


Placental Stem Cells from Domestic Animals: Translational Potential and Clinical Relevance

Cell Transplantation
2018, Vol. 27(1) 93–116
© The Author(s) 2018
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/0963689717724797
journals.sagepub.com/home/cll


B. Barboni¹, V. Russo¹, P. Berardinelli¹, A. Mauro¹,
L. Valbonetti¹, H. Sanyal¹, A. Canciello¹, L. Greco¹, A. Muttini¹,
V. Gatta¹, L. Stuppia², and M. Mattioli³

Abstract

The field of regenerative medicine is moving toward clinical practice in veterinary science. In this context, placenta-derived stem cells isolated from domestic animals have covered a dual role, acting both as therapies for patients and as a valuable cell source for translational models. The biological properties of placenta-derived cells, comparable among mammals, make them attractive candidates for therapeutic approaches. In particular, stemness features, low immunogenicity, immunomodulatory activity, multilineage plasticity, and their successful capacity for long-term engraftment in different host tissues after autotransplantation, allotransplantation, or xenotransplantation have been demonstrated. Their beneficial regenerative effects in domestic animals have been proven using preclinical studies as well as clinical trials starting to define the mechanisms involved. This is, in particular, for amniotic-derived cells that have been thoroughly studied to date. The regenerative role arises from a mutual tissue-specific cell differentiation and from the paracrine secretion of bioactive molecules that ultimately drive crucial repair processes in host tissues (e.g., anti-inflammatory, antifibrotic, angiogenic, and neurogenic factors). The knowledge acquired so far on the mechanisms of placenta-derived stem cells in animal models represent the proof of concept of their successful use in some therapeutic treatments such as for musculoskeletal disorders. In the next future, legislation in veterinary regenerative medicine will be a key element in order to certify those placenta-derived cell-based protocols that have already demonstrated their safety and efficacy using rigorous approaches and to improve the degree of standardization of cell-based treatments among veterinary clinicians.

Keywords

placenta stem cells, regenerative medicine, cell-based therapy, domestic animals

Introduction

Stem cell-based regenerative medicine represents one of the most relevant challenges in the biomedical sciences. The scientific expectation toward regenerative medicine is related to its potential in producing a paradigm shift in medicine. With few exceptions (i.e., antimicrobials and hormone replacement therapy), traditional medicine has been concerned with treatment of symptoms of disease but rarely the correction or reversal of the pathology itself.

However, whenever possible, medical therapy should also contain a component of disease correction. Correction of a disease process can be accomplished through several mechanisms. The therapy applied can itself replace or reverse the disease-causing process. The cell-based approach, if effective, would directly replace the degenerated tissues of the patient by providing an immediate and direct functional effect. One of the mechanisms of action prompted by cell transplantation is to

stimulate disease correction by facilitating the body's innate regenerative pathways. Another way to enhance repair of damage is via inhibition of events that actually prevent natural

¹ Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

² Medical Genetics, University "G. d'Annunzio" of Chieti Pescara, Chieti, Italy

³ Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale," Teramo, Italy

Submitted: March 31, 2017. Revised: June 2, 2017. Accepted: June 23, 2017.

Corresponding Author:

Valentina Russo, Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Campus Coste Sant'Agostino, Via R. Balzarini 1, 64100 Teramo, Italy.
Email: vrusso@unite.it



regeneration from occurring. Cell-induced regeneration may, indeed, proceed through many processes even if 2 major mechanisms are recognized: the *in situ* transdifferentiation toward the lineage of host damaged tissues and/or the improvement of paracrine factors that modulate the proliferation/differentiation of resident progenitor cells. In both cases, the repair of the damaged tissues and the restoration of organ functions can be achieved. Since increasing evidence supports the hypothesis that stem cell therapy has the capacity to provide the complex processes involved in tissue regeneration it is now moving from translation to clinical practice¹⁻³. However, there are still knowledge gaps and safety concerns regarding stem cell-based therapies. Improving the research value of large animal models may represent one key challenge to favor the progress of regenerative medicine and to facilitate eventual use of them in medical clinical translational research.

Veterinary Stem Cell-Based Therapy: Domestic Animals between Translational Models and Patients

Veterinary medicine has an important role in the translational process offering the missing link between basic science and human clinical applications. Many diseases encountered in humans also pose a problem in veterinary patients with similar pathology and etiopathogenesis. These diseases certainly raise the interest in regenerative medical treatments on the veterinary side but, at the same time, offer a perfect model for human patients, much better than laboratory animals that do not accurately reproduce in full the complexity of disease conditions.

Tight cooperation between basic science and human and veterinary medicine would therefore not only be beneficial for veterinary patients but would drive the field of regenerative medicine forward for the benefit of human patients. This concept is clearly supported from past preclinical studies performed on canine models that have been instrumental in advancing hematopoietic stem cell protocols for human oncology. Translational preclinical studies have allowed the development of bone marrow (BM)-based therapy⁴⁻⁶ and optimization of this technique over 4 decades^{7,8}. Success in numerous animal models of disease and emerging achievements in human clinical trials allow scientists to realize that stem cell-based regenerative medicine will soon be possible. A remaining challenge is collaboration among different experts such as veterinarians, biologists, geneticists, physicians, and other scientific health and environmental professionals operating using a "One Health" approach⁹.

Animal Models for Stem Cell-Based Regenerative Medicine: Mice versus Domestic Animals

It is generally accepted that use of companion (dog, cat, and horse) and farm animals (sheep, goat, bovine, and pig) has an

enhanced ability over laboratory mammals for predicting clinical efficacy of new medical devices, pharmacological therapies, and cell organ-based surgery¹⁰. It should be noted that the nomenclature regarding animals is quite confusing. In this review, companion and farm animals will be referred to as domestic animal models whether they are used as clinical patients or in preclinical/translational settings.

Although the use of rodents, especially genetically altered mice, maintains a central role in the study of stem cell biology, no rodent models have better advantages compared to domestic animals with regard to system physiology and anatomy, which are more similar to humans. Indeed, domestic animals and humans share a similar basal cellular metabolism rate of cells, a longer life span, comparable organs in size, and physiology¹¹⁻¹⁵. Moreover, unlike laboratory rodents, the health conditions of domestic animals are determined by lifestyle influences. They are outbred and thereby continually exposed to environmental factors that underlie several diseases (cancer, diabetes, etc.) or traumatic defects. Similar to humans, many dogs and horses are expected to undertake an athletic (e.g., sport horses and agility dogs) or a working career (service dogs). This increases the incidence of chronic musculoskeletal disorders that continue to be a therapeutic, diagnostic, and clinical challenge in medicine. Another relevant aspect that increases the scientific interest in nonrodent animal models is that they suffer from a variety of spontaneously occurring diseases that reproduce the human phenotype and etiology. In particular, some domestic animals are currently used to study human genetic diseases. Indeed, they may be spontaneously affected by genetic disorders induced by a single gene defect or due to the complex interaction between gene expression and environmental conditions¹⁶⁻¹⁸. Alternatively, genetically induced conditions are also inducible in nonrodent animals to reproduce debilitating degenerative disorders (e.g., Alzheimer and Huntington diseases, cystic fibrosis, and muscular dystrophy) by targeting specific genomic sites¹⁹. Thus, the use of domestic animals has a tremendous potential for validating and advancing the crucial field of regenerative medicine.

Even if domestic animals offer numerous advantages, major limitations remain. For example, specie-specific reagents are less available, such as antibodies, growth factors, and fully annotated expression microarrays. There is a lack of centralized resources where cells are characterized and stored, reagents made available, and databases maintained for the wider biomedical community. If these drawbacks still represent a barrier for researchers, several strategies can be implemented to overcome these limitations.

Important research institutions like the National Institutes of Health (NIH) are trying to overcome these limitations by developing a publicly available website and annual meetings that provide investigators and program officers with an online resource to disseminate information such as what species are available from which particular centers, service/expertise available at each site, and so on in order to

facilitate collaboration. Indeed, having better integrative and informatics tools will likely have a greater and more innovative impact on biomedical research.

All of these efforts can be strengthened by the awareness that research using domestic animals will complement the use of mice, leading to more comprehensive studies that can then be applied to humans.

Stem Cell–Based Therapy Application in Veterinary Medicine

The impact of stem cell-based regenerative medicine in veterinary clinics offers interesting insights. Several private companies, spin-offs, and university departments (Vet-stem; VetBiologics; UCDAVIS; Riddle and Rood; RENOVOCyte; Biologics Medivet; Celavet; Therapies, ART Advanced Regenerative; Laboratories, Fat-Stem, etc.) are engaged in a widespread stem cell service at providing cells isolated from patient's tissue samples with or without an amplification step, or, alternatively, to sell kits that allow for in-house cell isolation from tissues. Such a service, originated in North America, has now extended to Asia (Histostem Co., Ltd., South Korea) and Europe (Belgium Fat-Stem Laboratories) and supports cell-based treatments for thousands of animals^{20,21}. However, this empirical use of cell products applied in a variety of pathological conditions mainly in horses, dogs, and cats has not really enhanced knowledge on the properties and mechanisms of these innovative therapeutic procedures for the care of animals. The major clinical outcomes generated by this widespread practice are mainly represented by anecdotal and case reports. Although the animal cell products have been commercially available since 2003, few studies have documented the scientific improvement promoted by the injection of autologous cells collected by adipose tissue (AT). As an example, 2 double-blinded controlled and multicenter studies performed on 21²² and 39 dogs²³ affected by coxofemoral osteoarthritis (OA) and 2 clinical trials involving 14 recruited dogs with humeroradial joint OA²⁴ and 10 dogs with severe hip OA²⁵ have been published.

A widespread use of stem cells in veterinary clinics is the consequence of a complete absence of legislation. For this reason, cell therapy may be adopted using an empirical approach without holding a solid scientific basis. Veterinarians can either prepare the necessary cell-based therapy products themselves or, alternatively, obtain the stocks from suppliers located in their respective country or from abroad. Although, at least in Europe, stem cell–based pharmaceuticals for veterinary use have been widely ignored by legislators, no special regulations have been issued compared to stem cell–based pharmaceuticals for human use. The existing legislation is incomplete and leaves too many loopholes for unproven stem cell–based pharmaceuticals for veterinary use. It is likely that in the future, regulations on veterinary use of cell therapy will be modeled after the established legislation for cell therapy for humans. However, in

the meantime, the popular appeal of stem cell–based therapies, and their widespread commercialization, has led to their application for many conditions in veterinary patients for which there are little to no evidence-based preclinical animal studies or even supporting *in vitro* data. Therefore, enthusiasm for stem cell therapies as a powerful treatment strategy for the repair and regeneration of tissue injury and disease must be tempered until experimental evidence is sufficient to supersede anecdotal reports. In the absence of any regulations, however, no such cell-based therapy protocol has been certified so far and we are missing out on the opportunity to expand the evidence-based preclinical and clinical trials on veterinary patients, which may accelerate the advancement of regenerative medicine applications for several mammalian species²⁶.

The current European Union (EU) and national legislations on veterinary medicine need to be reformed in order to bring about legislative improvements, which can facilitate the development of cell-based pharmaceuticals for human use. The legal requirements of manufacturing, marketing, and application of cell-based veterinary pharmaceuticals are not as well developed as the requirements for chemical pharmaceuticals. Stem cell–based veterinary pharmaceuticals are medicinal products in the sense of the pharmaceutical laws of the EU. For that reason, such medicinal products principally require official approval for their manufacture and an official marketing authorization for their placement on the market before being used by the veterinarian. The manufacture, marketing, and use of cell-based veterinary pharmaceuticals without manufacturing approval and marketing authorization, is permitted only in certain exceptional cases determined by EU and individual Member State law. Violations of this requirement may have consequences for the respective veterinarian under criminal law and under the code of professional conduct in the respective Member States²⁶.

Therefore, legislation is desirable in order to be able to certify the safety and efficacy of cell-based treatments, to standardize protocols, and to make comparable clinical outcomes between centers. Obviously, legislation such as that applied to stem cell–based pharmaceuticals in medicine may completely hinder veterinary research. Animal health, indeed, does not receive public funding and does not affect the economy the way human medicine does. However, the lack of any legislation limits the ability to control the clinical effects of innovative therapeutic approaches and it neutralizes the positive effects that could arise from dynamic market competitiveness.

Stem/Progenitor Cell Sources for Veterinary Regenerative Medicine

Several preclinical and clinical studies have been performed on domestic animals, offering important insights on cell-based tissue regenerative mechanisms. Most of the information has been derived from canine and equine models which have been chosen based on their impact on veterinary

Table 1. Cell-Based Regenerative Medicine in Dog.

| Stem/progenitor cells source | Experimental and spontaneous diseases treated with cell-based protocols |
|---|---|
| Hematopoietic Stem Cells - Bone marrow ^{19,118,119} - Peripheral blood cells ^{120,121} | Hematologic cancer disorders ^{120,149} |
| Mesenchymal Stem Cells - Bone marrow ^{68,80,122-125} - Adipose tissue ^{68,80,123,126-132} - Umbilical cord ^{68,78-80,133-139} - Skeletal muscle ¹³⁹ - | Musculoskeleton - Bone ^{67,68,150-161} - Intraarticular ^{22-25,162,163} - Muscle ^{164,165} |
| Amniotic derived Cells ^{134,135,140-143} | Neurogenic ^{78-80,166-171} |
| Embryonic Stem Cells ¹⁴⁴ | Cardiac ¹⁷²⁻¹⁷⁹ |
| induced Pluripotent Stem Cells ¹⁴⁵⁻¹⁴⁸ | Ophthalmology ^{106,107} Urologic ¹⁸⁰ |

medicine (animal patients) and from ovine or porcine models mainly adopted as translational models. As detailed in Table 1, the stem/progenitor cells isolated so far in canine model are of various origins, but the most characterized are mesenchymal stem cells (MSCs) isolated from BM, AT, and umbilical cord (UBC). Additionally, other stem cell sources have been thoroughly investigated recently such as amniotic cells and to a lesser extent, embryonic and induced pluripotent stem (iPS) cells (Table 1). Scientists have focused mainly on treatment of canine musculoskeletal, cardiac, and nervous system disorders. Moreover, the dog has represented for decades the ideal translational model for optimization of cell transplantation in hematologic cancers, whereas, more sporadically, it has been adopted for the treatment of ophthalmologic and urologic disorders (Table 1).

The horse is another domestic animal in which stem cell therapy has been extensively studied and applied either to treat experimental or spontaneous diseases (Table 2).

MSCs derived from BM and AT are the chief cell types used in equine research and clinic trials, however many other stem/progenitor cells sources have been taken into account and relevant results have been obtained to date (Table 2). Stem cell-based protocols have been mostly developed so far to deal with pathologies related to sports medicine such as tendon, ligament, and cartilage/joint disorders or, to a lesser extent, bone defects. It is not surprising that musculoskeletal disorders represent the main clinical target in horses, a species largely involved in athletic competition.

Sheep represent another domestic animal model for designing translational experiments aimed at verifying the effectiveness of stem cell-based therapy for musculoskeletal disorders. Sheep, indeed, are considered a valuable medium-

Table 2. Cell-Based Regenerative Medicine in Horse.

| Stem/progenitor cells source | Experimental and spontaneous diseases treated with cell-based protocols |
|---|--|
| Mesenchymal stem cells - Bone marrow ^{94,181-191} - Peripheral blood cells ^{181,187,192} - Adipose tissue ^{93,181-185,189,191,193-196} - Synovial membrane ¹⁹⁷⁻¹⁹⁹ - Peridontal ligament ²⁰⁰⁻²⁰² - Umbilical cord ^{75,185,203-218} - Tendon derived stem cells ²¹⁹⁻²²¹ | Musculoskeleton - Tendon ^{55,58,59,92,96,101,103,104,190,193,212,224,236-260} - Bone ^{261,262} - Osteoarticular ^{75,182,191,263-278} - Muscle ²⁵⁷ |
| Amniotic derived Cells ^{55,101,110,203,204,222-227} | Wound ^{110,279} |
| Embryonic Stem Cells ²²⁸⁻²³² | Ophthalmology ^{108,109} |
| induced Pluripotent Stem Cells ^{148,231,233-235} | |

sized translational mammal. In this model, evidence of regenerative potential of amniotic cells, MSCs isolated from BM and from UBC, as well as the embryonic stem cells have been documented (Table 3). Importantly, the ovine model offers the opportunity for investigating prenatal surgical stem-based treatments. Many rigorous preclinical studies of in utero stem cell transplantation have confirmed that the clinical use of stem cells can be adopted to ameliorate prenatal congenital diseases, thereby offering new innovative therapeutic approaches (Table 3).

In addition, animal iPS cells represent powerful biological models for assessing human iPS therapeutic applications. There was an innovative breakthrough in the field of stem cell research with the isolation of iPS cells from humans and mice. Several studies on various animal cellular systems (Tables 1–3) suggest that the basic pluripotency network appears to be conserved among different species, allowing derivation of iPS cells from a variety of domestic animal species.

Placenta-Derived Stem Cell Application in Domestic Animal Models

In addressing the complex scenario described above, increasing attention has been focused on the placenta as a possible source of progenitor/stem cells. The embryonic origin of placenta-derived cells (PCs) explains the evidence of their retained high plasticity, with the possibility of providing progenitor/stem cells that are capable of differentiating into multiple cell types²³⁹. Meanwhile, the fact that the placenta is fundamental for maintaining physiologically feto-maternal tolerance during pregnancy suggests that cells present in placental tissue may simultaneously have low immunogenicity and immunomodulatory activities. The confirmation for this is the absence of MHC class I molecules and the low expression of MHC class II, allowing these cells to be effectively employed in immunocompetent transplanted organisms^{224,240}. Furthermore, these

Table 3. Cell-Based Regenerative Medicine in Ovine Translational Animal Model.

| Stem/progenitor cells source | Experimental and spontaneous diseases treated with cell-based protocols |
|---|---|
| Mesenchymal stem cells - Bone marrow ²⁸⁰⁻²⁸² - Adipose tissue ²⁸⁰ - Peridental ligament ²⁸¹ - Umbilical cord ^{283,284} - Skeletal muscle ²⁸⁰ | Musculoskeleton - Tendon ^{30,32,54,60,292} - Bone ^{69,70,74,280-281} - Osteoarticular ⁷⁶ |
| Amniotic derived cells ^{29,41,56,58,70,87-89,91,285-289} | Wound ⁸⁶ |
| Embryonic Stem Cells ²⁹⁰ | Prenatal ^{87-89,91} |
| induced Pluripotent Stem Cells ^{148,291} | |

cells have also been shown to secrete soluble factors involved in pathophysiological processes that may aid tissue repair, such as cytokines which have immunomodulatory and anti-inflammatory effects^{215,241} as well as angiogenic factors associated with wound healing^{216,242}, growth factors related to cell proliferation and differentiation²⁴³, and antiapoptotic and antioxidative factors²⁴⁴. These key aspects make cells from placenta ideal candidates for developing cell therapy protocols that encourage PC allo-transplantation or xenotransplantation in different domestic animal models. Indeed, autologous transplantation is more feasible with other stem cell sources (i.e., BM-MSCs, AT-MSCs, etc.); hence, the patient can benefit from his or her own stem cells. With PCs, allo-transplantation and xenogeneic transplantation are more realistic than autologous, but it has been well documented that PCs from different animal models possess high genetic stability and marked immunomodulatory properties. Furthermore, given that the placenta is generally discarded after birth or can be frequently collected at the slaughterhouse for several domestic animals, the derived tissues are largely available, thus the recovery of cells does not involve any invasive procedures and their use does not pose any ethical concerns^{239,245-249}.

According to cell origin, PCs can be distinguished in:

- amniotic-derived cells from which can be identified amniotic epithelial cells (AECs), amniotic MSCs (AMSCs), and amniotic fluid MSCs (AFMSCs);
- UBC-derived stem cells from which can be isolated umbilical cord blood (UCB), umbilical cord blood MSCs (UCBMSCs), and umbilical cord matrix MSCs (UCMMSCs).

Despite the extensive literature available, domestic animal PCs are not easily comparable, especially considering

the current lack of common protocols and the different gestational stages that can highly affect the native biological properties. Indeed, gestational age is a key factor capable of influencing morphological and functional properties of PCs. For instance, Barboni et al.²²⁵ demonstrated that gestation considerably affected the expression of surface markers, as well as the expression and localization of pluripotency markers of ovine AECs. Moreover, their differentiation ability changed with the gestational age, affecting cell plasticity and the degree of global DNA methylation, which increased in term gestation amnia, thus probably affecting the outcome of cell transplantation therapies. Therefore, innovative approaches on stem cell aging in preclinical models are essential before their application for clinical translation²⁵⁰.

In addition, methodological aspects make animal cell research results frequently noncomparable²³⁹. The major criticisms are the absence of commercially species-specific reagents and of a complete protein/genome database that is required for methodological conception and comprehensive procedures for monitoring and sharing results. Moreover, all culture protocols result in mixed cell preparations and obtaining specialized cells in sufficient quantities and purity is still a challenge especially for PC-MSCs. For all these reasons and limitations, continuous and careful updates on research and breakthroughs using domestic animal PCs may help bring about reproducible results and allow for comparison among groups, to focus on the conserved biological properties among species, and to better understand the mechanisms underlying the regenerative efficacy.

Starting from this premise, this review aims to provide an overview of the contribution of PC-based therapies by considering the scientific evidence arising either from pre-clinical or clinical trials performed on domestic animals.

Preclinical Studies to Test the Regenerative Properties of PCs in Domestic Animal Models

The properties displayed by PCs have led scientists to seek to take advantage of these types of stem cells by studying their therapeutic potential in domestic animal models of different diseases. In this regard, successful results on domestic animal preclinical models have been reported for the treatment of neurogenic disorders, myocardial infarction (MI), wound healing, prenatal diseases, and tendon bone and cartilage defects.

Several domestic animal models have been used to design preclinical experiments. Relevant for PCs is the ovine model where allo- and xenotransplantation settings have been performed. Ovine species play a major role in musculoskeletal regenerative and prenatal preclinical trials, because of its high translational value due to the similarities with humans in terms of weight, mechanical exertion, and reproductive gestational outcomes¹¹⁻¹⁴.

Musculoskeletal Preclinical Studies

Tendon Injuries

Tendon injuries are a common cause of disease in both human and veterinary medicine. Currently, in our society, more than 30 millions of musculoskeletal lesions occur and most of them involve tendons and ligaments. In the United States and in Europe, the economic impact of tendon and ligament morbidity is around €140 billion each year²⁵¹. With the increase in life expectancy, tendon-related disorders will increase worldwide with a huge economic impact on the sanitary system²⁵². The incidence is up to 25% considering the aging of the population, the increasing prevalence of metabolic disorders^{253,254}, and the increase in life expectancy²⁵⁵. Tendinopathies also have clinical relevance in veterinary medicine: 46% of racehorses suffer from tendon pathologies and their reduced sporting performance generate a negative economic impact estimated worldwide to be €400 billion^{256,257}. Tendon injuries are among currently incurable diseases and the poor pronoses are often exacerbated by a high incidence of recurrences²⁵⁸.

Tendons can be exposed to trauma during sports activities, but they can also be affected by overuse or aging. The most commonly injured are Achilles and patellar tendons in humans or superficial digital flexor tendons (SDFTs) in horses with pathologies ranging from degenerative tendinopathies, partial tears, up to complete ruptures^{259–261}. These injuries are difficult to manage because adult tendons do not regenerate spontaneously but result in a fibrotic scar with poor tissue quality and mechanical properties, frequently resulting in long-term pain, discomfort, and disability²⁵⁸. Given the frequency and the increasing cost of treating injuries, as well as the relatively poor results obtained through surgical intervention, new and innovative strategies have become more appealing. In this context, in recent years, PCs have attracted increasing attention as a possible source of stem cells that may be useful for clinical application in tendon regenerative medicine^{141–143,216,217,226,239,249,262}. In particular, our research group has increased the role of amniotic-derived cells^{142,143,215–218,226,262} by carrying out preclinical studies adopting either allotransplantation or xenotransplantation approaches²¹⁸ on a validated ovine experimentally injured calcaneal tendon model (Fig. 1). These studies have demonstrated that ovine AECs have the ability to support tendon regeneration and an early recovery of the biomechanical properties of the tissue²¹⁶. Through these studies, the mechanisms underlying tendon regeneration have begun to be elucidated. Transplanted AECs support tendon regeneration partly through a paracrine stimulation of the damaged host tissue. AECs modulate the production of critical growth factors (i.e., vascular endothelial growth factor [VEGF] and transforming growth factor beta1 [TGFβ1])²¹⁶ and of the immunomodulatory cytokines^{215,218} involved in healing processes. Amniotic-derived cells enhance, innate regenerative mechanisms, which was confirmed by greater recruitment of tenocytes involved in the organization of the extracellular matrix and a more active

remodeling of supporting tissues such as blood and nervous system. Interesting, data obtained under allotransplantation and xenotransplantation settings are converging in order to confirm a direct role of AECs in the process of tendon regeneration exerted through their in situ transdifferentiation (Fig. 2). Indeed, the molecular chimerism obtained in xenotransplantation settings confirmed that the cells, which survived within the host tissue for 28 d, modulated their gene profile by upregulating 48 human species-specific genes. The functional analysis of these genes revealed that they are involved in epithelial-mesenchymal transition (*KDM6B*, *NR2F2*), inflammatory response (*CCRL2*) and extracellular matrix synthesis (*COL1A1*)²¹⁸ as indicated in Fig. 2). The relevance of these genomic results has been reinforced by evidence that AECs differentiated toward tenocyte-like cell-synthesized collagen type I (COLI), thus contributing to tissue regeneration through a direct release of major tendon extracellular matrix proteins^{142,216} (see Preclinical studies and Clinical Trials boxes in Fig. 2). Moreover, allotransplanted AECs not only modulated the phases of tissue regeneration but also guided a specific process of healing. In particular, cell injection was associated with a specific centripetal process of tissue regeneration that started close to the healthy portion of the tissue and progressively affected the core of the wound site. This dynamic process of tendon healing was accompanied by the migration of transplanted AECs that were always recorded in proximity to the front of extracellular matrix deposition. These cells enhanced collagen synthesis and participated in the process of cell and matrix alignment. Among AEC regenerating mechanisms, immunomodulatory effects seem to exert a central role: AEC tendon transplantation induced a reduction in leukocyte infiltration and modulation of the recruitment of macrophages (Mφ) M1, pro-inflammatory, and M2, pro-regenerative, phenotype in favor of the latter ones²¹⁵ (see Preclinical Studies box in Fig. 2). The immunogenic role switched on in AEC-transplanted tendons may have a role in accelerating early healing and in preventing fibrosis²¹⁵ (Fig. 1). Altogether, these findings support the idea that AECs are one of the most promising stem cell sources for achieving tendon regeneration. They are indeed able to direct tendon healing by stimulating a prompt recovery of tissue function without any preliminary transfection.^{263–265} Another clinical advantage offered by AECs is that they promoted tendon regeneration without any undesirable in situ cell differentiation (osteogenic or chondrogenic) as observed after MSC transplantation^{263,266}. For both of these reasons, AECs may represent a source of progenitor/stem cells that can be quickly obtained for clinical practice. Tendon regeneration was also confirmed using green fluorescent protein (GFP)-nucleofected AFMSCs by Colosimo et al.²¹⁷ Notably, not only were limb tendons regenerated with AFMSCs but also diaphragmatic tendons. Indeed, Kunisaki et al.²⁶⁷ demonstrated neonatal diaphragmatic tendon repair with the use of an AFMSC-based engineered tendon that led to improved structural outcomes when compared with equivalent fetal

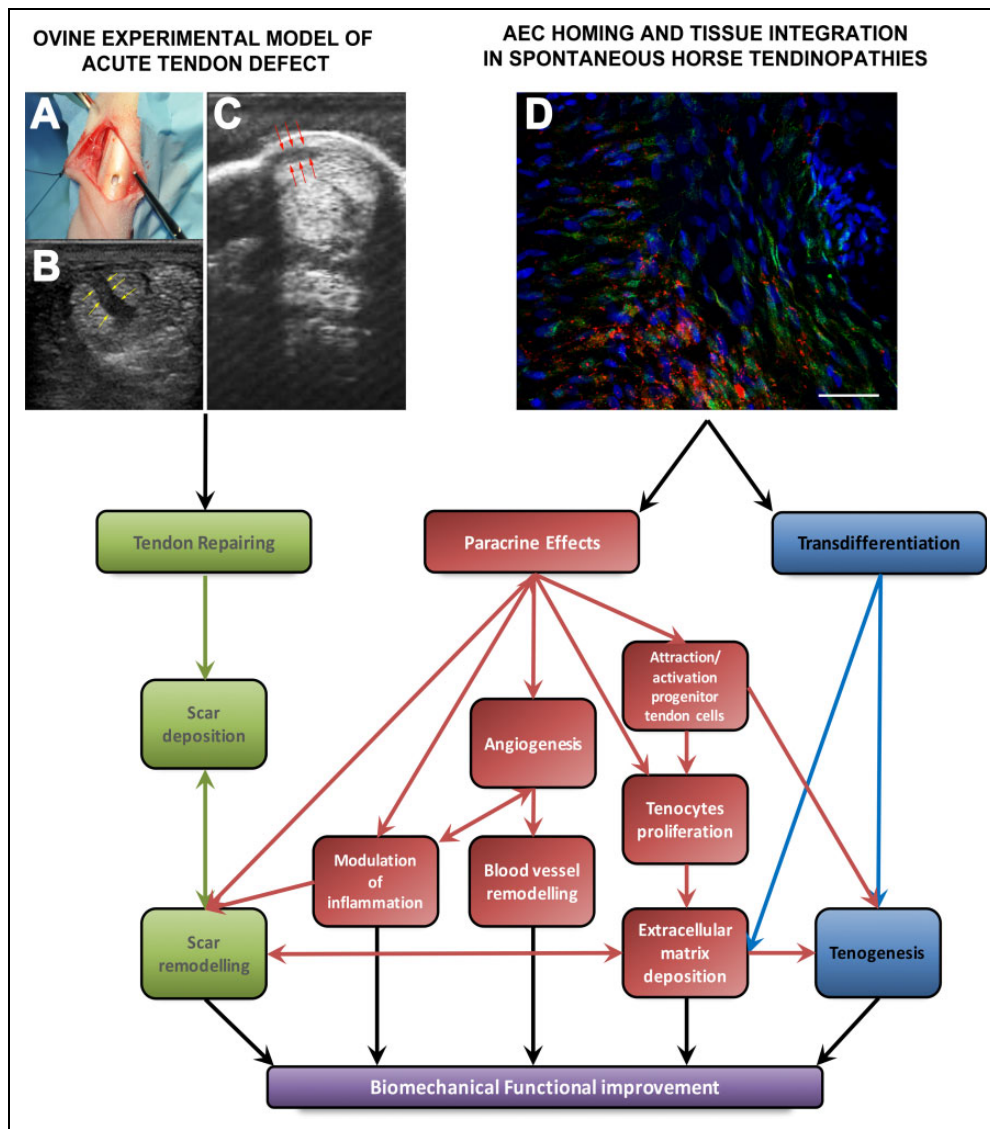


Figure 1. Potential mechanisms involved in tendon regeneration promoted by amniotic epithelial cell (AEC) transplantation. The figures and scheme described the potential mechanisms through which AEC-based therapies contribute to tendon regeneration as outlined in preclinical/translational studies and clinical trials performed on domestic animal models (i.e., sheep and horses). The scheme summarized that AEC transplantation induces an early functional recovery of the biomechanical properties of the damaged tendon by synchronizing angiogenesis, inflammation, and extracellular matrix deposition/remodeling. The early stage of tendon repair is also supported by the increased attraction and activation of resident progenitor cells as well as by the in situ transdifferentiation of transplanted AECs. The images on the left (A, B, and C) show the ovine experimental model of calcaneal tendon defect (equivalent to the Achilles tendon in human). (A) The defect was generated through the mechanical removal of a fixed volume of tissue. (B) Ultrasound image example of tendon immediately after the tissue removal (yellow arrows). (C) Ultrasound image example of the defect regeneration 14 d after AEC transplantation (red arrows). Inside of the defect, the deposition of new tendon fibers was evident through the improvement of the echogenicity score. The image on the right (D) displays the persistence of AECs injected into a spontaneous horse superficial digital flexor (SDFT) tendinopathy at day 60. The viability of AECs in host tissue has been demonstrated by loading the cells with a vital membrane dye (PKH26; Sigma-Aldrich, St. Louis, MO, USA). Cell labeling persisted after transplantation and it is identifiable as a red fluorescence localized on cell membrane around the blue nuclei (4',6-diamidino-2-phenylindole [DAPI] counterstaining fluorescent dye [Sigma-Aldrich, St. Louis, MO, USA]). The host damaged tissue is visualized by the faint green fluorescence that indicated the low density of collagen type I (COL1, Chemicon Int., Billerica, MA, USA) in the extracellular matrix. Scale bar = 50 μ m.

myoblast-based and acellular grafts. Similar results were obtained by Turner et al.²⁶⁸ who demonstrated tendon diaphragmatic repair with a clinically viable autologous tendon engineered with AMSCs with efficacy analyses performed up to ovine adulthood.

Bone Defects

Stem cell-based therapy for bone regeneration is an emerging treatment. PCs have the potential to be utilized to mainly treat craniofacial bone defects or major bone injuries. In particular, preclinical studies carried out on canine and

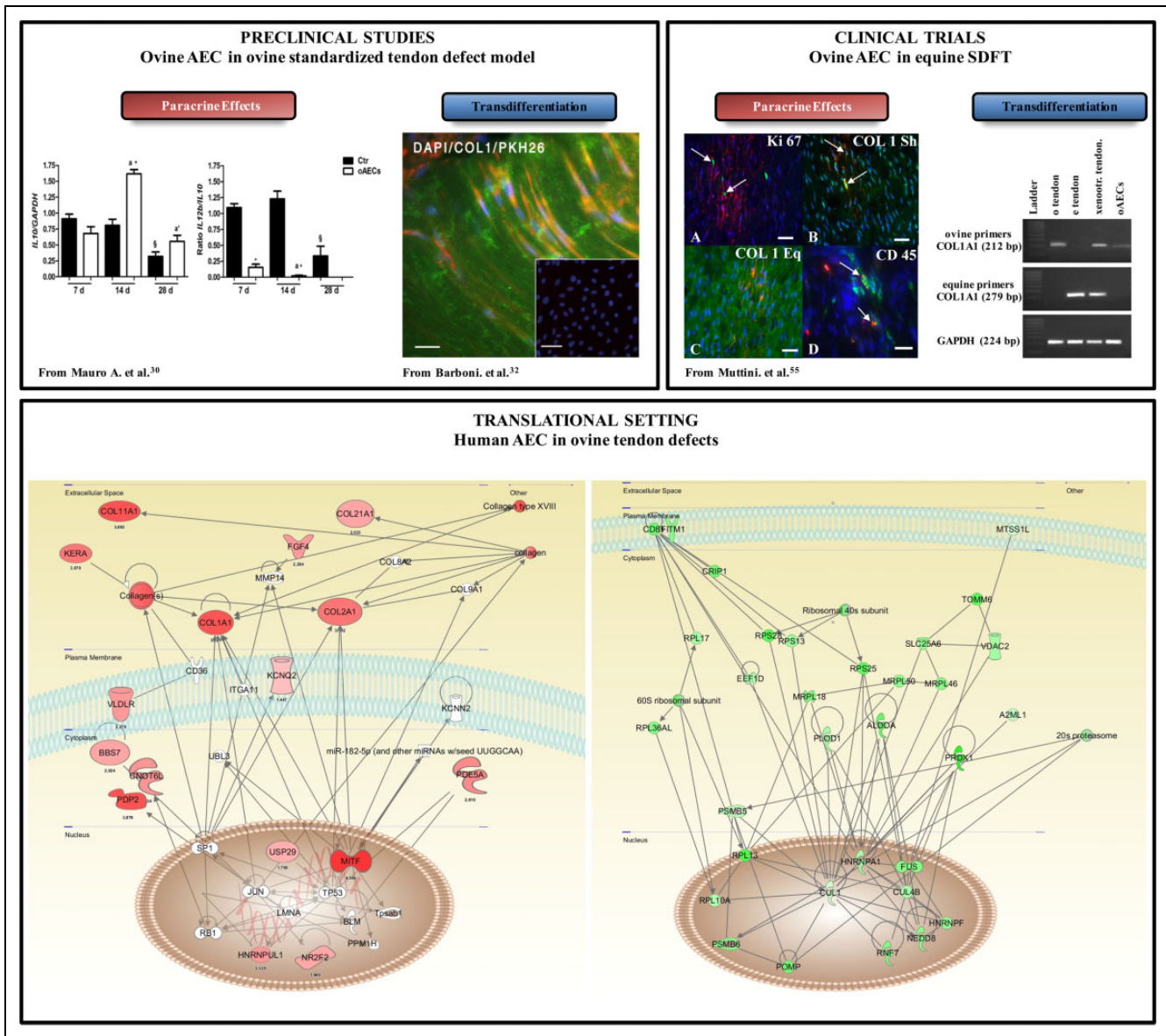


Figure 2. Regenerative mechanisms involved in tendon healing after amniotic epithelial cell (AEC) transplantation. The 3 boxes summarized the major scientific data clarifying the mechanism promoted by AECs for tendon regeneration in preclinical (ovine amniotic epithelial stem cells [oAECs]) into ovine damaged tendon: left top box), clinical settings (oAECs into equine spontaneous tendinopathies: right top box), and translational (human amniotic epithelial stem cells [hAECs]) into ovine tendon: bottom box). In all of the experimental settings, both paracrine and in situ differentiation data have been documented. (Left top box) The preclinical studies had documented the immunomodulatory influence of AECs through the higher expression of anti-inflammatory cytokines recorded in host tissue 28 d after transplantation (see histograms). The AEC in situ transdifferentiation (right image) was confirmed by immunohistochemistry. In particular, the image shows some PKH26-positive oAECs recorded in the experimental injured calcaneal tendons. Some of the AECs showed a fusiform shape and started to synthesize collagen type I (COLI). The latter event was demonstrated by the colocalization of the green (anti-COLI; Chemicon Int., Billerica, MA, USA) and red (PKH 26; Sigma-Aldrich, St. Louis, MO, USA) fluorescence. The inserted box shows a group of freshly isolated AECs before transplantation that are negative for COLI. The cells were identified by the DAPI (Sigma-Aldrich, St. Louis, MO, USA) counterstained nuclei (blue fluorescence in small insert). In both the images, the scale bars is 50 μ m. (Right top box) The clinical trials were performed using ovine AECs to cure superficial digital flexor tendons (SDFT) spontaneous tendinopathies diagnosed in sportive horses. The effect of oAEC treatments had been mainly documented on the basis of the positive clinical outcomes and of the athletic performances follow-up carried out for 18 mo after cell transplantation. However, after 60 d, 1 patient died for causes unrelated to the treatment and allowed us to collect more detailed information. Left images (A, B, C, and D) are examples of PKH26-labeled AEC (red fluorescence) paracrine effects obtained with immunohistochemical analyses. (A) The proliferative marker Ki-67 (Dako Cytomation, Denmark) was observed either in oAECs (PKH26-positive cells: cells indicated with arrows) or in several neighboring endogenous proliferating cells. (B) Flattened ovine AECs (PKH26-positive cells) parallel to the longitudinal axis of the horse tendon fibers were observed. Some of them colocalized within the cytoplasm species-specific ovine COLI (oCOLI; Chemicon Int., Billerica, MA, USA) antibody (cells indicated with arrows). (C) PKH26-positive oAECs were also identified among the equine COLI (eCOLI; Abcam, Cambridge, UK) fibers (green fluorescence). (D) CD45; AbD Serotec, Oxford, UK marker (green fluorescence) was used to record the leukocyte infiltration and to identified ovine

ovine models have demonstrated that 3-dimensional scaffolds engineered with PCs are able to repair different types of bone defects. Indeed, Jang et al.⁵⁴ have demonstrated that the orthotopic implantation of canine UCBMSCs mixed with beta-tricalcium phosphate (β -TCP) was able to enhance osteogenesis in a dog diaphyseal radius defect model. Additionally, UCBMSCs were applied to a dog with nonunion fracture. Histomorphometric analysis revealed a significant increase in new bone formation at 12 wk after implantation, indicating that a mixture of UCBMSCs and β -TCP is a promising osteogenic material for repairing bone defects. Moreover, Kang et al.³² carried out another in vivo orthotopic implantation assay on radial diaphysis of Beagle dogs by demonstrating that MSCs derived from AT, BM, UCB, and UCM have similar osteogenic capacities even higher than cell-free implants. However, clinical application is more feasible for the MSC source that can be most easily and noninvasively collected such as UCB and UCM.

Similar successful results on bone regeneration were obtained by implanting ovine AECs into a sheep tibia defect and into a maxillary sinus lift model^{220,221}. The labeled AECs survived in the experimental tibia lesions for 45 d and supported consistent bone neof ormation and reduced the infiltration of inflammatory cells, thus showing the potential applications in osteogenic regenerative medicine for this type of PCs. Notable mechanistic advantages have been gained in oral bone regeneration settings. All of these pre-clinical studies were carried out in a sheep model by mimicking the sinus augmentation lift human maxillofacial procedure. The surgical experimental protocol for extraoral maxillary sinus augmentation has been previously validated and tested for its translational value²⁶⁹ by comparing size, structural, and functional parameters with humans^{270,271}. Furthermore, ovine AECs were able to enhance bone regeneration after maxillary sinus augmentation when implanted for 45 and 90 d with synthetic bone substitutes²²¹. AEC allotransplantation provided prompt scaffold integration in host tissue. Moreover, sinus explants displayed a reduced fibrotic reaction, a limited inflammatory response, and an accelerated process of angiogenesis when sinus lift was made with engineered AEC scaffolds. The prompt recovery of homeostasis in cell-treated sinuses may contribute to the increased bone deposition and the widespread presence of bone

nucleation foci. Additionally, using the maxillary sinus lift model, ovine AECs exerted a relevant paracrine role to modulate VEGF expression and pro- and anti-inflammatory cytokine expression, thus successfully guiding tissue regeneration. AECs directly participated in bone deposition, as suggested by the presence of ovine AECs entrapped within the newly deposited osteoid matrix and by their ability to switch-on the expression of bone-related genes when transplanted into host tissues. Analogously, AFMSCs have demonstrated a similar efficacy role in supporting bone regeneration in maxillary sinuses. Berardinelli et al.²²² engineered a commercial magnesium-enriched hydroxyapatite/collagen scaffold for orthopedic purposes and demonstrated that ovine AFMSCs may improve bone regeneration by persisting for 90 d postimplantation.

Joint/Cartilage Injuries

Sporadic research linked to the use of PCs for experimental joint defects are available to date. Of interest, the preclinical study aimed to verify in horses the safety of allogeneic UCB intra-articular transplantation¹²¹. It has been demonstrated that there were minimal local responses, such as joint swelling or lameness after UCM and UCBMSCs intrasynovial injection, in healthy horses thus offering the first biological paradigm for the allogeneic use of these cells. Recently, amniotic membrane (AM) samples have also been tested in vivo for their regenerative role in full-thickness femoral cartilage defects²²³.

Soft Tissue Preclinical Studies

Neurogenic Disorders

Spinal cord injury (SCI) produces progressive cell death, axonal degeneration, and functional loss in multiple motor, sensory, and autonomic system neurons²⁷². All preclinical research on PC-based SCI regenerative medicine has been carried out in dogs using UCBMSCs. The first study performed allogeneic UCBMSC transplantation for SCI induced by balloon compression at the first lumbar vertebra. In this research, Lim et al.⁴⁵ found that transplantation of the UCBMSCs resulted in recovery of nerve function with a significant improvement in the tissue conduction velocity based on the somatosensory evoked potentials. In addition,

Figure 2. (continued) phagocytated PKH26-positive AECs (merged green and red fluorescence and indicated by the arrows). Scale bar = 25 μ m. Right box (transdifferentiation). The *oCOL1* expression performed with species-specific primers was used to verify the differentiation of oAECs after xenotransplantation into the equine SDFT. Reverse transcription-polymerase chain reaction (RT-PCR) analysis, performed 60 d posttransplantation, confirmed the presence of *oCOL1* gene expression in the equine host tissue thus documenting the in situ specialization of the ovine transplanted AECs. (Bottom box) A translational setting was designed by transplanting human AECs into an ovine calcaneal tendon defect for 28 d. Taking advantage of genomic chimerism (human vs. ovine), an active in situ specialization and a paracrine role of hAECs were substantiated by the microarray analysis. Ingenuity Pathway Analysis (IPA)-inferred top network for modulated gene data set analysis was generated for upregulated (red network) and downregulated (green network) transcript data set to disclose functional networks based on their connectivity and enrichment statistics. Color legend spans from dark to light, which reflect more or less downexpression, respectively. Genes labeled in white are not modulated. The network is constructed following the subcellular localization of the genes. (Left image) The majority of the upregulated transcripts support human AEC specialization after transplantation. (Right image) By contrast, a more generic biological role may be associated with the function of downregulated genes.

Park et al.⁴⁶ compared the effects of UCBMSCs at different transplantation time points after SCI induction. These authors identified the best interval between SCI and cell injection, demonstrating that transplantation of UCBMSCs 1 wk after injury induction was more effective in improving clinical signs and neuronal regeneration by reducing fibrosis. The analyzed tissues showed an increased expression of neuronal markers. More recently, Ryu et al.³³ performed a comparative study by using MSCs derived from AT, BM, Wharton's jelly, and UCB. All sources of MSCs survived for 8 wk and reduced interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2) levels, which may have promoted neuronal regeneration in the spinal cord. Although there was no significant difference in functional recovery among the different MSC groups, interestingly, UCBMSCs induced higher nerve regeneration, neuroprotection, and anti-inflammatory activity.

Myocardial Infarction (MI)

MI causes tissue death, and the important goal in this field of regenerative medicine is to replace lost tissue²⁷³. Only 2 preclinical studies on MI using domestic animal models have been conducted, both in porcine models. One study carried out by Sartore et al.²⁷⁴ demonstrated the ability of autotransplanted AFMSCs to improve cardiac functional recovery after acute ischemic myocardium experimental defects in a porcine model. The regenerative role was exerted 30 d after transplantation through the transdifferentiation of the cells toward the vascular tissue lineage whereas, by contrast, evidence of *in situ* cardiomyocyte differentiation has been observed. The surviving AFMSCs downregulated mesenchymal cell markers with the exception of smooth muscle and endothelial antigens but did not express any major cardiac markers such as troponin.

Recently, Kimura et al.²⁷⁵ demonstrated the therapeutic potential of porcine GFP-transfected AMSCs on a chronic myocardial ischemia model. The AMSCs survived after allogeneic transplantation performed in an immune-competent animal, by gaining the *in situ* cardiac phenotype through either transdifferentiation or cell fusion, differently from AFMSCs.

Wound Healing

Cutaneous wound healing requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation and extracellular matrix deposition, angiogenesis and remodeling. However, this orderly progression of the healing process is impaired in many chronic diseases²⁷⁶. Preclinical studies carried out to test PC regenerative properties for wound healing have been conducted in goat and sheep models. Azari et al.²⁷⁷ investigated the effects of allotransplanted UCMMSCs on the cutaneous wound healing process. A histopathological study revealed a complete re-epithelialization after 7 d,

whereas in control samples, the wounds still showed an incomplete process. An interesting experiment was carried out by Klein et al.²³⁶ who investigated wound healing in fetal lambs. Fetal wound healing involves minimal inflammation and limited scarring. During this study, fetuses received an intra-amniotic infusion of labeled autologous AMSCs, clarifying their direct role in accelerating wound closure and enhancing the extracellular matrix profile rather than the release of soluble factors. The mechanisms highlighted still need to be fully elucidated and hold valuable clues for wound healing and the development of MSC-based regenerative strategies, both perinatally and later in life.

Prenatal Preclinical Studies

Many rigorous preclinical studies have focused their attention on the *in utero* PC transplantation to ameliorate prenatal congenital disease. All of these studies have been carried out using a sheep model. Shaw et al.²²⁷ were the first to demonstrate the safety of *in utero* AFMSC autologous transplantation. In this study, GFP-transduced AFMSCs were injected into the peritoneal cavity of each fetal sheep donor. GFP-positive cells were detected in fetal tissues including liver, heart, placenta, membrane, UCB, adrenal gland, and muscle, demonstrating that autologous AFMSCs have widespread organ homing and can offer an alternative treatment for prenatal congenital diseases. These authors were also able to establish the hematopoietic potential of GFP⁻ sheep AFMSCs selected for CD34 (GFP-CD34⁺ AFMSCs). After autologous *in utero* transplantation these cells colonized hematopoietic organs and peripheral blood, confirming their potential for the development of cell-based protocols to treat congenital hematopoietic diseases²²⁸.

Prenatal studies have also been conducted to treat airway pathologies. Particularly, Gray et al.²²⁹ have shown that AMSC-engineered airways may become an option for perinatal airway repair. Fetal lambs with tracheal defects were implanted with expanded/labeled autologous AMSCs engineered in the de-cellularized leporine tracheal segment. Lambs that survived to term could breathe at birth. Engineered constructs exhibited full epithelialization, displaying a pseudostratified columnar epithelium, a significantly greater degree of increase in elastin levels after implantation than acellular grafts.

Severe congenital tracheal anomalies, namely, long segment stenosis, atresia, and agenesis, represent another typology of unsolved prenatal diseases^{267,278}. Engineered cartilaginous grafts with GFP-AFMSCs have been used for fetal tracheal repair by evaluating their effect to term. Respiratory functional tests combined with morphological evidence of the presence of fluorescent protein-positive cells lined with pseudostratified columnar epithelium and remodeled into a predominantly fibrous cartilage pattern were the most relevant results obtained. By contrast,

implants alone did not show any significant changes in glycosaminoglycans, collagen, or elastin content at harvest. Thus, these findings demonstrated that AFMSCs can be a practical cell source for engineered tracheal reconstruction.

The in utero PCs transplantation technique was also used for the treatment of prenatal congenital cardiac malformations. Weber et al.²³⁰ carried out prenatal heart valve interventions aimed at the early and systematic correction of congenital cardiac malformations. In this experiment, fetal implantation was carried out in utero into the pulmonary position of prenatally engineered biodegradable polyglycolic acid-poly-4-hydroxybutyrate (PGA-P4HB) composite heart valves with autologous ovine AFMSCs. Tissue-engineered heart valves showed in vivo functionality with intact valvular integrity and absence of thrombus formation, thus providing evidence that this approach may serve as an experimental basis for future human prenatal cardiac interventions and a promising treatment option in maternal-fetal care.

Clinical Application of PCs in Veterinary Regenerative Medicine

Domestic animal PC clinical application is limited to date and has been used mainly to treat tendinopathies in horses and ocular surface reconstruction both in horses and in dogs.

Musculoskeletal Applications

Tendinopathy

Tendinopathies of the SDFT is a significant cause of lameness and often a career-ending event in Thoroughbred horses because of its high incidence, prolonged recovery period, and high rate of recurrence^{97,110,144,145,262,279}. Afflicted horses are prone to distal limb injury due to hyperextension of the metacarpal joint during racing or riding; thus, the SDFT represents the highest frequency of injury in racehorses²⁸⁰. After injury, the equine SDFT heals via a process of fibrosis, but the scar tissue that forms is functionally deficient compared to that for the normal tendon and it is the predisposing factor of the high incidence of recurrences²⁸¹⁻²⁸³. However, recently in equine medicine, PCs have been used to treat tendon injuries. The most widely used cells for this purpose are UCB and amniotic-derived cells. The first clinical trial was performed with horse AMSCs to investigate their therapeutic potential and cell tolerance in vivo, when allogeneically injected into spontaneous tendon injuries. The study resulted in a quick reduction in tendon size and ultrasonographic (US) cross-sectional area measurements¹⁴⁶. The same group also conducted a series of clinical studies that confirmed the efficacy of the amniotic-derived stem cells in curing SDFT tendinopathies demonstrating their better clinical outcome over BMMSCs²⁸⁴. In the same year, Lange-Consiglio et al.¹⁴¹

demonstrated that the conditioned medium obtained from horse AMSCs can also be useful for cell therapy applications in tendon diseases, hypothesizing that these cells may promote tendon repair mainly via paracrine-acting molecules targeting inflammatory processes rather playing a direct regenerative role. This study identified AMSC-conditioned media as a novel therapeutic biological cell-free product for treating horse tendon and ligament diseases.

Another source of amniotic-derived cells has been used with success in the treatment of equine spontaneous tendinopathies. Muttini et al.¹⁴² have demonstrated that ovine AEC xenotransplantation was able to improve the clinical outcome in 15 horses with acute SDFT lesions. In particular, US controls showed infilling of the defect and early good alignment of the fibers, and 12 of the 15 horses resumed their previous activity during the 18 mo after treatment. The clinical data were also substantiated by histological analyses carried out on a treated SDFT of a horse who died for unrelated causes. The results demonstrated that ovine AECs contribute to tendon healing. The recovered transplanted cells were indeed able to deposit ovine COLI in the repaired area, as revealed by using ovine-specific primers and antibodies that did not cross-react with equine COLI. These cells also confirmed their low immunogenicity, as they were able to survive in the healing site for 60 d.

The effective role of ovine AEC treatment has been confirmed in an experimental trial carried out on 2 horses with acute and 1 horse with chronic spontaneous SDFT tendinopathies. Muttini et al.¹⁴³ used xenotransplantation with ovine AECs demonstrating that, after 180 d, they were able to induce an almost complete restoration of normal tendon architecture with an optimal alignment of tendon fibers. AECs represent to date the only cell source used for the treatment of tendinopathies where experimental, preclinical, translational, and clinical studies have been combined to demonstrate the efficacy and safety of these stem cell-based protocols.

Studies using UBC-derived stem cells have also demonstrated their efficacy in healing SDFT tendinopathies¹⁴⁷. Additionally, equine UCBMSCs¹⁴⁸ have also demonstrated a therapeutic effect in clinical cases of desmitis of the suspensory ligament and of the inferior check ligament and tendinitis of the deep digital flexor tendon.

Soft Tissue Applications

Ophthalmology

The positive results in human and veterinary medicine have led to the use of the AM as a clinic dressing protocol to promote healing of epithelial tissues^{248,285}. AM transplantation indeed has an effective clinical role in veterinary ophthalmology for its avascular and strong structure and for the large presence of growth factors (mainly antiangiogenic and anti-inflammatory) that are able to prevent or decrease fibrosis during healing.

Indications for its use are steadily growing from previously human experience and include grafting/patching to replace diseased, missing, or excised tissue. Alternatively, AM has been used as a substrate for the expansion of epithelial cells for transplantation for organs or tissues such as the cornea. In this context, AM transplantation has been demonstrated to preserve the integrity of the globe, optimize the visual outcome, and minimize scarring in severely diseased corneas.

Based on these findings, xenogeneic use of AM was adopted in companion animals as replacement for full-thickness corneal defects (18-treated dogs)⁹⁴ and to treat keratomalacia (1 dog)⁹⁴, fibrous histiocytoma (1 dog)⁹⁴, or symblepharon (1 cat)⁹⁵. In all of these clinical cases, the patients experienced reduced ocular pain by recovery of vision and tissue architecture although some degree of graft rejection was observed⁹⁴.

In the same year, AM was also allotransplanted in horses affected by corneal ulceration and severe keratomalacia²⁰⁵. The treatment preserved vision and maintained the structural integrity of the globe by maximizing cosmesis in the eyes. The validity of AM transplantation for ocular surface reconstruction in horses was then definitively confirmed by Plummer et al.²⁰⁶ who conducted a retrospective study on 58 equine clinical cases.

Wound Healing

In domestic animal patients, the use of AM to promote wound healing has been less investigated than in humans to date with the exception of 1 paper by Iacono et al.¹⁹³ which compared AFMSCs and platelet rich plasma (PRP) gel treatments in severe decubitus ulcers by demonstrating that the combination of AFMSCs plus PRP promoted a faster healing in aseptic neonatal foal.

Conclusions

Although PCs do not represent the most widespread used progenitors/stem cells in veterinary science and medicine, they probably are the most promising and scientifically solid cell source studied so far^{239,249}.

Rigorous investigations have begun to clarify the biological properties of PCs in domestic animals by confirming a high degree of conservation among species and reinforcing the idea of that experimental data between species remain robust and can be compared.

One valuable biological characteristic of PCs are their paracrine effects. Several studies using human and animal amniotic-derived cells demonstrated that either the native or induced secretomes contain an array of modulatory molecules and proteins capable of recapitulating most of the regenerative processes involved in the recovery of tissue homeostasis after cell transplantation²⁸⁶. A large number of human and animal studies confirm a conserved paracrine effect of amniotic-derived cells in the modulation of inflammatory and antifibrotic mechanisms. More

recently, new perspectives on application of cells for in antiaging and antitumor therapies owe to the secretory activities of AECs²⁸⁷.

Domestic animal PCs, in addition, have offered advanced insights in relevant challenges related to the ex vivo negative effects. Indeed, although most PCs can be maintained ex vivo and expanded, the yield and recovery of them can be quite variable and their biological characteristics appear to be dependent on the genotype, gestational age of the donor, and the collection/amplification methods^{225,288–290}. These data introduce some important caveats related to cell culture that may limit the comparison of results among research groups and, as a consequence, the translation of animal and human studies into clinical trials. Data recently obtained on domestic animal AECs offers practical solutions to preserve the native phenotype during in vitro cell amplification, and culture methods have been proposed that may improve the biological regenerative properties of expanded AECs thus increasing the comparability of cell transplantation protocols²⁹¹.

The standardization of PC protocols represents, in addition, a prerequisite for developing comparable preclinical treatments. Of utmost importance is that the effectiveness of a treatment designed to replace or regenerate tissues depends on a correct balance between the inherent response of the recipient and the quality of the treatment itself.

The elucidation of the properties of PCs combined with the robust evidence of their safety and regenerative efficacy represents the proof of concept of their therapeutic use^{239,292}.

Based on the evidence discussed, PC-based therapy is now used in veterinary medicine for musculoskeletal disorders, mainly in athletic horses^{141,146,239}.

The clinical translation of the PC-based treatments has been validated by a large amount of data collected in pre-clinical settings, which demonstrated that PC transplantation in different animal models promoted common mechanisms leading to regeneration. The strategies adopted by PCs to combat pathological phenotypes include the exogenous cell graft persistence, the direct replacement of the dysfunctional cells through the in situ tissue-specific lineage transdifferentiation (e.g., tendon- and bone-derived lineage cells), as well as the improvement of endogenous regenerative milieu realized through the release of pro-angiogenic, pro-neurogenic, anti-inflammatory, and antifibrotic factors^{142,216–218,221,274}. However, what is not clear is how long exogenous cells remain viable and active, whether they change significantly with time and/or stimulation in vivo, and how dynamic changes may influence biomolecule production and immunogenicity. The potential extent and impact of PC viability and functionality and the delineation of local and systemic immune responses continue to be vital areas of study.

The biological mechanisms and application of domestic animal PCs to date appears to be more consistent than other sources of progenitor/stem cells that are more commonly used in veterinary medicine such as MSCs

derived from adult tissues. On the basis of the data gathered from preclinical studies and clinical trials, PC-based treatment may have a wide range of applications for treating diseases that affect domestic animals either during adulthood or prenatally.

In light of the robustness of the basic experimental and clinical data obtained using PCs, it is not surprising if the use of these cells moves toward translation to clinical practice in human patients.

Future Perspectives

The demonstration of both safety and efficacy is paramount for translation to clinical applications. However, legislation may mandate delays in translating a method or treatment for veterinary regenerative medicine. Regulating drugs or biologics for veterinary medicine in a manner similar to that in human medicine may not be ideal and in the next future, a legislation in this operative context is desirable in order to be able to certify the safety and efficacy of different cell-based protocols and to standardize these treatments among clinicians.

It has been thoroughly researched and well-supported in preclinical and clinical multicenter studies that PCs possess superior regenerative potential compared to conventional cell-based treatments for horse tendinopathies. In addition, given the high economic value of horses, protocols for cell banking may be highly beneficial for this valuable source of stem cells. This is a practice that vets should consider when preparing for the birth of a foal. PC banking allow for a fast and readily-available supply of stem cells for when an injury occurs, which will give the horse the best chance of a successful recovery. Horses that would have PCs already available would therefore be at a considerable advantage following an SDFT injury. Furthermore, PC cryopreservation is crucial since this would allow for the generation of a quality-controlled stock of cells, transport of cells among investigators, and avoidance of the need for expensive and time-consuming continuous culture.

Furthermore, the consistent availability of data on domestic animal PCs could lead to the use of this cell source and others to treat unexplored animal diseases, which cannot currently be addressed by drug therapy. Detailed characterization of PCs may be also useful insolving emerging and challenging technological issues related to, for example, accuracy and efficacy of cell injection site and doses, cell migration track, and off-target and monitoring long-term cell engraftment. The resolution of these applicative aspects is crucial for standardization of cell-based protocols and for increasing the predictive validity of regenerative medicine applied to human and animal diseases.

Authors' Note

B. Barboni and V. Russo provided equal contribution to this work.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by MIUR with the action PRIN 2015 prot. 20157FF4KM_002 and by the European Community with a MSCA-ITN-2015-EJD: REP-BIOTECH proposal number: 675526 grant.

References

1. Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. *Regen Med.* 2010;5(1):121–143.
2. Jackson WM, Nesti LJ, Tuan RS. Mesenchymal stem cell therapy for attenuation of scar formation during wound healing. *Stem Cell Res Ther.* 2012;3(3):20.
3. Pederiva F, Ghionzoli M, Pierro A, De Coppi P, Tovar JA. Amniotic fluid stem cells rescue both in vitro and in vivo growth, innervation, and motility in nitrofen-exposed hypoplastic rat lungs through paracrine effects. *Cell Transplant.* 2013;22(9):1683–1694.
4. Deeg HJ, Storb R. Canine marrow transplantation models. *Curr Topics Vet Res.* 1994;1:103–114.
5. Thomas ED. A history of haemopoietic cell transplantation. *Br J Haematol.* 1999;105(2):330–339.
6. Thomas ED, Storb R. The development of the scientific foundation of hematopoietic cell transplantation based on animal and human studies. In: Thomas ED, Blume KG, Forman SJ, editors. *Hematopoietic cell transplantation.* 2nd ed.; Blackwell Science: Maiden, Massachusetts; 1999. p. 1–11.
7. Sykes M. Hematopoietic cell transplantation for tolerance induction: animal models to clinical trials. *Transplantation.* 2009;87(3):309–316.
8. Trobridge GD, Kiem HP. Large animal models of hematopoietic stem cell gene therapy. *Gene Ther.* 2010;17(8):939–948.
9. Kahn LH, Kaplan B, Monath TP, Steele JH. Teaching “one medicine, one health.” *Am J Med.* 2008;121(3):169–170.
10. Dehoux JP, Gianello P. The importance of large animal models in transplantation. *Front Biosci.* 2007;Sep 1;12:4864–4880.
11. Wagner JL, Storb R. Preclinical large animal models for hematopoietic stem cell transplantation. *Curr Opin Hematol.* 1996;3(6):410–415.
12. Bruns J, Kampen J, Kahrs J, Plitz W. Achilles tendon rupture: experimental results on spontaneous repair in a sheep-model. *Knee Surg Sports Traumatol Arthrosc.* 2000;8(6):364–369.
13. Wang JH. Mechanobiology of tendon. *J Biomech.* 2006;39(9):1563–1582.
14. McCarty RC, Gronthos S, Zannettino AC, Foster BK, Xian CJ. Characterisation and developmental potential of ovine bone marrow derived mesenchymal stem cells. *J Cell Physiol.* 2009;219(2):324–333.
15. Parker HG, Shearin AL, Ostrander EA. Man’s best friend becomes biology’s best in show: genome analyses in the domestic dog. *Annu Rev Genet.* December 2010;44:309–336.

16. Lunney JK. Advances in swine biomedical model genomics. *Int J Biol Sci.* 2007;3(3):179–184.
17. Kuzmuk KN, Schook LB. Pigs as a model for biomedical sciences. In: Eotheschild MF, Ruvinsky A, editors. *The genetics of the pig*. 2nd ed. 2011. p. 426–444.
18. Switonski M. Dog as a model in studies on human hereditary diseases and their gene therapy. *Reprod Biol.* 2014;14(1):44–50.
19. Volk SW, Theoret C. Translating stem cell therapies: the role of companion animals in regenerative medicine. *Wound Repair Regen.* 2013;21(3):382–394.
20. Fortier LA, Travis AJ. Stem cells in veterinary medicine. *Stem Cell Res Ther.* 2011;2(1):9.
21. Cyranoski D. Stem cells boom in vet clinics. *Nature.* 2013;496(7444):148–149.
22. Black LL, Gaynor J, Gahring D, Adams C, Aron D, Harman S, Gingerich DA, Harman R. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. *Vet Ther.* 2007;8(4):272–284.
23. Cuervo B, Rubio M, Sopena J, Dominguez JM, Vilar J, Morales M, Cugat R, Carrillo JM. Hip osteoarthritis in dogs: a randomized study using mesenchymal stem cells from adipose tissue and plasma rich in growth factors. *Int J Mol Sci.* 2014;15(8):13437–13460.
24. Black LL, Gaynor J, Adams C, Dhupa S, Sams AE, Taylor R, Harman S, Gingerich DA, Harman R. Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. *Vet Ther.* 2008;9(3):192–200.
25. Vilar JM, Batista M, Morales M, Santana A, Cuervo B, Rubio M, Cugat R, Sopena J, Carrillo JM. Assessment of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells in osteoarthritic dogs using a double blinded force platform analysis. *BMC Vet Res.* Jul 1, 2014;10:143.
26. Faltus T, Brehm W. Cell-based veterinary pharmaceuticals—basic legal parameters set by the veterinary pharmaceutical law and the genetic engineering law of the European Union. *Front Vet Sci.* 2016;3:101.
27. Csaki C, Matis U, Mobasheri A, Ye H, Shakibaei M. Chondrogenesis, osteogenesis and adipogenesis of canine mesenchymal stem cells: a biochemical, morphological and ultrastructural study. *Histochem Cell Biol.* 2007;128(6):507–520.
28. Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. *Cell Transplant.* 1997;6(2):125–134.
29. Suter SE. Collection of peripheral blood CD34+ progenitor cells from healthy dogs and dogs diagnosed with lymphoproliferative diseases using a baxter-fenwal CS-3000 plus blood cell separator. *J Vet Intern Med.* 2011;25(6):1406–1413.
30. Huss R, Lange C, Weissinger EM, Kolb HJ, Thalmeier K. Evidence of peripheral blood-derived, plastic-adherent CD34(-low) hematopoietic stem cell clones with mesenchymal stem cell characteristics. *Stem Cells.* 2000;18(4):252–260.
31. Escobar C, Grindem C, Neel JA, Suter SE. Hematologic changes after total body irradiation and autologous transplantation of hematopoietic peripheral blood progenitor cells in dogs with lymphoma. *Vet Pathol.* 2012;49(2):341–343.
32. Kang BJ, Ryu HH, Park SS, Koyama Y, Kikuchi M, Woo HM, Kim WH, Kweon OK. Comparing the osteogenic potential of canine mesenchymal stem cells derived from adipose tissues, bone marrow, umbilical cord blood, and Wharton's jelly for treating bone defects. *J Vet Sci.* 2012;13(3):299–310.
33. Ryu HH, Kang BJ, Park SS, Kim Y, Sung GJ, Woo HM, Kim WH, Kweon OK. Comparison of mesenchymal stem cells derived from fat, bone marrow, Wharton's jelly, and umbilical cord blood for treating spinal cord injuries in dogs. *J Vet Med Sci.* 2012;7(12):1617–1630.
34. Volk SW, Wang Y, Hankenson KD. Effects of donor characteristics and ex vivo expansion on canine mesenchymal stem cell properties: implications for MSC-based therapies. *Cell Transplant.* 2012;21(10):2189–2200.
35. Kisiel AH, McDuffee LA, Masaoud E, Bailey TR, Esparza Gonzalez BP, Nino-Fong R. Isolation, characterization, and in vitro proliferation of canine mesenchymal stem cells derived from bone marrow, adipose tissue, muscle, and periosteum. *Am J Vet Res.* 2012;73(8):1305–1317.
36. Volk SW, Diefenderfer DL, Christopher SA, Haskins ME, Leboy PS. Effects of osteogenic inducers on cultures of canine mesenchymal stem cells. *Am J Vet Res.* 2005;66(10):1729–1737.
37. Bertolo A, Steffen F, Malonzo-Marty C, Stoyanov J. Canine mesenchymal stem cell potential and the importance of dog breed: implication for cell-based therapies. *Cell Transplant.* 2015;24(10):1969–1980.
38. Neupane M, Chang C-C, Kiupel M, Yuzbasiyan-Gurkan V. Isolation and characterization of canine adipose-derived mesenchymal stem cells. *Tissue Eng.* 2008;14(6):1007–1015.
39. Reich CM, Raabe O, Wenisch S, Bridger PS, Kramer M, Arnold S. Comparison of canine adipose and bone marrow-derived mesenchymal stem cells. Paper presented at World Conference on Regenerative Medicine. *Regen Med Suppl;* 2009, Vol. 4, No. 6 (suppl 2).
40. Arnold S, Wenisch S. Adipose tissue derived mesenchymal stem cells for musculoskeletal repair in veterinary medicine. *Am J Stem Cells.* 2015;4(1):1–12.
41. Marx C, Silveira MD, Nardi NB. Adipose-derived stem cells in veterinary medicine: characterization and therapeutic applications. *Stem Cells Dev.* 2015;24(7):803–813.
42. Lee KS, Kang HW, Lee HT, Kim HJ, Kim CL, Song JY, Lee KW, Cha SH. Sequential sub-passage decreases the differentiation potential of canine adipose derived mesenchymal stem cells. *Res Vet Sci.* 2014;96(2):67–275.
43. Martinello T, Bronzini I, Maccatrozzo L, Mollo A, Sampaolesi M, Mascarello F, Decaminada M, Patrino M. Canine adipose-derived-mesenchymal stem cells do not lose stem features after a long-term cryopreservation. *Res Vet Sci.* 2011;91(1):18–24.
44. Vieira NM, Brandalise V, Zucconi E, Secco M, Strauss BE, Zatz M. Isolation, characterization, and differentiation

- potential of canine adipose-derived stem cells. *Cell Transplant*. 2010;19(3):279–289.
45. Lim JH, Byeon YE, Ryu HH, Jeong YH, Lee YW, Kim WH, Kang KS, Kweon OK. Transplantation of canine umbilical cord blood-derived mesenchymal stem cells in experimentally induced spinal cord injured dogs. *J Vet Sci*. 2007;8(3):275–282.
 46. Park SS, Byeon YE, Ryu HH, Kang BJ, Kim Y, Kim WH, Kang KS, Han HJ, Kweon OK. Comparison of canine umbilical cord blood-derived mesenchymal stem cell transplantation times: involvement of astrogliosis, inflammation, intracellular actin cytoskeleton pathways, and neurotrophin-3. *Cell Transplant*. 2011;20(11–12):1867–1880.
 47. Seo MS, Jeong YH, Park JR, Park SB, Rho KH, Kim HS, Yu KR, Lee SH, Jung JW, Lee YS, et al. Isolation and characterization of canine umbilical cord blood-derived mesenchymal stem cells. *J Vet Sci*. 2009;10(3):181–187.
 48. Filioli Uranio M, Dell'Aquila ME, Caira M. Characterization and in vitro differentiation potency of early-passage canine amnion- and umbilical cord-derived mesenchymal stem cells as related to gestational age. *Mol Reprod Dev*. 2014;81(6):539–551.
 49. Lee KS, Nah JJ, Lee BC, Lee HT, Lee HS, So BJ, Cha SH. Maintenance and characterization of multipotent mesenchymal stem cells isolated from canine umbilical cord matrix by collagenase digestion. *Res Vet Sci*. 2013;94(1):144–151.
 50. Filioli Uranio M, Valentini L, Lange-Consiglio A, Caira M, Guaricci AC, L'Abbate A, Catacchio CR, Ventura M, Cremonesi F, Dell'Aquila ME. Isolation, proliferation, cytogenetic, and molecular characterization and in vitro differentiation potency of canine stem cells from foetal adnexa: a comparative study of amniotic fluid, amnion, and umbilical cord matrix. *Mol Reprod Dev*. 2011;78(5):361–373.
 51. Seo MS, Park SB, Kang KS. Isolation and characterization of canine Wharton's jelly-derived mesenchymal stem cells. *Cell Transplant*. 2012;21(7):1493–1502.
 52. Lee KS, Cha SH, Kang HW, Song JY, Lee KW, Ko KB, Lee HT. Effects of serial passage on the characteristics and chondrogenic differentiation of canine umbilical cord matrix derived mesenchymal stem cells. *Asian Aust J Anim Sci*. 2013;26(4):588–595.
 53. Rizzi R, Bearzi C, Mauretto A, Bernardini S, Cannata S, Gargioli C. Tissue engineering for skeletal muscle regeneration. *Muscles Ligaments Tendons J*. 2012;2(3):230–234.
 54. Jang BJ, Byeon YE, Lim JH, Ryu HH, Kim WH, Koyama Y, Kikuchi M, Kang KS, Kweon OK. Implantation of canine umbilical cord blood derived mesenchymal stem cells mixed with beta-tricalcium phosphate enhances osteogenesis in bone defect model dogs. *J Vet Sci*. 2008;9(4):387–393.
 55. Dehghan MM, Eslaminejad MB, Motallebizadeh N, Ashrafi Halan J, Tagiyar L, Soroori S, Nikmahzar A, Pedram M, Shahverdi A, Kazemi Mehrjerdi H, et al. Transplantation of autologous bone marrow mesenchymal stem cells with platelet-rich plasma accelerate distraction osteogenesis in a canine model. *Cell J*. 2015;17(2):243–252.
 56. Marx C, Silveira MD, Selbach I, da Silva AS, Braga LM, Camasola M, Nardi NB. Acupoint injection of autologous stromal vascular fraction and allogeneic adipose-derived stem cells to treat hip dysplasia in dogs. *Stem Cells Int*. 2014; August 11, 2014: 391274.
 57. Crovace A, Favia A, Lacitignola L, Di Comite MS, Staffieri F, Francioso E. Use of autologous bone marrow mononuclear cells and cultured bone marrow stromal cells in dogs with orthopaedic lesions. *Vet Res Comm*. 2008;32(1):39–44.
 58. Cui L, Liu B, Liu G, Zhang W, Cen L, Sun J, Yin S, Liu W, Cao Y. Repair of cranial bone defects with adipose derived stem cells and coral scaffold in a canine model. *Biomaterials*. 2007; 28(36):5477–5486.
 59. Umeda H, Kanemaru SI, Yamashita M, Ohno T, Suehiro A, Tamura Y, Hirano S, Nakamura T, Omori K, Ito J. In situ tissue engineering of canine skull with guided bone regeneration. *Acta Otolaryngol*. 2009;129(12):1509–1518.
 60. Arinzech T, Peter S, Archambault M, van den Bos C, Gordon S, Kraus K, Smith A, Kadiyala S. Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. *J Bone Joint Surg*. 2003;85-A:1927–1935.
 61. Yuan J, Zhang WJ, Liu G, Qi ZL, Liu W, Cui L, Cao YL. Repair of canine mandibular bone defects with bone marrow stromal cells and coral. *Tissue Eng Part A*. 2010;16(4):1385–1394.
 62. Weng Y, Wang M, Liu W, Hu X, Chai G, Yan Q, Zhu L, Cui L, Cao Y. Repair of experimental alveolar bone defects by tissue-engineered bone. *Tiss Eng*. 2006;12(6):1503–1513.
 63. Bruder SP, Kraus KH, Goldberg VM, Kadiyala S. The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg Am*. 1998;80(7):985–996.
 64. Yuan J, Cui L, Zhang WJ, Liu W, Cao Y. Repair of canine mandibular bone defects with bone marrow stromal cells and porous beta-tricalcium phosphate. *Biomaterials*. 2007;28(6): 1005–1013.
 65. Muschler GF, Matsukura Y, Nitto H, Boehm CA, Valdevit AD, Kambic HE, Davros WJ, Easley KA, Powell KA. Selective retention of bone marrow-derived cells to enhance spinal fusion. *Clin Orthop Relat Res*. 2005;(432):242–251.
 66. Crovace A, Staffieri F, Rossi G, Francioso E. Implantation of autologous bone marrow mononuclear cells as a minimal invasive therapy of Legg-Calvé-Perthes' disease in the dog. In *World Conference on Regenerative Medicine*. Regen Med Suppl; 2009, Vol. 4, No. 6 (suppl 2).
 67. Xiang Z, Hu W, Kong Q, Zhou H, Zhang X. Preliminary study of mesenchymal stem cells-seeded type I collagen-glycosaminoglycan matrices for cartilage repair. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi*. 2006;20(2):148–154.
 68. Guercio A, Di Marco P, Casella S, Cannella V, Russotto L, Purpari G, Di Bella S, Piccione G. Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. *Cell Biol Int*. 2012;36(2):189–194.
 69. Nitahara-Kasahara Y, Hayashita-Kinoh H, Ohshima-Hosoyama S, Okada H, Wada-Maeda M, Nakamura A, Okada T, Takeda S. Long-term engraftment of multipotent mesenchymal stromal cells that differentiate to form myogenic cells in dogs with Duchene muscular dystrophy. *Mol Ther*. 2012; 20(1):168–177.

70. Brown GS, Harman RJ, Black LL. Adipose-derived stem cell therapy for severe muscle tears in working German shepherds: two case reports. *Stem Cell Discov.* 2012;2(2):41–44.
71. Park SB, Seo MS, Kim HS, Kang KS. Isolation and characterization of canine amniotic membrane-derived multipotent stem cells. *PLoS One.* 2012;7(9):e44693.
72. Choi SA, Choi HS, Kim KJ, Lee DS, Lee JH, Park JY, Kim EY, Li X, Oh HY, Lee DS, Kim MK. Isolation of canine mesenchymal stem cells from amniotic fluid and differentiation into hepatocyte-like cells. *In Vitro Cell Dev Biol Anim.* 2013;49(1):42–51.
73. Kim EY, Lee KB, Yu J, Lee JH, Kim KJ, Han KW, Park KS, Lee DS, Kim MK. Neuronal cell differentiation of mesenchymal stem cells originating from canine amniotic fluid. *Hum Cell.* 2014;27(2):51–58.
74. Fernandes RA, Wenceslau CV, Reginato AL, Kerkis I, Migli MA. Derivation and characterization of progenitor stem cells from canine allantois and amniotic fluids at the third trimester of gestation. *Placenta.* 2012;33(8):640–644.
75. Jung DL, Ha J, Kang BT, Kim JW, Quan FS, Lee JH, Woo EJ, Park HM. A comparison of autologous and allogeneic bone marrow-derived mesenchymal stem cell transplantation in canine spinal cord injury. *J Neurolog Sci.* 2009;285(1-2):67–77.
76. Sergiano K, Sakai D, Hiyama A, Tamura F, Tanaka M, Mochida J. Effect of cell number on mesenchymal stem cell transplantation in a canine disc degeneration model. *J Orthop Res.* 2010;28(10):1267–1275.
77. Nishida H, Nakayama M, Tanaka H, Kitamura M, Hatoya S, Sugiura K, Harada Y, Suzuki Y, Ide C, Inaba T. Safety of autologous bone marrow stromal cell transplantation in dogs with acute spinal cord injury. *Vet Surg.* 2012;41(4):437–442.
78. Ding F, Wu J, Yang Y, Hu W, Zhu Q, Tang X, Liu J, Gu X. Use of tissue-engineered nerve grafts consisting of a chitosan/poly(lactic-co-glycolic acid)-based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. *Tissue Eng Part A.* 2010;16(12):3779–3790.
79. Ganey T, Hutton WC, Moseley T, Hedrick M, Meisel HJ. Intervertebral disc repair using adipose tissue-derived stem and regenerative cells. *Spine.* 2009;34(21):2297–2304.
80. Adel N, Gabr H. Stem cell therapy of acute spinal cord injury in dogs. *Third world congress of regenerative medicine. Regen Med.* 2007;2(5):523.
81. Vaags AK, Rosic-Kablar S, Gartley CJ, Zheng YZ, Chesney A, Villagómez DAF, Kruth SA, Hough MR. Derivation and characterization of canine embryonic stem cell lines with in vitro and in vivo differentiation potential. *Stem Cells.* 2009;27(2):329–340.
82. Van der Spoel TIG, Jansen of Lorkeers S, Agostoni P, van Belle E, Gyöngyösi M, Sluijter JPG, Cramer MJ, Doevendans PA, Chamuleau SAJ. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. *Cardiovasc Res.* 2011;91(4):649–658.
83. Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Haggstrom J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *J Vet Intern Med.* 2009;23(6):1142–1150.
84. Silva GV, Litovsky S, Assad JAR, Sousa ALS, Martin BJ, Vela D, Coulter SC, Lin J, Ober J, Vaughn WK, et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation.* 2005;111(2):150–156.
85. Vela DC, Silva GV, Assad JAR, Sousa ALS, Coulter S, Fernandes MR, Perin EC, Willerson JT, Buja LM. Histopathological study of healing after allogeneic mesenchymal stem cell delivery in myocardial infarction in dogs. *J Histochem Cytochem.* 2009;57(2):167–176.
86. Bartunek J, Croissant JD, Wijns W, Gofflot S, de Lavareille A, Vanderheyden M, Kaluzhny Y, Mazouz N, Willemsen P, Penicka M, et al. Pretreatment of adult bone marrow mesenchymal stem cells with cardiomyogenic growth factors and repair of the chronically infarcted myocardium. *Am J Physiol Hear Circ Physiol.* 2007;292(2):1095–1104.
87. Perin EC, Silva GV, Assad JAR, Vela D, Buja LM, Sousa ALS, Litovsky S, Lin J, Vaughn WK, Coulter S, et al. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. *J Mol Cell Cardiol.* 2007;44(3):486–495.
88. Hou YB, Zou CW, Wang Y, Li DC, Li QB, Li HX, Zhang HZ, Zhang Q, Fan QX. Establishing a new electrical conduction pathway by anastomosis of the right auricle and right ventricle assisted by mesenchymal stem cells in a canine model. *Transplan Proc.* 2011;43(10):3980–3986.
89. Minguell JJ, Florenzano FM, Ramírez MR, Martínez RF, Lasala GP. Intracoronary infusion of a combination of bone marrow-derived stem cells in dogs. *Exp Clin Cardiol.* 2010;15(2):17–20.
90. Shimada H, Nakada A, Hashimoto Y, Shigeno K, Shionoya Y, Nakamura T. Generation of canine induced pluripotent stem cells by retroviral transduction and chemical inhibitors. *Mol Reprod Dev.* 2010;77(1):2.
91. Luo J, Suhr ST, Chang EA, Want K, Ross PJ, Nelson LL. Generation of leukemia inhibitory factor and basic fibroblast growth factor-dependent induced pluripotent stem cells from canine adult somatic cells. *Stem Cells Dev.* 2011;20(10):1669–1678.
92. Lee AS, Xu D, Plews JR, Nguyen PK, Nag D, Lyons JK. Preclinical derivation and imaging of autologously transplanted canine induced pluripotent stem cells. *J Biol Chem.* 2011;1628(37):32697–32704.
93. Kumar D, Talluri TR, Anand T, Kues WA. Induced pluripotent stem cells: mechanisms, achievements and perspectives in farm animals. *World J Stem Cells.* 2015;7(2):315–328.
94. Barros PS, Garcia JA, Laus JL, Ferreira AL, Salles Gomes TL. The use of xenologous amniotic membrane to repair canine corneal perforation created by penetrating keratectomy. *Vet Ophthalmol.* 1998;1(2–3):119–123.
95. Barros PS, Safatle AM, Godoy CA, Souza MS, Barros LF, Brooks DE. Amniotic membrane transplantation for the reconstruction of the ocular surface in three cases. *Vet Ophthalmol.* 2005;8(3):189–192.

96. Yoo JH, Park C, Jung DI, Lim CY, Kang BT, Kim JH, Park JW, Kim JH, Park HM. In vivo cell tracking of canine allogenic mesenchymal stem cells administration via renal arterial catheterization and physiopathological effects on kidney in two healthy dogs. *J Vet Med Sci.* 2011;73(2):269–274.
97. Smith RK, Korda M, Blunn GW, Goodship AE. Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. *J Equine Vet Sci.* 2003;35(1):99–102.
98. Ahren BJ, Schaer TP, Terkhorn SP, Jackson KV, Mason NJ, Hankenson KD. Evaluation of equine peripheral blood apheresis product, bone marrow, and adipose tissue as sources of mesenchymal stem cells and their differentiation potential. *Am J Vet Res.* 2011;72(1):127–133.
99. Frisbie DD, Kisiday JD, Kawcak CE, Werpy NM, McIlwraith CW. Evaluation of adipose-derived stromal vascular fraction or bone marrow-derived mesenchymal stem cells for treatment of osteoarthritis. *J Orthop Res.* 2009;27:1675–1680.
100. Vidal MA, Robinson SO, Lopez MJ, Paulsen DB, Borkhse-nious O, Johnson JR, Moore RM, Gimble JM. Comparison of chondrogenic potential in equine mesenchymal stromal cells derived from adipose tissue and bone marrow. *Vet Surg.* 2008;37(8):713–724.
101. Vidal MA, Walker NJ, Napoli E, Borjesson DL. Evaluation of senescence in mesenchymal stem cells isolated from equine bone marrow, adipose tissue, and umbilical cord tissue. *Stem Cells Dev.* 2012;21(2):273–283.
102. Toupadakis CA, Wong A, Genetos DC, Cheung WK, Borjes-son DL, Ferraro GL, Galuppo LD, Leach JK, Owens SD, Yelloley CE. Comparison of the osteogenic potential of equine mesenchymal stem cells from bone marrow, adipose tissue, umbilical cord blood, and umbilical tissue. *Am J Vet Res.* 2010;71(10):1237–1245.
103. Fortier LA, Nixon AJ, Williams J, Cable CS. Isolation and chondrocytic differentiation of equine bone marrow-derived mesenchymal stem cells. *Am J Vet Res.* 1998;59(9):1182–1187.
104. Koerner J, Nestic D, Romero JD, Brehm W, Mainil-Varlet P, Grogan SP. Equine peripheral blood-derived progenitors in comparison to bone marrow-derived mesenchymal stem cells. *Stem Cells.* 2006;24(6):1613–1619.
105. Vidal MA, Kilroy GE, Johnson JR, Lopez MJ, Moore RM, Gimble JM. Cell growth characteristics and differentiation frequency of adherent equine bone marrow-derived mesenchymal stromal cells: adipogenic and osteogenic capacity. *Vet Surg.* 2006;35(7):601–610.
106. Vidal MA, Kilroy GE, Lopez MJ, Johnson JR, Moore RM, Gimble JM. Characterization of equine adipose tissue-derived stromal cells: adipogenic and osteogenic capacity and comparison with bone marrow-derived mesenchymal stromal cells. *Vet Surg.* 2007;36(7):613–622.
107. Pacini S, Spinabella S, Trombi L, Fazzi R, Galimberti S, Dini F, Carlucci F, Petrini M. Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. *Tissue Eng.* 2007;13(12):2949–2955.
108. Frisbie DD, Kawcak CE, McIlwraith CW. Evaluation of bone marrow derived stem cells and adipose derived stromal vascular fraction for treatment of osteoarthritis using an equine experimental model. *AAEP Proc.* 2006;52:420–421.
109. Bussche L, van de Walle GR. Peripheral blood-derived mesenchymal stromal cells promote angiogenesis via paracrine stimulation of vascular endothelial growth factor secretion in the equine model. *Stem Cells Transl Med.* 2014;3(12):1514–1525.
110. Nixon AJ, Dahlgren LA, Haupt JL, Yeager AE, Ward DL. Effect of adipose-derived nucleated cell fractions on tendon repair in horses with collagenase-induced tendinitis. *Am J Vet Res.* 2008;69(7):928–937.
111. Del Bue M, Ricco S, Ramoni R, Conti V, Gnudi G, Grolli S. Equine adipose-tissue derived mesenchymal stem cells and platelet concentrates: their association in vitro and in vivo. *Vet Res Comm.* 2008;32(1):51–55.
112. Vidal MA, Walker NJ, Napoli E, Borjesson DL. Evaluation of senescence in mesenchymal stem cells isolated from equine bone marrow, adipose tissue, and umbilical cord tissue. *Stem Cells Dev.* 2012;21(2):273–283.
113. Arnhold S, Wenisch S. Adipose tissue derived mesenchymal stem cells for musculoskeletal repair in veterinary medicine. *Am J Stem Cells.* 2015;4(1):1–12.
114. Marx C, Silveira MD, Nardi NB. Adipose-derived stem cells in veterinary medicine: characterization and therapeutic applications. *Stem Cells Devel.* 2015;24(7):803–813.
115. Stewart A, Chen YJ, Caporali EH, Stewart A. Isolation and chondrogenic differentiation of cells isolated from equine synovial fluid. In *World Conference on Regenerative Medicine. Regen Med Suppl; 2009, Vol. 4, No. 6 (suppl 2).*
116. Murata D, Miyakoshi D, Hatazoe T, Miura N, Tokunaga S, Fujiki M, Nakayama K, Misumi K. Multipotency of equine mesenchymal stem cells derived from synovial fluid. *Vet J.* 2014;202(1):53–61.
117. Zayed M, Caniglia C, Misk N, Dhar MS. Donor-matched comparison of chondrogenic potential of equine bone marrow- and synovial fluid derived mesenchymal stem cells: implications for cartilage tissue regeneration. *Front Vet Sci.* January 18, 2017;3:121.
118. Staszuk C, Gasse H. Primary culture of fibroblasts and cementoblasts of the equine periodontium. *Res Vet Sci.* 2007;82(2):150–157.
119. Warhonowicz M, Staszuk C, Rohn K, Gasse H. The equine periodontium as a continuously remodeling system: morphometrical analysis of cell proliferation. *Arch Oral Biol.* 2006;51(12):1141–1149.
120. Staszuk C, Mensing N, Hambruch N, Häger JD, Pfarrer C, Gasse H. Equine periodontal ligament: a source of mesenchymal stem cells for regenerative therapies in the horse? In *World Conference on Regenerative Medicine. Regen Med Suppl; 2009, Vol. 4, No. 6 (suppl 2).*
121. Carrade DD, Owens SD, Galuppo LD, Vidal MA, Ferraro GL, Librach F, Buerchler S, Friedman MS, Walker NJ, Borjesson

- DL. Clinicopathologic findings following intra-articular injection of autologous and allogeneic placentially derived equine mesenchymal stem cells in horses. *Cytherapy*. 2011;13(4):419–430.
122. Lovati AB, Corradetti B, Lange Consiglio A, Recordati C, Bonacina E, Bizzarro D, Cremonesi F. Comparison of equine bone marrow-, umbilical cord matrix and amniotic fluid-derived progenitor cells. *Vet Res Commun*. 2011;35(2):103–121.
 123. Iacono E, Brunori L, Pirrone A, Pagliaro PP, Ricci F, Tazzari PL, Merlo B. Isolation, characterization and differentiation of mesenchymal stem cells from amniotic fluid, umbilical cord blood and Wharton's jelly in the horse. *Reproduction*. 2012;143(4):455–468.
 124. De Schauwer C, Meyer E, Cornillie P, De Vlieghe S, van de Walle GR, Hoogewijs M, Declercq H, Govaere J, Demeyere K, Cornelissen M, et al. Optimization of the isolation, culture, and characterization of equine umbilical cord blood mesenchymal stromal cells. *Tissue Eng Part C Methods*. 2011;17(11):1061–1070.
 125. Paebst F, Piehler D, Brehm W, Heller S, Schroeck C, Tárnok A, Burk J. Comparative immunophenotyping of equine multipotent mesenchymal stromal cells: an approach toward a standardized definition. *Cytometry A*. 2014;85(8):678–687.
 126. Mohanty N, Gulati BR, Kumar R, Gera S, Kumar P, Somasundaram RK, Kumar S. Immunophenotypic characterization and tenogenic differentiation of mesenchymal stromal cells isolated from equine umbilical cord blood. *In Vitro Cell Dev Biol Anim*. 2014;50(6):538–548.
 127. De Schauwer C, Piepers S, Van de Walle GR, Demeyere K, Hoogewijs MK, Govaere JL, Braeckmans K, Van Soom A, Meyer E. In search for cross-reactivity to immunophenotype equine mesenchymal stromal cells by multicolor flow cytometry. *Cytometry A*. 2012;81(4):312–323.
 128. Guest DJ, Ousey JC, Smith MR. Defining the expression of marker genes in equine mesenchymal stromal cells. *Stem Cells Cloning*. November 2, 2008;1:19.
 129. De Schauwer C, van de Walle GR, Piepers S, Hoogewijs MK, Govaere JL, Meyer E, van Soom A. Successful isolation of equine mesenchymal stromal cells from cryopreserved umbilical cord blood-derived mononuclear cell fractions. *Equine Vet J*. 2013;45(4):518–522.
 130. Carrade DD, Lame MW, Kent MS, Clark KC, Walker NJ, Borjesson DL. Comparative analysis of the immunomodulatory properties of equine adult-derived mesenchymal stem cells. *Cell Med*. 2012;4(1):1–11.
 131. Reed SA, Johnson SE. Equine umbilical cord blood contains a population of stem cells that express Oct4 and differentiate into mesodermal and endodermal cell types. *J Cell Physiol*. 2008;215(2):329–336.
 132. Corradetti B, Lange-Consiglio A, Barucca M, Cremonesi F, Bizzarro D. Size-sieved subpopulations of mesenchymal stem cells from intervacular and perivascular equine umbilical cord matrix. *Cell Prolif*. 2011;44(4):330–342.
 133. Hoynowski SM, Fry MM, Gardner M, Leming MT, Tucker JR, Black L, Sand T, Mitchell KE. Characterization and differentiation of equine umbilical cord-derived matrix cells. *Biochem Biophys Res Commun*. 2007;362(2):347–353.
 134. Lange-Consiglio A, Corradetti B, Rutigliano L, Cremonesi F, Bizzarro D. In vitro studies of horse umbilical cord matrix-derived cells: From characterization to labeling for magnetic resonance imaging. *Open Tissue Eng Regen Med*. 2011;4:120–133.
 135. Passeri S, Nocchi F, Lamanna R, Lapi S, Miragliotta V, Giannessi E, Abramo F, Stornelli MR, Matarazzo M, Plenteda D, et al. Isolation and expansion of equine umbilical cord-derived matrix cells (EUCMCs). *Cell Biol Int*. 2009;33(1):100–105.
 136. Co C, Vickaryous MK, Koch TG. Membrane culture and reduced oxygen tension enhances cartilage matrix formation from equine cord blood mesenchymal stromal cells in vitro. *Osteoarthritis Cartilage*. 2014;22(3):472–480.
 137. Martino NA, Lange-Consiglio A, Cremonesi F, Valentini L, Caira M, Guaricci AC, Ambruosi B, Sciorsci RL, Lacalandra GM, Reshkin SJ, et al. Functional expression of the extracellular calcium sensing receptor (CaSR) in equine umbilical cord matrix size sieved stem cells. *PLoS One*. 2011;6(3):e17714.
 138. Durgam SS, Stewart AA, Caporali EH, Karlin WM, Stewart MC. Effect of tendon derived progenitor cells on a collagenase-induced model of tendinitis in horses. In *World Conference on Regenerative Medicine*. *Regen Med Suppl*; 2009, Vol. 4, No. 6 (suppl 2).
 139. Giai Via A, Frizziero A, Oliva F. Biological properties of mesenchymal stem cells from different sources. *Muscles Ligaments Tendons J*. 2012;2(3):154–162.
 140. Po Yee Lui P, Tik Wong O. Tendon stem cells: experimental and clinical perspectives in tendon and tendon-bone junction repair. *Muscles Ligaments Tendon J*. 2012;2(3):163–168.
 141. Lange-Consiglio A, Rossi D, Tassan S, Perego R, Cremonesi F, Parolini O. Conditioned medium from horse amniotic membrane-derived multipotent progenitor cells: immunomodulatory activity in vitro and first clinical application in tendon and ligament injuries in vivo. *Stem Cells Dev*. 2013;22(22):3015–3024.
 142. Muttini A, Valbonetti L, Abate M, Colosimo A, Curini V, Mauro A, Berardinelli P, Russo V, Cocciolone D, Marchisio M, et al. Ovine amniotic epithelial cells: in vitro characterization and transplantation into equine superficial digital flexor tendon spontaneous defects. *Res Vet Sci*. 2013;94(1):158–169.
 143. Muttini A, Russo V, Rossi E, Mattioli M, Barboni B, Tosi U, Maffulli N, Valbonetti L, Abate M. Pilot experimental study on amniotic epithelial mesenchymal cell transplantation in natural occurring tendinopathy in horses. *Ultrasonographic and histological comparison*. *Muscles, Ligaments and Tendons J*. 2015;5(1):5–11.
 144. Dyson SJ. Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992–2000). *Equine Vet J*. 2004;36(5):415–419.
 145. de Mattos Carvalho A, Alves AL, de Oliveira PGG. Use of adipose tissue-derived mesenchymal stem cells for experimental tendinitis therapy in equines. *J Equine Vet Sci*. 2011;31(1):26–34.

146. Lange-Consiglio A, Corradetti B, Bizzaro D, Magatti M, Ressel L, Tassan S, Parolini O, Cremonesi F. Characterization and potential applications of progenitor-like cells isolated from horse amniotic membrane. *J Tissue Eng Regen Med.* 2012;6(8):622–635.
147. Kang JG, Park SB, Seo MS, Kim HS, Chae JS, Kang KS. Characterization and clinical application of mesenchymal stem cells from equine umbilical cord blood. *J Vet Sci.* 2013;14(3):367–371.
148. Van Loon VJ, Scheffer CJ, Genn HJ, Hoogendoorn AC, Greve JW. Clinical follow-up of horses treated with allogeneic equine mesenchymal stem cells derived from umbilical cord blood for different tendon and ligament disorders. *Vet Q.* 2014;34(2):92–97.
149. Lange-Consiglio A, Corradetti B, Meucci A, Perego R, Bizzaro D, Cremonesi F. Characteristics of equine mesenchymal stem cells derived from amnion and bone marrow: in vitro proliferative and multilineage potential assessment. *Equine Vet J.* 2013;45(6):737–744.
150. Dowling BA, Dart AJ, Hodgson DR, Smith RKW. Superficial digital flexor tendonitis in the horse. *Equine Vet J.* 2000;32(5):369–378.
151. Schnabel LV, Lynch ME, van der Meulen MC, Yeager AE, Kornatowski MA, Nixon AJ. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res.* 2009;27(10):1392–1398.
152. Godwin EE, Young NJ, Dudhia J, Beamish IC, Smith RKW. Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. *Equine Vet J.* 2011;44(1):25–32.
153. Guest DJ, Smith MRW, Allen WR. Equine embryonic stem-like cells and mesenchymal stromal cells have different survival rates and migration patterns following their injection into damaged superficial digital flexor tendons. *Equine Vet J.* 2010;42(7):636–642.
154. Watts AE, Yeager AE, Kopyov OV, Nixon AJ. Fetal derived embryonic-like stem cells improve healing in a large animal flexor tendonitis model. *Stem Cell Res Ther.* 2011;2(4):4–16.
155. Nixon AJ, Watts AE, Schnabel LV. Cell- and gene-based approaches to tendon regeneration. *J Shoulder Elbow Surg.* 2012;21(2):278–294.
156. Hertel D. Enhanced suspensory ligament healing in 100 horses by stem cells and other bone marrow components. *Proc Am Assoc Equine Practice.* 2001;47:319.
157. Guest DJ, Smith MR, Allen WR. Monitoring the fate of autologous and allogeneic mesenchymal progenitor cells injected into the superficial digital flexor tendon of horses: preliminary study. *Equine Vet J.* 2008;40(2):178–181.
158. Smith RK. Mesenchymal stem cell therapy for equine tendinopathy. *Disabil Rehabil.* 2008;30(20-22):1752–1758.
159. Taylor SE, Smith RK, Clegg PD. Mesenchymal stem cell therapy in equine musculoskeletal disease: scientific fact or clinical fiction? *Equine Vet J.* 2007;39(2):172–180.
160. Leppänen M, Miettinen S, Mäkinen S, Wilpola P, Katiskalahti T, Heikkilä P, Tulamo RM. Management of equine tendon and ligament injuries with expanded autologous adipose-derived mesenchymal stem cells: a clinical study. In World Conference on Regenerative Medicine. *Regen Med Suppl;* 2009, Vol. 4, No. 6 (suppl 2).
161. Smith RK, Webbon PM. Harnessing the stem cell for the treatment of tendon injuries: heralding a new dawn? *Br J Sports Med.* 2005;39(9):582–584.
162. Crovace A, Lacitignola L, De SR, Rossi G, Francioso E. Cell therapy for tendon repair in horses: an experimental study. *Vet Res Commun.* 2007;31(1):281–283.
163. Smith R, Young N, Dudhia J, Kasashima Y, Clegg PD, Goodship A. Effectiveness of bone-marrow-derived mesenchymal progenitor cells for naturally occurring tendinopathy in the horse. In World Conference on Regenerative Medicine. *Regen Med Suppl;* 2009, Vol. 4, No. 6 (suppl 2).
164. Brehm W. Equine mesenchymal stem cells for the treatment of tendinous lesions in the horse—cellular, clinical and histologic features. International Bone-Tissue-Engineering Congress, Hannover, Germany, 7–9 November 2008, Engineering Part A. July 2009, 15(5):O-1-O-29. Mary Ann Liebert, Inc. publishers.
165. Mountford DR, Smith RK, Patterson-Kane JC. Mesenchymal stem cell treatment of suspensory ligament branch desmitis; post mortem findings in a 10 year old Russian Warmblood gelding—a case report. *Pferdeheilkunde.* 2006;5 (September/October);22:559–563.
166. Carvalho ADM, Badial PR, Alvarez LE, Yamada L, Borges AS, Deffune E, Hussni CA, Garcia Alves AL. Equine tendonitis therapy using mesenchymal stem cells and platelet concentrates: a randomized controlled trial. *Stem Cell Res Ther.* 2013;4(4):85.
167. Vandenberghe A, Broeckx SY, Beerts C, Seys B, Zimmerman M, Verweire I, Suls M, Spaas JH. Tenogenically induced allogeneic mesenchymal stem cells for the treatment of proximal suspensory ligament desmitis in horse. *Front Vet Sci.* 2015;2:49.
168. Dahlgren LA. Fat-derived mesenchymal stem cells for equine tendon repair. In World Conference on Regenerative Medicine. *Regen Med Suppl;* 2009, Vol. 4, No. 6 (suppl 2).
169. Caniglia CJ, Schramme MC, Smith RK. The effect of intraligamentary injection of bone marrow derived mesenchymal stem cells and bone marrow supernatant on collagen fibril size in a surgical model of equine superficial digital flexor tendonitis. *Equine Vet J.* 2012;44(5):587–593.
170. Marfè G, Rotta G, De Martino L, Tafani M, Fiorito F, Di Stefano C, Poletini M, Ranalli M, Russo MA, Gambacurta A. A new clinical approach: use of blood-derived stem cells (BDSCs) for superficial digital flexor tendon injuries in horses. *Life Sci.* 2012;90(21-22):825–830.
171. Chaudhury S. Mesenchymal stem cell applications to tendon healing. *Muscles Ligaments Tendons J.* 2012;2(3):222–229.
172. Renzi S, Ricco S, Dotti S, Sesso L, Grolli S, Cornali M, Carlin S, Patruno M, Cinotti S, Ferrari M. Autologous bone marrow mesenchymal stromal cells for regeneration of injured equine

- ligaments and tendons: a clinical report. *Res Vet Sci.* 2013; 95(1):272–277.
173. Smith RK. Stem cell technology in equine tendon and ligament injuries. *Vet Rec.* 2006;158(4):140.
 174. Sole A, Spriet M, Padgett KA, Vaughan B, Galuppo LD, Borjesson DL, Wisner ER, Vidal MA. Distribution and persistence of technetium-99 hexamethyl propylene amine oxime-labelled bone marrow-derived mesenchymal stem cells in experimentally induced tendon lesions after intratendinous injection and regional perfusion of the equine distal limb. *Equine Vet J.* 2013;45(6):726–731.
 175. McDuffee L. Osteoprogenitors in bone repair. In *World Conference on Regenerative Medicine. Regen Med Suppl;* 2009, Vol. 4, No. 6 (suppl 2).
 176. Milner PI, Clegg PD, Stewart MC. Stem cell-based therapies for bone repair. *Vet Clin North Am Equine Pract.* 2011;27(2): 299–314.
 177. Wilke MM, Nydam DV, Nixon AJ. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res.* 2007;25(7):913–925.
 178. Ahern BJ, Parvizi J, Boston R, Schaer TP. Preclinical animal models in single site cartilage defect testing: a systematic review. *Osteoarthritis Cartilage.* 2009;17(6):705–713.
 179. Koch TG, Betts DH. Stem cell therapy for joint problems using the horse as a clinically relevant animal model. *Expert Opin Biol Ther.* 2007;7(11):1621–1626.
 180. Brommer H, van Weeren PR, Brama PA. New approach for quantitative assessment of articular cartilage degeneration in horses with osteoarthritis. *Am J Vet Res.* 2003;64(1):83–87.
 181. Goodrich LR, Nixon AJ. Medical treatment of osteoarthritis in the horse—a review. *Vet J.* 2006;171(1):51–69.
 182. Litzke LE, Wagner E, Baumgaertner W, Hetzel U, Josimovic-Alasevic O, Libera J. Repair of extensive articular cartilage defects in horses by autologous chondrocyte transplantation. *Ann Biomed Eng.* 2004;32(1):57–69.
 183. Frisbie DD. Future directions in treatment of joint disease in horses. *Vet Clin North Am Equine Pract.* 2005;21(3):713–724.
 184. Broeckx S, Zimmerman M, Crocetti S, Suls M, Mariën T, Ferguson SJ, Chiers K, Duchateau L, Franco-Obregón A, Wuertz K, et al. Regenerative therapies for equine degenerative joint disease: a preliminary study. *PLoS One.* 2014;9(1):e85917.
 185. McIlwraith CW, Frisbie DD, Rodkey WG, Kisiday JD, Wery NM, Kawcak CE, Steadman JR. Evaluation of intra-articular mesenchymal stem cells to augment healing of microfractured chondral defects. *Arthroscopy.* 2011;27(11):1552–1561.
 186. Lee HY, Kopesky PW, Plaas A, Sandy J, Kisiday J, Frisbie D, Grodzinsky AJ, Ortiz C. Adult bone marrow stromal cell-based tissue-engineered aggrecan exhibits ultrastructure and nanomechanical properties superior to native cartilage. *Osteoarthritis Cartilage.* 2010;18(11):1477–1486.
 187. McCarthy HE, Bara JJ, Brakspear K, Singhrao SK, Archer CW. The comparison of equine articular cartilage progenitor cells and bone marrow-derived stromal cells as potential cell sources for cartilage repair in the horse. *Vet J.* 2012;192(3): 45–351.
 188. Ferris D, Frisbie D, Kisiday J, McIlwraith CW. In vivo healing of meniscal lacerations using bone marrow-derived mesenchymal stem cells and fibrin glue. *Stem Cells Int.* 2012;2012:691605.
 189. Ferris D. Clinical evaluation of bone marrow-derived mesenchymal stem cells in naturally occurring joint disease. In *World Conference on Regenerative Medicine. Regen Med Suppl;* 2009, Vol. 4, No. 6 (suppl 2).
 190. Fortier LA, Potter HG, Rickey EJ, Schnabel LV, Foo LF, Chong LR, Stokol T, Cheatham J, Nixon AJ. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *J Bone Joint Surg Am.* 2010;92(10):1927–1937.
 191. Sandoval JA, López C, Carmona JU. Therapies intended for joint regeneration in the horse. *Arch Med Vet.* 2013;45(3): 229–236.
 192. Trumble TN, Trotter GW, Oxford JR, McIlwraith CW, Cammarata S, Goodnight JL, Billingham RC, Frisbie DD. Synovial fluid gelatinase concentrations and matrix metalloproteinase and cytokine expression in naturally occurring joint disease in horses. *Am J Vet Res.* 2001;62(9):1467–1477.
 193. Iacono E, Merlo B, Pirrone A, Antonelli C, Brunori L, Romagnoli N, Castagnetti C. Effects of mesenchymal stem cells isolated from amniotic fluid and platelet-rich plasma gel on severe decubitus ulcers in a septic neonatal foal. *Res Vet Sci.* 2012;93(3):1439–1440.
 194. Corradetti B, Correani A, Romaldini A, Marini MG, Bizzaro D, Perrini C, Cremonesi F, Lange-Consiglio A. Amniotic membrane-derived mesenchymal cells and their conditioned media: potential candidates for uterine regenerative therapy in the horse. *PLoS One.* 2014;9(10):e111324.
 195. Coli A, Nocchi F, Lamanna R, Iorio M, Lapi S, Urciuoli P, Scatena F, Giannesi E, Stornelli MR, Passeri S. Isolation and characterization of equine amnion mesenchymal stem cells. *Cell Biol Int Rep.* 2011;18(1):e00011.
 196. Violini S, Gorni C, Pisani LF, Ramelli P, Caniatti M, Mariani P. Isolation and differentiation potential of an equine amnion-derived stromal cell line. *Cytotechnology.* 2012; 64(1):1–7.
 197. Gulati BR, Kumar R, Mohanty N, Kumar P, Somasundaram RK, Yadav PS. Bone morphogenetic protein-12 induces tenogenic differentiation of mesenchymal stem cells derived from equine amniotic fluid. *Cells Tissues Organs.* 2013;198(5): 377–389.
 198. Park SB, Seo MS, Kang JG, Chae JS, Kang KS. Isolation and characterization of equine amniotic fluid-derived multipotent stem cells. *Cytotherapy.* 2011;13(3):341–349.
 199. Spaas JH, Broeckx S, Van deWalle GR, Poletti M. The effects of equine peripheral blood stem cells on cutaneous wound healing: a clinical evaluation in four horses. *Clin Exp Dermatol.* 2013;38(3):280–284.
 200. Li X, Zhou SG, Imreh MP, Ahrlund-Richter L, Allen WR. Horse embryonic stem cell lines from the proliferation of inner cell mass cells. *Stem Cells Dev.* 2006;15(4):523–531.
 201. Desmaris J, Demers SP, Suzuki J, Laflamme S, Vincent P, Laverty S, Smith LC. Trophoblast stem cell marker gene

- expression in inner cell mass-derived cells from parthenogenetic equine embryos. *Reproduction*. 2011;141(3):321–332.
202. Guest DJ, Allen WR. Expression of cell-surface antigens and embryonic stem cell pluripotency genes in equine blastocysts. *Stem Cells Dev*. 2007;16(5):789–796.
203. Fortier LA. Equine embryonic stem and induced pluripotent stem cells. Paper presented at World Conference on Regenerative Medicine. *Regen Med Suppl*; 2009, Vol. 4, No. 6 (Suppl 2).
204. Guest DJ, Li X, Allen WR. Establishing an equine embryonic stem cell line. Paper presented at World Conference on Regenerative Medicine. *Regen Med Suppl*; 2009, Vol. 4, No. 6 (2).
205. Lassaline ME, Brooks DE, Ollivier FJ, Komaromy AM, Kallberg ME, Gelatt KN. Equine amniotic membrane transplantation for corneal ulceration and keratomalacia in three horses. *Vet Ophthalmol*. 2005;8(5):311–317.
206. Plummer CE, Ollivier F, Kallberg M, Brooks D, Barrie K, Utter M, Gelatt K. The use of amniotic membrane transplantation for ocular surface reconstruction: a review and series of 58 equine clinical cases (2002–2008). *Vet Ophthalmol*. 2009;12(1):17–24.
207. Nagy K, Sung HK, Zhang P, Laflamme S, Vincent P, Agha-Mohammadi S. Induced pluripotent stem cell lines derived from equine fibroblasts. *Stem Cell Rev*. 2011;7(3):693–702.
208. Khodadadi K, Sumer H, Pashaiasi M, Lim S, Williamson M, Verma PJ. Induction of pluripotency in adult equine fibroblasts without c-MYC. *Stem Cells Int*. 2012;2012:ID429160.
209. Donadeu X, Breton A, Diaz C. Transgene-induced reprogramming of equine fibroblasts. In World Conference on Regenerative Medicine. *Regen Med Suppl*; 2009; 2009, Vol. 4, No. 6 (suppl 2).
210. Niemeyer P, Fechner K, Milz S, Richter W, Suedkamp NP, Mehlhorn AT, Pearce S, Kasten P. Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. *Biomaterials*. 2010;31(13):3572–3579.
211. Mrozik KM, Zilm PS, Bagley CJ, Hack S, Hoffman P, Gronthos S, Bartold PM. Proteomic characterization of mesenchymal stem cell-like populations derived from ovine periodontal ligament, dental pulp, and bone marrow: analysis of differentially expressed proteins. *Stem Cells Dev*. 2010;19(10):1485–1499.
212. Kon E, Muraglia A, Corsi A, Bianco P, Marcacci M, Martin I, Boyde A, Ruspantini I, Chistolini P, Rocca M, et al. Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones. *J Biomed Mater Res*. 2000;49(3):328–337.
213. Jäger M, Bachmann R, Scharfstädt A, Krauspe R. Ovine cord blood accommodates multipotent mesenchymal progenitor cells. *In Vivo*. 2006;20(2):205–214.
214. Fadel L, Viana BR, Feitosa ML, Ercolin AC, Roballo KC, Casals JB, Pieri NC, Meirelles FV, Martins Ddos S, Miglino MA, et al. Protocols for obtainment and isolation of two mesenchymal stem cell sources in sheep. *Acta Cir Bras*. 2011;26(4):267–273.
215. Mauro A, Russo V, Di Marcantonio L, Berardinelli P, Martelli A, Muttini A, Mattioli M, Barboni B. M1 and M2 macrophage recruitment during tendon regeneration induced by amniotic epithelial cell allotransplantation in ovine. *Res Vet Sci*. 2016;105(April):92–102.
216. Barboni B, Russo V, Curini V, Mauro A, Martelli A, Muttini A, Bernabò N, Valbonetti L, Marchisio M, Di Giacinto O, et al. Achilles tendon regeneration can be improved by amniotic epithelial cell allotransplantation. *Cell Transplant*. 2012;21(11):2377–2395.
217. Colosimo A, Curini, Russo V, Mauro A, Bernabò N, Marchisio M, Alfonsi M, Muttini A, Mattioli M, Barboni B. Characterization, GFP gene nucleofection, and allotransplantation in injured tendons of ovine amniotic fluid-derived stem cells. *Cell Transplant*. 2013;22(1):99–117.
218. Russo V, Berardinelli P, Gatta V, Muttini A, Stuppia L, Parolini O, Mattioli M, Barboni B. Cross-talk between human amniotic derived cells and host tendon supports tissue regeneration. *J Tissue Eng Regen Med*. 2014;8(Suppl. 1):142.
219. Crovace A, Lacitignola L, Francioso E, Rossi G. Histology and immunohistochemistry study of ovine tendon grafted with cBMSCs and BMMNCs after collagenase-induced tendinitis. *Vet Comp Orthop Traumatol*. 2008;21(4):329–336.
220. Mattioli M, Gloria A, Turriani M, Mauro A, Curini V, Russo V, Tetè S, Marchisio M, Pierdomenico L, Berardinelli P, et al. Stemness characteristics and osteogenic potential of sheep amniotic epithelial cells. *Cell Biol Int*. 2012;36(1):7–19.
221. Barboni B, Mangano C, Valbonetti L, Marruchella G, Berardinelli P, Martelli A, Muttini A, Mauro A, Bedini R, Turriani M, et al. Synthetic bone substitute engineered with amniotic epithelial cells enhances bone regeneration after maxillary sinus augmentation. *PLoS One*. 2013;8(5):e63256.
222. Berardinelli P, Valbonetti L, Muttini A, Martelli A, Peli R, Zizzari V, Nardinocchi D, Vulpiani MP, Tetè S, Barboni B, et al. Role of amniotic fluid mesenchymal cells engineered on MgHA/collagen-based scaffold allotransplanted on an experimental animal study of sinus augmentation. *Clin Oral Investig*. 2013;17(7):1661–1675.
223. Garcia D, Longo UG, Vaquero J, Forriol F, Loppini M, Khan WS, Denaro V. Amniotic membrane transplant for articular cartilage repair: an experimental study in sheep. *Curr Stem Cell Res Ther*. 2014;10(1):77–83.
224. Barboni B, Curini V, Russo V, Mauro A, Di Giacinto O, Marchisio M, Alfonsi M, Mattioli M. Indirect co-culture with tendons or tenocytes can program amniotic epithelial cells towards stepwise tenogenic differentiation. *PLoS One*. 2012;7(2):e30974.
225. Barboni B, Russo V, Curini V, Martelli A, Berardinelli P, Mauro A, Mattioli M, Marchisio M, Bonassi Signoroni P, Parolini O, et al. Gestational stage affects amniotic epithelial cells phenotype, methylation status, immunomodulatory and stemness properties. *Stem Cell Rev*. 2014;10(5):725–741.
226. Muttini A, Mattioli M, Petrizzi L, Varasano V, Sciarrini C, Russo V, Mauro A, Cocciolone D, Turriani M, Barboni B. Experimental study on allografts of amniotic epithelial cells

- in calcaneal tendon lesions of sheep. *Vet Res Commun.* 2010; 34(1): S117–S120.
227. Shaw SW, Bollini S, Nader KA, Gastaldello A, Mehta V, Filppi E, Cananzi M, Gaspar HB, Qasim W, De Coppi P, et al. Autologous transplantation of amniotic fluid derived mesenchymal stem cells into sheep fetuses. *Cell Transplant.* 2011;20(7):1015–1031.
 228. Shaw SW, Blundell MP, Pipino C, Shangaris P, Maghsoudlou P, Ramachandra DL, Georgiades F, Boyd M, Thrasher AJ, Porada CD, et al. Sheep CD34+ amniotic fluid cells have hematopoietic potential and engraft after autologous in utero transplantation. *Stem Cells.* 2015;33(1):122–132.
 229. Gray FL, Turner CG, Ahmed A, Calvert CE, Zurakowski D, Fauza DO. Prenatal tracheal reconstruction with a hybrid amniotic mesenchymal stem cells-engineered construct derived from decellularized airway. *J Pediatr Surg.* 2012; 47(6):1072–1079.
 230. Weber B, Emmert MY, Behr L, Schoenauer R, Brokopp C, Drögemüller C, Modregger P, Stampanoni M, Vats D, Rudin M, et al. Prenatally engineered autologous amniotic fluid stem cell-based heart valves in the fetal circulation. *Biomaterials.* 2012;33(16):4031–4043.
 231. Zhu X, Wang X, Cao G, Liu F, Yang Y, Li X, Zhang Y, Mi Y, Liu J, Zhang L. Stem cell properties and neural differentiation of sheep amniotic epithelial cells. *Neural Regen Res.* 2013; 8(13):1210–1219.
 232. Mauro A, Turriani M, Ioannoni A, Russo V, Martelli A, Di Giacinto O, Nardinocchi D, Berardinelli P. Isolation, characterization, and in vitro differentiation of ovine amniotic stem cells. *Vet Res Commun.* 2010;34(1): S25–S28.
 233. Di Tomo P, Pipino C, Lanuti P, Morabito C, Pierdomenico L, Sirolli V, Bonomini M, Miscia S, Mariggio MA, Marchisio M, et al. Calcium sensing receptor expression in ovine amniotic fluid mesenchymal stem cells and the potential role of R-568 during osteogenic differentiation. *PLoS One.* 2013; 8(9):e73816.
 234. Weber B, Kehl D, Bleul U, Behr L, Sammut S, Frese L, Ksiazek A, Achermann J, Stranzinger G, Robert J, et al. In vitro fabrication of autologous living tissue engineered vascular grafts based on prenatally harvested ovine amniotic fluid-derived stem cells. *J Tissue Eng Regen Med.* 2016; 10(1):52–70.
 235. Kunisaki SM, Fuchs JR, Steigman SA, Fauza DO. A comparative analysis of cartilage engineered from different perinatal mesenchymal progenitor cells. *Tissue Eng.* 2007; 13(11):2633–2644.
 236. Klein JD, Turner CG, Steigman SA, Ahmed A, Zurakowski D, Eriksson E, Fauza DO. Amniotic mesenchymal stem cells enhance normal fetal wound healing. *Stem Cells Dev.* 2011; 20(6):969–976.
 237. Notarianni E, Galli C, Laurie S, Moor RM, Evans MJ. Derivation of pluripotent, embryonic cell lines from the pig and sheep. *J Reprod Fertil Suppl.* 1991;43:255–260.
 238. Liu J, Balehosur D, Murray B, Kelly JM, Sumer H, Verma PJ. Generation and characterization of reprogrammed sheep induced pluripotent stem cells. *Theriogenology.* 2012;77(2): 338–346.
 239. Barboni B, Russo V, Berardinelli P, Muttini A, Mattioli M. 12 applications of placenta-derived cells in veterinary medicine. In: *Placenta The Tree of Life.* Boca Raton, FL: CRC Press-Taylor & Francis; 2016. p. 217.
 240. Li X, Bai J, Ji X, Li R, Xuan Y, Wang Y. Comprehensive characterization of four different populations of human mesenchymal stem cells as regards their immune properties, proliferation and differentiation. *Int J Mol Med.* 2014;34: 695–704.
 241. Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea.* 2000;19(3):348–52.
 242. Steed DL, Trumpower C, Duffy D, Smith C, Marshall V, Rupp R, Robson M. Amnion-derived cellular cytokine solution: a physiological combination of cytokines for wound healing. *Eplasty.* April 7, 2008;8:e18.
 243. Kakishita K, Elwan MA, Nakao N, Itakura T, Sakuragawa N. Human amniotic epithelial cells produce dopamine and survive after implantation into the striatum of a rat model of Parkinson's disease: a potential source of donor for transplantation therapy. *Exp Neurol.* 2000;165(1):27–34.
 244. Liu Y, Mu R, Wang S, Long L, Liu X, Li R, Sun J, Guo J, Zhang X, Guo J, et al. Therapeutic potential of human umbilical cord mesenchymal stem cells in the treatment of rheumatoid arthritis. *Arthritis Res Ther.* 2010;12(6):R210.
 245. Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A. Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. *Immunology.* 2009;127(1):26–39.
 246. Parolini O, Caruso M. Review: preclinical studies on placenta-derived cells and amniotic membrane: an update. *Placenta.* 2011;32(Suppl 2): S186–S195.
 247. Bailo M, Soncini M, Vertua E, Signoroni PB, Sanzone S, Lombardi G, Arienti D, Calamani F, Zatti D, Paul P, et al. Engraftment potential of human amnion and chorion cells derived from term placenta. *Transplantation.* 2004;78(10): 1439–1448.
 248. Parolini O, Soncini M, Evangelista M, Schmidt D. Amniotic membrane and amniotic fluid-derived cells: potential tools for regenerative medicine? *Regen Med.* 2009;4(2):275–291.
 249. Cremonesi F, Corradetti B, Lange Consiglio A. Fetal adnexa derived stem cells from domestic animal: progress and perspectives. *Theriogenology.* 2011;75(8):1400–1415.
 250. Raggi C, Berardi AC. Mesenchymal stem cell, aging and regenerative medicine. *Muscles Ligaments Tendons J.* 2012;16(2/3):239–242.
 251. Abbah S, Spanoudes K, O'Brien T, Pandit A, I Zeugolis D. Assessment of stem cell carriers for tendon tissue engineering in pre-clinical models. *Stem Cell Res Ther.* 2014;5(2):38.
 252. Longo UG, Lamberti A, Maffulli N, Denaro V. Tendon augmentation grafts: a systematic review. *Br Med Bull.* 2010;94: 165–188.

253. Oliva F, Piccirilli E, Berardi AC, Frizziero A, Tarantino U, Maffulli N. Hormones and tendinopathies: the current evidence. *Br Med Bull.* 2016;117(1):39–58.
254. Oliva F, Piccirilli E, Berardi AC, Tarantino U, Maffulli N. Influence of thyroid hormones on tendon homeostasis. *Adv Exp Med Biol.* 2016;920:133–138.
255. Zeugolis D, Chan J, Pandit A. Tendons: engineering of functional tissues. In: Pallua N, Suscheck C, editors. *Tissue engineering.* Springer, Berlin: Heidelberg; 2011. p. 537–572.
256. Williams RB, Harkins LS, Hammond CJ, Wood JL. Racehorse injuries, clinical problems and fatalities recorded on British racecourses from flat racing and National Hunt racing during 1996, 1997 and 1998. *Equine Vet J.* 2001;33(5):478–486.
257. Alves AG, Stewart AA, Dudhia J, Kasashima Y, Goodship AE, Smith RK. Cell-based therapies for tendon and ligament injuries. *Vet Clin North Am Equine Pract.* 2011;27(2):315–333.
258. Sharma P, Maffulli N. Biology of tendon injury: healing, modeling and remodeling. *J Musculoskelet Neuronal Interact.* 2006;6(2):181–190.
259. Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. *J Bone Joint Surg Am.* 2005;87(1):187–202.
260. Duerden JD, Keeling JJ. Disorders of the Achilles tendon. *Curr Orthop Pract.* 2008;19:253–259.
261. Järvinen TA, Järvinen TL, Kääriäinen M, Kalimo H, Järvinen M. Muscle injuries: biology and treatment. *Am J Sports Med.* 2005;33(5):745–764.
262. Muttini A, Salini V, Valbonetti L, Abate M. Stem cell therapy of tendinopathies: suggestions from veterinary medicine. *Muscles Ligaments Tendons J.* 2012;2(3):187–192.
263. Hoffmann A, Pelled G, Turgeman G, Eberle P, Zilberman Y, Shinar H, Keinan-Adamsky K, Winkel A, Shahab S, Navon G, et al. Neotendon formation induced by manipulation of the Smad8 signalling pathway in mesenchymal stem cells. *J Clin Invest.* 2006;116(4):940–952.
264. Chen X, Yin Z, Chen JL, Shen WL, Liu HH, Tang QM, Fang Z, Lu LR, Ji J, Ouyang HW. Force and scleraxis synergistically promote the commitment of human ES cells derived MSCs to tenocytes. *Sci Rep.* 2012;2:977.
265. Hou Y, Mao Z, Wei X, Lin L, Chen L, Wang H, Fu X, Zhang J, Yu C. The roles of TGF-beta1 gene transfer on collagen formation during Achilles tendon healing. *Biochem Biophys Res Commun.* 2009;383(2):235–239.
266. Awad HA, Butler DL, Boivin GP, Smith FN, Malaviya P, Hui-bregtse B, Caplan AI. Autologous mesenchymal stem cell-mediated repair of tendon. *Tissue Eng.* 1999;5(3):267–277.
267. Kunisaki SM, Fuchs JR, Kaviani A, Oh JT, LaVan DA, Vacanti JP, Wilson JM, Fauza DO. Diaphragmatic repair through fetal tissue engineering: a comparison between mesenchymal amniocyte- and myoblast-based constructs. *J Pediatr Surg.* 2006;41(1):34–39.
268. Turner CG, Klein JD, Steigman SA, Armant M, Nicksa GA, Zurakowski D, Ritz J, Fauza DO. Preclinical regulatory validation of an engineered diaphragmatic tendon made with amniotic mesenchymal stem cells. *J Pediatr Surg.* 2011;46(1):57–61.
269. Valbonetti L, Berardinelli P, Scarano A, Piattelli A, Mattioli M, Barboni B, Vulpiani MP, Muttini A. Translational value of sheep as animal model to study sinus augmentation. *J Craniofac Surg.* 2015;26(3):737–740.
270. Grageda E, Lozada JL, Boyne PJ, Caplanis N, McMillan PJ. Bone formation in the maxillary sinus by using platelet-rich plasma: an experimental study in sheep. *J Oral Implantol.* 2005;31(1):2–17.
271. Scarano A, Piattelli A, Pecora G, Petrizzi L, Valbonetti L, Varasano V, Iezzi G. A histomorphometric comparison of anorganic bovine bone (ABB) and calcium sulfate (CaS) used in sinus augmentation procedures: a study in sheep. *J Osseointegration.* 2010;2(2):38–44.
272. Meng XT, Li C, Dong ZY, Liu JM, Li W, Liu Y, Xue H, Chen D. Co-transplantation of bFGF-expressing amniotic epithelial cells and neural stem cells promotes functional recovery in spinal cord-injured rats. *Cell Biol Int.* 2008;32(12):1546–1558.
273. Murphy SV, Atala A. Amniotic fluid and placental membranes: Unexpected sources of highly multipotent cells. *Semin Reprod Med.* 2013;31(1):62–68.
274. Sartore S, Lenzi M, Angelini A, Chiavegato A, Gasparotto L, De Coppi P, Bianco R, Gerosa G. Amniotic mesenchymal cells autotransplanted in a porcine model of cardiac ischemia do not differentiate to cardiogenic phenotypes. *Eur J Cardiothorac Surg.* 2005;28(5):677–684.
275. Kimura M, Toyoda M, Gojo S, Itakura Y, Kami D, Miyoshi S, Kyo S, Ono M, Umezawa A. Allogeneic amniotic membrane-derived mesenchymal stromal cell transplantation in a porcine model of chronic myocardial ischemia. *J Stem Cells Regen Med.* 2012;8(3):171–180.
276. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells.* 2007;(10):2648–2659.
277. Azari O, Babaei H, Derakhshanfar A, Nematollahi-Mahani SN, Poursahebi R, Moshrefi M. Effects of transplanted mesenchymal stem cells isolated from Wharton's jelly of caprine umbilical cord on cutaneous wound healing; histopathological evaluation. *Vet Res Commun.* 2011;35(4):211–222.
278. Airway Reconstruction Team. Recent challenges in the management of congenital tracheal stenosis: an individualized approach. *J Pediatr Surg.* 2005;40(5):774–780.
279. Richardson LE, Dudhia J, Clegg PD, Smith R. Stem cells in veterinary medicine—attempts at regenerating equine tendon after injury. *Trends Biotechnol.* 2007;25(9):409–416.
280. Kasashima Y, Takahashi T, Smith RK, Goodship AE, Kuwano A, Ueno T, Hirano S. Prevalence of superficial digital flexor tendonitis and suspensory desmitis in Japanese thoroughbred flat racehorses in 1999. *Equine Vet J.* 2004;36(4):346–350.
281. Crevier-Denoix N, Collobert C, Pourcelot P, Denoix JM, Sanaa M, Geiger D, Bernard N, Ribot X, Bortolussi C, Bousseau B. Mechanical properties of pathological equine superficial digital flexor tendons. *Equine Vet J Suppl.* 1997;(23):23–26.
282. Dahlgren LA, Mohammed HO, Nixon AJ. Temporal expression of growth factors and matrix molecules in healing tendon lesions. *J Orthop Res.* 2005;23(1):84–92.

283. Williams IF, Heaton A, McCullagh KG. Cell morphology and collagen types in equine tendon scar. *Res Vet Sci.* 1980;28(3):302–310.
284. Lange-Consiglio A, Tassan S, Corradetti B, Meucci A, Perigo R, Bizzaro D, Cremonesi F. Investigating the efficacy of amnion-derived compared with bone marrow-derived mesenchymal stromal cells in equine tendon and ligament injuries. *Cytotherapy.* 2013;15(8):1011–1020.
285. Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. *Br J Ophthalmol.* 1999;83(4):399–402.
286. Di Germanio C, Bernier M, de Cabo R, Barboni B. Amniotic epithelial cells: a new tool to combat aging and age-related diseases? *Front Cell Dev Biol.* 2016;4:135.
287. Di Germanio C, Bernier M, Petr M, Mattioli M, Barboni B, de Cabo R. Conditioned medium derived from rat amniotic epithelial cells confers protection against inflammation, cancer, and senescence. *Oncotarget.* 2016;7(26):39051–39064.
288. Izumi M, Pazin BJ, Minervini CF, Gerlach J, Ross MA, Stolz DB, Turner ME, Thompson RL, Miki T. Quantitative comparison of stem cell marker-positive cells in fetal and term human amnion. *J Reprod Immunol.* 2009;81(1):39–43.
289. Stadler G, Hennerbichler S, Lindenmair A, Peterbauer A, Hofer K, van Griensven M, Gabriel C, Redl H, Wolbank S. Phenotypic shift of human amniotic epithelial cells in culture is associated with reduced osteogenic differentiation in vitro. *Cytotherapy.* 2008;10(7):743–752.
290. Alcaraz A, Mrowiec A, Insausti CL, García-Vizcaíno EM, Ruiz-Canada C, López-Martínez MC, Moraleda JM, Nicolás FJ. Autocrine TGF- β induces epithelial to mesenchymal transition in human amniotic epithelial cells. *Cell Transplant.* 2013;22(8):1351–1367.
291. Canciello A, Parolini O, Barboni B. Progesterone prevents epithelial-mesenchymal transition of ovine amniotic epithelial cells and enhances their immunomodulatory properties. *Eur Cell Mater.* 2016;31(1):170.
292. Caruso M, Evangelista M, Parolini O. Human term placental cells: phenotype, properties and new avenues in regenerative medicine. *Int J Mol Cell Med.* 2012;1(2):64–74.