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RESEARCH REGARDING THE LIMBS VASCULARISATIONS AND INNERVATION IN AFRICAN OSTRICH (*Struthio camelus*)

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SUMMARY

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Anatomy (*ana*=through and *tomein*=cutting), defined as a fundamental, core science of medicine of any kind, appeared in the distant past as a necessity of knowing and understanding animal and vegetal organisms. Human anatomy and animal anatomy have contributed in time to the evolution of some pre-clinical and clinical subjects such as anatomo-pathology, semiology and most importantly, surgery.

Due to the fact that during the past three decades the global population increased considerably, the consumption and the necessity for sources of food have also increased. Because of this, new species of animals were domesticated for meat consumption, but also for ornamental or sportive purposes. Out of the recently domesticated species, the African ostrich stands out as a species whose morphological area is still not completely known. This is one of the reasons for which the present doctorate thesis focuses on a detailed study of the vascularisation and innervation of the limbs in this species, with a considerable amount of anatomical differences compared to other birds.

The thesis is structured according to imposed standards in a first part which includes bibliographical studies, with about 40 pages, and a second part of personal research made up of 171 pages with 77 original photographs.

Part I of the thesis, entitled "**Bibliographical Study"** includes three chapters.

The first chapter is called "**The origin of ostriches, fundamental aspects regarding the anatomy and physiology of ratites".** This chapter includes data referring to the ancestors of this species, whose morphology has been reconstituted based on bones and bone fragments, as well as fossilised eggs. Some anatomical particularities of the *Struthio camelus*, the more common of the two living species of the *Struthio* genus, were also mapped out. The skull, reduced in size by comparison to the rest of the body, the lack of a sternal careen, the decrease in the size of bone wings, the exaggerated development of the pelvic limb bones and the reduction of the number of fingers are all defining elements of an ostrich's skeletal structure. The weight, size and growth rhythm of the ostrich are neatly superior to all other orders. Interesting morphological characteristics are presented, such as specifics of the digestive, respiratory and uro-genital systems, of which the following are worth a mention: the ostrich's tongue is rounded at the apex and forms, on the dorsal side, a sort of recessus, the ingluvies is absent in all

ratites, the ceca are enormous, the colon is exceedingly long, the liver does not have a bile and the copulative organ of males, called a phallus, is very developed in this species, with a size of 20 cm flaccid and up to 40 cm when erect. The phallus is also just a copulative organ, not urinary like in most species.

This chapter also describes, in short, the phylogenetic evolution of the order.

The second chapter is called **"The evolution of limbs in Tetrapodes vertebrates"**, which initially describes the general structure of the limb skeleton in these vertebrates, reflecting on their evolution in relation to their position on the phylogenetic scale, as well as the function they accomplish. The chapter also integrates a table regarding the homologation of the general nomenclature with the terminology used in human anatomy.

Following that, the development of the limbs from a phylogenetic point of view is tackled, starting from amphibians and ending with mammals. In the case of amphibians, Urodela have all limbs, closer to the fundamental type, while Anura, more advanced from a phylogenetic point of view usually present an adaptation typical for jumping locomotion, which strongly influences the structure of the posterior limb. In the case of reptiles, they have conquered, just like mammals later on, both the sea (*ichthyosauri*, *plesiosauri*, *mosasauridae*) and the aerial environments (*pterosauri*), aside from the terrestrial one. Here is why the members of this class, even accounting for the extinct groups, have adaptations parallel to the mammalian skeleton. The anterior limb structure in birds is profoundly modified to aid with flight. In ratites (*Acarenata*), in general, the autopodial skeleton has a tendency to regress as a result of the loss of flight capacity. Finally, the particularities of limbs in mammals are briefly described, in correlation to their functional modifications.

The third chapter is titled **"Aspects regarding the ontogenetic development of the blood vessels and the spinal nerves"**.

Regarding the arterial system, the differences between the development of the aortic arches in some domesticated animals compared to humans are noted. The way these arches form major arteries is also explained. This chapter also tackles the mechanism which leads to the definite settlement of the unique trajectory of the caudal laryngeal nerves. The venous system develops under the influence of specific growth factors similarly to the arterial system. In early embryological stages three major pairs of veins are formed: vitelline, umbilical and cardinal veins.

The evolution of each pair is described in detail.

Regarding the nervous system, the focus was on the development of the spinal nerves. Detailed descriptions are also made of the ways through which the neuroblasts from the basal plate differentiate during the evolutionary process, developing cytoplasmic processes and turning into motor neurons, as well as the ways through which the sensory components of the spinal nerves differentiate themselves from neuroblasts in the ganglions of the dorsal roots. A complex graph is included, which shows a transversal section through the spinal cord, in order to facilitate the understanding of the structure and distribution of these nerves.

Part II, entitled "Personal Research", presents in detail **the fourth chapter, the aim and objectives of the research.**

The fifth chapter is titled **"Materials and methods"**. It contains information regarding the biologic material as well as the tools used, and it also describes the working methods in detail, methods applied according to their purpose (bone preparation, muscle identification, the injection with plastic contrasting material of the vessels, etc.)

The sixth chapter titled **"Results and discussions"** is, in turn, divided in four subchapters, each of them finalised through a series of partial conclusions which will serve for presenting the final conclusions.

The first subchapter describes **the anatomical basis of the limbs** in *Struthio camelus.* Anatomical particularities of the limb bones are presented, focusing on identifying, describing and homologating structures in accordance with the international anatomical nomenclature. Some aspects that are not encountered in other species were described, such as the absence of the clavicle and the melding of the scapula to the coracoid bone. The stylopodium is represented by a fine, thin, long humerus, these attributes correlated to the weak development of the muscles which it provides support to. The zeugopodial bones – the radius and the ulna- are small by comparison to the bird's size. However, they are well developed, and so is the autopodial segment.

The anatomical basis of the pelvic limb compensates for the poor development of the thoracic one. Aside from the fact that it is the only bird species in which the coxal bone, through its pubis, manages to create a true symphysis, the bones of the other anatomical regions are extremely powerful, structurally modelled and adapted functionally to sustain the most massive flightless bird that currently inhabits Earth.

The second subchapter tackles aspects regarding **the myology of the appendicular segments** in the African ostrich. As should be, the wing musculature is insignificant. First of all the powerful pectorals that exist in flying birds are represented in this case by a single muscle. Even though a numeric reduction of the arm muscles has not been noticed, most of them are wide muscles, with a reduced size on a transversal section, thus incapable of realising actual traction force. The autopodial muscles are relatively well represented. The reduction of the connective muscles and relative development of the musculature towards the wing's distal extremity is correlated with flight incapacity, namely to some muscles' actions, involved in some behavioural rituals such as nuptial dances. The pelvic limb muscles are certainly some of the strongest propulsive muscles in the entire vertebrate series. Aside from the aforementioned role, these also ensure the connection of the pelvic limb to the trunk through the belt, but also the sustenance of the bipedal animal with the largest weight out of all currently living ones. The tendons of these muscles, in the distal region of the limb, act largely similar to those in equines, having a series of characteristics which correspond morpho-functionally with the passive apparatus of these mammals.

The third subchapter treats the most important aspects regarding **the vascularisation and innervation of the thoracic limb in *Struthio camelus***. The vessel and nerve descriptions are incredibly detailed, the text being supported by 12 original photographs, making it easy to read and understand. Some of the more important particularities are presented next.

Regarding arterial vascularisation the main artery pertaining to the thoracic limb was identified and dissected- the axillary artery- alongside its terminals: the brachial and the profound brachial arteries. Out of these, the brachial artery is the most voluminous and the longest, disposed parallel to the entire caudo-medial edge of the humerus. It is the easiest vessel to find in practice. As for the venous system, the most important veins of the wing are the radial and the ulnar veins. They are the roots of the brachial vein. However, the most important vein that can also be used in regards to blood draws or intravenous administrations is the basilic vein. At the level of the arm, the basilic vein is placed at a distance from the humerus, in the distal half of the region, afterwards coming in intimal contact with the cranio-ventral face of the humerus.

Wing innervation is ensured by nerves originating from the brachial plexus, which includes a dorsal and a ventral fascicle. The dorsal fascicle ends with the radial and the axillary nerve. The ventral fascicle of the brachial plexus ends with the cutaneous brachial ventral, the bicipital and the medial-ulnar nerve. In this subchapter the trajectories of the cutaneous nerves were described for the first time, so the ones interested can intervene successfully in the case of performing anaesthesia, either experimentally or therapeutically. Another interesting aspect is the fact that in 20% of the cases, out of the ventral fascicle, an independent and very well represented nerve emerges, nerve which gives off branches for the skin on the medial side of the arm and branches which meld in the periosteum of the medial face of the humerus. This previously undescribed branch can be considered, through its topography and distribution, a veritable medial brachial cutaneous nerve.

The last subchapter is entitled **"Research regarding the vascularisation and innervation of the pelvic limb in *Struthio camelus***. This subchapter has 30 original photographs. It has been observed that the arterial vascularisation of the pelvic limb in the ostrich is realised through terminal branches and collaterals of the external iliac artery and the ischiatic artery. In regards to the distribution of the external iliac artery, the first interesting aspect was encountered in the terminals of the coxal cranial artery, which is, in turn, a branch of the femoral artery. In the case of these terminals (ascendant and descendant branches) individual discrepancies were encountered. Thus, in 40% of the cases, the ascendant branch is very poorly represented and it is supplied in this case by a developed branch, appearing at approximately 1 cm from the cranial coxal artery, as direct collateral of the femoral artery. The ischiatic artery also provides a series of new aspects. Such an example is the distribution of the caudal coxal artery, its first collateral, the discovered variants being described in detail in this subchapter. The main terminal of the ischiatic artery, the popliteal artery, emits the cranial and the caudal tibial arteries. Compared to existing literature data, differences in the distribution of two of the branches of the cranial tibial artery were observed. Proximal to the tarso-metatarsus, the tibial cranial artery continues as a common dorsal metatarsal artery, which emits the lateral and medial plantar tarsal arteries. The dorsal metatarsal common artery continues as the most important artery of the tarso-metatarsal area, going alongside the tendon of the common digital extensor muscle, finishing through the III and IV proper digital arteries. For the first time, a series of arterial anastomoses in the tarso-metatarsal area were described.

Generally, the veins are satellite to the arteries. However, through thorough dissection, some differences were noticed. As an example the tibial cranial vein is reminded, which does not have a trajectory through the interosseous distal tibio-fibular space, however climbing to the cranio-lateral edge of the fibula, until it reaches the superior third of the calf, where it recurves caudally, to finally fuse with the caudal tibial vein, creating the popliteal vein.

The innervation of the pelvic limb is ensured by nerves originating from the lumbar plexus and sacral plexus. The lumbar plexus results from the weaving of the ventral branches of the sinsacral spinal nerves II, III, IV and V, the last one contributing with a fine branch at forming the sacral plexus. The three nerves of the lumbar plexus are:

the cranial coxal nerve, the femoral nerve and the obturator nerve. The sacral plexus at ostriches is made of the ventral branches of the sinsacral spinal nerves V-XI. The first five roots form the cranial trunk of the plexus while the last two form the caudal trunk. From the cranial sacral plexus four nerves emerge: the ilio-tibular never, the ischio-femoral nerve, the ischiatic nerve and the iliofibular nerve.

Some individual particularities previously unmentioned in specialty literature are also described.

Frequently (30% of the cases) it has been noticed that the coxal cranial nerve appears as two distinct branches, the ventral branch (inconstant) being mainly sensory; it distributes to the skin which covers the iliotibial cranial muscle, thus working to enhance the function of the cranial femoral cutaneous nerve.

The medial sural nerve, originating from the tibial nerve, ends in two branches: the lateral and the medial branch. In most cases the lateral branch tackles the *flexus hallucis longus* muscle. The study has proven that there are about 20% of the cases where the nerve divides precociously, tackling the extremities of the muscle through the formed branches. The subchapter includes other variations and unique aspects, always mentioning, in the case of sensory nerves, the election place favourable for anaesthesia.

This thing allows performing interventions, should it be the case, using local anaesthesia.

The study ends through a chapter which includes a number of 16 conclusions.