



Applications of Mesenchymal Stem Cells in Treatments of Domestic Animals

D.V. Makakov¹ S.V. Nadezhdin¹ Murtadha Abbas²
Ismael Raheem Al-Muhana³

¹*The National Research University "Belgorod State University"
(Belgorod, Russia)*

² *University of Kufa, Faculty of Veterinary Medicine, Department of Public Health, Iraq.*

³ *University of Kufa, Faculty of Veterinary Medicine, Department of Microbiology, Iraq.*

Corresponding author: MA, e-mail: murtadhaa.alghazali@uokufa.edu.iq, ORCID: [0000-0002-4486-6290](https://orcid.org/0000-0002-4486-6290)

Co-authors: SVN, e-mail: nadezhdin@bsu.edu.ru, ORCID: [0000-0002-6249-2464](https://orcid.org/0000-0002-6249-2464) ; DVM, e-mail: maklakovdaniil12@gmail.com, ORCID: [0000-0002-9974-0433](https://orcid.org/0000-0002-9974-0433); IRA, e-mail: mailto:ismaelr.almhana@uokufa.edu.iq, ORCID: [/0000-0002-9986-599X](https://orcid.org/0000-0002-9986-599X)

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Abstract

Mesenchymal stem cells: they have received great attention in the field of regenerative medicine due to their ability to differentiate into different cell types and their ability to secrete a wide range of biologically active molecules that repair and regenerate tissues. Stem cells are usually isolated from bone marrow, adipose tissue, or other adult tissues and can be grown in culture media to generate large numbers of cells. Stem cells are widely used in veterinary medicine: for the treatment of bone diseases, diseases of the respiratory and digestive systems, immune diseases. The therapeutic effect of stem cells is due to the anti-inflammatory and immunomodulatory effects of the cells themselves and their excretory products. In general, the continuous investigation and development of Veterinary key therapies is essential to improve animal health and well-being, promote Translational Medicine, and thereby benefit human health. Mesenchymal stem cells have been studied as a potential treatment for various gastrointestinal diseases in cattle, including inflammatory bowel disease and Colitis. Stem cells can be used to replace damaged or diseased tissue by differentiating between specific cell types required for repair and regeneration. Stem cells can also secrete factors that promote tissue regeneration, which can enhance their therapeutic potential. Diverse alternatives provide an attractive option for the possibility of developing clinical applications of stem cells in veterinary medicine.

Keywords: Bone marrow stem cell, cell-based therapy, mesenchymal stem cells, paracrine effects, therapeutic application, veterinary medicine.

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INTRODUCTION

Mesenchymal stem cells are a type of multipotent stem cell that can differentiate into several cell types, including bone, cartilage, fat, and muscle cells. MSCs were first identified in bone marrow but have since been found in many other tissues, including adipose tissue, placenta, and umbilical cord [1].

MSCs have several characteristic features that distinguish them from other types of stem cells. Firstly, they are adherent cells, meaning that attach to plastic surfaces when cultured in vitro. Secondly, they express specific surface markers, such as CD73, CD90, and CD105, but lack expression of hematopoietic markers, such as CD45 and CD34. Thirdly, they have the capacity to differentiate into several cell types under appropriate conditions, including osteoblasts, chondrocytes, adipocytes, and myocytes [1,2]. MSCs also have immunomodulatory and anti-inflammatory properties, which make them attractive as a therapeutic agent. MSCs can modulate the function of various immune cells, including T cells, B cells, and natural killer cells, and can inhibit the production of pro-inflammatory cytokines. MSCs also secrete a variety of trophic factors, such as growth factors, cytokines, and chemokines, that can promote tissue repair and regeneration [3]. The properties of MSCs make them a promising candidate for regenerative medicine and cell-based therapies. MSCs have been used in pre-clinical and clinical studies to treat a wide range of conditions, including osteoarthritis, diabetes, liver failure, and cardiovascular diseases. However, much more research is needed to fully understand the mechanisms of action of MSCs and optimize their use in clinical applications

[4]. The world of science is still very much interested in the topic of stem cells. In the simplest way, they can be characterized as cells that have the ability to self-renew and differentiate into other types of cells. [5,8]. These cells are present in every living organism from the moment the ovum is fertilized until the moment of death. Their presence allows the body to develop and maintain the number of somatic cells in balance. They also enable the regeneration of organs and tissues by replacing somatic cells that deteriorate over time or are damaged [7,9,11]. The discovery and development of methods for obtaining stem cells allowed us to come much closer to implementing the age-old dream of mankind regarding replacing sick and worn-out cells and/or tissues with new ones, that are grown in the laboratory. The importance of stem cells in medicine was emphasized by the Nobel Prize award that acknowledged two scientists, John Gurdon and Shinya Yamanaka for the development of the so-called induced pluripotent stem cells. They are reprogrammed somatic cells that acquire the features of stem cells [12,13]. Thanks to numerous studies on stem cells in various fields of science, it is possible to use them in human [14] and animal medicine [15,16]. The characteristic features of stem cells mentioned by numerous authors include their simple structure and lack of differentiation; self-renewal, which allows one to maintain a constant population of cells throughout the life of an organism; asymmetrical cell division that results in the formation of a larger stem cell and a smaller cell undergoing further linear differentiation: the ability to differentiate into cells of various tissues; and the expression of proteins c specific to undifferentiated cells, i.e., c-kit, Thy1 [2,4,14,15].

Sources and methods of obtaining MSCs

Bone marrow:

Initially discovered in 1976 by Friedenstein, BM-MSCs were first described as undifferentiated MSCs in 1987 [17,18]. Subsequently, BM became the main source of multipotent stem cells. However, their procurement requires a highly invasive and painful procedure involving heavy use of anesthesia; moreover, the cell yield, longevity and potential for differentiation diminishes with donor age [19]. Compared with MSCs derived from other sources, BM-MSCs possess a longer duplication period, reach senescence earlier and constitute only 0.01–0.001% of nucleated marrow cells [19,20]. Nevertheless, one advantage of BM-MSCs over other cell types is their relatively short culture time [11,21].

The role of inflammation in the pathogenesis of arterial hypertension, particularly in activating the sympathetic nervous system, has been recently identified in a study using a murine model [22]. Penn et al. observed that BM transplantation from spontaneously hypertensive rats into normotensive rats resulted in a marked increase in blood pressure; however, treating the rats with minocycline, a second-generation semisynthetic tetracycline and microglial activation inhibitor with anti-inflammatory effects, reversed the hypertension [22]. The underlying mechanism involves an increase in the production of inflammatory cells and cytokines which translates into a stronger neuroinflammatory response and increased activity of the sympathetic nervous system. The association between haematopoietic (i.e. BM-derived) stem cells and hypertension indicates that BM-MSC therapy may have potential applications in treating obesity, diabetes mellitus (DM) and hypertension [22].

In a clinical context, attempts have been made to use BM-MSCs to alleviate heart failure, address cardio myocyte loss to improve heart function and prevent end-

stage heart failure. Although clinical trials in both human and animal models have been promising, a meta-analysis of 10 large-scale randomised phase two trials using BM-derived cells showed negligible benefits after an acute myocardial infarction [25,30]. In preclinical and clinical studies, intravenous, intracoronary or intramyocardial routes represent the main options for cell delivery within minutes to a few months after a myocardial infarction [25,30]. However, protocol variations impede the accurate interpretation of preclinical and clinical trial results [29,30]. Forest et al. compared cellular retention and engraftment with in vivo imaging in a porcine model using different administration routes [28]. Following an intracoronary injection, the safest and easiest delivery method, $34.8 \pm 9.9\%$ of cells were detected in the heart after one hour; this proportion declined precipitously to $6.0 \pm 1.7\%$ after 24 hours. Inconsistent results regarding the benefits of BM cell therapy in cardiac function may be due to variability in cell survival, engraftment and differentiation [27].

Currently, several ongoing clinical trials are being conducted to establish the usefulness of BM-MSCs, especially in the context of ischaemic heart failure. Diastolic left ventricular (LV) dysfunction represents a frequent complication after acute myocardial infarction and a major risk factor for the development of heart failure, independent of preserved LV ejection fraction Schaefer et al. conducted a follow-up study of 60 patients with ST-segment elevation acute myocardial infarction treated by BM-MSC transfer [30]. The time trend of the diastolic function and the E/A ratio remained unchanged in the experimental BM-MSC group relative to the control group, in which the E/A ratio decreased over the course of 60 months. However, the duration of the improvement noted in the experimental group did not

exceed five years [30]. Importantly, no arrhythmogenic or tumourigenic effects have been observed in the treatment of myocardial infarction with BM-MSCs [7]. In allogeneic (i.e. haematopoietic) stem cell transplantation, graft-versus-host disease (GVHD)—in which immunocompetent donor cells recognise and attack tissues in an immunocompromised host—is a significant cause of morbidity and mortality [11]. Traditional management entails immunosuppression with corticosteroids as first-line therapy, a treatment that produces sustained responses in <40–50% of patients with GVHD [31]. However, corticosteroid-refractory patients require effective second- and third-line treatment options [32]. The allogeneic transplantation of BM-MSCs can, when obtained from major histocompatibility complex-matched donors (i.e. a mother or twin), induce immunosuppression in the recipient and thus be used to treat GVHD. This effect was initially evaluated in murine models wherein MSCs inhibited naive and memory antigen-specific T cells [33]. BM-MSCs have also been used to treat systemic lupus erythematosus (SLE), multiple sclerosis (MS), autoimmune encephalomyelitis, asthma, allergic rhinitis and pulmonary fibrosis. Except for SLE and MS, these applications have all been tested in animal models [7]. The efficacy and safety of using BM-MSCs in GVHD patients is being tested in ongoing clinical trials based on growing evidence highlighting the key role of BM-MSCs in modulating inflammation [34].

In the digestive system, BM-MSCs can improve liver cirrhosis and liver failure caused by hepatitis B infections, with positive changes according to chronic liver disease staging and prognostic scales [35]. Other important applications of BM-MSCs are in orthopaedics. For example, BM aspirates from the iliac crest have been used to treat atrophic diaphyseal non-unions, leading to callus formation [9,12]. Placing BM-MSCs on scaffolds has been shown to regenerate meniscus tissue in rabbits and

BM-MSCs placed on conduits or grafts have been used to treat nerve defects, with better results than cell-depleted grafts [9]. Furthermore, BM-MSCs have been transplanted to various injured tendon sites, resulting in enhanced tissue repair, especially for injuries of the patellar tendon [9].

Another important application of BM-MSCs is in the treatment of spinal cord injuries. When mixed with minocycline, BM-MSCs have resulted in improved spinal cord injuries in a rat model. As such, these cells may represent a promising approach for neuroprotection following spinal cord injury. BM-MSCs have also been used to treat osteogenesis imperfecta in a mouse model, in which BM-MSCs from a disease-free mouse were infused into an affected mouse. This treatment promoted the differentiation of migrated cells into osteocytes and synthesised standard amounts of type I collagen, partially reverting the disease phenotype. Other BM-MSC applications include muscular dystrophy, physical defects and rotator cuff injuries [9].

Adipose tissue:

The main advantage of using AT as an MSC source is convenience, as human subcutaneous AT is usually abundant throughout the body and is a byproduct of cosmetic and therapeutic liposuction procedures. It is estimated that approximately 98–100% of cells obtained from AT are viable [5,36]. The morphological, phenotypical and functional characteristics of AT-MSCs are similar to those of BM-MSCs. Besides their stability in long-term cell cultures, AT-MSCs can expand effectively in vitro and possess high multilineage differentiation potential. The stromal vascular AT fraction produces more stromal elements than the mononuclear BM fraction, even though these stromal elements may represent other lineages, such as endothelial, haematopoietic and pericyclic origins [48–50]. Thus, AT represents a more practical autologous source of MSCs for tissue

engineering than BM. However, one limitation of AT-MSCs is that certain donor characteristics, such as age, can affect the expansion and differentiation of AT-MSCs, particularly into osteogenic and chondrogenic lineages, although the adipogenic lineage is not affected [36].

Besides their ability to differentiate and self-renew, AT-MSCs secrete several cytokines and growth factors with anti-inflammatory, antiapoptotic and immunomodulatory properties. Examples include vascular endothelial growth factor, hepatocyte growth factor and insulin-like growth factor, all of which are involved in angiogenesis and tissue repair. These characteristics render AT-MSCs prime candidates for treating ischaemic diseases [5].

Additionally, due to the immunomodulatory effects of human AT-MSCs, AT is an excellent source of MSCs for allogeneic transplants and, as AT-MSCs do not express major histocompatibility complex type II antigens, the risk of rejection is minimised. AT-MSCs regulate the function of T cells by promoting the induction of suppressor T cells and inhibiting the production of cytotoxic T cells, natural killer cells and proinflammatory cytokines (including tumour necrosis factor- α , interferon- γ and interleukin [IL-12]). Furthermore, these cells secrete soluble factors, such as IL-10, transforming growth factor- β and prostaglandin E₂, making these cells immunosuppressive [5,11].

It should be noted that the p53 protein plays a key role in the differentiation of human AT-MSCs cells, affecting their osteogenic and adipogenic potential by suppressing the osterix, runt-related transcription factor and peroxisome proliferator-activated receptor (PPAR) genes [39]. In various malignancies, p53 function is limited, thus offering the possibility of using cancer cells from mutant MSCs to investigate treatment targets [39,40] A recent study by Inatani et al. compared the differentiation potential of

MSCs from normal AT and atypical lipomatous tumour/well-differentiated liposarcoma (ALT/WDL) [37]. The osteogenic differentiation potential in ALT/WDL-MSCs was greater than that of the normal AT group, although normal AT produced more fatty acid droplets during adipogenic differentiation than ALT/WDL [37]. This type of study offers an interesting approach to therapeutics in that it postulates using differentiation as a means of treatment. This concept has also been explored with the use of troglitazone in patients with liposarcoma, defective proliferation, apoptosis and differentiation. Histological tumour changes were observed in a small follow-up study of patients with myxoid and pleomorphic liposarcomas; six months after the administration of PPAR- γ ligand, troglitazone, the researchers observed microvesicular cytoplasmic lipid accumulation and diminished proliferation according to Ki-67 expression [38]. Although imaging indicated that the tumour size increased as a result of intracellular lipid accumulation, with a corresponding increase in cellular volume, no new tumour sites were found. Thus, it may be possible to modify the pathophysiology of cancer and reduce the proliferation of malignant cells by promoting cellular differentiation [38].

Moreover, AT-MSCs may be useful for myocardial regeneration. In one trial, AT-MSC application resulted in improved cardiac function, elevated perfusion and a reduction in the extension of scar tissue [7]. AT-MSCs have also been used extensively for dermatological and cosmetic purposes. These cells promote wound healing by enhancing the secretion of type I collagen, upregulating the expression of type III collagen and fibronectin and downregulating matrix metalloproteinase-1 in fibroblasts, they have also been shown to accelerate granulation tissue and capillary formation and epithelisation in DM-induced tissue damage. Moreover, AT-MSCs have been

used for wrinkle reduction, skin whitening (by inhibiting melanin synthesis), as antioxidants and for the promotion of hair growth [13].

Furthermore, AT-MSCs have accelerated skeletal muscle regeneration in a rat model. Interestingly, AT-MSCs express various neuronal and Schwann cell markers, such as myelin protein zero, myelin basic protein and peripheral myelin 22, suggesting that they may possess a myelin-generating capacity. These cells have also been used to treat nerve defects and neuropathic pain in rats with promising results. Finally, AT-MSCs have been used to treat collagenase-induced tendinitis in horses, improving tendon organisation and successfully treating rotator cuff injuries [9].

Dental pulp:

Dental pulp (DP)-derived MSCs specialise into odontoblasts, which produce dentin [42]. These cells are obtained from pulp tissue from the third molar and are extracted by an enzymatic digestive process. Overall, DP-MSCs are easy to cryopreserve and, similarly to AT-MSCs, possess immunomodulatory properties. Since DP-MSCs are derived from the neural crest, they have ectomesenchymal origins and both ectodermal and mesenchymal lineages. Hence, in addition to differentiating into osteo/chondroblasts and adipocytes, DP-MSCs can also differentiate into neuronal lineages. When cultured in a three-dimensional (3D) dentin scaffold, DP-MSCs can differentiate into corneal epithelial cells, melanocytes and induced pluripotent stem cells, rendering them highly useful in regenerative medicine research [41,42,43].

The most promising clinical applications for this stem cell source involve the correction of metabolic diseases and the treatment of liver diseases with high mortality rates, such as cirrhosis and hepatocellular carcinoma [42,43]. Over time, DP-MSCs have become the preferred alternative to harvesting stem cells during hepatic transplantation. DP-MSCs have

also been used in the field of bone regeneration. When DP-MSCs are cultured on hydrogels, they can spontaneously differentiate into both odontogenic and osteogenic phenotypes [43,44].

A specific type of DP-MSCs, known as periapical cyst (PCy)-MSCs, have gained special attention because of their extensive proliferative potential, cell surface marker profile and ability to differentiate into various cell types, such as osteoblasts, adipocytes and neurons [16,17,43,44]. Importantly, these cells are easily collected from surgically removed PCys, thus allowing for the reuse of biological waste. They have also been used in the field of bone regeneration. Due to their neural plasticity, these cells represent an interesting option for treating neurodegenerative diseases [16].

Birth-derived tissues:

Currently, umbilical cord blood (UCB) is not universally accepted as a source of MSCs [45]. Nevertheless, the vast abundance of UCB, availability of donors, ease of procurement and reliability of sample collection and lack of transmission of Herpesviridae viruses are advantages of this particular cell source [45]. Furthermore, as UCB-MSCs are less mature than other types of adult stem cells, they do not elicit a strong immune rejection response in an allogeneic recipient, tolerating mismatches of HLA. Conveniently, UCB-MSCs can be cryopreserved in vast quantities for later cultivation and research [45]. The doubling time of these cells is similar to that of BM-MSCs (i.e. every 30 hours). Overall, UCB-MSCs possess osteogenic, chondrogenic, adipogenic and myogenic differentiation potential [45]. In humans, the intravenous administration of UCB-MSCs has been successfully used to treat GVHD and SLE [11]. Additionally, UCB-MSCs have been implanted to treat sciatic nerve defects and in post-crush and transection animal models with good results [9].

Recently, Wharton's jelly (WJ) has garnered attention as a potential source of

MSCs. The properties of WJ-MSCs are different from those derived from other sources, with WJ-MSCs exhibiting a higher proliferative potential independent of culture conditions [18]. Nevertheless, when cultured in the presence of serum, these cells produce fewer extracellular matrix components than BM-MSCs and AT-MSCs. In terms of clinical applications, WJ-MSCs have been shown to be beneficial and safe in the treatment of myocardial infarctions, positively affecting the size of the infarction and LV function [7]. In one study, human WJ-MSCs were placed on a 3D scaffold in a conditioned medium obtained from a culture of human articular chondrocytes [18]. The WJ-MSCs underwent chondrogenic differentiation without the need for growth factors, with a high accumulation of glycosaminoglycans and expression of cartilage-related genes. These observations indicate that WJ-MSCs may be a good option for regenerating articular cartilage [18].

Amniotic fluid and placenta:

According to immunophenotypical analyses, the phenotype of cultured cells obtained from amniotic fluid (AF) is similar to that of BM-MSCs.58 Cultured cells obtained from this source can differentiate into mesenchymal lineages. The expansion of AF- and amnion-derived MSCs is similar, with no marked differences in cell counts, although the latter grow at a slower pace and with a lower yield than BM-MSCs [19,46].AF-MSCs demonstrate high self-renewal capacity (>300 cell divisions) and a doubling time of 36 hours. Furthermore, they have been shown to maintain a normal karyotype even at late passages [46]. Interestingly, AF-MSCs express octamer-binding transcription factor-4 (Oct-4) and stage-specific embryonic antigen-4 (SSEA-4) human embryonic stem cell markers, which is typical for underdifferentiated embryonic stem cells; this means that AF-MSCs are not primitive embryonic stem cells but maintain greater differentiation potential than most adult stem cells [19,46].

In addition to common mesodermal lineages, AF-MSCs can also differentiate into hepatocytes and neurons under specific culture conditions.19 While no human investigations using AF-MSCs have yet been reported, several animal models have studied various clinical applications, including bladder formation using AF-MSCs differentiated into muscle cells, the treatment of nerve injuries, blood vessel and heart valve formation and regeneration of the diaphragm, kidney, bone, lung, heart and cartilage.[19,20,46] As with UCB-MSCs, AF-MSCs have also been successfully implanted in sciatic nerve defects and post-crush and transection animal models [9].

The basement membrane of the amnion contains MSCs derived from the inner cell mass of blastocysts. In addition to not expressing HLA class II, human amnion-derived MSCs possess immunosuppressive factors. Other parts of the placenta, such as the decidua basalis and decidua parietalis, also contain MSCs; upon cultivation, these cells exhibit similar characteristics to amnion-derived MSCs. Placenta-derived MSCs (P-MSCs) also express embryonic stem cell markers such as c-Kit, sex determining region Y-box 2, Oct-4 and SSEA-4 and are able to differentiate into mesenchymal as well as hepatic, pancreatic and neuronal lineages [44]. P-MSCs have been shown to improve lung function and reduce pulmonary fibrosis in animal models and have been used in the treatment of skin and ocular diseases [21,48]. Interestingly, these cells have also been used to create an artificial amnion within a collagen scaffold [20]. Other clinical applications of P-MSCs in animal models include the formation of cardiac tissue and treatment of bleomycin-induced lung injuries, cartilage defects, inflammatory bowel disease, Duchenne muscular dystrophy, ischaemic stroke, inflammation and DM., One of the main benefits of P-MSCs is that they can be easily obtained following delivery without the need for a specific procedure; they also

offer distinct advantages in terms of proliferation and plasticity [21].

Mobilized peripheral blood:

Specific factors that control hematopoiesis were initially identified by their ability to support progenitor cells in culture assays. These factors have since been isolated and purified, allowing for the development of therapeutic agents, including colony-stimulating factors (CSFs) [49,50]. Filgrastim, a granulocyte-CSF, is a bacterially synthesized recombinant protein that acts on neutrophils. It was first used in cancer patients in 1991 as an adjunct for ameliorating chemotherapy-induced neutropaenia. Filgrastim has since been approved in over 70 countries for mobilising peripheral blood progenitor cells for transplantation [49,50]. Granulocyte-CSF binds selectively to specific cell-surface receptors, triggering the phosphorylation of janus kinase (JAK)1 and JAK2 protein tyrosine kinases and activating the Ras/mitogen-activated protein kinase pathway. A subcutaneous dose of filgrastim (10 µg/kg) results in a six-fold increase in neutrophil levels within 24–48 hours. Furthermore, filgrastim treatment reduces the neutrophil maturation time from five days to one day [50,51]. Peripheral blood stem cells may be mobilised from healthy donors using granulocyte-CSFs [9]. The underlying mechanism involves a number of adhesion molecules, including lymphocyte function-associated antigen 1, very late antigen-4, C-X-C motif chemokine receptor 4, c-Kit, CD44 and macrophage-1. These molecules facilitate the binding of stem cells to BM and their disruption allows for their release into the circulation. While the underlying mechanism is unclear, this process is mainly regulated by cytokines [52,53].

As a replacement for BM-MSCs, granulocyte-CSF-mobilised peripheral blood stem cells are convenient and easily accessible. They are similar to BM-MSCs in most aspects, as they can differentiate into mesenchymal lineages and have a

strong capacity to adhere to plastic culture flasks [21]. However, MSCs mobilised from the blood using granulocyte-CSF exhibit a 95-hour duplication time, which is longer than that of MSCs from other sources. Moreover, they adhere in a monolayer and to different scaffolds [21]. Their surface expression profile is similar to that of BM-MSCs with specific MSC markers. Their capability to differentiate into chondral and bone lineages is lower than that of BM-MSCs, while their capability to differentiate into an adipose lineage is higher [21]. As yet, no clinical trial on the use of these cells has been published.

Synovium and synovial fluid:

In humans, cartilage and synovium originate from a common pool of cells during synovial joint development. In the treatment of musculoskeletal injuries, synovial MSCs are an attractive method of meniscus regeneration. Synovium-derived MSCs (S-MSCs) are far superior to cells derived from the skeletal muscle and AT, as determined by their in vitro expandability, differentiation potential and epitope profiles [54]. When replated at a density of 50 cells/cm², these cells retain their proliferative ability up to passage 10, whereas cells from other sources proliferate only until passage seven. Furthermore, these cells possess remarkable adipogenic, chondrogenic and osteogenic potential comparable to that of BM- and periosteum-derived MSCs [54]. Interestingly, in vivo evidence suggests that the intra-articular injection of endogenous cells from the synovial membrane can contribute to the repair of partial-thickness cartilage defects in a mouse injury model [22]. S-MSCs have also promoted ligament regeneration in another animal model [23].

Interestingly, in one study, when S-MSCs were implanted into the deeper aspect of an osteochondral defect, they differentiated into osteocytes; however, when they were implanted into the superficial aspect, the cells differentiated into chondrocytes [9]. These observations

suggest that the microenvironment may play an important role in the multilineage differentiation potential of MSCs. Furthermore, S-MSCs may play a role in the endogenous repair of intra-articular injuries because the number of S-MSCs in synovial fluid are elevated in patients with osteoarthritic knees, cartilage defects, meniscus damage or intra-articular ligament injuries [9].

Endometrium:

In 2004, Chan et al. hypothesized that because the human endometrium regenerates from the lower basalis layer, it might contain small populations of epithelial and stromal cells that exhibit clonogenicity [55]. Human clonogenic endometrial cells exhibit the same in vitro properties as BM-MSCs. Human endometrial stromal cell cultures can differentiate in vitro, unlike other non-endometrial gynecological tissues [57,57].

There are three types of endometrial stem cells (EnSCs): epithelial progenitor cells, MSCs and endothelial progenitor cells.68 EnSCs can be isolated from the menstrual blood or from endometrial biopsies without the need for invasive procedures [57,58]. Furthermore, EnSCs have similar properties to those of AT- and BM-derived MSCs. The functional dysregulation of these cells may contribute to a range of pathologies, from endometriosis to endometrial carcinomas [25]. The proliferation potential of EnSCs is 6×10^{11} cells from a single cell and these cells can differentiate into adipogenic, chondrogenic and osteogenic lineages.67,68 EnSCs have several clinical applications, albeit limited to preclinical studies. Current models include Duchenne muscular dystrophy, muscle repair by EnSC transplantation into atrophied muscle fibres, limb ischaemia and myocardial infarction [57].

The proliferative ability of menstrual blood-derived MSCs (Men-MSCs) is very high and they can maintain a relatively stable karyotype over 40 passages. The doubling time of these cells

is 18–36 hours, which is fast when compared with other types of MSCs.69–71 Men-MSCs can be induced into multiple lineages, including chondrocytes, osteoblasts, adipocytes, smooth muscle cells, myocardial cells and hepatocytes. In an animal model of type 1 DM, the intravenous administration of these cells improved hyperglycemia [61]. In another animal model, Men-MSCs were shown to secrete various neuroprotective factors (e.g. vascular endothelial growth factor and brain-derived neurotrophic factor) which promote neuron survival and mitigate behavioural and histological changes, making them applicable for stroke treatment. Other potential applications of Men-MSCs include ulcerative colitis, endometriosis, endometrial carcinomas, pelvic organ prolapse and cardiac failure [26].

Skin:

Skin-derived precursors are able to differentiate into both neural and mesodermal cells. These cells can be harvested from the human foreskin or even from skin biopsies [62]. When cultured, they differentiate into neurons, glia and smooth muscle cells, including cells of the peripheral neural phenotype (consistent with cells originating from the neural crest), but hold limited potential for mesenchymal lineage differentiation [27,62]. One of the main advantages of this cell type is its proliferation rate. The skinderived MSCs (S-MSCs) of human newborns exhibit a higher proliferation rate during long-term cultures than human AT-MSCs and skin stromal cells [57,62]. Furthermore, stem cells can be obtained from several regions of the skin, including the epidermis (epidermal stem cells), interfollicular epidermis, hair follicles, sebaceous glands and subcutaneous AT. Each of these stem cell types expresses different markers [27,28].

Although S-MSCs are able to differentiate into neurons, from a functional point of view, these cells present an immature electrophysiological profile [28].

The implantation of S-MSCs into an adult mouse brain led to the discovery that these cells are capable of differentiating into astrocytes as well as insulin-producing pancreatic cells [28]. An important application of S-MSCs is skin regeneration after serious skin damage, such as that caused by burns, chronic ulcers and deep wounds, as well as anti-ageing therapy and repigmentation in vitiligo, alopecia and melanoma cases. Other potential clinical applications of S-MSCs currently being studied include spinal cord injuries, regeneration of the haematopoietic lineage after radiotherapy, bladder reconstruction and alopecia [28]. Undifferentiated hair follicle stem cells have also been used in murine models of sciatic and tibial nerve transections and crush injuries, with the animals exhibiting improved functional outcomes after cell administration [9].

Muscle:

The skeletal muscle and bone share a common mesodermal origin and these tissues exhibit regenerative capacities due to the presence of endogenous muscle stem cells, satellite cells and skeletal stem cells [63]. It is important to distinguish between satellite cells and muscle-derived MSCs (M-MSCs), as the former are committed to a myogenic lineage while the latter are satellite cell predecessors capable of multilineage differentiation. Other subtypes of M-MSCs include myoendothelial cells—which express surface markers of both endothelial and satellite cells—and traumatised M-MSCs which are rapidly adherent [63]. M-MSCs differentiate into mesenchymal tissues, including osteogenic, myogenic and even chondrogenic lineages. The most important and extensively researched clinical application of this cell type is for use in the treatment of muscular dystrophy [64].

Overall, M-MSCs have many clinical applications; for instance, upon transfection with the bone morphogenetic protein-4 gene, M-MSCs were able to differentiate into osteoblasts and fill a critical femoral defect in an animal

model.²⁸ These cells have also been used in a similar fashion for craniofacial regeneration. In an animal model, the implantation of M-MSCs with fibrin glue directly into osteochondral defects resulted in persistent repair of the defect site after 24 weeks [30]. These cells, when differentiated, express type II collagen and proteoglycan-rich extracellular matrix.

M-MSCs may also promote peripheral nerve regeneration, as shown in animal models of sciatic nerve injuries [9]. Another therapeutic application of M-MSCs is the promotion of skeletal and cardiac muscle regeneration. Several studies have supported the possibility that M-MSCs can augment muscle healing following injury; another study found that the injection of M-MSCs into the infarcted region of cardiac tissue resulted in improved function [29]. Additionally, M-MSCs can mediate a secondary mechanism of regeneration by promoting angiogenesis [29,31]. Other potential applications of M-MSCs include vascular regeneration, the treatment of urinary incontinence and the repair of vaginal tissue. M-MSCs have also been successfully used for treating rotator cuff injuries [9].

Division of MSCs:

There are many types of MSCs, occurring at different places and different periods of time during the life of an organism. They vary between themselves in their proliferation potential, the ability to differentiate into other types of cells in the body, the source of their origin, and in the relation to the recipient [2,7,65,66]. Taking into account the properties of MSCs in terms of their ability to differentiate into other types of cells, we distinguish totipotent, pluripotent, multipotent, and unipotent cells [2,7,66,67]. Totipotent stem cells are cells that exhibit unlimited dividing capacity. These cells can give rise to the entire body, which is due to the ability to differentiate into any type of cells that build the embryo and extra-embryonic tissues (placenta, umbilical cord). The totipotent cell is a fertilized ovum (zygote)

and cells obtained from the first germinal stage (morula), as evidenced by monozygotic twins produced from different blastomeres. Pluripotent stem cells are cells that can transform into any of the three germ layers: The endoderm, ectoderm, mesoderm, and the cells derived from them. Unlike the cells described earlier, they cannot give rise to the entire organism. Examples of pluripotent cells are the blastocyst germ cells, referred to as inner cell mass (ICM). Multipotent stem cells are cells that can differentiate into all types of cells that originate from the germ layer. Examples include hematopoietic stem cells found in the bone marrow or umbilical cord blood. Unipotent stem cells, also called precursor cells, show a targeted differentiation mechanism into a specific type of mature body cells. Under regular conditions, they allow the maintenance of a constant cell number in the tissues; an example here is the reproductive layer cells of the epidermis [2,7,67,68]. Taking into account the source of MSCs, we can distinguish embryonic, fetal, and adult types of cells. Embryonic stem cells (ESCs) obtained from the embryo of blastomeres are totipotent. On the other hand, for experimental purposes, cells from the blastocyst embryonic node, which are pluripotent, are most often used. These cells can transform into all types of cells in the organism, while at the same time showing unlimited self-renewal capacity in vitro. Fetal stem cells (FSCs) derive from fetal tissues, umbilical cord tissues (e.g., Wharton's jelly), umbilical cord blood, and amniotic cells. They show a multipotential character. Adult stem cells (ASCs) are also known as mature or somatic. They are undifferentiated cells with multi- or unipotent properties. They occur in the body in the postnatal period, giving the possibility of tissues and organs in which they occur [2,7,67,69]. MSCs can also be divided according to its relation to the recipient, distinguishing stem cells of autogenous, allogeneic, and xenogeneic

origin. Autogenous stem cells are currently the most popular among scientists and clinicians. These cells are isolated from their source from the donor patient (e.g., bone marrow, adipose tissue, cord blood) who is also the recipient. Then they get applied to the regenerating tissue or organ. This procedure aims to stimulate the repair of tissue damage by differentiating to the desired cell/tissue type [1,16]. Allogeneic stem cells are cells taken from another individual of the same species. They constitute the basis of therapy with the use of embryonic and mature stem cells. This method allows one to use an appropriate number of necessary cells without having to be bothered by time limitations [7,68,69]. This kind of cell can be obtained long before the implantation procedure and can also be multiplied and stored for quite some time. Using these cells as opposed to autogenous ones allows one to not expose the patient Biomolecules 2021, 11, 1141 3 of 20 to additional anesthesia and trauma during the collection of cells. The limitation may be having to have a donor. Xenogeneic stem cells are an alternative to autologous and allogeneic stem cells [69,70].

Numerous studies with the use of antlerogenic (xenogenic) stem cells, marked with the symbol MIC-1, showed a regenerative effect on the tissues of other mammals, such as rabbits [19,20] and horses [21].

ESCs, given as an ideal example of stem cells in the natural environment, have unlimited in vitro potential for self-renewal and differentiation into each type of cell in an organism. At the same time, it has not been possible to develop precise procedures for managing their differentiation and division at the site of administration into the body, which may lead, among others, to the development of neoplastic lesions observed in experimental animals. That and the difficulties of ethics human medicine is why the ESCs have not yet been used in clinical stem cells therapy [7,65,71].

The discovery and development of methods of obtaining adult stem cells made it possible to significantly avoid the problems mentioned above, allowing them to be used in clinical treatment. These cells are presumed to be present in all organs of the body [71]. This is evidenced by, inter alia, their presence in tissues with negligible regenerative abilities, such as nervous tissue [68]. Despite the small number of ASCs in the adult organism, difficulties in obtaining them, and their lower ability to self-renew and differentiate compared to ESCs, these cells have attracted great interest, arousing high hopes in regenerative medicine [7].

The best-known cells that have been used for years are hematopoietic stem cells of the bone marrow, the stroma of which also contains MSCs. They show the ability to adhere to the substrate and divide with the formation of fibroblast-like cells [2,3,70,73]. These cells, with the possibility of self-renewal and multipotential differentiation, are also obtained for clinical purposes from adipose tissue [70]. Despite the basic range of MSCs differentiation into chondrocytes, osteocytes, or adipocytes [2,3], under laboratory conditions these cells can also differentiate into cells such as myocytes, hepatocytes, or neurons [6,16,74].

Autogenous stem cells of pluripotent nature can be obtained by transforming adult somatic cells, for example, fibroblasts (cultured in vitro), and are an alternative to pluripotent cells of embryonic origin. They are obtained by introducing genes encoding transcription factors necessary for the development of embryonic cells (c-Myc, Klf 4, Oct 3/4, Sox 2) [8,73]. The resulting cells can differentiate similarly to embryonic cells and are referred to as induced pluripotent stem cells (iPSCs) [10,74]. Unfortunately, the effectiveness of this procedure is low, and the cells obtained in this way, administered to laboratory animals, caused

(similarly to ESC) the development of teratomas [75].

Applications of MSCs in Veterinary Medicine

Treatment of orthopedic conditions in companion animals:

Orthopedic conditions are common in companion animals, such as dogs and cats, and can significantly impact their quality of life. Mesenchymal stem cells (MSCs) have been studied as a potential treatment for various orthopedic conditions in companion animals, including osteoarthritis, ligament injuries, and bone fractures [76], use in many cases; 1. Osteoarthritis: Osteoarthritis is a degenerative joint disease that affects many companion animals. MSCs have been shown to have anti-inflammatory and regenerative effects on the joint tissue, which can improve the clinical symptoms of osteoarthritis. MSCs can be injected directly into the affected joint, and their effects can last for several months [77]. 2. Ligament injuries: Ligament injuries, such as cranial cruciate ligament (CCL) rupture, are common in dogs. MSCs have been studied as a potential treatment for CCL rupture, and early studies have shown promising results. MSCs can be injected into the site of the injury, and their regenerative effects can improve the healing process and reduce the risk of further injury [78]. 3. Bone fractures: Bone fractures are common in companion animals, and can be challenging to treat, especially in cases where there is a delay in healing. MSCs have been studied as a potential treatment for bone fractures and have been shown to have a positive effect on bone healing. MSCs can be injected directly into the site of the fracture, and their regenerative effects can promote bone healing and reduce the healing time [79], MSCs have shown promising results as a potential treatment for various orthopedic conditions in companion animals. Further studies are needed to determine the optimal dosing and administration of MSCs, as well as their long-term safety and efficacy [80].

Treatment of respiratory diseases in horse

Respiratory diseases are common in horses and can significantly impact their performance and overall health. Mesenchymal stem cells (MSCs) have been studied as a potential treatment for various respiratory diseases in horses, including chronic obstructive pulmonary disease (COPD), inflammatory airway disease (IAD), and equine asthma [23]; 1. COPD: COPD, also known as heaves, is a chronic respiratory disease that affects many horses. MSCs have been shown to have anti-inflammatory and regenerative effects on the lung tissue, which can improve the clinical symptoms of COPD. MSCs can be administered via inhalation or intravenous injection, and their effects can last for several weeks; 2. IAD: IAD is a common respiratory disease in young performance horses. MSCs have been studied as a potential treatment for IAD, and early studies have shown promising results. MSCs can be administered via inhalation or intravenous injection, and their regenerative effects can improve the lung tissue and reduce inflammation [81]; 3. Equine asthma: Equine asthma is a common respiratory disease in horses that is characterized by airway inflammation and constriction. MSCs have been studied as a potential treatment for equine asthma and have been shown to have a positive effect on lung function and clinical symptoms. MSCs can be administered via inhalation or intravenous injection, and their anti-inflammatory and regenerative effects can improve the lung tissue and reduce inflammation.

Treatment of gastrointestinal diseases in livestock

Gastrointestinal diseases are common in livestock and can have a significant impact on their health and productivity. Mesenchymal stem cells (MSCs) have been studied as a potential treatment for various gastrointestinal diseases in livestock, including inflammatory bowel disease (IBD) and colitis [82]; 1. IBD: IBD is a

chronic inflammatory disease of the gastrointestinal tract that affects many livestock species. MSCs have been shown to have anti-inflammatory and regenerative effects on the intestinal tissue, which can improve the clinical symptoms of IBD. MSCs can be administered via oral gavage or intravenous injection, and their effects can last for several weeks. 2. Colitis: Colitis is an acute or chronic inflammation of the colon that can affect many livestock species. MSCs have been studied as a potential treatment for colitis, and early studies have shown promising results. MSCs can be administered via oral gavage or intravenous injection, and their regenerative effects can improve the intestinal tissue and reduce inflammation [83].

Treatment of immune-mediated diseases in cats and dogs

Immune-mediated diseases are common in cats and dogs and can have a significant impact on their health and quality of life. Mesenchymal stem cells (MSCs) have been studied as a potential treatment for various immune-mediated diseases in cats and dogs, including inflammatory bowel disease (IBD), atopic dermatitis, and immune-mediated hemolytic anemia (IMHA); 1. IBD: IBD is a chronic inflammatory disease of the gastrointestinal tract that can affect cats and dogs. MSCs have been shown to have anti-inflammatory and regenerative effects on the intestinal tissue, which can improve the clinical symptoms of IBD. MSCs can be administered via intravenous injection or subcutaneous injection, and their effects can last for several months [83]; 2. Atopic dermatitis: Atopic dermatitis is a common skin disease in cats and dogs that is caused by an allergic reaction. MSCs have been studied as a potential treatment for atopic dermatitis, and early studies have shown promising results. MSCs can be administered via intravenous injection or subcutaneous injection, and their regenerative effects can improve the skin tissue and reduce inflammation; 3. IMHA:

IMHA is an immune-mediated disease that affects the red blood cells in cats and dogs. MSCs have been studied as a potential treatment for IMHA and have been shown to have a positive effect on the immune system and red blood cell count. MSCs can be administered via intravenous injection or subcutaneous injection, and their regenerative effects can improve the immune system and reduce the risk of further red blood cell destruction [84].

Mechanisms of action of MSCs anti-inflammatory and immune-modulatory effects

Mesenchymal stem cells (MSCs) have been shown to have anti-inflammatory and immunomodulatory effects, which make them attractive candidates for the treatment of a wide range of diseases.

MSCs can migrate to sites of inflammation and injury when they are introduced into the body. There they can modulate the immune response and promote tissue repair. MSCs have been shown to release various anti-inflammatory molecules, such as cytokines, chemokines, and growth factors, which can reduce inflammation and promote tissue regeneration. MSCs can also interact with various immune cells, such as T cells, B cells, and natural killer cells, and modulate their function, leading to a reduction in inflammation and an improvement in tissue repair [85].

MSCs can also have immunomodulatory effects, meaning that they can influence the immune system and its response to various pathogens and diseases. MSCs have been shown to suppress the function of immune cells that are responsible for attacking the body's own tissues, such as T cells and B cells. MSCs can also modulate the activity of immune cells that are responsible for fighting infections, such as natural killer cells and macrophages, leading to an improvement in the immune response [86].

Tissue repair and regeneration

Mesenchymal stem cells (MSCs) have been shown to have the ability to promote tissue repair and regeneration in various tissues and organs throughout the body. MSCs can differentiate into various cell types, including bone, cartilage, muscle, and fat cells, and can also produce growth factors and other molecules that promote tissue regeneration [87].

When MSCs are introduced into damaged tissues or organs, they can migrate to the site of injury and differentiate into the appropriate cell types to repair or regenerate the damaged tissue. For example, in bone repair, MSCs can differentiate into bone-forming cells called osteoblasts, which can stimulate the formation of new bone tissue. In cartilage repair, MSCs can differentiate into chondrocytes, which are the cells that produce cartilage tissue. In muscle repair, MSCs can differentiate into myoblasts, which are the cells that form muscle tissue. In addition to their differentiation capacity, MSCs also secrete various growth factors, cytokines, and chemokines that can stimulate tissue repair and regeneration. These molecules can promote angiogenesis (the formation of new blood vessels), recruit other cells to the site of injury, and stimulate the proliferation and differentiation of local cells.

The tissue repair and regeneration properties of MSCs make them attractive candidates for the treatment of various diseases and injuries, including bone fractures, cartilage damage, muscle injuries, and heart disease. However, more research is needed to fully understand the mechanisms underlying these effects and to determine the optimal dosing and administration of MSCs for different types of tissue repair and regeneration [88]. BM became the main source of multipotent stem cells. However, their procurement requires a highly invasive and painful procedure involving heavy use of anesthesia; Moreover, the cell yield, longevity and potential for differentiation

diminishes with donor age[89]. Compared with MSCs derived from other sources, BM-MSCs possess a longer duplication period, reach senescence earlier and constitute only 0.01–0.001% of nucleated marrow cells [90,91]. Nevertheless, one advantage of BM-MSCs over other cell types is their relatively short culture time [92,93]. The role of inflammation in the pathogenesis of arterial hypertension, particularly in activating the sympathetic nervous system, has been recently identified in a study using a murine model [38]. Penn *et al.* observed that BM transplantation from spontaneously hypertensive rats into normotensive rats resulted in a marked increase in blood pressure; however, treating the rats with minocycline, a second-generation semisynthetic tetracycline and microglial activation inhibitor with anti-inflammatory effects, reversed the hypertension [92]. The underlying mechanism involves an increase in the production of inflammatory cells and cytokines which translates into a stronger neuroinflammatory response and increased activity of the sympathetic nervous system. The association between haematopoietic (i.e. BM-derived) stem cells and hypertension indicates that BM-MSC therapy may have potential applications in treating obesity, diabetes mellitus (DM) and hypertension [93].

In a clinical context, attempts have been made to use BM-MSCs to alleviate heart failure, address cardio myocyte loss to improve heart function and prevent end-stage heart failure. Although clinical trials in both human and animal models have been promising, a meta-analysis of 10 large-scale randomised phase two trials using BM-derived cells showed negligible benefits after an acute myocardial infarction [93,94]. In preclinical and clinical studies, intravenous, intracoronary or intramyocardial routes represent the main options for cell delivery within minutes to a few months after a myocardial infarction. [94]. However, protocol

variations impede the accurate interpretation of preclinical and clinical trial results [41,42]. Forest *et al.* compared cellular retention and engraftment with *in vivo* imaging in a porcine model using different administration routes [93]. Following an intracoronary injection, the safest and easiest delivery method, $34.8 \pm 9.9\%$ of cells were detected in the heart after one hour; this proportion declined precipitously to $6.0 \pm 1.7\%$ after 24 hours [93]. Inconsistent results regarding the benefits of BM cell therapy in cardiac function may be due to variability in cell survival, engraftment and differentiation [94].

Paracrine effects

Paracrine effects refer to the effects of signaling molecules produced by one cell on nearby cells. Mesenchymal stem cells (MSCs) are known to produce a variety of signaling molecules, such as growth factors, cytokines, and extracellular vesicles, that can act on nearby cells and tissues to promote healing and tissue regeneration [95].

MSCs can secrete various growth factors, such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and transforming growth factor-beta (TGF- β), which can stimulate cell proliferation, differentiation, and migration. These growth factors can also promote angiogenesis, or the formation of new blood vessels, which is essential for tissue repair and regeneration [96].

MSCs can also produce cytokines, such as interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF- α), which can modulate the immune response and reduce inflammation. By secreting these cytokines, MSCs can promote tissue repair and regeneration while also reducing the risk of inflammation and tissue damage.

Extracellular vesicles, which are small membrane-bound structures released by cells, are another important paracrine signaling mechanism used by MSCs. These vesicles contain various signaling

molecules, such as microRNAs and growth factors, which can be taken up by nearby cells to promote tissue repair and regeneration.

Finally, the paracrine effects of MSCs are thought to be a major mechanism underlying their therapeutic potential. By producing a variety of signaling molecules that can act on nearby cells and tissues, MSCs can promote tissue repair and regeneration and reduce inflammation and tissue damage [97].

Challenges and limitations of MSC therapy in veterinary medicine

Safety concerns:

Although mesenchymal stem cells (MSCs) have shown great promise as a potential therapy for various diseases and injuries, there are still some safety concerns that need to be addressed. Some of the main safety concerns include the following:

1. Tumorigenic potential: There is a theoretical risk that MSCs could become cancerous or contribute to the formation of tumors. However, numerous studies have shown that MSCs have a low risk of transformation or tumorigenic potential.
2. Immune response: Although MSCs have immunomodulatory properties that can be beneficial in certain conditions, there is a risk that they could trigger an immune response in the recipient. This could lead to an unwanted inflammatory reaction or rejection of the transplanted cells.
3. Infection transmission: MSCs are typically harvested from either the patient's own tissues or from a donor, and there is a risk of infection transmission if appropriate screening and sterilization procedures are not followed.
4. Embolism: There have been reports of MSCs causing blockages in small blood vessels, which can lead to tissue damage or even death in rare cases.
5. Ethical concerns: There are ethical concerns surrounding the use of embryonic stem cells, which are typically derived from embryos that are no longer needed for reproductive purposes. However, MSCs can be obtained from adult tissues and

therefore do not raise the same ethical concerns [98]. To mitigate these safety concerns, researchers and clinicians are working to establish best practices for the collection, preparation, and administration of MSCs. In addition, clinical trials are ongoing to evaluate the safety and efficacy of MSCs in various therapeutic applications.

Standardization of protocols:

Standardization of protocols is a critical aspect of ensuring the safety and efficacy of mesenchymal stem cell (MSC) therapies in veterinary medicine. Standardization involves establishing consistent procedures for the collection, processing, and administration of MSCs, as well as defining the parameters for evaluating their effectiveness.

The International Society for Cellular Therapy (ISCT) has established guidelines for the characterization and quality control of MSCs. These guidelines include criteria for defining MSCs based on their immunophenotype, differentiation potential, and other characteristics. Adherence to these guidelines can help ensure that the cells used in MSC therapies are of a consistent quality and have the expected biological properties [99]. In addition, there are ongoing efforts to standardize the methods used to isolate and culture MSCs. These methods can vary widely depending on the tissue source of the cells and the specific conditions used for their expansion. Standardizing these methods can help ensure that the resulting cells are consistent in terms of their properties and potency. Standardization of protocols is also important for defining the optimal doses and routes of administration for MSC therapies. Dosage and delivery methods can have a significant impact on the safety and efficacy of MSC therapies, and standardized protocols can help ensure that these factors are carefully controlled [100]. All in all, standardization of protocols is essential for ensuring the safety and effectiveness of MSC therapies in veterinary medicine. By establishing

consistent procedures for the collection, processing, and administration of MSCs, researchers and clinicians can maximize the potential benefits of these therapies while minimizing the risks to animal health.

Cost-effectiveness:

The cost-effectiveness of mesenchymal stem cell (MSC) therapies in veterinary medicine is an important consideration, as it can influence their adoption and availability in clinical practice. The cost of MSC therapies can vary widely depending on several factors, including the source and type of cells, the processing and administration methods, and the regulatory requirements [101]. Despite the potential benefits of MSC therapies, they can be expensive compared to conventional treatments, such as surgery or medication. However, MSC therapies may be cost-effective in certain situations, such as treating conditions that have limited treatment options or that require extensive and costly long-term management.

One potential advantage of MSC therapies is that they may reduce the need for other costly treatments or interventions, such as repeated surgeries or hospitalizations. In addition, MSC therapies may have long-term benefits that can reduce the overall cost of care, such as improved tissue regeneration and reduced risk of complications.

The cost-effectiveness of MSC therapies can also be influenced by the regulatory environment and reimbursement policies. In some countries, MSC therapies may be subject to strict regulatory requirements that can increase their cost and limit their availability. In addition, reimbursement policies may vary depending on the specific condition being treated, the availability of alternative treatments, and other factors [102].

Future directions and conclusion

Potential for combination therapies:

Combination therapies involving mesenchymal stem cells (MSCs) and other treatments have the potential to improve the

effectiveness of treatment for a wide range of conditions in veterinary medicine. By combining MSCs with other therapies, it may be possible to enhance their regenerative and immunomodulatory properties and improve their overall therapeutic potential. One potential area for combination therapies is in the treatment of orthopedic conditions in companion animals. Studies have shown that combining MSCs with growth factors or scaffolds can enhance their ability to regenerate damaged tissues and promote healing. Similarly, combining MSCs with non-steroidal anti-inflammatory drugs (NSAIDs) or other pain medications may help to reduce inflammation and pain and improve the overall outcome of treatment [103]. In addition, combination therapies involving MSCs and other stem cell types are being explored as a means of improving their regenerative potential. For example, combining MSCs with induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) may help to enhance their ability to differentiate into various cell types and promote tissue repair. Combination therapies involving MSCs and other treatments are also being explored for the treatment of respiratory, gastrointestinal, and immune-mediated diseases in veterinary medicine. For example, combining MSCs with antibiotics or anti-inflammatory drugs may help to improve the efficacy of treatment for respiratory infections, while combining MSCs with probiotics or dietary supplements may help to improve gut health in livestock [104].

Research directions for optimizing MSC therapy:

As research on the use of mesenchymal stem cells (MSCs) in veterinary medicine continues to advance, there are several key directions that are being pursued to optimize the efficacy, safety, and accessibility of MSC therapy. First, Standardization of protocols: The development of standardized protocols for the isolation, expansion, and administration

of MSCs is crucial for ensuring consistency and reproducibility across studies. Standardization can help to reduce variability in outcomes and improve the reliability of results, ultimately facilitating the translation of MSC therapies into clinical practice, second; Optimization of dosing and delivery: The optimal dose and mode of delivery of MSCs may vary depending on the specific condition being treated, the type of cells used, and other factors. Researchers are exploring different dosing regimens and delivery methods to determine the most effective and efficient means of administering MSCs for various conditions, third; Identification of ideal sources of MSCs: There are several potential sources of MSCs, including bone marrow, adipose tissue, and umbilical cord tissue. Researchers are investigating the differences in properties and therapeutic potential of MSCs derived from different sources to identify the most effective and practical sources for specific applications, fourth; Enhancing homing and engraftment: One challenge of MSC therapy is ensuring that the cells reach and remain in the target tissues long enough to exert their therapeutic effects. Researchers are exploring ways to enhance the homing and engraftment of MSCs, such as by modifying their surface markers or by combining them with other therapies that promote tissue regeneration, fifth; Addressing safety concerns: Although MSCs are generally considered safe, there are still concerns about their potential to cause adverse effects such as immune rejection, tumorigenicity, and infection transmission. Researchers are investigating ways to mitigate these risks, such as by optimizing the selection and characterization of MSCs, improving quality control measures, and developing strategies for monitoring and managing potential side effects [105].

Importance of continued investigation and development of MSC therapy in veterinary medicine

The continued investigation and development of MSC therapy in veterinary medicine is critical for several reasons: first, Improved animal health and welfare: MSC therapy has shown promise as a safe and effective treatment for a variety of conditions in animals, including orthopedic injuries, respiratory diseases, and immune-mediated disorders. By further optimizing and refining this approach, veterinarians can provide better care and treatment options for their patients, ultimately improving animal health and welfare; second, Advancement of translational medicine: MSC therapy represents a prime example of translational medicine, in which research on the cellular and molecular mechanisms of disease is translated into practical therapies for patients. Continued investigation and development of MSC therapy in veterinary medicine can help to advance this field and facilitate the translation of new discoveries into clinical practice; third, Potential for human health applications: Because many of the conditions treated with MSC therapy in animals are similar to those seen in humans, this research may also have implications for human health. By further exploring the safety and efficacy of MSC therapy in veterinary medicine, researchers may gain valuable insights into the potential applications of this approach in human medicine, fourth, Economic benefits: By improving animal health and welfare, MSC therapy has the potential to reduce the economic burden of veterinary care and improve the productivity and profitability of livestock industries. Further development of this therapy may help to generate new economic opportunities and promote sustainable animal agriculture [106].

CONCLUSION

MSCs have been investigated for their therapeutic potential in a wide range of human and animal diseases, including orthopedic conditions, cardiovascular diseases, neurological disorders, and immune-mediated diseases. MSCs have been used to treat a variety of conditions in different species, including horses, dogs, cats, and livestock in veterinary medicine [107].

MSCs represent a heterogenous population of adult, fibroblast-like multipotent cells. MSCs have drawn much attention during the last decade in the field of regenerative medicine, mainly due to their capacity to differentiate into specific cell types, abundant production of soluble growth factors and cytokines, and hematopoiesis supporting properties. In addition, MSCs can migrate to the sites of inflammation and hold potent immunomodulatory and anti-inflammatory effects through cell and cell interactions between MSCs and lymphocytes or production of soluble factors. Therefore, the application of MSCs in many disease situations is full of possibilities for future clinical treatment. Phase III clinical trials have been run using MSCs for treatment of Graft versus Host Disease (GVHD), and MSCs product approval has been achieved for pediatric GVHD treatment in Canada and New Zealand (Prochymal®; Osiris Therapeutics). In addition, hundreds of clinical trials are being run using MSCs for treatment of several immune mediated diseases, including GVHD, aplastic anemia (AA), Crohn's disease (CD), rheumatoid arthritis (RA), and multiple sclerosis (MS). In this review we mainly focus on immunomodulation potential of MSCs and promising therapeutic application of MSCs in immune mediated diseases. Furthermore, we emphasize that

biological effectiveness of MSCs will be one of most important standards to determine the dose of MSCs infusion [108].

In addition, MSCs have been shown to have a direct effect on tissue repair and regeneration. They can differentiate into various cell types, including bone, cartilage, and muscle, and can secrete growth factors and cytokines that stimulate the growth and repair of damaged tissues. This property has been harnessed for the treatment of orthopedic conditions, such as joint injuries and fractures, as well as for tissue repair in other organs, such as the heart and liver. mesenchymal stem cells (MSCs) are adult stem cells found throughout the body that share a fixed set of characteristics. Discovered initially in the bone marrow, this cell source is considered the gold standard for clinical research, although various other sources—including adipose tissue, dental pulp, mobilised peripheral blood and birth-derived tissues—have since been identified. Although similar, MSCs derived from different sources possess distinct characteristics, advantages and disadvantages, including their differentiation potential and proliferation capacity, which influence their applicability. Hence, they may be used for specific clinical applications in the fields of regenerative medicine and tissue engineering. This review article summarises current knowledge regarding the various sources, characteristics and therapeutic applications of MSCs.

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

All authors equally contributed.

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