

Fetal membranes stem cells application in pets

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ABSTRACT

Background: Stem cells are precursor cells that have the capacity for self-renewal and could generate cells with characteristics similar to cells and differentiation, generating varied cell lines. Considering the plasticity of cells can be classified into totipotent, pluripotent or multipotent. According to the isolation period, the stem cells can be classified as embryonic, fetal and adult. In the embryo stage are considered totipotent because they can rebuild any tissue in the body and adulthood are considered multipotent, since they have a more limited plasticity. The fetal tissues and the fetus is a potential source for stem cells, since they expand more rapidly compared to the cells after birth. Stem cells of fetal membranes are derived from extra-embryonic tissues with high capacity to differentiate into various tissues. The cord blood stem cells have mesenchymal and hematopoietic, and mesenchymal cells have the potential to proliferate and differentiate into multiple cell lineages. The yolk sac in dogs is morphologically composed of three layers: a single layer of endoderm, a simple mesothelium, and intermediate to them, the vascular mesenchyme. Work identified a population of pluripotent cells in the yolk sac can differentiate into hematopoietic cells, however, can be isolated mesenchymal stem cells. In this review we aim to focus new isolations of cells from umbilical cord blood and yolk sac of dogs, reviewing the main literature on this species. The importance of using dogs out of work has intensified in recent years, since many diseases can manifest itself in a similar way to humans. Additionally, the dog is a pet, and interest in the treatment of diseases and improved quality of life of this species has been accentuated in veterinary medicine. Thus, identifying the cellular sources in the dog opens new horizons for preclinical studies and new therapies for veterinary medicine.

Review: This study is related to morphological biology multipotent stem cells, focusing its expansion and use in cell therapy in animal models that have different pathologies. A widely studied model for muscular dystrophy is the GRMD (Golden Retriever Muscular Dystrophy), which is homologous to DMD (Duchenne Muscular Dystrophy) that affects humans. It is a recessive genetic disease, X chromosome which affects approximately 1 in every 3500 boys. It is characterized by a progressive muscle degeneration, resulting from the absence or reduction in the production of dystrophin protein present in the sarcoplasmic membrane of muscle fibers.

Conclusion: The use of cells derived from fetal tissue are strong candidates for veterinary regenerative medicine, since they have high capacity for cellular differentiation. The use of fetuses and fetal tissues of humans still has limitations, so the dog is a viable alternative for studies of fetal stem cells. Thus, it is extremely important to know the characteristics of morphology and proliferation of cells derived from fetuses and fetal annexes canines, including yolk sac and umbilical cord as well as know the feasibility of clinical application of these cells in preclinical testing in animal models and eventually in human medicine, thus contributing to regenerative medicine.

Keywords: stem cells, fetal membranes, dogs, therapy.

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I. INTRODUCTION

II. MATERIALS AND METHODS

III. STEM CELL POTENTIAL AND THEIR APPLICATIONS

I. INTRODUCTION

Transplantation of hematopoietic stem cells derived from umbilical cord has been widely used in various types of pathologies, including metabolic disorders, acquired immunodeficiencies, and hematological disorders [1].

Ontogeny, hematopoiesis begins in the ventral aorta and the fetal yolk sac, and a second time they reach the bone marrow at the end of the second trimester. It is known that stem cells from umbilical cord blood contains hematopoietic progenitor cells in large numbers [1]. Already, some authors stated that the number of nucleated cells and progenitor cells in cord blood is lower than expected [2] showed that these umbilical cord cells have a greater capacity for expansion compared to the bone marrow in a particular medium. Several researchers said that the CD34+ cells from cord blood have a greater proliferative potential and require different growth factors of those CD34+ bone marrow [1]. The number of Colony Forming Units related to granulocytes, erythrocytes, monocytes and megakaryocytes (CFU - GEMM) as well as the proliferative capacity of these cells appear to be reasonably high in cord blood of newborns compared to peripheral blood of adults [10].

Studies indicate that progenitor cells derived from umbilical cord blood that express the membrane antigen CD34+ does not have the same phenotypic features of cells extracted from bone marrow of adults, and also indicate that the CD34+ cells extracted from umbilical cord blood have a high proliferative capacity [1]. But these authors also claim that little has been studied on the morphofunctional characteristics of these cells.

Some authors state that the amount of these progenitor cells derived from umbilical cord blood is insufficient for a successful transplant, and suggest the holding of an expansion of these cells “*in vitro*” in a culture medium with specific cytokines [2]. This process aims to increase the number of hematopoietic

progenitor cells, reducing the time of cell reconstitution after transplantation. Spherical clusters of cells expressing several markers of HSCs were observed inside the major arteries within the aortic ganglia (AGM) and extra-embryonic (vitelline and umbilical) in mouse embryos [4,5,13] and human [7,15,16], and it is believed that stem cells represent the definitive hematopoietic lineages. Definitive HSCs are also formed in the yolk sac. Although it was concluded in recent studies that correlated the hematopoiesis of the yolk sac to the AGM that definitive HSCs formed in the yolk sac only contribute to primitive hematopoiesis [12], and these cells have a definitive hematopoietic potential.

Cells isolated from the yolk sac at day 9 may be grafted, and have the ability to repopulate the recipient animals after transplantation, the liver of newborn mice [19].

The correlation of morphological synthesis, uptake, transport and erythropoiesis are found in the yolk sac of the dog at the end of pregnancy and decrease very close to delivery. In the developing embryo, hematopoiesis and early vascular structure are identified as blood islands of the yolk sac. Blood islands are formed of mesoderm aggregates that have migrated in the early training [9].

The outer cells are differentiated into endothelial cells and the internal, primitive blood. The termination of the association between developmental hematopoiesis and endothelial cells suggests that they depart from a common progenitor, the hemangioblasts [4] and also shown in dogs by our group [9].

Research focused on the canine model and its tissues, to describe new sources of stem cells and innovative use in therapy for treating genetic and acquired diseases were evaluated extensively by our laboratory, focusing so thorough and appropriate description of new sources of stem cells canine, as well as new treatments for diseases in veterinary medicine and to extrapolate future called translational medicine [6,8,17].

II. MATERIALS AND METHODS

For the extraction and analysis of morphological stem cells were used 20 newborn

animals affected by muscular dystrophy or normal GRMD obtained from the kennel (Golden Retriever Muscular Dystrophy - Brazil, Department of Surgery, Faculty of Veterinary Medicine, University of São Paulo).

The newborn animals are monitored, and soon after birth proceeded to collect the cord blood, by puncturing the umbilical vein. In this act, the samples were divided into two aliquots, one was placed in vacountainers 9 mL EDTA, and the second in eppendorfs, with the same anticoagulant solution for genotyping of newborn dogs and quantification of the amounts of the enzyme creatine kinase (CK).

Samples collected before proceeding to the separation of “pellet” of white blood cells, blood smears were made on slides and stained for cell overview and estimate the percentage of populations of white blood cells (trypan blue). In addition, the cells were analyzed by flow cytometry to quantify apoptosis and percentage of distinct populations of progenitor cells with different surface epitopes of the CD lines. Fragments of the placenta, the yolk sac and amnion were processed for cell culture. The culture of these cell lines was performed at 37°C and 5% CO₂, with renewal of medium every 48 or 72 h, and tested different culture media to obtain the best solution for development, growth, maintenance and expansion these cells. Test curve of cell growth and differentiation were performed, and morphological analysis of the general cultivation.

III. STEM CELL POTENTIAL AND THEIR APPLICATIONS

The results so far have been very satisfactory, showing that these cellular sources can generate important information in the field of cellular and gene therapy with mesenchymal stem cells.

The immunocytochemistry was performed using the protein vimentin, a cytoplasmic protein of the cytoskeleton that supports cellular organelles, and is present in large quantities in mesenchymal cells [11,14]. Despite the positive labeling will require further experiments with other markers of stem cells, since vimentin can also be found in high amounts in tumor cells [18].

The structure of the yolk sac was marked by vimentin and demarcated the potential of their blood islets (Figure 1A) and its products, in case the hemangioblasts. Further details of this cell type was described by our group [17], and its potential for tissue production of classical mesenchymal lineages (Figure 1B) or hematopoietic lineages.

The growth curve of cells from amniotic membrane showed an initial drop in the number of cells, stabilizing that amount in subsequent counts. As the count is done every 72h, it is possible that this is not the appropriate time for new trypsinization of cells, many do not resist the process and die. This could explain why that number is apparently stabilized, as if there were no cell multiplication. During the daily observation of the optical microscope used for counting cells we noticed that many cells are killed in the days following trypsinization. The placenta was difficult as canine cell culture conditions and maintains (Figure 1C), however, the canine tissues and their cell culture and this process seems to be more fragile. However, it was possible to establish this simple line amniotic membrane (Figure 1D), with unique characteristics and its potential (Figure 1E-F).

The use of these cells from fetal tissue is strong candidates for new possibilities for regenerative therapy in veterinary medicine.

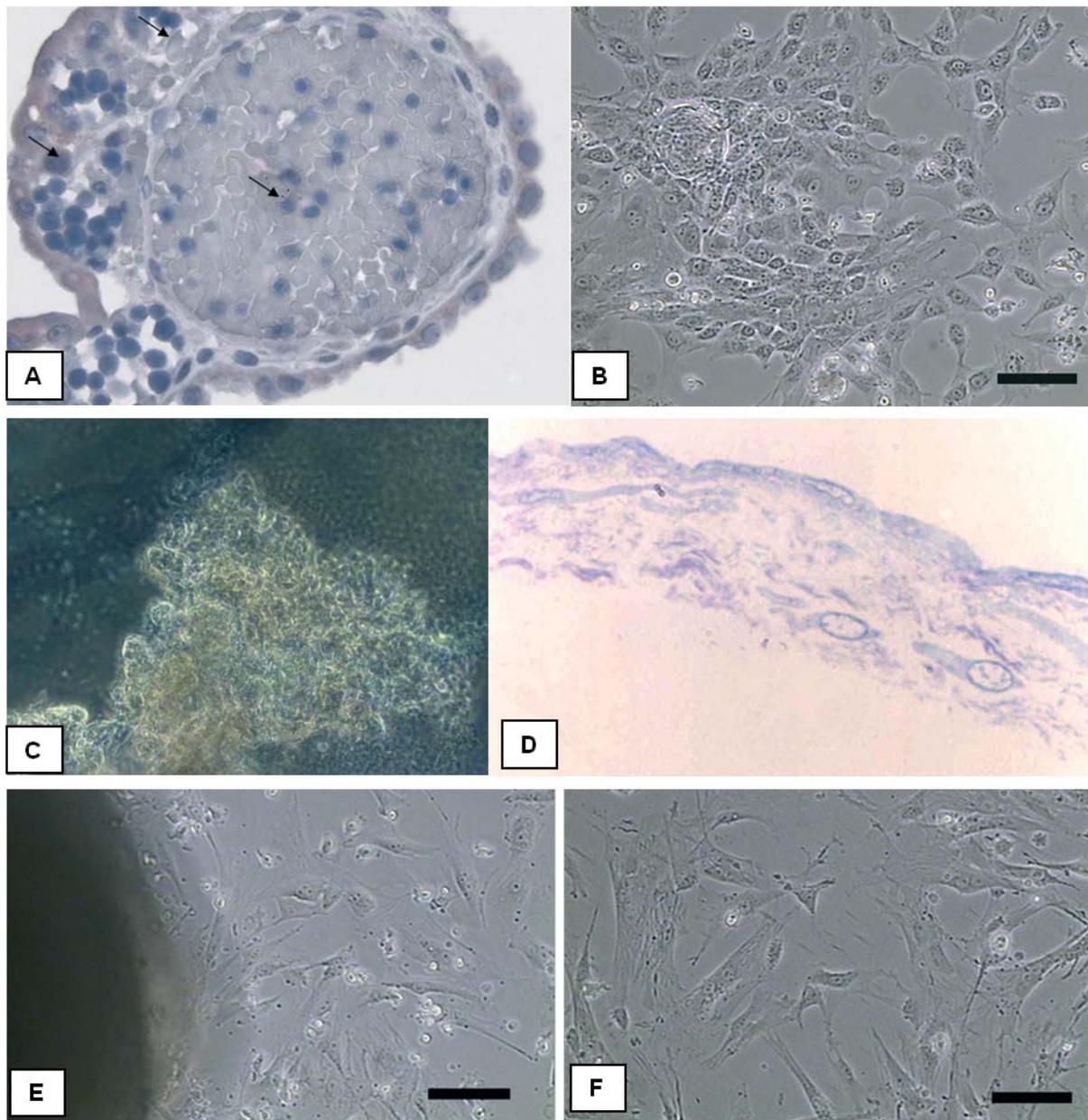


Figure 1. A: Photomicrography of canine yolk sac at middle gestation. Vimentin stain showing the mesenchymal progenitor cells at epithelium layer. Arrows indicate normal erythrocytes inside blood island of this tissue. B: Typical stem cells from canine yolk sac. C: Placental cell culture at term pregnancy canine placenta. D: Semi thin section of dog amnion showing simple layers of tissue. E-F: Mesenchymal stem cell from canine amnion.

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