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XI Congresso Nazionale Associazione Italiana dei Morfologi Veterinari

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ABSTRACT

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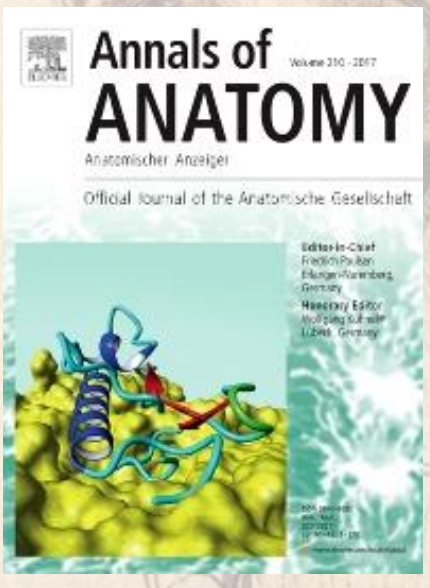

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La Segreteria Scientifica e la Segreteria Organizzativa si riservano il diritto di apportare qualsiasi variazione al programma che si dovesse rendere necessaria per ragioni tecniche e/o scientifiche

Session I - Skin, Bone, Muscle

1.1 Treating skin wounds in Veterinary Medicine: conventional versus innovative therapies

Gomiero C.[1], Martinello T.[1], Perazzi A.[2], DeBenedictis G.M.[2], Ferro S.[1], Zuin M[3], Martines E.[3], Brun P.[4], Maccatrozzo L.[1], Broeckx S.Y.[5], Spaas J.H.[5], Chiers K.[6], Iacopettil.[2], Patruno M.[1]

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The loss of skin integrity may induce important dysfunctions and, in large wounds, it is difficult to obtain the *restitutioadintegrum*. The aim of this study was to verify the efficacy of conventional and innovative topic treatments on skin regeneration. To achieve this goal different types of investigations were performed. Six lesions were surgically created on the back of six healthy adult sheep; every single wound was destined to different conventional (acemannan gel, manuka honey, hyaluronic acid) or innovative treatments such as allogeneic mesenchymal stem cells and plasma gas. The sixth wound was the placebo.Biopsies were collected at time T0, T15 and T40 days. Lesions were clinically evaluated. Histological examinations considered re-epithelization and epidermal thickness. Immunohistochemistry for the evaluation of inflammation, vascularization, and cell proliferation was performed using CD3, CD20, MHCII, von Willebrand factor and KI67 antibodies. Real time-PCR investigated transcripts such as VEGF, TGF-beta 1, Vimentin, Collagen 1α1 and hair Keratin.Clinically, the lesions treated with MSCs healed more rapidly with respect to the placebo. From the second week onwards, all wounds did not show presence of fluid and exhibited a dry and clean secondary layer. MSCs treatment reached the optimal healing since the new tissue presented a mature organization and skin annexes. To sum up, MSCs isolated from peripheral blood can be used *in vivo* to regenerate wound healing suggesting their involvement not only to improve the healing of primary intention but also for the stimulation of secondary intention.

1.2 Effects of Epidermal Growth Factor on horse skin from different anatomical locations

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Healing can be impaired in horses depending on the anatomical location of the wound. Wounds on the limb may develop exuberant granulation tissue that does not re-epithelialize, evolving in aberrant scarring, known as “proud flesh”. This preliminary ex vivo study investigates the effects of Epidermal Growth Factor (EGF) on epidermal thickness and re-epithelialisation of cultured horse skin.Skin strips were obtained from a euthanized horse exempt of dermatological lesions. From the mentioned strips, six-mm full-thickness punch biopsies were obtained from body and limb skin and cultured in a serum free medium, then treated for 6 days (D): 10ng/mL or 20 ng/mL Epidermal Growth Factor (EGF) either alone or with 10µg/mL Dexamethasone (DMS). Culture medium and treatments were replaced every other day. Epidermal thickness and the length of neoformed peripheral epidermis (µm) were measured at D3, D5 and D7.The higher dose of EGF modified epidermal thickness in skin from both locations at D5: a statistically significant increase was observed for body skin while a decrease was observed for limb skin. Analogously, a significant increase of the neoformed peripheral epidermis (epithelial tongue) was observed at D5 for body skin; although a decrease was seen in limb skin, it failed to reach statistical significance. Statistically significant changes were not observed at both D3 or D7.Our findings confirm the differences between body and limb skin morpho-physiology reported in horses. Moreover, while body skin responded to EGF as hypothesised, limb skin showed an unexpected response.

1.3 Platelet-derived growth factor-A is expressed by hair follicle epithelium in cashmere goats

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Platelet-derived growth factor A (PDGF-A) is implicated in hair follicle (HF) morphogenesis and hair cycle. It is considered an extrinsic signal, secreted by intradermal adipose tissue that can activate the hair follicle stem cells. Furthermore, PDGF-A is produced by the epidermis and follicular epithelium in humans. In this study, PDGF-A expression was investigated in the skin of the cashmere goats with the aim to correlate this molecule with HF stem compartment. Skin samples were collected from seven goats during different times according to hair cycle: in particular, sampling were performed at early anagen, anagen, early catagen and last catagen. For the immunohistochemistry, a mouse monoclonal anti PDGF-A antibody (SCBT) was used. Immunostaining was observed in some structures of the goat skin including the epidermis, HFs, blood vessels, arrector pili muscles and myoepithelial cells of the sebaceous gland. In particular, positive cells were localized in the basal layer of the epidermis and of the HF outer root sheath. As regard HF, the staining extended from the infundibulum to the isthmus while no reaction was detected in the bulb. A stronger immunoreaction was observed in the bulge region of regressive HFs compared to anagen phase. The expression of PDGF-A by the follicular epithelium and the increased immunoreaction during the regressive phase of the hair cycle let us to suppose that the PDGF-A, secreted by the HF, is involved in the anagen activation during the hair cycle.

1.4 Morphological findings in skeletal muscle fibres of congenital pseudomyotonia affected cattle

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Cattle congenital pseudomyotonia (PMT) is a genetic autosomal recessive disorder affecting skeletal muscles, clinically characterized by stiffness and delayed muscle relaxation. PMT is caused by a defect of *ATP2A1* gene, coding for the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA1). The genetic defect leads to amino acid substitution in the SERCA1, the main protein component of the non-junctional sarcoplasmic reticulum (SR). In skeletal muscle fibres, SERCA1 allows relaxation by removing calcium from cytosol to restore resting calcium concentration. During the last ten years, cattle PMT has been described in the Chianina and Romagnolaitalian breeds, in the Belgian Blue breed (where the pathology was named "congenital muscular dystonia1") and as a single case in a Dutch Improved Red and White (VRB) double-muscled phenotype calf. Different *ATP2A1* mutations have been found in these various breeds, showing that cattle PMT is genetically heterogeneous. *In spite ofgenetic heterogeneity*, bovine pathological muscles from all breeds examined were characterized by a striking, selective reduction of SERCA1 protein content at SR membranes. We have provided evidence that in pathological muscle fibres, triads as well as non-junctional SR are normal in size and distribution, are not depleted of their main protein markers and that SERCA1 mutants described until now, although expressed at low level, are correctly targeted to SR membranes. Moreover, we have found that only pathological muscles from the double-muscled VRB calf are characterized by a broad distribution of mitochondrial markers as a result of a possible compensatory response aimed at lowering excessive cytoplasmic calcium concentration.

1.5 Evaluation of *in vivo* osteogenic activity induced in the peri-implant bone by a peptidomimetic titanium functionalisation

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Osteoblast cell adhesion to the extracellular matrix is established through two main pathways: one is mediated by the binding between integrin and a minimal adhesion sequence (RGD) on the extracellular protein, the other is based on the interactions between transmembrane proteoglycans and heparin-binding sequences found in many matrix proteins. The aim of this study is the in vivo osteogenic properties evaluation of peri-implant bone in response to a biomimetic titanium surface functionalized with the dimeric form of the adhesive, retro-inverso DHVP peptide, reproducing the vitronectin 351-359 sequence. The experimental plan is based on a bilateral study design of Control and DHVP implants inserted respectively in the right and left femur distal metaphysis of Wistar rats (n=16) and evaluated after 15 days. Fluorescent bone vital markers were used to monitor the osteogenic activity over time. Bone was treated as undecalcified sample in order to maintain the bone to implant interface. Histological, static and dynamic histomorphometry analysis were performed on three concentric and subsequent circular Regions Of Interest (ROI) of equivalent thickness (220 μm), ROI1 adjacent to the interface, ROI2, the middle, and ROI3 the farthest. The data indicated that these functionalized implants have higher bone apposition rate and a larger and rapid osteoblast activation in terms of mineralising surface within ROI1 compared to the Control. These higher osteoblast recruitment and activation leads to a greater bone to implant contact reached for DHVP samples. This represents an initial stimulus of the osteogenic activity that might result in faster and better osteointegration process.

1.6 Distal femur and proximal tibia: normal bone features in pig

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Pigs are commonly employed as model in bone-related research. Swine femur and tibia have been often used in interspecies etiopathogenesis studies of fractures, osteoarthritis and femoral head avascular necrosis. Moreover, the pig represents a model in cell regenerative medicine. Nevertheless, the literature about normal bone features in pigs is haphazard and incomplete, making it difficult to interpret the results in comparative studies.The present work aims to define benchmarks on the microscopic structure, composition and density of the pig distal femur and proximal tibia. For this purpose, five 80-100 kg swine hind limbs were collected from slaughtered animals. Cortical and cancellous bone of the middle/distal femur and proximal/middle tibia were microscopically analysed; bone mineral density (obtained with Dual-energy X-Rays Absorptiometry) was also evaluated. Statistical analysis was performed by one way/two way ANOVA. Despite the small sample size, the homogeneous results seem encouraging and may represent reference parameters for microscopic architectural features such as type of bone, cortical thickness, osteonal and Haversian canals diameter, area and perimeter, bone area, trabecular width, trabecular number and trabecular separation and, eventually, for bone mineral density. The identification of normal parameters is essential for the interpretation of results from research in the field of applied anatomy and regenerative medicine; normal parameters are also crucial in the design and engineering of bone substitutes, implants and scaffolds.

1.7 Meniscus maturation in the swine model: role of endostatin in cellular differentiation

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The development of an engineered meniscus derives from the need to regenerate a tissue which is largely unable to self-repair with consequent loss of functionality. Hence a deeper knowledge of the native meniscus morphology and biomechanics in its different regions, including molecules involved in regulation of the maturation process, is essential. The meniscus is a complex tissue, displaying great regional variation in extracellular matrix components and in vascularization, as a result of several biomechanical stimuli. Its biochemical composition is modulated to adapt the tissue to the different functions that are required throughout growth, until a “mature” phase is reached in adulthood. The aim of this work is to evaluate the biological role of Endostatin in the regulation of angiogenesis as in the fibro-chondrogenic differentiation of neonatal meniscal cells in the pig. The swine is an attractive model for meniscal repair studies, as its knee joint is closely comparable to the human one in terms of anatomical structure, vascularization, and healing potential. Our preliminary data show that Endostatin contributes to the acquisition of chondrocyte phenotype in an undifferentiated but committed cellular population. Thus, a better understanding of the role of Endostatin in cell metabolism might lead to a deeper knowledge of the events regulating meniscus maturation. These findings may be crucial for the development of an engineered scaffold able to induce meniscal cell differentiation by releasing Endostatin-rich microspheres.

1.8 Biometrical study to evaluate the relationship between body parameters and winter feeding resources in central Italy Apennine roe deer population

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The study analyses the biometrical data obtained from animals pertaining to class 0 (0-11 months) and 2 (over 2 years), in roe deer population of central Italy Apennine. The biometrical analysis was performed on body and cranial parameters of 234 roe deer obtained by selective shooting. For the mandible morphometric analysis, 58 samples were treated with the GeoGebra’s program. To evaluate the relationship between size/shape of body structure/parameters and the feeding resources available during the autumn-winter period, an environmental category (derived from the carrying capacity of forest ecosystems relative to the hunting zone/district) was attributed to each specimen. Statistical analysis was performed by ANOVA. Shape variables were generated using Generalized Procustes TPS Analysis program. In class 0 the analysis showed no significant differences neither between sex nor among environmental categories. In class 2 the analysis showed significant differences for four body parameters (but not for the cranial ones) between sex; among environmental categories the mandible length showed significant differences, in addition there is an increasing trend for tooth line and body weight from lower to higher environmental category. Males pertaining to class 2 showed an increasing trend in the tooth line related to environmental category; in females, this parameter had similar values in the different environmental categories, while the mandible length showed significant differences inversely linked to environmental category. Shape analysis showed a more open mandibular angle in class 0 subjects living in the hunting zone characterized by highest feeding resources; however, additional data occur to confirm this statement.

Session II - Nervous system and Sense Organs I: Fish

2.1 Localization of cholecystokinin in the zebrafish retina from larval to adult stage

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The peptide hormone cholecistokinin (CCK) plays a key role in the central and peripheral nervous system. It is known to be involved in the digestive physiology and in the regulation of food intake. Moreover, the CCK expression has been detected also in the retina of different vertebrates, including fish, although its biological activity, in this organ, remains to be elucidated. In literature no data are so far available about the CCK-immunoreactivity in the zebrafish retina. Therefore, the aim of the study was to investigate the distribution of CCK in the zebrafish retina during development, from 3 days post hatching (dph) until adult stage, using Immunohistochemistry, in order to elucidate the potential role of this protein in the development and maintenance of normal retinal homeostasis. The cellular distribution of CCK in the retina was similar from 3 dph to 40 days post fertilization (dpf) when immunoreactivity was found in the outer nuclear layer, in the outer plexiform layer, in the inner plexiform layer and, to a lesser extent, in the ganglion cell layer (GCL). Immunohistochemical localization at 50 dpf was observed in the inner nuclear layer and ganglion cell layer, whereas in the adult stage it was restricted to ganglion cell layer. Our results demonstrate for the first time the occurrence of CCK in the zebrafish retina since larval to adult stage with a different pattern of distribution, suggesting different roles of CCK during retinal cells maturation.

2.2 Neuromast hair cells retain the capacity of regeneration during heavy metal exposure

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The neuromast is the morphological unit of the lateral line of fishes and is composed of a cluster of central sensory cells (hair cells) surrounded by support and mantle cells. Heavy metals exposure leads to disruption of hair cells within the neuromast. It is well known that the zebrafish has the ability to regenerate the hair cells after damage caused by toxicants. The process of regeneration depends on proliferation, differentiation and cellular migration of sensory and non-sensory progenitor cells. Therefore, our study was made in order to identify which cellular types are involved in the complex process of regeneration during heavy metals exposure. For this purpose, adult zebrafish were exposed to different heavy metals (Arsenic, Cadmium and Zinc) for 72h. After 24 h of exposure, immunohistochemical localization of S100 (a specific marker for hair cells) in the neuromasts highlighted the hair cells loss. The immunoreaction for Sox2 (a specific marker for stem cells) was observed in the support cells, after exposure to Arsenic and Zinc, while in mantle cells after exposure to Cadmium. After 72h of exposure the hair cells were regenerated, showing immunoreaction for S100 protein. At the same exposure time to the three metals, Sox2 immunoreaction was expressed in support and mantle cells. Our results showed for the first time the regenerative capacity of hair cells, not only after, but also during exposure to heavy metals, demonstrated by the presence of different stem cells that can diversify in hair cells.

2.3 Age-related changes of orexin system in the brain of the model organism *Nothobranchius furzeri*.

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The orexin system is functionally and anatomically well conserved during the evolution, and plays key roles in regulating sleep, metabolism, feeding, anxiety, reward and addiction. In vertebrates, orexin synthesis is relatively confined to neurons located in the hypothalamus. Age-related decline in the orexin system has been widely documented in mammals, including humans, and parallels physiological alterations in body weight regulation and sleep. Orexin system has been characterized in teleost fish, such as zebrafish and goldfish, also based on the neuroanatomical homology of the hypothalamic region across vertebrates. We propose to investigate the orexin system in the brain of *Nothobranchius furzeri*, the vertebrate with the shortest lifespan ever described under laboratory conditions. The short lifespan is coupled to rapid age-dependent functional decline and expression of cellular and molecular changes comparable to those observed in other vertebrates, including humans. At this aim, we examine the pattern of distribution of Orexin A, B and orexin 2 receptor (OX2R) in the brain of young and old specimens (respectively at 3 and 27 weeks old), to: (a) clarify if changes in Orexins and OX2R-ir occur between younger and older; (b) determine any regional differences or effects of age. Our results show that OXA is localized in some groups of neurons, and OXA and OX2R are widely distributed in nervous fibres throughout the whole brain. OXB is scarcely detectable either in neurons and fibres. An age-related reduction in the distribution of OXA and OX2R has been highlighted, and confirms the involvement of orexin system in the N. furzeri ageing.

2.4 Zebrafish: an attractive vertebrate model to study orexin and endocannabinoid morphological interaction in the brain

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Orexin-A (OX-A) and Orexin-B (OX-B) are neuropeptides widely expressed among vertebrates. They act through orexin receptors (OX1R and OX2R) to regulate numerous physiological functions, such as energy homeostasis, food intake, sleep/wake cycle, learning and memory. The endocannabinoids, mainly the 2-arachydonoil glycerol (2-AG), control similar biological processes by binding the cannabinoid receptor 1 (CB1). Phylogenetic analyses of the zebrafish have demonstrated that orexin and endocannabinoid systems are structurally and functionally highly conserved with the mammalian counterpart. In zebrafish, a common OX precursor gives rise to OXA and OXB, like in mammals, but only the OX2R has been identified. OX neurons are located in the hypothalamus and send projections to many brain regions, which express OX2R with a pattern of expression similar to CB1. We and others have found a structural and functional interaction between orexin and endocannabinoid systems with OX-A being a strong enhancer of 2-AG synthesis in mammals models. Since a similar OX2R and CB1 neuronal networks and functions has been described in zebrafish and mammals our aim is to provide a compelling morphological study of the OX2R/CB1 interaction in the brain of zebrafish by exploiting the unique properties of this animal model like small size, transparency, neuroanatomical and physiological homology to mammals in order to understand this interaction in the control of physiological function such as feeding, energy homeostasis, learning and memory.

2.5 Neurogenesis and aging: localization of novel genes in the short-lived fish *Nothobranchiusfurzeri*

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Adult neurogenesis is a dynamic and highly regulated process observed in all vertebrate species. In mammals, adult neurogenesis is spatially restricted into two specific neurogenic brain regions: subgranular zone and subventricular zone. At opposite, in teleost fish extensive adult neurogenesis has been detected in sixteen niches, mostly associated with the ventricular system. *Nothobranchius furzeri* is an emerging teleost model organism for aging research. It is characterized by a very short lifespan, strictly related to rapid growth, accelerated sexual maturation and age-related changes. One of the aging hallmarks is the decay of the adult neurogenesis. Here, we investigate the involvement of positive selected genes in neurogenic niches of *N. furzeri* during aging. After searching for positive selection signatures in protein-coding sequences, we decided to undertake in-depth studies of the Inhibitor of DNA binding 3 (ID3) and two α- 1-chain collagen genes, respectively COL4A1 and COL25A1. Bioinformatics investigations show that ID3, COL4A1 and COL25A1 display analogous regulation in four different species (*Danio rerio, Mus musculus, Homo sapiens* and *Nothobranchius furzeri*), during aging. To localize these genes, we performed In Situ Hybridization (ISH) experiments on brain cryosections of young (5 weeks) and old (27 weeks) male animals, belonging to the long-lived strain MZM – 04 /10. The expression levels of ID3, COL4A1 and COL25A1 confirms the bioinformatic data. The neuroanatomical localization, at the two investigated stages, reveals that ID3, COL4A1 and COL25A1 are mostly expressed in telencephalon, optic tectum and cerebellum, which are considered neurogenic areas also in this model organism.

Session III - Nervous system: Mammals

3.1 Spatial distribution of peptidergic and non-peptidergic nociceptors in mouse dorsal root ganglia: a cluster story.

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Dorsal root ganglia (DRGs) are formed by pseudounipolar neurons, receiving sensory information from periphery and conveying it to the central nervous system, and satellite glial cells (SGCs), surrounding the neurons. Among the latter, the nociceptors play a primary role in the transmission of pain information. Nociceptors are typically classified in non-peptidergic neurons, binding the isolectin B4 (IB4), and peptidergic neurons, containing neuropeptides such as the calcitonin gene-related peptide (CGRP). Albeit the functional profile of these neurons has been duly investigated, little is known on their mutual spatial relationship as well as with SGCs and whether this has an impact on their function in health and disease. SGC ensheathment has, in fact, an important role in mediating neuronal response to inflammatory and pathological events.Here we used a combination of confocal imaging and 3D software-based analysis to investigate the spatial distribution of peptidergic and non-peptidergic nociceptors in normal mice and in a model of diabetic neuropathy.We developed software to automatically perform segmentation and 3D rendering of labeled neurons in whole DRGs, as well a quantitative characterization of immunopositivity. Our results highlight that the non-peptidergic IB4+ neurons, but not the peptidergic CGRP+ ones, tend to cluster in groups of three-four, without significant changes between normal and diabetic mice. SGCs were identified by a glutamine synthetase antibody and their distribution quantified. Preliminary results suggest a decrease of the SGC ensheathment around nociceptors in diabetes, which may lead to a functional crosstalk between clustered neurons in pathological settings.

3.2 Analisys of synaptophysin expression in the heterozygous reeler mouse cerebellum: a gender related study

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Reelin is an extracellular protein involved in neuronal migration, correct dendrite outgrow and synapse formation in the adult forebrain and cerebellum. The heterozygous Reeler mice (*Reln*$^{+/-}$) are haplodeficient for the *Reln* gene and display 50% reduced levels of Reelin in the brain. They show subtle anatomical and functional alterations, and are phenotypically indistinguishable from normal mice. Our previous studies indicated that a total lack of Reelin is correlated with a decrease in the number of synaptic contacts and spine density in the homozygous Reeler mouse (*Reln*$^{-/-}$) cerebellum. Here, by means of high resolution immunofluorescence on LR-White semithin sections, we have quantified the distribution of the small synaptic vesicle marker synaptophysin in the cerebellar cortex of adult *Reln*$^{+/-}$ and control mice of both sexes. Our results indicate that there is a significant decrease of synaptophysin immunoreactivity in the *Reln*$^{+/-}$ male cerebellar cortex. Furthermore, a gender specific increase in synaptophysin labeling was found in the granular layer of females irrespective of genotype.Our data show that *Reln*$^{+/-}$ male mice display synaptic disarray of their cerebellar cortex. These findings confirm the crucial role of Reelin in the formation and maintenance of the correct synaptic networks in the cerebellum.

3.4 A quantitative approach to measure and rank the brain cytoarchitecture: the cerebellar case study

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We developed a computational and statistical method that allows to measure, compare and rank the cytoarchitecture of defined brain regions, across different populations considering factors such as sex, age, species. In this approach, we combined image analysis of digitized Nissl-stained sections, computational neuroanatomy and non-parametric methodology as synergistic tools for the assessment of neuronal cell morphology, to elaborate quantitative information on cytoarchitecture of the nervous tissue. We set up an automated software tool capable of systematically organizing and analyzing morphometric data. The software focuses on the measures of the neural cells, establishing a series of shape and spatial descriptors relative to three domains, named size, irregularity and proximity (cell density) respectively. To assess brain cytoarchitecture, we design a nonparametric multivariate method to model morphological data using the statistical dispersion scatter based indicator MAD (Mean Absolute Deviation), as measure of the neuronal complexity. In this regard, we present a case study showing the accuracy of this multidisciplinary procedure in the cytoarchitectonic investigations of sexual dimorphism of the vermal lobules VI-VII, comparing male, female and the pseudo-hermaphrodite freemartin in the Bostaurus cerebellum.

3.5 Trafficking and release of Orexin-A are increased in the LH target areas of the brain in obese mice

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Hypothalamic circuits are affected by synaptic rewiring induced by leptin. Orexins (also known as hypocretins) are peptides produced by a small number of neurons exclusively localized in the perifornical (PeF) and lateral hypothalamic areas of the brain. Orexins play an important role in feeding and energy expenditure by monitoring humoral and neural indicators of energy balance. By exploiting obese mice as a model of leptin signal deficiency (ob/ob mice or mice fed with high fat diet, i.e. HFD mice) we found an endocannabinoid-mediated dishinibition of orexin neurons with consequent elevation of orexin A trafficking and release to many LH target areas like ARC, PVN, DMH, septal area, hippocampus, nucleus accumbens (NAcc) and ventral tegmental area (VTA). Since orexin-A acts via OX-1R, a Gq coupled protein receptor (GPCRs), by enhancing intracellular Ca+2 influx and membrane depolarization, here we sought to investigate the putative effect of the aberrant OX-A trafficking and release to dopamine trafficking which underlies the rewarding circuitry of NAcc and VTA in obese mice. These results are of special relevance since the aberrant excitation of OX-A neurons during obesity could trigger a vicious cycle by enhancing dopamine synthesis thus inducing food and drug seeking behaviors, hyperphagia, body weight gain and dysfunctional metabolism.

SESSION IV - Reproductive system, Digestive System

4.1 Urocortin and CRH-receptors in the epididymis of normal and cryptorchid dogs

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In dogs, cryptorchidism is a common disorder and unilateral cryptorchidism is more frequent than bilateral. Urocortin (UCN) is a corticotrophin-releasing hormone (CRH)-related peptide which was observed to affect several functions in male genital organs. The aim of the present study was to investigate the expression of UCN, and its receptors CRHR1 and CRHR2 by immunohistochemistry, Western blot and real-time RT-PCR in the epididymis of the normal and cryptorchid dog. The results showed that UCN, CRHR2 and CRHR1 were expressed in normal and cryptic epididymal duct. UCN-and CRHR2-Immunoreactivities (IRs) were distributed in the principal cells of the caput, corpus and cauda of the normal and cryptic epidydimal duct. In the epididymis of the cryptorchid dog, the lumen of the epididymal duct was relatively small compared to that in the normal epididymis, and interstitial tissue was abundant. In addition, only a few spermatids were observed in the lumen of the epididymal duct. CRHR1-IR was distributed in the vascular smooth musculature and in fibromuscular cells surrounding epididymal tubules of the normal and cryptorchid dog. UCN and CRHR2 mRNA expression levels were higher in the cryptic than in normal epidydimal duct. These results suggest that UCN and its receptors might play a role in regulating the maturation and storage of spermatozoa.

4.2 Chromatin remodeling and histones modifying enzymes in bovine oocyte: interpretation of trascriptomic data meets morphology

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Before meiotic resumption, the chromatin enclosed within the nucleus of prophase I arrested mammalian oocytes undergoes morphological and functional changes that are crucial for meiotic progression and early embryonic development. Histones post-translational modification such as acetylation and methylation, imposed by histone modifying enzymes, are considered important hallmarks in these processes. However, the study of histone modifications during oogenesis is hindered by the lack of antibodies specific to all the different histone modifications and histones modifiers and by the inaccurateness of protein determination in low-amount samples. Conversely, the availability of transcriptomic platforms applicable to oocytes generated, in the last decade, a large amount of data, revealing the expression of histones modifiers. However the interpretation of gene profiling data is not straightforward in oocytes, since in these cells transcripts can be stored for several days before being used for protein synthesis. Hence we propose an integrated experimental and bioinformatics approach to overcome this limitation. Specifically, changes in the transcript levels of histone modifying enzymes coming from different microarray analyses were combined and compared with the corresponding phenotype investigated by immunocytochemistry, whenever the antibody specific to the modified histone residue was available. This combined approach enabled the formulation of precise hypotheses on the role of specific enzymes responsible for histone's acetylation and methylation signature and it will be expanded to other modifications. This strategy can be particularly useful in large mammalian species that rarely allow the application of genetic manipulation to confirm experimental hypothesis. (Supported by OECD program JA00091594 and NSERC-CRSNG-Canada).

4.3 Ovary of *Coturnixcoturnix japonica*: immunochemical and immunohistochemical detection of Neurotrophin system

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In birds, like in mammals, neurotrophins (NGF, BDNF, NT-3) and their specific Trk receptors (TrkA, TrkB, TrkC) exert their effects also on reproductive system. The aim of this study is to explore the involvement of neurotrophins and related receptors in ovaries of Coturnixcoturnix japonica. With this aim, ovaries from adult and embryos (from 4th to17th day of incubation) were collected. Western blotting analysis on adult ovary showed the presence of neurotrophins and their Trk receptors. Immunohistochemical investigation in adult ovaries underlined different localization of Neurotrophins and Trk receptors in oocytes cytoplasm, follicular and stromal cells, and also in the nervous component. In embryo ovaries from the 9th day of incubation, only NGF, NT-3 and Trk receptors were differently distributed in cortical and medullary areas. By double staining NGF/TrkA and BDNF/TrkB were colocalized in oocytes, follicular and stromal cells, in both adult and embryo ovaries. Taken together these results confirm an involvement of neurotrophin system in the quail ovary physiology: this system, promoting the oocyte development and maturation in adult and during development, plays a critical role in oogenesis and follicologenesis, similarly to previous results reported in birds and mammalian species.

4.4 Expression of Orexin A and its receptors in the porcine ovary

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Orexin A (OXA), and B are two neuropeptides mainly synthesized in the lateral hypothalamus, from a common precursor called prepro-orexin and involved in different functions such as regulation of food intake, energy balance, reproductive activities, sleep and wakefulness. Their action is mediated by binding G-protein-coupled receptors, termed orexin receptor 1 (OXR1) and orexin receptor 2 (OXR2). As orexins and their receptors were previously described also in different tissues and organs outside the brain, we investigated their presence in the porcine ovary. Using double labelling immunofluorescence techniques, we observed OXA and its receptors in granulosa, thecal and luteal cells, moreover in *corpora albicantia,* atretic follicles and interstitial cells of the porcine ovary. These last three localizations had never been investigated in previous research. OXA and OXR2 appeared more diffused in the cytoplasm and OXR1 associated to the nuclear envelop of the granulosa cells, while in thecal, luteal and interstitial cells, all the substances appeared associated to cytoplasmic vesicles. We also proved, by real-time Polymerase-Chain-Reaction, that granulosa cells of large follicles and luteal cells express the prepro-orexin gene. In vitro administration of OXA stimulated the growth and induced an increased production of oestradiol in granulosa cells. The progesterone production was instead not affected in granulosa cells, while inhibited in luteal cells. Taken together these findings demonstrate that porcine ovary contains different cells types able to secrete and/or internalize OXA, thus suggesting both autocrine and paracrine modalities of action of OXA. Moreover, OXA could be involved in modulating ovarian steroidogenesis and follicle growth.

4.5 Probiotic supplementation affects the glycan composition of mucins secreted by Brunner's glands of the pig duodenum

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Brunner's glands (BGs) are mucus-secreting glands located in the submucosa of the duodenum of mammals and also discontinuously extending in the jejunum of pig and big herbivores. The mucus secreted by BGs has little digestive capacity, is viscous and protects the mucosal surface from abrasive contents entering from the stomach. Mucin composition and secretion can be stimulated or inhibited by several factors including the diet. In this study, we compared the carbohydrate composition of BGs mucins from domestic pigs fed with a basal diet (n=3) and with a dietary probiotic complex (Sivoy™, SLAB51 - Mendes SA, Lugano, Switzerland) (n=3). The investigation was performed on duodenum pieces removed from the 5 cm caudal part of the pyloric region, immediately fixed in 4% buffered paraformaldehyde, and embedded in paraffin wax. Dewaxed sections were stained by means of the conventional lectin histochemistry for glycoconjugate characterization. The glands of all specimens exhibited secretory granules containing neutral and carboxyl but not sulpho-acidic mucins. Probiotic treated animals displayed i) increase in glycoproteins with carboxyl groups, ii) appearance of complex-type N-glycans, iii) decrease in O-glycans (mucin-type) terminating with galactoseβ1,3N-acetylgalactosamine and in high mannose N-glycans terminating with lactosamine and fucose, and iv) no effect on N-acetylgalactosamine, N-acetylglucosamine and sialic acids terminating glycoproteins. Since probiotic treatment did not affect the morphology of BGs and resulted in a significant increase in the growth performance, the observed glycan changes in the GBs could be related to a positive effect of the probiotic on the physicochemical properties of pig gut.

AMV Scholarship 2015

Ss.1 Role of Dab1 in cerebellar circuitry and motor learning

Cocito Carolina

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Disabled-1(Dab1) is one of the cytosolic effectors of the Reelin pathway. In the cerebellum, Reelin and Dab1 are expressed by the post-mitotic granule cells and are responsible for the proper migration and maturation of Purkinje neurons (PNs). The role of these two proteins during the adulthood is unclear; although it has been demonstrated that Dab1 is required for synaptic plasticity and associative learning in the hippocampus, no data are available in the cerebellum. At this level, the circuitry among PNs, climbing fibers and parallel fibers is involved in the motor learning and displays a certain degree of synaptic plasticity. To assess whether the reduction and/or the total lack of Dab1 could perturb this circuitry, levels of the NR2A and NR2B subunits of NMDA receptors, and levels of the GluR1 subunit of the AMPA receptors were investigated by the use of western blot in the cerebellum of a group of adult heterozygous (*Dab1*$^{+/-}$) and homozygous (*Dab1*$^{-/-}$) *scrambler* mice. Levels of the GluR1 subunit were significantly lower in *Dab1*$^{-/-}$ mice respect to the control whereas no significant differences were found in the levels of the NMDA receptor subunits, NR2A and NR2B. However, it worth to be mentioned that all the receptor subunits in exam tend to decrease in the mutants. These results support the role of Dab1 and Reelin in the cerebellar synaptic plasticity.

Ss.2 Changes in the expression of *trkA* and *p75* in the brain of *Nothobranchius furzeri* upon ageing

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In course of ageing, mammalian brain is characterized by marked changes in the expression levels of neurotrophin receptors, TrkA and p75. Specifically there is a progressive increase in the level of p75 and a parallel decrease in the level of TrkA. This switch is physiologically relevant in the pathogenesis of neurodegeneration. Nerve growth factor (NGF), its receptors tropomyosin receptor kinase (Trk) family member TrkA and p75 neurotrophin receptor, are well conserved in vertebrates evolution. Upon neurotrophin binding, the receptors can elicit their function independently (TrkA mediates the survival and neurite outgrowth-promoting effects of NGF, and p75 promotes apoptosis) or interact to potentiate NGF action. Few data are available on TrkA and p75 in fish models, only p75 has a role in axonal growth and synaptic plasticity in zebrafish brain development. This study is addressed to explore the role of these receptors in the aged brain of *N. furzeri,* an excellent model for ageing studies. The brain of this fish displays typical ageing biomarkers, as mammals. In addition, previous studies have demonstrated the occurrence of neurotrophins system in *N. furzeri* adult brain. The results show that, in old specimens, the two receptors are expressed in the brain, although at low levels, and are both down-regulated. *trkA* and *p75* positive neurons are differently expressed throughout the brain regions, with *trkA* more widespread, and are clearly less numerous when comparing the same regions of young and old animals. These data confirm that *trkA* and *p75* activity is reduced in the aged brain of *N. furzeri*.

Plenary lectures

Pl. 1 Two photon microscopy: a window with a vie on brain function and pathology

Gian Michele Ratto *(NEST, Istituto Nanoscienze CNR and Scuola Normale Superiore, Pisa, Italy).*

Pl. 2 Considerations on Nomina anatomica veterinaria from latin to italian

Rino Panu *(Dipartimento di Scienze Medico-Veterinarie, Università di Parma)*

Mariella Bonvicini *(Dipartimento di Discipline Umanistiche, Sociali e delle Imprese Culturali, Università di Parma)*

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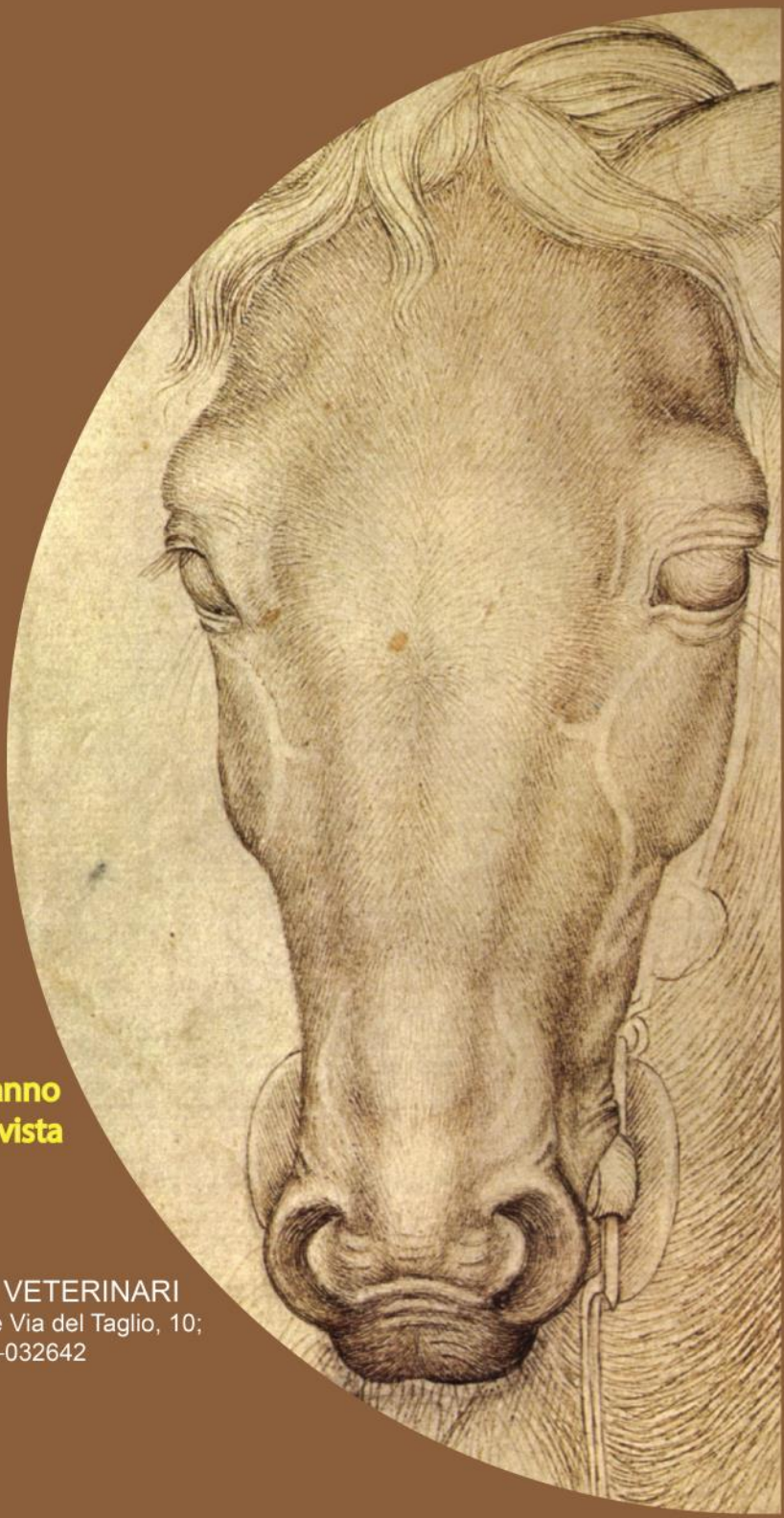
- coffee break e light lunches
- attestato di partecipazione



Gli ABSTRACT dei contributi scientifici e una selezione di articoli in extenso saranno pubblicati su un numero speciale della rivista ANNALS OF ANATOMY

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