Associazione Italiana
Morfologi Veterinari

# ROMA

***21-22 Maggio 2015***

**Palazzina dell'Auditorio**
**Via della Lungara 230**

**X CONGRESSO AMV**
**Roma, 2015**

# *Abstract delle comunicazioni scientifiche*

## *X CONGRESSO NAZIONALE*
## *Associazione Italiana Morfologi Veterinari*

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**Gli abstract dei contributi scientifici sono pubblicati in forma preliminare dopo revisione da parte del Comitato Scientifico e prima della pubblicazione definitiva sulla rivista**
***Annals of Anatomy***

## P.1 Muscle diversification and plasticity

***Francesco Mascarello***
*Dipartimento di Biomedicina Comparata ed Alimentazione*
*Università degli Studi di Padova*

## P.2 In vivo molecular imaging during mammary tumor progression to identify new biomarkers useful for clinical applications

***Isabella Manni[1], Cristina Avvantaggiati[2], Luisa De Latouliere[1], Gabriele Toietta[1], Tania Battisti[1], Maria Giulia Rizzo[1], Paolo Ciana[2], Giulia Piaggio[1]***
*[1]Experimental Oncology Area, National Cancer Institute Regina Elena, Rome, Italy*
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By using a mouse model engineered to express the luciferase gene in cells undergoing proliferation (MITO-Luc reporter mice) we have studied the dynamics of cell proliferation in mouse model of sporadic and genetic carcinogenesis of the mammary gland. The results of this study demonstrated that the evolution of the majority of tumorigenic processes of breast cancer in mice share common molecular steps in their progression. In vivo imaging, gave us the opportunity to exactly define when this molecular switch occurs and where (e.g. if other tissues participate to the process). Since this process occurs much before the tumour appearance, we have now the unprecedented opportunity to identify the timing of the very initial molecular changes responsible for tumour onset. With the purpose to identify clinical useful biomarkers for early diagnosis, our major aim is now to characterize the expression of a specific miRNA profile, in breast tissues and in serum, in the transformation process stages identified through in vivo imaging.

## SESSION 1: Cytology, differentiation and cell engineering

### 1.1 Mechano-sensing influences morphology and differentiation efficiency during epigenetic conversion of fibroblasts into insulin-producing cells

***Brevini TAL**, Pennarossa G, Santoro R, Maffei S, Zenobi A, Pesce M, Gandolfi F*

Fibroblasts can be epigenetically converted into insulin-secreting cells (EpiCC), using the epigenetic modifier 5-aza-cytidine (5-aza-CR), followed by a three-step pancreatic induction protocol. Here we investigate if the use of a thin polyacrylamide-based (PAA) gel substrate with soft stiffness may increase the efficiency of differentiation and the acquisition of a more mature phenotype.

Murine skin fibroblasts were plated either on standard plastic dish (group A) or on PAA gel with soft (1kPa) stiffness (group B). Cells were exposed to 1 μM 5-aza-CR for 18 hours, and then subjected to pancreatic induction for 10 days. At the end of differentiation all EpiCC modified their typical fibroblast elongated shape and acquired an epithelioid morphology. However, while group A cells remained monolayer, group B cells formed three-dimensional spherical structures, reminiscent of in vitro cultured pancreatic islets. Group B cells also showed a significant increase of pancreatic hormone-positivity (82.83±6.8% vs. 26.86±5.8%) and became mono-hormonal (65.33±2.5%). In contrast, 100% of group A cells remained poly-hormonal. Glucose triggered insulin release was significantly higher in B EpiCC (262.57±0.79 mU/μgDNA) than in A (171.22±0.9 mU/μgDNA).

The data presented demonstrate that 3D stiffness regulates cytoskeletal and adhesion mechanics during cell conversion. A soft substrate can drive cell response both at the morphological as well as at the functional level. It increases hormone release and encourages the acquisition of a mono-hormonal mode, which is associated with a mature pancreatic phenotype. This suggests that cell mechano-sensing and biomechanical properties, specifically stiffness-sensing mechanisms, influence cell commitment.

### 1.2 Acid-sensing ion channel 2 is at the basis of proprioception

***Guerrera MC**, Abbate F, Vàsquez G, Germanà GP, Levanti MB, Germanà A, Vega JA*

Mechanosensory neurons convey to the central nervous system touch, vibration, pressure sense, and proprioception. They project to the periphery and form different kinds of mechanoreceptors, in the skin, joints and skeletal muscles. The manner in which they sense mechanical signals is still not fully elucidated, but electrophysiological experiments have suggested that this may occur through the activation of ion channels that gate in response to mechanical stimuli. The acid-sensing ion channels (ASICs), especially ASIC2, function as mechanosensors or are required for mechanosensation. The ASIC2 is expressed in both mechanosensory neurons and cutaneous mechanoreceptors. We have used immunohistochemistry for ASIC2, and image analysis, to investigate the distribution of ASIC2 in mouse lumbar dorsal root ganglia, as well as in mechanoreceptors of the rectus femoris and gastrocnemius muscles. In lumbar dorsal root ganglia ASIC2 immunoreactive neurons were all intermediate or large sized (mean diameter ≥20 μm to 60 μm), and no ASIC2 was detected in the satellite glial cells. ASIC2 positive axons were observed in association with muscle spindles in the two muscles analyzed. Based on their morphology and topographical distribution within the muscle spindles, they must be regarded as the sensory axons, innervating the proprio-mechanoreceptors responsible for the afferent component of muscle tone. Nevertheless, motoneurons also display ASIC2. Moreover positive immunostaining was detected in the external capsule of muscle spindles. The present results demonstrate the distribution of ASIC2 in the mouse in the peripheral proprioceptive system, and suggest the involvement of ASIC2 in proprioception.

### 1.3 Alternative cell sources for tendon engineering

***Di Giancamillo A**, Deponti D, Peretti G, Domeneghini C*

Tendons regeneration is a very critical issue in the field of tissue engineering, because their connective tissues display a low healing potential. In order to find new solutions for tendon regeneration in both veterinary and human medicine, several models have been developed combining different cell populations and different biomaterials. The choice of an opportune cell population is a fundamental step for engineering a tissue. The cell source has to be competent for a specific phenotype and it has to be characterized by a minimally invasive approach for its isolation. According to this characteristics different cell populations were studied for their tenogenic potential, in particular tenocytes, dermal fibroblast and mesenchymal stem cells derived from adipose tissue (ASCs). In the present study, these cell populations were compared to each other considering their synthetic profile, both in monolayer culture and in combination to a collagen sponge in order to understand if dermal fibroblast or ASCs can be considered suitable alternative cell sources to native tenocytes for engineered tendons. The data showed that these populations share the same synthetic profile of tenocytes, characterized by the expression of collagen type 1 and collagen type 3; moreover, these cells population were able to colonize and survive into a collagen sponge, as well as the tenocytes, and a new matrix were produced. In conclusion dermal fibroblast and ASCs can be considered suitable cell population for tendon tissue engineering.

## 1.4 The fusion of MyoD transcription factor with the TAT peptide induces, in contioned medium, equine stem cells towards a myogenic lineage

***Martinello T**, Gomiero C, Negro A, Topel O, Sacchetto R, Patruno M*

The trans-activating transcriptional activator TAT is a small peptide essential for viral replication that possesses the property of entering the cells from the extracellular milieu, acting as a membrane shuttle. In order to produce myocytes from undifferentiated Mesenchymal Stem Cells (MSC) we present an innovative and safe methodology based on the fusion of the transcription myogenic factor MyoD and the TAT sequence, that promotes the cellular internalization without the use of viral vectors. The nucleotide sequence encoding MyoD was amplified from a cDNA library; the amplified products were cloned in the plasmid pTAT-Ngl to generate the fusion protein TAT-MyoD containing the translocation domain of the HIV-1 protein TAT. The fusion protein was subsequently expressed and purified adapting standard recombinant techniques. In our study we have used undifferentiated MSC collected from peripheral blood of a donor horse (pbMSC). We have tested different incubation times to detect when the protein fused with TAT was fully internalized in the nucleus of pbMSC. We have demonstrated, using immunohistochemical methods, that MyoD-TAT protein can be observed in the cell nucleus after few hours from the inoculation. Finally, we have evaluated the differentiation of pbMSC: for a myogenic induction cells were not only exposed to MyoD-TAT but also to mouse myoblast cell line C2C12 in a co-culture system. The differentiation of pbMSC towards the myogenic lineage was analysed using immunofluorescent specific antibodies.

## 1.5 A canine serum free skin culture model for basic research studies and testing therapeutics

***Miragliotta V**, Pirone A, Lenzi C, Ricciardi MP, della Valle MF, Abramo F*

Canine skin full thickness culture may offer unique opportunities both for overcoming the concerns on the use of animals for biomedical research and to study drug mechanism of action in a biologically relevant environment.

Normal skin was obtained from donor dogs referred for mastectomy. Biopsy samples were cultured in triplicate in Williams'E medium supplemented with penicillin/streptomycin, insulin, hydrocortisone and glutamine. General morphological features of epidermis, dermis and adnexa, morphometric assessment of epidermal thickness and keratinocyte proliferation (via Ki67 immunostaining) were assessed at day 1, 4 and 7. The effect of Epidermal Growth Factor (EGF) was also tested. Palmitoylethanolamide (PEA) was used to counteract mast cell degranulation induced by compound 48/80.

General morphological features of skin anatomical structures were well maintained up to day 7, except adnexa which showed early sign of vacuolization mainly in the proliferative compartment of the bulb; scattered pyknotic nuclei were visible in the epidermis at day 7. Epidermal thickness decreased from day 0 to day 7. Keratinocyte proliferation decreased from day 1 to day 4 excluding peripheral areas of the cultured biopsies; no Ki67 positive nuclei were visible at day 7. Treatment with EGF induced the formation of "epithelial tongues" at the periphery of cultured biopsies. Mast cell degranulation induced by compound 48/80 was inhibited by PEA.

The main concern of this model appeared to be inter-experimental variability. Once thoroughly standardized this method may help in dissecting canine skin morpho-physiology and has the potential of characterizing the biological effects of dermatologic therapeutics.

## 1.6 Epithelial-like stem cells isolated from equine epidermis show regenerative capacities in vitro and in vivo

***Patruno M**, Broeckx S, Martinello T, Gomiero C, Maccatrozzo L, Spaas J*

Stem cells have been found in hair follicles and dermis of mammals although also the epidermis contains a subpopulation of undifferentiated progenitor cells. However, only limited information concerning epidermis-derived epithelial-like stem/progenitor cells (EpSCs) is available, especially in a large animal model. In this research the purified cells from equine epidermis were characterized as EpSCs by means of positive expression for CD29, CD44, CD49f, CD90, Casein Kinase 2b, p63, and Ki67, low expression for cytokeratin (CK)14, negative expression for CD105, CK18, Wide CK, and Pan CK; in vitro, cells were differentiated toward keratinocytes and adipocytes fates. Moreover, to evaluate the regenerative capacities of EpSCs in vivo, six full-thickness skin wounds were performed in a 5-years old gelded horse: three were treated with allogeneic EpSCs and autologous platelet-rich-plasma (EpSC/PRP-treated) while three were treated with carrier fluid alone (PRP-treated). After 30 days the skin of EpSC/PRP-treated wounds was significantly thinner and exhibited increases in vascularization, elastin content, follicle-like structures and more restricted granulation tissue than the PRP-treated wounds, confirming that the stem cell treatment improved tissue repair after the clinical application. Recent data concern the comparison of autologous versus allogeneic EpSCs for treating the skin of our equine wound model (six French trotter mares).

## SESSION 2: Reproductive system

### 2.1 Morphometry and differential expression of carbohydrate residues in the sheep oviductal isthmus after superovulation

***Accogli G**, Lacalandra GM, Silvestre F, Binetti F, Caira M, Desantis S*

Isthmus, the caudal portion of the oviduct, consists in finger-like mucosal folds surrounded by a well-developed muscle wall. At the base of the folds there are narrow crypts which contribute to sperm selection and polyspermy control. Specific oviductal glycoproteins (OGPs) exposed on the apical surface of epithelial cells promote sperm-isthmus binding, sperm storage, sperm survival and capacitation state. Moreover, OGPs secreted by non-ciliated cells accumulate as a mucus-like substance in the lumen of the isthmus and associate with sperm and early embryos. Production and secretion of OGPs depend on the sex steroid fluctuations. Since the superovulation induces a lower fertilization rate after natural mating compared to the intrauterine insemination, we studied its effects on the isthmus epithelium using morphological analysis and lectin histochemistry. Isthmi from ovariohysterectomized ewes were fixed in neutral formalin, embedded in paraffin wax and sections processed for morphological and lectin histochemistry studies. The height of normal isthmus lining epithelium was significantly taller in the mucosal folds than in the crypts. Superovulation significantly decreased the epithelial height of the folds. Only non-ciliated cells from control ewes contained apical protrusion suggesting apocrine secretory activity. The quantitative evaluation of lectin staining revealed that superovulation reduces N- and O-linked asialoglycans but increases terminal fucose, N-acetylglucosamine, galactose, lactosamine, α2-3- and α2-6-linked sialic acids. Analysis of lectin binding distribution showed increase in difference of the glycan pattern between crypts and mucosal folds epithelium in the superovulated ewes. Further researches are needed to determine the effect of these changes in the sheep isthmus physiology.

### 2.2 Brain-derived neurotrophic factor (BDNF) and its receptor TrkB, during oocyte development in zebrafish

***Cacialli P**, Pellegrini E, Kah O, Castaldo L*

Brain-derived neurotrophic factor (BDNF) is a member of neurotrophin family, whose other components are nerve growth factor (NGF), neurotrophin (NT)3, NT 4/5 and limitedly to fish, NT 6/7. BDNF has been conserved during the vertebrate evolution. The primary amino acid sequences of zebrafish (Danio rerio) and human BDNF are 91% identical. BDNF signaling is transduced by TrkB receptor. In zebrafish there are two genes encoding for TrkB receptor. It is largely known that BDNF and TrkB promotes neuronal growth, differentiation, survival and synaptogenesis. BDNF, such as the other components of neurotrophin family, also acts on non neuronal cell populations. In the ovary, BDNF is involved in mammalian oocyte development, early embryo cleavage and blastocyst formation. To date, no data concerning BDNF and TrkB in teleost fish ovary are available. Thus, this study aims to investigate, by means of immunohistochemistry, the presence and distribution of BDNF and TrkB in the ovary of zebrafish, a teleost fish widely used as vertebrate model. In zebrafish, oocytes undergo five developmental stages. In early stages (I-II) no immunoreactivity to BDNF and TrkB expression was observed. From stage III onward BDNF was detected in the follicle cell layer, and TrkB appeared only in the stage V in thecal cells. These preliminary findings represent the first description of BDNF involvement in teleost fish oocytes development. The occurrence of BDNF in the follicular cells and TrkB in the thecal cells of oocytes stage V suggests a paracrine mode of action.

### 2.3 Functional assessment of Progesterone Receptor Membrane Component 1 (PGRMC1) activity during bovine oocyte meiosis

***Lodde V**, Tessaro I, Franciosi F, Raucci F, Terzaghi L, Sivelli G, Peluso JJ, Modina S, Luciano AM*

Previous studies suggest that PGRMC1 plays essential roles during bovine oocyte meiosis since it localizes to the centromeres at metaphase-I and II and concentrates between the separating chromosomes at ana/telophase-I. To confirm this hypothesis, we conducted a series of functional studies in which PGRMC1 activity was disturbed by using several experimental approaches and the effect on meiosis-I was assessed. In a first set of experiments, treatment with the PGRMC1 inhibitor (AG205) significantly affected oocyte meiosis in a dose dependent manner by: 1) decreasing the % of oocytes that extruded the first polar body (PB-I); 2) affecting meiotic progression by decreasing the % of oocytes that reached MII-stage and increasing the % of oocytes showing aberration of chromosome segregation that often resulted in the formation of scattered DNA within the cytoplasm. In a second set of experiments, a siRNA-mediated gene silencing approach was used to reduce oocytes PGRMC1 expression. Overall, PGRMC1 down-regulation mirrored AG205 effects, by 1) significantly reducing the % of oocytes extruding PB-I; 2) increasing the % of oocytes showing aberrant meiotic figures and DNA scattering. Finally in a third set of experiments, injection of an antibody to PGRMC1 had a more pronounced effect. In fact, this treatment significantly impaired the formation of MI plates and completion of meiosis-I. The present findings are consistent with PGRMC1 localization and with a putative role in both chromosomes separation and cytokinesis. Funding: FP7-PEOPLE-2011-CIG, contract n.:303640-Pro-Ovum and Fondo Piano di sviuppo UNIMI linea B – Giovani ricercatori - Grant n.:15-6-3027000-54.

## 2.4 Neurotrophins and their specific receptors in the oviduct of Japanese quail (Coturnix coturnix japonica)

***Maruccio L**, Castaldo L, D'Angelo L, Gatta C, Lucini C, Cotea C, Solcan C, Nechita EL*

Neurotrophins (NGF, BDNF and NT3) and their specific receptors (TrKA, TrKB and TrKC) were studied in the oviduct of three months old quails. The neurotrophin family mainly controls the development and maintenance of neuronal populations in the central and peripheral nervous system, but also acts on reproductive system.
The oviduct, distinguished in infundibulum, magnum, isthmus, uterus and vagina, was sampled for western blotting and immunohistochemistry analyses. Western blotting analyses revealed bands consistent with predicted molecular weights of neurotrophins and Trk receptors. Immunohistochemistry showed NGF positivity in surface epithelium and in ductal and/or glandular cells along all segments, and BDNF and NT3 positivity in cells of surface and ductal epithelium in magnum and isthmus. TrKA positivity was mainly detected in cells of surface and ductal epithelium along all segments, and TrKB only in surface and ductal cells of magnum. Moreover NGF, TrKA and TrKC positivity was always detected in the nervous components along all segments. These results suggest a role of neurotrophins related to the specific functions of the different oviductal tracts.

## 2.5 Urocortinergic system in the testes of normal and cryptorchid dogs

*Squillacioti C, De Luca A, **Liguori G**, Alì S, Germano G, Vassalotti G, Navas L, Mirabella N*

*Cryptorchidism is the most common disorder of the sexual development in dogs, occurring in* 13% of the males. Unilateral cryptorchidism is more frequent than bilateral and the right testicle seems to be more frequently affected. 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 normal and cryptic testis of the dog. The results showed that UCN, CRHR2 and CRHR1 were expressed in normal and cryptic testes. In normal testes UCN- and CRHR2-immunoreactivities (IR) were distributed in germ and interstitial Leydig cell. In cryptic testes, UCN- and CRHR2-IRs were found in gonocytes and in interstitial Leydig cells. CRHR1-IR was distributed in the vessel smooth musculature and in the fibromuscular cells encircling testicular tubules. UCN and CRHR2 mRNA expression levels were higher in the cryptic than in normal testes.
These results suggest that UCN and its receptors might play a role in regulating the spermatogenesis and hormonal activity of interstitial Leydig cells. The increase of UCN and CRHR2 expression observed in cryptic testes remains to be further investigated.

# SESSION 3: Digestive system, Bone, Response to toxicants

## 3.1 Plasma cortisol levels and expression of glucocorticoid receptors and oxidative stress markers in the fish Ombrina boccadoro exposed to transportation

***Boscolo Papo M**, Bertotto D, Negrato E, Maccatrozzo L, Poltronieri C, Caberlotto S, Radaelli G*

Transportation of fish is a common practice among aquaculture facilities and it is known to cause stress influencing the release of catecholamines and corticosteroids as well as the expression of oxidative stress markers. It includes a complex of factors such as handling, air exposure, constraint and low oxygen levels that induce in fish an increase in metabolic rate, overexertion and in general a rapid deterioration of water quality. The aim of the work was to investigate muscle cortisol levels the expression of glucocorticoid receptors in liver and muscle and the cellular localization of HSP70, 8-OHdG, HNE and NT in several tissues of the teleost fish Ombrina boccadoro (Argyrosomus regius) exposed to transport stress. Fish transport has been carried out by a commercial truck and lasted 48 hours. Fish were sampled before and after loading and during and at the end of transport event (14 and 48 hours after departure). Muscle cortisol levels were investigated by a specific radioimmunoassay protocol, whereas the expression of glucocorticoid receptors was studied by Real Time PCR. Oxidative stress biomarkers were investigated by an immunohistochemical approach but no differences were detected between stressed and control animals. Both muscle cortisol and glucocorticoid receptor levels strongly increased after loading but decreased during transport indicating adequate transportation conditions. Aquaculture specialists will benefit from the present work by taking into consideration the importance of cortisol levels as well as of glucocorticoid receptor expression as stress indicators during transport and the *importance of loading procedure to reduce stress during transport management.*

## 3.2 Regulation of taste signaling molecules by high protein diet in the pig gastrointestinal tract

***Mazzoni M**, De Giorgio R, Bombardi C, Grandis A, Vallorani C, Giancola F, Bianco F, Sternini C, Clavenzani P*

The discovery that taste receptors (TRs) and signaling molecules are expressed in the gastrointestinal tract (GIT) mucosa suggests nutrient-mediated chemosensing mechanisms influencing GI physiology via the release of endocrine messengers. TRs mediate gustatory signaling by interacting with α-gustducin (Gαgust) and α-transducin (Gαtran) subunits. This study was aimed to investigate Gαtran and Gαgust immunoreactive (-IR) cell changes in the pig GIT exposed to high protein diet (HP) for 3 and 30 days, respectively. In the stomach, Gαgust and Gαtran-IR cells contained 5-HT and ghrelin (GHR) while in the small and large intestine IR cells colocalized with 5-HT, CCK and PYY, respectively. In the pyloric mucosa, the mean number of Gαtran-IR cells were 111.5±25 (CTR), 144.8±12 (HP3), and 218.5±23 (HP30) (CTR vs HP3 and HP30 $P<0.05$; HP-3 vs HP30 $P<0.001$); Gαgust-IR cells were 126.9±26 (CTR), 158.4±24 (HP3), 214±26 (HP30) (CTR vs HP30 and HP3 vs HP30 $P<0.05$). In the pyloric mucosa, Gαtran/5-HT-IR cells were 107.8±28 (CTR), 141.3±14 (HP3) and 207.5±22 (HP-30) (HP30 vs CTR and HP3 $P<0.01$), similarly the Gαgust/5-HT-IR cells were 127.3±44 (CTR), 161±13 (HP3) and 203.3±24 (HP30) (HP30 vs CTR and HP3 $P<0.05$). In pyloric mucosa, Gαtran/GHR-IR cells were 76±22 (CTR), 106±24 (HP3) and 191.5±15 (HP30) (HP30 vs CTR and HP3 $P<0.01$) likewise Gαgust/GHR-IR cells were 64.5±23, 111.8±33, 189.5±34 in CTR, HP3 and HP30, respectively (HP30 vs CTR and HP3 $P<0.05$). We demonstrated that HP (30d > 3d) evoked an increased density of Gαgust/Gαtran in 5-HT- and GHR -IR cells lending support to TR-mediated effects in metabolic homeostasis and satiety mechanisms.

## 3.3 Investigation on skeletal development in small-sized breed newborn dogs: anatomic and radiographic findings obtained by spontaneously died animals

***Modina SC**, Veronesi MC, Andreis ME, Lodde V, Bolis B, di Giancamillo M*

Species-specific measurements and ranges for normal skeletal growth are reliable reference data for various studies, including those related to age determination and disorders of growth and nutrition. In dog, limited information is available about normal skeletal growth characteristics and when present, they refer mostly to Beagles as animal model (Helmsmuller, 2013). Because of the great number of pure breed dogs the main limitation of these studies relies on the need of homogeneous and sizeable populations; moreover each subject must be submitted to serial and multiple investigations that, although weakly invasive, are inadvisable in puppies, according to animal welfare rules. Finally, some analyses are not possible on living individuals. In this research we considered the possibility of using 27 remains of spontaneously died puppies (0-28 days) as a consistent population to study the skeletal development of small-sized breed dogs during the first month of life. These puppies belonged to different pure breeds and were categorized according to the standard breed adult body weight < 7 kg (Brianza, 2006). Anatomic and radiographic measurements of limb bones length and of skull diameters were positively correlated with weight and age of the subjects and weight was positively correlated with radius bone mineral density (Spearman bivariate test, $P<0.01$). Histological evidences confirmed the presence of the limb secondary ossification centres observed by x-rays and detailed the onset of their formation. These data suggest that cadavers of young animals may represent a useful tool to study skeletal development and possible disorders in dog.

## 3.4 Effect of a mixture of short and medium chain fatty acids esterified to glycerol in milk replacer on weaning calves rumen papillae development

***Ragionieri L**, Cacchioli A, Ravanetti F, Botti M, , Ivanovska A, Righi F, Quarantelli A, Panu R, Gazza F*

The supplementation of sodium butyrate in milk replacers (MR), commonly used to anticipate weaning, affects both small intestine and rumen development, determining earlier solid feed intake which leads to better performances in cattle. The present study aims to determine the effects of a mixture of short and medium chain fatty acids esterified to glycerol (SMCFA) added to MR on rumen papillae development of weaning calves offered starter diet at libitum.

Eight bull calves (10-days-old) were divided in two balanced groups: Control (C) and Treated (T). The latter received 0.2% SMCFA in MR besides starter diet. Animals were slaughtered at the age of 72 days.

Histomorphometric analysis performed on samples from the cranio-dorsal sack of the rumen did not indicate differences in the mean number of papillae or in their total surface/cm2 of mucosa but rather in their shape. T group had more wide, short tongue-like or irregular shaped papillae and many interposed papillae compared to C group whose narrow, tongue-to-finger-like papillae, often showed secondary papillae or irregular branches. In T group, enhanced proliferation of the epithelial cells of the stratum basale, spinosum and granulosum was observed in the interpapillar region, while C group showed more cellular layers in the stratum corneum at the base of the papillae and in the interpapillar region. SEM analysis confirmed the different morphology of the papillae in the two groups and, at high magnification, revealed the presence of more dense cytoplasmic protrusions covering horn cell surfaces in T group.

### 3.5 First spine of Atlantic bluefin tuna cranial dorsal fin: structure and bone resorption in wild and captive-reared specimens

*Santamaria N, Bello G, **Zupa R**, Passantino L, Pousis C, Di Comite M, Cicirelli V, Lacalandra GM, Carrassi M, Corriero A*

*The Atlantic bluefin tuna (ABFT), Thunnus thynnus, is one of the most promising fish* candidate for aquaculture. The ABFT, a highly migratory fish, is provided with a full set of fins, which play different roles in the swimming mechanics. The two dorsal fins are supported by spiny rays (spines) at the front and soft rays (rays) at the rear. The aims of the present study were to investigate the structure of the first spine of the cranial dorsal fin and to compare its bone apposition/resorption patterns in wild and captive-reared individuals. Cross-sections of this spine were cut by a low speed diamond saw; some of them were ground, polished and mounted on glass slides, whereas others were fixed in 4% paraformaldehyde, decalcified in 10% EDTA, dehydrated in ethanol and embedded in paraffin. Total, compact bone and reabsorbed bone surfaces were measured on undecalcified sections. Paraffin-embedded sections were further cut into 10-µm sections, which were stained with histological and conventional histochemical methods. Spine cross sections are characterized by several peripheral concentric compact bone layers delimiting an internal trabecular zone. New bone tissue is continuously apposed at the periphery of the spine while a progressive bone resorption occurs on the inside. The fraction of spine compact bone progressively decreases as fish age and size increase. The rates of spine bone resorption were significantly higher in captive-reared specimens. This abnormality adds to a number of anomalies occurring in reared ABFT and already reported in the literature.

### 3.6 Histological and immunohistochemical features of gastrointestinal tract, alveolar macrophages and blood leukocytes of pigs fed with polyphenols

*Varricchio E, Lombardi V, Paolucci M, Viola C, Coccia E, Romania S, Pasquale V, Maruccio L, Arcamone N, Avallone L, **Russo F***

In the last years, great importance has been given to the anti-oxidant and anti-pathogens effects of polyphenols. In this report, we describe the effect of polyphenols derived from wastewater olive-oil production on blood leukocytes and alveolar macrophages primary cell cultures and on gastrointestinal tract of pigs. Pigs were fed with the addition of polyphenols to standard diet for three months until their regular slaughter. Superoxide anion assay was performed on leukocytes, extracted and cultured from peripheral blood, and alveolar macrophages, isolated and cultured following Brockmeier method. In addition, histological samples of gastrointestinal tract (from stomach to rectum) were analyzed by ematoxylin-eosin staining (to evaluate the height and length of mucosal epithelium and the wideness of leukocytes infiltrate) and immunohistochemical method (using antibody against cyclooxygenase-2/COX-2). The results obtained show low levels of superoxide anion in the samples collected from the experimental group with respect to the control, while no significant differences were observed in the gastrointestinal tract, with the exception of leukocytes infiltrate in caecum-colon samples of treated animals. Finally, COX-2 immunopositive cells were found exclusively in the samples collected from control group. We suggest that the addition of polyphenols to standard diet reduces the presence of superoxide anion in pig peripheral leukocytes and alveolar macrophages and improves the immune response in the adult pig gut. In conclusion, we propose a possible re-use of this agri-food industry waste, otherwise highly polluting, as feed additives for farm animals.

## SESSION 4: Nervous system I: Fish

### 4.1 NT-4 mRNA and protein in the central nervous sytem of Nothobranchius furzeri

***D'Angelo L**, de Girolamo P, Avallone L, Cellerino A, Paolucci M, Varricchio E, Lucini C*

Neurotrophin-4 (NT-4) is a member of the nerve growth factor family, and is structurally and functionally related to other neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and the fish specific neurotrophin-6 (NT-6). Neurotrophins share approximately 50% homology in amino acid sequences and are known to have individual functions in limited regions and at specific ages. The molecular phylogeny of neurotrophin sequences suggests that the duplication of neurotrophins ancestor genes, occurred in fish, led to the formation of NT-4/5 and BDNF from an intermediate ancestor. NT-4, as all other neurotrophins, interact with two distinct receptors: TrkB, high affinity receptor, and p75 low-affinity receptor (p75NTR).

In the present survey, we identified and cloned the gene encoding NT-4 in the teleost N. furzeri, a model species for aging research. We studied the neuroanatomical localization of NT-4 mRNA and protein in the whole brain of adult specimens, respectively by in situ hybridization and immunohistochemistry.

NT-4 mRNA was abundantly and diffusely expressed in the telencephalon, and sparsely or in restricted areas of diencephalon, mesencephalon and rhomboencephalon. NT-4 protein was localized in few neurons of the telencephalon, in some nuclei of diencephalon, and in restricted areas of mesencephalon and rhomboencephalon. The neuroanatomical survey demonstrates that NT-4 is present in the adult brain of teleost N. furzeri, and mRNA and protein are differently distributed. These differences could indicate that the site of synthesis of NT-4 might not be the site of action.

## 4.2 Central regulation of food intake during aging in the teleost fish Nothobranchius furzeri

***Montesano A**, Arcamone N, Genade T, De Girolamo P*

The mechanisms deputed to energetic control have been selected by ancestral diets resulting from the nutrient disposal during the evolution. They are regulated by a network of molecules controlling metabolic needs and influence caloric intake. Also the course of aging adds significant modifications to energy homeostasis and fuel metabolism in.

An emerging model for aging research, a teleost fish Nothobranchius furzeri, the vertebrate with the shortest lifespan described in laboratory, is employed to investigate the regulation of Neuropeptide Y (NPY) and Orexin A and B in the hypothalamus. The study is carried out on specimens at different age stages and fed with different diets.

Immunohistochemical analyses revealed the localization and co-labeling of NPY, Orexins A and B in some hypothalamic areas, as the dorsal hypotalamus (Hd) and diffuse inferiore lobe (DIL). The co-labeled neurons were identified as serotoninergic.

In addition, the median lifespan is prolonged of more than one week in subjects treated with hypocaloric diet. In the latter group, western blot analyses revealed high expression of NPY in the brain.

These observations suggest that the role of NPY in food intake regulation is modulated during aging and hypocaloric regime, and is linked to the action of Orexin A. Furthermore, the co-presence of NPY and Orexin A in serotoninergic neurons could indicate the involvement of the two peptides also in the circadian rhythm regulation.

## 4.3 Chemosensory proteins in the chemosensory organs of adult zebrafish

***Randazzo B**, Abbate F, Ciriaco E, Montalbano G, Madrigrano MF, Levanti MB*

The three major chemosensory systems in teleosts are the gustatory system, the olfactory system and the diffuse cutaneo-mucus chemosensory system. All are devoted to the evaluation of chemical environment, i.e. food contents and pheromonal detection. The morphofunctional organs of these senses are the taste buds, the olfactory epithelium and the solitary chemosensory cells, respectively. In the last decade electrophysiological and molecular studies in non-vertebrates and vertebrates have identified several ion channels as responsible for detecting a range of thermal, chemical or mechanical stimuli. The capability of sensitive cells to detect and to codify the specific stimuli is due to the combination of different ion channels. To elucidate this topic in fishes we have systematically investigated the occurrence of different ion channels involved in chemodetection in mammals (acid-sensing ion channels 1 to 4 (ASIC 1-4), transient-receptor potential ion channels TRPA1, TRPC2, TRPM8, TRPVR1 and TRPV4 in taste buds, olfactory epithelium and the skin of adult zebrafish. In taste buds positive immunostaining for ASIC4, TRPA1, TRPVR1, TRPV4 was detected whereas ASIC2 was found in the nerve supplying them. Regarding the olfactory epithelium , ASIC1, ASIC3 and ASIC4 immunolabeled the cilia of the olfactory epithelium while ASIC2 was detected in the cilia of the non-sensory epithelium; discrete population of sensory neurons also displayed immunoreactivity for TRPC2, TRPVR1, TRPV4 and TRPM8. Finally, the diffuse cutaneous chemosensory cells only displayed TRPA1 immunoreactivity. The present results contribute to the knowledge of the molecular basis of chemosensitivity in teleosts.

## 4.4 Putative mechanoproteins in the cephalic neuromast of the adult zebrafish

***Madrigrano M**, Randazzo B, Scopitteri T, Cavallaro M, Montalbano G, Germanà A, Vega JA, Laurà R*

The neuromasts are the functional unit of the lateral line system in fishes that function as mechanosensors. The mechanisms underlying the detection of the mechanical stimuli remain poorly understood at the molecular level, and no information is available in neuromasts. Mechanotransduction requires a mechanical stimulus to be converted into an electrical signal, and this occurs through the activation of ion channels that gate in response to chemical stimuli. At present members of the degenerin /epithelial sodium channels (DEG/ENaC), the transient receptor potential (TRP) channel, and the two pore domain potassium (K2p) channel super-families are considered as putative mechanotransducer channels. Nevertheless, only few members of these super-families have proved mechanotransducer properties in vertebrates. In any case the ion channels involved in mechanotransduction are expected to be expressed in the mechanoreceptors where they occur. We have used simple and double immunohistochemistry to systematically investigate the occurrence of putative mechanoproteins αe γENaC subunits,ASIC1-4, TRPC6, TRPV1 and 4,TRPM8 in the cephalic neuromasts of the adult zebrafish. Specific immunoreactivity for ASIC2 was observed in nerves supplying neuromast while the sensory hair cells displayed immunoreactivity for TRPV4, but not for TRPV1, TRPC6 and TRPM8. A subpopulations of hairy cells also displayed αENaC subunit immunostaining, and ASIC3 was occasionally found in these cells. All together the results strongly suggest that, similarly as it occurs in mammals, putative mechanoproteins are localized in the mechanosensory system of the teleost.

## SESSION 5: Nervous system I: Mammals

### 5.1 The number of Purkinje neurons and their topology in the cerebellar vermis of the normal mouse and the Reln haplodeficient mouse

***Lossi L**, Magliaro C, Cocito C, Bagatella S, Ahluwalia A*

The *Reeler* heterozygous mice (*Reln*$^{+/-}$) are haplodeficient in the gene (*Reln*) encoding for the Reelin glycoprotein (Reln) and, similarly to autistic or schizophrenic human subjects, display reductions in brain/peripheral Reln. They are, however, still poorly characterized in structural and functional terms, as the alterations in the cytoarchitecture of the brain may be subtle, and difficult to demonstrate by current histological approaches. In *Reln*$^{+/+}$ (controls) and *Reln*$^{+/-}$ adult mice (P60) of both sexes (n = 16), we have analyzed the number and topological organization of the Purkinje neurons (PNs) in five vermal lobules that intervene in the processing of different types of functional inputs to the cerebellum [central and culmen (sensory-motor), tuber (cognitive), uvula (default mode network), and nodulus (emotive/vestibular)]. Animals were crossed with L7GFP mice so that the GFP-tagged PNs could be immediately identified in cryosections, which were photographed with a digital camera and processed using NEMO [NEuron MOrphological analysis tool - *Front Neuroinform* (2013) 7:2] for quantitative topological and statistical analyses.

We defined diversity indices to identify the lobules most subject to variations in PN arrangement and numbers after 2-way ANOVA. Our analysis suggest the existence of gender differences in the topology of PNs in both *Reln*$^{+/+}$ and *Reln*$^{+/-}$ mice, with diversity indexes indicating loss of PN alignment particularly in *Reln*$^{+/-}$ versus *Reln*$^{+/+}$ females.

Therefore image processing combined with statistical analyses can reveal previously unforeseen gender and genotype-related structural differences in the cerebellum. They may be clues for the definition of novel biomarkers in human psychiatric disorders.

### 5.2 Programmed cell death in the postnatal cerebellar development of the Reeler mouse

***Castagna C**, Merighi A, Lossi L*

Programmed cell death (PCD) was demonstrated in neurons and glia in normal brain development, plasticity, and aging, but also in neurodegeneration. Autophagy, characterized by cytoplasmic vacuolization and activation of lysosomal hydrolases, and apoptosis, portrayed by chromatin and nuclear condensation, are the two most common forms of PCD. Their underlying intracellular pathways are partly in common and a population of neurons can die following both modalities, according to the type of death-triggering stimulus. Reelin is an extracellular protein necessary for proper neuronal migration and brain lamination. In the mutant Reeler mouse, its absence causes neuronal mispositioning, impairment of dendrite outgrowth and reduced numbers of synapses throughout the CNS, with a notable degree of cerebellar hypoplasia that was tentatively related to increased PCD. We have carried out an ultrastructural analysis on the occurrence and type of postnatal PCD affecting the cerebellar neurons in normal and Reeler mice. In the forming cerebellar cortex, PCD took the form of apoptosis or autophagy and mainly affected the granule cells. Numbers of apoptotic neurons were comparable in both mouse strains at P0-P5, while in mutants they increased at P10 and became significantly higher at P15. The number of autophagic neurons in Reeler mice decreased from birth to P5, was significantly lower than in controls at P10 and increased thereafter. Therefore cerebellar neurons undergo different types of PCD and a Reelin deficiency affects the type and degree of neuronal death during cerebellar development.

### 5.3 An improved method for in vitro morphofunctional analysis of dorsal root ganglia in the normal and diabetic mouse

***Ciglieri E,** Ferrini F, Boggio E, Salio C*

Nociceptive sensory neurons in dorsal root ganglia (DRGs) are the first-order neurons along the pathway that conveys pain to the cerebral cortex. Physiological pain has a protective role, which is disrupted in several pathologies leading to abnormal inflammatory or neuropathic pain. The latter is among the most common complications of diabetes in humans. Here, we describe an in vitro procedure for combined morphofunctional analysis of mouse DRGs. We have applied this protocol to study the neurochemistry and functional properties of DRG neurons in normal CD1 mice and in mice that were made diabetic after a single intraperitoneal injection of streptozotocin (150 mg/Kg) at P30. All animals were sacrificed at P60, the DRGs were acutely excised in ice cold oxygenated Krebs solution, and the connective tissue was dissolved by incubation in 5-10 mg/mL collagenase. Acutely excised DRGs were then used for patch-clamp recording and immunocytochemical staining, as the elimination of the connective capsule facilitated the access of the recording pipette and the penetration of primary antibodies in whole-mount preparations. This method makes possible to imagine the entire ganglion cytoarchitecture at the confocal microscope without sectioning. After immunostaining with neuronal (CGRP and IB4) and non-neuronal (GFAP) markers fine confocal-aided 3D reconstructions were obtained. Electrophysiological recordings confirmed an increased excitability of DRG neurons from diabetic mice, which was also confirmed after pERK immunostaining. This method is a flexible in vitro approach that preserves the neuroanatomical relationships between individual neurons at the same time allowing a functional analysis of their electrophysiological properties.

## 5.4 BDNF and GDNF expression characterizes discrete populations of nociceptors

*Salio C,* ***Ferrini F***

The brain derived neurotrophic factor (BDNF) and the glial cell line-derived neurotrophic factor (GDNF) are growth factors promoting the survival and differentiation of sensory neurons and intervening in the control of nociceptive neurotransmission.

We have studied the localization of BDNF and GDNF in parallel with other established markers of the nociceptors in the lumbar dorsal root ganglia (DRGs) of P21 mice.

Our results can be summarized as follows:

1) BDNF and GDNF are detected in distinct populations of small- to medium-sized DRG neurons, with BDNF three times more expressed than GDNF [186.4±1.7 BDNF-immunoreactive (IR) cells/DRG vs 57.7±0.3 GDNF-IR cells/DRG; n= 3 mice]; 2) A subset of BDNF-expressing neurons and a subset of GDNF-expressing neurons are of the peptidergic type; 3) BDNF-IR neurons are a subpopulation of calcitonin gene-related peptide (CGRP)-IR neurons (41.3±0.4%), which are also positive for substance P (SP) (42.3±0.1%) but not for somatostatin (SST); 4) GDNF-IR neurons are a subpopulation of CGRP-IR neurons (95.8±0.1%), which are also positive for SST (67.9±2.1%) but not for SP; 5) Both growth factors do not colocalize with IB4, a marker of non-peptidergic nociceptors.

Our results show the existence of two distinct subpopulations of peptidergic CGRP-IR nociceptors, either expressing BDNF (plus SP) or GDNF (plus SST). Together with previous functional observations in the dorsal horn, these data suggest the existence of two independent subpopulations of nociceptors that may be one of the anatomical substrates for discrimination of specific painful stimuli modalities.

## 5.5 Calcitonin Gene-Related Peptide (CGRP) expression in the spinal cord and spinal ganglia of the Bottlenose Dolphin (Tursiops truncatus)

***Rambaldi A****, Grandis A, Mazzoni M, Tagliavia C, Clavenzani P, Cozzi B, Bombardi C*

CGRP is a neuropeptide especially involved in pain transmission. The localization of CGRP in the nervous system of different species has been extensively investigated, but no data are available on its distribution in the spinal cord and spinal ganglia of Cetaceans. Samples of spinal cord and spinal ganglia from three bottlenose dolphins (Tursiops truncatus) were used for this study. The tissue was stained with histological and immunohistochemical methods. Morphometric analysis of motor neurons and primary afferent neurons (PANs) of spinal ganglia was performed. In addition, the percentage of ir-neurons in both spinal cord and ganglia has been calculated. In the spinal cord the CGRP immunoreactivity was localized in small interneurons of laminae I-II and in large motoneurons of lamina IX. The immunostaining in motoneurons was mainly localized in the somata and appeared as a specific granular pattern. In the spinal cord intensely labelled fibers were observed in laminae I, II and X. In spinal ganglia the majority of CGRP-ir PANs showed a small or medium-sized somata and represented the 40 % of the total neuronal population. The abundance of ir-fibers in laminae I and II is in agreement with the role played by this neuropeptide in the transmission of nociceptive stimuli. Ir-fibers have also been observed in lamina X, which receives visceral afferent fibers; thus CGRP could be also involved in modulation of visceral sensibility. The presence of the CGRP in motoneurons suggests an interaction between these cells and skeletal muscle mediated by this neuropeptide.

Questa pagina è lasciata intenzionatamente vuota per eventuali appunti

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