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COMPREHENSIVE REVIEW

Adipose-Derived Stem Cells in Veterinary Medicine: Characterization and Therapeutic Applications

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Mesenchymal stem cells, considered one of the most promising cell types for therapeutic applications due to their capacity to secrete regenerative bioactive molecules, are present in all tissues. Stem cells derived from the adipose tissue have been increasingly used for cell therapy in humans and animals, both as freshly isolated, stromal vascular fraction (SVF) cells, or as cultivated adipose-derived stem cells (ASCs). ASCs have been characterized in different animal species for proliferation, differentiation potential, immunophenotype, gene expression, and potential for tissue engineering. Whereas canine and equine ASCs are well studied, feline cells are still poorly known. Many companies around the world offer ASC therapy for dogs, cats, and horses, although in most countries these activities are not yet controlled by regulatory agencies. This is the first study to review the characterization and clinical use of SVF and ASCs in spontaneously occurring diseases in veterinary patients. Although a relatively large number of studies investigating ASC therapy in induced lesions are available in the literature, a surprisingly small number of reports describe ASC therapy for naturally affected dogs, cats, and horses. A total of seven studies were found with dogs, only two studies in cats, and four in horses. Taken as a whole, the results do not allow a conclusion on the effect of this therapy, due to the generally small number of patients included, diversity of cell populations used, and lack of adequate controls. Further controlled studies are clearly needed to establish the real potential of ASC in veterinary medicine.

Isolation and Characterization of Adipose-Derived Stem Cells

It is currently well established that all tissues and organs have their own compartments of stem cells. Their primary function is to replace cells that are lost during normal wear and tear, but they are also very important in the repair of tissues damaged by disease and trauma. This natural capacity of adult stem cells is the basis for their use in cell therapy protocols.

Actually, adult stem cells have been therapeutically used for half a century, in bone marrow transplantation for hematological diseases. Around 15 years ago, when studies focused on the nature of other types of adult stem cells, their potential for treating non-hematological diseases, such as heart diseases, began to be explored. The initial studies used the same procedure, bone marrow transplantation, so that the results were interpreted as an evidence for the great plasticity of the bone marrow stem cells [1]. Soon after that, stromal stem cells present in the bone marrow were acknowledged as the main therapeutic agent for non-hematological diseases [2].

The possibility to isolate stromal cells from the bone marrow was first demonstrated in the late 1960s [3]. Subsequent studies showed that these adherent cells were capable of renewing themselves and displayed trilineage differentiation potential (adipogenic, osteogenic, and chondrogenic), and the term mesenchymal stem cell (MSC) was popularized in the early 1990s [4].

MSCs are defined as adult stem cells of mesodermal origin and great plasticity. They reside in a perivascular niche, which explains their presence in virtually all tissues and organs [5], and are believed to derive from pericytes, perivascular cells with cellular processes that wrap endothelial cells in blood vessels [6]. Tissue-resident MSCs are poorly characterized, so that what is known about these cells is more due to their performance in vitro than in vivo. To help standardize the study of these cells, the International Society for Cellular Therapy established minimum criteria for their definition. The first relates to their capacity to proliferate as adherent cells in culture systems. The second characteristic refers to the ability of in vitro differentiation into osteoblasts, adipocytes, and/or chondroblasts. Finally, the cells must be positive for CD73, CD90, and CD105, and negative (>95% of the cells in culture) for CD14 or CD11b, CD34, CD45, CD19, or CD79 alpha [7].

Although MSCs first attracted attention due to their ability to differentiate into various cell types, they were later found to be capable of secreting a large number of bioactive

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molecules, which are responsible for trophic, antiapoptotic, immunomodulatory, angiogenic, and antiscar effects [8,9]. Their therapeutic potential is currently explained by the production of these bioactive molecules, which provide a regenerative microsystem and result in a regenerative response that restores the normal morphology and function of the tissue. MSCs have a further interesting characteristic, related to the capacity to exert immunoregulatory effects on cells of adaptive and innate immunity, such as T and B lymphocytes, dendritic cells, natural killer cells, and monocytes [10].

The bone marrow was the first source of MSCs to be used, but the fact that these cells are present in all types of tissues suggested that other tissues could be more easily available, and in 2001 they were identified in the adipose tissue [11]. The tissues are processed differently: bone marrow is centrifuged through Ficoll-Hypaque to generate the mononuclear fraction, whereas the adipose tissue is digested with an enzyme such as collagenase to generate the stromal vascular fraction (SVF). Among many other cell types, stem cells are present in these two fractions, and mesenchymal-type stem cells have been shown to be up to 300 times more abundant in the adipose tissue than in the bone marrow [12]. Minimal criteria to define the stem cells present in the SVF [adipose-derived stem cells (ASCs)] have recently been proposed by the International Federation of Adipose Therapeutics and International Society for Cellular Therapy [13]. Similar to the criteria for defining MSCs, ASCs are characterized by growing as adherent cells in culture, displaying trilineage differentiation potential, and being phenotypically positive for CD13, CD73, CD90, and CD105, and negative for CD31, CD45, and CD235a. CD34 in ASCs is still a controversial marker of ASCs, and has been defined as ''primary unstable positive…present at variable levels'' [13]. It is possible that this marker is present in freshly isolated cells, but lost after long-term cultivation [14]. Our group reported recently a greater potential of osteogenic differentiation of ASCs than bone marrow MSCs [15]. However, in a careful review on in vivo and in vitro studies comparing the osteogenic potential of bone marrow- and adipose tissue-derived MSCs, Liao and Chen described controversial results [16].

ASCs are attracting great attention for their potential application in cell therapy [17] and tissue engineering procedures [18–20]. In a recent review, Lim et al. described the characterization of human ASCs, the production and banking of clinical-grade cells, legal and regulatory issues of cell therapy in different countries, and details on the 87 ASC clinical trials currently registered in clinicaltrial.gov [21]. The present review aims to discuss the characterization and clinical use of ASCs in veterinary medicine.

Canine, Feline, and Equine Adipose-Derived Stem Cells

Adipose-derived cultivated stem cells have been characterized in different animal species. Following the criteria proposed for the definition of human ASCs [13], the characteristics more frequently investigated are their proliferation as adherent cells, the potential to differentiate along the osteogenic, adipogenic, and chondrogenic lineages, and their immunophenotype. Immunophenotyping is more limited due to the lack of many of the antibodies necessary for the definition of the surface profile in some of the species, but cross-reactive antibodies can be useful [22].

Although the implementation of methods to isolate, expand, and characterize ASCs has provided initial information allowing their use in cell therapy and tissue engineering procedures, there are still issues with inconclusive or controversial results. Our understanding on the biology of these cells is incomplete, and further studies are needed for the design of adequate protocols in regenerative medicine.

Canine adipose-derived stem cells

Wu et al. were the first to report the establishment of preadipocytes from the canine SVF [23]. Conditions for the cultivation of adherent cells were described, and the SVF isolated from several adipose sites were characterized for adipocyte differentiation: inguinal > abdominal-subcutaneous > perirenal > omental. Since then, a number of studies have described the isolation and characterization of canine ASCs. Cultures were mainly characterized for the frequency of stem cells, morphology, proliferation and differentiation. Neupane et al. reported for the first time the adaptation of methods to isolate human ASCs for the derivation of cells from canine adipose tissues, showing their expansion as adherent cells, expression of pluripotency genes, and differentiation into osteoblasts and adipocytes [24]. Later studies showed that, besides being capable of in vitro expansion for extended periods with stable population doubling and low senescence levels, the cells have the potential to differentiate into adipogenic, chondrogenic, myogenic, and osteogenic lineages, present a surface profile characteristic of mesenchymal origin, and express pluripotency genes, including *Nanog*, *Oct4*, and *SOX2* [25,26]. The recent production of canine-specific antibodies allowed a more detailed characterization of the surface profile of canine ASCs showing that, similar to bone-derived MSCs, the cells are positive for CD44, CD90, and MHC I, and negative for CD14, CD29, and MHC II [27].

Some aspects that are important for the clinical use of stem cells are being investigated, although the data are still scarce. Chung et al., for instance, observed that cultivation of canine ASCs with reduced oxygen levels inhibits cell proliferation and differentiation, which should be considered when using the cells in a potentially hypoxic environment such as a fracture site [28]. The possibility to avoid the use of fetal bovine serum (FBS) was explored by the cultivation of canine ASCs with a serum-free medium supplemented with a serum substitute [29]. The adipogenic and osteogenic differentiation potential was not modified, but the proliferation rate was higher in the culture medium with FBS. Cryopreservation of ASCs, which is also relevant in the clinical setting, has been shown not to affect the original morphology, immunophenotype, and differentiation potential of the cells, in spite of inducing a slight decrease in the proliferation ratio and telomerase activity [30].

Another important aspect refers to the level of in vitro expansion of stem cells before use. Although Vieira et al. reported the expansion of ASCs up to 10 passages without karyotypic alterations [25], Lee et al. showed that the population doubling level and differentiation potential of canine ASCs decreased after the fifth passage [31]. This study also showed a progressively decreased expression of canine ASC

markers along passages, as well as a decreased osteogenic differentiation potential. These results suggest that canine ASCs should not be expanded further than five passages before therapeutic use.

Finally, the establishment of optimal shipping conditions is important for the delivery of stem cells to distant places. Bronzini et al. analyzed the impact of several experimental conditions on the number, gene expression, acquired resistance to apoptosis, and beta-galactosidase activity of canine ASCs, observing that only time and temperature influenced ASC number during the experimental shipping conditions [32]. The results indicate that canine ASCs should be delivered in phosphate buffered saline at room temperature, in a period of 9–12 h.

Interestingly, canine ASCs have attracted attention as donor cells for somatic cell nuclear transfer. One of the key factors affecting the efficiency of the procedure is the tissue origin and extent of differentiation of the nuclear donor, and fibroblasts have shown efficacy of cloning [33]. However, fetal fibroblasts proved more competent, due to higher ability to proliferate in culture and be transfected [34]. As the differentiation status of the donor cells is important for the success of cloning, stem cells are natural candidates for the procedure. Oh et al. reported for the first time the production of cloned beagles by nuclear transfer of ASCs [35], and the procedure has been improved since then by modification of variables such as the culture medium used to expand ASCs [36].

Feline adipose-derived stem cells

Very few studies have investigated the isolation and characterization of feline mesenchymal or ASCs. In the first reports describing the isolation of feline ASCs, Quimby et al. [37] and Webb et al. [38] observed that ASCs had normal mesenchymal-type morphology, trilineage differentiation potential, and were easier to culture and expand than bone marrow-derived MSCs. Immunophenotyping showed that the cells were positive for CD44, CD90, and CD105, and negative for CD4 and MHC class II. These results were confirmed in a later study with a larger antibody panel, showing that the cells were positive for CD44, CD90, CD105, and alpha-smooth muscle actin, and negative for CD14, CD34, and CD45 [39]. This study also showed that feline ASCs have the potential to differentiate into smooth muscle cells.

A few other studies have described feline bone marrow-derived MSCs [40–42]. Kono et al. further reported on the generation of feline mature adipocyte-derived dedifferentiated fat cells from the floating adipocyte fraction obtained after collagenase digestion of mature adipose tissue [39]. All these cell populations exhibit similar morphology, immunophenotype, and proliferation and differentiation potential.

Equine adipose-derived stem cells

Although equine bone marrow-derived MSC began to be investigated in the 1990s [43], the first study on equine ASCs was published in 2007 [44]. The methods were based on the techniques described for human ASC isolation, and resulted in the establishment of cultures with spindle-shaped cells able to differentiate into the adipogenic and osteogenic lineages. Other studies reproduced or extended these results.

Although highly variable, the proliferation rate of equine ASCs is higher than that of bone marrow-derived cells [45–47]. Other factors, including serum, autologous platelet lysate, basic fibroblast growth factor, focused extracorporeal shock wave therapy, and exposure to a static magnetic field, have been shown to stimulate the proliferation of equine ASCs [48–53]. Equine ASCs display trilineage differentiation potential, but their chondrogenic and osteogenic potential seems to be lower than that of bone marrow MSCs in conventional cultures [9,49,54,55], or for cells associated to biomaterials [56,57]. Supplementation of the culture medium with vitamin C, platelet-rich plasma (PRP), and B-cell activating factor have been shown to alter the differentiation potential of equine ASCs in vitro [58,59].

Although initial immunophenotyping studies were limited due to the low number of equine-specific antibodies available [60], these cells are now well characterized as positive for CD29, CD105, CD44, CD90, CD140b, and CD164 and with low or negative expression of CD34, CD45, CD73, and MHC II [47,61–64]. Interestingly, Ranera et al. described the absence of CD44 and CD105 in equine ASCs [65]. Penny et al. suggested that CD90 and CD34 can be seen as positive and negative markers, respectively [66].

Equine ASCs were used for the investigation of the in vivo niche of MSCs [63]. Similar to bone marrow-derived MSCs [5], a perivascular niche was suggested for ASCs, and a relationship between MSC frequency and blood vessel density was identified.

The potential of equine ASCs for tissue engineering has also been explored. The analysis of chondrogenic differentiation of equine ASCs on 3D biodegradable poly(lactide-co-glycolide) matrices showed changes in mechanical properties of tissue constructs, including the extracellular matrix content [67]. ASCs were shown to differentiate into chondrogenic, osteogenic, and adipogenic lineages and form extracellular matrix on hydrogel [56] and collagen scaffolds [57, 68–70].

Types of Adipose-Derived Stem Cells Used for Therapy

As in humans, therapeutic protocols in veterinary medicine use MSCs for non-hematological diseases. The cells are generally isolated from the adipose tissue, and are thus characterized as ASCs. Two basic types of approaches can be used (Fig. 1), as described below.

Stromal vascular fraction

The adipose tissue is composed of mature adipocytes embedded into an extracellular matrix where several other cell types are also present [71]. The incubation of the fragmented adipose tissue (or liposuction material) with an enzyme such as collagenase results in the digestion of the extracellular matrix and release of the cells [72]. The suspension is then centrifuged to separate the floating population of mature adipocytes from the pellet which contains the remaining cells and is called the SVF. The frequency of the ASC in the SVF has been estimated in around 2%–10% [73]. An automated image cytometry method for canine SVF analysis was recently established, and validated by comparison with other methods such as hemocytometer or flow cytometer counting [74].

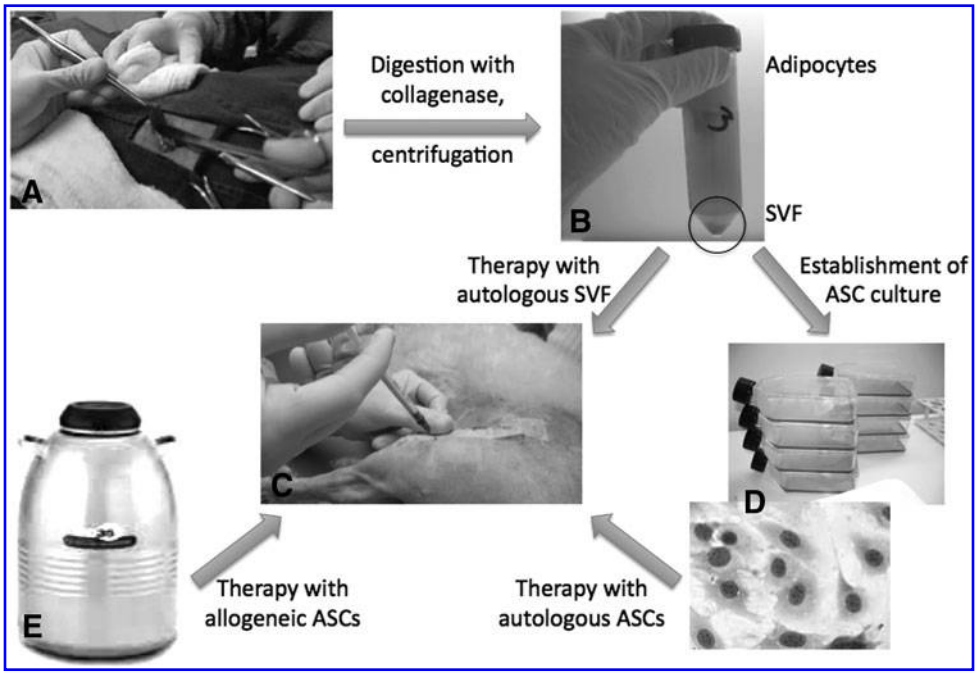

FIG. 1. Procedures for therapy with stem cells from adipose tissue. **(A)** Subcutaneous adipose tissue is collected from the patient. **(B)** After mincing, incubation with collagenase and centrifugation, mature adipocytes are separated from the stromal vascular fraction (SVF), which contains stem cells and other cellular components. **(C)** The SVF may be used directly for treating the patient, by systemic injection or at the site of injury. **(D)** The SVF may also be used for the establishment of adipose-derived stem cell (ASC) cultures, which are used for treating the patient in an autologous context. **(E)** Cryopreserved ASCs may also be used, after a short culture period, to treat patients in an allogeneic context.

In this therapeutic approach, a sample of subcutaneous adipose tissue is collected from the patient (most generally, from the inguinal region in dogs and cats, and from the tail base in horses) and transported to the laboratory, where the SVF is isolated. Viable cells are counted, and generally a sample is separated for further characterization. The cells are then suspended in a culture medium or saline for administration to the patient, in numbers and volumes to be determined.

Adipose-derived stem cell

For isolation of ASCs, the SVF is resuspended in a culture medium complemented with FBS, plated in culture flasks or dishes, and incubated at 37°C in a humidified atmosphere containing 5% CO_2. Nonadherent cells are removed 48 h later, and the adherent layer is refed every 3 or 4 days, being split and expanded as necessary. ASCs thus represent a purified population of the adherent stem cells present in the adipose tissue, since all other cell types are removed or die with time. Long-term cultivation of ASCs before therapeutic use is not recommended, since the cells may lose their progenitor characteristics [75].

As discussed below, the use of allogeneic ASCs has been proposed, since these cells have immunoregulatory properties [76,77]. This would allow the use of species-specific, allogeneic cryopreserved ASCs, avoiding the need for collection of adipose tissue from the patient.

In this second approach, when autologous ASCs are used, the adipose tissue is collected 2 or 3 weeks before treatment, and the animal receives the cultivated cells. When allogeneic cells are used, there is no need for further intervention to collect adipose tissue from the patient.

Adipose-Derived Stem Cell Therapy in Veterinary Medicine

For many years, different species of animals were used as models for human diseases in preclinical stem cell therapy studies. Starting around 10 years ago [78], stem cell therapy began also to be used in animal patients. A number of companies (Table 1) have established around the world, offering MSC therapy for dogs, cats, and horses. In most countries, cell therapies in veterinary patients are not controlled by regulatory agencies [79]. Although the use of unproven therapeutic approaches is better accepted in animals than in humans, there is a general expectancy for the establishment of regulations for these procedures for veterinary patients [80].

A few institutional clinical trials are being developed with cell therapy for veterinary patients, including for instance Veterinary Cancer Trials (Veterinary Cancer Society); Colorado State University's James L. Voss Veterinary Teaching Hospital; Veterinary Clinical Investigations Center (University of Pennsylvania); and UC Davis Veterinary Medical Teaching Hospital. A relatively large number of studies have described the results of stem cell therapy for artificially induced conditions in dogs, cats, and horses [81,82]. The use of animals as models for diseases, with surgically or chemically induced lesions, can provide valuable information on specific aspects of available therapies. In one of these studies, for instance, lesions were induced in the superficial digital flexor tendon (SDFT) of both forelimbs of healthy horses, by intratendinous injection of collagenase [83]. Autologous ASCs labeled with nanocrystals were implanted in one of the limbs, and biopsies of both forelimb SDFTs were performed 2 weeks later. Fluorescence microscopy of the biopsies revealed the presence of labeled cells only in the treated SDFTs showing that, despite some migration of labeled cells to the blood stream (13 ± 18 labeled cells in 1×10^6 peripheral blood mononuclear cells, 72 h after treatment), the cells homed to the injured tissue where they were injected. While these information are important, induced lesions cannot adequately reproduce the conditions present in spontaneously affected patients. Acquired diseases or trauma affect many more aspects of the

Table 1. Some of the Main Companies Offering Adipose-Derived Stem Cells for Veterinary Medicine Applications

Company	*Country*	*Electronic contact*
Animal Cell Therapies	United States	www.actcells.com
Austinburg Veterinary Clinic	United States	www.austinburgvetclinic.com
Australian Veterinary Stem Cells	Australia	www.australianstemcells.com.au
BIO–Bio Tecnologia Animal	Brazil	www.labbiocell.com
CellMed Medicina Regenerativa Ltda	Brazil	www.cellvet.com.br
Celltrovet Medicina Veterinária Regenerativa	Brazil	www.celltrovet.com.br
Fat Stem	Belgium	www.fat-stem.be
Innova Celulas Madres	Costa Rica	www.es-es.facebook.com/InnovaCelulasMadre
Medivet America	United States	www.medivet-america.com
RenovoCyte	United States	www.renovocyte.com
Stemcellvet UK	England	www.stemcellvet.co.uk
Stemlogix, LLC	United States	www.facebook.com/stemlogix
Stemvet NZ Ltd.	New Zealand	www.stemvet.co.nz
Tierärztliche Klinik für Pferde Grosswallstadt	Germany	www.pferdeklinik-grosswallstadt.de/
Vet-Stem Regenerative Veterinary Medicine	United States	www.vet-stem.com
Vetbiobank	France	www.vetbiobank.com
Veterinaria Portal Mayor	Chile	www.veterinariaportalmayor.cl

organism, so that the answer to an experimental therapy may be very different. Furthermore, it is now well established that, in humans [84,85] as well as in animals [86,87], autologous stem cell compartments are affected by several conditions of the organism, including age and disease.

The need for a separate analysis of the therapeutic potential of adipose tissue- and bone marrow-derived MSCs in veterinary medicine is stressed by in vitro studies that show differences between these two cell populations. ASCs show higher proliferative potential [34,35,42–44] and lower chondrogenic and osteogenic potential [31,42,49,57] than bone marrow MSCs. A lower osteogenic potential is also shown by a different level of expression of the *MMP2* gene, which is 3.5-fold higher in bone marrow- than in adipose-derived cells (27). Furthermore, these cells seem to use different types of immunoregulatory mechanisms [88]. Some controversial results are still reported [45,60,89], indicating the need for further characterization of these cell populations. However, these results suggest the existence of biological differences which probably have consequences for their activity in the therapeutic setting.

Therefore, the present review focused on the reports describing the results of adipose-derived stem cell therapy in spontaneously occurring diseases in veterinary patients. In spite of the thousands of animals reported as treated by stem cell companies around the world, a surprisingly small number

Table 2. Veterinary Clinical Studies in Dogs with Autologous or Allogeneic Stromal Vascular Fraction Cells or Cultivated Adipose-Derived Stem Cells

Ref.	*Pathology*	*Treatment*	n	*Control*	*Evaluation*	*Conclusion*
26	Osteoarthritis of humeroradial joints	Aut ASCs + PRP	4	No	Clinical, 30 days	Treatment induced functional improvements
89	Hip dysplasia	Aut SVF or allo ASCs	9	No	Clinical, up to 30 days	Treatments induced clinical improvement, with a possible advantage of autologous SVF
90	Coxofemoral joint osteoarthritis	Aut SVF	18	Yes	Orthopedic, up to 90 days	SVF induced significant clinical improvement
91	Chronic osteoarthritis of humeroradial joints	Aut SVF	14	No	Orthopedic, up to 180 days	SVF induced clinical improvement
92	Atopic dermatitis	Aut ASCs	5	No	Clinical, up to 12 weeks	Treatment had no effect
93	Chronic osteoarthritis associated with hip dysplasia	Aut ASCs + PRP	8	No	Gait analysis using a force platform, up to 180 days	Treatment reduced lameness
94	Severe osteoarthritis	Aut ASCs	14	No	Gait analysis using a force platform, up to 180 days	Treatment reduced lameness, but effect was not maintained after 90 days

PRP was also used in some of the studies. The number of patients (*n*) and the existence of a control group (control) are also indicated. ASCs, adipose-derived stem cells; Allo, allogeneic, Aut, autologous; PRP, platelet-rich plasma; SVF, stromal vascular fraction.

Table 3. Veterinary Clinical Studies in Cats with Autologous or Allogeneic Cultivated Adipose-Derived Stem Cells

Ref.	*Pathology*	*Treatment*	n	*Control*	*Evaluation*	*Conclusion*
37	Chronic kidney disease	Aut ASCs	3	No	Glomerular filtration rate (GFR) through nuclear scintigraphy, up to 30 days	Treatment induced modest improvement in GFR
95	Chronic kidney disease	Allo ASCs, in different doses	9	No	Biochemical and nuclear scintigraphy analyses, up to 8 weeks	Adverse gastric and respiratory reactions related to the infusion of higher numbers of cryopreserved cells immediately after thawing, but not after the cells were cultivated; no clinical improvement was observed

The number of patients (*n*) and the existence of a control group (control) are also indicated.

of studies have been published in indexed scientific journals. The PubMed and SciELO databases were searched up to July 2014 using the subject keywords mesenchymal, adipose, stem, cell combined with canine, dog, feline, cat, equine, or horse in individual searches. The publications found in this search are summarized below.

Adipose-derived stem cell therapy in dogs

A total of seven studies describing adipose-derived stem cell therapy in dogs, published by five research groups, were found in the literature (Table 2). Six of them treated orthopedic conditions, reporting in general ''clinical improvement'' after the therapy. The efficacy of the treatments was more quantitative in only two of these studies, in which a force platform measure lameness due to osteoarthritis [93,94]. In one study, atopic dermatitis was treated with no effect [92].

Several other limitations are observed in these reports. Only one of the studies used allogeneic cells, but although no adverse effects were observed in the patients, a detailed search for the existence of immunologic reactions was not conducted [89]. The other studies used autologous ASCs or SVF cells, but only one of them included a control group, reporting significant differences in clinical assessment scores [90]. In two reports, ASCs were combined with PRP, without a control for the effect of cells alone. One of these studies [93] was conducted by a group that, in sequence, published a similar report with ASCs alone [94]. In both studies the treatment improved limb functions, whereas this effect was prolonged beyond 6 months when ASCs were combined with PRP, and it started to decrease after 3 months when the cells were used alone.

Adipose-derived stem cell therapy in cats

Only two studies, by the same group, were found with adipose-derived stem cell therapy in spontaneously affect cats, with very inconclusive results (Table 3). Animals were treated for chronic kidney disease, with assessment of results by biochemical and nuclear scintigraphy analyses. No control groups were included. The first study used autologous ASCs resulting in modest improvement of

Table 4. Veterinary Clinical Studies in Equines with Autologous or Allogeneic Cultivated Adipose-Derived Stem Cells

Ref.	*Pathology*	*Treatment*	n	*Control*	*Evaluation*	*Conclusion*
50	Tendonitis	Allo ASCs + PRP	16	No	Clinical and ultrasonographic, up to 240 days	Treatment induced functional recovery in 14 animals
96	Superficial digital flexor tendonitis	Allo ASCs + PRP	19	No	Clinical and ultrasonographic, up to 24 months	Treatments induced complete regeneration of injured tendons ($n = 19$) and return to previous level of competition ($n = 17$)
97	Endometriosis	Allo ASCs	6	Yes	Histopathology, immunohistochemistry, confocal microscopy, up to 60 days	Treatments induced positive remodeling of endometrial tissue
98	Bone spavin	Aut ASCs	16	Yes	Clinical and scintigraphic, up to 180 days	Treatment induced improvement in clinical conditions and inflammatory reaction

PRP was also used in some of the studies. The number of patients (*n*) and the existence of a control group (control) are also indicated.

renal function in two of the four cats treated. In the second study, no clinical improvement was observed after treatment of nine cats with allogeneic ASCs. Adverse reactions were seen in animals receiving higher doses of cryopreserved cells injected immediately after thawing, but not when the cells were used at the same doses, but after a short cultivation period.

Adipose-derived stem cell therapy in horses

Four reports, all with cultivated ASCs, described the use of adipose stem cells in horses (Table 4). Two of them, conducted by the same group, treated tendonitis with allogeneic ASCs combined with PRP. Clinical and ultrasonographic analyses showed regeneration of injured tendons and functional recovery, but the absence of a control group limits the significance of the results in terms of the efficacy of ASCs.

The third study analyzed the role of allogeneic ASCs in promoting endometrial tissue remodeling in mares with endometriosis. Four animals were treated and two were used as controls, with histopathology and immunohistochemical results showing positive remodeling of endometrial tissue in treated mares. The limitation of this study is the small number of patients included.

In the fourth study, 10 horses with early symptoms of osteoarthritis were treated with autologous ASCs, and 6 animals were used as controls (3 receiving pharmacological therapy, and 3 only limited in movement). Results were evaluated by comprehensive clinical examination, showing that stem cell therapy had a more beneficial effect, on long-term evaluation, than routine steroid usage.

Conclusion and Future Perspectives

Equine and canine ASCs are well characterized, whereas feline ASCs are still poorly known. Overall, the immunophenotype, proliferation and differentiation potential, and expression of pluripotency genes are similar to the characteristics described for human cells. However, as the SVF is also frequently used for cell therapy, further studies are needed to understand in greater detail the composition of these preparations and their interaction with injured tissues.

The perspective to use allogeneic ASCs for cell therapy is highly interesting, for allowing the production of off-the-shelf cells. A comparison of the therapeutic properties of autologous and allogeneic bone marrow MSCs in canine spinal cord injury showed that both cell types had beneficial effect, which, however, lasted longer when autologous cells were used [76]. The immunomodulatory properties of canine ASCs were demonstrated in vivo after local and systemic repeated delivery of allogeneic cells in healthy dogs [99]. Similar to human ASCs [13], animal cells express low levels of MHC class I and no MHC class II [27,83], and are thus expected to escape immune recognition. Although this explains the apparently successful use of allogeneic cell therapy, allogeneic MSC rejection has already been reported, and might be explained by the presence of MHC class I antigens and by the fact that class II molecules may be induced by IFN-γ, for instance [100].

None of the studies included in this review conducted a detailed investigation of the immunological status of the treated patients. Since there is a tendency to increase the use of allogeneic ASCs in veterinary cell therapy, it is also of great importance to investigate and define potential immune responses that may occur.

The small number of published studies describing the results of ASC therapy in spontaneously affected veterinary patients calls attention to the need of a better understanding of these interventions, to help with the establishment of legal and regulatory issues of cell therapy in veterinary medicine. The published studies do not allow a conclusion on the effect of ASC therapy, or a comparison with bone marrow-derived MSCs, due to the generally small number of patients treated, diversity of cell populations used, combination with PRP, and lack of adequate controls.

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