IJAE
Vol. 116, n. 3: 153-206, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists (AMV)

Bologna 26-28 May 2011

Main Lectures and Abstracts

Edited by
Rino Panu

http://www.fupress.com/ijae

CONTENTS

IJAE
Vol. 116, n. 3: 158-xx, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

In memoriam –Professor Vera Pedini –1959-2010

Professor **Ceccarelli Piero**

Dear colleagues, I never would have imagined that I would be commemorating Vera - in fact, I would have thought the opposite. Unfortunately, fate had other plans. Hence, I am here, commemorating her on behalf of the Group of Veterinary Anatomy of Perugia, which includes Paola Coliolo and Gabriella Mancini.

Vera Pedini was born in Perugia on January 18, 1959. She graduated *cum laude* in Agricultural Science at the University of Perugia in 1980/81, and her supervisor, Professor Fagioli, was a constant presence in her academic career from then on. In fact, he was a member on the commissions of her examinations for both associate professor in 1998, and, subsequently, full professor in 2006.

Normally, in a commemoration, one reviews the academic and scientific achievements of the person who has passed. To this end, Vera's notable and highly qualified scientific studies include: mammals major and minor salivary glands, of which she investigated the morphology, ultrastructure (under the initial guidance of Prof. Anna Maria Gargiulo), and histochemical characteristics of secretion, with special reference to the typization of glycoconjugates; the study of fish digestive systems; the identification of endocrine cells in various systems of fish and mammals; and, most recently, stem cells from hair follicles and adipose tissue. However, I believe that the leitmotif of Vera's research can be indentified in the typization of surface glycoconjugates, demonstrated by her prolific activity as an international peer reviewer. Moreover, this topic was at the heart of her relationship with Paola Scocco, which began as a scientific collaboration, but became a solid and sincere friendship.

As a teacher, she was very appreciated by colleagues and students alike, at both the undergraduate and postgraduate levels, as well as higher levels of specialization. Nevertheless, I like to remember her as a person and a friend. Vera embodied intelligence, sincerity, irony, cheerfulness, and openness. Her contribution to the scientific, professional, and personal growth of the youngest members of the Group of Veterinary Anatomy of Perugia was essential, as was her contribution to a collaborative and serene work relationship for everyone involved. No less important, though, was her capability to understand academic trends, which made her a valued confidant. Every morning, possibly over a cup of coffee, we would decide what to do in the section, the department, and the faculty. For a long time, and sometimes even now, I tried to imagine how Vera would comment, and, more importantly, how she would have proposed to resolve the delicate and critical steps that involved the small but, and I say this without false modesty, valuable Group of Veterinary Anatomists of Perugia.

IJAE
Vol. 116, n. 3: 159-xx, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Main Lectures

The upper respiratory tract of dolphins

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Summary

The functional anatomy of the respiratory system of dolphins has been scarcely studied. Specifically, the capacity of the system to resist pressure changes during diving has not been fully understood. Here we shortly describe the upper respiratory tract of dolphins based on three common species, the bottlenose dolphin *Tursiops truncatus*, the Risso's dolphin *Grampus griseus*, and the striped dolphin *Stenella coeruleoalba*. We emphasize the keymorphological features that represent evolutionary adaptations to life in the water, and, furthermore, also present a model of the tracheo-bronchial tree based on mechanical characterization and subsequent computational simulation of its biomechanical behaviour. Comparisonswith the goat allowed us to determine how different structures may respond to diving-related pressure.

Key words

Respiratory system; dolphin; diving; breath-holding, finite element model.

Introduction

The respiratory system of cetaceans is modified to allow complete gas exchange during the short surface intervals between dives (Ito et al., 1967; Ponganis et al., 2003). The nose is turned into a blowhole that requires voluntary opening, the nasal cavities are substituted by a series of nasal sacs and the larynx has a peculiarly angular shape to convey inspired air into the short trachea, placed parallel to the long axis of the body. Based on a series of evidences, it is now widely accepted that,during descent, the air progressively escapes the dolphin lung till complete organ collapse, believed to take place between 70 (Ridgway and Howard, 1979) and 150 meters of sea water (msw), (Fahlman et al., 2009). The blowhole and nasal sacs are not modified by increases of the external pressure, but recent studies demonstrated, at least in human breath-holding deep divers (>100 msw), that the trachea is progressively deformed and flattened possibly to curtail the inner dead space (Lindholm and Nyren, 2005; Fitz-Clarke, 2007). The tracheo-bronchial structure of dolphin slacks the dorsal *musculus trachealis*, the cartilaginous rings are irregular and intertwined (Fanning and Harrison, 1974), and the tracheal submucosa is lined by an extensive cavernous venous plexus of veins (Cozzi et al., 2005). The whole structure must stand increasing and potentially long lasting external pressures (for detailed description of pressure effects see Fahlman et al., 2006; Fitz-Clarke, 2009). Since in cetaceans a series of muscular sphincters intervene to shut tightly the terminal ramifications of bronchioles after the air escapes the lungs during descent,

the gas flow that thus moves upward leaves the trachea and larynx to deal with pressure changes.

Here we examined the upper respiratory tract of three species of dolphins common in the Mediterranean waters, namely the bottlenose dolphin *Tursiops truncates* (Montagu, 1821), the Risso's dolphin *Grampus griseus* (G. Cuvier, 1812), and the striped dolphin *Stenella coeruleoalba* (Meyen, 1833). We also describe a computational finite element model of the tracheal bifurcation of the bottlenose dolphin that allowed us a functional comparison with the trachea of the goat under simulated diving conditions.

Materials and Methods

Animal tissues

This study is based on cetacean tissues stored in the Mediterranean marine mammal tissue Bank of the University of Padova (http://www.mammiferimarini.sperivet.unipd.it/), that presently preserves 10 specimens of *Grampus griseus*, 58 of *Stenella coeruleoalba* and 82 of *Tursiops truncatus*. The goat tracheas were obtained at local abattoirs working under the European Communities Council directive concerning animal welfare during the commercial slaughtering process (86/609/EEC). The cetacean tracheas, larynxes and samples of the blowhole and air sacs were either fixed in buffered formalin for anatomical evaluations or frozen for stress-strain relationship detection byclassical biomechanical tests (Fung, 1993), aimed at implementing a reliable computational model (for details see Bagnoli et al, 2011).

Computational model

Two 3-D geometrical computational models representing the tracheo-bronchial bifurcation of a bottlenose dolphin and a goat were developed based on histology measurements. These models were discretized with 8-nodes hexahedral elements and used for numerical analyses (ABAQUS Inc., Providence, RI, USA) aimed at simulating the effect of water pressure applied on the airways during diving.

Experimental uni-axial tensile tests (Synergie 200 MTS Axial machine, 2003 MTS Systems Corporation, Eden Prairie, MN, USA) performed on samples cut from the tracheo-bronchial trees of 4 adult bottlenose dolphins and 1 goat, provided the stress-strain mean curves for cartilage, connective tissue and muscle (goat only), to be implemented in the finite element model (Bagnoli et al., 2011).

Boundary conditions were assigned to simulate *in vivo* loading conditions: pre-tensioning (3% pre-stretch in longitudinal direction) and external uniform pressure load (0 to 100 msw). The presence of air in the upper airways at different depths was simulated to highlight the contribution of air in maintaining the airways lumen pervious. The alveolar collapse phenomenon was also reproduced, resulting in an air flow of $27 \cdot 10^{-6}$ kg/s (Fitz-Clarke, 2007) from the alveoli to the upper airways.

Results

Structure of the upper airways

The blowhole of the three species shows the classical crescent shape of the external lid that leads Fig. 1A), upon opening, into a series of chambers that in the living

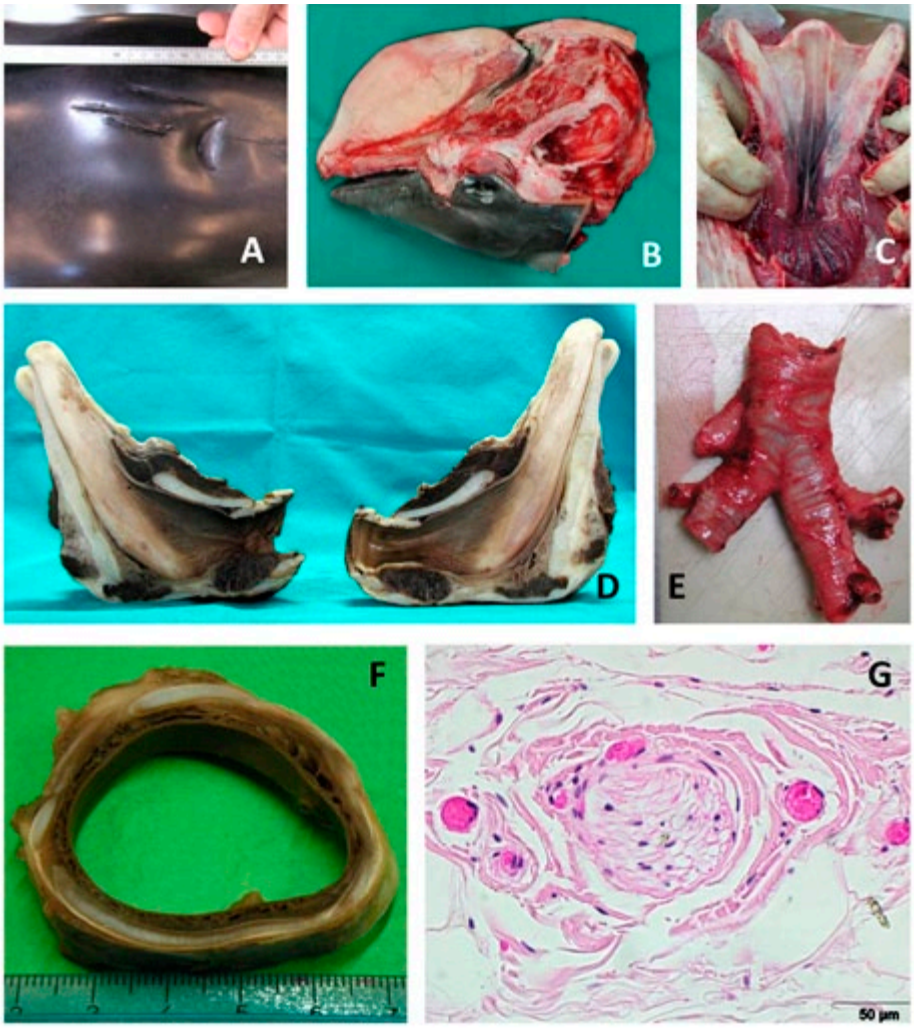

Figure 1 – Images of the upper respiratory tract of dolphins. A), Blowhole of a bottlenose dolphin; B) Section of the head of a Risso's dolphin showing the blowhole opening into the nasal sacs; C) Laryngeal tonsil of a bottlenose dolphin; D) Sagittal section through the trachea of a bottlenose dolphin; E) Trachea and early bronchial subdivisions of a Risso's dolphin; F) Thick section of the trachea of a bottlenose dolphin showing the huge vascular venous plexus in the submucosa; G) Innervation of the submucosal venous plexus of the trachea of a bottlenose dolphin.

animal modulate the whistles typical of each species (Fig. 1B). The larynx shows the well described "duck bill shape" (Fig. 1 D) and presents a well evident laryngeal tonsil in the basal folds of the organ (Fig. 1 C). The trachea is composed by irregular but semi-continuous rings (Fig. 1 E), and is more robust in the bottlenose and Risso dolphins than in the striped dolphin, depending also on the increasing size of the individual specimens. In all three species the trachea shows no *musculus trachealis* and displays a peculiar venous network in the submucosa (Fig. 1 F) endowed with a rich innervation (Fig. 1 G). For further details on the vascular plexus and relative innervations see Cozzi et al. (2005).

Computational model outcomes

Comparison between the mechanical behaviour of the goat (Fig. 2 A) and dolphin (Fig. 2 B) airways highlights the lower collapsibility of the dolphin structure due to higher stiffness, muscle lack and irregularly shaped cartilaginous rings. The goat trachea appears to be almost completely collapsed (Fig. 2 A2) when an external pres-

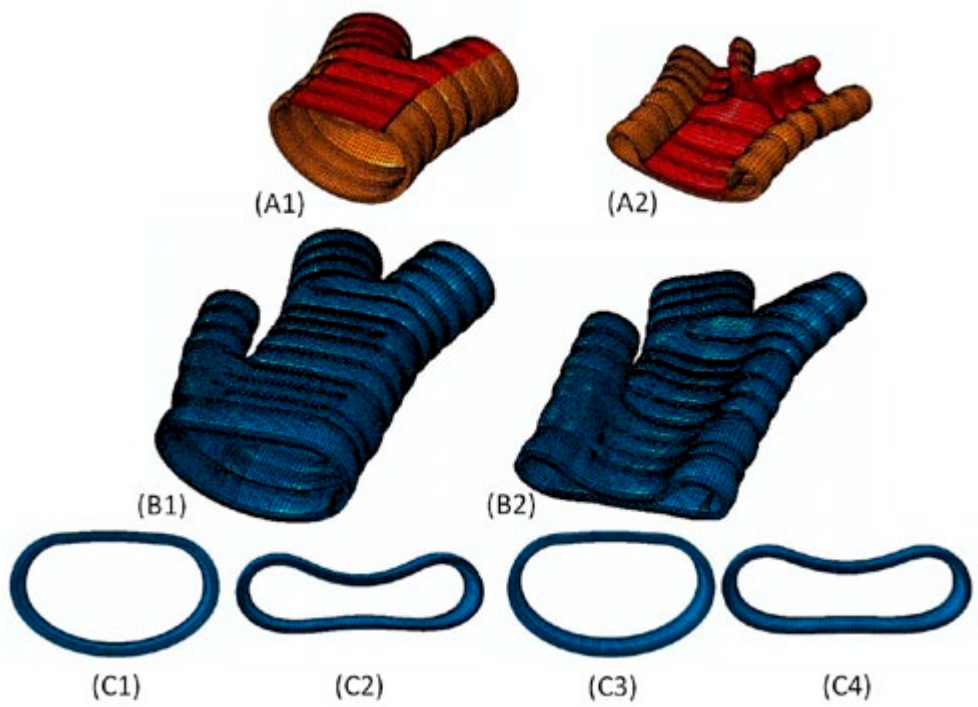

Figure 2 – Finite Element Model outcomes. Comparison between goat (A) and bottlenose dolphin (B) models: unloaded (A1, B1) and loaded (A2, B2) configurations (pressure 98 kPa).C1–C4: Dolphin airways deformation (single tracheal ring); C1,unloaded at 0 msw; C2, with trapped air at 10 msw depth; C3, with trapped air and flow from the collapsed alveoli at 10 msw depth; C4, with trapped air and flow from the collapsed alveoli at 80 msw depth.

sure of 98 kPa (about 10 msw) is applied, due to the inward displacement of the soft muscular region into the lumen of the cartilaginous rings. Considering the presence of air within the trachea during simulated dives, we noted that at the same pressure of 98 kPa, the dolphin airways appear to be slightly less collapsed without air (Fig. 2 B2), while the lumen is still open, even though deformed, when the presence of air inside the airways is accounted for (Fig. 2 C2; compare with the unloaded shape of Fig. C1). At a pressure of 98 kPa, alveolar air flow completely prevents the collapse of the tracheo-bronchial tree (Fig. 2 C3). A pressure of 784.5 kPa (about 80 msw) (Fig. 2 C4) induces the same tracheal deformation obtained at 98 kPa without considering air flow (Fig. 2 C2).

Discussion

The structure of the upper respiratory tract of the three species of dolphins clearly indicate an increase of the general resistance and stiffness in comparison with terrestrial mammals. The larynx, and especially the trachea with its continuous rings and absence of *musculus trachealis*, represent a robust mean for gas transfer, since the relatively large lumen allows a fast flow during surface breathing. On the other hand, the presence of vascular lacunae in the tracheal submucosa, resembling a corpus cavernosum-like structure, is puzzling, given the potential danger of gas exchanges between the pressurized air content of the lumen and the huge submucosal veins.

The computational model of the tracheo-bronchial tree of the bottlenose dolphin enabled us to replicate diving pressure conditions of the isolated organ. Our data confirm previous studies on terrestrial mammals that showed smooth muscle collapse when the internal airway pressure becomes negative (Begis et al., 1988; Costantino et al., 2004; Bagnoli et al., 2007). Our model also indicates that the absence of the *musculus trachealis*, the stiffer mechanical properties of the cartilage, and the peculiar conformation of the cartilaginous rings indeed contribute to keep the tracheo-bronchial tree of the bottlenose dolphin open under pressure. This evolutionary adaptation may explain the differences with human breath-holding deep divers (see also Meyers et al., 1980; Rains et al., 1992; Bostrom et al., 2008). The air trapped into the airways is fundamental to delay the collapse of the trachea with respect to what is observed in the organ of the goat.

Therefore the different anatomy and stiffer mechanical properties of the upper respiratory tract of the dolphin must be considered an adaptation to an external environmental characterized by ever-changing pressure. Comparisons with the goat upper airways may explain structural differences and performance limits displayed by terrestrial *vs.* aquatic mammals during breath-holding diving.

References

Bagnoli P., Cozzi B., Zaffora A., Acocella F., Fumero R., Costantino M.L. (2011) Experimental and computational biomechanical characterisation of the tracheo-bronchial tree of the bottlenose dolphin (*Tursiops truncatus*) during diving. J. Biomech.44: 1040–1045.

Bagnoli P., Tredici S., Seetharamaiah R., Brant D.O., Hewell L.A., Johnson K., Bull J.L., Costantino M.L., Hirschl R.B. (2007) Effect of repeated induced airway collapse during total liquid ventilation. ASAIO J. 53: 549-555.

Begis D., Delpuech C., Le Tallec P., Loth L., Thiriet M., Vidrascu M. (1988) A finite-element model of tracheal collapse. J. Appl. Physiol. 64: 1359-1368.

Bostrom B.L., Fahlman A., Jones D.R. (2008) Tracheal compression delays alveolar collapse during deep diving in marine mammals. Resp. Physiol. Neurobiol. 161:298-305.

Costantino M.L., Bagnoli P., Dini G., Fiore G.B., Soncini M., Corno C., Acocella F., Colombi R. (2004) A numerical and experimental study of compliance and collapsibility of preterm lamb tracheae. J. Biomech. 37: 1837–1847.

Cozzi B., Bagnoli P., Acocella F., Costantino M.L. (2005) Structure and biomechanical properties of the trachea of the striped dolphin *Stenella coeruleoalba*: evidence for evolutionary adaptations to diving. Anat. Rec. A Discov. Mol. Cell. Evol. Biol. 284: 500-510.

Fahlman A., Hooker S.K., Olszowka A., Bostrom B.L., Jones D.R. (2009) Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-holding diving: The Scholander and Kooyman legacy. Resp. Physiol. Neurobiol. 165: 28-39.

Fahlman A., Olszowka A., Bostrom B.L., Jones D.R. (2006) Deep diving mammals: dive behaviour nd circulatory adjustments contribute to bends avoidances. Resp. Physiol. Neurobiol. 153: 66-77.

Fanning J.C., Harrison R.J. (1974) The structure of the trachea and lungs of the South African bottlenose dolphin. In: Harrison R.J.: Functional anatomy of marine mammals, Vol. 2; Academic Press, London-New York. Pp. 231-252.

Fitz-Clarke J.R. (2007) Mechanics of airways and alveolar collapse in human breath-hold diving. Resp. Physiol. Neurobiol. 159: 202-210.

Fitz-Clarke J.R. (2009) Lung compression effects on gas exchange in human breath-hold diving. Resp. Physiol. Neurobiol. 165: 221-228.

Fung Y.C. (1993) Biomechanics: Mechanical properties of living tissue. Springer-Verlag, Berlin.

Ito T., Kobayashi K., Takahashi Y. (1967) Histological studies on the respiratory tissue in the dolphin lung. Arch.Histol.Jpn. 28: 453-470.

Lindholm P., Nyrén S. (2005) Studies on the inspiratory and expiratory glossopharyngeal breathing in breath-hold divers employing magnetic resonance imaging and spirometry. Eur. J. Appl. Physiol. 94: 646-651.

Meyers A.D., Bishop H.E., Peters, S. (1980) Biomechanical characteristics of the human trachea. Otolaryngol. Head Neck Surg. 88: 409-411.

Ponganis P.J., Kooyman G.L., Ridgway S.H. (2003) Comparative diving physiology. In: Brubakk A.O., Neuman T.S.: Bennett and Elliott's Physiology and Medicine of Diving, Saunders, Edinburgh. Pp. 211-226.

Rains J.K., Bert J.L., Roberts C.R., Paré P.D. (1992) Mechanical properties of human tracheal cartilage. J. Appl. Physiol. 72: 219-225.

Ridgway S.H., Howard R. (1979) Dolphin lung collapse and intramuscular circulation during diving: evidence from nitrogen washout. Science 206: 1182-1183.

IJAE
Vol. 116, n. 3: 165-xx, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Hic est locus ubi mors gaudet succurrere vitae: the utility of morphologic disciplines in the study of cetaceans' pathology

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Summary

The role played by morphologic disciplines and investigations in the study of the causes of death as well as of the pathology of cetaceans is of paramount relevance. In this respect, an absolutely paradigmatic example is that represented by *Morbillivirus* infections, which during the last 25 years have caused a number of dramatic epidemics among free-ranging pinnipeds and cetaceans worldwide.

Key words

Stranded cetaceans; pathologic anatomy; histopathology; immunohistochemistry; pathogenesis; morbillivirus; Mediterranean Sea; Italy.

The contribution given by anatomo-histopathology, along with histochemistry, immunohistochemistry and ultrastructural pathology, to the assessment of the cause(s) of stranding and death of cetaceans is of paramount relevance. A similarly crucial role is also played by stranded cetaceans as strategic indicators of the health and conservation status of their "conspecifics and heterospecifics" living in the open sea. With reference to the aforementioned concepts, a highly paradigmatic example is with no doubt that represented by *Morbillivirus* infections. As a matter of fact, in the last 25 years at least 10 distinct morbilliviral epidemics have occurred among several free-ranging pinniped and cetacean populations and species throughout the world. Five new members of the *Morbillivirus* genus have been identified from affected animals in the context of these epidemics, namely "*Phocine/Phocid Distemper Virus*" (PDV), "*Porpoise Morbillivirus*" (PMV), "*Dolphin Morbillivirus*" (DMV), "*Monk Seal Morbillivirus*" (MSMV) and "*Pilot Whale Morbillivirus*" (PWMV), with PMV, DMV and PWMV having been more recently gathered under the common denomination of "*Cetacean Morbillivirus*" (CeMV). Different strains of "*Canine Distemper Virus*" (CDV) were also responsible for the two mass die-offs of Bajkal seals (*Phoca sibirica* or *Pusa sibirica*) and Caspian seals (*Phoca caspica* or *Pusa caspica*) in 1987-'88 and in 1997-2001, respectively. Apart from the two aforementioned outbreaks, other dramatic morbilliviral epidemics were, in chronological order, those involving common seals (*Phoca vitulina*) in the North Sea during 1988 (and subsequently, in an even more dramatic manner, during 2002), bottlenose dolphins (*Tursiops truncatus*) along the eastern USA

seaboard during 1987-'88, striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea throughout 1990-'92 and Mediterranean monk seals (*Monachus monachus*) along the coast of Mauritania in 1997, respectively. The severe epidemic which occurred between 1990 and 1992 in the Mediterranean Sea basin was followed, throughout 2006-2008, by a less dramatic mortality event in the same area, with the latter affecting not only striped dolphins but also pilot whales (*Globicephala melas*). At *post-mortem* examination, a macroscopic lesion frequently encountered in both *Morbillivirus*-infected pinnipeds and cetaceans is represented by a more or less severe bilateral pneumonia, with consolidation, congestion and *oedema* of both lungs, which fail to collapse. Histologically, a non-suppurative broncho-interstitial pneumonia, characterized by type II pneumocyte hyperplasia and intrabronchial, intrabronchiolar and endoalveolar "*Warthin-Finkeldey*"- type syncytia, as well as a multifocal, non-suppurative encephalitis, associated with a severe and generalized lymphoid tissue depletion, are commonly observed. Furthermore, eosinophilic viral inclusions are often detected, at both intracytoplasmic and intranuclear level, within bronchial and bronchiolar epithelial, pulmonary syncytial, neuronal and other cell types. These inclusions, together with lymphoid and other cellular elements, are often found immunohistochemically positive for morbilliviral antigens. Although much high quality scientific work has been carried out during the last 20-25 years in the context of *Morbillivirus* infections in aquatic mammals, there still remain a number of relevant issues requiring further research activity. Among these, hitherto unsolved questions concern the origin and the evolutionary phylogeny of such viruses, as well as their host range (including also terrestrial mammals), pathogenicity, ecology and epidemiology. In this respect, it should be also underlined that, apart from the lack of detailed scientific information regarding the pathogenesis of *Morbillivirus* infections in aquatic mammals, such infections may represent useful comparative pathology models in the study of similar disease conditions in man and terrestrial mammal species. Finally, another crucial issue is that regarding the potential synergistic effects, if any, exerted by a number of environmental pollutants, with special emphasis on certain organochlorines (PCBs, dioxins, 4-4'DDE, etc.) and heavy metals (Hg, Pb, Cd, etc.), in modulating the pathogenic and pathogenetic activity of morbilliviruses in susceptible aquatic mammal species.

Dedication

The present Lecture is dedicated to the memory of Elena Petrizzi, a marvellous Baby Angel quietly resting in Heaven and now living inside other three children, to whom Elena's organs were generously donated by her Parents, Lucio and Chiara, one Father Angel and one Mother Angel for whom I and we all pray to God every day.

IJAE
Vol. 116, n. 3: 167-xx, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Main Lectures

Carlo Ruini *junior* (1530-1598) and the story of his *'Anotomia del cavallo et suoi rimedi'*

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Abstract

This report short news about Ruini family and the public life of Charles JR, author of the famous treatise '*Anotomia del cavallo e suoi rimedi*'. The treatise is mentioned for his seven editions, the translated versions and the most sensational imitations

Keywords

Carlo Ruini junior, anatomy, horse

The treatise '*Anatomia del cavallo et suoi rimedi*' (Anatomy and medicine of the Horse) by Carlo Ruini (1530-1598) was published in 1598 in Bologna by the Rossi brothers press. With this work, whose undisputed merit lies in a systematic treatment of anatomy based on experimental observation of an animal, Ruini gave animal medicine the scientific basis which human medicine had enjoyed since the publication in 1543 of Andrea Vesalius's '*De umani corporis fabbrica'*. In contrast to Vesalius and the post-Vesalian anatomists who consolidated his work, Carlo Ruini was not a member of the academic classes but belonged to a small circle of senatorial families who governed Bologna.

The Ruini family was originally from the Reggio Emilia region. Its social rise dates from 1511 when Carlo Ruini *senior* (grandfather of the author of the treatise), who was a Doctor in Law, moved to Bologna to teach Law at the University. His fame spread rapidly and widely, to the extent that the Bolognese Senate doubled his salary to ensure he would not transfer to another university. The prestige of the Ruini family grew and strengthened thanks to a shrewd matrimonial policy adopted by the head of the family for his son Antonio, who in 1525 married a daughter of the extremely wealthy Bolognese senator Ercole Felicini. In turn, our Carlo *junior*, son of Antonio, further reinforced the family's standing by marrying Vittoria Pepoli, a member of one of the most noble and influential Bolognese senatorial families who had attended the Hapsburgh and Papal courts. The Ruini family's entry into the circle of Bolognese senatorial families occurred in 1584 when the seat left vacant on the death of the last Felicini passed to his descendant Carlo Ruini *junior*.

Carlo Ruini junior, like his father Antonio, did not study at the University – this was normal at the time for the sons of grand families. However, he had received an excellent education from private tutors, and this was re-inforced by his contacts with University circles in Bologna, especially with the highly respected Claudio Betti, and

Achille Bocchi, lecturer in Greek & Humanities. His wide-ranging cultural interests are also shown by his active participation in the Typographical Society founded in Bologna in 1572.

The confirmed power of the new senatorial Ruini family was most clearly demonstrated by the impressive size of the splendid mansion that Carlo *junior* had built on land chosen for this purpose by his father. The Ruini mansion, extended in the 17th century by the Ranuzzi family and in the following century by the Balocchi family, is today better known as the Palazzo di Giustizia because it houses the court offices.

The very thorough archival research carried out in 1984 by Mario Fanti & Rosa Chiossi clearly revealed Carlo Ruini *junior*'s passion for horses, as the mansion that he built boasted a far greater number of stables compared to those present in the mansions of the other senators. It is this passion for horses that provides the true context for Ruini's anatomical work, which is as important for the scientific evolution of veterinary medicine as it was both misunderstood by the academic world outside which it was conceived and carried out, and plagiarised over two centuries by dishonest authors who succeeded in deceiving many of its critics. These ill-informed critics on occasion went as far as denying Ruini's authorship of the treatise with arguments which were shaky from the outset because of confusion between Ruini and his grandfather of the same name, the most absurd being that a lawyer (who they supposed to be our Carlo) was incapable of conceiving and producing a work such as *'l'Anatomia del cavallo'*. They even suggested that the Senator could have acquired a manuscript by Leonardo, citing as evidence the woodcut illustrations which adorn the treatise, and thereby demonstrating their ignorance of the artistic environment in Bologna in the 16th century. This was rich in not only painters but also expert illustrators and engravers trained in the Carracci academy, as shown by the splendid illustrations in Ulise Aldrovani's magnificent treatise on Natural History.

Ruini's treatise was published in Bologna in 1598, just a few months after the unexpected early death of its author, who thus never had the satisfaction of seeing the printed version or the opportunity to defend it.

Perhaps to make it more widely available, the year after Ruini's death his son Ottavio gave the treatise to the Venetian printer Gaspare Bindoni who in 1599 published a second edition. This differs from the first only in the frontispiece and dedication letter. Bindoni also published the 3rd and 4th editions in 1602 and 1607 respectively; these are extremely rare and none are to be found in libraries in Italy. The most widely available editions are the two printed in 1618 in Venice by Fioravante Prati. The seventh and last edition appeared in Venice, printed Lorenzo Basegio in 1707-1708.

In addition to the seven Italian editions there are the translated versions: Uffenbach's German edition published in Frankfurt in 1603, and the 1647 French version prepared by Jourdain (reprinted in 1654, 1655 and 1667). There was also a falsified version by Franchini who, passing himself off as a descendant of Ruini, also claimed to be its author on the basis of quite untenable evidence.

The plagiarised version by Franchini was followed by others of the same ilk: from *The Anatomy of an horse* by Snape (1683) to *Anatomia et Medicina equorum nova* (1715) by the German Trichter, *The Farrier's new guide* (1720) by the Englishman Gibson, *Anatomie generale di Cheval* (1734) by the Frenchman De Garsault and finally *Le parfait connoissance des Chevauxs* (1734) by the Frenchman De Saunier, the most brazen and

arrogant of the plagiarists.

Among admirers of Ruini's work we should note Francesco Liberati Romano who, in his 1664 treatise *La perfezione del cavallo,* states that all his knowledge of of the topic came from Ruini. De Solleysel is also full of praise for Riuini in his *Le Parfait Mareschal* of 1664; similarly Cuvier and Albert von Haller in the 18th century, and Alessandrini and Ercolani in the 19th century. The scientific contribution of Ruini's 'Anatomia' was the theme of the interesting inaugural lecture given by Angelo Cesare Bruni in 1952 on the occasion of the unveiling of the plaque mounted by Valentino Chiodi in the Anatomy theatre of the old veterinary school in Bologna. Ruini's work was unknown to Claude Bourgelat, author of *Eléments de l'art vétérinaire.* In this work he comments on the difficulties encountered in anatomical studies of animals but cites only numerous anatomists who, following Vesalius, concentrated on dissection of humans.

Finally, it is worth emphasising that Ruini, a cultured man who would certainly have had no difficulty in writing his treatise in Latin, wrote it in Italian knowing that the principal readers of his work would not be in the academic world but men of a lower class such as grooms and farriers who were responsible for the care of animals.

His greatness and modernity lie in having undertaken a body of work which on account of its scope, complexity of content and methodological organisation must have occupied him for years - but without the support of the academic world which would have further increased his reputation. Certainly he was stimulated not only by his passion for horses but also by the knowledge that the flourishing economy of his time depended on their health and well-being. In other words, he anticipated by two centuries the reasons which lead to the opening of the first schools of veterinary medicine.

Bibliography

A.C. Bruni (1952) L'opera anatomica di Carlo Ruini. In: Inaugurazione della lapide a Carlo Ruini presso l'Istituto di Anatomia Comparata degli animali domestici della Facoltà. La Nuova veterinaria 8: 1-7.

M. Fanti, R. Chiossi (1984) Ricerche su Carlo Ruini (1530-1598). Li Causi Editore, Bologna.

IJAE
Vol. 116, n. 3: 170, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

The claustrum of the bottlenose dolphin

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Key words

Claustrum, Cytoarchitecture, Bottlenose dolphin.

The claustrum is a subcortical nucleus present in the mammalian brain. The precise functional significance of this thin gray structure, composed by a dorsal and a ventral part, all comprised between the extreme and the external capsules, remains largely unknown. In most mammalian species, the claustrum is reciprocally connected with the cerebral cortex, and the claustrocortical and corticoclaustral projections show a topographical organization (Dinopoulos, J Comp Neurol 316, 1992; LeVay, J Neurosci 1, 1981). However, an important and specific role is presently associated to the projections and reciprocal connections with the visual occipital cortex. As widely known, the location and cytoarchitecture of the visual cortex are strikingly different in cetacea, where visual stimula are referred to a dorsal para-sagittal area of the temporo-parietal lobe.

The sensory-related cortical areas of the cetacean neocortex have a five-layer organization and lack an internal granular layer (layer IV), and these latter peculiar characteristics may influence the claustrum and result in a different organization of the nucleus. The aim of our study is therefore to describe the cytoarchitectonic and the main neurochemical features of the claustrum in the bottlenose dolphin *Tursiops truncatus*, and compare the results with the structure of the human claustrum taken as reference.

IJAE
Vol. 116, n. 3: 171, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Extrapineal melatonin production in the bottlenose dolphin (*Tursiops truncatus*): an immunohistochemical study

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Key words

Melatonin, Bottlenose dolphin , HIOMT.

Melatonin is involved in many physiological actions and especially in the regulation of circadian rhythms. In mammals the pineal gland is the main site of production. However, the unequivocal existence of a pineal gland in adult cetaceans is still a matter of debate, being documented in some species but unclear in others. Gross dissection of a series of twenty-four bottlenose dolphin brains did not reveal the presence of the gland (with one doubtful exception). Melatonin was recently quantified in circulating blood by our group for the first time in the same species. Data showed a significant monthly variation, thus suggesting a circannual rhythm. To investigate the site of extra-pineal melatonin production, we have focussed on the retina, the lacrimal complex and the gut Hydroxy-indole-O-methyl-transferase (HIOMT) was chosen as a biomarker, since it is the ultimate enzyme involved in melatonin synthesis and therefore highly specific for melatonin-producing tissues. We used a validated primary antibody raised in rabbit against human HIOMT to find immunoreactive cells. In the retina a strong immunoreactivity was observed in gangliar cells and in some gangliar cell fibers. In the lacrimal complex only some adenomers were marked, while in the gut we observed a positive staining on the apical surface of enterocytes.

IJAE
Vol. 116, n. 3: 172, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Orexigenic and anorexigenic peptides in digestive apparatus of *Tursiops truncatus*

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Key words

Tursiops truncatus, Food intake, Gastrointestinal tract.

In this study we have examined the presence of some peptides involved in food intake like leptin, oxerins and nesfatin in the gastrointestinal tract of *Tursiops truncatus*. Samples of the three chambers and proximal, middle and distal intestine, were been used. The specimens were derived from Banca per i Tessuti dei Mammiferi Marini del Mediterraneo, University of Padua. Paraffin sections of 7 μm thickness were labeled by avidin-biotin immunohistochemical technique. The primary antibodies employed were raised against: chromogranin A, protein gene product (PGP) 9.5, leptin, orexin A (OXA), orexin 1 (OX1R) and 2 (OX2R) receptors and nesfatin. The presence of chromogranin A immunoreactive (ir) cells was observed in the mucosal epithelium of the III chamber and proximal and middle intestine. PGP 9.5 ir neurons and fibers were localized in the mioenteric and submucosus plexuses of all gastrointestinal segments. Leptin ir neurons were found in some plexuses of the III chamber and middle intestine so that numerous OXA, OX1R and OX2R ir ones. Nesfatin ir cells were identified in the some glands of the third chamber. In this report, for the first time, was described the immunolocalization of leptin, orexins and nesfatin in the *Tursiops truncatus* digestive apparatus. These results could provide interesting information on feeding behavior of these cetacean and add new data to the little known world

IJAE
Vol. 116, n. 3: 173, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Distribution of calbindin-D28k immunoreactivity in the deep nuclei of the bottlenose dolphin *(Tursiops truncatus)* amygdaloid complex

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Key words

Amygdaloid complex, Calbindin-D28k, Bottlenose dolphin.

The amygdala plays a key role in emotional learning and behavior. Calbindin-D28k (CB) is a calcium-binding protein located in a variety of neuronal cell types in many regions of the brain. Despite previous studies in rat (Kemppainen, J. Comp. Neurol. 426, 2000) and Primates (Sorvari, Neuroscience 75, 1996), there are no reports concerning the distribution of CB immunoreactivity in the dolphin amygdala. Thus, we studied the distribution, morphology, and size of CB-immunoreactive (IR) neurons as well as the distribution of CB-IR neuropil in the deep nuclei of the bottlenose dolphin amygdala. The results obtained showed that in the deep nuclei CB-IR neurons were numerous and could be divided into two major cell types: pyramidal and nonpyramidal neurons. Pyramidal cells were large, lightly stained and without an evident immunostained dendritic tree. Nonpyramidal neurons could be subdivided into three types on the basis on the shapes and sizes of their somata and on their dendritic morphology. Type 1 neurons had a small spheroidal somata from which arose thin primary dendrites. Type 2 neurons were large cells with angular somata and evident primary dendrites. Type 3 neurons had a fusiform somata originating the primary dendrites from their opposite pole. These data suggest that in the deep nuclei neuronal microcircuit activity is controlled also by different cell types that contain CB.

IJAE
Vol. 116, n. 3: 174, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

The orexinic system in the swine digestive tract

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Key words

Orexins, Pig, Immunohistochemistry

In the last years, the presence of the orexinic system in the digestive tract of men and different animal species (Kirchgessner, Neuron, 1999; Näslund, Am J Physiol Gastroinest Liver Physiol, 2002) led to the hypothesis of its local participation in the control of the digestive functions. From these bases and due to the lack of specific information on pigs, we looked at the presence of this system in the digestive tract pf swine by using immunohistochemistry techniques. The two peptides, OXA and OXB, were evidenced prevalently in the basal third of the stomach body glands. This study, made on serial sections, allowed us to hypothesize that the two peptides were synthesised by the same cell and this hypothesis was confirmed by the co-localization reaction. Moreover, in the stomach the study of the two receptors allowed us to observe only the receptor type 1, mainly localized in the basal third of the glands. By contrast, the same immunohistochemical researches done on the intestinal different tract, did not give us positive results. In conclusion, our studies allow us to demonstrate the presence of the orexinic system in the swine stomach and to hypothesize that, also in this animal specie, the orexins could be locally produced as well as act.

IJAE
Vol. 116, n. 3: 175, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Glial cell line derived neurotrophic factor and its receptors in the gut of five teleost species

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Key words

Teleosts, Gut, GDNF and its receptors

Glial cell line-derived neurotrophic factor (GDNF) binds to specific GDNF family receptor (GFRa1) protein, forming receptor complex that signals through the RET receptor tyrosine kinase. GDNF can also signal through GFR1a RET independently. GDNF supports the survival of central, peripheral and autonomic neuron populations, but it was also seen to act on non-neuronal tissues. In the gut, GDNF appears to play multiple roles in enteric neuron development, including survival, proliferation, and differentiation. GDNF seems to be chemoattractive to enteric neural crest-derived cells. Furthermore, mice lacking GDNF, GFRa1 or RET, lack enteric neurons in the small and large intestine.

Five species of adult teleost were used for this study: bass, gilt-head, scorpionfish, trout and zebrafish. Specimens of pyloric caeca (which are lacking in zebrafish) and intestine were fixed in Bouin's and included in paraffin wax. Microtomic sections were immunocytochemically stained by EnVision and double immunofluorescence method.

GDNF, GFRa1 and RET immunoreactivity was visualized in the gut of all five species. Both endocrine cells and neurons of the enteric nervous system resulted positive to the antisera employed and in some cell populations GDNF, GFRa1 and RET appeared differently co-localized.

IJAE
Vol. 116, n. 3: 176, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Morphological and molecular changes of pig skin fibroblasts exposed to 5-aza cytidine and addressed to subsequent pancreatic differentiation

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Key words

Reprogramming, Morphology, Fibroblast

Recent experiments demonstrate that terminally differentiated cells can be induced to de-differentiate in vitro and increase their plasticity in response to synthetic molecules capable of reverting cells from their lineage commitment to a more pluripotent state. In response to specific conditions de-differentiated cells can then be re-addressed to a different cell type. However only scattered information are available on the morphological changes and the ultrastructural remodelling required when cells transit along the differentiation pathways, adopting the modifications needed in order to adequately adapt to a different and specific state.

In the present study we prepared skin fibroblast primary cultures and exposed them to 5-aza-cytidine (5-aza), an inhibitor of DNA methylation, to increase cell plasticity. We then investigated the ability of 5-aza treated cells (5-azatC) to be re-addressed to pancreatic beta cell-like cells (r-betaC), in response to specific differentiation conditions. Ultra-structural modifications were evaluated in parallel with molecular analysis for the expression of differentiation stage-specific markers.

Elucidation of the morphological changes that take place in cells undergoing de-differentiation events is essential for a better understanding of the biology of these process paving the way to the successful use of stem cells in regenerative medicine and tissue replacement therapies.

IJAE
Vol. 116, n. 3: 177, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

A novel model of non-aganglionic congenital megacolon in the rabbit: genetic and morpho-functional characterization

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Key words

Megacolon, Interstitial cells of Cajal, Enteric neurons.

The pathogenesis of congenital non-aganglionic megacolon (CNAM) is unknown. Aim: To characterize a new non-rodent model of CNAM caused by an incomplete dominant allele at the English spotting locus (En) in homozygous En/En rabbits. Methods: Compared to the littermate controls (en/en), En/En rabbits can develop the CNAM. Ascending colon of controls (n=4) and En/En (n=6) was processed for double label immunohistochemistry (Hu, substance P [SP], neural nitric oxide synthase [nNOS], c-Kit for interstitial cells of Cajal [ICC]) and electron microscopy (EM). DNA was used for candidate gene analyses. RNA extracted from colon specimens was used for gene expression analysis Results: En/En rabbits showed reduced body weight and massive colonic distension. Sequencing and genotyping showed complete co-segregation of KIT gene polymorphisms with En phenotypes. KIT gene expression level in En/En rabbits was only 5-10% vs en/en. The ascending colon showed a decreased number of Hu- and SP-immunoreactive (IR) neurons (950±110 vs 1440±120 and 76±14 vs 160±24, respectively; $P<0.05$) and a trend to a reduced nNOS-IR in En/En. Kit-IR ICC networks were altered and EM showed neuronal and ICC abnormalities in En/En. Conclusions: Neuronal and ICC network alterations contribute to this CNAM phenotype. KIT mutations may account for ICC abnormalities.

IJAE
Vol. 116, n. 3: 178, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Gastric mucosa-associated lymphoid tissue (MALT) in conventional piglet

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Key words

Piglet, Gastric MALT, DiI.

Mucosal-associated lymphoid tissue (MALT) is the initial inductive site for mucosal immunity. It consists of organized lymphoid tissue which may occur as isolated or aggregated lymphoid follicles (LFs) and interfollicular areas. In the pig stomach the gastric MALT has been intensely studied in experimentally-infected pigs, but few data are available in healthy, non-gnotobiotic or germ-free animals. In the present study we described the gastric MALT in conventional piglets by means of histological and immunohistochemical stains. The majority of LFs were located in the cardiac mucosa and in the transition from the cardiac to the oxyntic mucosa. Here the LFs were mainly located in the submucosa and reached the mucosa and we called them submucosal lymphoid follicles (SLFs). In the pyloric mucosa and in the transition sites from the cardiac to the pyloric mucosa, LFs were located in the mucosa and we called them mucosal lymphoid follicles (MLFs). In SLFs, a compartmental organization of T and B lymphocytes was present; on the contrary, in the MLFs, the T and B cells were intermingled. In the epithelium overlying the lymphoid tissue, numerous T lymphocytes and some cells immunoreactive to cytokeratin-18 were observed. Following the application of the fluorescent tracer DiI into the SLFs of the diverticulum, enteric neurons located in the submucosal plexus were labeled.

IJAE
Vol. 116, n. 3: 179, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Expression and distribution of RET receptor in the brain of adult zebrafish brain

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Key words

Zebrafish, Brain, RET receptor.

RET is a member of the receptor tyrosine kinase superfamily, which transduces signaling by family of glial cell line-derived neurotrophic factor (GDNF) ligands. RET exists in two isoforms: C (short) and A (long), whose amino acid sequences show high similarities across vertebrates. The aim of the present study is to investigate the expression and cell localization of RET in the brain of zebrafish, a well established model organism for studies on development and gene functions. To achieve these goals RT-PCR and immunohistochemistry were employed.

RT-PCR revealed the expression of C-RET mRNA isoform. RET immunoreactivity (IR) against C-RET was observed in neurons in different areas of central nervous system. In particular, the IR was observed in the telencephalon, either in olphactory bulbs and in dorsal and ventral telencephalic regions; in the diencephalon, mainly in some thalamic nuclei; in the mesencephalon, in the optic tectum; in the rhombencephalon, in cerebellum and medulla oblongata.

The results demonstrate that RET is expressed in the major regions of the adult zebrafish brain. The extensive distribution, consistent with data from mammalian brain, highlights the usefulness of zebrafish, to elucidate further aspects of RET role in central nervous system.

IJAE
Vol. 116, n. 3: 180, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

A model to study aromatase P450, estrogen receptors expression and Ca^{2+} homeostasis in the developing bovine brain

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Key words

Aromatase, Estrogen receptors , Ca^{2+} homeostasis.

Estradiol (E2) exerts many important effects in a variety of physiological processes acting by genomic or non-genomic mechanisms, influencing cell morphometry, neuronal survival, dendritic branching, and synaptic patterning in the developing brain. More information on aromatase P450 (P450Arom) and estrogen receptors (ERs) expression is necessary to better understand the mechanisms of E2 action. In this work we determined the expression profiles of P450Arom and ERs in the hypothalamus and fetal bovine frontal cortex by quantitative Real Time PCR throughout fetal development. The data showed that the expressions of both ERs are strongly correlated during pregnancy and increase in the last stage of gestation. On the contrary, the expression of P450Arom has no correlation with ERs expression and is not developmentally regulated.

Recent experimental evidences highlight an involvement of E2 also in the regulation of intracellular Ca^{2+} homeostasis. Therefore we focused our attention on an immortalized endothelial-like cell line derived from fetal bovine cerebellum. Effects of E2 were tested with different exposures of the cells to the hormone. Ca^{2+} measurements were performed by Ca^{2+} probes (Cameleons) targeted to cytosol, endoplasmic reticulum and mitochondria. Data show that mitochondrial Ca^{2+} uptake is significantly decreased by exposure to E2 for 48 hours.

IJAE
Vol. 116, n. 3: 181, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Observations on the location of sensitive and motor neurons related to the pig urinary bladder trigone

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Key words

Fluorescent tracer, Pig, Urinary bladder trigone, Sensitive and Motor neurons.

The urinary bladder trigone (UBT) is a limited area in which the majority of vessels and nervous fibres penetrate into the urinary bladder and where neuronal fibres and intramural neurons are more concentrated. Moreover the UBT represent an elective site for cancers origin and proliferation, both in veterinary and humane medicine. So we think that the knowledge of the UBT nervous pathways is crucial for clinical involvement.

The aim of our study was to localize the sensitive and motor neurons related with the porcine UBT. The research was carried out on 3 male intact pigs, in which 50 μl of the retrograde neuronal tracer Fast Blue (FB) were injected. After a 15 day survival time, the animals were euthanized and the following samples were collected: the spinal cord (SC), the bilateral spinal ganglia (SG) and sympathetic trunk ganglia (STG) from T1 to Co1, the bilateral cranial (CrMG) and caudal mesenteric ganglia (CMG) and the microganglia of pelvic plexus(PG). Labelled cells were found in the the sacral (S3-S4) SC, bilaterally in the SG from L2 to Co1, in the STG from L1 to S2, in the CMG and in the PG. On the whole, the obtained results allowed us to document a broad extension of the labelled neurons projecting to a little area, such as the UBT.

IJAE
Vol. 116, n. 3: 182, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

A morphometric and neurochemical study of spinal ganglion neurons innervating the porcine *(Sus scrofa)* urinary bladder trigone

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Key words

Retrograde tracer, Sensory neurons, Immunohistochemistry.

Porcine lumbo-sacral spinal ganglion (SG) neurons were neurochemically characterized by the use of five neuronal markers: calcitonin gene-related peptide (CGRP), substance P (SP), neuronal nitric oxide synthase (nNOS), neurofilament 200kDa (NF200), and isolectin B4 (IB4) from *Griffonia simplicifolia*. In addition the phenotype and the area of SG neurons innervating the urinary bladder trigone (UBT) were evaluated by coupling retrograde tracer technique and immunoistochemistry. Lumbar and sacral SG neurons were immunoreactive (IR) for CGRP (30±3% and 29±3%, respectively), SP (26±7% and 23±6%, respectively), nNOS (21±4% and 26±6%, respectively), NF200 (75±14% and 81±7%, respectively), and labeled for IB4 (40±13% and 38±3%, respectively). UBT sensory neurons, which were distributed from L2 to Ca1 SG levels, presented a segmental localization, showing their major density in the L4-L5 and S2-S4 SG. Lumbar and sacral UBT sensory neurons expressed similar percentage of NF200-IR (60% and 66%, respectively) and IB4-labeling (64% and 58%, respectively) but showed meaningful different immunoreactivity for CGRP, SP, and nNOS (62%, 41%, 21% *vs* 16%, 27%, 6%, respectively), it thus indicating a probable different role of lumbar and sacral pathways in sensory transmission from the UBT. Also data related to cell size reinforced this hypothesis, being lumbar UBT SG neurons significantly larger than the sacral ones (1112±624 μm^2 vs. 716±421 μm^2).

IJAE
Vol. 116, n. 3: 183, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

BDNF expression in the nervous system of zebrafish embryo

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Key words

Zebrafish, BDNF, Nervous system.

The Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophins family and plays a survival- and growth-promoting actions on a variety of neurons, including synaptic development and neuronal plasticity. recently a potential role of BDNF in the pathogenesis and treatment of both neurological and psychiatric disorders has been suggested. The organization of the zebrafish BDNF gene is identical to that of the mammalian one and its expression has been demonstrated throughout zebrafish embryonic development. Moreover, in zebra-fish the BDNF expression has been showed in different sensory systems and recently a possible involvement of BDNF in the huntington's disease model has been proposed. The zebrafish embryo is a model for developmental biology and genetics because of its conserved organization of organs and tissues. in this study we demonstrated using single and double in situ hybridization the expression of BDNF in the zebrafish nervous system in the early stages of development. Particularly, the BDNF expression was localized in different brain districts, in the retina, in the inner ear and in the lateral line system. our results, taken together, suggest the involvement of BDNF in the zebrafish nervous system development and these observations could be useful for further investigations using zebrafish as an experimental model in the study of neural diseases.

IJAE
Vol. 116, n. 3: 184, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Gliocites (*gliocyti*) in the buffalo choroid plexuses

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Key words
Buffalo; GFAP; Wnt3.

By means of ultrastructural and immunocytochemical studies are identified and typed glial cells in the choroid epithelium of buffalo choroid plexuses. In previous researches carried out by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are evidenced between epithelial choroid cells another cell type with numerous extensions like nervous cells. By TEM in the cytoplasm of these cells are resulted present elongated mithocondria, microtubules, endoplasmic reticulum and lysosomes. Additionally the extensions of these cells are resulted covered with a thin plasmatic membrane, and are constituted mainly of microfilaments and microtubules. These extensions are distributed between the epithelium choroid cells. By SEM the like nervous cells showed a body of varied forms of 2-7μm diameter, that emits extensions of 400-500nm of uniform thickness and various lenght. In this study by SEM immunogold technique have been identified these like nervous cells using following antibodies against: GFAP and Wnt3. The samples treated with antibody against GFAP, have evidenced an immunopositivity in ovoidal cells distributed in choroid plexuses epithelium. The same immunopositivity was evidenced in the samples traited with antibody against Wnt3. In conclusion the like nervous cells, are gliociti and are cells responsible of the polarity epithelial maintenance, important for the cerebrospinal liquid formation.

IJAE
Vol. 116, n. 3: 185, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Histological, ultrastructural and immunocitochemical characterization of the post-natal development in the reeler mouse

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Key words

Neurons, Apoptosis, Autism.

Reelin, a protein necessary for the correct neuronal migration in several brain areas with laminar architecture, is missing in the reeler mouse (*reeler*$^{-/-}$). Therefore, the mutant mouse is an appropriate model to study the mechanisms involved in the neuronal migration and cortical lamination and it is considered a good model for neurological disorders such as autism. The aim of this study was to characterize the development of the cerebellum of the reeler mouse from birth to adulthood. The cerebellum of these animals is reduced in size (both in vermis and hemispheres), folia are completely missing and the lamination of the cortex is incomplete. In particular, the Purkinje neurons fail to migrate and to form a well-defined layer in the cortex and can be detected deeply in the white matter, intermingled with the deep cerebellar nuclei neurons and with some ectopic glial cells. The proliferation/death rate typical of the post-natal development of the wild type mouse is also alterated: the immunocitochemical analysis by means of neuronal markers (Pax-6, NeuN, Smi-32, calbindin) demonstrates that the more affected cells are the Purkinje neurons. Ultrastructural analysis indicates that the synaptic connections are altered in terms of morphology and position.

Honda et al. (2011) Neurochem. Res. 21
Myiata et al. (2010) Neural Dev. 1
Badea et al. (2007) Neuroimage 15

IJAE
Vol. 116, n. 3: 186, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

A comparative assessment of the effectiveness of adult stem cells (MSCs) and platelet rich plasma (PRP) applied on tendon lesions: preliminary results

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Key words

Adult stem cells, PRP, Tendon regeneration.

In veterinary as well as in human medicine the tenodesmic lesions play a great interest because of their high incidence and entail long periods of rest. The therapeutic protocols recently used in the treatment for these lesions are not able to achieve a real "*restitutio ad integrum*".

In sport horses, tendon and ligament injuries are a frequent cause of lameness and recently many novel therapies, such as the use of Platelet Rich Plasma (PRP) and mesenchymal stem cells (MSCs), have been proposed to treat these pathologies. In the present project we are evaluating the efficacy of the application of: PRP (group 1), autologous MSCs transfected with the reporter gene GFP (group 2) and PRP combined with MSCs (grioup 3) in experimentally injured tendons of sheep (DM n°97/2010-B). The lesions have been performed with the collagenase enzyme in the deep digital flexor tendon (*flexor digitorum profundus)* of pelvic limb in three animals for each group. The effectiveness of this treatment has been assessed clinically *in vivo* and histologically *post mortem*. Moreover, the in vivo integration of the MSCs injected in the injured tendon has been verified by several approaches.

The preliminary results of our research indicates a better quality of tissutal regeneration, especially after the use of MSCs while the clinic evaluation is satisfactory also after the combined use of PRP and MSCs.

IJAE
Vol. 116, n. 3: 187, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Influence of different culture media on growth kinetics and phenotypic features of equine adipose-derived multipotent mesenchymal stromal cells (MSCs)

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Key words

Culture medium, Horse, MSCs.

Multipotent mesenchymal stromal cells, also known as mesenchymal stem cells (MSCs), are a population of adult stem cells with a broad potential in cell-based regenerative therapies. With the rapid increase of their use in experimental clinical procedures, it has become evident that an urgent need does exist to define a common operative protocol of isolation and culture, aimed at optimizing their *in vivo* efficacy (Ho, Cytotherapy, 10, 2008).

The aim of the present study was to evaluate the best culturing conditions of MSCs isolated from equine adipose tissue and cultivated with two different media: Earle-MEM and D-MEM, both with the addition of Fetal Calf Serum, antibiotics and antimycotic. At each passage the cells were counted and Cell Doubling (CD), and Doubling Time (DT) values were calculated by means of established formulae. In addition, the expression of CD90, CD44 and CD73 was assessed by flow cytometry on both the cell populations. Finally, cell morphology was evaluated at light and at transmission electron microscopy. Our data suggest that different media may yield significant difference in growth kinetics and in morphology of cultured MSCs. As a consequence, the choice of culture media has to be carefully take into account since a variable efficacy *in vivo,* due to different culture conditions, can not be excluded.

IJAE
Vol. 116, n. 3: 188, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Cytomorphological evaluation of horse adipose-derived mesenchymal stem cells after different storage conditions

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Key words

Mesenchymal stem cells; Storage; Horse.

Transplantation of mesenchymal stem cells (MSCs) is a promising therapy and it is known that, in the horse, these cells are already used for teno-desmic injuries treatment. However, till now, knowledge about the MSC behaviour is scarce (Koch, Can. Vet.J., 50, 2009) and, in particular, data on the effects of the cell manipulation before transplantation are lacking.

In this work we studied MSCs maintained at different temperatures for different periods of time simulating the phase of conservation before their use. Cryopreserved MSCs were thawed, resuspended in a medium devoid of fetal calf serum ($1x10^6$cc/ml) and divided in five aliquots. After a maintenance at 4°C or at room temperature for 0, 24 and 48 hours, MSCs were used for the following trials: cell viability, duplication time, Colony-Forming Unit Assays, karyotype evaluation, adipogenic and osteogenic differentiation, expression of MSC marker CD44 and CD90. Result evaluation seems to suggest that all tested manipulation were unable to induce appreciable modifications on MSC behaviour. All the aliquots were clonogenic, could differentiate along the osteogenic and adipogenic lineage and expressed high levels of CD44 and CD90 antigens. We suppose that the tested conditions of maintenance do not change the characteristics of horse adipose-derived MSCs as regards the considered parameters.

IJAE
Vol. 116, n. 3: 189, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Expression analysis of ten myosin heavy chain isoforms in human extraocular muscles

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Key words

Myosin heavy chain, Special muscles, Expression.

Human extraocular muscles (EO) express, together with skeletal MyHC isoforms (type 1, 2A, 2X), the following: developmental isoforms (Embryonic and Perinatal) all life long; a cardiac isoform, present in other particular muscles as masseter and laryngeal; EO isoform that is muscle specific; 2B isoform found in a recent research only in these muscles. The type M isoform, typical of masticatory muscles of some species such as carnivores and non human primates, is not express. Moreover, in EO muscles another isoform was identified, the slow tonic, but not characterized yet at genomic level. Recently Schiaffino and collaborators (Rossi, J Physiol 588.2, 2010) studied two new genes concluding that MYH14/7b correspond to slow tonic and MYH15 gene to ventricular isoform of chicken. Another author found, on the contrary, that the slow tonic is codified by MYH15 gene (Rahnert, Cell Tissues Organs 191, 2010). Our results, assessed by Realtime PCR and electrophoresis, comparing skeletal, laryngeal, EO, masseter and atrium muscles, tend to link MYH15 gene to slow tonic (express only in EO), while MYH14/7b to a chicken embryonic isoform, express in atrium, laryngeal and EO. MyHC expression is muscle dependent and its regulation is under the control of several factors. Recently Harrison (Skeletal Muscle 1, 2011) correlated the lack of 2B expression in human skeletal muscles to the promoter region that differ from the mouse promoter (that express the 2B). We aligned promoter sequence of other species and our results do not confirm his assumptions. EO muscles are, therefore, extremely complicated, able to express, also at protein level, 10 of 11 isoforms identified at genomic level and the problem of this complexity remain open yet.

IJAE
Vol. 116, n. 3: 190, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Melanomacropage centres as indicator of Atlantic bluefin tuna (*Thunnus thynnus* L.) "Health state"

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Key words

Bluefin tuna, Liver, Melanomacropage centres.

Fish liver is constituted of hepatocyte cords pervaded by a network of sinusoids. in fish liver, macrophages tend to give rise to melano-macrophage centres (MMCs). The aim of this study was to: a) characterize histochemically Atlantic bluefin tuna (*Thynnus thynnus* L.) (ABFT) MMCs; b) evaluate the use of MMCs as indicator of health status. Liver samples were taken from: a) wild adult males captured by traditional traps in Sardinia and Morocco; b) captive adult males experimentally reared in sea cages in Spain; c) captive juvenile males commer-cially reared in Croatia. The samples were fixed in 10% formalin, dehydrated in ethanol and embedded in paraffin wax. Sections were stained with: haematoxylin-eosin, α-naphtyl acetate esterase (AnAE) for macrophages; Mallory's method for lipofuscin/ ceroid, Perl's stain for he-mosiderin; antibodies against vitellogenin (VTG) and cytocrome 450P1A (CYP1A) mono-ossigenase. MMCs showed lysosomial activity and contained lipofuscins/ceroids and hemosiderin. MMCs density was higher in the Croatian group in comparison to the other two fish groups. Moreover, individuals with hepatocytes immunopositive to VTG and CYP1A were found only in the Croatian group, thus indicating the exposure of these fish to environmental pollutants. This study indicates a role of MMCs as metabolic dumps and confirmed their utility as biomar-ker of fish health state.

IJAE
Vol. 116, n. 3: 191, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Cytochrome P4501A (CYP1A) as a biomarker to evaluate the environmental quality in venice lagoon

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Key words

Expression, CYP1A, Biomonitoring.

The Cytochrome P4501A (CYP1A) induction is widely employed for environmental biomonitoring, especially in marine organisms. In the present study, the levels of CYP1A was evaluated in the fish species *Zosterisessor ophiocephalus* sampled in three different sites (Porto Marghera, Val di Brenta and Caroman) of the Venice Lagoon. The expression of CYP1A mRNA, evaluated by Real-Time PCR, was significantly higher in both males and females from the Porto Marghera site. By immunohistochemistry, cellular localization of CYP1A was detected in liver, kidney and ovary. In liver, the strongest immunopositivity was found in males from all sites, whereas the detoxified fish exhibited a faint immunoreactivity. By Western blot, the CYP1A antiserum recognized in the liver a band corresponding to the CYP1A protein. The antibody strongly reacted in males from all sites excepted for control group, whereas weakly reacted in females revealing a marked sex differences in response to environmental pollution. In all sites, the CYP1A gene expression and its protein was increased as evidenced by Real-Time PCR results, as well as by Western blotting and IHC. These results indicated that pollutants are bio-available as evidenced by the biological study and may have an harmful effects on aquatic organisms such as *Z. ophiocephalus*.

IJAE
Vol. 116, n. 3: 192, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Ultrastructural changes induced by silicon-stabilized tricalcium phosphate in cultured sheep bone marrow mesenchimal stem cells

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Key words

Stem cells, Ultrastructure, Sheep.

Bone marrow mesenchymal stem cells (BM-MSCs) in combination with bioceramics have been used to repair bone lesions but the effects of these biomaterials on BM-MSCs are lit-tle known. This study reports the changes observed in the ultrastructure of sheep BM-MSCs incubated with silicon-stabilized tricalcium phosphate (TSP). MSCs were isolated from bone marrow of iliac crest and cultured according to Crovace et al. (VCoT, 21, 2008). TSP was ad-ded to some cultures and cells were incubated for 10 days. Control and TPS-incubated cells were fixed with GTA, post-fixed in oso4 and embedded in Epon 812. Control and TSP cultures consisted of differently electron-dense polygonal cells containing euchromatic nuclei with prominent nucleoli. in control coltures, the electron-lucent cells were characterized by dilated rEr cisternae whereas the moderately electron-dense cells showed dark bodies as prominent features. Small blebs and filopodia were present on the surface of light and dark cells, respec-tively. in TSP cultures, the light cells showed large surface blebs, peripheral cytoplasm poor in organelles which were packed with contractile filaments around the nucleus. The dark elongated cells displayed pseudopodia and filopodia, peripheral vacuoles, dense bodies, round or elongated mitochondria. These findings demonstrate that TSP modifies differently the ultrastructure of cell types constituting the BM-MSCs of sheep.

IJAE
Vol. 116, n. 3: 193, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Oxidative-induced microtubular changes in a rat schwann cell line, under experimental hyperglycemic conditions

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Key words

Oxidative stress;Ttubulin; Schwann cell line.

The neuropathies of the peripheral and central nervous systems can be caused by hyperglycemia, a consequence of the deregulation of glucose, resulting in an increase in reactive oxygen species, with subsequent cellular lesions and impairment of cytoskeletal components (Maritim et al. 2003; Allen et al. 2005; Drel et al. 2006; Gadau et al., 2009). Microtubules, one component of the cytoskeleton, are composed of heterodimers of α- and β-tubulin. Tubulins, mainly α-tubulin, may receives diverse post-translational modifications , consisting in tyrosination, detyrosination, acetylation, nitrotyrosination, polyglycylation, polyglutamylation, phosphorilation, palmytoylation (Westermann and Weber, 2003; Fukushima et al., 2009). In the present work attention has been paid on the possible nitrosative effect of high glucose conditions on microtubular network in a rat shwannoma cell line. Cells incubated for 72hrs in the presence of 180mM D-glucose revealed alterations in cell morphology, growth rate and catalase activity in comparison with controls. In addition, an increase in acetylated α-tubulin and nitrotyrosine and a downregulation of total, tyrosinated and detyrosinated α-tubulin was found with Western blot analysis and immunofluorescence. No significant changes in other cytoskeletal components such as actin and GFAP was seen. Our work underlined that high glucose can selectively affects microtubular network in schwannoma cells, exerting its detrimental effect either through a nitrosylation of tubulin or through the possible inhibition of deacetylase enzymes, with subsequent impairment of microtubular functionality.

IJAE
Vol. 116, n. 3: 194, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Swine meniscus characteristics: differences from young to adult

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Key words

Meniscus, Histology, Swine.

The menisci play important roles in the biomechanics of the knee joint. The meniscal tissue has a poor healing potential, partly due to the absence of vasculature in the inner portion. Due to the presence of both collagen I and II, the meniscus has properties of both fibrous and cartilaginous tissue. The term fibrochondrocytes has been introduced to identify the meniscus cells: their phenotype is known to be close to that of chondrocytes in the inner meniscus and fibroblasts in the external area, but a clear distinction between the different phenotypes with particular association to their development with increasing age is still missing. This work was aimed to study the characteristics of swine meniscus cells by analyzing three different areas. The results obtained lead to the conclusion that meniscus maturation, from young to adult, is accompanied by changes in cell phenotype; in the early stages of life the cells from the intermediate and outer part are still immature and far from a chondrocyte-like phenotype. In adult age all meniscus cells assume a mature and specialized phenotype: cells of the external area maintain a fibroblasts-like phenotype, while cells of the intermediate and inner areas develop a chondrocyte-like phenotype. Both in young and adult pigs, menisci show an inner avascular zone and an intermediate and external vascular zones.

IJAE
Vol. 116, n. 3: 195, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Fetal ossification in the buffalo: immunocytochemical study

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Key words

Buffalo foetus, CD133, Wnt3.

The first nuclei of ossification where observed in the buffalo fetuses of 3cm CRL (crown-rump lenght) in the higher and lower jaw, frontal, ribs, scapula, humerus, tibia and ulna. In the fetuses of 5cm and 7cm CRL the first nuclei of ossification where observed also in: zygomatic bone, incisive bone, nasal, cervical and thoracic vertebrae, sacrum, first caudal vertebrae, splint bones, III phalanx, ilium, ischium, femur, fibula and metatarsal bones. In the fetuses of 10cm CRL the primary ossification is completed. On the 10-12cm CRL fetuses is applied the technique of the immunogold to the scanning electron microscopy (SEM) using the following primary antibodies against CD133 and Wnt3. The samples treated with antibody against CD133, marker for multipotent stem cells, have evidenced the presence of positive cells along the deep layer of the periosteum, that they could represent the stem cells implicate in the bone formation. Intense immunopositivity was observed to the border with the cartilage, where the trabecular bone invade the cartilage and the ossification progresses to interstitial of growth. The similar positivity was observed in the samples treated with antibody against Wnt3, that have evidenced the presence of immunopositive cellular elements in the deep layer of the periosteum and on the surface of the trabecular bone. Then in this study we have demonstrated that the ossification may depend on the presence of osteogenetic stem cells, located in the deep layer of the periosteum, but also on the other factors (Wnt3), that they could further the ossification along the periosteum and in the trabecular bone.

IJAE
Vol. 116, n. 3: 196, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Diversity of glycoconjugate pattern in the bladder urothelium of horse and donkey revealed by lectin histochemistry

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Key words
Urothelium, Glycohistochemistry, Equidae.

The glycoconjugates expressed on the mucosal surface of urinary bladder act as a barrier against invasion by pathogenic microorganisms and injury from toxic substances in the urine. Also, they serve as a major source for soluble urinary glycoproteins, which can actively modify the urine composition. Glycoconjugate pattern characterizes distinct cellular populations, cell differentiation and maturation, as well as cell morpho-functional changes in both normal and pathological conditions. The informations on the bladder glycoconjugates suggest that glycosaminoglycans are only a minor component at urothelial level compared with glycoproteins. In this research we characterized the glycoconjugate pattern in the bladder epithelium by lectin histochemistry, comparatively in the horse and donkey. Tissue fragments were fixed in 4% (w/v) neutral formalin and then embedded in paraffin wax. Sections were stained with a panel of twelve lectins, in combination with KOH saponification and sialidase digestion. The entire urothelium reacted with SNA, KOH-sialidase (s)-PNA, s-SBA, Con A, GSA II in horse bladder, and showed additional binding sites for MAL II and GSA I-B_4 in the donkey one. The urothelium luminal surface bound MAL II, DBA, LTA in the horse and DBA and LTA I in the donkey. In addition, the urothelium of the horse bladder stained with PNA, SBA and GSA I-B_4 in the basal and luminal regions and with UEA I in the adluminal zone. These results demonstrate remarkable inter-specific difference of the glycoprotein pattern expressed in the urothelium of two Equine species, despite the very close taxonomic vicinity. It is noteworthy that glycans terminating with Galβ1,3GalNAc (PNA reactivity) and Fucα1,2Galβ1,4GlcNAcβ (UEA I affinity) lack in the epithelium lining the donkey urinary bladder.

IJAE
Vol. 116, n. 3: 197, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

From biodiversity safeguard to wood fire prevention: the anatomy applied to the animal well being

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Key words

Rumen, keratinization, BCS.

Anatomical researches, supporting eco-vegetational investigation focussed on the pastural ecosystem biodiversity maintainance, allowed the validation of BCS as representative parameter of morphofunctional modifications which are strictly related to ecological-productive variations of the grassland systems (Ceccarelli, *It J Anat Embryol* 114, 2009). A cause of wood fire primer is the presence of necromass in the wood/pasture fringes characterized by a strong covering of *Brachypodium rupestre* (*Br. r.*), a tall grass lowly palatable for ovine because of its silicate rich and high fibrousness leaves. However, sheep closed in fences on *Br. r.* highly covered zone, exploit all forage resources, preventing the fire primer. So, a sheep experimental group was taken to graze for twenty days on *Br. r.* highly covered plot; during this period, the ruminal mucosa keratinization degree and the body state modifications (BCS and body weight) were monitored in order to determine the animal stay length without negatively affect their well being. Data were then compared with those of sheep control group grazing on a natural semi-mesophylic pasture. Experimental group showed a body weight mean decrease of 1,79 kg and a marked BCS decrement (-1,10). In control group BCS slightly decreased (-0,13) and body weight increased of 0,62 kg. Keratinization degree changed more in experimental group (17,2%-31,7% in rumen atrium, 20%-37,3% in rumen ventral sac) than in control one (17%-19,5% in rumen atrium, 20,2%-22,1% in rumen ventral sac). Considering that the high keratinization degree was quickly reached, while the most negative effects on BCS and body weight occurred after twenty days of grazing on *Br. r.* pasture, it seems advisable for animals to stay on this pasture not more than 10-15 days.

IJAE
Vol. 116, n. 3: 198, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Anatomical and endoscopic studies of the extracranial internal carotid artery in the horse

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Key words

Gross anatomy, Horse, Internal carotid artery.

Routine endoscopy of the guttural pouches in neonatal foals occasionally shows atypical aspects of the extracranial internal carotid artery. On the contrary, such aspects are not observed in adult horses. In order to verify whether they can be attributable to the so-called dolichoarteriopathies described in humans (Beigelman et al., Angiology 61, 2010), an endoscopical investigation was carried out on 50 foals younger than 2 weeks of age and a dissection was performed on 15 horses of different age (young and adults) to expose, bilaterally, the internal carotid artery. Both the endoscopy and the anatomical study confirmed the presence of differently shaped internal carotid arteries in subjects aged less than one month. These anomalies were similar to the aspects belonging to the dolichoarteriopathies, and precisely, "tortuosity, kinking and coiling" (Weibel e Fields, Neurology 15, 1965). The presence of dolichoarteriopathies only in young animals together with the total absence in adults, prompt to consider these forms as a developmental stage of the arteries. The gradual lengthening of the neck and the caudally expansion of the guttural pouches cause a distension of the vessel and their acquisition of the definitive aspect. On the base of this hypothesis, tortuosity, kinking and coiling in foals should be considered a simple anatomical variability and not a pathological event.

IJAE
Vol. 116, n. 3: 199, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Morphological assessment *"in vivo"* of the orbital vessels in dog and cat by multidector computed tomography angiography

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Key words

Orbital vessels, MDCT-angiography, Dog and Cat.

Five mesaticephalic dogs and five cats with no evidence of ocular or cardiovascular disease were included in this study. A 16-multidetector-row computed tomography (16-MDCT) was used. A freestanding workstation was used to generate two-dimensional reformatted images and three-dimensional volume rendered models of the orbital vasculature and surrounding structures.

Results of MDCT-angiography have been compared with results from vascular corrosion casts and plastinated specimens of the region.

The study provided excellent arterial and venous opacification and identification. The external and internal ophthalmic arteries, external ethmoidal artery and its muscular branches, long and short posterior arteries, superficial temporal and malar arteries with their branches, external ophthalmic veins and the anastomotic branch, the ophthalmic plexus, could all be identified.

This preliminary data suggest MDCT angiography may represent an advanced imaging modality to in vivo assess the complex orbital vascular network. In addition, it can be an useful tool for educational support, providing accurate mapping of individual venous and arterial anatomy and variations.

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IJAE
Vol. 116, n. 3: 200, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Does an improper placental vascularization in pregnancies from in vitro produced sheep embryos compromise heart development?

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Key words

Sheep embryos, Placental vascularization, Heart development.

Pregnancies obtained by various assisted reproduction techniques (ART) across several species show compromised foetal growth and placental disfunction. A severely reduced placental vascular development is incompatible with placental function. Also placental defects involve cardiovascular development. In this study we examined: a) the vascular growth in developing placentas from *in vitro* produced sheep embryos (IVP) in comparison to *in vivo* developed controls (CTR). The levels of transcripts involved in vascular growth (FGF2, FGF2R, VEGF, ANG1, ANG2, TIE-2) in placentas collected on days 22 and 26 of pregnancy, the number, area and diameter of placental vessels were analyzed; b) the heart development by histological analyses in related foetuses.

A reduced expression of FGF2, ANG2 and TIE-2 ($p<0.05$), indicative for defective maturation of vessels and lower vessel number (0.78 ± 0.02 fold relative to CTR; $p<0.01$), area (0.22 ± 0.04 fold relative to CTR; $p<0.05$) and diameter (0.55 ± 0.05 fold relative to CTR; $p<0.01$) was observed in IVP placentas. An underdeveloped ventricular wall was noted in hearts from IVP embryos either on day 22 (0.64 ± 0.06 fold relative to CTR; $p<0.01$) and 26 (0.72 ± 0.06 fold relative to CTR; $p<0.05$). Similar reduction of ventricular wall thicknesses and similar placental vascular defects were observed in mouse lacking HOXA13, which is a transcription factor of TIE-2. Relevantly, we observed a severe reduction of HOXA13 transcript ($p<0.01$) in IVP placenta on day 22. In conclusion our study demonstrates that heart development in IVP embryos was retarded. This defect is consistent with the lower expression of factors regulating placental vessel growth.

IJAE
Vol. 116, n. 3: 201, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Parthenogenetic embryos display aberrant centriole number, decreased apoptotic activity and abnormal autophagic ability

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Key words

Parthenote, Centriole, Autophagy.

Mature oocytes can be activated in vitro leading to the generation of parthenotes who will develop in culture forming blastocysts morphologically indistinguishable from those derived from fertilized eggs. However many aspects of parthenote biology still need to be elucidated. Here we describe experiments where we carried out a careful comparative analysis between pig parthenogenetic and biparental embryos. Immunocytochemical characterization and ultrastructural analysis demonstrated that parthenogenetic cells are affected by centrosome amplification and suggest that this is determined by the lack of paternal centriole, normally contributed by the sperm at fertilization. The higher incidence of multiple centrioles did not induce cell cycle arrest in blastomeres that progressed normally along development and did not trigger programmed cell death process. Indeed we show that parthenogenetic embryos activate a series of adaptive mechanisms that allow them to proliferate and differentiate. In particular Real Time PCR showed down-regulation of the p53/p21 pathway, indicating a decrease in apoptotic activity. A further adaptive strategy was highlighted using Lysotracker red and LC3 staining as well as electromicroscopy evaluation and suggested a massive increase of autophagic activity in parthenotes.

IJAE
Vol. 116, n. 3: 202, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

European master in comparative morphology (EUCOMOR). A curriculum to be developed… by 2013

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A project granted within the Life Long Learning Programme – Erasmus Curriculum Development – Year 2010 is described. This project aims at building an European Master curriculum in Comparative Morphology (120 ECTS credits) providing in depth knowledge and practical skills in embryology, gross and microscopic anatomy of farm, pet and laboratory animals. The programme addresses the need for highly qualified graduates in the field of comparative morphology. The international and multidisciplinary approach of the programme enables students to become familiar with aspects of comparative morphology in research (e.g. neuroanatomy of mice) and will result in increasing student's career opportunities beyond an academic or research environment (e.g. diagnostic imaging). To date no Master programme (national nor European) covers these aspects. A network of universities active in comparative morphology together with the EAVA will develop a curriculum that aims to result in expert morphologists. With this Master's degree we envisage a role for the expert in comparative morphology in teaching animal morphology and in research. The international character of the master programme offers those involved extra-curricular competences that are helpful for teaching, research and provides them with an international network.

IJAE
Vol. 116, n. 3: 203, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Chronic mastitis is associated with altered ovary morphology in dairy cattle

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Key words

Mastitis, Follicle, Bovine.

We investigated the effect of chronic mastitis on the ovarian reserve and follicular dynamic. Ovaries and milk samples were collected from 74 cows at the time of slaughter and the degree of infection was determined. The ovaries of animals belonging to the two groups with lowest (n=8) and highest (n=9) degree of mastitis were examined. Primordial, primary and secondary follicles were counted on similar surface area for each animal. The extension of ovarian stroma was determined with Masson trichrome staining whereas *Bandeiraea simplicifolia*-I lectin (BSL-I) was used to visualize endothelial cells and evaluate the extension of the vascular bed. Expression of GDF-9, a marker of oocyte development was assessed with a specific antibody. Results show that chronic mastitis is associated with a significant reduction of the ability of primary follicles to develop into secondary state and with a lower number of large antral follicles (>8 mm). The lower degree of follicular development was accompanied by a considerable reduction of the cortical vasculature and an increase of fibrotic tissue. Furthermore GDF-9 expression was significantly lower in the oocytes of animals with the higher degree of mastitis. Overall our results indicate that the ovary is a primary target of mastitis-related reproductive problems. We hypothesize that udder pathogens may act disrupting the intraovarian cytokine network.

IJAE
Vol. 116, n. 3: 204, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Captivity affects spermatogenis in atlantic bluefin tuna (*Thunnus thynnus* L.)

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Key words

Bluefin tuna, Germ cells, Proliferation.

The knowledge of gametogenesis is of paramount importance to develop a reliable technology for Atlantic bluefin tuna (*Thunnus thynnus* L.) (ABFT) rearing in captivity. The aims of this study were: a) to evaluate the capacity of male ABFT, confined in captivity before puberty, to finalize spermatogenesis; b) to compare germ cell proliferation between wild and captive ABFT. Testis samples were taken from: a) 13 juvenile ABFT reared in the North Adriatic Sea (Croatia); b) 30 adult ABFT reared in the central and western Mediterranean (Spain, Malta and Italy); c) 20 adult wild ABFT captured by tuna traps in Italy and Morocco. Samples were fixed in 10% formalin, dehydrated in ethanol and embedded in paraffin. Proliferating germ cells were identified through the immunohistochemical detection of proliferating cell nuclear antigen (PCNA). The first spiniform ray of the first dorsal spine was taken from the juvenile fish in order to estimate the age through the count of annual discontinuities. Juvenile ABFT captured before puberty were able to finalize spermatogenesis starting from 3 years of age. Germ cell proliferation was delayed in captive-reared ABFT specimen compared to wild individuals. These results seem to indicate that testis maturation can be anticipated in ABFT caught before puberty, but spermatogenesis is somewhat damaged in adult fish reared in captivity compared to wild individuals.

IJAE
Vol. 116, n. 3: 205, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

8th Meeting of the Italian Association of Veterinary Morphologists – Abstracts

Orexin A and receptor 1 for orexins in the rat testis: a morfo-functional study

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Key words

Orexin A, Receptor 1 for orexins, Genital tract.

The orexin A (oxA) and B a two peptides discovered in the rat hypothalamus and successively found in the gastrointestinal and genital tract of various mammals. They derive from the proteolytic cleavage of the common precursor pre-proorexin and bind two membrane receptors defined receptor 1 (ox1r) and 2 for orexins. The first receptor is specific for oxA and the second binds both the peptides with equal affinity. While the functions played by the hypothalamic orexins have been partially studied, those played peripherically are almost unknown. Here we describe presence and steroidogenic effect of oxA and ox1r in the rat testis by immunohistochemistry, western blotting, RT-PCR and in vitro cultured tissue slices.

OxA-immunoreactivity has been found in Sertoli cells and spermatids. These latter were ascribed to the stages of the germ cell developing cycle ranging from the VIIth to the XIVth. Ox1r-immunoreactivity has been found in leptotene and pachytene spermatocytes and in immature (round) and mature (elongated) spermatids. The presence in the testis of oxA and ox1r and the expression of the relative mRNAs were demonstrated by the biochemical techniques employed. The addition of oxA to in vitro cultured tissue slices showed that the peptide consistently enhanced the basal testosterone production of the rat testis.

IJAE
Vol. 116, n. 3: 206, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Disorders of sex development in the dog: morphologic, genetic and cytogenetic examination in two cases of true hermaphroditism (XXTH)

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Key words

Dog, Intersexuality, Histology.

Investigations of sexual development disorders in dogs and cats are important to identify new mutations, allowing us to eliminate inherited disorders from breeding populations, while contributing to the understanding of mammalian sexual development and differentiation. This work was aimed at giving a deeper insight into peculiar cases of intersexuality, happening in the canine specie, known as XX true hermaphroditism (XXTH), so classified by having both testis and ovarian tissue in one or both gonads in the presence of a XX chromosome constitution. Clinical, histological and genetic approaches were used in the study of a Cocker Spaniel dog 8 months old and a 3 years old mixed breed Pitbull. A normal female karyotype (78,XX) was noticed and polymerase chain reaction (PCR) failed to detect SRY gene on genomic DNA obtained from peripheral blood lymphocytes in both dogs. Female phenotype, hypertrophic clitoris and male behaviour were shown by both dogs. The reproductive tract was removed by standard surgical methods for ovariohysterectomy, and processed for histology. Vaginoplastic was carried out for reconstruction of a normal female phenotype. Histological examination of the genitalia showed bilateral ovariotestis in both cases: the gonad showed immature testicular parenchyma containing seminiferous tubules, Sertoli cells and Leydig cells but no signs of spermatogenesis, together with differently developed ovarian follicles containing oocytes. Genital tracts either of Wolffian and Müllerian origin co-existed in both subjects. Both cases belong to the very rare cases in which testicular tissue develops in the absence of the key gene SRY. Up to date very few genetic events have been associated to this abnormal sexual differentiation: SOX9 over-expression and RSPO1 mutation, nevertheless none of them have been found in dogs.