SUMMARY

The doctoral thesis entitled **"Research on auto and allo-articular regenerative therapy in dogs and rats"** was developed within the Doctoral School of Veterinary Medicine of the "Ion Ionescu de la Brad" University of Life Sciences in Iași.

The work comprises 175 pages and consists of two main parts, according to the current regulations. 285 bibliographic sources from the national and international specialized literature, relevant to the research topic, were consulted for the writing of the thesis. Accumulated data were presented in 41 figures and 8 tables.

The originality of the research results from the development of a protocol for the isolation and cultivation of mesenchymal stem cells from the marrow, in two species, respectively dog and rat. Unlike other doctoral theses in Romania where the studies were carried out on standardized human cell lines, this thesis describes both the process of isolating mesenchymal stem cells and their processing, until obtaining stable cultures that show the characteristics of the mesenchymal stem cells (adhesion to plastic, differentiation towards chondrogenic lines, presence of specific markers).

Also, as a novelty, we evaluated the grafting capacity of these cells, by histopathological evaluation, in rats.

In addition, following clinical work in two small animal practices in the United Kingdom, it is described the use of platelet-rich plasma therapy and adipose-derived mesenchymal stem cell therapy in the treatment of canine patients diagnosed with osteoarthritis.

The first part, entitled "The Current Status of Knowledge" includes three chapters (X pages) accumulating information available in the literature regarding the pathogenesis of osteoarthritis and the diagnostic methods used in veterinary medicine. One of the chapters summarizes the data accumulated worldwide in the field of regenerative medicine, and the last chapter describes the current options in veterinary medicine used in the management and treatment of osteoarthritis.

Part II, entitled "Personal Contributions", is structured into seven chapters (X pages) and presents the purpose and objective of the research, the materials and methods used, the results obtained, the interpretation of the results, as well as discussions and conclusions based on them.

The thesis also contains own data, published in specialized magazines from Romania and abroad.

The first chapter of the second part of the thesis (Chapter IV) presents the purpose and objectives of the research, such as:

- evaluation of the incidence of osteoarticular diseases in domestic carnivores from a studied population;

- establishing the diagnostic methods used in osteoarthritis;

- obtaining experimental protocols for harvesting and isolating stem cells from dogs and rats;

- comparative description of two mesenchymal stem cell cultivation protocols;

- highlighting the grafting capacity and characterization of the cell culture obtained;

- description of the therapy protocol with mesenchymal stem cells in osteoarthritis in dogs;

- evaluation of clinical cases in which platelet-rich plasma therapy was applied, as part of the treatment protocol in dog osteoarthritis;

Chapter V, entitled "RESEARCH ON THE INCIDENCE OF OSTEOARTICULAR DISEASE IN DOGS", had the aim of evaluating the incidence and distribution by species of the locomotor disorders in domestic carnivores, aiming at an assessment of morbidity according to breed and sex in dogs. In order to achieve this objective, the data from the consultation register of a practice in the United Kingdom, over a period of 19 months, were analyzed. The analyzed data included information related to the distribution by pathologists of the cases that benefited from a radiological examination, the majority of cases with locomotor disorders being diagnosed with osteoarthritis.

The results highlighted the fact that from the total number of patients taken into the study, most locomotor disorders were reported in dogs, the interested population being middle-aged to elderly and female.

Chapter VI, entitled "RESEARCH ON THE RADIOLOGICAL DIAGNOSIS OF OSTEOARTHRITIS IN DOGS" sought to highlight the characteristic radiological aspects of the most frequently diagnosed locomotor disorders in dogs. The study emphasizes the usefulness of the radiological examination in establishing the diagnosis of osteoarthritis in dogs, by highlighting the information gained on its account, such as: changes in the joint surfaces, reduced joint space, increased bone density or joint deformations. In addition, to obtain a diagnosis of certainty, most cases benefit from complementary examinations such as: hematological and biochemical profile, quantitative evaluation of C-reactive protein, cytological and microbiological examination of synovial fluid and histopathological examination.

Chapter VII, entitled "IMMUNOHISTOCHEMICAL AND HISTOPATHOLOGICAL RESEARCH IN RATS" is structured on three subchapters.

In the first two subchapters, the aim of the study was to obtain stable mesenchymal cell cultures, starting from the first step, their harvesting and isolation. The study describes different methods of harvesting bone marrow from both dogs and rats. For both species, harvesting was carried out under conditions of maximum sterility, a primordial condition, since cell cultures do not possess their own immunity.

The third subchapter, entitled "Research on the cultivation of mesenchymal stem cells, their characterization, inoculation on experimental models and evaluation of engraftment capacity", describes comparatively two methods of cultivation of rat mesenchymal stem cells. After obtaining stable cell cultures, by performing four passages, their characteristics were evaluated, such as: adhesion to plastic, initially fibroblastiform, then stellate morphology. To demonstrate the cartilaginous regenerative potential of mesenchymal stem cells used as an allo-graft, we created a cartilaginous lesion on the femoral trochlea in rats, where we instilled stem cells, evaluating the cartilaginous regenerative potential through histopathological examination.

Also, another aim of the study was to identify the presence or the absence of cell differentiation markers specific to rat mesenchymal stem cells, an aspect highlighted by immunohistochemistry.

Chapter VIII, "RESEARCH ON AUTOLOGOUS THERAPY WITH MESENCHIMAL STEM CELLS OF ADIPOSE ORIGIN IN DOGS" represents the clinical summary of the cases in which the auto-transplantation of mesenchymal stem cells was part of the treatment protocol for osteoarthritis. The study took place over a period of three years and 10 months at a private pet clinic in the UK. In the present study, we described the adipose tissue harvesting technique as well as the inoculation technique of the obtained cell suspension. We also dynamically followed the way in which the degree of lameness improved, through clinical examination correlated with the observations of the owners. This protocol has previously been described in the UK specialist literature, but we have not found any equivalent in Romania.

Based on this study, we stated conclusions regarding the average age of patients affected by osteoarthritis, which in this study was 6.15 years, the youngest patient suffering from osteoarthritis being 3 years old, and the oldest 9 years old, the distribution on sexes: in the present study, the percentage of affected males (62.5%) being higher than that of females (37.5%), which are the most frequently affected joints, namely the stifle joint (50%), followed by the elbow joint (37.5%) and the clinical effectiveness of this treatment: in 50% of the patients studied, autologous adipose stem cell therapy resulted in lameness resolution 2 months after administration, and in the other cases a significant decrease in the degree of lameness was recorded.

For chapter IX, entitled "RESEARCH ON THE USE OF PLATELET-ENRICHED PLASMA IN VETERINARY MEDICINE", the study was carried out over a period of approximately 6 years, respectively October 2018-September 2024, the analyzed subjects being patients from two small animal practices in the UK: Black Sheep Vets in Louth, Lincolnshire and Fivelands Veterinary Center in Birmingham.

In this study, two methods of obtaining platelet-rich plasma are described: the V-PET system (PALL Life Sciences) and the PRP-HA system (cell therapy sciences), which are licensed for use in dogs. The V-PET system separates the platelet-rich plasma by filtering the blood using gravity and a special filter, while the PRP-HA system, a formula that additionally has hyaluronic acid is based on centrifugation.

In the patients described in this study, the males were predominantly affected, in a percentage of 57.14%, the results obtained being different from the specialized literature, where females were described as being more frequently affected by osteoarthritis. The most commonly affected breed was the Labrador Retriever, at 21.42%, followed by the Golden Retriever and Shih Tzu, both at 14.28%. All three breeds are popular in the UK, but also frequently affected by osteoarthritis. The improvement in the degree of lameness varied between patients, in 64.28% of them the degree of lameness was 0 at 2 months after administration, while in 35.72% of patients the lameness was reduced to grade 1 (normal gait with slight lameness when trotting).

Chapter X, entitled "General conclusions" presents the most important conclusions that have been highlighted following the research carried out, as well as suggestions and recommendations for veterinarians interested in providing high standards of treatment to patients suffering from osteoarthritis, but also for doctors who are passionate about the laboratory and the study of mesenchymal stem cells.