Associazione Italiana
Morfologi Veterinari

ROMA

23-24 Maggio 2013

Palazzina dell'Auditorio
Via della Lungara 230

IX CONGRESSO AMV
Roma, 2013

IX CONGRESSO NAZIONALE

Associazione Italiana Morfologi Veterinari

Abstract delle comunicazioni scientifiche

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IX CONGRESSO AMV
Roma, 2013

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Gli abstract dei contributi scientifici sono pubblicati in forma preliminare prima di essere sottoposti a revisione per la pubblicazione definitiva sulla rivista Annals of Anatomy.

1.1 Post-natal development of synaptic connections in the cerebellar cortex of the *Reeler* mouse

***Castagna C**, Aimar P, Alasia S, Gambino G, Lossi L*

Reelin, an extracellular protein that promotes neuronal migration in the brain areas with laminar architecture, is missing in the Reeler mouse (*reeler* $^{-/-}$). Several studies indicate that reelin is also necessary for correct dendrite outgrow and synapse formation in the adult forebrain of mice and humans. More specifically, a Reelin deficit is correlated with a decrease in the number of synaptic contacts and spine density in hippocampus. Our previous observations at the light level in the postnatal and adult *reeler* $^{-/-}$ mouse cerebellum demonstrated a reduction in size, foliation and cortical lamination. We have characterized here the development and synaptic organization of the cerebellar cortex in P0, P5, P10 and P15 Reeler mice and wild type controls by TEM. Our ultrastructural studies confirmed the existence of deep alterations in cortical architecture with difficulties in identifying the inhibitory interneurons and altered migration of the Purkinje cells that remained deeply embedded in the white matter, intermingled with other cortical neurons and the glial cells. There is a general decrease in the density of the synapses made by the parallel fibers, in the number of the climbing fiber boutons on the Purkinje cells, and in mossy fiber rosettes. Some Purkinje cells are devoid of axo-somatic contacts and surrounded by glial laminae. Examination at different post-natal ages indicates that synaptogenesis requires a longer time in mutant mice but shows very similar morphological features, although decreased in total numbers when compared to controls. Our results confirm that reelin affect synaptic connectivity in post-natal mouse cerebellum.

1.2 The brain of the horse

***Cozzi B**, Ballarin C, Pirone A, Granato A*

Most of the studies on the brain of the horse are descriptive reports on the surface configuration of *sulci*, *gyri* and brain weight of single individuals.

For the present study we have sampled 131 horse brains, removed either at the slaughterhouse (# 105) or in the necropsy room of the Dept. of Comparative Biomedicine and Food Science of the University of Padova (# 26). Fresh brains removed at the abattoir were weighed and the data used together with the body weight to produce an *Encephalization quotient* with the formula $EQ=E_i/E_e$ [$E_e=E_i/0.12P^{0.67}$ where E_i and P represent the mean weight of the brain and body, respectively] (Jerison, 1973). Brains removed in the necropsy room were fixed in buffered formalin and used to calculate the percentage weight of the single vesicles. Tissue blocks were also removed respectively from the left *gyrus post-cruciatus* and the left *gyrus occipitalis*: Nissl stained sections were used to obtain automatic threshold algorithm-generated images of the density and "weight" of the cortical layers; immunohistochemistry for calcium-binding proteins was performed to analyze the selected motor and cortical areas. Archival Rhesus monkey brains were used for comparison.

The data obtained show that the weight of the horse brain corresponds to what may be expected for an animal of its mass. Analyses of the cortical column indicate the presence of an apparently 5-layered cortex, with consistent reduction of layer 4. Comparisons with the motor and visual cortex of the monkey reveal a quite different structure with prevalence of pyramidal cells.

1.3 Fos and pERK immunoreactivity in spinal cord slices: comparative analysis of *in vitro* models for testing putative antinociceptive molecules

***Ferrini F**, Russo A, Salio C*

To detect central neuron activation, expression of the transcription factor Fos and phosphorylation of the protein kinase ERK (pERK) can be visualized by immunocytochemistry. These approaches have been extensively used to quantify the activation of nociceptive neurons in the spinal dorsal horn (DH) following peripheral stimulation *in vivo*. Here we propose an alternative and simplified *in vitro* model to investigate Fos and pERK expression based on the stimulation of acutely dissected spinal cord slices to mimic acute inflammatory changes in DH. Transverse slices were obtained from postnatal (P8-P12) CD1 mice and were treated for 5 min with capsaicin (CAP, 2mM). CAP induces a strong release of glutamate from primary afferent terminals which, in turn, excites spinal DH neurons. Since ERK phosphorylation and Fos expression occur following different time frames, two distinct protocols were used to detect their activation. Thus, for studying Fos immunoreactivity CAP-treated slices were left for 3 hours in Krebs solution after stimulation. Instead, for studying pERK immunoreactivity slices were maintained in Krebs solution for only 15 min after stimulation. Both Fos and pERK were significantly up-regulated following CAP challenge. To validate our model we tested the efficacy of octreotide (OCT, 1mM), a synthetic antinociceptive analogue of somatostatin, in preventing the CAP effect on Fos and pERK expression. After CAP, OCT reduced the response to both Fos and pERK. Our data validate the use of Fos and pERK immunoreactivity *in vitro* to investigate the activation of spinal nociceptive pathways and testing potentially antinociceptive molecules.

1.4 Morphological analysis of neurons and astrocytes in primary cultures of fetal bovine cerebellum in response to estradiol exposure

Suman M, Montelli S, Ballarin C, ***Peruffo A***

Estradiol (E2) synthesized de novo by the enzyme aromatase ($P450_{arom}$) acts on neural cells as a neurotrophic factor regulating neurogenesis; cell growth and differentiation; neurotransmitter plasticity and neurochemical expression; exerting its effect in different ways depending on the topographic area of action.New findings over the past decade have established that the cerebellum has an active steroidogenic activity, especially during development. We focused our attention on the trophic effect of E2 on neurons and astrocytes in primary cultures obtained from fetal bovine cerebellum. We estimated the area and the perimeter of neuronal and glial cell bodies, as well as the number, spatial extent, and branching complexity of constituting segments. Differences between cells obtained from male or female fetuses were also considered. Our results suggest that E2 produces a strong trophic effect on neurons but not on astrocytes. Furthermore, we observed that E2 was a stronger activator of neuronal arborizations in primary cerebellar cultures obtained from female fetuses. The wide range of effects associated with sexual brain differentiation is the result of a complex response cascade which is difficult to explore by in vivo research in a large uniparous mammal with a long gestation period. The in vitro model that we propose may represent a performing and standardized system to study morphological changes induced by steroid through brain growth and differentiation.

1.5 Neural stem cells in the cerebral cortex of the domestic ruminant brain

Scala G

The presence of neural stem cells in the cerebral cortex of domestic ruminant (buffalo, cattle and sheep) brains was showed by immunogold-labelling scanning electron microscope (SEM) analysis. Using specific markers, such as CDI33, p53 family (p53, p63 and p73), and MDM2, the respective immunoreactivities were shown in adult neural stem cells in some layers of cerebral cortex. Samples of the cerebral cortex (rostral pole, caudal pole, and dorsal-medial border) were incubated for 1 hr with normal goat serum, subsequently with the primary polyclonal antibodies overnight at 4°C, and then with gold-conjugated goat anti-rabbit IgG for 1 hr at room temperature. The specimens were then fixed by 2.5% glutaraldehyde, subjected to silver enhancement, dehydrated, and examined with a LEO 435 VP at variable pressure in the backscattered mode. The presence of immunogold-positivity was detected in the cerebral cortex as follows:

- Either the molecular or plexiform layers show several spheroid cells with an intense anti-CDI33-immunopositivity;
- The external pyramidal layer shows isolated pyramidal cells with a strong anti-p53 family positivity in form of particles, scattered in perikaryon and dentrites of the neurons.

1.6 Sympathetic innervation of the porcine urethral muscle

Botti M*, Ragionieri L, Gazza F, Bo Minelli L, Panu R*

The striated perineal urethral muscle (UM) is involved in the voluntary control of the micturition that necessitates complex interactions between afferent and efferent (autonomic and somatic) pathways to storage and periodically eliminate the urine. Our aim was to define the site, cross sectional area and phenotype of sympathetic trunk ganglia (STG) neurons projecting to the porcine UM, combining the retrograde neuronal tracer Fast Blue (FB) and the double immunohistochemical labeling. The research was carried out on 3 male intact pigs, in which we counted a total number of 4992.67±834.35 (mean ± S.E.M., n=3) FB+ neurons distributed in the bilateral T12-S3 STG. These neurons were significantly larger in lumbar STG than the sacral ones. Moreover we highlighted the presence of Dopamine b hydroxylase (DbH), Vesicular Choline Acetyl Transferase (VAChT), neuronal Nitric Oxide Synthase (n-NOS), Calcitonine Gene Related Peptide (CGRP), Leu-Enkephaline (LENK), Neuropeptide Y (NPY), Substance P (SP), Vasoactive Intestinal Polypeptide (VIP) and Somatostatine (SOM) and their possible co-existence with Tyrosine Hydroxylase (TH) in both lumbar and sacral FB+ neurons. In particular lumbar and sacral STG neurons expressed similar percentages for TH, SP and CGRP, but showed significantly different levels of immunoreactivity for NPY, VIP, VAChT, LENK, nNOS, DbH and SOM.

Taken together, these data indicate a probably different contribution of lumbar and sacral pathways in the sympathetic transmission to the boar UM.

2.1 Mechanisms involved in inter-lineage conversion and differentiation of porcine fibroblasts

***Brevini TAL**, Pennarossa G, Maffei S, Gandolfi F*

We previously demonstrated that porcine fibroblasts exposed to 5-aza-cytidine (5-aza-CR), an inhibitor of DNA methylation, increase their plasticity and can be re-addressed to pancreatic lineage.

Here we investigate the mechanisms involved in the acquisition of a higher plasticity state by 5-aza-CR treated fibroblasts. Furthermore we characterized the cellular and molecular events driven by the differentiation protocol following thereafter. Cells were analyzed at different time points: untreated fibroblasts, after 5-aza-CR exposure and then on day 1-2-3-4-5-6-7 of pancreatic induction. DNA global methylation modifications were evaluated with an antibody against 5-Methylcytidine used for DNA dot blot analysis and immunolocalization studies. Cells were also immuno-characterized with primary antibodies against Vimentin, Oct4, Nanog, Sox17 and Hnf4, and gene expression level changes were evaluated in parallel. Our results show that 5-aza-CR induced a decrease in 5- Methylcytidine positivity that gradually returned to the levels observed in untreated fibroblasts within 3 days. Consistently, a down-regulation of vimentin was observed, together with an increase of Oct4 and Nanog, which remained clearly expressed until day 4 and were down-regulated thereafter. At the same time Sox17 and Hnf4, involved in the induction of definitive endoderm and primitive gut tube, respectively, displayed a reverse trend with a signal becoming gradually more intense.

Our observations demonstrate that 5-aza-CR effect on DNA methylation is transient and initial levels are restored within 3 days. The combined and sequential action of the molecule with an induction protocol enables efficient inter-lineage conversion and controlled cell differentiation.

2.2 Membrane vesicles derived from horse multipotent mesenchymal stromal cells (MSCs): a morphological and functional study

***Pascucci L**, Dall'Aglio C, Alessandri G, Petrini S, Ceccarelli P*

Multipotent mesenchymal stromal cells (MSCs) assumed a great interest in the last decades, due to several distinctive features such as the ability to home to damaged tissue, the modulation of immune response and the participation in regenerative processes. The observation that only a small number of transplanted MSCs integrate and survive in host tissues, has highlighted the possibility that they may predominantly act by a paracrine mechanism. MSCs actually produce a plethora of trophic factors, cytokines and signaling molecules able to create the optimal environmental conditions for tissue regeneration, including neoangiogenesis, inhibition of apoptosis, activation of resident stem cells (Horwitz, 2009). This paracrine action may be determined, at least in part, by the horizontal transfer of molecules through membrane-bounded vesicles (MVs) released as exosomes from the endosomal compartment, or as shedding vesicles from the cell surface (Gyorgy, 2011). In this study, we isolated by ultracentrifugation (100,000g) MVs derived from supernatants of equine adipose-derived MSCs and we characterized them by transmission and scanning electron microscopy. An evaluation of their ability to affect angiogenesis was subsequently performed by means of *in vitro* functional assays. Electron microscopy revealed that MV morphology and size are quite heterogeneous. We additionally observed that MVs may affect migration of endothelial cells and formation of neovessels. As a consequence of these results, it could be hypothesized that MVs released from MSCs have the potential to be exploited in novel therapeutic approaches as an alternative to cell-based procedures.

2.3 New regenerative strategies for tendon repair and regeneration

*Martinello T, Perazzi A, Iacopetti I, Vindigni V, Bassetto F, **Patruno M***

In recent years tissue engineering techniques and cell-based therapies improved regenerative outcomes. Both approaches are important for treating frequently occurring conditions as traumatic rupture or tendinopathies and attempt to improve the intrinsic repair mechanism that mimics embryonic tendon development. For instance, the injection of mesenchymal stromal cells (MSC) and/or platelet rich plasma (PRP) into the core of a damaged tendon aims to enhance and support regenerative mechanisms that occur naturally in the animal body. Moreover, our results confirmed once more the great "flexible" nature of MSC since they can be injected in a decellularized tendon, obtained from cadaveric tissues, and survive in the resulting scaffold for a couple of weeks. In this report we summarize the most significant results and future strategies of our research into two main sections, one devoted to bioscaffold technology for curing complete tendon tears (Martinello T. et al 2012) and one dedicated to the use of adult stem cells and PRP for treating overstretching lesions of tendons (Martinello T. et al 2013, Renzi S. et al 2013).

We underline the fact that MSC obtained from different tissutal sources are safe and have the potential to enhance functional recovery in equine injuries although molecular mechanisms are still to be elucidated. Likewise, the real efficacy of PRP is still under debate in the orthopaedic field of both human and veterinary medicine. Therefore, we suggest increasing the number of clinical and experimental cases in a long-term follow-up period for evaluating the re-injury percentages and analyse the histological and molecular parameters of the healed tissues (Patruno M & Martinello T, *in press*).

2.4 Cell behavior and cellular uptake of SiC/SiO2 nanowires in two different cell models

Cacchioli A, ***Ravanetti F****, Pinelli S, Alinovi R, Galletti M, Rossi F, Attolini G, Salviati G*

Nanomedicine is the application of nanotechnology to medicine and in particular to the diagnosis and treatment of diseases. Our goal is to develop a nanosystem, composed by 3C-SiC/SiO2 nanowires (NWs) and superparamegnetic Fe3O4 nanoparticles, functionalized with partially-fluorinated Tetraphenylporphyrins, for concurrent photodynamic therapy, hyperthermia and magnetic resonance imaging as deep cancer therapy. A549 and THP-1 cell lines were chosen as model to investigate both the cytoxicity and the uptake of the SiC/SiO2 NWs at increasing concentrations. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to analyse the cellular uptake of NWs. Both MTT and Tripan Blue assay showed that NWs were slightly toxic only at the highest concentration tested. For the SEM and TEM analysis morphological and ultrastructural parameters were qualitatively and quantitatively considered and no significant alteration in either A549 or THP-1 cells occurred. Concerning the cell to nanowires interactions the SEM analysis showed cytoplasmic protrusions resulting in the contact of both a single NW and/or NWs agglomerates with the cell membrane. The TEM analysis showed the effective internalization of NWs in both A549 and THP-1 cells through two different mechanisms: pinocytosis and phagocytosis. In both cell lines the NWs close to the cell membrane proximity were uptake by short cytoplasmic processes and internalised into the cell as small vesicles with a pinocytosis mechanism. Moreover, THP-1 cells showed the presence of cytoplasmic processes which cover the NWs indicates an aspecific phagocytosis mechanism. Finally, regardless the internalisations mechanisms, big vesicles overcrowd of NWs were the result of their compartmentalisation within cells.

2.5 Expression of CYP4 and GSTr genes in *R. philippinarum* exposed to benzo(a)pyrene

Boscolo Papo M*, Maccatrozzo , Bertotto D, Negrato E, Binato G, Pascoli F, Radaelli G*

Bivalve mollusks, such as *R. philippinarum,* are often used as bioindicators of environmental pollution. The Polycyclic Aromatic Hydrocarbon (PAH) Benzo(a)Pyrene (BaP) is an important contaminant, commonly present in the marine environment. Pollutants are generally metabolized by enzymes of phase I, mainly CYPs enzymes, and by conjugation enzymes of phase II like GST. In this study, we investigated by Real Time PCR the expression of CYP4 and GSTr (GST class rho) in the digestive gland of *R. philippinarum* subjected to BaP exposure. To evaluate the effect of both acute exposure and depuration, mollusks were exposed to different concentrations of BaP (0.03 mg/l, 0.5 mg/l, 1 mg/l) and after 24h of exposure half of the animals were sampled and the remaining clams were subjected to a 24h depuration. The exposure of clams to all tested BaP concentrations has been confirmed by the Fluorous-HPLC analysis. Moreover, F-HPLC analysis evidenced that after depuration, BaP concentrations decreased in animals subjected to 0.03 mg/l and 0.5 mg/l exposures but did not decrease in animals subjected to 1 mg/l exposure. The analysis of GSTr expression showed a significant increase of mRNA in animals subjected to 1 mg/l exposure, whereas the analysis of CYP4 expression did not evidence differences among treatments. Moreover, the expression of both genes did not exhibit any differences after the depuration treatment. The results demonstrate that BaP significantly affected the expression of GSTr mRNA in the digestive gland of *R. philippinarum* and suggest that GSTr gene could play an important role in the biotransformation of BaP.

3.1 Anatomo-topographic and morphometric study of the jejunal arteries in the cat

Grandis A*, De Sordi N, Spiteri J, Canova M, Gardini A, Bombardi C*

The jejunal arteries are not profoundly studied in veterinary medicine, especially those regarding cats. Consulting the literature, it was found that most authors referred to carnivores or treated only the dog, while only few studies were specifically related to the cat. In particular, it is not well known how the jejunal vasculature is distributed along the jejunum. The purpose of this study was to clarify the number of jejunal arteries and their respective length, the correspondent number of orders of divisions, the length of the cranial mesenteric artery, the length of the jejunum and, finally, the distribution of the arteries in each respective third of the jejunum.

From the present study, it was seen that the number of jejunal arteries, varied from a minimum of 6 to a maximum of 11. These numbers are independent from the length of the cranial mesenteric artery as well as from the length of the jejunum. Furthermore, it was found that the shortest jejunal arteries were more represented in the first third of the jejunum. They presented the highest number of orders of divisions and of terminal branches. On the long run, both parameters, tended to decrease as they approached the terminal tract of the jejunum, and contemporarily, the number of jejunal arteries, increased. From this different distribution of jejunal arteries, it is possible to speculate a different sensitivity of the jejunal tracts against ischemia.

3.2 Expression and distribution of leptin and ghrelin in the digestive apparatus of DIO (diet-induced obesity) zebrafish

***Maruccio L**, Russo F, Arcamone N, Mania M, Randazzo B, Ciriaco E*

In this report we analyzed the expression and distribution of leptin and ghrelin in the digestive apparatus of DIO zebrafish. Leptin is anorexigenic peptide while ghrelin is orexigenic one. They act as antagonists. In this study in order to develop an obesity phenotype adult zebrafish were divided into two dietary groups. The control group was fed with *Artemia* (5 mg cysts/fish/day) once per day. The overfeeding group was fed three times per day with *Artemia* (60 mg cysts/fish/day). For calorie restriction, the zebrafish was fed with *Artemia* (2.5 mg cysts/fish/day) for 2 weeks after being overfed for 8 weeks. At the end of this period, after 24h of fasting, the zebrafish digestive apparatus were sampled for immunohistochemistry, western blotting and qRT-PCR techniques. Leptin and ghrelin immunoreactivity were found in the enteric nervous system and neuroendocrine cells in overfeeding and control zebrafish intestine. The number of immunopositive cells is greater in DIO zebrafish than in control ones. In both groups leptin immunoreactive nervous fibers were found around ducts and vessels of liver. The presence of proteins were confirmed by western blotting analysis. By qRT-PCR, leptin and ghrelin mRNA levels are higher in the overfeeding zebrafish intestine and in control zebrafish liver. The immunological detection of ghrelin and leptin in control zebrafish are in agreement with literature data. In DIO zebrafish the results are similar to those found for obese mammals. In conclusion, this study represent a starting point to investigate the mechanisms involved in the regulation of appetite and energy balance in DIO zebrafish.

3.3 Enteroendocrine profile of α-transducin immunoreactive cells in the gastrointestinal tract of the European sea bass (*Dicentrarchus labrax)*

***Mazzoni M,** Latorre R, Gatta PP, Chiocchetti R , Vallorani C, Bonaldo A, De Giorgio R, Sternini C, Clavenzani P*

In vertebrates, chemosensitivity of nutrients in the gastrointestinal (GI) tract is likely to involve the activation of taste receptors coupled with G-protein subunits, including α-transducin ($G_{\alpha tran}$) and α-gustducin ($G_{\alpha gust}$). This study was aimed at characterizing the cells expressing $G_{\alpha tran}$-immunoreactivity throughout the mucosa of the sea bass GI tract. $G_{\alpha tran}$-IR cells in the stomach were 15.7 ± 2.2, while there were 3-4 IR cells / 200 folds and 1-2 IR cells / 200 folds in the cranial and middle-caudal portions of the intestine, respectively. Some $G_{\alpha tran}$ immunoreactive cells in the stomach contained $G_{\alpha gust}$. Gastric $G_{\alpha tran}$ cells co-expressed ghrelin, obestatin and 5-hydroxytryptamine immunoreactivity. In contrast, $G_{\alpha tran}$ cells did not contain somatostatin, gastrin/cholecystokinin, glucagon-like peptide-1, substance P, and calcitonin gene-related peptide immunoreactivity in any investigated segments of the sea bass gastrointestinal tract. Specificity of $G_{\alpha tran}$ and $G_{\alpha gust}$ antisera was determined by Western blot analysis, which identified two bands at the theoretical molecular weight of ~45 and ~40 kDa, respectively, in sea bass gut tissue as well as in positive tissue, and by immunoblocking with the respective peptide, which prevented immunostaining. The results of the present study demonstrate that G proteins involved in chemosensory transmission are expressed in the sea bass GIT enteroendocrine system. Nutrients may elicit the release of different bioactive messengers (mainly peptides), which directly, or via neural reflexes, contribute to the control of GIT functions and nutrient intake of this fish.

Orexigenic peptides in bottlenose dolphin enteric nervous system

***Russo F**, Gatta C, Varricchio E, Russolillo MG, Giurisato M, Cozzi B*

In this report, we described the immunological detection of orexin system and neuropeptide Y (NPY) in the enteric nervous system (ENS) of bottlenose dolphin. Orexin A (OXA) and orexin B (OXB) derive from the same precursor prepro-orexin and bind two ligands: orexin 1 receptor (OX_1R) and orexin 2 receptor (OX_2R). They are notoriously involved in the regulation of food intake, and together with NPY increase food uptake. Samples of gastrointestinal tract of three animals, stored at the Mediterranean marine mammal tissue bank of the University of Padova, were analyzed by immunohistochemistry and western blotting analysis. Numerous OXA- and NPY- immunoreactive neurons and nervous fibers in submucosal and myenteric plexuses of a) mainstomach; b) pyloric stomach; and c) duodenum were detected. OX_1R immunopositive neurons and nervous fibers in the ENS of all gastrointestinal tracts were found. OX_2R immunoreactive nervous cells and fibers in the ENS of gastric compartments were observed. The presence of prepro-orexin and orexin receptors was detected and confirmed by western blotting analysis. The immunolocalization of orexin system in the gastrointestinal tract is considerably different from species to species. In terrestrial mammals, they perform many digestive functions. In fact, they regulate gastroduodenal secretion and induce motility of the different intestinal tracts. The presence of the orexin system and NPY in the ENS of bottlenose dolphin maybe interpreted as an additional mechanism of action through which the ENS can operate to control gut functions of this polygastric sea mammal.

4.1 Nerve Growth Factor in the adult brain of a teleostean model for ageing research: *Nothobranchius furzeri*

***D'Angelo L**, Castaldo L, Cellerino A, de Girolamo P, Lucini C*

Nerve growth factor (NGF) is a pluripotent mediator, originally considered important in neuronal homeostasis and pathophysiology, later it was also implicated in the ageing processes. In mammals, NGF is viewed as important for the survival of forebrain cholinergic neurons, involved in cognitive function, and appears to degenerate with age and markedly diminish in Alzheimer's disease. The age-related decrease of NGF occurs particularly in memory-linked areas such as the hippocampus. Also in non mammalian vertebrate models, NGF changes in age-related degeneration and increases in various pathological lesions. This study analyzes the localization of NGF in the brain of *N. furzeri,* a model for ageing research. Experiments of *in situ* hybridization (ISH), by using a LNA probe, and immunohistochemistry (IHC), by employing an antibody mapping at the N-terminus of the mature chain of NGF of human origin, were performed. NGF mRNA expression was observed in the neuronal perikarya of all major regions of the brain, with the highest rate of signal probe in the telencephalon (mainly in the subpallial telencephalon). The protein was detected in the cytoplasm of neurons and, to a less extent, in fibers, both diffused throughout the telencephalon, diencephalon, mesencephalon and rhomboencephalon. The widespread distribution of NGF mRNA and protein suggests that the NGF system may modulate numerous physiological functions also in the adult brain of fish. The present survey constitutes a baseline study to enhance the understanding of the mechanisms underlying the role of NGF during ageing processes.

4.2 Brain derived neurotrophic factor in the retina of *Nothobranchius furzeri*

***Gatta C**, Castaldo L, Cellerino A, de Girolamo P, Lucini C, D'Angelo L*

Brain-derived neurotrophic factor (BDNF) is an important neuroprotective factor belonging to the neurotrophin family whose effects are mediated by TrkB receptor. This functional system is involved in the biology of the vertebrate retina. BDNF exerts its survival-promoting effects on retinal photoreceptors and is critically involved in a variety of retinal degenerative diseases in animal models. It has been suggested also to aid in retinal recovery after reattachment and in ganglion cell regeneration following optic nerve injury. Recently, the teleost *Nothobranchius furzeri* has emerged as a model for neurobiological and age research, due to the exceptionally short lifespan, age-dependent cognitive/behavioral decline and expression of age-related biomarkers. The aim of this study is to investigate BDNF pattern of expression in the retina of the *N. furzeri*. The occurrence of BDNF was detected by in situ hybridization (ISH) and immunohistochemistry (ICH), by employing respectively a riboprobe and an antibody mapping an epitope of an internal region of BDNF. BDNF mRNA was expressed in cell bodies of all the layers of the retina, especially in the photoreceptors. BDNF protein was observed in cell processes of all retinic layers, mainly of the outer plexiform layer, but it was lacking in photoreceptors. These results suggest a retinal synthesis of BDNF and a highly trafficking of the protein by retrogradal/anterogradal transport in the retinal network. In conclusion, BDNF fairly appears to be involved in the retinal functions of *N. furzeri*.

4.3 The BDNF/TrkB system in the zebrafish retina under normal and experimental conditions

***Guerrera MC**, Sanchez Ramos C, CoboT, Madrigrano M, Vega JA, Levanti MB, Germana P, Germanà A, Ciriaco E*

The BDNF/TrkB system regulates different features of neuronal development and, particularly, plays a key role in the molecular mechanisms underlying the development, differentiation and maturation of photoreceptors and retinal nerve circuits. Therefore, the aim of this study was to analyze the role of the BDNF/TrkB system in the biology of the zebrafish retina used as an experimental model. The expression levels and the cellular localization of BDNF and TrkB in the zebrafish retina under normal conditions and after exposure to different lighting conditions, natural photoperiod (control), continuous exposure to different wavelengths (white light, white blue-and blue), and darkness for 10 days have been analyzed using qRT-PCR, Western blot and immunohistochemistry techniques. The results demonstrated that the expression of BDNF and TrkB in the retina of zebrafish remains constant from the first stages of development to adulthood, while the exposure to different wavelengths causes a decrease of BDNF mRNA and of BDNF immunostaining. On the contrary the expression of TrkB mRNA was upregulated and TrkB immunoreactivity increased. The exposure to continuous darkness determines a decrease of the expression levels of mRNAs for BDNF and TrkB and the absence of the immunoreaction for BDNF but not for the TrkB. The results, taken together, demonstrate that light regulates the expression of the BDNF/TrkB system in the zebrafish retina and might contribute to better understand different aspects of the complex pathophysiology of retinopathies induced by light.

4.4 Dose-dependent effect of aminoglycoside treatments on hair cells of transgenic ET4 zebrafish

***Montalbano G**, Abbate F, Laurà R, Madrigrano M, Mania M, Randazzo B, Germanà A*

In human, aminoglycoside treatment disrupts inner ear hair cells leading to hearing and balance disorders. It is well known that the mammalian inner ear does not present regenerative capacity while the fish hair cells located in the inner ear and in the lateral system have an high capacity of regeneration. The hair cells of the lateral line system of zebrafish resemble for their morphology and molecular aspect those present in the human inner ear. For this reason the zebrafish lateral line offers several advantages that make it a powerful animal model for studying hair cells regeneration and ototoxicity drug screening. In the present study we examined the dose-dependent effects of two aminoglycosides, neomycin and gentamicin, in the hair cells of the lateral line in the ET4 transgenic line. The fishes were treated with the aminoglycosides for 24h at different dose levels. Immediately after treatment the morphological observation and the counts of the neuromast hair cells have been made on L1 (First Lateral Neuromast located in the trunk) and T1 (First Terminal Neuromast located in the fin). The results show that neomycin and gentamicin have different effect on the hair cells death at the same concentration, showing also different toxicity in L1 neuromast and T1 neuromast. The toxicity observed in the hair cells of T1 neuromast was less than in L1 neuromast, with particular reference to the gentamicin treatment. The morpho-functional evidence of this data provides a strong support to the use of the zebrafish as a pre-clinical indicator of drug-induced ototoxicity.

4.5 Mature olfactory crypt neurons in adult zebrafish express S100 protein and lack TRPV4

***Parisi V**, Guerrera MC, Abbate F, Garcia Suarez O, Vina E, Vega JA, Germanà A*

The peripheral olfactory system of vertebrates contains olfactory receptor neurons (ORNs) that convey information to the central nervous system. In teleosts three types of ORNs have been identified denominated ciliated, microvillous, and crypt neurons. Crypt neurons can be distinguished on the basis of their morphology, the expression of different families of odorant receptor molecules, the presence of receptors for neurotrophic factors, the occurrence of cytosolic Ca^{2+}-binding proteins and ion channels, and the central projections to the central nervous system. The zebrafish is an attractive model for studying the cellular and molecular basis of behaviours in which the olfaction plays a key role. S100 protein and the ion channel TRPV4 have been detected in heterogeneous olfactory cells and both are candidates to be present in crypt neurons. Here we have investigated the occurrence of S100 protein and TRPV4 in the olfactory crypt neurons of adult zebrafish (*Danio rerio*) using double immunofluorescence associated to laser confocal microscopy. Calretinin (CR) was used as a specific marker for all crypt neurons. An additional ultrastructural study was carried out. CR positive cells were found in the sensory segment of olfactory lamellae while S100 positivity was restricted to a subpopulation of superficial mature crypt neurons, and non mature crypt cells were CR+/S100P-. Therefore, the acquisition of S100 protein is an evidence of crypt neurons maturation. Conversely TRPV4 identified ORNs but never co-localise with CR, thus excluding the presence of this ion channel in the crypt neurons. The functional significance of these findings remains to be elucidated.

5.1 Morphological and glycan features of the dromedary oviduct epithelium

***Accogli G**, Monaco D , El Bahrawy KA, El-Sayed A, Ciannarella , Beneult B, Lacalandra GM, Desantis S*

The mammalian oviduct is not a simple conduit of the female reproductive tract, but it plays an essential role in the mammalian reproduction. Its epithelial non-ciliated cells secrete glycoproteins in the lumen, which, together with a selective transudate of serum, constitute the oviduct fluid in which gamete transport, maturation, fertilization and early embryonic development occur. Morphology and glycan composition of oviduct from one-humped camel (*Camelus dromedary*) have been scarcely studied in comparison with other livestock species. In this study we investigated the regional differences in the dromedary oviduct by means of scanning electron microscopy (SEM) and lectin histochemistry. Oviducts from mature phase dromedaries were cut into small pieces and processed for SEM and lectin histochemistry. Morphometrical analysis revealed that the epithelial height gradually increased from infundibulum to utero-tubal junction (from 18,19 ± 45 µm to 23,12 ± 52 µm). At SEM numerous, region-specific and elaborated branched folds of the mucosa were observed and the lining epithelium consisted of ciliated and non-ciliated cells, although in the fundus of the folds the ciliated cells predominated. Non-ciliated cells were more numerous in the isthmus and the utero-tubal junction and most of them showed blebs protruding from the apical surface. N- and O-linked sialoglycans (MAL II,SNA and KOH-sialidase-PNA reactivity) were described throughout the oviduct luminal surface. They were synthesized and secreted by the non-ciliated cells that in their supranuclear cytoplasm of isthmus and utero-tubal junction tracts also bound PNA, revealing the O-linked mucin type asialoglycans. This lectin binding pattern indicates a species-specific glycan content.

5.2 Immunohistochemical identification of leptin and its receptor in bovine testis

***Dall'Aglio C**, Mercati F, Scocco P, Ceccarelli P*

Leptin is a protein encoded by the Obese gene and mainly produced by fat cells, which, interacting with its receptor in the hypothalamus, controls metabolism and appetite. The observation that mice genetically deprived of leptin, in addition to being obese, are also sterile and that the exogenous administration of leptin to these same animals is capable of restoring fertility, has led to the hypothesis that this hormone may play a control on reproduction. Recently, some studies have shown the presence of leptin and its receptor in human and rat testis. Starting from these observations, we investigated the presence of leptin and its receptor in the testis of cattle using immunohistochemical techniques. In particular, leptin was detected in cells of the epithelium of the seminiferous tubules mainly in the basal third within the Sertoli cells, spermatogonia and spermatocytes. The localization of the immuno-positivity that affects only the nucleus is very peculiar and reflects what is present in the literature concerning rats. On the contrary, the Leydig cells are negative. Instead with regards to the receptor, positivity is localized in the cytoplasm of the Leydig cells while the epithelial cells of the seminiferous tubules are negative. In conclusion, the identification of leptin and its receptor in the testis of cattle reflects what has already been described in humans and in laboratory animals and highlights, also in this animal species, the importance of this molecule in the control of gonadal function.

5.3 The urocortinergic system in the rat epididymis

***Liguori G**, De Luca A, Squillacioti C, Paino S, Langella E, Alì S, Mirabella N*

Urocortin (UCN), a 40 amino acid peptide, is a corticotrophin-releasing hormone (CRH)-related peptide. The biological actions of CRH family peptides are mediated via two types of G protein-coupled receptors, CRH type 1 receptor (CRHR1) and CRH type 2 receptor (CRHR2). The biological effects of these peptides are mediated and modulated not only by CRH receptors but also via a highly conserved CRH-binding protein (CRHBP). The aim of the present study was to investigate the expression of UCN, CRHR1, CRHR2 and CRHBP by immunohistochemistry, western blot and real- time RT-PCR in the rat epididymis. The results showed that UCN, CRHR1 and CRHR2 were expressed in all segments of the rat epididymis, whereas CRHBP was not expressed. Specifically, UCN- and CRHR2- immunoreactivities (IRs) were distributed in epididymal epithelial cells of the caput, corpus and cauda. CRHR1-IR was distributed in the fibromuscular cells surrounding the epididymal tubules and in smooth musculature of the blood vessels throughout the organ. UCN and CRHR2 mRNA expression levels were higher in the caput and corpus than in the cauda, while CRHR1 mRNA levels were higher in the cauda than in the caput and corpus. These results suggest that UCN, CRHR1 and CRHR2 are expressed in the rat epididymis and that CRH-related peptides might play multiple roles in maturation and storage of spermatozoa by regulating, via CRHR2, the growth of epididymal epithelial cells, epididymal hormonal secretion and passage of spermatozoa throughout the epididymis by inhibiting the contractility of epididymal fibromuscular stromal cells via CRHR1.

A5.4 ssessment of cellular damage in sheep ovaries subjected to different freezing methods

***Maffei S**, Pennarossa G, Brevini TAL, Gandolfi F*

Whole ovary cryopreservation is currently under study for preserving female fertility but may result in DNA damage, even in the absence of evident morphological changes.

We used immunolabelling and image analysis to evaluate the expression of γH2AX (marker of DNA damage) and of RAD51 (marker of DNA repair) molecules that allow a sensitive assessment of cellular damage, and compare the effect of conventional (CF) and directional freezing (DF) on sheep whole ovary.

Fresh controls and thawed samples previously subjected to CF or DF freezing, were cut in 2x2x1 mm pieces and cultured for 7 days. They were then fixed in formalin, embedded in paraffin and sectioned. Slides were incubated over-night with primary antibodies and then with fluorescent secondary antibodies for 45 minutes. Sections were observed under an Eclipse-E600 microscope. Images were acquired with Nis-Elements-Software and analyzed using ImageJ-Software.

As expected, no γ-H2AX and RAD51 signals were detected in fresh control tissue. Conversely, γ-H2AX signal was observed in frozen samples, immediately after thawing. Its intensity was significantly stronger in CF than in DF samples. After 7 days of culture, γ-H2AX signal decreased in DF ovaries concomitantly with an increase of RAD51. By contrast, CF ovaries showed no signal for RAD51 leaving the rate of DNA damage unchanged.

The results obtained show that cryopreservation induces DNA damage with both methods, however, the ability of DF tissue to repair DNA damage demonstrates that this method allows a better preservation than CF.

5.5 The presence of neurotrophins and their specific receptors in adult and developing Japanese quail ovaries

***Nechita E-L**, Arcamone N, Maruccio L, Solcan C, Cotea C*

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are members of neurotrophins family, which are involved in growth, maintaining, differentiation and survival of neurons in the central and peripheral nervous system. The activity of these neurotrophins is elicited in systems other than nervous, such as reproductive and endocrine systems. In mammals, neurotrophins and their receptors TrkA, TrkB, TrkC play a critical role in oogenesis and folliculogenesis, promoting the follicular development and oocyte maturation, also during early embryonic development. In this study we report the presence of NGF, BDNF, NT-3 and their specific receptors in adult and embryonic quail gonads. The investigations were conducted on embryonic stages from 4th to 17th day of incubation and on adult laying quail ovaries. Immunohistochemical Envision technique was used. The immunoreactivity (ir) was observed from 6th day of embryonic development. Neurotrophins and their receptors were differently distributed in cortical and medullary areas. The localization of NGF, NT3 and Trk receptors-ir was differently observed in cytoplasm of oocytes, follicular cells and stromal cells, both in adult and embryonic quail ovaries from the 9th day of incubation, while BDNF ir was observed only in adult. The results obtained confirm the involvement of neurotrophins during the development of gonads in quails, as well as in mammals. Furthermore, the occurrence of neurotrophins and the receptors could provide new insights on their possible paracrine and/or autocrine mode of action in quail ovary during development and folliculogenesis.

5.6 Intercellular bridges functionally connect parthenogenetic cells

***Pennarossa G**, Maffei S, Gandolfi F, Brevini TAL*

We previously reported that parthenogenetic cells display abnormal centrosome and spindle formation resulting in chromosome malsegregation and a high incidence of hypoaploidy. Unexpectedly this is not accompanied by a correspondingly high rate of apoptosis. We hypothesize that a series of adaptive mechanisms make this possible. The presence of intercellular bridges may represent one of such mechanism and would provide a strategy for mutual exchange of missing cell products, alleviating the unbalanced chromosome distribution.

The presence of intercellular bridges was investigated in pig parthenogenetic cells by transmission and scanning electron microscopy. For the former, cells were fixed in 2% glutaraldehyde and post-fixed in 1% osmic acid. After standard dehydration, samples were embedded in an Epon-Araldite 812 mixture, sectioned and observed with a Jeol 1010 electron microscope. For scanning electron microscopy cells were covered with a 9 nm gold film by flash evaporation of carbon and examined with a SEM-FEG Philips XL-30 microscope. Functional trafficking activity was demonstrated with fluorescent 10-kDa dextran. The tracking molecule was injected into the cytoplasm of a single cell with FemtoJet Microinjector and its movement was monitored.

Ultra-structural analysis of parthenogenetic cells showed the presence of intercellular bridges that ensured cytoplasmic continuity among cells. Furthermore extensive movement of 10-kDa dextran demonstrated functional intercellular trafficking through these canals suggesting their use for transfer of mRNAs, proteins and ribosomes among cells. Our results demonstrate that parthenogenetic cells present a wide network of functional intercellular bridges that may constitute an adaptive mechanism to support normal cell functions.

6.1 Anatomical study of the muscles of the shoulder, arm and forearm in three species of wild birds

***Canova M**, Bedoni C, Rambaldi F, Grandis A, Clavenzani P*

In the past many authors have focused on the anatomical study of the wing in order to correlate anatomical details with the peculiarities of flight in different species. In spite of the limited information about the anatomy of the thoracic limb in European avian species, we decided to investigate these structures in three species presenting a different kind of flight spread throughout the Italian territory: the Grey Heron (*Ardea cinerea*), the Eurasian Buzzard (*Buteo buteo*) and the Common Kestrel (*Falco tinnunculus*).

We performed a stratigraphic dissection of the wing in different subjects of the species examined.

Comparing the results of this study with those found in literature for similar species, we observed many peculiarities which have not been previously described. The most relevant was that involving the *Coracobrachialis caudalis* muscle, the *scapular* and *humeral anchor* and the *Extensor radialis carpi* muscle. The *Coracobrachialis caudalis* muscle in the Grey Heron is composed of two different heads instead of the typical one observed by Vanden Berge (1970) in other *Ciconiiformes*. Regarding the *scapular* and the *humeral anchor* the different development found through the species suggests a correlation between these structures and the kind of flight. Concerning the *Extensor radialis carpi* muscle, the differences we found in the number of bellies could support Nair's hypothesis (1954) about a correlation between the heads of this muscle and different types of flight such as soaring of flapping. These deductions should be confirmed by further studies in wind tunnel and electromyography.

6.2 Structure and innervation of retia mirabilia of Bottlenose dolphin (*Tursiops truncatus*)

***Gardini A**, Chiocchetti R, Giancola F, Mazzoni M, Bombardi C*

The cetaceans *retia mirabilia* represent a vascular network that supplies a preferred vascularization to organs that require high amounts of oxygen during diving. The present study, conducted in the perispinal *retia mirabilia* of bottlenose dolphin (*Tursiops truncatus*), analyzed: nature of the blood vessels; innervation by the tyrosine hydroxylase (TH)-, neuronal nitric oxide synthase (nNOS)-, and substance P (SP)-immunoreactive (IR) fibers; co-localization in the nerve fibers of the antigens mentioned above. The results demonstrated that the perispinal *retia mirabilia* consists of arterial vessels (muscular arteries and arterioles) and, minimally, of venous vessels. In all types of vessels, the innervation was supplied mainly from TH-IR fibers. Even nNOS-IR fibers were numerous, especially at the level of arterioles; on the contrary, SP-IR fibers were observed in a small number of vessels. The fibers were located mainly in the *tunica adventitia*, but also between *media* and *adventitia tunicae*. This research indicates that the perispinal *retia mirabilia* of bottlenose dolphin is innervated by sympathetic system (TH-IR fibers) and primary afferent neurons of the spinal ganglia (nNOS- and SP-IR fibers).The sympathetic system probably induces vasoconstriction and vasodilation by increasing and lowering its level of activity, respectively. Sensory fibers, especially through the local release of nitric oxide, are probably involved in the regulation of vasodilatory processes. Since the sympathetic fibers appear in greater numbers than those containing nNOS and SP, it is reasonable to hypothesize that the vasodilatory activity of primary afferent fibers can be realized only by means of a simultaneous inhibition of sympathetic tone.

6.3 Expression of Myosin Heavy Chain isoforms in laryngeal muscles of mammals of veterinary interest in comparison with humans

***Maccatrozzo L**, Toniolo L, Cancellara P, Patruno M, Reggiani C, Mascarello F*

The larynx of mammals is characterized by five intrinsic laryngeal muscles with complex movements involved in respiration, airway protection and phonation. These muscles, differently from limb and trunk muscles that derived from somites, originate from the fourth and the sixth branchial arches. In horses and cows the laryngeal muscles express only the three adult skeletal MyHC isoforms (type 1, 2A and 2X) (Toniolo et al., 2005; Rhee et al., 2009); other species (dog, cat and tiger) express also a faster isoform, not detectable in skeletal muscles, the 2B isoform (Wu et al., 1998; Bergrin et al., 2006; Toniolo et al., 2007). To make matter more complicated, in species where the 2B isoform is present in skeletal muscles (rats and rabbits), another isoform presumably faster is present, the EO MyHC (Lucas et al., 1995; Briggs and Schachat, 2000). In the laryngeal muscles of rats and humans a different isoform (IIL MyHC) was described (DelGaudio et al., 1995; Toniolo et al. 2008) but it is unclear if this new isoform corresponds to the EO of rats or to the 2B of humans or to the two "novel/ancient" isoforms identified by Rossi et al. (2010) in EO muscles. Combining RNA expression, SDS-gel electrophoresis and immunoblotting we have finally demonstrated that the L isoform of humans does not correspond to the type 2 isoforms (2A, 2X, 2B, EO, embryonic and perinatal cluster), to the cardiac isoforms (alpha and beta/1), to the M isoform and to the two novel isoforms and therefore is probably a new isoform.

6.4 Leptin receptor identification in canine skin

***Mercati F**, Pascucci L, Scocco P*

Leptin is a polypeptide secreted by adipose tissue regulating appetite and energy consumption. It acts by binding with a specific receptor that is expressed in various tissues suggesting that leptin might exert diverse biological functions other than energy metabolism. Accordingly, leptin acts as a mitogen for a growing number of cell types, including the epidermis where it was detected together with its receptor. In particular, leptin strongly stimulate a proliferative response of keratinocytes during skin repair. Moreover, leptin and its receptor were detected in hair follicle where it may be involved in the control of hair follicle morphogenesis. At present, this hormone is considered a novel therapeutic factor to improve severely disturbed wound-healing conditions. To evaluate the action of leptin in the skin of the dog, we investigated the presence and localization of leptin receptor by using immunohistochemical technique. Through the application of a polyclonal antibody, we observed the expression of the receptor in the cells of the basal layer of the epidermis, in the hair follicles and in the apical membrane of the apocrine sweat gland cells. As regards hair follicles, the positivity was observed in the cells of the outer root sheath both in growing and regressive phase. The identification of leptin receptor suggests an important role of leptin in the metabolism of the epidermis and skin structures in canine species.

6.5 A morphometric study using Image Analysis to evaluate time-dependent changes in the bovine skin. A scientific approach to a practical problem

Montelli S *, Peruffo A*

We realized a new computational approach to implement classical histological analysis to evaluate the progressive degradation of bovine skin tissues. This automated method was performed to highlight the different skin components (epidermal cells, nuclei, collagen and elastic fibers, extracellular matrix) and evaluate if the histological structures present in the fresh skin are preserved also in refrigerated and salted skins. In the industrial process of tanning, the study of time-dependent progressive changes and post-mortem degradation of the bovine skin are of crucial importance to obtain an high quality tanned leather. We applied a custom-designed Image-J technology that enables us to analyze objectively the images taken from slides of tissue treated with histological techniques. We were able to consider which changes are induced in the skin by biotic and abiotic agents during the skin biological degradation before the start of tanning. Our results showed that collagen and elastin fibers were present and organized in fibers. The main effects of degradation relate to a) time-dependent decrease in the number of nuclei present in the tissue, and b) the loss of carbohydrate acids in salted skins.

6.6 Spleen of domestic ruminants: morpho-structural and immunocytochemical study

Scala G, ***Paino G****, Pelagalli GV*

Using the light microscope (LM) and scanning electron microscope (SEM) we showed some morpho-structural and immunocytochemical peculiarities of the vascular formations located on the internal surface of the lienal vein (*V. lienalis*) origin in the spleen of domestic ruminants (buffalo, cattle and sheep). Vascular formations showed by SEM an ovoid or spheroid shape, were 300 µm - 3 mm sized, and were observed to be single or amassed. By LM and SEM, vascular formations contained lymphocytes, incorporated into reticular cell system, and covered with a thin layer of flat cells. This layer in some areas highlights openings that favor the release of lymphocytes into lienal vein for ultimate local defense. For the immunogold-labeling SEM analysis, vascular formations were removed from the spleen immediately after sacrifice, incubated with normal goat serum, and subsequently with primary polyclonal antibodies (podoplanin, Wnt3, SOX9, CD133, and p53 family) in PBS overnight at 4°C. After washing in PBS, samples were incubated with gold-conjugated goat anti-rabbit IgG, fixed in 2% glutaraldehyde, subjected to silver enhancement, dehydrated, and examined under LEO 435 VP at variable pressure in the backscattered mode. Vascular formations showed intense immunopositivities to the used antibodies, a sign of the intense functional activity elicited by substances displaying antigenic capacities. The spleen of domestic ruminants shows wider defensive functional roles than other typical functions.

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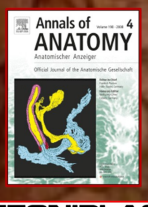

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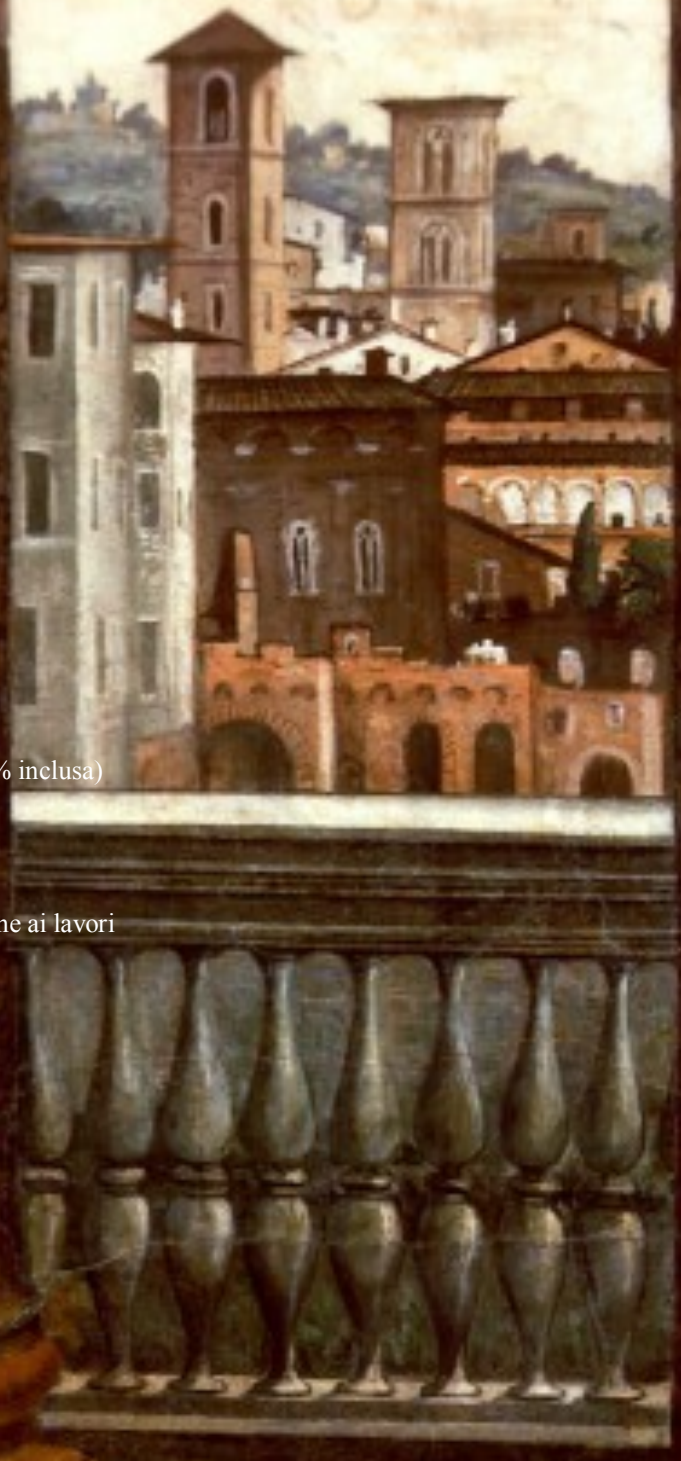

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Villa Farnesina: SALA DELLE PROSPETTIVE affreschi di Baldassarre Peruzzi 1519.
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