UNIVERSITY OF AGRICULTURAL SCIENCES AND
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DOCTORAL SCHOOL OF VETERINARY MEDICINE

ABSTRACT OF THE HABILITATION THESIS

THE IMPORTANCE OF ANATOMICAL RESEARCH AS A STARTING POINT IN MEDICAL STUDIES

DOMAIN: VETERINARY MEDICINE

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The habilitation thesis presents the most important results of my academic and scientific activity obtained after doctoral studies and until now, as well as the development plan of the academic career. The thesis is structured in three parts: the first part makes a short presentation of the professional, scientific and academic career focused on the professional, didactic and research fields; the second part presents the results of scientific research in the field of morphology and translational medicine, as the main topic of this thesis; and the third part presents the development plan of the academic teaching and research career.

The first part briefly presents the professional and academic evolution after my integration in the Anatomy team discipline since 2003. I promoted through the competition the hierarchical steps, from assistant professor to associate professor in 2016. I carried out the didactic activity within the same disciplines by teaching practical work and Anatomy courses. The didactic activity was completed by the scientific and research activity, materialized by the elaboration of numerous scientific works, didactic manuals and specialized books.

The second part presents the most important researches and their results obtained after accomplishment of the doctoral thesis. This part is structured on two main chapters, which represent the most important research directions approached, each chapter being divided into several subchapters.

The first chapter entitled *Scientific contributions to the systematization of lymphatic drainage of healthy and tumoral mammary glands in females' dog*, presents the results of studies aimed at investigating the superficial lymphatic drainage in the region of mammary gland in females' dog.

Starting from the premise that lymphatic vessels are essential in the systematization of lymphatic drainage, in subchapter 1.2 was briefly presented their importance in maintaining homeostasis, the role of lymphatic endothelium in modulating the immune response and their importance in the presence of diseases involving the lymphatic system. Regarding tumor lymphangiogenesis, the results of my studies show that this multifactorial process occurs due to the interaction between tumor cells, lymph endothelial cells and immune cells. All these processes take place and can be identified morphologically at the local level.

Subchapter 1.3 presents studies on the *Morphology of lymphatic drainage of healthy mammary glands in female's dog.*

The identification of the lymphatic network of mammary glands and lymphatic vessels that become afferents of regional lymph nodes is presented in subchapter 1.3.1. The lymphatic efferents from the mammary glands arise from the union of the lymphatic vessels coming from the deep perilobular network with those of the superficial network. Thus, two locations are identified in which these

anastomoses are performed, namely, one at the level of the mammary areola and the second at the periphery of the mammary gland. The methods for injection of the revealing solutions of the lymphatic vessels and lymph nodes were based on these considerations.

A separate subsection presents the morphological and imaging techniques used in the studies conducted to perform the lymphatic mapping of the mammary glands. The tropism of the blue dye Evans and the ultrasound contrast agent SonoVue, for the lymphatic tissue determined their use for the identification, of both, the lymphatic vessels and the lymph nodes draining the mammary glands.

Cranial lymphatic drainage of healthy mammary glands is highlighted in subchapter 1.3.2. The main lymph center that drains healthy mammary glands are the axillary lymph center through the proper axillary lymph nodes and accessories lymph nodes. This lymph center is constant in presence, but variable in terms of the number of lymph nodes that make it up, their size or even their afferents and efferents lymphatic vessels. Studies have shown the existence of lymphatic vessels which bypass the axillary lymph nodes reaching the cranial sternal lymph node or even the deep caudal cervical lymph nodes. Regarding the cranial abdominal mammary gland, strict caudal lymphatic drainage was demonstrated, in addition to the established direction, namely both cranial and caudal. This pattern is presented in studies from the subchapter 1.3.3.

Subchapter 1.3.4. presents the results of studies that analyzed *The caudal lymphatic drainage of healthy mammary glands, lymph centers involved and lymphatic connections*. Therefore, the essential lymph center involved in this drainage is the inguino-femoral or superficial inguinal, through the superficial or mammary lymph nodes. It consists of one, two or even three lymph nodes, joined or just close one to each other, which invariably drain the healthy caudal mammary glands. Moreover, it was observed that some of the lymphatic vessels cross the median plane and make the connection between the two contralateral inghino-femoral lymph centers, which demonstrates the presence of lymphatic connections between the contralateral superficial inguinal lymph nodes. At the same time, it should be mentioned the existence of vessels that bypass the mammary lymph nodes, to drain into the following lymphcenters such as the deep ilioinginal or profound inguinal lymph center or even iliosacral lymph center, more precisely in the iliofemoral or medial iliac lymph nodes, which commonly receive direct afferents from the superficial inguinal lymph nodes.

Subchapter 1.4 entitled *Morphology of lymphatic drainage of mammary glands with tumors in females' dog*, presents the most relevant results obtained in studies that evaluated the impact of the presence of malignant mammary tumors on lymphatic drainage. The presence of tumors of the mammary glands determines the modification of both the lymphatic routes but also the recruitment of other lymph

centers compared to those known to perform the lymphatic drainage of the mammary gland. For this reason, subchapter 1.4.1 presents *The importance of the concept of sentinel lymph node of mammary glands with tumors in females dog.* The sentinel lymph node is not specific to the type or location of the tumor, but it is specific to each individual and more than anything, its location is extremely variable. Under these conditions, no exact assessments can be made of a particular condition or belonging to a defined or predetermined lymph node draining a tumor located in a particular place. Therefore, it is imperative to identify and evaluate with the greatest accuracy the real sentinel lymph node and corresponding to the tumor drainage basin. The final goal of my research in the field of drainage of mammary glands with tumors was to achieve a noninvasive evaluation of the sentinel lymph nodes that drain these glands.

The cranial and caudal lymphatic drainage of the mammary glands with tumors is presented in subchapter 1.4.2. and 1.4.3. After peritumoral administration of the dye and the contrast agent, were identified as sentinel lymph nodes, other lymph nodes that are not established as specific to the drainage of the mammary glands, respectively cranial sternal lymph nodes or deep caudal lymph nodes. The bypass lymphatic vessels of the regional lymph nodes were well highlighted by both methods of investigation used in these studies. Dissemination of tumor cells often in the form of tumor thrombi causes obstruction of these lymphatic vessels or the formation of metastatic islands in the sentinel lymph nodes. In subjects in whom the presence of metastases was detected in the sentinel lymph nodes, the afferents from the tumor were much more numerous, and the anastomoses between the tumor lymphatics of the adjacent glands were much more common.

Assuming that the summation of classical ultrasonographic methods, available to practitioners, can increase the accuracy of diagnosis, my studies have analyzed and described the normal and pathological aspects of lymph nodes that drain healthy and tumoral mammary glands in females' dog. These aspects are presented in subchapter 1.5 - *Imaging studies on lymph nodes that drain the mammary glands. The usefulness of an ultrasonographic algorithm in the evaluation of sentinel lymph nodes that drain the tumoral mammary glands in bitches*. The ultrasonographic techniques used were two-dimensional ultrasound, Doppler technique, CEUS (contrast enhanced ultrasound) and real-time elastography, each technique being described diagnostic performance.

The results obtained when applying these techniques are presented in separate subchapters. Thus, it was shown that each solitary technique has a guiding role of the diagnosis, but assigning to each statistically significant parameter in differentiation, a certain score and summing the four techniques we obtained the highest accuracy in identifying metastases in lymph nodes.

In current practice, in dogs with mammary tumors, the current surgical procedure involves mastectomy and local lymphadenectomy, most often without a prior evaluation of the lymph nodes. By applying the proposed algorithm, it can be assessed not only the presence or absence of metastases in the lymph nodes, but also it could be identified the true sentinel lymph nodes, because in the presence of a tumor, lymphatic drainage can be unpredictable. In this way, unjustified excision of benign (unaffected) lymph nodes and deprivation of a certain territory of lymphatic drainage can be avoided.

The second chapter includes *Detailed anatomical studies of small animals used as experimental models in medical research and the clinical significance of these features.* It briefly presents the results of studies that have analyzed the peculiarities of different anatomical systems in rabbits, guinea pigs, chinchillas and laboratory rats, which make them experimental models specific to medical research.

Pointing out the morphological features of each segment, in this chapter, we provided the explanation of some clinical aspects often encountered in the pathology of rabbits, guinea pigs and chinchillas, which are directly related to certain morphological features. The anatomical-clinical details of each segment of the digestive tract are detailed in subchapters 2.2 - *Morphological features of the prediaphragmatic digestive tract in domestic rabbits, guinea pigs and chinchillas. Anatomo-clinical details from the studies performed* and 2.3 - *Comparative studies of the postdiaphragmatic digestive tract in domestic rabbits, guinea pigs and chinchillas. The anatomical-clinical importance of morphological features.*

Subchapter 2.4 entitled - *Comparative studies of different anatomical systems in rabbits, laboratory rats, guinea pigs and chinchillas* present the most important studies on the comparative morphology of the liver and bile ducts in laboratory rats, rabbits, chinchillas and guinea pigs.

The third part of this thesis contains the Academic Career Development Plan and is about the future. The near future is based on what we have achieved so far and the desire for development. These elements are the foundation of building the more distant future. My entire activity will be oriented towards progress and obtaining the best results in teaching and research careers. In a field that is believed to be monotonous, namely that of anatomy, there is place for improvement, a different approach to how to learn and exploit what is known or what has remained unexplored.

The main objective of my professional life is the ability to combine the quality of a teacher with that of a researcher and diagnostician, together with finding the much-needed balance between the three. This balance is the secret of prosperity and the motivation that gives me strength.