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the neurogenic process in the adult brain.
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AN INNOVATIVE STRATEGY IN SELECTING OOCYTES WITH HIGH IMPLANTATION POTENTIALITY FOR INTRACYTOPLASMIC SPERM INJECTION PROCEDURE

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The aim of the research was to investigate the apoptosis rate of individual cumulus cell-oocyte complexes (COC), associated to the level of pAKT, to verify the difference between oocytes who produce embryos able to reach the blastocyst stage compared with embryos arrested during the *in vitro* culture. It was demonstrated that DNA fragmentation in cumulus cells was remarkably lower in patients who achieved a pregnancy after ICSI cycles, related to the quality of oocytes and embryos[1,2]. AKT pathway plays a critical role in the regulation of cell survival, and most growth factors activate this pathway[3]. The study focused on 53 patients, involved after informed consent. In this prospective and randomized study, it has been measured the DNA fragmentation rate and the level of pAKT in cumulus cells of individual COC for each follicle containing a mature oocyte. Normo-responder patients have been selected. DNA fragmentation rate in cumulus cells has been examined with the use of a TUNEL assay *in situ*. pAKT has been examined by immunological assay *in situ*. Statistic of molecule expression and DNA fragmentation was tested through the repeated measures ANOVA test of log-transformed variables. Out of 255 MII oocytes, 197 were fertilized and the derived embryos had the following evolution: 117 completed the development to blastocyst (day 5or 6) and were transferred in uterus, 57 were vitrified at blastocyst stage and 23 were arrested during *in vitro* culture at different stage of cleavage. In conclusion we found a statistical difference between the DNA fragmentation rate of cumulus cells between the arrested embryos compared to the transferred and vitrified blastocysts ($p=0.004$), confirming that apoptotic rate of the cumulus cells could be considered as a marker of oocyte competence. Likewise we a found statistical significance between oocytes resulting in transferred blastocyst and arrested embryos in the ratio pAKT/TUNEL ($p=0.043$). Therefore, the ratio pAKT/TUNEL could be considered also a marker of oocyte quality. More studies are needed to confirm these data and to determine the how these molecular pathways are involved on the oocyte competence.

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IMMUNOLOCALIZATION OF COCAINE-AMPHETAMINE REGULATED TRANSCRIPT (CART) IN BILIARY EPITHELIUM

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Cholangiocytes proliferation can be modulated by several neuroendocrine factors[1]. The peptide cocaine-amphetamine-regulated transcript (CART) has various physiological functions and is widely expressed in many organs[2]. CART increases the survival of hippocampal neurons by up-regulating Brain Derived Neurotrophic Factor (BDNF)[3]. Recent study has detected the expression of BDNF and of its two receptors (TrKB and p75NT) in cholangiocytes of rat liver and their involvement in proliferation rate of biliary tree[4]. Aim of this study was to investigate the expression of CART in the rat biliary epithelium. Male Wistar rats were divided into normal (n=6) and BDL (n=6) group. All rats were sacrificed after 1 week. Liver samples from both BDL and normal group were collected to perform light microscopy and immunohistochemistry for CART, CK19 (cytokeratin-19) and PCNA (proliferating cell nuclear antigen). Our results show an increased expression of CART together with the growth of intrahepatic bile ductal mass (IBDM) and of cholangiocytes proliferation in BDL. CART may be implicated in the remodeling of biliary epithelium during cholestasis through key mediators of cell survival and proliferation, as well as through BDNF activated pathways.

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EFFECTS OF POLYSACCHARIDE A PRODUCT BY *BACTEROIDES FRAGILIS* ON ENDOTHELIAL PROGENITOR CELLS

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Endothelial progenitor cells are produced in the bone marrow and have the hemangioblast as common precursor to hematopoietic stem cells. They have been identified in 1997 in the peripheral blood stream. The EPC are able to migrate, proliferate and differentiate into mature endothelial cells and to determine the formation of new blood vessels even in the postnatal period. These cells can be infected by *Bartonella henselae*, a Gram-negative pathogen, aetiological agent of a series of human diseases known as bartonellosis. Polysaccharide A pro-

duced by *Bacteroides fragilis*, a human intestinal bacterium, has immunoregulatory properties, among which is proven in mice to provide protection from inflammation caused by *B. henselae*. We have infected the EPC with *B. henselae*, *B. fragilis* WT or *B. fragilis* PSA (not able to produce the polysaccharide A), or co-infected with *B. henselae* and *B. fragilis*, WT or PSA respectively, to assess whether the polysaccharide A had a role in the response of these cells to the infection. After the ultrastructural characterization of bacterial strains used in our experiments, *Bacteroides* ability to infect EPC was assessed by CLSM. Both bacterial strains are internalized already at 24h after infection and at a multiplicity of infection of 100. By TEM it was observed that *Bacteroides* in contrast to *Bartonella*, are internalized activating cytoplasmic lysosomes that digest them. *B. henselae* instead infects those cells forming invasomes in which they continues to proliferate. So polysaccharide A accentuates the macrophagic features that these cells have in their early differentiation stage. Analysis of genic expression using real time PCR from total RNA extracted by the cell cultures have shown the up-regulation of the gene coding for the IL-10 anti-inflammatory cytokine in cells co-infected with *B. fragilis* WT and *B. henselae* compared to the cells infected only with *B. henselae*. The main inflammatory cytokines secretion evaluated by ELISA has also evidenced an increase of IL-10 produced by cells co-infected with *B. fragilis* WT and *B. henselae*. These cells react to the co-infection in the same manner of macrophages. These data assert that the EPC does not play only a key role in vasculogenesis, but in an adult organism they could also play an essential role in the immune response. The EPC may collaborate with macrophages and T cells $CD4^+$, to the inflammatory process triggered by the polysaccharide A after infection.

FUNCTIONAL MORPHOLOGY OF THE ALIMENTARY CANAL OF THE ASCIDIAN *BOTRYLLUS SCHLOSSERI*: A HISTOCHEMICAL CHARACTERISATION

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A variety of histochemical assays were carried out on the monolayered epithelium of the alimentary canal of the colonial ascidian *Botryllus schlosseri* with the aim of increasing the knowledge on the physiology of the filter-feeding nutrition of tunicates. The endostyle of the branchial pharynx, the oesophagus, the saccular stomach and the U-shaped intestine divided into proximal, mid- and distal (or rectum) intestine and associated with a pyloric gland in its mid-tract, were examined as regards the distribution of enzymatic activities for both digestion (amylase, acid phosphatase, 5'-nucleotidase, aminopeptidase M, lipase, non-specific esterase) and absorption (alkaline phosphatase, Ca^{2+}- and Mg^{2+}-ATPases), the presence of storage substances (glycogen, proteins, lipids) and the quality of mucous substances. The argentaffin reaction revealed the presence of endocrine cells scattered in all tracts with the exception of endostyle and mid-intestine. The alimentary particles enter the pharynx, where they are trapped and rolled up by the mucous net secreted by the glandular cells of the endostyle producing different types of mucins. Along this tract, a pre-digestion of polysaccharides occurs and the epithelium is protected by an antimicrobic peroxidase activity. The digestible string is transported towards the post-pharyngeal gut by the activity of the ciliated mucous cells. The oesophagus plays the role of food progression. The stomach, with its many folds in which are located various cell types, carries out functions of extracellular digestion (zymogenic cells), absorption and intracellular digestion (vacuolated cells), osmoregulation (plicated cells of the pyloric caecum), and storage of lipids and glycogen (ciliated mucous cells). These functions, with the exception of the extracellular digestion and osmoregulation, continue in both proximal and mid-intestine, where the peroxidase activity reappears. The pyloric gland reveals hydrolytic activities and storage of glycogen and proteins. In the distal intestine, weak digestive and absorption activities still occur; faecal pellets are formed and then ejected through the anal opening in the atrial cavity and then into the common cloacal chamber of the colonial system. *B. schlosseri* can represent a model species for the development of histochemical methods which will be extended to other species belonging to other classes of tunicates showing more or less tissue complexity due to different specialisations.

PHOSPHORYLATED TAU IN PURKINJE NEURONS. NEUROARCHITECTURAL ALTERATIONS OF CEREBELLAR CORTEX IN PROLIDASE DEFICIENT MICE

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Many enzymes differently contribute to extracellular matrix (ECM) remodeling, being implicated in various physiological and pathological processes. The ECM of the Central Nervous System (CNS) surrounds neurons and glial cells providing a unique microenvironment. ECM not only has a passive structural property, but also actively influences cell proliferation, migration, differentiation, axonal guidance, synaptogenesis and homeostatic plasticity, during both development and adulthood. One special family-member enzyme linked to ECM remodeling is prolidase. The absence of prolidase activity characterizes a rare human autosomal recessive disorder called Prolidase Deficiency (PD) (OMIM 170100). The PD phenotype varies widely, spanning from the principal clinical feature, ulcers mainly located on the lower part of legs and feet, to different degrees of mental retardation. In 2008, a PD mouse model was found, name dal mice. The heterozygous and homozygous mice had cerebral and cerebellar morphological defects with a disorganized ECM profile. In this study, we further investigated the neurodegenerative aspects manifested by dal mice, focusing on the cerebellum and considering as principal marker the phosphorylated form of Tau (p-Ser^{422}). The Tau protein belongs to the family of Microtubule Associated Proteins (MAPs) and in its phosphorylated form is present in a lot of neurodegenerative disorders, like Alzheimer's disease and in other tauopathies. In order to identify when the alterations start to arise, our study had taken in consideration some critical phases of cerebellar postnatal development (postnatal day 10, 21, 30 and 60). Some of the Purkinje neurons were positive for phosphorylated Tau in both principal branches and cell soma. In those neurons, using MAP2 antibody, we observed a less extended, disorganized and thicker dendritic tree branches, as well as a decreased immunopositivity for calbindin, marker of morphology/functionality of Purkinje neurons. The surrounding basket cells axons, highlighted with neurofilaments marker, were found less structured and had fewer contacts with Purkinje neurons. Since prolidase is involved in ECM remodeling and integrity, it is essential for the correct neu-