Morphological studies in the buffalo as a contribution to biotechnological methodologies in the animal production

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ABSTRACT - The researchers in the morphological area of the Faculty of Veterinary Medicine of Naples are long since carrying out investigations on the buffalo. This is due to the paucity of data in the literature of the field as well as scientific interest for many aspects of the biology of this species like the seasonality of the sexual cycle. The studies that have been done are numerous and regard many different fields as the chromosome map, spermatogenesis, histogenesis of the endocrine pancreas, blood circulation and innervation, and the structure and ultrastructure of several organs. In the present lecture, more recent and interesting results are reported regarding the blood circulation and peripheral innervation of several organs and the structure an ultrastructure of tracts of the digestive system. In particular, the following topics are presented: the evolution of the coronaric circle during the embryonic development; the hypophyseal circulation; the morphology of sensitive corpuscles in the external genital organs and in the reticular groove. In addition, data regarding the structure, the ultrastructure of the stomach wall and the role of each mucosal layer in the stomach functions are reported. The results of these morphological studies constitute a scientific background which is essential in the field of the applicative biotechnology.

Key words: Innervation, Buffalo, Stomach, Microcirculation.

INTRODUCTION - In the last years, the increase of the buffalo breeding in Italy, in Campania particularly, has raised the request of information, explanation and notices regarding the buffalo biology, morphology, pathology, nutrition, reproduction and the management of buffalo breedings. The necessity of a deeper knowledge in the field has encouraged for a long time the researchers of the faculty of Veterinary Medicine of Naples in undertaking investigations regarding many aspects of the biology of the water buffalo. The effort implemented by the researchers in the several fields during the last decades was great and full of difficulties because of the lacking in the region of a species similar to the buffalo which could represent a reference from a scientific point of view. As it is well known, the buffalo has peculiar life habits and a seasonal sexual activity. In order to reveal morphological and functional diversities, the results of the studies in the buffalo have been frequently compared by the researchers with those reported in other ruminant species, in particular, with those reported in the bovine. In the field of morphology, the pioneer was Prof. Antonio de Girolamo who gave an important contribution in the field of the buffalo biology with numerous and detailed studies.
undertaken by himself and by his pupils. The most important studies regard the anatomy of the tongue (Da Girolamo 1950), spermatogenesis (Da Girolamo 1952), innervation of the lingual papillae (Da Girolamo 1954), histogenesis of the endocrine pancreas (Da Girolamo 1956) and the chromosome map (Da Girolamo 1957). The researchers of the morphological area have planned, in the last few years, a broad study program focusing on topics which have had a poor attention by other researchers and, in particular, on the reproduction. The studies deals with several morphological subjects: macro- and microanatomy of the digestive, respiratory and urogenital systems; blood circulation and innervation; structure and ultrastructure of organs playing particular functions. The results of these studies have provided useful information in the understanding of the role of each structural component during the life and, in the same breath, increased the value of morphological investigations. These investigations, in many cases, costitute a fundamental scientific background which is essential in the biotechnological and applicative field. The studies made during the years in the field of the buffalo morphology are numerous, however, in the present lecture, only the most important and more recent data are reported. These data deal with the cardiocirculatory system and the peripheral innervation of some organs, and the structure and ultrastructure of several sections of the digestive system.

MATERIAL AND METHODS - The present lecture summarizes the results of several studies. The most important techniques used in these studies are reported as follows:
1. The investigations on the cardiocirculatory system were performed by using specimens injected with Neoprene Latex or histological sections obtained from organs injected with Indian Ink.
2. The investigations on the peripheral innervation were performed by using Ruffini's gold and Silver staining methods and, recently, histochemical and immunohistochemical methods.
3. The investigations on the digestive system were performed using several methods. Common histological stainings were used to study the palatine tonsils. Scanning and transmission electron microscopy and histological techniques were used to study lingual papillae and the stomach. Vascular corrosion cast techniques were used to study the microcirculation.

RESULTS AND CONCLUSIONS - Cardiocirculatory system. Among the investigations carried out on the buffalo, those dealing with the circulation of some organs (kidney, heart, hypophysis, placenta) are the most interesting. They were accomplished when the data regarding these topics were completely absent in the ruminant species.
a) Comparative investigations (Rosati, 1958) on the morphology of the kidney blood circulation showed that the angioarchitecture of the buffalo kidney is very similar to that of the bovine. Interesting data were reported about the interlobular, afferent and efferent arteries and the glomerular vascular network.

The interlobular arteries branch directly the afferent arteries or divide before branching them. The glomerular vascular network has a diverse morphology based on the branching patterns of the glomerular vessels: the afferent arteriole is single or, otherwise, branch off collaterals before forming the glomerular ansae. Often, these ansae form anastomoses between them.
b) The distribution and route of collateral and terminal branches of coronary arteries have been described during the embryonic life as well as in the adults by Caputo et al. 1967. These authors revealed interesting organogenetic aspects occurring in these vessels. They showed the presence of intra- and inter-coronaric anastomoses during early and middle phases of the embryo life. These anastomoses gradually decrease in number, diameter and extension. The coronary arteries, as the cardiac muscle increase in size, lose, from a morpho-functional point of view, their capability to form an efficient collateral circulation and, finally, become “functionally terminally” arteries. The collateral anastomatic circle of these arteries become inadequate to provide the blood supply in the case of occlusion of a main arterial branch.

c) A comparative study on the hypophyseal blood circulation has been undertaken (Paino et al., 1981) in the bovine and buffalo. The blood supply is provided by two systems: a rostral one which is formed by vessels directed to the *pars tuberalis*, median eminence and to the superior tract of the infundibular pedicle; and an aboral one which supply posterior, intermediate and Wulzen lobes. The vessels in the rostral system, median eminence, infundibular pedicle and *pars tuberalis* form vascular arches which, in turn, give rise to thin branches. These branches converge into fan-shaped venules located in the distal portion of the anterior lobe. This peculiar vascular arrangement constitutes the hypophyseal portal system. This system connects the blood circulation of some hypothalamic areas with the sinusoidal network which encircles the cells of some regions of the *pars distalis*. A similar pattern of vascular arrangement has been described in the humans and has been related to the capability of the vascular ansae to regulate the secretion of adenohypophyseal hormones.

d) In study carried out on some ruminant species (sheep, bovine and buffalo), the arrangement of placental arterial and venous vessels has been comparatively described (Pelagalli et al. 1973). In the buffalo, the artery and vein supplying the chorion in the pregnant horn branch
collaterals (cotyledonar branches) to the right and left. In addition, going up towards the apex of the horn, the cotyledons and their vessels become smaller and smaller. In the buffalo, the cotyledonar vascularization pattern is more simple than in the bovine and the vessels are lager.

In all the three species the uterine caruncles and the caruncle-cotyledon relationships have a particular morphology. In the buffalo, smallest caruncles are round-shaped and have a location similar to that observed in the sheep. Largest caruncles are oval-shaped and the caruncle surface which is in contact with the cotyledons is lightly convex. On the contrary, in the bovine, this surface is prominent and convex.

Somatic an visceral innervation

The studies carried out on the somatic and visceral innervation of the buffalo are numerous and, primarily, deal with the digestive and urogenital systems. Among these studies, those carried out on the sensitive and autonomic innervation of the uterus (Giordano, Rosati, 1956), and bladder (Rosati, 1956) are noteworthy.

a) Neuro-histological studies were carried out on the reticular groove (Paino et al., 1976). In these studies, the arrangement of the striated portion of the musculature of the reticular groove has been described. As it is well known, this striated portion originates from the esophageal striated musculature and spreads out towards the reticular groove and rumen. It plays an important role in the function of the proventriculus and, consequently, in the rumination process. The distribution of the myenteric nerve fibres has been described in both the smooth and striated portion of the reticular groove musculature. In addition, it has been described that the submucous plexus in the reticular groove is formed by nerve fibres which for the most part originate from the myenteric plexus. The submucous plexus has been found to contain only single, scattered ganglion cells. Sensory nerve fibres end in the tunica submucosa and, more frequently, in the lamina propria of the mucosa with arabesque-like free expanded endings or Ruffini’s corpuscles. The Ruffini’s corpuscles form often other free nerve endings or other corpuscles of the same type. The
morphology of these sensory nerve endings is very similar to that described by Rosati e Pelagalli (1956) in the equine esophagus and by Vesce (1968) in the reticular groove of the sheep. These data are very interesting and in contrast with those reported by Grau e Walter (1957) who denied the presence of any kind of sensory innervation in the esophagus and reticular groove.

b) Very detailed studies have been carried out on the innervation of both male and female external genital organs (Pelagalli, Cecio, 1957; Cecio, Pelagalli, 1958).

Numerous expanded nerve endings were found. These nerve endings varied in morphology and were often connected between them by ultra- or inter-ending fibres. In addition, poecil-like nerve fibres were found. These fibres formed different receptor types or expanded free endings which, in turn, were grouped in a “bouquet”-like fashion. The presence of these kind of relationships between the receptors suggests that the genital sensitivity is generated by both local and diffuse stimuli.

Ruffini’s and Pacini’s corpuscles were also found. Sometimes, Pacini’s corpuscles were found to have a Timofew’s apparatus which was for the most part formed by very thin periaxonal amielinic fibres.

Button-like nerve endings were described in both male and female external genital organs. These nerve endings were formed by thick nerve fibres which had neither pre-ending narrowings nor corpuscle coating. This kind of nerve ending has had never been reported in other species so that it is considered specific for the buffalo.

Figure 3. Nerve corpuscles in the external male genital organs of the buffalo. A inter-ending genital corpuscles, B nerve ending with a Timofeew apparatus.

c) Recently (Mirabella et al. 2003), an histochemical and immunohistochemical study has been undertaken on the innervation of the vas deferens and male accessory genital glands. A relationship was found between the neurochemical content of the nerve fibres and the seasonality of the reproductive activity. A marked decrease of the noradrenergic innervation were described during the spring-summer when the reproductive activity is low. The results of this study show that the reproductive activity in the male buffalo is regulated by neuro-endocrine mechanisms occurring between the androgen hormones and the noradrenergic innervation, and provide useful suggestion for a pharmacological approach to the buffalo infertility.
Digestive system

The alimentary canal is one of the most studied sections of the buffalo anatomy. This is due to the significance of the physiology of digestion and of the feeding habit.

In the recent years, investigations were carried out on the lingual papillae, palatine tonsil and stomach. Some of these investigations, performed using modern histochemical, immunohistochemical and electron microscopy techniques, provided important results from a functional and biotechnological points of view.

a) The morphology of lingual papillae was described by Scala et al., 1993. Circumvallate papillae were 15-18 in number and have a complex arrangement which is related to their role in the digestion. These papillae have only one vallum which is frequently crossed by small grooves. These grooves facilitate the contact between the food particles and the taste buds located on the edge of the central portion. The central portion is single or subdivided in several parts. It is known that the only contact of the food particles with the lingual mucosa is capable to stimulate salivation.

Figure 4. Circumvallate papilla of the buffalo tongue. CP central part, S groove, V groove on the vallum.

b) A comparative study has been carried out on the palatine tonsil in the bovine and buffalo (Pelagalli et al 1983). The morphology of palatine tonsil differs between these two species. In the bovine, this tonsil has a globose shape and is divided in two symmetric parts. In the buffalo, the surface of the palatine tonsil appears to be divided in 6-8 lobes by the presence of grooves. These grooves are formed by connective septa which penetrate the organ.
The tonsil sinus opens in the oropharynx and is large. It is single in the buffalo. The tonsil sinus collects the openings of the tonsil crypts. The crypts show several series of division and are more numerous in the buffalo than in the bovine.

The lymphatic tissue has nodular and not-nodular parts. The connective framework which supports the lymphatic tissue is formed by reticular fibres which originate from the capsule septa. The gland portion is of the mucous type and is distributed among the lymphatic tissue. In the bovine, this portion is located at the periphery of the tonsil or outside of it. In the buffalo, the gland portion is always distributed within the organ. The excretory ducts open in the tonsil sinus or crypts.

The lining epithelium of the tonsil sinus and crypts is pavement stratified and shows zones in which the “lympho-epithelial symbiosis” phenomenon occurs. This phenomenon has also been described in other species and is observable in those zones of the epithelium where a great lymphatic infiltration occurs. The epithelium shows a change in its structure: it noticeable grows thinner, the epithelial cells separate between them thus allowing the epithelial cells themselves and the lymphatic ones to reach the lumen of crypts and the sinus. The presence of the “lympho-epithelial symbiosis” is related to the function of the tonsil. The tonsil plays a role in the local immune response and, hence, it reacts to local noxious stimuli by introducing a great quantity of lymphocytes in the tonsil sinus and, consequently in the saliva.

c) A detailed study has been carried out on the stomach (Pelagalli and Scala, 1997). Several morphological features, including the innervation and microcirculation, were investigated. The tunica mucosa of the ruminant stomach has multiple roles. In the abomasum, it has the same roles as in the stomach of a monogastric species. In the proventriculus, the tunica mucosa is involved in the absorption of the free fatty acids and fluids at level of rumen papillae, reticulum cristae and omasum laminae. The proventriculus plays a pivotal role in the ruminations as it provides a large amount of nutrients which are released by feed during the microbial fermentation.

The digestion of feed by means of a microbial fermentation occurs also in the large bowel of monogastric species. However, in these species, this kind of energy production is less effective than in ruminants. Ruminants, in addition, are capable to digest the same microorganisms, in the abomasum and intestine, which are responsible of the fermentation with further energy production.

The horny and granulose layers of the epithelium of the proventricular mucosa are very dense and constitute a barrier against noxious particles which are contained in the proventricular cavities. Intercellular spaces are numerous in the basal and spinous layers and are involved in the absorption mechanisms of free fatty acids and fluids.

The vascularization of the lamina propria also is involved in these mechanisms. A dense vascular network has been observed in the lamina propria of the proventricular mucosa. This network is particularly dense in the omasum where it forms dilated vascular loops in the subepithelial layer. These vascular loops increase the contact surface between the capillaries and the neighbouring cells thus facilitating the absorption of trophic substances as well as free fatty acids and fluids.

In the reticulum, the *muscularis mucosae* plays an important role in the contraction of the reticular crista. The contraction of the reticular cristae and that of the reticular wall
are synchronous. The synchronism in the contraction of the reticular cristae is due to the arrangement of the musculature of the cristae. This arrangement has been previously described by Rosati and Pelagalli (1960). The musculature of the omasal laminae is formed by three layers: a central layer which originates from the tunica muscularis of the omasum, and two lateral longitudinal layers which derive from the muscularis mucosae. The large and medium laminae slide one upon the other thus mincing the feed particles which are packed between the interlaminar spaces. Horny papillae which are distributed on the surfaces of the laminae facilitate this process. The peristaltic movements of the omasal musculature squeeze the fluids between laminae. This fluids are partially absorbed by the laminae themselves. In the abomasum, the lamina propria is formed by inner submucosal and outer layers. The outer layer is located just beneath the basal zone of the gastric gland. This basal zone is passed through by large and small perpendicular connective septa. The large ones envelop at the base clusters of gastric glands and delimit the gastric lobules. The small ones penetrate among the glands for a short tract.

Figure 5. Buffalo stomach. a omasal laminae, b basal layer of the epithelium of the omasal mucosa, c vascular casts of the lamina propria in the omasal mucosa, d arrangement of periglandular capillaries in the abomasums. M muscularis mucosae, B basal cells, C blood capillaries, N periglandular network.
As it regards the reticular groove, it can be deduced that the smooth musculature is the major responsible of the contraction. The striated musculature, which is present only in some tracts of the groove, constitutes a sort of connection with the esophagus and plays an important role in the ruminations.

The relationships between the microcirculation of the lamina propria and the deep layers of the epithelium in the proventriculus are ample at level of the rumen papillae and in the omasum. In the abomasum, the vascular network which encircles the gland adenomeres has not only a role in the trophism of this glands but also regulate their secretory activity.

All the investigations which has been carried out on the stomach gave useful suggestions for applicative purposes.

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REFERENCES - de Girolamo, A., 1950. Sull’anatomia della lingua di bufalo. (Anatomy of the Buffalo tongue). Riv. di Medicina Veter. E Zootecnia 2(11-12): 315-331. de Girolamo, A., 1952. Ricerche sulla spermatogenesi di Bubalus buffalus. 1-Osservazioni preliminari. (Spermatogenesis in Bubalus buffalus. I- Preliminary observations). Quad. Anat. Prat. 7(1-2): 1-15. de Girolamo, A., 1952. Ricerche sulla spermatogenesi di Bubalus buffalus. 2-Osservazioni sull’evoluzione dell’acido ribonucleico negli elementi germinali e trofici dei tubuli. (Spermatogenesis in Bubalus buffalus. II- Ribonucleic acid evolution in the germinal and trophic elements of the tubules). Quad. Anat. Prat. 7(1-2): 1-11. de Girolamo, A., 1954. Ulteriori osservazioni sulla innervazione delle papille linguali di bufalo (Innervation of lingual papillae in the buffalo). Quad. Anat. Prat., Serie 9,14: 1-13. de Girolamo, A., 1956. Primi risultati sulla istogenesi e sulla morfologia del pancreas endocrino di Bufalo (First results on the histogenesis and morphology of the Buffalo endocrine pancreas). Atti Soc. Itl. Anat. 17. de Girolamo, A., 1957. Uber das veraelten der Chromosomem beim Buffel (The chromosomal map in Bubalus bubalis). Acta Anatomica, 26. Rosati, P., 1958. Sulla morfologia comparata del circolo sanguigno nel rene lobato (Ricerche sul bufalo). (Comparative morphology of the vascularization in lobed kidney. A study in Buffalo). Quad. Anat. Prat. 14(1-2):1-47. Caputo G, 1964. Sulle connessioni vascolari anastomotiche nel circolo arterioso e venoso cardiaco in cuori embrionali e fetali di alcuni ruminanti. Rilievi morfologici e funzionali. Quad. Anat. Prat. 20(1-4): 61-84. Caputo, G., Rascio, L., Finelli, R., 1967. Ricerche sul circolo coronario arterioso nel cuore di bufalo adulto. (Arterial coronary circulation in the buffalo heart). Quad. Anat. Prat. 23(1-2): 1-27. Paine, G., Langella, M., Caputo, G., 1981. Vascular feature of the hypophysis in Bubalus buffalus. Acta Anatomica, 110(3):206-218. Pelagalli, G., V., Mastronaro, M., Potena, A., 1973. La circolazione placentare in alcuni Ruminanti.Acta Medica Vet., 19:1-2. Giordano, G., Rosati, P., 1956. Sulla innervazione vegetativa dell’utero nel Bufalo (Vegetative innervation of the buffalo uterus). Atti Soc. Ital. Sci. Veter. 10:453-458. Rosati, P., Giordano, G., 1956. L’innervazione della vescica nel bufalo (Bubalus buffalus) (Innervation of the Bubalus bladder). Boll Soc. Ital.
Biol. Sper. 32(7):666-668. Paino, G., Langella, M., Budetta, G., 1976. Studio neuro-istologico della doccia esofagea in alcuni ruminanti. (Neuro-histological study of the reticular groove in some ruminants). Acta Med. Vet. 22:23-29. Rosati, P., Pelagalli, G., V., 1956. Ricerche nell’innervazione sensitiva dell’esofago negli Equini. Acta Med. Vet. 2:5. Vesce, F., 1968. L’innervazione della doccia esofagea in Ovis aries. Acta Med. Vet. 14:211-222. Grau, H., Walter, P., 1957. Ueber die feinere innervtion der Varmergenn der Wieder-Kauer. Acta Anat. 31:21-25 Pelagalli, G., V., Cecio, A., 1957. L’innervazione sensitiva dei genitali esterni maschili di bufalo (Sensitive innervation of the male buffalo external genitalia). Acta Med. Vet. 3(6):481-509. Cecio, A., Pelagalli, G., V., 1957. L’innervazione sensitiva dei genitali esterni femminili di bufalo (Sensitive innervation of the female buffalo external genitalia). Acta Med. Vet. 4(1):69-97. Mirabella, N., Squillacioti, C., Varricchio, E., Genovese, A., Paino, G., 2003. Innervation of the vas deferens and accessory male genital glands in the water buffalo (Bubalus bubalis). Neurochemical characteristics and relationships to the reproductive activity. Theriogenology 59:1999-2016. Scala, G., Pelagalli, G., V., Vittoria, A., de Girolamo, P., 1993. Etude Morpho Structurale ds papilles linguales chez le Buffle (Bubalus bubalis). Anat. Histol. Embriol.,22. Pelagalli, G., V., Langella, M., Coltella, G., 1983. Aspects morphologiques et structuraux de la tonsille palatine du beuf Bos taurus et du buffle (Bubalus bubalis). Zbl. Vet. Med C. Anmat. Histol. Embryol. 12: 253-265 Pelagalli, G., V., Scala, G., 1997. Morphostructural and ultrastructural characteristics of buffalo stomach. Bubalus bubalis, Journal of Buffalo Science and Technique Suppl. N.3. Rosati, P., Pelagalli, G., V., 1960. Sull’anatomia microscopica dello stomaco dei poligastri. Ricerche in Bubalus bubalis. Acta Med. Vet. Suppl n° 6.