http://dx.doi.org/10.4322/rbcv.2014.374

Stem cells derived from bone marrow and preclinical trials in Veterinary Medicine*

Células-tronco derivadas da medula óssea e suas aplicações na Medicina Veterinária

Vanessa Cristina de Oliveira,** Aline Fernanda de Souza,** Fabio Sergio Cury,** Felipe Perecin,***
Daniele dos Santos Martins,*** Carlos Eduardo Ambrósio***

Abstract

The bone marrow is the largest reserve of stem cells into the body, consisting of stromal cells/mesenchymal niches and hematopoietic system. These cells can differentiate as different lineages as osteogenic, chondrogenic and adipogenic. The bone marrow stem cells population has widely used and an attractive target for potential therapeutic treatment for preclinical trials, due its high plasticity of differentiation. Thus, with this review we aimed to show an overview of stem cells and mainly bone marrow cells with basic concepts, as well as their potential new venues for treatment in regenerative veterinary medicine.

Keywords: bone marrow, stem cell, plasticity and application.

Resumo

A medula óssea é a maior reserva de células-tronco do corpo, cujo consistem em células mesenquimais e hematopoiéticas. Estas células têm a capacidade de diferenciar-se em varias linhagens, como osteogênicas, condrogênicas e adipogênicas. A população de células-tronco derivadas da medula óssea tem sido um alvo atraente para tratamentos terapêuticos e ensaios pré-clínicos, devido sua elevada plasticidade e capacidade de diferenciação. Assim, esta revisão objetiva mostrar uma visão geral das células-tronco e principalmente conceitos básicos sobre as células da medula óssea, bem como os seus potenciais e novos espaços para o tratamento regenerativo na medicina veterinária.

Palavras-chave: medula óssea, células-tronco, plasticidade e aplicação.

Introduction

In recent decades the knowledge about stem cells has evolved significantly, particularly on its expandability capacity, differentiation and self-regeneration. These properties make the stem cells unique as a potential tool in the treatment of diseases that can not be treated in the traditional way (Pranke, 2004).

The blood progenitor cells were the first stem cells to be characterized with success, these cells were characterized as “fibroblastics stem cells” able to form colonies with high capacity for replication. From the 60’s it was obtained the information that adult organisms have the self-regeneration ability in some tissues such as skin, intestinal epithelium and mainly blood cells that are constantly renewed and destroyed (Friedenstein et al., 1974). The bone marrow cells are very studied for application in regenerative veterinary medicine because of their diversity, cellular plasticity and low tumorigenesis. Recent studies demonstrate that bone marrow cells not only reconstitute blood cells, but also contribute in muscle, brain, liver, heart and vascular endothelium formation. The isolation, quantification and expansion of these cells allow that cell therapy can be used to try success in treatments like pathologies that affect humans and animals (Barker et al. 2003).

Thus, through this review we propose to elucidate and describe adult bone marrow stem cells, limiting the study in mesenchymal and hematopoietic lineages showing their applicability in veterinary medicine.

Stem cells

In general, stem cells are defined as cells that have the ability to differentiation and self-renew by indefinite periods during the life of an individual and when this cells are in appropriate conditions and in a microenvironment with correct signs, it can differentiate into several lineages with characteristics and specialized functions such as myocytes, neurons and hepatocytes (Kerkis et al, 2001;. Verfaillie et al, 2002;. McBeath et al, 2004;. Pardo, 2005; Semb, 2005, Wang et al, 2005;. Arce et al. 2007; Brevini et al, 2007;. Kuijk et al, 2010).

Stem cells can be classified according its plasticity, being necessary to know which embryonic tissue these different cells are originated. Therefore we can classify them into: totipotent, pluripotent, multipotent and unipotent cells. The totipotent term refers to the potential to generate a fully functional organism, or correspond to a complete embryo that is able to originate embryonic cells and extra-embryonic tissues. Pluripotent stem

*Recebido em 13 de junho de 2014 e aceito em 30 de setembro de 2014.
**Department of Surgery, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, Brazil.
***Department of Veterinary Medicine, Faculty of Animal Sciences and Food Engineering - University of São Paulo, Pirassununga/SP, Brazil. Correspondence to: ceambrosio@usp.br (C. E Ambrósio).

cells have the ability to originate progenitors cells forming any embryonic germ layers: mesoderm, endoderm and ectoderm. Because of these cells lineage, its type is very used in research for therapeutic purposes, medical and commercial applications (Martin, 1981; Evans et al, 2003; Wang et al, 2005; Arce et al, 2007; Brevini et al, 2007;. Yu et al, 2008;. Kuijk et al, 2010). The multipotent stem cells are present in adults, which have limited potential because of their origin that can give only one of the three germ layers to the precursors listed: for example, stem cells that give rise to tissues derived exclusively as pancreatic endoderm cells or lung tissue (Brown, 2005; Wang et al, 2005;. Arce et al, 2007). Finally the category that corresponds the unipotent stem cells, represent the cells that just can generate daughter cells that differentiate along a single cell line, or only produce one mature cell type (Kuijk et al., 2010).

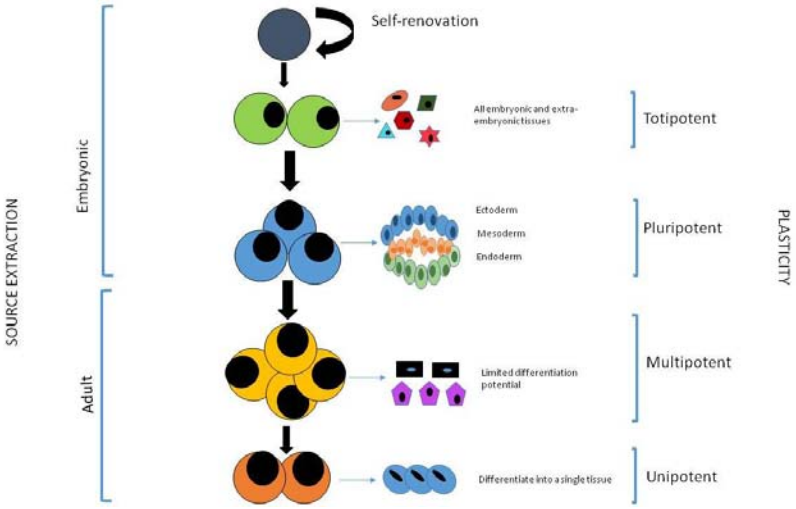

Figure 1: Schematic drawing showing the ability of differentiation and self-renewal of stem cells. It can be classified in two forms - first according to cell plasticity: totipotent, pluripotent, multipotent and unipotent. Second, according to source extraction: embryonic (Totipotent and Pluripotent) and adult (Multipotent and Unipotent).

The origin of the stem cells extraction can be obtained from two tissues: embryonic stem cells (ES cells or ESCs) and adult stem cells (ASCs). Thus, adult stem cells were the first to be well characterized, from the 60s decade, when studies demonstrated that adult organisms have the ability to self-regeneration of some tissues such as the skin, intestinal epithelium and mainly blood cells, where its cells are constantly destroyed and renewed, however, this can decrease its capacity to differentiate (Friedenstein et al, 1974; Orlic et al, 2001; Verfaillie et al, 2002; Wagers & Weissman, 2004; Arce et al, 2007). Embryonic stem cells are classified as totipotent or pluripotent due their ability to differentiate into other cellular tissues being possible obtain it since the earliest stages of embryo formation, when the fertilized egg is a compact sphere or morula (Odorico et al., 2001; Smith, 2001; Verfaillie et al, 2002; Arce et al, 2007).

According to the lineage, embryonic stem cells are derived from the blastocyst and germ embryonic stem cells after blastocyst implantation, being similar in many aspects. Both are able to replicate and divide in cultures showing no chromosomal alterations, and express a number of markers characteristic of totipotent progenitors that facilitate their identification. The only difference is that embryonic germ cells have their potential more limited than embryonic stem cells, being in advanced stage (Odorico et al, 2001;. Saito et al, 2002;. Verfaillie, 2002; Semb, 2005; Arce et al, 2007).

One of the advantages of using embryonic stem cells in research, treatments and therapies is the ability to proliferate indefinitely, being able to generate a great variety of cell groups when stimulated by mechanisms that control the differentiation, allowing a wide manipulation in vitro (Verfaillie et al, 2002;. Arce et al, 2007). However, these cells are highly undifferentiated, so it is possible that it induce the formation of certain tumors as teratomas, besides several ethical implications correlated in its use, that is a very important point to be considered (Pear et al, 2000;. Doss et al, 2004).

Bone marrow

In adults, the bone marrow is the largest reserve of stem cells, containing a heterogeneous mix of different cell types, as well as fibroblast, reticular cells and an osteoprogenitor and multipotent mixed cells (Carlson 2009). When the bone marrow cells are cultured, it is possible observe different characteristic as non-adherent and adherent cells. The non-adherent cells will originate the hematopoietic cells (HSCs), a variety of progenitors blood cells and cells of the immune system comprising the lymphoid system, erythroid and myeloid (Damme et al. 2002). The adherent cells are stromal cells that have the ability to differentiate (in vitro) into cartilage, bone, and adipocytes cells (Bianco et al. 2001).

Commonly the bone marrow cells and mesenchymal stem cells (MSCs) are often described like the same kind of cells because the similarity of both populations is in the endosteum surface of trabecular bone and the periosteum. The endosteum and MSCs invade the medullar cavity of the periosteum during fetal development, when the cartilage of the endochondral bone is destroyed and vascularized. Thus, the bone marrow MSCs differentiate into osteoblasts, reticular cells, and adipocytes, being able to renew the maintenance of a population of MSCs (Stocum, 2001).

Another kind of adherent cells from bone marrow stromal cells are multipotent adult progenitor cells (MAPCs) or mesodermal progenitor cell (MPCs). These cells are obtained from the mononuclear cells fraction of bone marrow by immuno-magnetic depletion of hematopoietic cells (Reyes et al. 2001). MAPCs have the high proliferative capacity and are able to differentiate into distinct mesenchymal lineages, as well as in endothelial, epithelial and in blood cells (Blau et al. 2001).

Thus, through of many purification methods, it is possible to obtain distinct cell populations with different and multiple lines that have greater plasticity and therapeutic potential.

Bone marrow cells: Hematopoietic and Mesenchymal

As previously mentioned above, the bone marrow cells have numerous cell lines and among these lines we have the mesenchymal and hematopoietic cells.

Among the cellular variability found in bone marrow, the hematopoietic cells are the better characterized. Hematopoietic cells were first described by Friedenstein et al. (1976), which characterized these cells as fibroblast stem cells capable to form colonies. These cells have fibroblastoid morphology with high replication capacity and after appropriate stimulus can differentiate into all cell types (Largeault, 2004).

Hematopoietic cells reside in the bone marrow of adult mammals (typically from 0.01% to 0.05%), and may also circulate in the peripheral blood, where its concentration is even lower (~ 0,001 %) (Abdelhay et al. 2009).

Thus, the prior characterization has shown that hematopoietic cells are capable of regenerating tissues and may be a promising source for cell therapy. The first regenerative veterinary medicine studies were performed by Till & Mcculloch in 1961, and showed the reconstitution of the hematopoietic system of lethally irradiated mice.

Hematopoietic bone marrow cells can be isolated from semisolid systems cultures with methylcellulose that contain many growth factors that determine different types of hematopoietic progenitors cells through the colony formation (Dexter et al. 1977).

The first animal bone marrow research, done in dogs, showed the presence of hematopoietic stem cells, which both have the same morphological and immunophenotypic features (Bruno et al, 1999; Bruno et al, 2001). This discovery allowed an amplification in canine therapy, being an important specie model for providing pre–clinical information to humans because of its high homology physiological (Nakage & Santana , 2006).

The mesenchymal stem cells were described by Friendenstein (1970) as fibroblast precursors of bone marrow. After this, Caplan (1991) and Colter (2000) purified and characterized the bone marrow cells, resulting in mesenchymal lineages of cells. This kind of cell is considered multipotent, because it has the capacity to propagate and differentiate into multiple lineages of osteocytes, chondrocytes, adipocytes and neurogenic (Romanov, 2003; Bydlowski et al, 2009).

The mesenchymal stem cells have high plasticity and ability to originate mesodermal and not mesodermal tissues, has also immunoregulation and immunosuppressive characteristics that expand the possibilities for therapeutic use. MSCs also secrete a variety of pro and anti-inflammatory besides the growth factors, through these bioactive molecules, provide inflammatory response modulation, restoration of vascular supply and adequate tissue repair, contributing to tissue homeostasis and immunological under physiological conditions. These cells can induce others cells (present in the tissue) to secrete others soluble factors that stimulate the differentiation of undifferentiated cells, favoring the repair process (Miller et al, 2010; Bydlowski et al, 2009).

The mesenchymal bone marrow cells are located in the stromal fraction, these stromal cells provide support for the cells growth and differentiation. Constitute a very small population in fresh adult bone marrow, being only about 0.01 % to 0.0001 % of nucleated cells (Bydlowskil et al., 2009).

The bone marrow cells are adherent to plastic and have heterogeneous characteristics, similar to fibroblast morphology. These cells are able to express undifferentiated markers such as OCT4, NANOG and SSAE, although their differentiation capacity have more limitation, have the capacity of propagating in culture and are not immunogenic, so can be used in allogeneic transplants (Bydlowskil et al ., 2009).

Applications and clinical implications of bone marrow cells in veterinary medicine

The use of stem cells (clinically and experimentally) is very common in veterinary medicine, in therapeutic applications, mainly in horses and dogs in musculoskeletal disorders treatment. It is also used in new assisted reproductive technologies for spermatogonial stem cells application to preserve endangered animals species, it can also generate transgenic animals for pharmaceutical and biomedical models production (Fortier & Travis, 2011).

Bjornson et al. (1999) demonstrated that bone marrow transplantation, marked genetically in immunodeficient mice, showed that steam cells derived from bone marrow migrate to induced muscle degeneration areas, submitted in myogenic differentiation and participating in damaged fibers regeneration. Genetically modified and derived from bone marrow, the myogenic progenitors could potentially be used to target therapeutic genes in the muscle tissue, providing an alternative strategy for the muscular dystrophies treatment.

Aiming to isolate, identify and analyze dogs MSCs capacity of differentiation, Tharasanit et al. (2011), removed bone marrow aspirates from four dogs and cultured MSCs to examine two cell lines for CD34, CD44 and CD90 expression by flow cytometry. In vitro, MSCs differentiation from mesodermal lineages (bone, cartilage and adipose tissues) and ectodermal (neurons) was made by osteogenic, chondrogenic, adipogenic and neurogenic media techniques respectively. Based on histological and genomic expressions, these cells demonstrated the ability to differentiate into bone, cartilage and adipose tissue. The conclusion was that MSCs can be isolated from dog's bone marrow and have the differentiation ability in vitro in specific mesodermal lineages.

Feitosa et al. (2010) examined the sheep bone marrow mesenchymal stem cells effects and human dental pulp in sheep with induced osteonecrosis in the femoral head, after the transplant the mesenchymal cells injected into the femoral head, it was concluded that these cells were viable in the transplantation of a new place, have proliferated in a short time and apparently favors bone region of injured tissues.

Kim et al. (2013) evaluated if the mesenchymal bone marrow cells allogeneic transplant could accelerate the cure of skin wounds and relieve inflammation, it was used Beagle dogs with skin wounds, and applied mesenchymal cells. They observed that the treated wounds with mesenchymal cells showed a faster lock, increase of collagen, increased cell proliferation and a reduction in post-inflammatory cytokines.

Olsson (2009) did a tendon lesion treatment using hydrolyzed collagen implant and as lyophilized with MEC, embedded or not with bone marrow mononuclear cells marked with nano crystal in Achilles common tendon of dogs experimental surgical lesion. Their results showed that mononuclear bone marrow cells did not influence the quality of the tendon tissue regenerative process in the period up to 30 days post-surgical evaluation, and proposed new studies about the effect of the application of mononuclear cells for a longer time reviewed with the same extracellular matrix presented.

Barros et al. (2001) studied the bone marrow percutaneous autografting in bone repair of segmental defects produced on Radio in New Zealand rabbits procedure, the percutaneous grafting was taken five days after the induced faults, whereas in group I, each animal received 1.0 ml of whole bone marrow in a radius (treatment), immediately after the iliac crest suction. In group II, after centrifugation and aspiration of bone marrow (2.0 ml), 1.0 ml of the sediment was injected in one radius (treatment). Radiographic evaluation conducted every seven days for five weeks showed that bone marrow percutaneous grafting resulted in early radiopacity in the bone defect region in both groups compared with the control, but this bone repair process in rabbits is still an early phenomenon and its benefits needs to be analyzed with greater intensity in the first and second week of grafting.

Chang (2013) evaluated mice bone marrow cells and found that these cells contribute to revascularization and improved in several ischemic tissues and these cells migrate to damaged sites in the eye. He also reports that these cells hold promise for the treatment of retinal diseases.

Pinto (2009) examined the cerebral malaria treatment effect with bone marrow cells in mice infected with Plasmodium berghei. The experiment consisted in administering small fraction of femur and tibia bone marrow infused, after its purification in the ophthalmic plexus of mice. The results showed that bone marrow cells injection had no effect on animals parasitemia, but was capable to extend their survival.

Alves-de-Morais et al. (2013) investigated if bone marrow cells therapy would be able to modify the behavioral effects of electrolytic lesion of the dorsal hippocampus in mice, and if these cells also have an effect in nervous tissue damage correction. The behavioral results showed that transplantation of mononuclear bone marrow cells in animals with hippocampus damage, caused changes in exploratory pattern, causing a disinhibition, but there was no regeneration of injured tissues.

In cardiology Branco et al. (2009) evaluated the intrapericardial infusion technique of bone marrow mononuclear cells in pigs induced with myocardial infarction, the results showed that infarcted animals showed different cellular distribution in cardiac tissue. The cell concentration was greater acute myocardial infarction places, suggesting that these cells can interact increasing cellular migration and survival of the injured myocardium cells.

Alexander et al. (2009) evaluated the negative cell lines impact obtained from mice bone marrow in a model of subtotal renal ablation. The results were that the infusion of these cells attenuated all markers of renal injury in a model of early disease.

Zhou et al. (2013) evaluated β-TCP (tricalcium phosphate) combined with stromal cells from autologous bone marrow to use in canine orbital wall repair. Defects measuring 10 mm in diameter were created in the medial orbital walls in 12 dogs, the results showed that the β-TCP with bone marrow stromal cells combination have been successfully to restore bone functionality, which can perform the orbital wall repair and bone regeneration.

Kaufmann (2008) studied the mice adult bone marrow stem cells influence, in erectile function in animals with cavernous nerve lesion, the results proved that seeded cells application in silicone tube promote the cavernous nerve regeneration and promote the reestablishment of erectile function.

Ribeiro (2009) studied the bone repair in dehiscence defects around treated implants using autogenous bone marrow cells, isolated or in combination with the bone regeneration technique guided in dogs. Bone marrow was obtained from the iliac crest of eight adult male beagle dogs. Derived bone marrow cells (MCs) were isolated, cultured in vitro and phenotypically characterized with respect to their osteogenic properties. The results point (within the limits of this study), that bone marrow cells used isolated promoted good results in relation to bone formation within the implant threads, but its associated with guided bone regeneration (GBR) did not promoted additional benefits on bone formation in peri-implant dehiscence defects.

In a study to improve haploidentical stem cell transplantation protocols, Zorn et al. (2011) using canine models, transfused peripheral blood CD6 stem cells with depletion 6 days post not modified bone marrow transplantation in an attempt to acquire immunological tolerance. The experiment showed a significant improvement in graft and chimerism, despite improvements in clinical applications are still being discussed.

Nakamuta (2008) evaluated the cardiac retention efficacy of transplanted bone marrow cells according the time after infarction, the results showed that the animals that bone marrow cells was established by intramyocardium 24h after ischemia in cardiac perfusion with or without fibrin had a significant improvement in cardiac performance.

Zhang W at al. (2013) compared the osteogenic potential of stem cells from adipose tissue and bone marrow stem cells to evaluate which one would be more efficient in the maxillary sinus floor raising of dogs, and evaluate which would help in faster bone formation. The results of fluorescence labeling for canine sinus showed that bone marrow stem cells had more efficiency in bone regeneration and faster process. Concluding a greatest utility of bone marrow stem cells compared with adipose tissue stem cells in the maxillary sinus increase with implant placement.

Table 1: Researches of Bone marrow cells applications in veterinary medicine

Author	Year	Specie	Result
Bjornson, et al	1999	Mice	Positive
Barros, et al	2001	Rabbits	In study
Kaufmann	2008	Mice	Positive
Nakamuta	2008	Rat	Positive
Olsson	2009	Dogs	Negative
Pinto	2009	Mice	Negative
Branco, et al	2009	Pigs	Positive
Alexander, et al	2009	Mice	Positive
Ribeiro	2009	Dogs	Negative
Feitosa, et al	2010	Sheep	Positive
Tharasanit, et al	2011	Dogs	Positive
Zorn, et al	2011	Dogs	In study
Kim, et al	2013	Dogs	Positive
Chang	2013	Mice	In study
Morais, et al	2013	Mice	Negative
Zhou, et al	2013	Dogs	Positive
Zhang, et al	2013	Dogs	Positive

Concluding remarks

We can conclude through this literature review that bone marrow is a promising source of stem cells used in the treatment of numerous hematological diseases (cell therapy), but there are still some difficulties to be faced in relation to manipulation, differentiation and application of this cells. However this kind of therapy has been improving more and more, through the researches and applications with experiments in animals and humans, creating future expectations in solving several diseases.

Refer

ABDELHAY, E.S.F.W.; BRAGA-PARAGUAÇU, F. H.; BINATO, R.; BOUZAS L.F.S. Células-tronco de origem hematopoética: expansão e perspectivas de uso terapêutico. *Revista Brasileira de Hematologia e Hemoterapia*. v. 31, p. 2-8, 2009.

ABE, S.; BOYER, C.; LIU, X.; WEN, F.Q.; KOBAYASHI, T.; FANG, Q.; WANG, X.; HASHIMOTO, M.; SHARP, J.G.; RENNARD, S.I. 2004. Cells derived from the circulation contribute to the repair of lung injury. *American Journal of Respiratory and Critical Care Medicine*.v. 170, n.11, p.1158-1163, 2004.

ALEXANDRE, C.S.; VOLPINI R, A.; SHIMIZU, M.H.; SANCHES, T.R.; SEMEDO, P.; DI JURA, V.L.; CÂMARA, N.O.; Seguro A. C& Andrade L.Lineage-negative bone marrow cells protect against chronic renal failure. *Stem Cells.* v. 7, n. 3, p. 682-692, 2009.

ALVES-DE-MORAES, L.B.C.; RIBEIRO-PAES, J.T.; LONGO, B.M.; FERRAZOLI, E.G.; ANDRADE, T.G.C.S. Effect of the bone marrow cell transplantation on elevated plus- maze performance in hippocampal- injured mice. *Behavior Research Methods* . v. 248, p. 32-40, 2013.

ARCE, S.R.A.; MOSQUEDA, M.L.J.; GAONA, H.V.; MAS, M.C.R.; CORTES, M.A. C.; RIOS, M.A.M. Qué son las células troncales o "células madre. *Veterinária Mexico*. v. 38, p. 81-104, 2007.

BAKSH, D.; SONG, L.; TUAN, R.S. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *Journal of Cellular and Molecular Medicine*. v. 8, n. 3, p. 301-316, 2004.

BARKER, R.J.; BRADLEY, L.C., BRADLEY, R.D.; DRAGOO, J.W.; ENGSTROM, M.D.; HOFFMANN, R.S.; JONES, C.A.,; REID, F.; RICE, D.W.; JONES, C. Revised checklist of North American mammals north of Mexico. *Occasional Papers of the Museum of Texas Tech University*. v. 230, n. 229, p. 1-22, 2003.

BARROS, S.V.S.G.; DEL CARLOS, R.J.; VILORIA, M.I.; CALVÃO, S.R.; FILHO, A.M.; OLIVEIRA, D.R. Auto-Enxerto Percutânea de Medula Óssea II, Reparação de Falhas Segmentares Produzidas no Rádio de Coelhos. *Ciência Rural.* v. 31, n. 4, p. 627-632, 2001.

BIANCO, P.; COSSU, G. Uno, nessuno e centomila: Searching for the identity of mesodermal progenitors. *Experimental Cell Research*. v. 251, n. 2, p. 257- 263, 1999.

BIANCO, P.; RIMINUCCI, M.; GRONTHOS, S.; ROBEY, G.P. Bone Marrow Stromal and Potential Applications. *Stem Cells.* v. 19, n. 3, p. 80-192, 2001.

BJORNSON, C.R.; RIETZE, R.L.; REYNOLDS, B.; MAGLI, M.C.; VESCOVI, A.L. 1999. Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. *Science*.v. 283, n. 5401, p. 534-537, 1999.

BLAU, H.M.; BRAZELTON, T.R.; WEIMANN J.M. The evolving concept of a stem cell: entity or function? *Cell*. v.105,n. 7, p. 829-841, 2001.

BRANCO, E.; FIORETTO, E.T.F.; CABRAL, R.; PALMERA, C.A.S.; GREGORES, G.B.; STOPIGLIA, A.J.; MAIORKA, P.C.; LEMOS, P.A.; CAMPOS, C.; TAKIMURA, C.; RAMIRES, J.A.F.; MIGLINO, M.A. 2009. Myocardial homing after intrapericardial infusion of Bone Marrow Mononuclear Cells. *Arquivo Brasileiro Cardiologia*. v. 93, n. 3, p. 37-40, 2009.

BREVINI, T.A.L.; TOSETTI, V.; CRESTAN, M.; ANTONINI, S.; GANDOLFI, F. Derivation and characterization of pluripotent cell lines from pig embryos of different origins. *Theriogenology*. v. 67, n. 1, p. 54-63, 2007.

BRUNO, B.; NASH, R.A.; WALLACE, P.M.; GASS, M .J.; THOMPSON, J.S.; TORB, R.; MCSWEENEY, P.A. 1999. $CD34^+$ selected bone marrow grafts are radioprotective and establish mixed chimerism in dogs given high dose total body irradiation. *Transplantation*. v. 68, n. 3, p. 338-344.

BRUNO, B.; GOENER, M.A.; NASH, R.A.; STORB, R.; KIEM, H.P.; MCSWEENEY, P.A. Purified canine $CD34^+$Lin- marrow cells transduced with retroviral vectors give rise to long-term multi-lineage hematopoiesis. *Biology and Blood Marrow Transplantation, Baltimore*. v. 7, n. 10, p. 543-551, 2001.

BYDLOWSKIL, S.P.; DEBES, A.A.; MASELLI, L. M.F.; JANZ, F.L. 2009. Características biológicas das células-tronco mesenquimais. *Revista Brasileira de Hematologia e Hemoterapia*, v. 31, p. 25-35.

CAPLAN, A.L. Mesenchymal stem cell. J. Orthopaetic Research. v. 9, p. 641- 650, 1991.

CARLSON, B.M. 2009. Stem Cell Anthology: stem cell biology, tissue engineering cloning, regenerative medicine and biology. 1° ed. Academic Press Elsevier. 424 p.

COLTER, D.C.; CLASS, R.; DIGIROLAMO, C.M.; PROCKOP, D.J. Rapid expansion of recycling stem cell in cultures of plastic – adherent cells from human bone marrow. *Proceedings of the National Academic Sciences of United States of America*. v. 97, n. 7, p. 3213- 3218, 2000.

CHANG, L.K. Bone marrow stem cells in retinal disease. Stem cell Biology and Regenerate Medicine.v. 6, p. 99-105, 2013.

DEXTER, T.M.; ALLEN, T.D.; LAJTHA, L.G. Conditions controlling the proliferation of hematopoietic stem cell in vitro. *Journal of Cellular Physiology.* v. 91, n. 3, p. 335-344, 1977.

DOSS, M.X.; KOEHLER, C.I.; GISSEL, C.; HESCHELER, J.; SACHINIDIS, A. Embryonic stem cells: a promising tool for cell replacement therapy. Journal of Cellular and Molecular Medicine v. 8, n. 4, p. 465-473, 2004.

EVANS, H.; SACH, W.O Prenatal development of domestic and laboratory mammals: growth curves, external features and selected references. 1 ed. Anatomy Histology and Embryology. 1973, 45 p.

FEITOSA, M.L.T.; FADEL, L.; BELTRÃO-BRAGA, P.C.B.; WENCESLAU, C.V.; KERKIS, I.; KERKIS, A.; BIRGEL JUNIOR, E.H.; MARTINS, J.F.M.; MARTINS, D.S.; MIGLINO, M.A.; AMBRÓSIO, C.E. Successful transplant of mesenchymal stem cells in induced osteonecrosis of the ovine femoral head: preliminary results. *Acta Cirurgica Brasileira*.v. 25, n. 5, p. 416-422, 2010.

FORTIER, L.A.; TRAVIS, A.J. Stem cells in veterinary medicine. *Stem Cell Research & Therapy*. v. 2, n. 1, p. 2-6, 2011.

FRIEDENSTEIN, A.J.; GORSKAJA, J.F.; KALAJINA, N.N. Fibroblast precursor in normal end irradeated mouse. hematopoietic organs. *Experimental Hematology*. v. 4, n. 5, p. 267-274, 1974.

GOUVEIA, C.H.; SCHULTZ, J.J.; BIANCO, A.C.; BRENT, G.A. Thyroid hormone stimulation of osteocalcin gene expression in ROS 17/2·8 cells is mediated by transcriptional and post-transcriptional mechanisms. *Journal of Endocrinology.* v. 170, n. 3, p. 667-675, 2001.

HWANG, W.S.; RYU, Y.J.; PARK, J.H.; PARK, E.S., LEE, E.G.; KOO, J.M.; JEON H.Y.; LEE, B.C.; KANG, S.K.; KIM, S.J.; AHN, C.; HWANG, J.H.; PARK, K.Y.; CIBELLI, J.B.; MOON, S.Y. Evidence of a Pluripotent Human Embryonic Stem Cell Line Derived from a Cloned Blastocyst. *Science*. v. 303, n. 5664, p. 1669-1674, 2006.

KAUFMANN, O.G. *Avaliação da função eretil após a reconstituição do nervo cavernoso com o uso de células-tronco adultas da medula óssea: estudo experimental em ratos*. 2008. 62 f. Tese (Doutorado)- Programa de Pós-Graduação da Faculdade de Medicina da Universidade de São Paulo, São Paulo, 2008.

KERKIS, A.; SOUKOIAN, M.; KERKIS, I.; MERKEL, C.; MELLO, M.R.B.; PEREIRA, L.V. Células tronco-embrionárias e a geração de modelos animais para doenças genéticas humanas. *Biotecnologia Ciência & Desenvolvimento*. v. 20, p. 20-25, 2001.

KIM, J.K.; LEE, J.H.; LYOO, Y.S.; JUNG, D.I.; PARK, H.M. The effects of topical mesenchymal stem cell transplantation in canine experimental cutaneous wounds. *Veterinary Dermatology*. v.. 24, n. 2, p. 242-253, 2013.

KUIJK, E.W.; LOPES, S.M.C.S.; GEIJSEN, N.; MACKLON, N.; ROELEN, B.A.J. The different shades of mammalian pluripotent stem cells. *Human Reproduction Update*. v. 17, n. 2, p. 254-257, 2010.

LARGEAULT, A.F. Embriões, células-tronco e terapias celulares: questões filosóficas e antropológicas. *Estudos Avançados.* v. 18, n. 51, p. 227-245, 2004.

MARTIN, G.R. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proceed of the Nation Academic of Science of the United States of America*. v. 78, n. 12, p.7634-7638, 1981.

MCBEATH, R.; PIRONE, D.M.; NELSON, C.M.; BHADRIRAJU, K.; CHEN, C.S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Developmental Cell*. v. 6, n. 4, p. 483-495, 2004.

MONTEIRO, B.S.; ARGOLO, N.M.N.; CARLO, R.J.D. Mesenchymal stem cell. *Ciência Rural* v. 40, n.1, p. 238-245, 2010.

NAKAGE, A.P.M.; SANTANA, A.E. Células-tronco hematopoiéticas em cães. *Ciência Rural*. v. 36, n. 1, p. 325-29, 2006.

NAKAMUTA, J.S. Terapia celular para isquemia cardíaca: efeitos da via de administração, do tempo pós-lesão e do uso de bipolimero para rentenção das células e função miorcárdica. 2008. 225 f. Tese (Doutorado) - Programa de Pós-Graduação da Faculdade de Medicina de São Paulo, São Paulo, 2008.

ODORICO, J.S.; KAUFMAN, D.; THOMSON, J. Multilineage differentiation from human embryonic stem cell lines. *Stem Cell*, v. 19, n. 3, p. 193-204, 2001.

OLSSON, D.C. *Transplante de células-tronco com a fração total de células mononucleares autógénas da medula óssea na lesão iatrogênica aguda de tendão calcâneo de cães.* 2009. 106 f. Tese (Doutorado) - Programa de Pós-Graduação em Medicina Veterinária – Universidade Federal de Santa Maria (UFSM), 2009.

ORLIC, D.; KAJSTURA, J.; CHIMENTI, S.; LIMANA, F.; JAKONIUK, I.; QUAINI, F.; GINARD, B.N.; BODINE, D.M.; LERI, A.P. Mobilized bone marrow cells repair the infracted heart, improving function and survival. Proceedings of the National Academy of Sciences of the United States of America, v, 98, n, 18, p. 1344–1349, 2001.

PARDO, V.M.R. Células Madre: Conceptos Generales y Perspectivas de Investigación. *Universitas Scientiarum*, v, 10, n, 1, p. 5-14, 2005.

PERA, M.F.; REUBINOFF, J.; TROUNSON, A. Human Embryonic Stem Cells. *Journal of Cell Science*, v. 113, n. 1, p. 5-10, 2000.

PINTO, H.C.S. *Efeito do tratamento da malária cerebral com células da medula óssea de camundongos infectados pelo Plasmodium berghei* ANKA. 2009. 91 f. Dissertação (Mestrado) - Universidade estadual de Campinas – Instituto de Biologia de Campinas, 2009.

PRANKE, P. A importância de discutir o uso de células-tronco embrionárias para fins terapêuticos. *Ciência e Cultura*, v. 56, n. 3, p. 33-38, 2004.

REYES, M.; LUND, T.; LEUVIK, T.; AGUIAR, D.; KOODIE, L.; VERFAILLIE, C.M. Purification and in vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood Journal*, v. 98, n. 9, p. 2615-2625, 2001.

RIBEIRO, F.V. *Avaliação da regeneração óssea em defeitos peri-implantares de deiscência tratados com uma abordagem combinada associando células derivadas da medula óssea e regeneração óssea guiada*. 2009. 60 f. Tese (Doutorado) - Faculdade de Odontologia de Piracicaba - Universidade Estadual de Campinas, 2009.

ROMANOV, Y.A.; SVINTSITSKAYA, V.A.; SMIRNOV, V.N. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells*, v. 21, n. 1, p.105-110, 2003.

SAITO, S.; UGAI, H.; SAWAI, K.; YAMANATO, Y.; MINAMIHASHI, A.; KUROSAKA, K.; KOBAYASHI, Y.; MURATA, T.; OBATA, Y.; YOKOYAMA, K. Isolation of embryonic stem-like cells from equine blastocysts and their differentiation in vitro. *Letters*, v, 531, n, 3, p. 389-396, 2002.

SEMB, H. Human embryonic stem cells: origin, properties and applications. *Acta Pathologica, microbiológica, immunológica Scandinavica*, v, 113, n, 11, p. 743-750, 2005.

SMITH, A.G. Embryo-derived stem cells: of mice and men. *Annual Review of Cell and Developmental Biology*, v, 17, n, 1, p. 435-462, 2001.

STOCUM, L.D. Stem cells in regenerative biology and medicine. *Wound Repair and Regeneration*, v, 9, n, 6, p. 429- 442, 2001.

THARASANIT, T.; PHUTIKANIT, N.; WANGDEE, C.; SOONTORNVIPART, K.; TANTRAJAK, S.; KAEWAMATAWONG, T.; SUWIMONTEERABUTR, J.; SUPAPHOL, P.; TECHAKUMPHU, M. Differentiation Potentials of Canine Bone Marrow Mesenchymal. *Stem Cells*. Thai J Vet Med, v, 41, n, 1, p. 79-86, 2011.

TILL, J.E.; MCCULLOCH, E. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiation Research*, v, 14, n, 2, p. 213-22, 1961.

VAN DAMME, A.; DRIESSCHE, V.T.; COLLEN, D.; CHUAH, M. Bone Marrow Stromal Cells as Targets for Gene Therapy. *Current Gene Therapy*, v, 2, n, 2, p. 195-209, 2002.

VERFAILLIE, C.M. *Adult stem cells: assessing the case for pluripotency. Trends in Cell Biology*, v, 12, n, 11, p. 502-508, 2002.

VERFAILLIE, C.M.; PERA, A.; LANSDORP, P.M. Stem cells: hype and reality. *Hematology American Society of Hematology Education Program*, v, 2002, n, 1, p. 369-387, 2002.

ZHANG, W.; ZHANG, X.; WANG, S.; XU L.; ZHANG M.; WANG, G.; JIN, Y.; ZHANG, X.; JIANG, X. Comparison of the Use of Adipose Tissue–Derived and Bone Marrow–Derived Stem Cells for Rapid Bone Regeneration. *Journal of Dental Research*, v, 92, n, 12, p. 1136-1141, 2013.

ZHOU, H.; DENG, Y.; BI, X.; XIAO, C.; WANG, Y.; SUN, J.; FAN, X. Orbital wall repair in canines with beta-tricalcium phosphate and induced bone marrow stromal cells. *Journal of Biomedical Materials Research B Applied Biomaterials*, v, 101, n, 8, p. 1-9, 2013.

ZORN, J.; SCHWAMBERGER, S.; PANZER, W.; ADLER, H.; KOLB, H.J. Transplantation of CD6-depleted peripheral blood stem cells after DLA-haploidentical bone marrow transplantation contributes to engraftment and tolerance in a preclinical model of stem cell transplantation. *Veterinary Immunology Immunopathology*, v, 144, n, 2, p. 27-35, 2011.

WAGERS, A.J.; WEISSMAN, I.L. Plasticity of adult stem cells. *Cell*, v, 116, n. 5, p. 639-648, 2004.

WANG, L.; DUAN, E.; SUNG, L.; JEONG, B.S.; YANG, X.; TIAN, X.C. Generation and Characterization of Pluripotent Stem Cells from Cloned Bovine Embryos**.** *Biology of Reproduction*, v. 73, n, 1, p. 149-155, 2005.

YU, X.; JIN, G.; YIN, X.; CHO, S.; JEON, J.; LEE, S.; KONG, I. Isolation and Characterization of Embryonic Stem-Like Cells Derived From In Vivo-Produced Cat Blastocysts. *Molecular Reproduction and Development*, v. 75, n, 9, p. 1426-1432, 2008.