1; Boaventura, GT1, Pantaleão, JAS1; Guzmán-Silva, MA1.

1 Fluminense Federal University, RJ, Brazil

There is no consensus on the effect of Hormonal Therapy on the body weight during the post-menopausal period.

The aim of this study is to assess the effect of tibolone (T), estradiol (E2) or combined estradiol/progesterone (E2Pg) Hormonal Therapy (HT) on body weight, food intake and feed efficiency in rats.

The assay was approved by the Ethics Committee on Animal Research PROPPi/UFF (10/2010). Twenty-five rats weighing 200.1 ± 2.9 g were randomly divided into five groups ($n=5$) as follows: OVX+T, OVX+E2, OVX+E2Pg, OVX+pl, SHAM+pl. Animals were ovariectomized (OVX) or sham operated, and after 28 days they were treated daily by gavage (0.5ml) containing T (2mg/ml), E2 (1mg/ml), E2Pg (1mg/ml and 0.5mg/ml) or carboxymethylcellulose (0.5mg/ml) as placebo (pl) for 35 days. Body weight and food consumption were registered and feed efficiency was calculated. Results were evaluated by Mann-Whitney or Kruskal Wallis and Dunn's post hoc test with significance level set at $P < 0.05$.

Body weight gain, food intake, and feed efficiency after ovariectomy were significantly lower in the SHAM group compared to OVX ($P < 0.01$). During HT, rats with hormonal treatment showed loss of body weight and control rats showed gain of body weight. Differences in body weight were significant in the comparison of OVX+T with OVX+pl ($P < 0.05$) and SHAM+pl ($P < 0.01$); also, OVX+E2Pg was significantly different from the SHAM+pl group ($P < 0.05$). Furthermore, the food intake was significantly lower in OVX+T and OVX+E2 compared to OVX+pl ($P < 0.01$) and SHAM+pl ($P < 0.05$). Feed efficiency results showed similar pattern to body weight, being negative in groups submitted to hormonal treatment and positive in the control groups. Tibolone and E2Pg lead to reduction in body weight reversing the usual gain in menopause, probably by the reduction of food intake.

B12-154 - Differences in salivary α -amylase levels among women with different taste sensitivities

Sequeira, M.1; Rodrigues, L.1; Costa, A.R.1,2;

Pinheiro, C.1,3; Antunes, C.2,4; Lamy, E.1,5

1Instituto de Ciências Agrárias e Ambientais Medi-

terrânicas (ICAAM), Universidade de Évora, Portu-

gal

2Departamento de Química, Universidade de Évora, Portugal

3Departamento de Zootecnia, Universidade de Évora, Portugal

4Centro de Neurociências e Biologia Celular (CNC), Universidade de Coimbra, Portugal

5Química Orgânica, Produtos Naturais e Agro-Alimentares (QOPNA), Universidade de Aveiro, Portugal

Background: Saliva is the main component of taste receptor cells external environment, and consequently it may have a decisive role in taste perception. Taste sensitivity varies among different individuals. Sensitivity to the compound n-6-propylthiouracil (PROP) has been considerably studied and besides the known influence of genetic background, the contribution of perireceptor environment is not completely clear yet. Salivary α -amylase (one of the main proteins of saliva) is involved in carbohydrate digestion and its enzymatic activity may change the levels of sugars present in the mouth, influencing food perception. Objectives: To evaluate differences in salivary total protein content and α -amylase activity and expression among individuals with different PROP taste sensitivities. Methodology: Sixty seven female women (18-30 years old) were classified in one of the three groups of taste sensitivity (non-taster, medium-taster or super-taster), according to the perceived intensity for PROP, using Labeled Magnitude Scales. Saliva was collected without stimulation. Flow rate was calculated by dividing total volume for the 5 minutes collection. Bradford method was used for total protein assessment. Dinitrosalicylic acid assay was used for measuring the starch-hydrolyzing activity of salivary α -amylase, while the expression of this enzyme was evaluated by Western blot. Results: 20.9% of the subjects were classified as non-tasters. The three groups presented similar saliva flow rates and total protein content was not significantly different although a tendency for lower protein concentration in medium-tasters individuals was observed. Salivary α -amylase activity (U/min) was higher in super-tasters ($P < 0.05$). Salivary α -amylase activity (U/min) was higher in super-tasters ($P < 0.05$) without any significant differences in expression. Conclusions: In women individual differences in saliva composition can contribute to the different taste sensitivity. One of the differences appears to be α -amylase enzymatic activity. The reason for this deserves to be elucidated, as well as the potential involvement of others salivary proteins.

Keywords: Saliva; Salivary amylase; Taste sensit

B13-120 - Optimal concentration for hormonal therapy by subcutaneous injection in female rats

Couto, BB 1; Henriques, HN 1; Azeredo, VB 1; Pantaleão, JAS 1; Guzmán-Silva, MA 1.
1 Fluminense Federal University, Brazil

There is no consensus about the ideal dose of Hormone Therapy (HT) to obtain proper hormonal effect by subcutaneous administration in rats. The aim of this study is to determine the optimal concentration for s.c. HT injection in Wistar rats.

The experimental protocol was approved by the Ethics Committee on Animal Research PROPPi/UFF (10/2010). Thirty rats weighing 199.9 ± 4.8 g were randomly divided into six experimental groups ($n = 5$). Tibolone was administered at high (310 μ g/ml THi) and low dose (155 μ g/ml TLo). Estradiol was administered at high (250 μ g/ml EEHi) and low dose (125 μ g/ml EELo). Corn oil (0.1ml) was administered to control (C) and sham (S) groups. The s.c. injections were performed three times/week for 30 days. All rats were ovariectomized (OVX) 56 days before starting the experiment, except in group S (sham operated). Vaginal cytology was performed periodically. Finally, the animals were euthanized, uteri were weighed and thoracic and abdominal mammary glands were collected for histological analysis. Parameters to assess HT influence were vaginal cytology similar to persistent estrus and uterine weight. Data were compared using Kruskal Wallis and Dunn's post hoc test, with significant level at $P < 0.05$. Absolute and relative uterine weight showed significant increase ($P < 0.05$) in S compared to groups which did not use estradiol (THi, TLo and C). Higher absolute and relative uterine weight ($P < 0.05$) was noticed in EEHi compared to C group. Persistent estrus was not observed at the vaginal cytology in either group. The mammary glands morphology was similar in all HT treated groups and C showing atrophy.

To get a constant hormonal effect in female rats, the HT s.c. administration should be in higher doses than the one used in this experiment, 310 μ g/ml of tibolone and 250 μ g/ml estradiol.

Key words: hormonal therapy, ovariectomy, rats.

B14-100 - MDMA and its metabolites inhibit 5-HT concentration-response curves in the human internal mammary artery.

Pimentel, I.1; Guerra, F.1; Fonseca, D.1; Carvalho, F.2; Fernandes, E.3; Ferreira, L.P.4; Antunes, M.5; Cotrim, M.D.1

1Center for Pharmaceutical Studies (CEF), Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Portugal.

2REQUIMTE Department of Toxicology, Faculty of Pharmacy, University of Porto, Portugal.

3REQUIMTE Department of Physical-Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

4REQUIMTE Department of Chemistry, Faculty of Sciences and Technology, University of Caparica, Portugal.

5Cardiothoracic Surgery, University Hospital of Coimbra (CHUC-Huc), Portugal.

Introduction: 3,4-methylenedioxymethamphetamine (MDMA) is a drug with high potential of abuse among young adults. The use of MDMA is associated with cardiovascular toxicity and vasoconstriction. MDMA metabolism involves N-demethylation to 3,4-Methylenedioxyamphetamine (MDA) and both are O-demethylated to N-methyl- α -methyldopamine (N-Me- α -MeDA) and α -methyldopamine (α -MeDA), respectively. The presence of glutathione (GSH) forms glutathionyl adducts 5-(GSH)- α -MeDA and 5-(GSH)-N-Me- α -MeDA. These metabolites have proved to be more toxic than MDMA.

Objective: The aim of this work was to evaluate the contractile effects of MDMA and its metabolites in the human internal mammary artery (HIMA).

Methodology: HIMA segments were collected from patients undergoing coronary artery bypass grafting surgery. The vessels were cut into rings (3-4 mm), mounted in organ baths and maintained at 37°C in Krebs-Henseleit solution, aerated with 95%O₂-5%CO₂. After an equilibration period of 2 hours, changes in isometric force were measured using PowerLab® data acquisition package. Concentration-response curves were obtained for MDMA and respective metabolites. To test MDMA and its metabolites effects on 5-HT concentration-response curves (0.1-30 μ M) several concentrations were used. Results are shown in milliNewton (mN).

Results: Under the present experimental conditions, only MDMA, α -MeDA and N-Me- α -MeDA showed a contractile effect on the HIMA with a % of maximum contraction of 1.98 mN, 2.97 mN and 0.95 mN, respectively. In the HIMA, a 30-min preincubation with MDMA, α -MeDA, N-Me- α -MeDA, 5-(GSH)- α -MeDA and 5-(GSH)-N-Me- α -MeDA significantly shifted the 5-HT concentration-response curve to the right with a concentration-dependent reduction of the maximum response. The maximal % of reduction for MDMA, α -MeDA, N-Me- α -MeDA, 5-(GSH)- α -MeDA and 5-(GSH)-N-Me- α -MeDA was 81.35%, 61.93%, 75.40%, 56.78% and 39.20%, respectively.

Conclusions: Results show that MDMA and its metabolites inhibit the 5-HT concentration-response curves and this inhibition is concentration dependent. Results also show that MDMA, α -MeDA and N-Me- α -MeDA are partial agonists but 5-(GSH)- α -MeDA and 5-(GSH)-N-Me- α -MeDA are antagonists.

Keywords: MDMA, metabolites, vascular reactivity.

B15-101 - Vascular effect of Oxalis pes-caprae L. leaves extract in human internal mammary artery.

Gaspar, M.1; Fonseca, D.1; Campos, M.G.2; Frigerio, C.2; Antunes, M.3; Cotrim, M.D.1

1Center for Pharmaceutical Studies (CEF), Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Portugal.

2Center for Pharmaceutical Studies (CEF), Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Coimbra, Portugal.

3Cardiothoracic Surgery, University Hospital of Coimbra (CHUC-Huc), Portugal.

Introduction: In the present work, we studied a leaves extract of Oxalis pes-caprae L., a winter an-

nual geophyte from South Africa which is very common in temperate areas like Portugal. The main compounds found in the flavonoid fraction of the extract were flavones based derivatives of luteolin and apigenin. Several authors described an influence of luteolin and apigenin in noradrenaline (NA) and 5-hydroxytryptamine (5-HT) induced vascular contractions.

Objectives: The purpose of this study was to screen the bioactivity of an *Oxalis pes-caprae* L. leaves extract, evaluating a possible involvement on NA and 5-HT concentration-response curves in human internal mammary artery (HIMA).

Methodology: The HIMA was harvested from patients undergoing coronary artery bypass graft surgery, with an age between 49 and 73. After an equilibration period of 2 hours, with a basal tension of 19.6mN, changes in isometric tension were measured using the PowerLab® data acquisition package. Concentration-response curves to an *Oxalis pes-caprae* L. leaves extract were performed to evaluate its contractile effect. 5-HT and NA concentration-response curves in HIMA were obtained using concentrations between 10⁻⁵ and 10⁻³ M. To test *Oxalis pes-caprae* L. leaves extract effect on the 5-HT and NA concentration-response curves, it was used a 0.164 mg/ml *Oxalis pes-caprae* L. leaves extract. All data are expressed as mean ± SEM.

Results: In the concentrations used, *Oxalis pes-caprae* L. leaves extract did not show contractile effect. *Oxalis pes-caprae* L. leaves extract caused a parallel shift of the NA concentration-response curve to the right with a reduction of the NA maximum contraction of about 58.44%.

Conclusions: Results showed non competitive antagonism effect of *Oxalis pes-caprae* L. leaves extract on NA concentration-response curves in HIMA. The structure activity relationship will be achieved in further studies.

Keywords: *Oxalis pes-caprae* L., human internal mammary artery, noradrenaline.

B16-102 - Contractile effects of MDMA and its metabolites in the human internal mammary artery under normothermic and hyperthermic conditions.

Guerra, F.1; Pimentel, I.1; Fonseca, D.1; Carvalho, F.2; Fernandes, E.3; Ferreira, L.P.4; Antunes, M.5; Cotrim, M.D.1

1Center for Pharmaceutical Studies (CEF), Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Portugal.

2REQUIMTE Department of Toxicology, Faculty of Pharmacy, University of Porto, Portugal.

3REQUIMTE Department of Physical-Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

4REQUIMTE Department of Chemistry, Faculty of Sciences and Technology, University of Caparica, Portugal.

5Cardiothoracic Surgery, University Hospital of Coimbra (CHUC-Huc), Portugal.

Introduction: MDMA metabolism involves N-demethylation to 3,4-methylenedioxymphetamine (MDA) and both are O-demethylated to N-methyl- α -methyldopamine (N-Me- α -MeDA) and α -methyldopamine (α -MeDA), respectively. The presence of glutathione (GSH) forms 5-(GSH)-

α -MeDA and 5-(GSH)-N-Me- α -MeDA. The major feature of clinical cases related to MDMA is hyperthermia.

Objectives: The aim of this work was to evaluate the contractile effects of MDMA and its metabolites in the human internal mammary artery (HIMA) under normothermic and hyperthermic conditions.

Methodology: HIMA segments were collected from patients undergoing coronary artery bypass grafting surgery. The vessels were cut into rings (3-4 mm), mounted in organ baths and maintained at 37°C or 40°C in Krebs-Henseleit solution, aerated with 95%O₂-5%CO₂. Changes in isometric force were measured using PowerLab® data acquisition package. Concentration-response curves were obtained for MDMA and respective metabolites. To test MDMA and its metabolites effects on 5-HT concentration-response curves (0.1-30 μ M) several concentrations were used. Results are shown in milliNewton (mN).

Results: Under the present experimental conditions, MDMA and α -MeDA elicited a contractile effect on the HIMA at 37°C (1.98 mN and 2.97 mN, respectively) and 40°C (8.03 mN and 11.56 mN, respectively). In the HIMA, a 30-min pre-incubation with MDMA and its metabolites at 37°C and 40°C significantly shifted the 5-HT concentration-response curve to the right with a reduction of the maximum response. The maximal % of reduction at 37°C for α -MeDA and 5-(GSH)- α -MeDA (61.93% and 56.78%, respectively) was higher than at 40°C (41.24% and 39.84%, respectively). The maximal % of reduction at 40°C for MDMA and 5-(GSH)-N-Me- α -MeDA (84.14% and 55.95%, respectively) was higher than at 37°C (81.35% and 39.20%, respectively).

Conclusions: Under hyperthermic conditions (40°C) the vascular reactivity is higher than under normothermic conditions for MDMA and some metabolites. These results show that at 37°C the cardiovascular risk exists, though it may be higher in hyperthermia.

Keywords: MDMA, metabolites, hyperthermia.

B17-103 - Influence of age and gender on human internal mammary artery reactivity.

Fonseca, D.1; Bacino, A.1; Guerra, F.1; Pimentel, I.1; Gaspar, M.1; Antunes, P.2; Antunes, M.2; Cotrim, M.D.1

1Center for Pharmaceutical Studies (CEF), Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Portugal.

2Cardiothoracic Surgery, University Hospital of Coimbra (CHUC-Huc), Portugal.

Introduction: Coronary artery bypass grafting (CABG) is performed in cases of ischemic heart disease and was one of the most important advances in cardiothoracic surgery in the 20th century. Vascular tone is regulated by many factors such as noradrenaline (NA), 5-hydroxytryptamine (5-HT), potassium chloride (KCl) and nitric oxide. Among other physiological characteristics, aging and gender have been associated to vascular tone differences between individuals. The combination of those factors can directly influence vascular reactivity and thus the prevalence and outcome of CABG.

Objective: The present study had the objective of

understanding the influence of age and gender in vascular reactivity, specifically in human internal mammary artery (HIMA).

Methodology: HIMAs were harvested from patients undergoing CABG. Experiments were performed in HIMA from 74 patients with an age between 41 and 82 years. Local research ethics committee approval for the use of discarded segments of HIMA was granted and numbered as PC-388/08. NA, 5-HT and KCl were used to evaluate HIMA reactivity. Results were converted from grams to mN (mN) and statistical analysis was performed using Student's t test and ANOVA. Results are presented as Mean \pm SEM.

Results: Results showed no statistically significant difference in HIMA reactivity according to the age of the patient. However, differences in HIMA reactivity associated with gender were demonstrated. Female patients had higher contraction, compared to male patients, to NA (12.794 mN and 5.986 mN, respectively) and KCl (5.043 mN and 4.000 mN, respectively), but not to 5-HT (2.828 mN and 4.459 mN, respectively). The differences were statistically significant, with $p < 0.05$.

Conclusions: Although there is no evidence of an influence of age, results showed that gender influences vascular reactivity. Regarding this subject, further studies are necessary to correlate other patient characteristics, for example concomitant diseases, with these differences in vascular reactivity. **Keywords:** age, gender, vascular reactivity.

B18-020 - Intestinal villi changes in experimental malnutrition

José Cabeças¹, Helena Moreno², Silvério Cabrita¹.

¹ Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

² Braga

Malnutrition is the condition that results from taking a unbalanced diet, with the lacking or excess of certain nutrients. A number of different nutrition disorders may arise, depending on which nutrients are under or overabundant in the diet. Malnutrition is more common in developing countries, but its also present in industrialized countries, caused by unhealthy diets with excess energy, fats and refined carbohydrates.

Thirty animals were randomly distributed by two groups of ten animals each. Group I was submitted to a standard feed with the following composition: 15,5% of total (gross) protein, 2,7% of total fat, 3,7% of total fiber, 5,5% of minerals and 58,5% of carbohydrates. The humidity was 12%. With this feed, by addition of saccharose, was made a modified feed that was submitted to the group II and the composition was: 7,75% of total (gross) protein, 1,35% of total fat, 1,85% of total fiber, 2,75% of minerals and 79,25% of carbohydrates. The humidity was 6%. The concentration of the components on the modified feed is much lower than on the standard feed, except for the carbohydrates.

All the animals were killed on the 30th day, by and overdose of anesthetics and the complete necropsy was performed. The small intestine was collected for histopathological processing and analysis.

Pictures of routine stained slides were taken and the small intestinal villi length measured, using the software ImageJ.

The intestinal villi were distributed into five classes (very small - VS, small - S, medium - M, big - B and very big - VB) using the average and standard deviation, for the control group, and the villi length were classified according to the following criteria: Medium villi (M), when the value varies between the range $[\mu-\delta; \mu+\delta]$, big villi (B), when the value varies between the range $[\mu+\delta; \mu+2\delta]$, small villi (S), when the value varies between the range $[\mu-\delta; \mu-2\delta]$, very big villi (VB), when the value is more than $\mu+2\delta$ and very small villi (VS), when the value is less than $\mu-2\delta$; The test groups were compared with the control one. In the control group the percentage of each class was: 2 VS; 16 S; 64 M; 16 B; 2 VB. In the group II the percentage of each class was: 37,37 VS; 32,32 S; 29,29 M; 1,09 B; 0 VB. We found significant changes between the control and the test groups, with an increase of the number of smaller intestinal villi in the animals submitted to the administration of semicarbazide. These results suggest that maybe an adequate collagen synthesis is necessary to maintain the intestinal villi length.

B19-081 - Prickly pearfruit

Pereira, P.1, Vicente, F.1

¹ Laboratório de Bioquímica, CiiEM, Instituto Superior de Ciências da Saúde Egas Moniz, Campus Universitário, Quinta da Granja, Monte de Caparica, 2829-511 Caparica Portugal.

Fruits had been extensively recognized as important antioxidant sources. Despite the broad variety of fruits available in the market, a quite significant number of species with a remarkable nutritive value and very high antioxidant activity remain unknown. Vitamin C and phenolic compounds are important antioxidant substances in fruit.

The present work intended to evaluate the vitamin C and flavonoid content of several varieties of Prickly pear fruits, comparing with other fruits and within the different prickly pear varieties found.

Samples included several unitary portions of two different prickly pear varieties: green lime with green pericarp and green lime pulp; and red with red pericarp and bright red pulp. These were harvested from wild *Opuntia* cactus trees located in Alentejo. Fruits were unpeeled, the pulp was extracted and manually homogenized, then frozen to -18°C until further analysis. Ascorbic acid was determined using the dichlorophenolindophenol (DCFH) method while total phenolic compounds were determined using the Folin-Ciocalteu method. Results revealed that red prickly pear fruits have significantly more vitamin C (28.78mg/100g edible fruit versus 18.58mg/100g of green lime variety). The average phenolic compound content in green lime fruits was 70.38mg/galic acid equivalents (GAE) while in red fruits the average phenolic compounds content was 75.28mg/100g.

According to these results, vitamin C content in prickly pear fruits is lower than in oranges, the most recognized vitamin C source among fruits, but it is higher than apple, pear and plum vitamin

C contents. In what refers to polyphenol content, prickly pear fruits revealed a very high content, significantly higher than apples, red grapes, peach and mango.

Further studies should discriminate better which phenolic compounds are present in this fruit and evaluate antioxidant capacity such as through in vitro studies.

Keywords: antioxidants; ascorbic acid; polyphenols; fruits

B20-081 - Food labeling on Europe: which changes to come on 2014 and 2016?

M. Bettencourt-Câmara¹, P. Pereira¹

¹Laboratório de Bioquímica, CiiEM, Instituto Superior de Ciências da Saúde Egas Moniz, Campus Universitário, Quinta da Granja, Monte de Caparica, 2829-511 Caparica Portugal.

A livre circulação de alimentos seguros e sãos The free food safe food circulation is a crucial aspect for European Internal Market and contributes to consumer's health and well being. The guarantee of consumer's interest's protection is a basic principal from food legislation (article 8 from European Regulation (CE) N 178/2002, from European Parliament and Council, 28th January 2002) and it obligates to provide the necessary information in order to allow conscientious and adequate food choices. This study intends to present the new food labeling rules, which application will be mandatory in 2014 and 2016. The introduced changes aim to improve consumer's perception and understanding about the information present in the label including in what refers to online food market which will be highlighted by then.

In the European Union, food labeling legislation begun in 1978, with Directive 79/112/CEE, relating to the labeling, presentation and advertising of foodstuffs. In the beginning of XXI century, Directive 2000/13/CE consolidated and updated rules about general labeling applying to every foodstuffs.

Nutritional labeling and allegations started to be regulated by Directive 90/494/CEE before the Regulation 1169/2011 which now legislates the information provided to consumers, updated the rules of general food labeling and also refers to nutritional allegations.

Keywords: general food labeling, nutritional labeling, EU legislation, consumer's food safety

B21-054 - Anti-inflammatory activity of *Ocimum campechianum* Mill

Souza, I.A.1.; Sousa, S.T.3; Ximenes, E.A. 1; Anjos, F.B.R.2; Silva, E.V.3; Silva, E.C.B.3; Caribé, R.A. 3 Cavalcanti, K.P.S. 4; Silva Neto5, J.F.; Jimenez, J.5

¹Department of Antibiotics-UFPE

²Department of Histology and Embryology-UFPE

³Department of Pharmaceutical Sciences-UFPE

⁴Hospital das Clinicas-Lika-UFPE-University of Coimbra

⁵Department of Morphology and Physiology-UFPE

Background: The *Ocimum campechianum* Mill

presents essential oils with a sensitive pharmacological action of character antifungal and antibacterial. Popularly called basil is the field, is distributed throughout tropical America and Brazil. Objectives: The plant material used was composed of the dried leaves of *O. campechianum* Mill as ethanolic extract (70%) obtained after maceration, and concentrated under reduced pressure to eliminate the organic solvent. In the test we used male Wistar rats, aged 60 days and weighing between 200-320g were divided into 3 groups of 6 animals (control-GC, GP-standard and test-GT). All animals were maintained under controlled lighting (cycle 12 hours light / dark), temperature (25 ± 3 ° C), PURINA-LABINA balanced feed and water ad-Labina libtum, being deprived of food 18 before the experiment. Edema was induced using 0.1 ml of 1% carrageenin injected into the subplantar region of the left rear paw. The volume of paws was measured before and 30 minutes after injection of carrageenan. Then we proceeded to the administration (oral and intraperitoneal) *O. campechianum* Mill extract (100mg/kg- GT) to the test group, indomethacin (10mg/kg-GP) and 0.9% saline (GC). The paw volumes were measured at constant intervals (1 hour) using a plethysmometer (Ugo Basile Italy Model 7150) for 6 hours. Results showed that animals administered orally and intraperitoneally with ethanolic extract of *O. campechianum* Mill and indomethacin showed a decrease in the volume of edema when compared to control animals. The reduction process was more significant edematogenic 4 hours after administration of the extract, and promoted a more pronounced inhibition than indomethacin. Based on the biphasic nature of the paw edema induced by carrageenan is possible to propose that the anti-inflammatory activity of the ethanol extract of *O. campechianum* Mill involves mediators of the late phase of edema.

Keywords: *Ocimum campechianum*; carrageenin; paw edema

B22-007 - Phytochemistry of *Aspidosperma pyrifolium* Mart.

Cavalcanti, K.P.S.1; Souza, I.A.2.; Anjos, F.B.R.3; Silva, E.V.4; Silva, E.C.B.4; Caribé, R.A.4; Guaraldo, P.4; Higino, J.S.4 ; Silva Neto5, J.F.; Jimenez, J.5

¹Hospital das Clinicas-Lika-UFPE-University of Coimbra

²Department of Antibiotics-UFPE

³Department of Histology and Embryology-UFPE

⁴Department of Pharmaceutical Sciences-UFPE

⁵Department of Morphology and Physiology-UFPE

Background: The genus *Aspidosperma* occurs from Mexico to Argentina. *Aspidosperma pyrifolium* Mart. is a tree up to 5 meters high, native of the Northeast of Brazil, also known as pereiro, stick-to-coaru and pequiá the woods. It is used in folk medicine as anti-inflammatory and used against cramps, dermatitis and urinary tract infections. Objectives: This study aims to determine the contents of favonóides, phenols and tannins in methanolic crude extract of *A. pyrifolium* Mart. Methodology: The methanolic crude extract was prepared from

the bark of *A. pyrifolium* Mart. After drying for 48 hours in incubator (42°C), the peel of the vegetable was processed in electric mill to obtain a powder. The extraction was performed in apparatus Soxhlet from 250g of dust with the addition of 600 mL of methanol for 12 hours. The extract was filtered and concentrated under reduced pressure (45°C) for subsequent analyzes. The analyzes for total phenols and residual were based on the methodology described by Folin & Ciocalteu (1927). The determination of total flavonoids was performed by the technique described by Peixoto Sobrinho et al. (2008) adapted for the species. Results: The results showed that the extract analyzed lacks tannins, but there is a large amount of phenolic compounds (1.425% of total extract) mainly flavonoids (7.701% of total extract). Conclusions: Flavonoids are a class of substances with a wide range of pharmacological activity. Consequently, our results demonstrate the potential of flavonoids obtained, which will be of great value to the continuity of research, evaluation perspectives with its use in inflammatory diseases, which will confirm its use in folk medicine.

Keywords: *Aspidosperma pyrifolium*, phytochemistry, flavonoids

Section C - Hot spot - Tuesday 11th December 10 h 30 min

Experimental Oncology Experimental Toxicology

C01-009 - Biomarker Investigation Related to mtDNA Copy Number in Lung Cancer. Bonifácio C1, Santos MJ1, Calvinho P2, Alarcão A3, Silva MR3, Dias JA3, Cabete A1, Wong LJ4, Carvalho L3, Grazina M1,3

1 CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal,

2 CHUC, Coimbra, Portugal,

3 Faculty of Medicine, University of Coimbra, Portugal,

4 Baylor College of Medicine, Houston, United States

Mitochondrial genome (mtDNA) is more susceptible to damage and acquires mutations at higher rate than nuclear DNA. Some types of cancer present increased mtDNA content, while others have been associated to decrease, compared to non-cancerous tissue from the same patient.

We have evaluated mtDNA copy number in lung tissue, derived from patients with lung cancer, to determine its role as a possible biomarker, according to smoking status.

It was observed that 67.6% of cases show a significant increase in mtDNA copy number in cancer lung tissue, compared to normal. Correlation analysis between clinical parameters and mtDNA copy number in normal tissue vs. tumor tissue shows increased of mtDNA content in normal tissue of active smokers, compared to passive smokers.

This result is original and suggests an important role of mtDNA in risk for lung cancer, according to smoking status

Keywords: Lung Cancer; Biomarker; mtDNA Copy Number

C02-110B - Influence of peritumoral microenvironment in the development of different tumor cell lines.

Carvalho¹, L.A.; Lavall¹,L.; Cenedezi¹, J.M.;Souza¹, J.P.C.; Viana² C.T.R.; Andrade² S.P.; Campos¹,P.P.

1 Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

2 Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

Introduction: The survival and proliferation of tumor cells depends on its interaction with tissue cells and extracellular matrix (ECM). Specific organic microenvironments establish the extension of the growth, angiogenesis, invasion and survival of these cells. Many types of tumor interact in different ways with the tissue microenvironment and new models could provide important contributions to the prediction of the tumor behavior. Objective: To analyse morphometric some parameter of the microenvironment around different tumors as the number of mast cells

and local angiogenesis. Methods: 5 groups of mice, 2 control groups animals Balb/c and C57BL/6 without tumor, 3 groups divided according to the type of tumor inoculated subcutaneously in the right flank region, cells of metastatic breast carcinoma (4T1), and cells of colon cancer (CT26) in Balb/c and melanoma cells (B16F10) in C57. The animals sacrificed after 15 days, the tumors were measured. Furthermore, tumors and adjacent skin were collected for histopathologic analysis. Results: Melanoma is the tumor that showed more growth 421.3 ± 66.07 ; CT26- 235.3 ± 16.63 ; 4T1- 45.26 ± 5.640 , although its adjacent tissue showed a lower number of mast cells (2.71 ± 0.08 ; CT26- 3.26 ± 0.13 ; 4T1- 4.63 ± 0.17) and a slight increase of angiogenesis (9.86 ± 0.38 ; CT26- 13.12 ± 0.61 ; 4T1- 4.42 ± 0.22). Nevertheless, colon tumor induced greater angiogenesis on adjacent tissue with a slight increase in the number of mast cells. The breast tumor, however, induced less angiogenesis and grew smaller, although number of mast cells in the surrounding tissue was higher. Conclusion: The tumor cell lines evaluated showed distinct profiles in the induction of angiogenesis and recruitment of mast cells in the peritumoral microenvironment. The role of mast cells and their correlation with angiogenesis differs between tumors studied. Thus, although there is a relationship between mast cells and tumor development, other factors are also related to the progression of tumors.

Key words: angiogenesis, tumor cell lines, mast cells

C03-110A - Model of chronic inflammation and tumor progression.

Carvalho¹, L.A.;Lavall¹,L.; Cenedezi¹, J.M.;Souza¹, J.P.C.; Viana² C.T.R.; Andrade² S.P.; Campos¹,P.P.

¹ Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

² Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

Introduction: Inflammation, defense mechanism of organisms, is an immediate response to tissue injuries caused by infections and other noxious stimuli such as cancer. In the intrinsic pathway, cancer develops through genetic events, including activation of various oncogenes and inactivation of tumor suppressor genes. The transformed cells produce inflammatory mediators, inducing an inflammatory microenvironment. In the extrinsic mechanism, infectious or inflammatory conditions increase the risk of developing cancer as gastritis, colitis, etc. However, more studies are needed to elucidate this interaction. Objective: Evaluate morphometric and biochemically the relationship between tumor development and responses as angiogenic and inflammatory in a chronically inflamed microenvironment and in the surrounding tissue. Methods: 24 C57Bl/6 mice were divided into 3 groups of 8 animals each. In the first a sponge was implanted in the subcutaneous tissue of the right flank; in the second melanoma cells (B16F10) were inoculated in the subcutaneous tissue of the right flank and in the third group, melanoma cells

were inoculated into the sponge matrix. The tumors and the sponge were measured, the animals sacrificed after 15 days, the tumor, the sponge and adjacent skin were collected for biochemical and histopathological analysis. Results: The sponge implant inoculated with tumor in its environment showed a greater mass 991.1 ± 164.1 mg, than B16F10 group 241.1 ± 80.96 mg and sponge group 266.2 ± 8.126 , although angiogenic parameters as hemoglobin content in implant with B16F10 cells 6.035 ± 0.3182 , sponge 9.6 ± 0.4 ; B16F10 - 14.4 ± 3.0 and inflammatory parameters MPO (0.10 ± 0.02 ; 0.29 ± 0.06 ; 0.45 ± 0.08); NAG (8.3 ± 1.3 ; 9.2 ± 1.5 ; 23.9 ± 3.8) were lower in this place. The analysis of the adjacent skin of this group showed increased angiogenesis evaluated by the number of vessels (15.0 ± 0.7 ; 7.9 ± 0.5 ; 10.9 ± 0.5) and less number of mast cells (3.9 ± 0.2 ; 4.7 ± 0.16 ; 5.9 ± 0.2) when compared with others. Conclusion: Environments chronically inflamed can act immunomodulating and decreasing the inflammatory response against the tumor cells allowing tumor development. Key Words: angiogenesis, chronic inflammation, melanoma.

C04-084 - Evaluation of plant extracts of family Annonaceae in Ehrlich tumor in vitro. Silva, A. G.1; Gomes, I.N.F. 1; Longatti, T.R. 1; Moreira, G.A. 1; Souza, C.M.2; Cassali, G.D.2; Santos, F. V. 1; Ribeiro, R.I.MA.1

1Laboratório de Biologia Celular e Mutagênese, Campus Centro Oeste D. Lindu, Universidade Federal de São João Del Rei, Brasil

2Laboratório de Patologia Comparada, Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Brasil

Introduction: Ehrlich tumor is a transplantable tumor of a malignant epithelium, which corresponds to mammary adenocarcinoma in female rats. The Ehrlich ascites tumor in mice proved to be an extremely useful tool for research in biology, growth and biochemistry of neoplastic cells. Brazil savannah is rich in plant species with the active compounds that can be investigated on the biological activity and its ability to inhibit cell proliferation. Annonaceae family has many compounds investigated in cancer models with good results, however, little is known about the possible medical application of the kind presented in this paper.

Objectives: The main purpose of this study was to investigate the effect of 3E1 extract in Ehrlich tumor cells.

Methodology: The male mouse balb/c used in this study were inoculated with 5×10^6 tumor cells suspended in 0,5 ml of phosphate buffered saline (PBS) solution (pH 7.4). The acitic fluid was collected using a syringe and the tumor cell count was performed in a Neubauer hemocytometer, using the Trypan Blue dye exclusion method. The ascitic fluid was collected in the day seven; three mL of them were targeted for cell culture (DEMEN). The cells were submitted to treatments with crude extracts of the plant 3E1 diluted in DEMEM at the following concentrations: 0.005, 0.0007 and 0.0003. The control was conducted with DEMEM. Cell viability was assessed every hour for nine hours.

Results: All concentrations of plant extract were effective in inhibiting tumor growth, while reducing the number of cells was dose dependent. Conclusion: The crude extract 3E1 inhibits cell proliferation and is cytotoxic. Furthermore, tests are already being conducted out with complementary fractions of this extract.

Key Words: Tumor Ehrlich, Cell Culture, Plant extracts.

C05-111 - Cryopreservation of Ehrlich tumor in economically viable solutions. Santos, K. M1; Maciel, F.P.S.1; Souto, C. M.1; Santos, F.V.1; Reis, D.C2; Cassali, G.D.2; Ribeiro, R. I. M. A. 1

1 Laboratory of Cell Biology and Mutagenicity - Universidade Federal de São João Del Rei – CCO - Dona Lindu, BRAZIL.

2 Laboratory of Comparative Pathology of the Institute of Biological Sciences - Universidade Federal de Minas Gerais, BRAZIL.

Introduction: Tumor Ehrlich is an experimental transplantable neoplasm that develops in several species of mice, in both the ascitic profile, as in the solid, representing excellent tool for studies of the behavior of neoplasms. The maintenance of the tumor still occurs in vivo. Thus, freezing of the cells would reduce the number of passages between animals, ensuring genetic stability and storage for a long period of experimentation. Objectives: Was evaluated the viability of the ascitic Ehrlich tumor cells in cryopreservation solutions alternatives. Methodology: The tumor cells were removed from mice inoculated seven days, washed with saline (0.9%), centrifuged ($3.000\text{rpm}/3\text{min}$) and added to nutrient mixture: 1 (Tris, citric acid, D-fructose, chicken egg yolk), 2 (coconut water, 25% sodium citrate and chicken egg yolk) and 3 (SBF in DEMEM) containing cryoprotectants (DMSO), B (Glycerol) and C (Trehalose) in different combinations totaling nine aliquot containing 1860×10^7 viable cells / ml. The samples were stored in cryogenic tubes, in triplicate. Viable cell counts were made using a Neubauer chamber (1% Trypan Blue) by two independent observers before and after freezing. The cooling of the aliquots was conducted at $2^\circ\text{C} / \text{min}$. until -180°C , remaining stored in liquid nitrogen for ten days. The samples were thawed by immediate immersion in water at 37°C . Results: After analysis of viable cells, the best results were the associations 1B, 2A and 3B, showing cell viability of 17%, 15% and 12%, respectively. The inoculations of 50×10^6 viable cells / ml 1B, 2A and 3B revealed they were transplantable and sufficient for the development of Ehrlich ascites tumor. The solutions 1B, 2A and 3B showed satisfactory preservation of tumor cells in the freezing process, demonstrating cryopresrvation role, practical technique and low cost.

Keywords: Ascitic tumor, cryoprotectants;

C06-126 - Immunohistochemical characterization of cells in pleomorphic adenomas of the

human salivary gland. Ribeiro, A. M.¹; Damasceno, K. A.¹; Ferreira, E.¹; Leitão, T. J.³; Soares, J. M. A.²; Ribeiro, R.I.M.A.³; Cassali, G. D.¹.

1 Comparative Pathology Laboratory, ICB – UFMG
2 São João de Deus's Hospital, Divinópolis - Brazil
3 Laboratory of Cellular Biology and Mutagenesis, UFSJ -Brazil

Background:The pleomorphic adenoma is the most frequently identified mixed tumor in the salivary glands and has a tendency to recur and also progress to malignancy. Neoplastic cells have great morphological variability, presenting epithelial or myoepithelial components that compromises the histological diagnosis and definition of the biologic profile of these tumors. **Objectives:** The aim was to evaluate the expression of P63 and Cytokeratin in pleomorphic adenoma with the purpose of characterizing the myoepithelial and luminal proliferation. **Methodology:** Twenty-two cases of pleomorphic adenoma were retrieved from Cytology and Pathological Anatomy of Divinópolis, Brazil (CIAP). All cases were submitted to immunohistochemical staining for P63 (4 A4, Neomarkers, 1:100) and Cytokeratin (AE1/AE3, Dako, 1:100) that was performed at Comparative Pathology Laboratory, ICB - UFMG. Immunostaining was evaluated in groups of cells (epithelial, spindle, mioepithelial-epithelioid, plasmocytoid) and areas of myxoid and condroid matrix.

Results: The analyzed tumors have shown a great morphological variability, presenting different cell's patterns within the same neoplastic lesion. Epithelial neoplastic cells, forming tubular structures, were observed in all tumors. However, plasmocytoid, spindle and myoepithelioid-epithelioid cells and myxoid and condroid matrix were observed only in five, 11, 21, 19 and 17 cases, respectively. In all cases cytoplasmatic immunostaining for cytokeratin was identified in epithelial duct and neoplastic epithelioid cells only. The p63 labeling was positive in nuclei of myoepithelial cells of normal ducts. The P63's positivity was observed in spindle, myoepithelial-epithelioid and plasmocytoid cells in 11/11, 11/20, 3/5 cases, respectively. In an interest way, a cytoplasmatic expression of cytokeratin and p63 was identified in plasmocytoid cells (2/5).

Conclusion: Through the labeling of cytokeratin and p63, myoepithelial and Epithelial identification was possible. However, the expression of those markers in some cells with similar morphological patterns can be related to the epithelial-myoepithelial differentiation in mixed tumors.

Keywords : neoplasms ,p63, cytokeratin AE1/AE3, mixed tumor

C07-034 - Prevalence of anal human papilloma virus infection in anal canal of women with squamous neoplasia of the lower genital tract.

Isabela Iside Gagliardo Soares¹, Vanessa Regina Rocha³, Luciana Lara dos Santos³, Karla Lima do nascimento¹, Eduardo Sérgio da Silva², Rosy Iara Maciel de A Ribeiro¹

1 Laboratório de Biologia Celular e Mutagênese, Universidade Federal de São João del-Rei, Campus Centro-Oeste Dona Lindu, Divinópolis, Brazil
2 Laboratório de Parasitologia, Universidade Fed-

eral de São João del-Rei, Campus Centro-Oeste Dona Lindu, Divinópolis, Brazil

3 Laboratório de Biologia Molecular, Universidade Federal de São João del-Rei, Campus Centro-Oeste Dona Lindu, Divinópolis, Brazil

Introduction: The association between human papillomavirus infection and cervical, vaginal, vulvar, anal, penile, and oropharyngeal neoplasia is well established. Women with a history of gynecological cancer can progress to anal cancer.

Aim of the study: The objectives of this study were to determine the prevalence of HPV infection through the collection of anal and cervical material analyzed by cytology and PCR.

Materials and Methods: We conducted a cross-sectional study with 91 patients of the Municipal Polyclinic and of the Hospital São João de Deus in the city of Divinópolis - Minas Gerais. The results of analysis of the samples was correlated with the results of analysis of the samples and with the socio-demographic characteristics of the population studied; compare the two methods used for viral detection.

Results: By cytology, it was observed the presence of cervical HPV in 76.9% of the patients, anal HPV was present in 48.4%, and 40.6% in the HPV was diagnosed in both places. HPV was detected by PCR in 71.4% of cervical and in 60.8% of the anal samples; there was concomitant infection in 45.1%. Comparing the two methods of viral detection, there was no statistical difference between the results obtained by cytology and by PCR in anal samples. Since the PCR was taken as the reference standard, it was observed that cytology obtained a sensitivity of 62.5% in the samples of the anus and 84.8% in cervical samples. The specificity was 61.9% in the anus and only 42.9% in the cervix. Therefore, It was concluded that there was a high prevalence of anal HPV infection in patients with genital squamous neoplasia. The high rate of infection in treated patients suggests that the anus can be a reservoir responsible for viral reinfection of the genital tract.

Conclusion: Both the PCR and cytology obtained good results for viral detection. Further studies are needed to define the best screening method.

Keywords: cervical cancer, anal cancer, human papillomavirus; prevalence.. Support from FAPESP, CNPq, CAPES. Presenting author: Eduardo Sérgio da Silva

C08-127K - Cytotoxic activity and Acute Toxicity of 5-Fluorouracil Incorporated Into the Metal Organic Framework Cu-BTC

Nascimento, S.C.1, Lucena, F.R.S.1, Rodrigues, M.D.1, Militão, G.C.G.1
1 Departamento de Antibióticos, UFPE, 50590-470, Recife - PE, Brazil.

Background: Polymeric controlled release technology has been an impact on different areas of medicine. Metal Organic Frameworks (MOFs) are crystalline structures formed by metal ions or clusters coordinated by organic molecules. One such MOF is Cu-BTC. Some potential applications for MOFs include gas storage containers, sensors, catalysts and recently, drug carriers and imaging agents for

several tumor cell targets.

Objectives: The goal of this work was evaluated the cytotoxic activity against human cancer cells and the improvement of the drug's toxicity.

Methodology: The cytotoxic activity was performed against four cell lines: NCI-H292 cells (mucoepidermoid carcinoma of the lung), MCF-7 cells (breast adenocarcinoma), HT29 cells (colon adenocarcinoma) and HL60 cells (promyelocytic leukemia) using the MTT Tests. The Acute toxicity was performed using the ANVISA protocols. R

Results: Was observed that 5-fluorouracil exhibited cytotoxic activity on MCF-7 and HL-60 cells. The IC₅₀ value for the MCF-7a nd HL-60 cells was very low (1,735 and 4,615 µg/mL) when the drug was associated with the MOF. The IC₅₀ values decreased significantly when the drug was incorporated into the network, suggesting that the network modulation the cytotoxic activity of the drug. In relation to the acute toxicity was verified the decrease of DL50 dose.

Conclusions: The MOF-associated drug was extremely cytotoxic against the human breast cancer cell line MCF-7 and human acute promyelocytic leukemia cells (HL60). This improvement of cytotoxic activity and the decrease of DL50 dose, suggests that the MOF modulates the cytotoxic activity and the acute toxicity of the drug.

Keywords: Acute Toxicity, Cytotoxic activity, Cu-BTC. License number of the Animal Ethics Committees: 230076.024149/2012-48

C09-118 – Ceftriaxone increases oxidative stress in multiple organs: Preclinical Evidence in sepsis. André Martins Galvão, MSc 1, Célia Maria Machado Barbosa de Castro, PhD, MD2, Mario Ribeiro Melo-Junior, PhD3, Nereide Stela Santos Magalhães, PhD4, Carlos André Miranda Filho5, Luciano Acordi da Silva, PhD6, Emílio Luiz Streck, PhD6, Armele, Dornelas de Andrade, PhD7, Maria Bernadete de Souza Maia, PhD8

1 Post-Graduate Program (PhD level) in Biology applied to Health Science at the Laboratory of Immunopathology KeizoAsami, Recife (PE), Brazil;

2 Department of Nutrition at University Federal of Pernambuco, Recife (PE), Brazil;

3 Department of Pathology at University Federal of Pernambuco, Recife (PE), Brazil;

4 Department of Pharmacology at University Federal of Pernambuco, Recife (PE), Brazil;

5 Department of Biomedicine at University Federal of Pernambuco, Recife (PE), Brazil;

6 Post-Graduate Program in Health Sciences (PhD level) at University of Extremo Sul Catarinense, Criciúma (SC), Brazil;

7 Department of Physiotherapy at University Federal of Pernambuco, Recife (PE), Brazil;

8 Department of Pharmacology and Physiology at University Federal of Pernambuco, Recife (PE), Brazil

Background: The bactericidal activity of cephalosporins result in the inhibition of cell-wall synthesis, but recent studies have revealed that antibiotics can promote the formation of reactive oxygen and nitrogen species which contribute to the misfolding

of proteins, mutations in genes, instability between iron-sulfur, dysregulation of iron and depletion of NADH. **Objective:** To elucidate the effect of ceftriaxone toxicity on production of oxidative stress and inflammatory response in multiple organs of rats subjected to sepsis. **Method:** Design: Randomized, controlled preclinical trial. **Setting:** Animal basic science laboratory. **Subjects and Interventions:** Five groups of Male Wistar rats underwent cecal ligation and perforation (CLP) or sham-operated. Twelve hours after the above procedure, the animals were treated with ceftriaxone (30mg/kg; i.m.; 6h/6h, 5 days), 0.9% saline (10 ml/kg, i.m., 6h/6h, 5 days) or ceftriaxone plus antioxidants (8 mg/ml of vitamin C, NAC and vitamin E, intratracheal, 24h/24h, 3 days). **Measurements:** superoxide anion, TBARS, protein carbonil, sulphhydryl groups, SOD and CAT activities, survival curves and histological analyses were assessed in heart, lung, liver, spleen and kidneys. **Results:** The ceftriaxone produced, in control and septic groups, high levels of superoxide anion, MDA, carbonyls and decreased sulphhydryl groups, SOD and catalase activities. When the antioxidants were administered the oxidative stress was prevented, including inflammatory infiltrate and edema and there was an increasing of survival rate in 15%. **Conclusion:** The results showed that ceftriaxone is, in part, linked to oxidative stress and the antioxidant administration reduced the levels of superoxide anion, lipid peroxidation and carbonyls and restored the content of sulphhydryl groups. New approaches on antibiotic formulations and antioxidant encapsulations could be carried out for further investigations.

Keywords: ceftriaxone; oxidative stress; antioxidants; multiple organ damage; sepsis. **Grants:** Brazilian Ministry of Science and Technology - Brazilian Research Council (CNPq), Brazilian Ministry of Education - CAPES and Research Foundation at the Pernambuco State (FACEPE). **Institution where this work was performed:** Laboratory of Immunopathology Keizo Asami (LIKA) at University Federal of Pernambuco, Brazil.

C10-135 - Cytotoxic action of geopropolis produced by *Melipona fasciculata* Smith on human laryngeal epidermoid carcinoma (HEp-2) cells. Mendes Araújo, M.J.A.1; Búfalo, M.C.1; Conti, B.J.1; Sforcin, J.M.1

1 Institute of Biosciences, Department of Microbiology and Immunology, UNESP, Botucatu-SP, Brazil

Background: Propolis is produced by Africanized honeybees from resinous materials of plants, wax and bee enzymes. Conversely, geopropolis is produced by indigenous stingless bees from resinous materials of plants, adding salivary secretions, wax, but also adding mud or clay. **Objective:** Since there are few works regarding geopropolis cytotoxic action towards tumor cells, the goal of this work was to investigate its possible cytotoxic action on human laryngeal epidermoid carcinoma (HEp-2) cells. **Methodology:** These cells were incubated with different concentrations (5, 10, 25, 50 and 100 µg) of geopropolis for different time periods (6, 24, 48 and 72 hours), and then the number of viable HEp-2 cells and morphologic evaluation were an-

alyzed. The same procedure was performed with geopropolis solvent (70% ethanol). As a positive control, carboplatin (Darrow-Vancel® Laboratories A/S) was used, since it is employed in the clinical treatment of several tumors, due to its high efficacy and low toxicity. Cell viability was assessed using the MTT test. Results: With respect to the HEP-2 cells viability, a significant decrease was observed after 6 h of incubation using 50 and 100 μg . After 24 h of incubation there was a significant decrease in cell viability from 25-100 μg . Geopropolis solvent had no effects on morphology and number of viable cells. Data showed that HEP-2 cells exhibited a fast growth and a large number of fusiform and elongated-shaped cells was observed. After incubation with geopropolis, there was a few number of round cells. Furthermore, in our assays conditions, carboplatin (400 and 500 mMol/l) exerted only a slight cytostatic effect on HEP-2 cells. Conclusions: Although almost all tumor cell lines seem to be sensitive to the action of propolis produced by Africanized honeybees, geopropolis produced by stingless bees showed only cytostatic action on HEP-2 cells. Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 2010/50064-7 and 2010/01028-8).

Keywords: HEP-2 cells; cytotoxic action; geopropolis

C11-228 - Intestinal villi changes in experimental administration of DMBA. Ana Isabel Ferreira¹, Ricardo Cabeças¹, Eduardo Costa¹, Rodrigo Farinha¹.

¹Experimental Pathology Service, University of Coimbra, Portugal

7,12-Dimethylbenz(a)anthracene (DMBA) is an immunosuppressor and a powerful organ-specific laboratory carcinogen. This substance is widely used in many research laboratories for studying cancer in rats. The aim of this study was to investigate the effect of DMBA in intestinal villi of eight-week old male Wistar rats. Test group (n=12) was submitted to three doses of 20 mg/Kg of DMBA by weekly gavage administration, in a period of four weeks. Control group (n=12) was submitted to no manipulation. Small intestine was collected from all rats for histopathological processing and analysis. Intestinal villi length was measured by ImageJ software. The intestinal villi were distributed into five classes (very small - VS, small - S, medium - M, big - B and very big - VB) using the average and standard deviation, for the control group, and the villi length were classified according to the following criteria: Medium villi (M), when the value varies between the range $[\mu-\delta; \mu+\delta]$, big villi (B), when the value varies between the range $[\mu+\delta; \mu+2\delta]$, small villi (S), when the value varies between the range $[\mu-\delta; \mu-2\delta]$, very big villi (VB), when the value is more than $\mu+2\delta$ and very small villi (VS), when the value is less than $\mu-2\delta$; The test group was compared with the control one. In the control group the intestinal villi in each class was: 2 VS; 16 S; 64 M; 16 B; 2 VB. In the test group, the intestinal villi in class was: 17.07 VS; 39.09 S; 35.37 M; 7.32 B; 1.22 VB.

Test group presented smaller intestinal villi than control group. The length of intestinal villi rang-

ing between 682.01 – 747.96 μm and 567.97 – 629.18 μm , for a confidence interval of 95%, respectively for the control group and test group. These results suggest that DMBA leads to an atrophy of intestinal villi.

Key words: DMBA, intestinal villi

C12-017 - Gut morphologic evaluation during DMBA experimental intoxication. Gustavo Barandas¹, Eduardo Costa¹, Ricardo Cabeças¹, Rute Duarte¹, Diana Reis¹, Silvério Cabrita¹.

¹Experimental Pathology Service, University of Coimbra, Portugal

The gut-associated lymphoid tissue (Gut) is a tissue devoted to the immune surveillance of the intestinal lumen, detecting antigens such as microorganisms and toxic chemicals. In this study we evaluate, using morphometry, the presence a morphologic changes during 7,12 - Dimethylbenz(a)anthracene (DMBA) per os experimental intoxication.

One group of ten male Wistar rats, 8 weeks old, was submitted to the administration of 20mg/kg DMBA by gavage during 4 weeks. Another group of ten male Wistar rats, 8 weeks old maintained during 4 weeks with no manipulation was used as a control group.

All animals were sacrificed at the end of 8 weeks period and a complete necropsy was performed. The slides of gut tissue, stained by HE, were studied by light microscopy and submitted to morphometric analysis to evaluate the lymphoid tissue of the small intestine, organized in patches.

Test group presented smaller gut-associated lymphoid tissue than control group. The area of gut-associated lymphoid tissue in the small intestine ranging between 427887 – 646311 μm^2 and 209522 – 327127 μm^2 , for a confidence interval of 95%, respectively for the control group and test group.

These results allow us to conclude that the observed gut-associated lymphoid tissue is changed on the administration of 7,12-Dimethylbenz(a)anthracene (DMBA).

C13-012 - BALT and dietary conditioning - on a neoplastic experimental model.

Ana Calado¹, Paula Pereira², Rodrigo Farinha¹, Silvério Cabrita¹

¹Experimental Pathology Service, University of Coimbra, Portugal

²Laboratory of Biochemistry, CiiEM, Institute of Health Sciences Egas Moniz, University Campus, Caparica, Portugal.

Introduction: Bronchus-associated lymphoid tissue (BALT) is composed by organized aggregates of lymphocytes that are located within the bronchial submucosa. These aggregates are randomly distributed along the bronchial tract but are consistently present around the bifurcations of bronchi and bronchiole and always lie between an artery and a bronchus [1]. There is typically a paucity of follicles within the BALT, most likely due to the fast transport of the immunological reaction to the draining regional lymph nodes. In contrast to lymph nodes,

BALT have a relatively high proportion of collagen and reticular fibrils [2]. BALT is an inductive site, which means that contains secondary lymphoid tissues in which IgA class switching and clonal expansion of B-cells occurs in response to antigen specific T-cell activation. The active participation of T-cells in immune responses is indicated by the presence of large numbers of natural killer (NK) cells in the lung, and the occurrence of T-cells with the suppressor/cytotoxic phenotype in the lamina propria and within BALT [1].

Exposure to polycyclic aromatic hydrocarbons (PAHs) such as dimethylbenz[a]anthracene, is mainly by polluted air, for example, through exposure to exhaust fumes from burning fossil fuel. These compounds are immunosuppressive, and especially affect humoral responsiveness. The main property of the PAH compounds is their carcinogenicity. Besides a direct action of the compounds in inducing neoplasia, it is speculated that induction of neoplasia is also related to their immunosuppressive effects. This immunosuppression causes a reduction of natural immunosurveillance for tumor cells. The cells mediating immune surveillance include natural killer cells.[3]

Enhanced histopathology (EH) of BALT is an important aspect of immunopathology that can be made with traditional sectioning methods of the lung tissue. EH was considered to provide a reasonable level of accuracy as a screening test to identify immunomodulatory compounds [4, 5].

Materials and Methods: 168 Sprague Dawley virgin female rats with 42 days were randomly distributed for seven groups of 24 animals each. Besides the control group, all the treatment groups received carcinogenic 1,12-dimethylbenzanthracene (DMBA) at the dose of 20mg diluted on 1ml of olive oil orally administered by gavage plus dietary modifications. In this regard, The group I received a standard diet of 300Kcal/100g and no carcinogen. The group II received carcinogen and standard diet. The group III received the carcinogen and standard diet supplemented with beans 40g/Kg of diet (341Kcal/100g). The Group IV received carcinogen and standard diet supplemented with olive oil (50ml/Kg of diet). The group V received carcinogen and fiber supplement four times higher than the standard diet for a total 250Kcal/100g. Group VI received carcinogen and a diet supplemented with fiber four times higher than the standard diet plus 19,95gr of beans and 25ml of olive oil on 1Kg of standard diet. Group VII received carcinogen and standard diet (300Kcal/100g) supplemented with 41Kcal of a Mediterranean lyophilized diet: potato (22g), sardine (32g), tomato (4,3g), pepper (3,5g), onion (0,196g) and olive oil (2g). The experience was 150 days long. The animals were ethically euthanized and the lungs were routinely fixed and stained with hematoxylin-eosin. BALT was evaluated in accordance with the guidelines of MALT enhanced histopathology [2].

Conclusion: Diet has a marked effect on BALT immunity on this carcinogen neoplastic model. Mediterranean die and caloric restrain had the most significant influence, olive oil and beans had inconsistent influence needing additional investigation.

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C14-012 - BALT and high focalized hyperthermia - experimental study.

Ana Calado¹, Bruno Colaço², Ana Portela³, Silvério Cabrita¹.

¹ Experimental Pathology Service, University of Coimbra, Portugal

² UTAD, Vila Real, Portugal

³ Faculty of Dentistry, University of Porto, Portugal

Introduction: The immune respiratory defenses are partially composed with a secondary type of lymphoid tissue distributed consistently around the bifurcations of bronchi and bronchiole and always lie between an artery and a bronchus. The rat has a developed active bronchus-associated lymphoid tissue (BALT) rich in reticular fibers, lymphocytes B and lymphocytes T and a large number of Natural Killer (NK) cells. The rat BALT is a mirror of systemic status. It increases in response to systemic antigens and decreases under toxic conditions like in the rat model of mammary tumors chemically induced by 7,12-dimethylbenz[a]anthracene (DMBA). This carcinogen has a lytic effect over NK cells and this mechanism decreases the respiratory immune system. The systemic treatments for mammary tumors are, in general, immunodepressants. The treatment of tumors based on focal hyperthermia by ferromagnetic ceramics has unknown effect on systemic immune system. BALT enhanced histopathology is a reasonable accurate and inexpensive method to evaluate BALT with consistent results.

Materials and methods: 36 virgin female Sprague Dawley rats with 50 days were randomly distributed over three groups of 12 animals each during 27 weeks. The group I had no manipulation; the group II was chemically induced by DMBA at the dose of 65mg/Kg orally administered by gavage using olive oil as the vehicle not exceeding 1ml per animal; the group III received the same carcinogenic treatment of group II, but at week 26 of the experiment the tumors were injected with a ferrimagnetic ceramic and stimulated by electromagnetic energy over 30 minutes reaching a temperature of 43°C, 5°C superior to the animal normal temperature. Each animal of group III received 3 hyperthermia treatments, all separated by 48h to avoid termotolerance. All animals were ethically euthanized at 27 weeks of experiment and a full necropsy study was performed. BALT was evaluated by enhanced histopathology. **Results:** The groups II and III had decreased BALT

of interfollicular areas when comparing to group I. There were no significant changes between the BALT of group II and group III.

Conclusions: DMBA is a carcinogen with depressant effect over BALT, but the tumoral treatment by focal hyperthermia with ferrimagnetic ceramic has no additional effect over BALT, which suggests that the immune status is not disturbed by this therapeutic approach.

C15-105 - Evaluation of gene expression in a model of experimental breast neoplasia

Gonçalves, L.M.1; Calado, A2; Santos, M.J.3; Grazina, M.3; Seixas-Travassos, F.4; Sivério Cabrita, A.2

1 University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

2 Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

3 Laboratory of Biochemical Genetics, Faculty of Medicine- University of Coimbra

4 Laboratory of Pathological Anatomy and Histology, UTAD, Vila Real, Portugal

Background: The uncontrolled cell proliferation, which represents the essence of neoplastic disease, involves not only deregulation proliferative but also the metabolic adjustment to sustain growth and cell division. **Objectives:** evaluating the variation of gene expression of three genes metabolism: citrate synthase (Krebs cycle), acetyl-CoA carboxylase α (fatty acid synthesis), and glucose-6-phosphatase (gluconeogenesis and glycogenolysis) in four groups of histological lesions and normal breast tissue. **Methodology:** Samples were taken from mammary glands of 10 female Sprague-Dawley previously induced with 7,12-Dimethylanthracene (DMBA) at a concentration of 65mg/Kg of animal. Section was taken for histological classification. After extraction of RNA and evaluated its integrity was performed a "two-step real-time PCR". Levels of expression were assessed by comparing expression levels of target genes with β -actin gene (endogenous control). The data were processed by ANOVA program statistician. **Results:** The citrate synthase gene expression is decreased along the progression of the neoplastic disease, allowing a significant distinction between benign and malignant neoplasms since the expression is far below the latter. The gene expression of acetyl CoA carboxylase α is slightly increased in benign and significantly lower in the malignant neoplasm. The gene expression Glucose-6-phosphatase has wide expression values from values approaching zero values to several times the normal. **Conclusion:** There is a significant difference between benign and malignant tumors for genes citrate synthase and acetyl CoA carboxylase α , but none of the genes shows expression differences between malignant non-invasive from invasive. The gene expression glucose-6-phosphatase allows no distinction between histological groups.

Keywords: experimental breast neoplasia, Metabolism, gene expression

study.

Miguel Costa ^{1,2}, Ana Calado ^{1,2}, Fernando Capela ¹, António Ramos ¹, Silvério Cabrita ¹

1 Faculty of Medicine - University of Coimbra, Experimental Pathology Service

2 University School Vasco da Gama

The seeds of Lathyrus species contain Oxalyl-diaminopropionic acid (ODAP), a neurotoxin analog to the neurotransmitter glutamate able to cause a neuromuscular condition generically known as lathyrism in human and animals (Grela et al., 2001; Hanbury et al., 2000). The modern source a lathyrigenic molecule known as semicarbazide, can be found on canned food. The industrial process leads to the contamination of food with semicarbazide released from the package (Chowdhury and Davis, 1998).

In this study we evaluate the rat morphology of bronchus-associated lymphoid tissue (BALT) associated with ingestion of food contaminated with Semicarbazide. In rats, BALT reacts to systemic antigens and toxics, being a good source evaluation of the immune status.

Thirty Wistar rats with 30 days were randomly distributed into three groups. Group I was submitted to no manipulation. Group II was supplemented with 3g of Semicarbazide on 1Kg of standard diet. Group III were submitted to the administration of food contaminated with 6g of semicarbazide 1Kg of standard diet.

At the end of 4 weeks of experiment the animals were ethically euthanized and a complete necropsy study was performed. The slides of lungs, stained by HE, were evaluated for BALT morphologic lesions by enhanced histopathology.

In group I we found mild increased of interfollicular areas. In group II the results were inconsistent. In group III was decreased interfollicular areas in size and number of lymphocytes.

According to these results we may conclude that semicarbazide exposure has citotoxic effect on BALT lymphocytes dependent on the dose of oral intake.

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C17-237 - Adipode Tissue Evaluation in DMBA Experimental Intoxication.

Patricia Jesus^{1,2}, Gustavo Brandas², Rute Duarte², Nuno P. F. Gonçalves¹, A.M. Silvério Cabrita²

1 Chemistry Department, University of Coimbra

2 Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

Dimetilbenzantracene DMBA is an environmental

pollutant present in cigarette smoke, automobile exhaust, grilled food, woodsmoke, etc.[1]. It is the one of the most potent carcinogenic polycyclic aromatic hydrocarbons, can induce in experimental animals not only skin tumors, but also lung and mammary cancers [2]. The objective of this study is molecular evaluation in the experimental intoxication of 7,12-dimethylbenzanthracene (DMBA) in adipose tissue.

Ten Wistar male rats, 8 weeks old were submitted to a quarantine period of one week, and then distributed by two groups: the test group, submitted to the administration of DMBA 20mg/kg/day, three times a week, for four weeks; and the control group, kept on the standard conditions. At the end of the assay time, all animals were ethically killed and the complete necropsy performed. Fragments of abdominal adipose tissue were collected for routine histopathological processing and examination, and for snap freezing in liquid nitrogen and preservation at -80°C. A liquid-liquid extraction protocol was used to isolate the lipophilic portion of the liver and adipose tissue and analyzed by HPLC, in standard conditions, at 40°C.

No DMBA was detected in the animal of the control group. In 5/5 of the animals (test group) it was possible to detect and quantify the DMBA in the adipose tissue. The results of this study, lead us to conclude that DMBA can become stored on the adipose tissue in several conditions.

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C18-019 - 1-naphtyl N-methylcarbamate Induced Colonic Carcinogenesis

Validated by PCNA and p53 Expression

Pedro Sá¹, Nuno Coelho¹, Ana Alarcão², Lia Teixeira², Pedro Barreto¹, Karen Cavalcanti³, Lina Carvalho², António Cabrita¹

¹ Experimental Pathology Service, Faculty of Medicine, University of Coimbra

² Anatomic Pathology Institute, Faculty of Medicine, University of Coimbra.

³ University Federal of Pernambuco, Recife, Brazil.

Introduction: The wide spectrum of Insecticide 1-naphtyl N-methylcarbamate (1NC) has been considered of low toxicity when applied according with the good practice rules. The present work was designed to evaluate morphology and proliferation rates of experimentally induced adenocarcinomas by 1NC and DMH.

Material and Methods: Thirty Wistar 8 weeks old male rats were randomly distributed into three groups (10 rats each), to be submitted to dimethylhydrazine (DMH) 15mg/week during 8weeks – Group II, to 1NC 60mg/liter in drinking water dur-

ing 8weeks – Group III, and without manipulation – Group I. All the animals were sacrificed by the end of the twentyeighth week. The macroscopical tumors were collected and FFPE; PCNA and p53 antibodies were applied to sections of all tumours by the streptavidin-biotin/HRP method.

Results: In Group I, rats had no macroscopic colonic alterations while in Groups II and III there were exophytic lesions with wider diameter between 2 and 4 cm. All were well differentiated adenocarcinomas except for one case 1NC induced that was a moderately differentiated adenocarcinoma. Proliferation rate validated by PCNA immunohistochemical study showed to be higher in Group III cases (1NC induced): 6 negative cases in Group II against 3 negative cases in Group III; the cut off applied + (< 25% positive nuclei), ++ (25 – 75% positive nuclei) and +++ (> 75% positive nuclei) demonstrated 5 cases of ++ and +++ in Group III and 1 case +++ in Group II as the other 3 cases had proliferation rate of +. For p53 nuclear immunostaining there were 6 positive 1NC induced cases against 4 in the DMH Group.

Conclusion: Despite the small sample explored in this preliminary study, 1NC revealed to induce more aggressive adenocarcinomas validated by an higher proliferation rate when compared with the DMH induced cases, probably with apoptosis blockage. In general, macroscopic tumors were pediculated and developed from deep crypts where the functional stem cells pool need further studies.

C19-054 - Acute toxicity of *Ocimum campechianum* Mill.

Souza, I.A.1; Cavalcanti, K.P.S.2; Anjos, F.B.R.3; Silva, E.V.4; Silva, E.C.B.4; Oliveira, M.V.G.4; Silva, B.G.4; Carvalho, R.R. 4; Silva Neto5; J.F.; Jimenez, J.5

¹Department of Antibiotics-UFPE

²Hospital das Clinicas-Lika-UFPE-University of Coimbra

³Department of Histology and Embryology-UFPE

⁴Department of Pharmaceutical Sciences-UFPE

⁵Department of Morphology and Physiology-UFRPE

Background: The genus *Ocimum* belongs *Lamiaceae* family with about 30 species originating from Africa and represents a rich and diverse source of essential oils. *Ocimum campechianum* Mill. is an aromatic herb popularly known as the Brazilian northeast as big basil, basil-the-field or basil crow's. It is distributed by South America and western India. Investigations into the ethnopharmacology *O. campechianum* Mill revealed that this species is widely used in folk medicine in some regions of Brazil and Mexico. **Objectives:** The objective of this study was to determine the acute toxicity of the hydroalcoholic (70%) of the leaves of *O. campechianum* by observing general parameters of toxicity. **Methodology:** We selected male Swiss albino mice (*Mus musculus*), weighing between 25-35g were divided into groups of six animals were kept in polypropylene cages with temperature of 25 ± 3 ° C cycles and light / dark 12/12 hours. The animals received water ad libitum and were deprived of food 12 hours before the experiment. The acute oral tox-

icity was evaluated according to Karber & Behrens (1964). Results: Intermediate doses were used between 625 and 300mg/kg of body weight, intraperitoneally. The main reactions observed were tremors, piloerection, estereoptipado movement suggestive of effects on the central nervous system. Also observed were writhing and increased respiratory rate suggestive of effects on the autonomic nervous system. After the administered doses set by the Karber method & Berens (1964) established the LD50 was 1017.4 mg/kg bodyweight. Conclusions: The results presented by the hydroalcoholic extract of the leaves of *O. campechianum* are considered of low toxicity. These results lead us to continued research of this plant by the importance of its use in folk medicine.

Keywords: *Ocimum campechianum*, acute toxicity, mice

C20-014 - Evaluation of espermatogonium compartment in a model of semicarbazide into-xication

Eduardo Costa¹, António Ramos², José Cabeças¹, Rodrigo Farinha¹, Fernando Capela-Silva², Silvério Cabrita¹

¹Experimental Pathology Service, Faculty of Medicine, University of Coimbra

²University of Évora

Lathyrism is a pathologic condition with deficient collagen synthesis, which can be induced in the lab animal by the administration of semicarbazide.

The aim of this study is to evaluate the seminiferous tubules morphologic changes, measuring the area occupied by the spermatogonia, in the experimental administration of semicarbazide.

Thirty Wistar male rats, 8 weeks-old were randomly distributed on two groups: the control group was kept without manipulation and the animals of test group were submitted to the administration of semicarbazide incorporated in the diet in the dose of 6g/kg, during 4 weeks. All the animals were sacrificed on the 30th day, by an im overdose of anaesthetics and a complete necropsy was performed.

The fragments collected in the necropsy were studied under light microscopy, stained by Haematoxylin & Eosin. The testicle's slides were submitted to morphometric analysis to evaluate the area occupied by spermatogonium compartment, using imageJ software.

The medium compartment of spermatogonium is $11.86 \pm 4.06 \mu\text{m}^2$ in control group and $14.06 \pm 4.97 \mu\text{m}^2$ in the test group. The percentage of the area of compartment of spermatogonium in the seminiferous tubules is 20.18 ± 4.88 in the control group and 39.06 ± 13.23 in the test group.

Comparing both groups, we observe more cases with medium area than on the test group. The medium compartment of spermatogonium ranging between $11.36 - 12.36 \mu\text{m}^2$ and $13.35 - 14.75 \mu\text{m}^2$, for a confidence interval of 95%, respectively for the control group and test group.

These results suggest that the spermatogenesis is changed on the administration of semicarbazide.

Section D - Hot spot: Tuesday 10 h 30 min

**Microbiology
Immunophysiology and Immunopathology
Inflammation and Infection
Parasitology
Traumatology**

D01-132 - Interleukin-10 (IL-10) and Tumor necrosis factor alpha (TNF- α) genotypes in Brazilian patients with Crohn's Disease. Fernandes, W. N.1, Santos, L. P.1, Lima, F. E. B. A.1, Telles, A. M. S.1, Martinelli, V. F.1, Angelo, H. D.2, Souza, P. R. E.2, Melo-Júnior, M.R.1

1Universidade Federal de Pernambuco (UFPE), Brasil.

2Universidade Federal Rural de Pernambuco (UFRPE), Brasil

Background: Crohn's Disease (CD) is a recurrent chronic inflammatory disease characterized by segmental transmural inflammation that may affect any part of the digestive tract. Polymorphisms in cytokines genes may play an important role in the development and clinical manifestation. Objectives: Due to this, there is a great interest in the identification of biomarkers that which could quantify the susceptibility and disease activity. Methodology: A case-control study of 49 CD cases and 132 CD-free adults controls, was performed to analyze whether or not the polymorphisms of the TNF- α gene promoter at positions -308 G/A and IL-10 gene promoter at positions -1082 G/A would alter the risk for CD and clinical manifestations. Genotyping was carried out by polymerase chain reaction, PCR products to TNF- α polymorphism were digested by NcoI restriction enzyme and fractionated after on 2% Agarose gel and visualized posteriorly staining by ethidium bromide, while the PCR products to IL-10 polymorphism were fractionated on 1.5% Agarose gel and visualized posteriorly staining by ethidium bromide. Results: There were not significant ($p>0.05$) differences in the distribution of the TNF- α and IL-10 gene polymorphisms between the CD and control groups. Moreover, no association was observed between genotypic distribution of CD patients and localization or behavior's disease. Conclusions: Our data support the hypothesis that the TNF- α /IL-10 genotypes did not play an important role in pathogenesis of Crohn's disease in Brazilian Northeast.

Key words: Crohn's Disease; Genetic Polymorphisms, Tumor necrosis factor alpha; Interleukin 10. Scientific and Clinical

D02-155 - Hydatid cyst genotyping (preliminary data) – laboratory contribution to epidemiology surveillance and disease prevention. Mateus, T.L.1,2,3, Ribeiro, J.N.3, Vieira-Pinto, M.M.4, Cardoso, M.5, Castro, A.2

1 Escola Universitária Vasco da Gama, Coimbra, Portugal;

2 Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal;

3 Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal;

4 CECAV-UTAD, Vila Real, Portugal;

5 ESAB-Instituto Politécnico de Bragança, Portugal

Background: Hydatidosis is an important zoonotic infection caused by the development of larval forms of *Echinococcus granulosus* (hydatid cysts) both in humans and in other animals. The symptoms are highly variable depending on the affected organs, if the cysts rupture the release of its contents precipitates an anaphylactic allergic reaction that can be fatal. To date there are 10 *E. granulosus* genotypes (G1 – G10) identified according to the host to which they are better adapted, and which are therefore more important in the perpetuation of the biological cycle of the disease. This genetic variation may have an influence on the antigenicity, the sensitivity to chemotherapeutic agents, thus identifying the genotype have implications for the development of vaccines, medicines and diagnostic tools. The sheep strain (G1), is most often associated with human infection, namely in Portugal.

Objectives: The aim of this work is to identify the animals hydatid cysts genotypes collected in Portugal.

Methodology: Hydatid cysts were collected during slaughter of domestic and wild ruminants and pigs. We removed hydatid fluid, protoscoleces or germinal membranes and analyzed by PCR. To identify the genotypes we analyzed gene fragments of mitochondrial DNA - NADH dehydrogenase and cytochrome c oxidase I - and finally were amplified and sent for sequencing.

Results: To date we have analyzed 14 cysts: 10 from cattle, 3 from sheep and 1 from wild boar. PCR confirmed that all samples were hydatid cysts. Cattle samples that are already genotyped are genotypes G1 and G2, the sheep ones are G1 and the wild boar is G7.

Conclusions: The diversity of genotypes found is remarkable. We confirm the existence of genotype G1 in sheep but also in cattle, apparently these two species are the most associated to the maintenance of the disease in humans.

D03-134 - Correlation between salivary anti-HCV antibodies and HCV RNA in saliva and salivary glands of patients with chronic hepatitis C. Caldeira, P.C.1; Oliveira e Silva, K.R.1; Silva, T.A.1; Grossmann, S.M.C.1; Teixeira, R.2; Carmo, M.A.V1.

1 Department of Oral Pathology and Surgery, School of Dentistry, Universidade Federal de Minas Gerais, Brazil.

2 Department of Medical Clinic, School of Medicine, Universidade Federal de Minas Gerais, Brazil.

Background: Chronic hepatitis C is a severe disease, caused by hepatitis C virus (HCV). The use of saliva as an alternative diagnostic tool for HCV infection through the detection of HCV RNA and anti-HCV antibodies is still not established.

Objective: To investigate the correlation between anti-HCV antibodies in saliva and detection of HCV RNA in saliva and salivary glands of patients with chronic hepatitis C.

Methods: A total of 180 saliva samples (131 non-stimulated and 49 stimulated) from 133 patients with chronic hepatitis C were tested by ELISA for

presence of anti-HCV antibodies. Results were compared with the detection of HCV RNA in saliva and salivary glands samples. Statistical analysis was performed.

Results: Anti-HCV antibodies could be detected in 47/180 (26.1%) saliva samples. In 11/47 (23.5%) of these, HCV RNA was also detected. From the 133/180 (73.9%) saliva samples with undetectable anti-HCV antibodies, 49/133 (36.8%) were positive for HCV RNA at least in one saliva sample. From the 64 patients from whom salivary gland samples were available, 17/64 (26.6%) had detectable anti-HCV antibodies in saliva, from which 2/17 (11.8%) also had HCV RNA in the salivary gland. From the 47/64 (73.4%) cases negative for anti-HCV antibodies in saliva, 10/47 (21.3%) were positive for HCV RNA in salivary gland. Statistical analysis revealed a significant association ($p=0.035$) between detection of anti-HCV antibodies and HCV RNA in stimulated saliva, but not for non-stimulated saliva. Conclusions: The results suggest that there is no correlation between the presence of anti-HCV antibodies in saliva and the detection of HCV RNA in saliva and salivary glands in patients with chronic hepatitis C. Nevertheless, as there was a statistically significant difference between detection of anti-HCV antibodies and HCV RNA in stimulated saliva, the study points toward the need for new research on mechanisms of HCV shedding in saliva. Keywords: HCV, hepatitis C, saliva.

D04-130A - Melanization in *Culex quinquefasciatus* after exposure to different fatty acid methyl esters (FAME). Alves, SN1; Ribeiro Neto, JA1; Pinto, MEA1; Lima, LARS1

1Campus Centro-Oeste Dona Lindu / Universidade Federal de São João Del Rey , Brasil

Introduction: *Culex quinquefasciatus* is a mosquito sucking, cosmopolitan, anthropophilic, widely distributed for the planet, especially the tropical and temperate regions. Currently, natural compounds that are effective, selective and non-toxic to people associated, including man, are searched in order to ally efficiency, low production cost and minimize in the appearance of resistance. In this sense, fatty acid methyl esters (FAME), obtained by transesterification of vegetable material, are being tested with some success. However, little was observed of the pathology in larvae of these mosquitoes.

Objective: This study aims to observe morphological aspects in larvae of *C. quinquefasciatus* after exposure to different FAME's.

Methods: Groups of 36 larvae were exposed to sublethal concentrations of four different FAME's for 12 hours. Each container had 12 larvae and the every hours 3 larvae from each solution were withdrawn for analysis with a stereomicroscope. The control group was exposed to dechlorinated water with 1% DMSO and also observed in stereomicroscope.

Results: All compounds promoted the appearance of pigments in the larvae after 3 hours of exposure. Conclusion: The pigments are observed as electro-dense granulate material the ultrastructural level and can compromise the development and synthesis of different substances in the larvae of insects.

Keywords: *Culex*, melanization, FAME

Support: CAPES, FAPEMIG, UFSJ

D05-125 - Histopathological analysis of the effect of 7-epi-clusianone isolated from *Garcinia brasiliensis* on Experimental Murine Schistosomiasis. Vaz, T.M.1; Ribeiro, T.D.1; Martins-Souza, R.L.1; Mattos, A.C.A.2; Araújo, N.2; Santos, M.H.3; Costa, É.D.M.4; Ferreira4, E.B.; Marques, M.J.1; Oliveira, E.O.1

1 Department of Pathology and Parasitology/Institute of Biological Sciences - Federal University of Alfenas, Brazil

2 Research Center René Rachou at Oswaldo Cruz Foundation – Minas Gerais, Brazil

3 Faculty of Pharmaceutical Sciences – Federal University of Alfenas, Brazil

4 Institute of Exact Sciences – Federal University of Alfenas, Brazil

Background: Schistosomiasis affects over 200 million people in the world. Praziquantel is the choice medication for its treatment, but literature data describe resistance to this drug. Citations of the anti-inflammatory action of *Garcinia brasiliensis* motivated this study.

Objectives: To evaluate the effects of 7-epi-clusianone isolated from *Garcinia brasiliensis* on experimental murine infection by *Schistosoma mansoni*.

Methodology: 24 from 27 female mice were infected with 200 *S. mansoni* cercariae. Sixty days after infection they were separated in groups with 6 animals each, under: no treatment; 200mg/Kg Praziquantel; 500mg/Kg 7-epi-clusianone; or excipient. A three-individual group was used as control. The animals were sacrificed 15 days after treatment. Liver, spleen, lung, heart, esophagi, stomach, small and large intestines, mesenteric lymph nodes and kidneys were collected, fixed in 10% formaldehyde and processed according to routine procedures. 5 μ thick histological cuts were stained with hematoxylin and eosin for descriptive analysis and with Masson trichrome for measuring the granulomas' area. Results: Infected groups morphological aspect did not differ. Multiple hepatic granulomas; sinusoidal ectasia; increased Kupffer cells; ductal proliferation; steatosis; portal vein lumen worms (except for animals treated with Praziquantel); pneumonia, bronchopneumonia and lung vasculitis; congested spleen, with granulomas and confluence with white pulp ; myocarditis; mesenteric and lung lymph nodes with granulomas; presence of eggs and mucosal, submucosal and muscular granulomas in the small and large intestines; kidney perivenular inflammation; and pancreatitis were observed. The non-parametric Kruskal-Wallis test (5% level of significance) did not show difference concerning the total number of granulomas (p -valor = 0.4861) or concerning the number of granulomas with eggs (p -valor = 0.8312).

Conclusions: There was no difference in the mean of granulomas with eggs (variance analysis according to a thoroughly randomized design, at 5% level of significance). Within the study conditions, 7-epi-clusianone did not interfere with the evolution of schistosomiasis lesions.

Key words: Schistosomiasis; treatment; 7-epi-clusianone.

D06-122 - Preliminary study of Anti-Inflamma-

tory Activity of 5-Fluorouracil Incorporated into the Metal Organic Framework Cu-BTC. Lucena, F.R.S.1, Silva-Ramos, E.H.1, Nascimento, S.C1
1Departamento de Antibióticos, UFPE, 50590-470, Recife - PE, Brazil.

Background: Metal Organic Frameworks or MOFs are crystalline structures consisting of metal ions or clusters coordinated by organic molecules, often rigid structures to form one, two or three-dimensional porous. Among its uses are the storage of gases, used as sensors, catalysis and more recently as carriers of drugs and imaging agents for many of them targeting tumor cells, new therapies against cancer.

Objectives: The goal of this work was evaluated if the antitumor drug 5-Fluorouracil has a good anti-inflammatory activity when incorporated into the MOF Cu-BTC.

Methodology: For this, was used the test to induce inflammation on the peritoneal cavity using 1% carrageenan. Were used doses of 25, 50 and 75 mg/kg to the incorporation containing 5-Fluorouracil + MOF CuBTC, to compare with the others groups formed by 1% carrageenan (0.1ml/10g of body weight), indomethacin (10mg/kg) and saline 0.9% (0.1 ml/10g). The total number of leukocytes present in the peritoneal cavity was counted using an automatic cell counter (Micros 60).

Results: The results showed that the incorporation reduced the number of leukocytes in 43.7, 64 and 66.8% when used the doses 25, 50 and 75 mg/kg respectively when compared with the negative control (carrageenan).

Conclusions: With these results we can conclude that the system (Cu-BTC+ 5- fluorouracil) presented a good anti-inflammatory activity for this system.
Keywords: Anti-inflammatory activity, Cu-BTC, 5-Fluorouracil. License number of the Animal Ethics Committees: 230076.024149/2012-48

D07-013 - Inflammatory reaction molecular characterization after intramuscular implantation of biomaterials for bone regeneration – cytokines transcript expression analysis

Figueiredo A.1,2, Santos M. J.3, Cabrita A.1, Grazina M.3, 4

1 Experimental Pathology Service, Faculty of Medicine, University of Coimbra, Portugal

2 Portuguese Catholic University, Health Sciences Department, Viseu

3 CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

4 Faculty of Medicine, University of Coimbra, Portugal

Introduction: It is well known that implantation of biomaterials induces a response from the living host that comprises many steps, including inflammation. This process mediates cellular response of the "implantation bed" cells, which in return influences the biomaterial integration, degradation and vascularization, by secreting modulatory cytokines and chemokines. This inflammatory reaction can jeopardize the clinical outcome. **Objectives:** The aim of this study is to characterize the inflammatory response, after intramuscular implantation of two biomaterials, by quantifying the genetic ex-

pression transcripts of the cytokines genes IL-1 β , IL-6 e TNF- α in the blood of Wistar rats, before and after the implantation procedure. **Materials and Methods:** Samples of two biomaterials (Osteobiol™ and Bonelike™) were implanted in the dorsal muscles of 10 Wistar rats. Immediately before and one week after the implantation procedure, blood was taken from the tail vein. The blood was collected to Paxgene Blood RNA Kit™ tubes for RNA preservation. Total RNA was extracted according to manufacturer's instructions. Quantity and quality of RNA samples were assessed using Nanodrop ND-1000 Spectrophotometer™ and Agilent 2100 Bioanalyzer™ equipments. Reverse transcription was performed with High Capacity RNA-to-cDNA Kit™ from Applied Biosystems™. Real-Time PCR was conducted with QuantiTect primer assays, from QIAGEN™, for the targets IL-1 β , IL-6 and TNF- α , using SYBR Green™. **Results/Conclusions:** This work is still in progress and we will present the complete results. The assessment of RNA quantity and quality are crucial steps that have to be guaranteed prior to the expression analysis assays. RNAses are all over and can easily cause RNA degradation, which does not prevent the obtaining of results, but compromises its validity. It is mandatory to evaluate the RNA integrity number (RIN) of RNA samples before converting it into cDNA, for the sake of achieving reliable results.

Keywords: inflammatory reaction, biomaterials, cytokines transcripts expression

D08-109 - Inflammatory response associated with the type of sterilizing implants of sponge in normal and diabetic rats

Cenedezi, J.M.1; Souza, J.C.1; Carvalho, L.A.1; Lavall,L.1; Socarrás, T.O.2; Andrade, S.P3; Campos, P.P.1

1 Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brasil

2 Departamento de Ciências Pecuárias Universidade de Córdoba, Colômbia

3 Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brasil

Introduction: The implantation of polyether polyurethane sponges in animals is a simple model, easy reproduction, and is a procedure well tolerated by the animal, allowing the biochemical and morphometric study of the inflammatory response. This biomaterial must be sterilized before being implanted, however, there is few studies about the implications on the host inflammatory response according to the type of sterilization. **Objective:** Analyze acute and chronic inflammatory markers in sponge implants sterilized by boiling and irradiation in normal and diabetic Wistar rats. **Methods:** Total of 16 male Wistar rats, divided into 4 groups of 4 animals, groups I and III were diabetics, II and IV control. It was administered, intravenously, single injection of streptozotocin (STZ) in the animals of groups I and III. Animals whose glucose level was above 200 mg / dl were considered diabetic. Polyether-polyurethane sponges were irradiated and implanted intra-abdominal in groups I and II. Other sponges were soaked for 12 hours in 70% etha-

nol and sterilized by boiling in distilled water before being implanted intra- abdominal in animals of Groups III and IV. Sponges were removed 10 days post-implantation and were used to analyze markers of neutrophilic inflammatory activity (MPO) and monocytes / macrophages 'activities (NAG). Results: NAG levels were significantly higher in group IV than in groups I, II and III, NAG values were 1121 ± 242.7 in group I, 908 ± 225.5 in II, 1314 ± 445.4 in III and 2087 ± 611.8 in IV. There was no significant difference between the groups in the amounts of MPO. Conclusion: The type of sterilization (irradiation or boiling) used in polyester polyurethane sponge implants influences the chronic inflammatory response, as sterilization by boiling shows higher levels of NAG compared with the method of irradiation. However, there is no influence on neutrophilic inflammatory response, since there was no static difference between the groups.

Key words: biomaterial, sterilizing, implants.

Section E - Hot spot: Tuesday 11th December 16 h

**Neuroscience and Noetic sciences
Anesthesiology, Pain and Acupuncture
Experimental Sport Science**

E01-128 - Complementary Treatment of Experimental Hyperthyroidism in Salivary Glands.

Alves da Silva, M.A.1,2,3,4, Nery da Silva, E.1,2, Araújo Félix Junior, J.1,2, Campos Oliveira, M.1,3, Souza Freitas, V.1,3, Farinha Henrique Marques, R.H.2, Silvério Cabrita, A.M.2

1Faculty of Dentistry, State University of Feira de Santana, Brazil

2Experimental Pathology Service, Faculty of Medicine, University of Coimbra, Portugal

3Oral Cancer Center, State University of Feira de Santana, Brazil

4Faculty of Medicine and Dentistry, University of Rome "La Sapienza", Roma, Itália

Hyperthyroidism is caused by excess synthesis and secretion of thyroid hormone by the thyroid, which increases the metabolic rate. The experimental model of hyperthyroidism is conducted by an administration of a high dose of levothyroxine for two weeks. The aim of this study is to contribute to the histomorphometric characterization of acini parotid gland area on experimental hyperthyroidism. 20 Wistar male rats were used, randomly distributed in four groups: control group, kept with no manipulation (Ctrl); group II, which received an administration of 250 mg/kg of levothyroxine by gavage 3 times a week (HT); group III received levothyroxine and acupuncture treatment (HT+AC); group IV, submitted only to the acupuncture treatment. The points used for puncture were: ST09, ST36, HC4, RM23. After two weeks, all the animals were sacrificed and fragments from parotid salivary glands were collected and analyzed using ImageJ®. The histopathological analysis shown no signs of glandular lesion. The frequency distribution descriptive statistical analysis shown more cases of smaller parotid gland acini on the acupuncture treated group. The group with experimentally induced hyperthyroidism, as the group with hyperthyroidism plus acupuncture treatment, presented more acini with major areas than the group kept with no manipulation. The results of the present study, although without statistical significance, lead us to think that acupuncture and hyperthyroidism, individually, affects the parotid salivary gland. This gland is affected by the acupuncture treatment of hyperthyroidism with a different distribution of the acini area when compared with the control group. Molecular and metabolic profiling of parotid gland in the studied conditions will be important to understand the morphometric analysis results.

Keywords: Salivary Glands; Morphometrics, Hyperthyroidism

E02-121 - Immunohistochemistry study of Metallothionein in Pleomorphic Adenoma of minor salivary glands. Ribeiro, D.C.1; Viana, A.C.V1; Florêncio, T.N.G1, Santos, V.T.1; Cançado, H.R.2; Aguiar, M.C.F.1

1Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Brazil

2Department of Head and Neck Surgery, Hospital da Baleia, Brazil

Background: Pleomorphic adenoma (PA) is the most common benign neoplasm of major and minor salivary glands. The tumor shows slow growth, however, PA can reach large dimensions, mainly when located in the parotid gland. The histological features are diversified with the involvement of epithelial-myoepithelial structures. Metallothionein (MT) is a cysteine rich protein present in myoepithelial cells of several benign and malignant neoplasms. MT has its function associated with DNA protection, oxidative stress and apoptosis. Objectives: The purpose of this study was to evaluate the expression of MT in PAs. Additionally, we investigated the association of the clinicopathologic features of the lesions with MT, and specifically, the correlation of Bcl-2 with MT in an attempt to evaluate the role of MT in the control of apoptosis in the PAs. Methodology: Thirty five cases of PA of minor salivary gland were selected and immunohistochemistry was performed for Bcl-2 and MT proteins. The expression of these proteins was analyzed by the "QuickScore" method. Results: The sample showed epidemiological characteristics similar of those described in the literature. We did not find association between the clinicopathologic characteristics of PAs and the immunorexpression of the proteins studied, but a correlation between Bcl-2 and MT was demonstrated. Conclusion: The results suggest that MT may have a role in the control of apoptosis in PA. Apoio: Fapemig PPM00516-11 e CNPq 475141/2010-5.

Keywords: pleomorphic adenoma, metallothionein, immunohistochemistry

E03-219 - Effect of aerobic exercise in rat gastrocnemius fiber changes and architecture. André A3, Moretto D1, Pinto-Pereira S1, Tonelli L1, Fones-Ribeiro CA1,2, Tavares P1,2

1Faculty of Sport Sciences and Physical Education, University of Coimbra, Portugal

2Faculty of Medicine-IBILI, University of Coimbra, Portugal

3Coimbra College of Health Technology of Coimbra, Portugal

Background: The maintenance of skeletal muscle architecture is essential for its contraction and performance. In previous works we demonstrated that aerobic exercise changes skeletal muscle fiber type.

Objectives: The aim of this study was to investigate the relationship between muscle fiber changes and muscle architecture.

Methodology: An exercise animal model was used. All the animals' experiments and treatments were in agreement with the national and international guidelines regarding the experimental use of animals. Eighteen male Wistar rats (~250 g) performed an aerobic training for 8 weeks in a treadmill. At the end of the training period the training efficiency was assessed by a resistance/velocity test. After training all the animals, an ultrasound analysis of

the medial gastrocnemius muscle was taken (GE, Hologic E; 12 MHz transducer). Then, the thickness of the same muscle was measured laterally and in its antero-posterior face. After these measurements all the animals were sacrificed by anesthetic overdose. The gastrocnemius muscle was removed, weight and stored for further analyses. To evaluate the gastrocnemius muscle fiber type the enzymatic method of ATPase detection, was performed. The transversal section area was calculated by using the formula described by Roy (1991).

Results: The aerobic training show a slight increase of the type II fibers and similar decreases in type I in gastrocnemius muscle. No changes were observed in pennation angle, muscle weight or on the thickness of the muscle. Moreover, no changes were observed in the ultrasound analysis. **Conclusions:** Our results showed no changes in muscle architecture despite of the slight change in fiber type and the increase in the performance. These findings suggested that aerobic training for eight weeks increase muscle rats performance without changing gastrocnemius architecture.

Keywords: Skeletal Muscle Architecture; exercise; Fibers type

E04-220 - Mobilization and differentiation of circulating rat endothelial progenitor cells (EPCs) after aerobic exercise training.

Pinto-Pereira S1, Moretto D1, Lopes A3, Paiva A3, Tonelli L1, André A4, Fontes Ribeiro CA1,2, Tavares P1,2

1 Faculty of Sport Sciences and Physical Education, University of Coimbra, Portugal

2 Faculty of Medicine -IBILI, University of Coimbra, Portugal

3 Histocompatibility Center of Coimbra, Portugal

4 Coimbra College of Health Technology, Coimbra, Portugal

Background: The processing of fiber transformation induced by aerobic exercise has been described to be associated to an increase in muscle capillaries. It has been postulated that circulating EPC's may contribute to this effect.

Objectives: The aim of this study is to investigate the influence of exercise on the number and differentiation of EPC's and its correlation with capillary density in soleus muscle.

Methodology: Male Wistar rats were under aerobic protocol training for 8 weeks. The control group was submitted to a mild exercise (during the same time protocol). Blood was collected from the jugular vein (after anesthesia) using 6% EDTA as anticoagulant at the end of the exercise protocol. EPCs were determined on whole blood samples by flow cytometry using antibodies anti-CD45, -CD34, -CD133 and -CD146. The histological analysis of soleus muscle included a HE and an ATPase staining to count and measure the different fiber types. The muscle capillaries were analyzed by alkaline phosphatase staining on frozen sections and expressed as C:F ratio. The VEGF rat serum levels were determined by an ELISA commercial kit. **Results:** The results showed an increase of C:F ratio in the soleus muscle of the trained group. Concerning EPC's in total blood, we found for immature cells (CD34+ and CD133+) maintenance of cell percentage after

training but the cells CD146+ decreased in the exercise group. This decrease was correlated with the C:F ratio. The cell number positive for CD146+ and KDR+ was lower in the exercise group indicating that more than one stem cell population may differentiate into endothelium cells. Rat VEGF increased in the exercise group.

Conclusions: These results suggested that exercise increases differentiation and migration of circulating EPCs through activation of VEGF to form new vessels.

Keywords: Exercise; Endothelium progenitor cells; VEGF

E05-221 - Effect of aerobic exercise on the rat soleus muscle fiber changes. Implications of NOs and VEGF.

Moretto D1, Pinto-Pereira S1, Tonelli L1, André A3, Fontes-Ribeiro CA1,2, Tavares P1,2

1 Faculty of Sport Sciences and Physical Education –University of Coimbra, Portugal

2 Faculty of Medicine-IBILI, University of Coimbra, Portugal

3 Coimbra College of Health Technology of Coimbra, Portugal

Background: It has been demonstrated that aerobic exercise may change skeletal muscle fiber type. However, the mechanisms underlying this change are still controversial. **Objectives:** This work aimed to study possible mechanisms associated with the exercise-induced changes in skeletal muscle fiber type. Thus, the relationship between nitric oxide synthases (NOS), VEGF and muscle fiber type change was studied, since NO regulates VEGF expression and VEGF has been shown to regulate NOS. **Methodology:** An exercise animal model was used. All the animals' experiments and treatments were in agreement with the national and international guidelines regarding the experimental use of animals. Eighteen male Wistar rats (~250 g) performed an aerobic training for 8 weeks in a treadmill. At the end of the training period the training efficiency was assessed, and blood collected from the jugular vein, after which all the animals were sacrificed by anesthetic overdose. The soleus muscle was removed and stored for further analyses. To evaluate the soleus muscle fiber type the enzymatic method of ATPase detection, was performed. The expression of the NOS isoforms was assessed by western-blotting using the antibodies against iNOS and cNOS (Santa Cruz). The serum VEGF concentration was quantify by an ELISA commercial kit (R&D systems). **Results:** The aerobic training increased the number of type I muscle fibers and reduced the type II number. These changes were associated with an increase in iNOS and, principally, cNOS expression. Concerning VEGF serum concentrations, a significant increase in the trained group was verified, which was also positively correlated with an increase in the capillary/fiber ratio. **Conclusions:** Our results showed an increase in the type I fiber type induced by aerobic exercise. They also suggest that iNOS and, principally, cNOS and VEGF are implicated in fiber type change induced by aerobic exercise.

Keywords: Skeletal muscle, NOs, VEGF

E06-011 – Acupuncture treatment of Experimental Seizures. Varão Nolasco¹, Fábio Almeida², Silvério Cabrita¹, Fontes Ribeiro³

¹Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

²Faculty of Medicine, University of Coimbra.

³Laboratory of Pharmacology and Experimental Therapeutics-IBILI, Faculty of Medicine – University of Coimbra, Portugal

Epilepsy is a disease that presents complex problems of analysis, research and therapeutic. Nowadays, the antiepileptic therapeutic is the result of research that, beginning at 1980, has developed, as the mechanisms of the neurobiology of epilepsy are better known. In spite of the evolution in pharmacological therapeutic and even neurosurgical, 25 - 30% of the epileptic disorders are refractory at actual conventional therapeutics. This put the challenge of the resort of complementary therapeutics as helpers of the actual therapeutics schemes. Among those therapeutics, the Acupuncture has been used with evident results in epileptic syndromes, namely in those with seizures symptomatology.

The aim of this study is to assess if the acupuncture has any interference in the neutralization of the epileptic seizures.

In this study, were used Wistar male rats with eight week aged. The animals were subjected to a seizures induction protocol with Pentilenotetrazol (PTZ). In one of the protocols (A protocol), was made any treatment; in one another, B protocol, was used the acupuncture at the acupoints: 06 PE, 03SI, 06SP, 03LV, 34GB, 14GV, 16VG and Yintang; in the third protocol (C protocol) was done intraperitoneal administration of physiological saline. The animals were observed during the thirty minutes following PTZ administration and the animal behaviour was registered in video.

The analysis of the observations let us to conclude that acupuncture, at the acupoints used, interfered with the temporal profile of the clinical picture and in the majority of the cases, it was efficient.

E07-238 - Physical Exercise: a neuroprotective strategy in a model of methamphetamine-induced monoaminergic toxicity? Dias A.I.1*, Mendes S.M.1*, Freitas, H.J.1, Neves, A.F.1, Viana S.D.1, Nunes S1, Pereira F.C.1, Fontes-Ribeiro C.A.1

* Both authors contributed equally to this work

¹Laboratory of Pharmacology and Experimental Therapeutics-IBILI, Faculty of Medicine – University of Coimbra, Portugal

Introduction: Physical activity exerts neuroprotective effects and improves neuronal recovery in several brain injuries. We and others showed that a single high-methamphetamine injection causes neurotoxicity in dopaminergic as well as in serotonergic brain systems in mice+rats. **Objectives:** In this study we evaluated the possible neuroprotective effects of exercise towards methamphetamine-induced monoaminergic neurotoxicity.

Methodology: Twenty-four C57BL/6 mice (male,

twelve weeks old) were divided into four groups: sedentary/saline, exercise/saline sedentary/methamphetamine (MA) and exercise/MA. The animals were submitted to an exercise protocol that consisted in treadmill running for 8 weeks, 40 min/day, 5 days a week. By the end of the exercise protocol, animals were exposed to a single injection of MA (30 mg/kg, i.p.) or saline 72h prior to sacrifice. Striatum and frontal cortex were collected and tyrosine hydroxylase (TH), glial fibrillary acidic protein (GFAP) levels (western-blot) as well as dopamine (DA), its metabolites (DOPAC and HVA) and serotonin (5-HT) levels (HPLC-ED) were quantified. **Results:** Expectedly, MA administration evoked DA, its metabolites and TH depletion in both tested brain regions ($p < 0.05$). Additionally, a robust astrogliosis was observed in striatum ($p < 0.01$) but not in frontal cortex. Although MA imposed 5-HT depletion in frontal cortex ($p < 0.05$), it failed to change this monoamine striatal levels ($p < 0.05$). Although exercise did not prevent monoaminergic degeneration ($p > 0.05$), it increased 5-HT levels in both frontal cortex and striatum ($p < 0.05$).

Conclusion: Exercise did not inhibit MA-induced neurotoxicity. However, treadmill running exhibited a trophic effect on serotonergic terminals on both frontal cortex and striatum that warrants further scrutiny.

Keywords: Neuroprotective; Methamphetamine; Exercise;

E08-222 - Neuroregenerative effect of exercise on monoaminergic neurodegeneration evoked by methamphetamine. Freitas H.J.1*, Neves A.F.1*, Dias A.I.1, Mendes S.M.1, Viana S.D.1, Nunes S.1, Pereira F.C.1, Fontes Ribeiro C.A.1

*Both authors contributed equally to this work

¹Laboratory of Pharmacology and Experimental Therapeutics-IBILI, Faculty of Medicine – University of Coimbra, Portugal

Background: Methamphetamine is an illicit psychostimulant drug widely abused in the world that leads to chronic neurodegenerative changes in monoaminergic brain regions. Exercise has stood out because of its rehabilitation properties in drug addiction settings.

Objectives: In this study, we sought to investigate the putative effect of physical exercise on neuroregeneration of monoaminergic terminals in the frontal cortex and striatum following methamphetamine (METH) injection in mice.

Methodology: A total of 24 C57BL/6 mice, twelve weeks old, were divided into four groups. The exercise groups were submitted to a seven weeks exercise regimen post-METH injection (30 mg/kg METH, i.p.). The sedentary groups were exposed to the treadmill as well. The animals were sacrificed 48 hours after the exercise protocol. Cortical and striatal total levels of dopamine, serotonin and their metabolites (HPLC-ECD) as well as tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) levels (Western Blotting) were determined as monoaminergic terminals and astrogliosis markers.

Results: Cortical and striatal neurodegeneration of dopaminergic terminals was still observed 7 weeks

following METH single injection as evidenced by the decreased values of dopamine/metabolites and TH enzyme. On the contrary, GFAP levels were normal in both regions. Although METH had depleted 5-HT in frontal cortex it failed to impact striatal 5-HT levels. Exercise not only restored striatal dopaminergic markers levels but also increased total 5-HT content. Although, exercise fostered 5-HT cortical regeneration it failed to restore cortical dopaminergic markers. Conclusions: This study is highly suggestive that exercise is endowed with neuroregenerative/trophic properties towards monoaminergic striatal and cortical terminals. The mechanism underlying this positive effect warrants further scrutiny.

Keywords: methamphetamine, exercise, neuroregeneration.

E09-007 - Phytochemistry of *Aspidosperma pyriforme* Mart.

Cavalcanti, K.P.S.1; Souza, I.A.2; Anjos, F.B.R.3; Silva, E.V.4; Silva Neto5, J.F.; Silva, E.C.B.4; Caribé, R.A. 4; Higino, J.S.4, Jimenez, J.5

1 Hospital das Clínicas –Lika-UFPE-Brasil-Universidade de Coimbra

2 Departamento de Antibióticos-UFPE-Brasil

3 Departamento de Histologia e Embriologia-UFPE-Brasil

4 Departamento de Ciências farmacêuticas-UFPE-Brasil

5 Departamento de Morfologia e Fisiologia Animal-UFPE-Brasil

Background: The genus *Aspidosperma* occurs from Mexico to Argentina. *Aspidosperma pyriforme* Mart. is a tree native of the Northeast of Brazil, also known as pereiro, stick-to-coaru and pequiá the woods. It is used in folk medicine as anti-inflammatory and used against cramps, dermatitis and urinary tract infections. Objectives: This study aims to determine the contents of flavonoids, phenols and tannins in methanolic crude extract of *A. pyriforme* Mart. Methodology: The methanolic crude extract was prepared from the bark of *A. pyriforme* Mart. After drying for 48 hours in incubator (42 °C), the peel of the vegetable mill was processed in electric mill for obtaining electric dust. The extraction was performed in apparatus Soxhlet from 250g the powder with the addition of 600 mL of methanol for 12 hours. The extract was filtered and concentrated under vacuum (45 °C) for subsequent analyzes. The analyzes for total phenols and residual were based on the methodology described by Folin & Ciocalteu (1927). The content of tannin is calculated as the difference between the level of phenols and total phenol content of residual non-complexed, since the tannins are removed from the middle through complexation with the caseine. The determination of total flavonoids was performed by the technique described by Peixoto Sobrinho et al. (2008) adapted for the species. Results: The results showed that the extract analyzed lacks tannins, but there is a large amount of phenolic compounds (1.425% of total extract) mainly flavonoids (7.701% of total extract). Conclusions: Flavonoids are a class of substances with a wide range of pharmacological activity. Our results demonstrate

the potential of flavonoids obtained, which will be of great value to the continuity of research, evaluation perspectives with its use in inflammatory diseases, which will confirm its use in folk medicine.

Keywords: Phytochemistry, *Aspidosperma pyriforme*, flavonoids

E10-021 - Lymphatic nodules evaluation in an experimental hyperthyroidism model

Rute Duarte¹, António Cabral¹, Gustavo Barandas¹, Rodrigo Farinha¹, Silvério Cabrita¹

¹ Experimental Pathology Service, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

Hyperthyroidism is a pathological condition in which there is an excessive production and action of the thyroid hormones. The objective of this work was to evaluate the lymphatic nodules in an experimental hyperthyroidism model with acupuncture. Fifteen rats with eight weeks were divided randomly into three groups, each of which with five animals: the group I – control group which received levothyroxine (C1: Ctrl HT); the group II – control group which received acupuncture (C2: Ctrl AC); and the group III – test group which received levothyroxine and acupuncture (T1: HT+AC). Lymph node samples were collected in the end of experience for histopathological study and none of the three groups showed pathological changes and not found differences in cellularity of lymph nodes.

Keywords: Wistar rat, Hyperthyroidism, Acupuncture, Lymphatic nodules.

E11-173 - Acupuncture evaluation in a model of administration of a hepatotoxic - preliminary results

Branca Carrito¹, Ana Calado¹, Miguel Costa¹, Rute Duarte¹, José Cabeças¹, Silvério Cabrita¹

¹ Experimental Pathology Service, Faculty of Medicine, University of Coimbra

Since several years that Acupuncture is well accepted in the scientific and clinical medium for the complementary treatment of some pathologic conditions. In the last decades an increasing number of studies have emerged in scientific reviews, mainly in supporting the use in pathology of locomotor system and pain. There are an increasing number of studies that suggest that Acupuncture can be useful in many other conditions. In this study we tried to search a possible benefit by the dry puncture during the exposition to thioacetamide.

The study was carried on the Wistar male rat, 8 weeks old. Submitted to the administration of 20 mg /Kg of thioacetamide, by intraperitoneal route, three times a week, during three weeks. The animals divided into two groups of 5 animals each. One group was submitted to no treatment, the control group, the other were submitted to the dry puncture, three times a week, of the traditional acupoints: 36ST and 06SP, in sessions of ten minutes.

Before the administration of the hepatotoxic, from all the animals were collected blood for evaluation of: Urea, cholesterol, albumin, SGPT, SGOT and

triglycerides.

The blood analysis was repeated by the end of the first week, before acupuncture of the test group and two weeks later, just before the necropsy examination.

There are minor differences between the groups for all the markers except for cholesterol, that is significantly higher in test group.

This results suggest that acupuncture in these acupoints have minor interference and others acupoints should be chosen.

E12-271 Chronic amphetamine regimen potentiates stress-induced bruxism: a new animal model

J. Fonseca¹, J. Coutinho¹ F.C. Pereira², M.J. Rodrigues¹, S. Cabrita⁵

¹ Dentistry area, Faculty of Medicine, University of Coimbra, Portugal

² Pharmacology and Experimental Therapeutics Service, IBILI, Faculty of Medicine, University of Coimbra, Portugal

³ Experimental Pathology Service, Faculty of Medicine, University of Coimbra, Portugal

Introduction: Central dopaminergic activity has been related to the etiopathogenesis of stress-induced bruxism. This para-functional masticatory activity is also a recognized side effect of dopamine modulating drugs namely amphetamine-type stimulants. However, there is a surprising lack of structured information on the central etiology of bruxism.

Objective: To evaluate the impact of chronic use of amphetamine on the non-functional masticatory movements (NFMM), stress-induced behavior and dental attrition. Serum creatinine kinase (CK) and cortisol levels were also assessed.

Materials and methods: 30 Wistar male rats (9 weeks of age) were divided as follows: Group I (control; GI) did not suffer any experimental manipulation; Group II was submitted to a stress induction protocol for 14 days including a single daily saline i.p. injection (GII); Group III was submitted simultaneously to stress and to escalating single daily doses of amphetamine ranging from 1.6mg/kg to 12 mg/kg i.p. (GIII). Dental marks close to the gingival margin in the lower incisors were drawn to evaluate the influence of the induction protocols in the dental attrition. Blood samples were collected in days 0, 7 and 14 to evaluate the serum CK and cortisol levels. Data were statistically analyzed using repeated-measures ANOVA and t tests with Bonferroni's corrections for specific group/time comparisons. **Results:** Amphetamine potentiated stress-induced increase in NFMM, stress-related behavior and consequently in dental attrition (GI: 6.4±0.1mm, GII: 6.8±0.1mm and GIII: 7.2±0.1mm; GIII vs GII p<0.01). The CK serum levels increased time-dependently in both GII and GIII groups (Day 14-GI: 1752±106U/L; GII: 2573±87U/L and GIII: 3417±100U/L; GIII vs GII P<0.0001). Our results further showed that the amphetamine potentiated the stress-induced increase in the CK serum levels. This might reflect amphetamine induced rhabdomyolysis. The GII and GIII cortisol levels were higher than the GI cortisol levels (p<0.0001) in all assessed periods. However, the GIII cortisol levels

were not significantly different from G2 in any time-point. It is also noteworthy that amphetamine produced a decrease in the body weight throughout the experiment (GIII_day 0: 27±5g; GIII_day 14: 260±6g; P<0.05).

Conclusions: A sensitizing dose d-amphetamine regimen potentiates stress-induced bruxism but not the increase of the cortisol, suggesting that the expression of NFMM attenuates the function of the hypothalamus-pituitary-adrenal axis. Further characterizing the ethiology of bruxism is essential for an integrative care of the amphetamine-type stimulants abusers.

Keywords: Bruxism; Amphetamine; Stress; Dental attrition

Section F - Hot spot: Tuesday 11th December 16 h

Experimental Dental Medicine Experimental Forensic Medicine

F01-157 - Carbonized bodies identification – The importance of fluorescence in dental composites. Figueiredo, C.1,3; Azenha, M.E.2

1Faculty of Medicine University of Coimbra, Portugal

2Department of Chemistry University of Coimbra, Portugal

3Portuguese Catholic University, Coimbra, Portugal

Introduction: The identification of carbonized bodies is a challenge in the practice of Legal Medicine, especially due to the lack of information that is preserved after the action of high temperatures. In such cases, all the information that can be collected from the corps assume vital importance.

Objectives: The aim of this study is to evaluate the fluorescence of dental composites at room temperature and when they are submitted to high temperatures. It was also an objective to compare the fluorescence differences between the three commercial brands.

Methodology: We have selected three brands of dental composites Voco®, Kerr® and Colthéne® with the commercial names of Grandio®, Herculite XRV® and Synergy D6® respectively. These materials have been subjected to a range of high temperatures (200°C, 250°C, 300°C and 500°C), during 1 hour. After that was analyzed the fluorescence with a Fluorometer (Spex Fluorolog, FL 3-22) and assessed all the emission and excited spectrums.

Results: The dental composites analyzed have some differences in fluorescence spectrum at room temperature. At 200°C, comparing the emission spectra with exciting λ of 400nm it is possible to distinguish the dental composites Herculite® from Voco®. At 250°C it is possible to distinguish, as well, from emission spectra the dental composite Herculite XRV® from Voco® and Synergy® (these last two materials have identical behavior). The fluorescence disappears, in all materials, at 300°C. All methods of dental identification that are based in the presence of fluorescence in dental composites will be inconclusive, at temperatures of 300 or more Celsius degrees.

Palavras-chave: Dental composites, Fluorescence, Carbonized bodies

F02-013 - DLX3 polimorfisms in patients diagnosed with taurodontism in the Catholic University's Clinic – Viseu. Figueiredo, A.1,2; Veiga, L.2; Seabra, M.2, Braga P.2

1Experimental Pathology Service, University of Coimbra, Portugal

2Pediatric Dentistry Department, Portuguese Catholic University, Viseu, Portugal

Introduction: The process of human dental devel-

opment is very complex, during which may occur some disturbs that could origin some unique dental manifestations. Taurodontism is an abnormality of the dental development, which is characterized by the elongation of the pulp chamber and by apical floor displacement of the pulp. The aim of this study was to assess the prevalence of Taurodontism in a sample of patients of the UDC-UCP (University's Dental Clinic of Viseu's Catholic University), and additionally assess the prevalence of polymorphisms in DLX3 gene in these patients and their relatives.

Materials and Methods: We conducted a pilot study in which we evaluated a sample of patients, aged 10-30 years, with panoramic radiography in the informatics file of the CDU Viseu, with the purpose to assess the existence of Taurodontism. After the selection of the panoramic radiographies, we used the Shifman & Chanannel classification to evaluate the dental pieces concerning their severity of taurodontism. Next we proceed to the collection of saliva samples of these patients and their relatives to search for the presence of polymorphisms in the DLX3 gene.

Results: The prevalence of Taurodontism in a sample of 1369 panoramic radiographies was 1,75%. From the 627 teeth analyzed, 6,54% were taurodontic. Hipertaurodontism had the greater number of case reports (58,5%). The teeth with higher prevalence were the premolars (51,2%).

Conclusion: The prevalence of taurodontism evaluated in the CDU's population is within the values founded in other studies. The higher degree of taurodontism is also identical to the values observed in other studies, yet the type of tooth was different. The lack of results in the search for polymorphisms in the DLX3 gene prompts us to suggest that changes to the methods of collection and DNA isolation (the use of commercial DNA extraction kits) are needed in order to successfully amplify the DLX fragments containing the mutation.

Keywords: taurodontism, DLX3.

F03-018 - Masticatory muscle aging - Morphometric analyses of the extracelular compartment of sectioned fibers. Silva L1, Fadigas G1, Barandas G1, Soares T2, Cabrita S1

1Experimental Pathology Service, Faculty of Medicine, University of Coimbra

2Dentistry School, Faculty of Medicine, University of Coimbra

Muscle has great plasticity, being easily changed according to it's function. This plasticity can lead to either atrophy or hypertrophy. Current knowledge allows us to know that this events alter the number of satellite cells nuclei.

In this study, 50 Sprague-Dawley (SD) rats of both genders were divided into five groups according to age as follows: Group I, animals with 10 days old, Group II, animals with 15 days old, Group III, animals with 20 days old, Group IV, animals with 60 days old and Group V, animals with 90 days old.

Masseter muscle fragments were collected in the necropsy and prepared with Feulgen stain preparation. Each slide obtained was randomly photographed, 10 captures per each slide, totaling 30

slides for each group of animals. The obtained images were submitted to morphometric analysis with Adobe Photoshop CS6 to evaluate the average number of nuclei throughout age.

Group I, the average number of nuclei is 2986.67 ± 764.48 varying between 2637.19 and 3336.19 for a 95% confidence interval. Group II, the average number of nuclei is 2651.29 ± 298.22 varying between 2423.53 and 2879.05 for a 95% confidence interval. Group III, the average number of nuclei is 2696.22 varying between 252.53 and 2872.35 for a 95% confidence interval. Group IV, the average number of nuclei is 3252.42 ± 874.8 varying between 2852.51 and 3652.33 for a 95% confidence interval. Group V, the average number of nuclei is 1251.77 varying between 1149.4 and 1354.14 for a 95% confidence interval.

These results show no significant alterations on the period between 10 and 60 days of age. From this time period, till 90 days of age, there is a significative decrease of nuclei number possibly in relation with the increase of ingestion and mastication usage.

F04-018 - Masticatory muscle aging - area morphometric analysis of sectioned fibers.

Luis Silva¹, Guilherme Fadigas¹, Gustavo Barandas¹, Silvério Cabrita¹, Maria João Rodrigues Rodrigues²
¹Experimental Pathology Service, Faculty of Medicine, University of Coimbra

²Dentistry School, Faculty of Medicine, University of Coimbra

The increase in muscle mass is designated as muscle hypertrophy and represents an increase of muscle fibers. Aging is a natural process that affects all living organisms and its tissues.

In this study, 50 Sprague-Dawley (SD) rats of both genders were divided into five groups according to age as follows: Group I, 10 days old animals, Group II, 15 days old animals, Group III, 20 days old animals, Group IV, 60 days old animals and Group V, 90 days old animals (young adult reference group). Masseter muscle fragments were collected during the animals complete necropsy and stained by hematoxylin and eosin. Three photographs (10X) were made by each preparation and submitted to morphometric analysis with Adobe Photoshop CS6 to evaluate the muscle cell prevalence of small fibers (SF) ($[\mu - \sigma; \mu + \sigma]$), medium fibers (MF) ($[\mu - \sigma; \mu - 2\sigma]$), big fibers (BF) ($[\mu + \sigma; \mu + 2\sigma]$) and very big fibers (VBF) ($> \mu + 2\sigma$) throughout time. The frequency distribution for the various groups was as follows: Group I – 54.17% SF, 45.83% MF, 0% BF, 0% VBF; Group II, 3.79% SF, 89.81% MF, 6.16% BF, 0.24% VBF; Group III, 0% SF, 73.83% MF, 24.30% BF, 1.87% VBF; Group IV, 3.06% SF, 79.59% MF, 13.27% BF, 4.08% VBF; Group V, 13.57% SF, 69.38% MF, 11.63% BF and 5.43% VBF. These results allow us to identify three chronologically defined stages of Sprague-Dawley masseter muscle adaptation to function.

F05-018 - Masticatory muscle aging - nuclear compartment morphometric analysis of sec-

tioned fibers. Luis Silva¹, Guilherme Fadigas¹, Gustavo Barandas¹, Silvério Cabrita¹, Maria João Rodrigues²

¹Experimental Pathology Service, Faculty of Medicine, University of Coimbra

²Dentistry School, Faculty of Medicine, University of Coimbra

The extracellular matrix has an important role on force transmission and passive elastic response. Fibrosis is the excessive production of extracellular matrix. The increased concentration of collagen and adipocytes, related with aging, alter the muscle contractile abilities.

In this study, 50 Sprague-Dawley (SD) rats of both genders were divided into five groups according to age as follows: Group I, animals with 10 days old, Group II, animals with 15 days old, Group III, animals with 20 days old, Group IV, animals with 60 days old and Group V, animals with 90 days old.

Masseter muscle fragments were collected in the necropsy and prepared with Masson's trichrome stain. Each slide obtained was photographed all around its area, in order to obtain one single image. The obtained images were submitted to morphometric analysis with Adobe Photoshop CS6 to evaluate the percentage of green pixels (connective tissue) and red pixels (muscle tissue) on the whole image.

Group I, the average area percentage of red pixels was $72.28\% \pm 2.24$ varying between 71.26% e 73.30% for a 95% confidence interval, the area of connective tissue represents an average value between 21.99% and 25.21% for a 95% confidence interval. Group II, the average percentage of muscle fibers represents $75.88\% \pm 1.91$ of the total area varying between 75.01% e 76.75% for a 95% confidence interval, the area of connective tissue represents $22.39\% \pm 1.75$ of the tissues total area, varying between 21.59% and 23.19% for a 95% confidence interval. Group III, the average percentage of muscle fibers represents $78.51\% \pm 1.00$ of the total area varying between 78.05% and 78.97% for a 95% confidence interval, the area of connective tissue represents $19.98\% \pm 1.03$ of the tissues total area varying between 19.51% and 20.45% for a 95% confidence interval. Group IV, the average percentage of muscle fibers represents $85.10\% \pm 0.69$ of the total area, varying between 84.78% and 85.42% for a 95% confidence interval, the area of connective tissue represents $13.71\% \pm 1.14$ of the tissues total area, varying between 13.19% and 14.23% for a 95% confidence interval. Group V, the average percentage of muscle fibers represents $91.21\% \pm 1.79$ of the total area, varying between 90.39% and 92.03% for a 95% confidence interval, the area of connective tissue represents $6.94\% \pm 1.00$ of the tissues total area, varying between 6.48% and 7.40% for a 95% confidence interval.

These results allow to identify two chronologically defined, at least, stages of masseter muscle adaptation to function.

F06-223 - The effects of hydrogen peroxide (6%) in gastric mucosa – an experimental study.

Paula A1, Dias I1, Ferreira MM1, Cabrita A1, Carrilho E1

1Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Introduction - The tooth whitening therapeutics can be performed at the clinic or ambulatory. It is the latest version of the technique that is the greatest danger of eating the products of bleaching.

Issues - To devise an experimental study on the action of these products on mucosa, especially in the gastric mucosa with or without non-tumor pathology. **Materials and Methods** - In this study we used 50 mice of the strain "Wistar-Han". In periods II and III, which took place on 2nd and 3rd weeks, the animals in groups II, III, IV and V were subjected to induction protocol not gastric tumor by administering a chemical compound (50% ethanol), and/or administration of a 6% hydrogen peroxide bleaching agent [Colgate® "Visible White"™] (Colgate Oral Pharmaceuticals Inc, New York, USA), by gavage. From the data collected were made statistical analysis of variance (ANOVA) and multiple comparison of pairs of means over time in a study by a t Student test. It was also performed a frequency analysis of the results of macroscopic observation of the gastric mucosa (statistical analysis with the software JMP Version 7, SAS Institute Inc., NC, USA). Differences were considered significant for a probability higher than 95% ($p < 0.05$).

Results - There was a decrease in body weight in animals of groups handled during the study period, which was most pronounced in groups IV and V-A. Changes in the spleen weight relative to body weight revealed no statistically significant changes. The gastric mucosa revealed in all groups manipulated lesions, which were more frequent in groups III and IV.

Conclusions - We can conclude that there seems to be some signs of toxicity in 3 to 4 days after administration of hydrogen peroxide 6%, and induction of gastric lesions and generalized moderate.

F07-036 - Study of Microleakage of a Experimental Restorative Material Through Radioisotopic Methods

EV Carrilho1, M Abrantes1, MF Botelho1, B Oliveiros1, A Paula1, T Afonso1, CM Marto1, D Rebelo1, JR Marques1, J Portugal2, P Ambrósio1, M Marques Ferreira1

1Faculty of Medicine, University of Coimbra, Coimbra, Portugal ;

2Faculty of Dentistry, University of Lisbon, Lisbon, Portugal

Aim: The purpose of this study was to evaluate the microleakage of the dental restorations using GCP Fill .The null hypothesis was that the type restorative system didn't have influence in what concerns to microleakage.

Method and materials: Sixty noncarious extracted human molars were selected for this study. The teeth were cut in two equal halves occlusogingivally. Class 5 cavities were prepared on the buccal or lingual surfaces of each tooth with gingival margin walls in enamel with cavity dimensions approximately 4 mm mesiodistally, 3 mm occlusogingivally and 3 mm depth. The specimens were divided randomly in 4 groups. Group 1 and 4 was restored with GCP Fill, while group 2 was restored with FiltekTM

Supreme (3M ESPE). The control cavities in group 3 weren't restored. The specimens were stored in distilled water at 37°C for 7 days and after thermocycling 500 cycles between 5°C and 55°C with a dwell time of 30 seconds. Two coats of nail polish were applied to the external surface around of each cavity except the negative control group, where the crowns were completely sealed with nail polish. The specimens were submersed in a solution of 99mTc-Perchnetate during 3 hours. The radioactivity was counted using a well-type gamma counter and a gamma camera.

Results: Results showed that there were no statistically significant differences ($P > 0.05$) among the materials with respect to microleakage scores.

Conclusion: Based on the results of this study, the use of GCP Fill doesn't reduce microleakage compared with conventional composites.

F08-070 - Emdogain® in the Socket of Autogenous Teeth Transplantation in the Dog. Does it Work? Manuel Marques Ferreira1; Maria Filomena Botelho2; Lina Carvalho3; Hugo Ferreira2; Eunice Virginia Carrilho1

1 Department of Dentistry, Faculty of Medicine, University of Coimbra, Coimbra, Portugal;

2 Department of Biophysics and Biomathematics, IBILI, CIMAGO, Faculty of Medicine, University of Coimbra, Coimbra, Portugal;

3 Department of Pathologic Anatomy, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Objective: This study aims to evaluate the periodontal regeneration, in autogenous tooth transplantation, with application of Emdogain® in the socket.

Methods: The study group comprised 3 Beagle dogs, in which 24 incisors and premolars were transplanted to the recipient sockets after mechanical reparation. The Emdogain® group (E), the saline group (S) and the control group (C) contained 12, 12 and 6 teeth, respectively. After surgery, clinical examinations were performed every week and animals were sacrificed 9 weeks later. Subsequently, decalcified sections were prepared for routine histological and immunohistochemical evaluation. Ordinal scores were analyzed using the t-student test ($p = 0.05$).

Results: All the transplanted teeth survived. No statistically significant difference was found among the complete healing in all treatment groups ($p = 0.303$). The mean occurrence of inflammatory and replacement root resorption was high with saline solution, compared with Emdogain® group, with significant difference ($p = 0.015$ and $p = 0.041$, respectively).

Conclusions: The Emdogain® does not seem to enhance the regeneration of periodontal tissues, but decrease the inflammatory and the replacement root resorption, in teeth transplantation.

Key words: autogenous tooth transplantation; beagle dogs; periodontal regeneration.

F09-224 - Ozone: New approach to treat dental caries. Joana Marques1, Anabela Paula1, Bárbara Oliveiros1, Teresa Gonçalves1, Manuel Ferreira1, Eunice Carrilho1

1 Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Introduction: Caries lesion is due to acid dissolution of enamel and/or dentin as a consequence of the metabolism of specific microorganisms, such as *Streptococcus mutans* and *Lactobacillus fermentum*. Ozone is a powerful oxidant that has the ability to eliminate bacteria, fungi and viruses. HealOzone is used in caries lesions, allowing the elimination of these microorganisms through exposure to ozone.

This work aims to assess the effectiveness of ozone in the elimination of cariogenic bacteria, followed with fluoride supplements provided by the manufacturer of HealOzone.

Materials and Methods: 60 teeth without caries were extracted. The experimental groups were immersed in artificial saliva with and without bacteria. In group V was applied the HealOzone (Kavo Dental) and were daily applied products of remineralization for 30 days. We perform the evaluation of caries with DIAGNOdent (Kavo Dental). At the end samples were collected for analysis and evaluation of bacterial activity by PCR. Statistical analysis was performed. All data analysis was carried out by application SPSS, version 18, and statistical tests were evaluated at significance of 5%.

Results and Discussion: In the initial measurement all groups had identical values, there was a statistically significant increase in the final evaluation, which shows that the bacteria studied were effective to produce caries in the teeth immersed in artificial saliva. After application of ozone and remineralizing products, the analysis of bacterial DNA, didn't detected bacterial DNA. Ozone was effective in the elimination of the study bacteria.

F10-104 - Periodontal ligament evaluation in a model of Diabetes and acupuncture treatment

Carlos Miguel Marto¹, Luís Silva¹, Gustavo Barandas¹, Cristina Brás¹, António Silvério Cabrita¹
1Experimental Pathology Service, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Introduction: Diabetes mellitus is a metabolic disorder characterized by defects in insulin secretion, action, or both, leading to chronic hyperglycemia and disturbances in the metabolism of carbohydrates, fat and proteins, like those from collagen connective tissue. Since collagen is the primary component of the periodontal ligament (PL) and alveolar bone (AB), marked changes in metabolism coincident with diabetes can affect the integrity and strength of the fibers of the PL and AB and the attachment between the two. Diabetes can also affect the bone remodeling resulting in decreased bone mineral density, osteopenia, osteoporosis, and an increased prevalence and severity of periodontal disease.

Aim: The aim of this study was to evaluate the effects of experimental diabetes in periodontal ligament and investigate the effect of acupuncture treatment.

Materials and Methods: 25 Wistar male rats were randomly distributed in five groups (n=5 each): group I: control group, no manipulation (Ctr); group II: received saccharose added in the diet (DBSA);

group III received a single injection of streptozotocin (65mg/kg) and were treated with acupuncture treatment (DBSA); group IV received a single injection of streptozotocin and no treatment was performed (DBSNA) and group V submitted only to acupuncture treatment (CtrA).

Rats in group II, III and IV became diabetic confirmed by measurement of blood glucose.

After 4 weeks, all the animals were sacrificed and mandible was collected. Hematoxylin-eosin coloration was performed and a morphometric analysis was performed in sections using software ImageJ®. **Results:** Animals in group II present more vascularization of periodontal ligament, with more areas of bone reabsorption and more hyalinization area. Animals in group III show more areas of bone reabsorption but areas of hyalinization are similar to control group. Group four show more vascularization and areas of bone reabsorption.

Conclusions: These results show that diabetes negatively affects periodontal ligament but acupuncture treatment may have some protective effect.

Keywords: Experimental diabetes; periodontal ligament; acupuncture treatment

F11-104 - Are Bleaching Products with Hydrogen Peroxide safe? A pilot study in Human Fibroblast Cells

Carlos Miguel Marto^{1,2}, Mafalda Laranjo², Ana Margarida Abrantes², João Casalta-Lopes², Ana Cristina Gonçalves³, Ana Bela Sarmento-Ribeiro³, Manuel Marques Ferreira¹, Maria Filomena Botelho², Eunice Carrilho^{1,2}

1Dentistry Area, Faculty of Medicine, University of Coimbra

2Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra

3Applied Molecular Biology Unit, Faculty of Medicine, University of Coimbra

Introduction: Because bleaching of vital teeth is an increasingly demanded therapy, it is necessary to evaluate its safety. Nowadays most of whitening products commercially available contains in its constitution hydrogen peroxide which is the active agent of the bleaching mechanism. Literature indicates that hydrogen peroxide and reactive oxygen species formed in its decomposition are able to reach the pulp and cause cell damage with varying degrees of intensity.

Materials and Methods: An in vitro study was performed using a human fibroblast cell cultures. The cells were exposed at different known concentrations of each commercial compound and hydrogen peroxide, with and without photo activation, at the times of 45 minutes, 24, 48 and 72 hours. Cell metabolism was evaluated using the MTT assay. Dose-response curves were traced. Also, cells exposed for 45 minutes at two known concentrations of each compound, with and without photo activation, were then analyzed by flow cytometry, testing cell viability, mitochondrial membrane potential and intracellular production of peroxides and superoxide. The Mann-Whitney test was used for statistical analysis.

Results and Discussion: Studies show that all the conditions examined have a cytotoxic effect on

cells which is dose-dependent. Time is also an important factor allowing cell recovery. The cytotoxic effect of hydrogen peroxide is continuous in time but when Zoom® is applied there is a recovery after the 45 minutes of application. When Zoom® is photo-activated increases its cytotoxic effect and the recovery of cell proliferation is only visible from 48 hours. The light of the bleach system has a cytotoxic effect per se. Cell death caused by the products tested occurs primarily by apoptosis induction and it was also verified a decrease in mitochondrial membrane potential. Our results reinforce the hypothesis that it is safe to use bleaching products based on hydrogen peroxide.

Keywords: bleaching; hydrogen peroxide; cytotoxicity; fibroblasts.

F12-161 - Masseter evaluation in experimental intoxication with Tioacetamide.

Joseide Araujo Félix 1,2; Eduardo Costa2; Felipe Pereira4; Rodrigo Farinha2; Ramos, M P1

1 Health Department - State University Of Feira De Santana, Brazil

2 Experimental Pathology Service, Faculty of Medicine, University of Coimbra

3 Chemistry Department, Faculty of Sciences and Technology, University of Coimbra

4 Faculty of Pharmacy, University of Coimbra

Muscle function is important for the stability of stomatognathic system, contributing to keeping the health of the temporo-mandibular system. Its dysfunction can be a cause or a consequence of the symptoms linked to temporo-mandibular dysfunction. Tioacetamide, a known hepatotoxic is a source of sulfur in the industry, used for organic and inorganic compounds synthesis and in the rubber production, as well as in metallurgy and for pesticide synthesis. Tioacetamide, is an experimental inductor of hepatic disease in the rat, producing lesions from necrosis to hepatic carcinoma, depending on time and dose.

The aim of this study is to evaluate the possible consequences of tioacetamide administration on the masseter muscle.

Twenty Wistar male rats, 9 weeks old, were distributed randomly by two experimental groups: control group, kept without manipulation and the test group, submitted to the administration of 20 mg/kg/day of tioacetamide, intraperitoneally, for four weeks. All the animals were killed by the end of the 4th week, and fragments of masseter muscle were collected for routine histopathological analysis, and frozen in liquid nitrogen for posterior molecular and metabolic studies. Ten pictures were taken of each slide of masseter muscle stained by hematoxylin e eosin. The morphometric analysis was performed with software ImageJ, for the measurement of masseter muscle fibers' areas in transverse cuts.

The frequencies distribution statistical test, shown no significative differences between test and control groups.

The analysis of the results of the morphologic analysis of masseter muscle on tioacetamide administration, shown no signs of morphologic lesion, but it is important to evaluate possible molecular or metabolic changes.

F13-104 - Periodontal ligament evaluation in an experimental hyperthyroidism model with acupuncture treatment

Carlos Miguel Marto1, António Cabral1, Gustavo Barandas1, Rodrigo Farinha1, António Silvério Cabrita1

1 Experimental Pathology Service, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Introduction: The thyroid is the major regulator of metabolism (especially energetic and adipose tissue metabolism) and affects all of the bodily functions. Thyroid dysfunction is the second most common glandular disorder of the endocrine system.

Hyperthyroidism is a condition caused by an increase in the production of thyroid hormones. The increase of body's metabolism can cause sudden weight loss, a rapid or irregular heartbeat, sweating, and nervousness or irritability. Oral manifestations include periodontal disease and an increase in bone turnover.

Treatment can be made with anti-thyroid medications and radioactive iodine to slow the production of thyroid hormones. Sometimes surgery to remove all or part of the thyroid gland is needed.

Aim: The aim of this study was to evaluate the effects of experimental hyperthyroidism in periodontal ligament and investigate the effect of acupuncture treatment as a treatment.

Materials and Methods: 32 Wistar male rats with 8 weeks old, were randomly distributed in four groups (n=8 each): group I: control group, no manipulation (Ctr); group II: received an administration of 250mg/kg of levothyroxine by gavage 3 times a week during two weeks (Ht); group III received only acupuncture treatment (Ac) and group IV received levothyroxine, as the group II, and acupuncture treatment (Ht+Ac).The points used for puncture were: 09ST, 36ST, 4HC, 23RM.

After 2 weeks, all the animals were sacrificed and mandible was collected. Hematoxylin-eosin coloration was performed and a morphometric analysis was performed in sections using software ImageJ®. Results: Animals in group II present several areas of root reabsorption and in some cases a small enlargement of periodontal ligament. Animals in group III equally shows areas of root reabsorption. Group IV show only few cases of root reabsorption and in small areas.

Conclusions: Results show an increase in root reabsorption in hyperthyroidism, which indicates inflammatory processes affecting periodontal ligament in this pathology. Acupuncture treatment seems to have some protective effect. More studies are needed to elucidate the protective effect of acupuncture treatment.

Keywords: Experimental hyperthyroidism; periodontal ligament; acupuncture treatment

F14-005 - Experimental model of pancreatic duct ligation with the use of patent blue dye

Daniel Cartucho, Gabriela Valadas

Surgical Service - Hospital Barlavento Algarve
CBME – Algarve University

Experimental models of pancreatitis have been de-

veloped since XIX century and have contributed to our understanding of the physiopathology and cell biology of the organ. Although not totally comparable to human disease, the refinement of the model is important for the comprehension of the pathologic process that, despite advances in the understanding of chronic pancreatitis, has no presently available causal treatment.

The 'pancreatic outflow obstruction' is referred to as the most critical event for disease onset. Existing experimental models for pancreatitis, based on duct obstruction, are not common. The reason for this is that in the small animal model the pancreatic duct is very difficult to identify, is technically challenging and only experienced surgical hands can minimize trauma.

In order to overcome this problem, the authors present an easy, low cost, method that makes the pancreatic duct more visible. Utilizing patent blue dye injected in a sub dermic location, it is possible to contrast the interior of the bile duct as well as the pancreatic duct. This color contrast permits a very selective pancreatic ligation without significant collateral damage to the organ. With this refinement, translation from the experimental model to the human situation permits possible benefits in the study and treatment of obstructive pancreatitis.

F15-277 - Lamendin method and physiological changes of root dentin - a forensic approach

Dyego Jhony Diniz Gonçalves Perminio¹, Marcela Leonel Mirandela², Paloma Suzart dos Santos Melo³, Ana Teresa Corte-Real⁴

¹ Universidade Federal Fluminense, Mobility student, Dentistry área, Faculty of Medicine, University of Coimbra, Portugal

² Universidade Federal de Santa Catarina, Mobility student, Mobility student, Dentistry área, Faculty of Medicine, University of Coimbra, Portugal

³ Universidade Estadual de Feira de Santana, Mobility student, Mobility student, Dentistry área, Faculty of Medicine, University of Coimbra, Portugal

⁴ LBMD, Faculty of Medicine, University of Coimbra, Portugal

The age estimation is an important component of forensic expertise in the identification and criminology, to match the chronological age. The mineralized dental tissues are more resistant to degradation and decay, and suffer less influence of external factors. The Lamendin method is used in anthropological practice, is an economical, practical and simple, and that does not preclude the use of other complementary methods. In this paper Lamendin method is applied to a single root tooth, with an without endodontic treatment, by a mathematical formula. Teeth were collected from Dental Medicine Clinic of Faculty of Medicine, University of Coimbra. Were analyzed 30 teeth witch 10 had endodontic teeth. The samples were analyzed at the Laboratory of Forensic Dentistry Faculty of Medicine of University of Coimbra. The parameters, gingival recession and root transparency, were analyzed in a light box and measured with digital calipers. Ours results were recorded by three observers. The physiological changes of root dentin witch support Lamendin method, in our study, were applied in one specific

situation, the tooth with endodontic therapy, and we conclude that was a sample for age estimation.

F16-254 - Bitemarks on food marks, natural teeth versus prosthetic elements

Alyne Silveira Pereira¹, Vanessa Paola Filter², Duarte Numo Vieira³, Ana Teresa Corte-Real⁴

¹ Universidade Federal de Santa Catarina, Mobility student, Dentistry area, Faculty of Medicine, University of Coimbra, Portugal

² Universidade Federal de Santa Maria, Mobility student, Dentistry area, Faculty of Medicine, University of Coimbra, Portugal

³ Faculty of Medicine, University of Coimbra and INMLCF,IP, Portugal

⁴ LBMD, Faculty of Medicine, University of Coimbra, Portugal

Bitemarks on food has its application in Forensic Dentistry, in exclusion or identification of the suspect of a crime scene. This paper aims to study the characteristics of the tooth marks left by natural and artificial teeth, in a particular type of food, the cheese.

7 patients were selected from Dental Medicine Clinic of Faculty of Medicine, University of Coimbra. Inclusion criteria were: prosthetic elements (removable and /or fixed) and natural teeth, at anterior superior dental arch and only natural teeth at opposing arch. A type of cheese (with 4 cm diameter and 1 cm thick) was bitten, without tearing of the material. Photographic record of the food bitten were made with metric scale ABFO^o 2. The images were analyzed with the software AUTOCAD[®] and registered, longitudinal and transversal, measures. The data were statistically analyzed.

We conclude that the distribution pattern of dental brands in food, from natural and artificial teeth, is different. This difference is most obvious for removable prosthetic elements.

The interpretation of results must be cautious since the greater the force applied during the bite, more evenly they are distributed between the right and left sides of the dental arch, the intention of the bitemark may be related to the applied load. Our study allows us to consider that differences in the characteristics of the tooth marks left on food may be related to prosthetic rehabilitation in the mouth at the time of the biting.

Section G - Hot spot: Wednesday 12th December 10 h 30 min

**Experimental Veterinary Sciences
Experimental Pathology Teaching
Experimental Models
Animal Facilities and Animal Welfare
Alternative Methods**

G01-158 - Changes in salivary α -amylase levels in an animal model of high fat diet induced obesity. Rodrigues L1, Mouta R1, Costa AR1,2, Capela e Silva F1,3, Pereira, A.1,4, Amado F4, Antunes C2,6, Lamy E1,5

1Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Portugal

2Departamento de Química, Universidade de Évora, Portugal

3Departamento de Biologia, Universidade de Évora, Portugal

4Departamento de Zootecnia, Universidade de Évora, Portugal

5Química Orgânica, Produtos Naturais e Agro-Alimentares (QOPNA), Universidade de Aveiro, Portugal

6Centro de Neurociências e Biologia Celular (CNC), Universidade de Coimbra, Portugal

Background: Obesity is often associated with choice of fat enriched foods, which may be related with changes in food perception. Being saliva a complex fluid with important physiological functions playing a role in food perception, studies about changes in salivary function associated with weight gain may be valuable for understanding this pathology.

Objectives: Our aim was to induce obesity in rats by feeding them with a high fat diet and to evaluate changes in salivary secretion. **Methodology:** 20 females Wistar rats were divided in two groups: one subjected to a diet enriched with sunflower oil (34.5% energy from fat) (N=13), and the other subjected to standard rodent chow (N=7), during 18 weeks. Body weights and food consumption were monitored weekly. Mixed saliva was collected after pilocarpine stimulation, before and at the end of the experimental period. Total protein concentration was determined using Bradford method. Dinitrosalicylic acid assay was used for measuring the starch-hydrolyzing activity of salivary α -amylase, while expression of this enzyme was evaluated by Western blot using an anti- α -amylase primary antibody.

Results: The animals subjected to the high fat diet responded differently in terms of weight gain and it was possible to divide them in obesity prone (OP) and obesity resistant (OR) subgroups. OP subgroup consumed a higher level of energy than OR and controls. The initial levels of α -amylase were positively correlated ($r=0.7$; $P<0.05$) with weight gain, being the OP subgroup who presented higher enzymatic activities and expression levels. After fat consumption animals from OR subgroup significantly increased their levels of this salivary protein. **Conclusions:** Salivary α -amylase can be an indicator of weight gain susceptibility, what deserves future attention. The increase in α -amylase levels in OR animals after 18 weeks of fat consumption suggest that animals may adapt to the lower carbo-

hydrate content of their diets.

Keywords: High fat diet, Saliva, Salivary amylase

G02-035 - Ductal carcinoma in situ of the breast: immunohistochemistry and hybridization analysis of HER-2 status in canine mammary model.

Ferreira, E.¹; Oliveira², N.S.; Silva, J.O.¹; Varaschin, M.S.²; Cassali, G. D.¹

¹Laboratory of Comparative Pathology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG

² Department of Veterinary Medicine, Federal University of Lavras, Lavras, MG

Background: The process of breast carcinogenesis suggests that ductal carcinoma in situ (DCIS) can progress to invasive carcinoma. The data relating cancer behavior to HER-2 status encourage the further exploration of links between molecular events and phenotypic diversity of breast cancer in its invasive and pre-invasive stages.

Methods: Thirty-two cases of DCIS were classified according to the veterinary and human mammary classification system (high, intermediate, and low grade). Immunophenotypic analysis of the HER-2 and E-cadherin status was performed. Positive cases for HER-2 (2+ and 3+) were analyzed for gene amplification by CISH. All analyses were performed at the Department of General Pathology, Federal University of Minas Gerais, Brazil.

Results: We identified 21 cases of high grade DCIS and 11 cases of low grade. Higher expression of HER-2 and E-cadherin were observed in carcinoma in situ of high grade ($p<0.05$). However, no correlation was found between these markers ($r=0.27$, $p=0.16$). Despite the presence of HER-2 hybridization, neoplastic cells showed typically two or four spots per nucleus, and the seventeen tumors overexpressing HER2 were considered nonamplified by CISH. **Conclusion:** The results suggest that overexpression of HER-2 may be associated with neoplastic progression of canine mammary carcinomas. However, these progression don't have relationship with HER-2 gene amplification.

Keywords: dog, tumor, neoplasms

G03-217 - Experimental canine model for peri-implantitis – a review.

Martins, O.1; Ramos, J.C.1; Baptista, I.1

1Dentistry School, Faculty of Medicine, University of Coimbra, Portugal

Background: Over 2 million dental implants are placed per year and that number is growing [1]. Some of these implants are associated to biological complications like peri-implantitis. Peri-implantitis is defined as an inflammatory process affecting hard and soft tissues around osteointegrated functional implant, resulting in supporting bone tissue lost [2]. Currently available evidence doesn't allow firm recommendations for surgical treatment of peri-implantitis [3] and we most likely will have to develop new strategies to surgically treatment. This will require the development of new experimental models that mimic the human naturally occurring peri-implantitis with respect to appearance and

progress. The dog was almost exclusively used for this purpose [4] with an experimental model based on ligature-induced peri-implantitis.

Objectives: The purpose of this review was to analyze the chronological evolution of the experimental peri-implantitis dog model in order to investigate the pathology outbreak.

Material and Methods: An electronic search of publications (up to 01 November 2012) was made on Medline (Pubmed), EBSCO Library (EBSCOhost) and Cochrane Library (All Cochrane Library Databases) databases. The key words and Boolean operators were: ("Peri-implantitis" OR "peri-implantitis (MeSH)" OR "Periimplantitis" OR "Perimplantitis") AND ("Animal" OR "Models, Animal (MeSH)"). Inclusion criteria were: english language, experimental dog studies, studies with data concerning peri-implantitis induction methodology. Exclusion criteria were: surgically created peri-implant osseous defects, less than two animals.

Results: From a total of 158 articles 50 were included. The ligature-induced peri-implantitis dog model has a great heterogeneity on: number/type of extracted teeth, duration of ligature period, methodology of peri-implantitis induction and oral hygiene measures.

Conclusions: the traumatic action produced by ligature doesn't mimic the natural occurring peri-implantitis in humans so it's important to explore new experimental models to induce this pathology in a canine model. Standardized procedures for this experimental model should be established.

Key-words: peri-implantitis, canine, experimental model.

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G04-225 - Kidney metallothionein expression in a rodent model of glucose-intolerance. Sofia, A.1; Veiga, E.1; Fialho, L.2; Costa, A.1,3; Lamy, E.3,4; Capela e Silva, F.3,5; Antunes, C.M.1,6

1Departamento de Química, Universidade de Évora, Portugal

2Departamento de Medicina Veterinária, Universidade de Évora, Portugal

3Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Portugal

4Química Orgânica, Produtos Naturais e Agro-Alimentares (QOPNA), Universidade de Aveiro, Portugal

5Departamento de Biologia, Universidade de Évora, Portugal

6Centro de Neurociências e Biologia Celular

(CNC), Universidade de Coimbra, Portugal

Background: Metallothionein (MT) is an ubiquitous, low molecular-weight non-enzymatic protein, inducible in various cell types under toxic conditions, inflammatory processes and acute stress. Although it mainly acts in tissues as a regulator of metal homeostasis such as zinc and copper, MT was found to be a potent antioxidant and adaptive (or stress) protein to protect cells and tissues from oxidative stress. Diabetes is a widespread disease, and its development and deleterious effects on various organs have been attributed to increased oxidative stress. **Objectives:** The objective of the present study was the evaluation of MT expression, by immunohistochemistry, in kidneys from glucose-intolerant rats (GIR) with normal fasting glucose levels, altered oral glucose tolerance test and fasting hyperinsulinemia.

Methodology: Adult Wistar rats with normal (controls, n=5) and impaired (GIR, n=5) glucose tolerance, were sacrificed, the kidneys collected and processed for immunohistochemistry. Immunohistochemistry was performed using the primary antibody against metallothionein by streptavidin-biotin-peroxidase. Sections were incubated (overnight, 4°C), with primary antibody at 1:50 dilution. As negative control adjacent sections were incubated without primary antibody. All the slides were examined under a Nikon Eclipse 600 light microscope using 200x magnification, for staining intensity [0 (absent); + (low); ++ (medium); +++ (high)] and location of immunoreactive cells.

Results: A weak and a strong nuclear and cytoplasmic immunoreactivity for metallothionein were observed in the proximal convoluted tubule of GIR and normal Wistar rats, respectively.

Conclusions: The results suggest a possible imbalance in the mechanisms of oxidation/reduction and a decrease in cellular antioxidant defenses associated with metallothionein in GIR occurring prior to overt diabetes. MTs might play an important role in the physiopathology of diabetic nephropathy.

Keywords: Kidney; Metallothionein; Glucose-intolerance

G05-225 - Kidney heat-shock protein 70 expression in kidneys from glucose intolerant Wistar rats. Sofia, A.1; Veiga, E.1; Fialho, L.2; Costa, A.1,3; Lamy, E.3,4; Capela e Silva, F.3,5; Antunes, C.M.1,6

1Departamento de Química, Universidade de Évora, Portugal

2Departamento de Medicina Veterinária, Universidade de Évora, Portugal

3Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Portugal

4Química Orgânica, Produtos Naturais e Agro-Alimentares (QOPNA), Universidade de Aveiro, Portugal

5Departamento de Biologia, Universidade de Évora, Portugal

6Centro de Neurociências e Biologia Celular (CNC), Universidade de Coimbra, Portugal

Background: Heat shock proteins (HSPs) is a group of different proteins, classified according to their

molecular weight, which can be induced by heat shock and other forms of pathophysiological stress, such as an oxidative injury. The functional mechanisms of HSPs are not clearly understood to date, but results suggest that HSPs protect stressed cells, serving as molecular chaperones. In diabetes, increased free radicals, due to hyperglycemia and glycated proteins, as well as reduced antioxidant capacity have been reported.

Objectives: The objective of the present study was the evaluation of Hsp70 expression, by immunohistochemistry, in kidneys from glucose intolerant Wistar rats (GIR) with normal fasting glucose levels, altered oral glucose tolerance test and fasting hyperinsulinemia.

Methodology: Groups of adult male (5 GIR and 5 control) were sacrificed, the kidneys collected and processed for immunohistochemistry. Immunohistochemistry was performed using the primary antibody against Hsp70 by streptavidin-biotin-peroxidase. Prior to immunostaining the sections were pretreated for antigen retrieval at 98°C in citrate buffer (pH 6.0) solution for 20 minutes. Sections were incubated (overnight, 4°C), with primary antibody at 1:50 dilution. As negative control adjacent sections were incubated without primary antibody. All the slides were examined under a Nikon Eclipse 600 light microscope using 200x magnification, for staining intensity [0 (absent); + (low); ++ (medium); +++ (high)] and location of immunoreactive cells. **Results:** A strong and a moderate staining intensity for HSP70 were observed in glomeruli from GIR and normal Wistar rats, respectively.

Conclusions: The increased oxidative stress in diabetes results from multiple factors, namely from glucose self-oxidation leading to the production of free radicals. The increase in HSP in renal tissue from GIR rats may reflect a response to increased oxidative stress in the early stages of diabetes development, as suggested by other authors for overt diabetes.

Keywords: Kidney; Heat-shock protein 70; glucose-intolerance.

G06-131 - Effect of chronic sleep loss on the organs weights, body mass, behavior and humoral immunity in rats Wistar

Fernandes, E.V.1; Filgueiras, G.B.2; Carvalho-Netto, E.2; Estanislau, C.2; Venancio, E.J.1

1Department of Pathological Sciences, Londrina State University, Brazil.

2Department of General Psychology and Analysis Behavior, Londrina State University, Brazil.

Background: Sleep has an important role in animal's physiological functioning. When normal sleep is prevented in rats, a series of harmful impacts may occur, for example, increase in anxiety-like behaviors and in susceptibility to infections. **Objectives:** Investigate the effects of chronic sleep deprivation on body and organs (adrenal, thymus and spleen) weight, as well on defensive behaviors in the elevated plus-maze and humoral immunity of rats.

Methodology: Twenty-eight male Wistar rats were randomly allocated to two groups; sleep restriction (n = 14) and control (n = 14). In order to evaluate the antibody production, half of the animals in each

group were submitted to the immunization protocol. During the 21 days of sleep restriction procedure (18 h daily by the multiple flower pot technique), rats were immunized twice (at days 01 and 15) subcutaneously with 100 µg of IgY in Freund's adjuvant. The blood samples were collected at 01, 08 and 22 days, and the levels of antibodies (IgM, IgG1 and IgG2a) were measured using enzyme-linked immune sorbent assay. On day 22, all animals were tested in the elevated plus-maze (EPM) for 5 min and subsequently were sedated for blood sample collections and euthanized. The thymus, spleen and adrenal were removed and weighed. **Results:** Our results demonstrate that sleep restriction provokes significant enhancement of adrenal weight and reduction on body mass of rats. No changes in weight were observed in other organs (thymus and spleen). The behavioral responses in the EPM and antibody production were not altered in the sleep restriction group. **Conclusions:** Sleep restriction may be related to animals' stress, as indicated by the increase in the adrenals weight. However, the sleep restriction was not able to change the humoral immunity of rats.

Keywords: Stress; Antibodies; Adrenal.

G07-226 - An experimental model of varicose veins – Preliminary results.

Pratas Balhau1, Luísa Guardão3. Costa Almeida2, Silvério Cabrita1.

1Experimental Pathology Service, Faculty of Medicine of Coimbra

2Vascular Surgery, Faculty of Medicine of Coimbra

3Faculty of Medicine of Oporto.

Until now there have been some difficulties in obtaining a good experimental model of varicose veins, as well a good histological characterization of varicose veins and the severity of their parietal lesions.

The aim of this study was to obtain more information towards the production of an experimental model of varicose veins using belier French giant rabbit's ears (with ears usually pending to the floor at all times).

Twelve male rabbits, three months-old at the beginning of the experiment were submitted to ligation of the auricular vein caudal of the left ear. The animals were sacrificed, four of them by the end of the first month, four after third months and the others by the end of the sixth post-operative month. The veins of both ears of all the animals where macroscopically and histologically studied and compared.

Macroscopically there was a minimal enlargement of the vein of the left ear beginning after the first month. Histological changes were minimal and inconstant. The results suggest that the veins changes induced by the performed ligation procedure should be better characterized by other parameters than those that have been used in the current study.

Section H - Hot spot: Wednesday 12th December 10 h 30 min

**Instrumentation and Applied Computation
Technical Procedures in Experimental Pathology
Experimental Surgery**

H01-014 - Automatic evaluation of movements during seizures. Eduardo Costa¹, Ricardo Cabeças¹, Silvério Cabrita¹.

¹Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

The analysis and quantification of seizures in experimental models needs to be carried out on a quantitative basis and not subject to subjective interpretation. To achieve this we propose the use of sensors placed within the animal and to transmit information in real time over a wireless system, to a computer.

We present a system, with the basic sensors, a gyroscope and an accelerometer that through communication protocol wireless bluetooth will communicate with the computer, where software is installed monitoring sensors.

Through these sensors will be possible to analyze the rat movements inside the cage to further identify the timing, intensity of seizures, and other characteristics.

H02-020 - Monitoring of temperature and humidity in the small animal cage environment

José Cabeças¹, Rodrigo Farinha¹, Silvério Cabrita¹

¹Experimental Pathology Service, Faculty of Medicine, University of Coimbra.

Monitorization of small animal's cages environmental variables is a major concern in animal experimentation. The access to variables such as the temperature and humidity of the cage allows greater control over animal welfare during an experimentation and can help improve the environmental conditions.

The purpose of this work is to create a condition for controlling the environment in the animal's cage, using temperature and humidity sensors connected to a microprocessor. The microprocessor saves the received data in the EEPROM (Electrically Erasable Programmable Read-Only Memory)

Using an array of sensors in one cage we monitored the environment inside the cage individually, managing the information from each sensor and processing that information.

We use cages with one animal and cages with two animals and were monitored during periods of two days.

From the results we concluded that the size of the cages its not enough, the animals need more space to have an environment with quality. In all "compartments" of the cage, specially near the food and water, the humidity values raised above the expected for small animal cage environment. The temperature had a few small changes during the monitorization.

Section I - Hot spot: Wednesday 12th December 10 h 30 min**Experimental Health Procedures****Experimental Integrate Medicine****Integrated Medicine in Pratical Clinics****101 - 239 - Energy healer procedure in an experimental model - preliminary results**

Maura Ferreira¹, Paulo Rodrigues-Santos², Vera Alves², Manuel Santos-Rosa², Silvério Cabrita¹

¹Experimental Pathology Institute, Faculty of Medicine, University of Coimbra

²Immunology Institute, Faculty of Medicine, University of Coimbra

Energy healer procedures have gained popularity over the past decades although involving controversial concepts and very often missing credible studies. The energy-healing interventions have gained popularity as a non-invasive and non-pharmacological approach, for relaxation, alleviation of anxiety and modifying the pain perception. There is very little evidence to support the application of these techniques within clinical practice and more clinical and laboratorial studies are necessary. The aim of this study is to evaluate the possible action of a Reiki procedure on the leukocytes of the rat.

A group of ten Wistar male rats, 8 weeks old were random divided into two small groups of 5 animals each. The group I was submitted to no manipulation and the animals of the group II were submitted to Reiki 15 min a day, three times per week during 8 weeks. After this period, blood where collected from all the animals and sent to evaluate lymphocytes and monocytes autofluorescence, without identifying the group of each animal. Intracellular autofluorescence is often dominated by the reduced pyridine nucleotides (NAD(P)H) and the oxidized flavins (FMN, FAD), both of which are potentially useful as cellular metabolic indicators. Autofluorescence has similar excitation and emission of Fluorescein isothiocyanate (FITC) and R-Phycoerythrin (RPE). Therefore, evaluation was performed measuring autofluorescence on a FITC/RPE dot plot using a BD FACSCanto II flow cytometer.

Average autofluorescence was significantly increased in the group II monocytes (0.76% vs. 0.10%; Mann-Whitney test $p=0.0238$). Lymphocytes from group II presented also increased autofluorescence (0.10% vs 0.04%) with no statistic significance.

Altogether, these preliminary results suggest an increased activity of leukocytes in animals submitted to Reiki procedure. Further characterization studies of immune response may elucidate the effects of energy healer procedures.

Section J - Hot spot: Wednesday 12th December 10 h 30 min

Teaching, Knowledge, motivation and research in Health Sciences

Ethics and Legislation

Others

J01-009 - Investigation of Citrulline as a Biomarker in Leigh Disease. Veríssimo C1, Simões M1, Pratas J1, Mendes C1, Macário MC2, Diogo L3, Grazina M1,4

1Center for Neuroscience and Cell Biology, University of Coimbra, Portugal,

2CHUC - Adult Neurology Department, Coimbra, Portugal,

3CHUC - Pediatric Hospital, Coimbra, Portugal,

4Faculty of Medicine, University of Coimbra, Portugal

Leigh syndrome, like other mitochondrial disorders, has no specific and sensitive biomarkers. High levels of lactate are frequently found, but not in all cases. Some reports indicate hypocitrullinemia as a possible biomarker of Leigh disease in cases with m.8993T>G mutation.

In this communication we present five cases of suspected Leigh syndrome. Our goal is to evaluate the role of citrulline as a possible biomarker in these cases.

Additionally, 4 of the five patients presented multiple oxidative phosphorylation defects, all having increased plasma alanine levels and 2 presented low citrulline concentration, with no correlation for the second, with the respiratory chain activity.

According to our results two patients suspected of Leigh syndrome presented low citrulline levels, associated with the m.8993T>G mutation. We suggest that when Leigh disease is suspected, evaluation of citrulline levels should be performed and, if hypocitrullinemia is detected, m.8993T>G mutation should be immediately investigated in blood.

Although our findings need confirmation in larger cohorts, we believe that hypocitrullinemia could be a helpful biomarker in Leigh disease, particularly related to the presence of m.8993T>G mutation. The quantification of this amino acid could also be valuable in other mitochondrial respiratory chain disorders such as MELAS and Pearson, as previously reported.

Keywords: Citrulline; Leigh Disease, mtDNA

J02-009 - Genetic profile of CYP2D6 pharmacogenetics in the Portuguese population. Albuquerque J1, Ribeiro C2, Naranjo ME3, Peñas-Lledó E3, Llerena A3, Grazina M1,2, CEIBA.FP Consortium of the Ibero-American Network of Pharmacogenetics and Pharmacogenomics RIBEF

1Faculty of Medicine, University of Coimbra, Portugal;

2CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal;

3CICAB Clinical Research Centre, Extremadura University Hospital and Medical School, Badajoz, Spain

Pharmacogenomics has been described as a part of the Biochemical Genetics which focuses on the study of the inter-individual variability, toxic effect and susceptibility to xenobiotics and/or endogenous substances, based on the genetic profile of the individual.

In the last years, the great variability in CYP2D6 gene has been described in individuals from different ethnicities (Spain, Cuba, Nicaragua, México and Ecuador). Up to date, the CYP2D6 has been one of the most investigated genes, coding for a protein that is highly involved in the metabolism of various substances. This gene is extremely polymorphic, which influences the enzymatic activity and originates a huge variability in the metabolism profile. Different metabolism profiles will influence the way how the xenobiotic/endogenous substances are processed, thereby influencing the disease risk, therapeutic efficacy and side effects, or toxicity of xenobiotics. We have analyzed 282 Portuguese healthy volunteers (65% females and 35% males, mean age 48 ± 20 years). The aim of this study was to characterize the CYP2D6 pharmacogenetics in the Portuguese population (gene copy number and alleles *2, *3, *4, *6, 10*, *17, using real-time PCR.

The allele and genotype frequencies were in Hardy-Weinberg equilibrium for all variants. The allele and genotype frequencies obtained were similar to others reported in European Populations, with exceptions, such as CYP2D6*4 (lower in the Portuguese population – 0.05) and CYP2D6*10 (higher in the Portuguese population – 0.08).

These data are important in the study of the genetic variability, to improve the effectiveness and safety in the exposition to xenobiotic substances, working also as a strong tool in clinical practice and in the development of an individualized pharmacotherapy.

Keywords: Pharmacogenomics; CYP2D6; Portuguese population

J03-009 - Experimental Evaluation for Predicting Pathogenicity of Novel Mitochondrial Genome Sequence Variations. Mafalda Bacalhau1,2, João Pratas2, Maria João Santos2, Carolina Ribeiro2, Mónica Vaz2, Cândida Mendes2, Marta Simões2, Cristina Rego1, Manuela Grazina1,2

1Faculdade de Medicina, Universidade de Coimbra

2Laboratório de Bioquímica Genética, Centro de Neurociências e Biologia Celular, Universidade de Coimbra

Mitochondrial disorders are a heterogeneous group of disorders that are caused by defects in the mitochondrial ATP production system.

This organelle has its own genome (mtDNA), that, when injured, leads to the formation of abnormal mitochondrial respiratory chain (MRC) subunits, jeopardizing the proper functioning of oxidative phosphorylation (OXPHOS) and therefore ATP syntheses. Then, mutations in any gene essential for integrity and function of CRM could cause structural and functional mitochondrial alterations.

The mtDNA point mutations include those that impair mitochondrial protein synthesis and those that affect any of the 13 MRC subunits encoded by

mtDNA. Given the fact that mtDNA is highly polymorphic, the pathogenic significance of a detected novel sequence alteration needs to be determined using a series of criteria before a genetic diagnosis can be established.

With this study, we aim to evaluate the pathogenicity of a novel mtDNA mutation (m. 14771C>A), previously detected in Biochemical Genetics Laboratory - CNC, University of Coimbra (Director: Professor Manuela Grazina), found in a Portuguese patient suspected of mitochondrial cytopathies.

In order to assess the impact of these sequence variations, the study was conducted to verify their absence in controls, by automated sequencing. Furthermore, the determination of its effect in transcripts expression levels by Real-Time PCR, in protein levels by Western blot and in MRC complexes activity by spectrophotometry, using primary culture fibroblasts, was also performed.

We have observed that the transcript expression levels are significantly decreased and protein levels are slightly diminished in patient fibroblasts for MT-CYTB gene and CYTB protein, respectively.

It is noteworthy that this study represents an original scientific contribution, allowing to analyze in more detail and precision the functional impact of the sequence variation. Therefore, according to published pathogenic criteria, we conclude that m.144771C>A sequence variation is "probably" pathogenic, being the possible cause for clinical phenotype.

Keywords: Mitochondrial DNA; Oxidative Phosphorylation; Pathogenicity; Mitochondrial Cytopathies

J04-130 - Virtual Atlas of Pathology in Ipad.

Alves, SN1; Soares, LF1; Leitão, TJ1; Thomé, RG2; Santos, HB1; Ribeiro, RIMA1

1Campus Centro-Oeste Dona Lindu / Universidade Federal de São João Del Rey, Brasil

2Campus Governador Valadares / Universidade Federal de Juiz de Fora, Brasil

Introduction: Using New Technologies of Information and Communication (NTICs) as a tool of teaching and learning, has for basic principle to raise the awareness of students and the school community, putting it in a new educational paradigm. The hypothesis systematizes the dynamics of teaching using electronic media inserted in pedagogical practices influencing student behavior and thus, these cognitive tools may develop transformation of students, improving the quality of classroom teaching and generating dissemination of knowledge.

Objectives: This study aims at a reflective reading focused on new pedagogical paradigm, supported in the use of tools derived from the NTICs applied in teaching contents of Pathology.

Methods: The material was prepared in UDK Engine platform - Mobile - in the form of an app for Ipad. The student through a virtual tour has access to images of slides, text, and sound content such as necrosis, edema and infarction. The images were distributed with the use increase of specific regions during a practical class may be a point of difficulty in teaching. The slides were photographed under a microscope (Axio, Zeiss) in the Laboratory

of Cell Biology and Mutagenesis (Campus Centro-Oeste Dona Lindu Federal University of São João del Rei in Divinópolis, MG, Brazil).

Results: Although there are different virtual atlas of pathology, in Brazil there have not been atlas developed for Ipad.

Conclusion: Several studies show that students increasingly use all social networks shared information on the web, however the possibility of insertion of these students in these environments a teaching of pathology will enable to improve the relationship with content.

J05-52 - Peri-implant defects in preclinical versus clinical models: etiology and anatomic characteristics - a review.

Baptista, I.P.1, Azinheira, A1, Martins, O1, Ramos, J.C.1; Matos, S.1

1Dentistry School, Faculty of Medicine, University of Coimbra, Portugal

Background: Peri-implantitis prevalence range between 38%-56% on a subject basis. Several surgical approaches have been suggested in order to solve this pathology but none is proven to be superior. Animal and experimental models have been used to test several surgical approaches, however we have limited data regarding the comparison of peri-implant defect characteristics between pre-clinical dog models and human natural occurring peri-implantitis bone defects.

Aim: Do a literature systematized review regarding the aetiology and morphology of periimplant defects in dogs (preclinical) and humans (clinical) comparing both types of characteristics. Methodology: The research was done in primary databases (PubMed/MEDLINE), mixed databases (EBSCOhost) and secondary databases (Cochrane Library). Keywords searched were "Peri-implantitis", "Humans" and "Animals" [MeSH Terms]. Literature was published between 1990 January and 2011 December, in English. Additionally, a manual search of literature was performed.

Results: This review included 93 articles of human, animal and both-types trials. The results analysis was done from the clinical, microbiological and radiographic point of view to understand the extrapolation possibility from preclinical to clinical results. In humans radiographic analysis revealed that peri-implant horizontal and vertical bone loss presented a saucer or crater-like shape. Intra-operative analysis revealed circumferential defects with or without buccal or lingual associated dehiscence's. In animals vertical and saucer bone loss is radiographically described. During per-operative period these animal defects are described as semi/circumferentials with or without dehiscences, however horizontal bone loss is only described in one study. Microbiological analysis suggests similar microorganisms found in animal and human defects, with highest variability in humans.

Conclusion: Animal peri-implant defects aetiology is exclusively by ligatures. Only in recent models "spontaneous" progression was caused by plaque accumulation. This ligature-induced aetiology is different from human bacterial etiology. Peri-implant morphology, in some aspects, appears to be similar

between this animal model and human peri-implantitis.

Keywords: “peri-implantitis”; “humans”; “animals”

J06-123 - 3D animations used for teaching Human Anatomy. Souza, D.B.S.1; Villarouco, F.M.O.1; Lima, V.J.M.1; Costa Sobrinho, A.V.1; 1Department of Anatomy - Universidade Federal de Pernambuco, Brazil

Background: The use of modern teaching resources like smart board, multimedia projector and digital libraries for example, has facilitated the demonstration and integration of content providing more dynamic and interactive lessons. However, more important than the technology is how it is used in everyday life, respecting the individual characteristics of people and disciplines. Anatomy is essentially image and its studies requires extensive reading of texts supported by pictures and a good capacity for three-dimensional perception by the students. The preview of each goal requires specific preparations of practice, and high amount of preparations. Most of the anatomical parts is obtained from cadavers and nowadays there are fewer cadavers available. Objective: Provide for the students of anatomy 3D animation films capable of complementing the theoretical and practical lessons. Methodology: The animations were built through the use of Blender software (version 2.63) and the 3D objects from the surface of the human body were obtained through MakeHuman software (version 0.9.1 RC1). We sought to answer at first, the demands of visualization of anatomical structures difficult to be understood by students, especially those found in the first contact with the discipline. Results: Animated films were produced showing the anatomical position, planes of delimitation and section even the axis of the human body. All of them performing a rotation body along the longitudinal axis. The animations were tailor made for each case. Conclusion: The use of animation films has allowed offer moving images of the human body to students. The animations of anatomical structures of the human body in motion are already under construction.

Keywords: Anatomy; 3D Animation

