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A new clinical approach: Use of blood-derived stem cells (BDSCs) for superficial digital flexor tendon injuries in horses

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ABSTRACT

Aims: In this study, we present an innovative therapy using stem cells that were obtained from the peripheral blood of racehorses affected by uninduced superficial digital flexor tendon (SDFT) injuries.
Main methods: Blood-derived stem cells (BDSCs) were generated from the blood samples of three horses in the presence of macrophage colony-stimulating factor (M-CSF). The racehorses received a single autologous BDSC treatment, which resulted in the successful repair of the tendons injuries.
Key findings: The results demonstrated that the BDSCs injection into the damaged tendon stimulated the regeneration of normal tissue. Furthermore, a relationship may exist between the speed and the quality of new tissue formation and the welfare and management of the treated animals.
Significance: This study demonstrates that stem cell technology offers new tools for tissue repair that in many cases is considered incurable, and provides additional evidence that BDScs injections increase the speed and quality of the regeneration process in different animal tissues.

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Introduction

In the veterinary field, the use of stem cells is gaining importance for exploring the potential of regenerative medicine in the treatment of horses with spontaneous tendinitis (Smith et al., 2003; Smith, 2004; Smith and Webbon, 2005; Richardson et al., 2007; Barreira et al., 2008; Fortier and Smith, 2008; Koch et al., 2008). In veterinary use, the most common sources of stem cells are animal adipose tissue or bone marrow. In both cases, the sampling is invasive, and the described protocols fail to provide a homogeneous stem cell population (Cho et al., 2005; Blatt et al., 2005). Our recent *in vivo* studies have suggested that blood-derived stem cells (BDSCs) can differentiate into a variety of adult cell types, including neurons (Marfe et al., 2011; Spaas et al., 2011). Furthermore, a number of experimental observations have shown that these cells can spontaneously repopulate different organs, such as muscle, bone, liver or heart, and generate differentiated cells in response to acute or chronic damage. These observations, which suggest that these stem cells are capable of contributing to tissue regeneration (Donovan and Gearhart, 2001), have ignited significant interest regarding the ability of cell therapy to repair damaged non-hematopoietic tissues. Large quantities of these cells that are injected into the peripheral blood are likely to lodge within severely damaged organs. The injection of stem cells into the peripheral blood represents a non-invasive and potentially effective approach to easily obtain large quantities of stem cells that are capable of lodging within damaged tissues (Gratwohl et al., 2007). The discovery of the therapeutic potential of stem cells offers new opportunities for the treatment of otherwise incurable diseases.

The digital flexor tendon sheath (DFTS) is a synovial structure that runs from the distal quarter of the metacarpus/metatarsus to the foot on the palmar/plantar aspect of the limb. The sheath contains the superficial and deep flexor tendons, the *manica flexoria*, digital manica and the palmar/plantar annular ligament, which forms the wall of the sheath as it passes between the proximal sesamoid bones of the metacarpo/tarsophalangeal joint. The *manica*

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flexoria is directly proximal to the palmar annular ligament, is attached to the superficial digital flexor tendon (SDFT), and forms a ring around the deep digital flexor tendon (DDFT) (Dyce et al., 1987). The tendon sheath attaches to the proximal part of the *manica flexoria*, which forms a blind-ending pouch. Consequently, this pouch has the following 4 potential spaces in the proximal DFTS: the length from palmar/plantar to the SDFT; the space between the SDFT and DDFT; the space between the DDFT and *manica flexoria*; and the length from the dorsal to the *manica flexoria* (Dowling et al., 2000; Smith, 2001). These structures can be involved in injuries that would be catastrophic for all types of sport horses. Racing statistics indicate that tendinitis is the most frequent cause of breakdown, and it is often a career-ending event in thoroughbreds (Rossdale et al., 1985; Kasashima et al., 2004). Injuries to the SDFT alone account for an estimated 8% to 30% of all racing injuries (Rooney and Genovese, 1981; Genovese, 1993; Goodship et al., 1994). Moreover, the recurrence of tendinitis after returning to competitions can be as high as 43% (Dyson, 1997, 2004). The horses used for competitive sports, including racing on flat surfaces or over hurdles (National Hunt), show jumping, endurance riding and dressage, can be affected by SDFTs (Richardson et al., 2007). Traditional treatments for tendinitis have included medical and surgical options (Dyson, 2004). However, retrospective studies showed that approximately 40% to 60% of the injured horses returned to athletic soundness for a short period after SDFT injury (Rossdale et al., 1985; Stashak, 1991; Rees et al., 2006). In fact, such injuries require considerable periods of rest to allow for healing and often recur when the horse returns to a full workload. In many instances, the horses never heal adequately enough to return to their previous level of competition (Stashak, 1991; Rees et al., 2006). Some reports support the use of multipotent cells in the repair of connective tissue diseases in horses (Smith et al., 2003; Wilke et al., 2007). Treatment with the immediate reinjection of bone marrow aspirates has been used for adult horses with tendon and ligament injuries (chronic suspensory disease) for many years (Herthel, 2001). Realistically, bone marrow aspirates contain few stem cells, and these stem cells are extensively diluted by the large volume of bone marrow-derived blood. Culture expansion increases the number of stem cells available for injection (Smith et al., 2003; Smith and Webbon, 2005; Wilke et al., 2007; Guest et al., 2008, 2010); however, this technique requires a delay of several weeks. Adipose tissue provides an alternative source of multipotent cells for culture expansion but may also provide benefits in the form of concentrated nucleated cell populations, many of which may be clinically relevant when compared with bone marrow (Zuk et al., 2002; Strem and Hedrick, 2005; Nixon et al., 2008). However, the effectiveness of concentrated nucleated cells that are prepared by digestion and centrifugation compared with the effectiveness for cultured multipotent stem cells is unknown. In addition, the treatment of tendonitis by stem cells was studied by Lacitignola et al. (2008) in horses. Tendonitis was induced by an injection of collagens into the superficial flexor tendons Three weeks later, bone marrow mesenchymal cells, bone marrow mononucleated cells, or fibrin controls were injected into the tendons. In the stem cell-treated groups, there was significantly improved healing histologically and improved fiber orientation compared to the control group. Watts et al. (2011) reported the use of an injection of fetal-derived embryonic-like stem cells for superficial flexor tendon injuries in horses. There was, improved tendon size, lesion size, and linear fiber pattern in the stem cell-treated lesions.

The aim of the present study was to develop a highly innovative and non-invasive method using autologous BDSCs to repair tendon injuries in horses affected by this pathology. Our results demonstrated that an *in vivo* treatment of tendons injuries using autologous stem cell therapy provides fast, effective and permanent tissue repair when compared to horses that are treated with conventional therapy.

Materials and methods

Blood-derived stem cells generation

The blood samples were collected from the lower limbs of the animals. BDSCs were generated as described previously (Patent n°:07 820 134.0-1222). Briefly, the nucleated blood cell fraction was isolated by ammonium chloride incubation (dilution 1:3 in NH_4Cl, 1 M), centrifuged at 400 ×*g* and washed several times with PBS (phosphate-buffered saline, pH 7.2, Oxoid, (Sigma-Aldrich St. Louis, MD, USA) to remove the majority of the erythrocytes. The cells were then re-suspended in 5 ml of PBS and incubated for 72 hours at 37 °C in the presence of 50 nM macrophage colony-stimulating factor (M-CSF, Sigma-Aldrich) and 5 μM gentamicin sulfate (Marfe et al., 2011; Spaas et al., 2011).

Flow cytometry analysis

Flow cytometry was used to characterize the stem cells phenotype. The cells were incubated at room temperature for 30 min with anti-human antibodies CD90-PE (phycoerythrin) (BD Biosciences, San Jose, CA, USA), CD117-APC (allophycocyanin) (BD Biosciences, San Jose, CA, USA) or matched isotype controls. Flow cytometry analysis was performed with a BD FACS Aria II cell sorter (BD Biosciences, San Jose, CA, USA).

In situ injection of "deprogrammed" blood-derived stem cells

For treatment, the CD90+ cells were sorted and re-suspended in PBS/gentamicin at a 5-μM concentration. After ultrasonographic localization of the SDFT, the cell suspension was slowly injected *in situ* using a 2.5 ml sterile syringe. Each animal received only autologous-derived stem cells.

In vivo study

This study was conducted in accordance with ethical procedures and policies approved by the Animal Care Committee' (ACC).

Three racehorses, 1 female (aged 20 years) and 2 males (aged 10 and 12 years) with a median age of 12 years (range 10–20) showed a typical core lesion in the BDSC-treated SDFTs upon ultrasonographic examination. A group of three male animals with a median age of 10 years (range 10–15) that were affected by the same disease were also included in this study. This group was treated with conventional therapy.

Results

Isolation of peripheral blood stem cells and flow cytometry analysis

In all of the samples tested, flow cytometry analysis revealed a clear expression of CD90 (PE) and CD117 (APC) on BDSCs that were obtained as described previously (Marfe et al., 2011; Spaas et al., 2011). To isolate the stem cells from peripheral blood, we incubated the nucleated blood cells from each horse with M-CSF for 72 hours as described (Marfe et al., 2011; Spaas et al., 2011). Within this period, the stem cell receptors were expressed on the surface of some cells. Because these cells were quickly dividing after 72 hours, there were a sufficient number of stem cells for sorting. In addition, such sorting resulted in the elimination of differentiated granulocytes and monocytes. Therefore, pure stem cells were obtained. Flow cytometry analysis revealed the clear expression of CD90 (PE) and CD117 (APC) on BDSCs obtained as described in the Materials and methods section after 72 hours in all of the samples tested. This phenotype suggests a commitment toward the hematopoietic stem cell lineage (Fig. 1). Furthermore, a small fraction (approximately 15%),

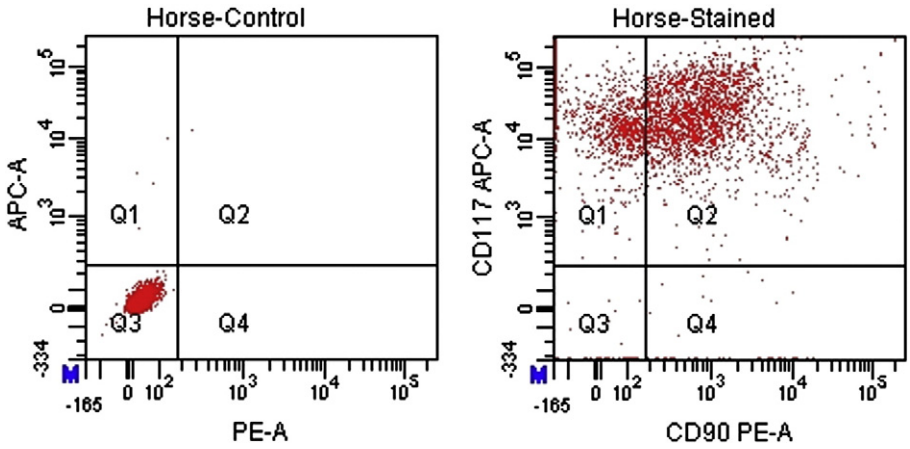

Fig. 1. Flow cytometry analysis of BDSCs before injection. Isotype-stained (left panel) and CD90 PE/CD117 APC-stained cells (right panel). Almost all of the cells are positive for CD117/CD90.

after sorting, showed the presence of stem cell embryonic markers, such as Sox2, Oct3/4, and Nanog (Table 1).

In situ injection of deprogrammed blood-derived stem cells

BDSCs were collected from the same animals as described in the Materials and methods section, and, after the isolation of the autologous peripheral blood stem cells, these cells were inoculated intravenously and locally into the horses. The ultrasonographic examination was performed before and after treatment for each case (Fig. 2).

Case 1. A 20-year-old female jumping-horse had an injury to the superficial flexor tendon. The injury at some points of the cross-sectional area reached 80% of the tissue. This horse had been treated with conventional therapy, but as shown by ultrasonographic examination, the disease had not regressed (Fig. 2a). Therefore, the horse was treated with an administration of BDSCs. Four months after the stem cell injection, the tendons appeared to be almost entirely repaired (over 90% filling), and the orientation of the fibers was perfectly parallel to the long axis of the tendon itself (Fig. 2b).

Case 2. A 10-years-old, gelding (that was used for dressage) had severe superficial digital flexor tendonitis. The lesion affected 80% of its tendon; the second horse had been treated with conventional therapy, and in this case, there was no regression of the lesion (Fig. 2c). In addition, the horse was treated with the administration of BDSCs intravenously and locally. After four months, the tendons appeared to be almost entirely repaired (over 97% filling); the fibers were correctly oriented, and parallel to the long axis of the tendon itself (Fig. 2d).

Case 3. A 12-year-old Italian gelding, relapsed with lesions in the right superficial flexor tendon during jumping activities. The lesions affected 30% of the tendon. This case was treated with vesicant eight months before BDSCs therapy without any improvement (Fig. 2e). For this reason, the horse was treated with local and systemic stem cell therapy as described above, and after five months, it was possible to observe the repair of the tendon with an almost 100% reduction of the lesion (Fig. 2d).

In addition, we observed a filling of the lesion and a correct pattern of fibers orientation, which suggested the continuance of a good healing process over this period. In all cases, we observed a reduction in the severity and size of the damaged area, and after three years, no recurrence was found in the treated horses that are presently still active.

On the contrary, the three other horses affected by the same disease and treated with conventional therapy alone showed fibrotic tendon images (Fig. 3a,c,e) and were re-injured within 3 to 6 months of recovery (Fig. 3b,d,f).

Table 1
Markers used to characterize and sort Blood Derived Stem Cells.

Marker	Hematopoietic stem cell (HSC)	Mesenchymal stem cell (MSC)	Blood Derived Stem Cell after sorting
CD14	−	−	−
CD34	+	−	−/+
CD90	+	+	+
CD117	+	−	+
Sox2	−	−	−/+
Oct3/4	−	−	−/+
Nanog	−	−	−/+

Discussion

Stem cells represent a promising treatment for certain degenerative or traumatic diseases because of their plasticity and differentiation capacity. Their use in equine veterinary medicine has been intensively studied in recent years, and their regenerative effect, mainly in light of tendon and ligament injuries, has been described in separate *in vivo* studies (Crovace et al., 2010). In fact, exercise-induced degeneration occurs in equine superficial digital flexor tendons. Increased stiffness, reduced collagen fiber diameter, altered fiber morphology, noncollagenous matrix composition and progressive central hypocellularity have been reported (Patterson-Kane and Firth, 2009). These changes are expected to contribute to tendonitis in athletic horses (Patterson-Kane and Firth, 2009) and are associated with a failure to return to the previous performance level and have a high incidence of recurrence (Smith, 2001). Racing is stressful to the SDFT, and the return to competitions without relapses appears to be a significant measure of functional recovery (Dyson, 2004). Many treatments have been used to facilitate the healing of these lesions, but currently, no treatment has consistently enhanced healing. The methods employed include prolonged periods of inactivity, controlled exercise programs, anti-inflammatory therapy, intra-lesion injections, and peritendinous injection of counterirritants, sclerosing agents, tendon splitting, annular ligament desmotomy, and superior check ligament desmotomy (McIlwraith, 2002).

In the present report, we have described the results obtained in three cases affected by SDFT and treated with the administration of autologous-derived BDSCs. After expansion and sorting, these stem cells were classified into three subpopulations: the first subpopulation was positive for two typical MSC markers, CD90 and CD105, but negative for CD73 (i.e., another MSC marker), which indicated a

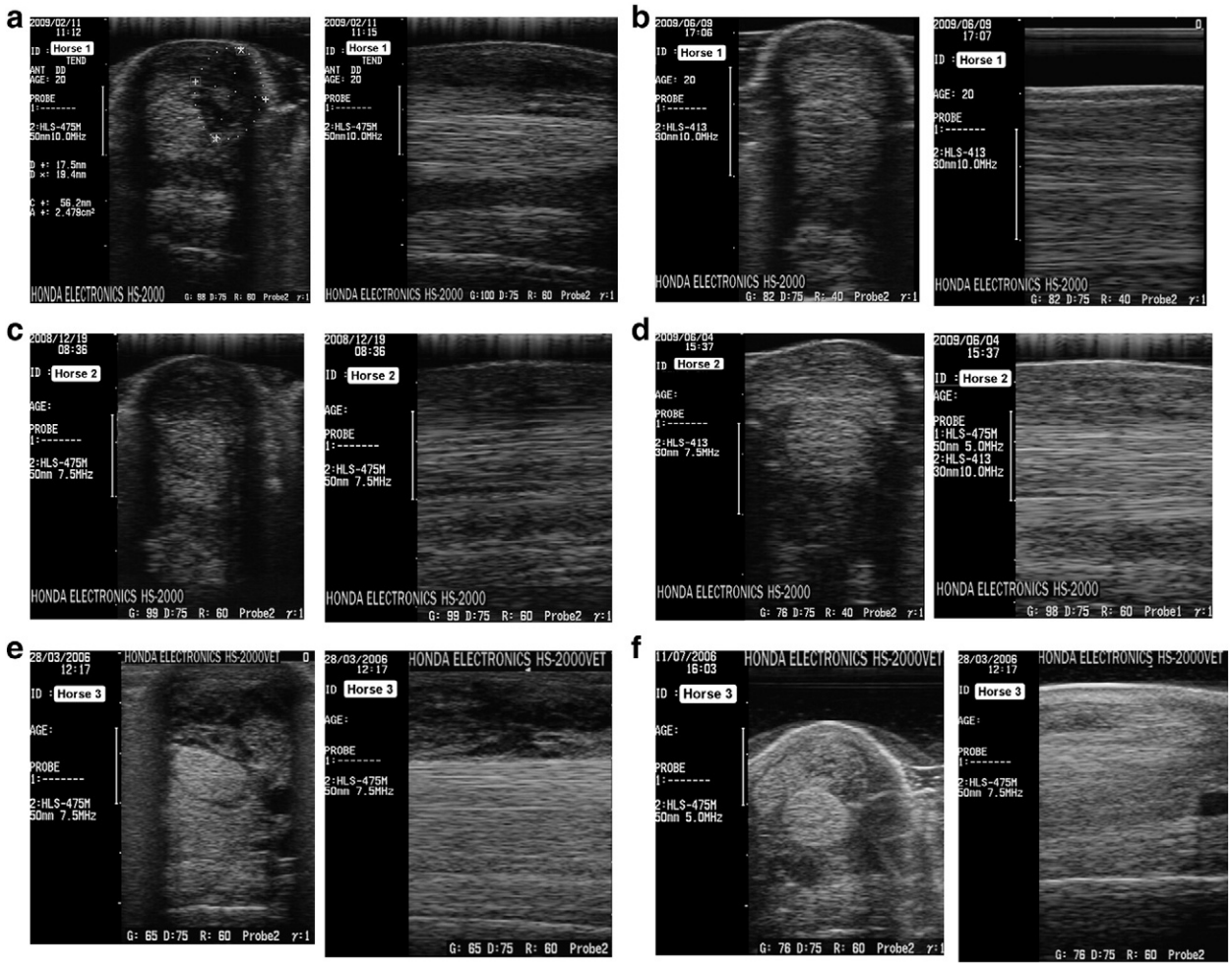

Fig. 2. Horse 1. Ultrasonographic images (7.5 to 12 MHz linear ultrasonographic transducer probe) of the superficial digital flexor tendon of a 20-year-old racehorse with severe superficial digital flexor tendonitis prior to BDSC injection and 4 months after treatment. The lesion affected 80% of the tendon.(a) Transverse and longitudinal images from level 4 (20 cm distal to the 3rd metacarpal bone) before inoculation.(b) Transverse images and longitudinal images (20–24 cm distal to the 3rd metacarpal bone) 4 months after injection.Horse 2. Ultrasonographic images of the superficial digital flexor tendon of a ten-year-old racehorse with severe superficial digital flexor tendonitis prior to BDSC injection and 4 months after treatment. The lesion affected 80% of the tendon.(c) Transverse and longitudinal images from level 4 (20 cm distal to the 3rd metacarpal bone) before inoculation,(d) transverse and longitudinal images (20–24 cm distal to the 3rd metacarpal bone) 4 months after injection.Horse 3. Ultrasonographic images of the superficial digital flexor tendon of a twelve-year-old racehorse with severe superficial digital flexor tendonitis prior to BDSC injection and 5 months after treatment. The lesion affected 30% of the tendon.(e) Transverse and longitudinal images from level 4 (20 cm distal to the 3rd metacarpal bone) before inoculation.(f) Transverse and longitudinal images (20–24 cm distal to the 3rd metacarpal bone) 5 months after injection.

commitment of the peripheral blood stem cells towards the MSC lineage; the second subpopulation was positive for CD90, CD117, and CD34, which are typical HSC markers; and the third subpopulation was positive for CD90 and CD117 as well as for Sox2, Oct3/4, and Nanog, which are pluripotent stem cell markers.

Here, we describe the clinical characteristics of the animals with SDFT. In the first case, different therapies were attempted without any success, and therefore, peripheral blood stem cells were used as the last treatment option. The second case was also treated with intravenous stem cell therapy. After four months, we observed a reduction in the severity and extent of the damaged area (over 90% for both animals). The third case, treated with conventional therapy, failed to show any improvement; the damaged lesioned area disappeared within five months but only after BDSC therapy.

In this study, we present a new clinical approach based on the isolation of blood-derived stem cells that were grown *in vitro* and then injected into the damaged tendon of the same donor horse. With this treatment, we can provide a non-invasive source of stem cells with potentially superior cellular characteristics compared with other stem cells used in veterinary medicine with respect to immune tolerance, proliferative potential and differentiation potency. As far as we know, the use of BDSCs for tendon repair has not been previously described, and extensive reports about their clinical applications in racehorses are lacking. Furthermore, horses treated with conventional methods may recover, but evidence supports a high re-occurrence of tendons injuries (Goodship et al., 1994; Rees et al., 2006). Therefore, all of the conventionally treated horses relapsed shortly after restarting training or competitive activities.

In conclusion, our results show that 30% to 80% of the damaged tendons can recover after a BDSCs injection. In addition, we observed that a greater proportion of normal tendon tissue is produced compared to other therapies (Stashak, 1991; Rees et al., 2006). Our current clinical experience has demonstrated that autologous BDSCs fail to cause any observed significant side effects by either the inoculation process or the formation of different normal tissues within the implantation site. In addition, BDSCs can be a reliable source of stem cells because their use is characterized by the ease of collection and minimal pain and because this process was less invasive compared to other methods described in literature (Wilke et al., 2007; Herthel, 2001; Zuk et al., 2002; Smith et al., 2003; Smith and Webbon, 2005; Strem and Hedrick, 2005; Wilke et al., 2007; Lacitignola et al., 2008; Nixon et al., 2008; Watts et al., 2011; MacLean et al., 2012).

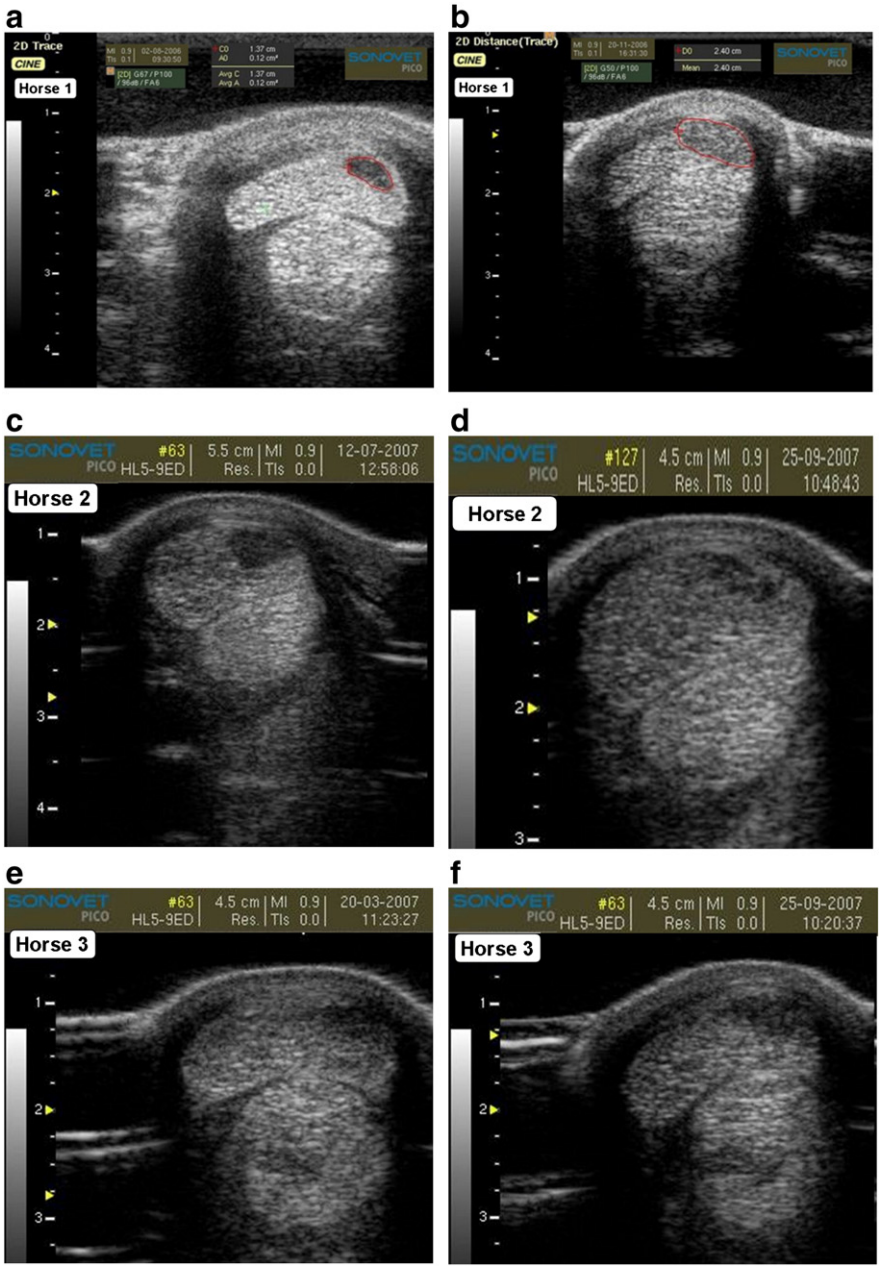

Fig. 3. Ultrasonographic images of lesions in the superficial digital flexor tendon of the three cases that were treated with conventional therapy.(a,c,e) Horses 1, 2 and 3 on the day of diagnosis;(b,d) Horses 1 and 2 after 3 months;(f) Horse 3 after 6 months.

Furthermore, the *in vivo* application of stem cells occurred without any side effects in all of the treated cases (Marfe et al., 2011; Spaas et al., 2011). After three years, none of the animals that were successfully treated have shown any side effects, such as rejection, infection or teratoma formation. Therefore, this procedure can significantly improve the healing process of damaged tendons when compared to animals treated with conventional therapies.

Conclusions

The clinical experience that we developed using our protocol has been encouraging in terms of the results, reliability and time of recovery. Of course, more evidence of efficacy, which is essential for full confidence in the technology to be achieved, is highly desirable. It is hoped that experience gained from treating horses with non-induced pathologies will provide sufficient supportive data to promote the application of this technology in humans.

Conflict of interest statement

The authors, M. P. and A.G., declare competing financial interests.

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References

Barreira APB, Alves ALG, Saito M, Amorim RL, Kohayagawa A, Menarim BC, et al. Autologous implant of bone marrow mononuclear cells as treatment of induced equine tendinitis. Int J Appl Res Vet Med 2008;6:46–54.

Blatt A, Cotter G, Leitman M, Krakover R, Kaluski E, Milo-Cotter O, et al. Intracoronary administration of autologous bone marrow mononuclear cells after induction of short ischemia is safe and may improve hibernation and ischemia in patients with ischemic cardiomyopathy. Am Heart J 2005;150:986.

Cho SW, Park HJ, Ryu JH, Kim SH, Kim YH, Choi CY, et al. Vascular patches tissue-engineered with autologous bone marrow derived cells and decellularized tissue matrices. Biomaterials 2005;26:1915–24.

Crovace A, Lacitignola L, Rossi G, Francioso E. Histological and immunohistochemical evaluation of autologous cultured bone marrow mesenchymal stem cells and bone marrow mononucleated cells in collagenase-induced tendinitis of equine superficial digital flexor tendon. Vet Med Int 2010;2010:250978.

Donovan PJ, Gearhart J. The end of the beginning for pluripotent stem cells. Nature 2001;414:92–7.

Dowling BA, Dart AJ, Hodgson DR, Smith RK. Superficial digital flexor tendonitis in the horse. Equine Vet J 2000;32:369–78.

Dyce KM, Sack WO, Wensing CGG. Textbook of veterinary anatomy. Philadelphia: W.B. Saunders Co.; 1987. p. 559–61.

Dyson SJ. Treatment of superficial digital flexor tendinitis: a comparison of conservative management, sodium hyaluronate, and glycosaminoglycan polysulfate. Proceedings. 43rd Annu Conv Am Assoc Equine Pract; 1997. p. 297–300.

Dyson SJ. Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992–2000). Equine Vet J 2004;36:415–9.

Fortier LA, Smith RK. Regenerative medicine for tendinous and ligamentous injuries of sport horses. Vet Clin North Am Equine Pract 2008;24:191–201.

Genovese RL. Prognosis of superficial flexor tendon and suspensory ligament injuries. Proceedings. 39th Annu Conv Am Assoc Equine Pract; 1993. p. 17–9.

Goodship AE, Birch H, Wilson AM. The pathobiology and repair of tendon and ligament injury. Vet Clin North Am Equine Pract 1994;10:323–49.

Gratwohl A, Baldomero H, Frauendorfer K, Urbano-Ispizua A, Niederweiser D. Results on the EBMT activity survey 2005 on hematopoietic stem cell transplantation: focus on increasing use of unrelated donors. Bone Marrow Transplant 2007;39: 71–87.

Guest DJ, Smith MRW, 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;40:178–81.

Guest DJ, Smith MR, 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 tendon. Equine Vet J 2010;42: 636–42.

Herthel DJ. Enhanced suspensory ligament healing in 100 horses by stem cell and other bone marrow components. Proceedings. 47th Annu Conv Am Assoc Equine Pract; 2001. p. 319–21.

Kasashima Y, Takahashi T, Smith RK, Goodship AE, Kuwano A, Ueno T, et al. Prevalence of superficial digital flexor tendonitis and suspensory desmitis in Japanese Thoroughbred flat racehorses in 1999. Equine Vet J 2004;36:346–50.

Koch TG, Berg LC, Betts DH. Concepts for the clinical use of stem cells in equine medicine. Can Vet J 2008;49:1009–17.

Lacitignola L, Crovace A, Rossi G, Francioso E. Cell therapy for tendinitis, experimental and clinical report. Vet Res Commun 2008;32:S33–8.

MacLean S, Khan WS, Malik A, Snow M, Anand S. Tendon regeneration and repair with stem cells. Stem Cells Int 2012. [Article ID 316281].

Marfe G, Massaro-Giordano M, Ranalli M, Cozzoli E, Di Stefano C, Malafoglia V, et al. Blood derived stem cells: an ameliorative therapy in veterinary ophthalmology. J Cell Physiol 2012;227:1250–6.

McIlwraith CW. Diseases of joints, tendons, ligaments, and related structures. In: Stashak TS, editor. Adams lameness in horses. (5th ed.). Philadelphia, PA: Lippincott, Williams, and Wilkins; 2002. p. 594–619.

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;69:928–37.

Patterson-Kane JC, Firth EC. The pathobiology of exercise-induced superficial digital flexor tendon injury in Thoroughbred racehorses. Vet J 2009;181:79–89.

Rees JD, Wilson AM, Wolman RL. Current concepts in the management of tendon disorders. Rheumatology (Oxford) 2006;45:508–21.

Richardson LE, Dudhia J, Clegg PD, Smith R. Stem cells in veterinary medicine-attempts at regenerating equine tendon after injury. Trends Biotechnol 2007;25:409–16.

Rooney JR, Genovese RL. A survey and analysis of bowed tendon in Thoroughbred racehorses. Equine Vet Sci 1981;1:49–53.

Rossdale PD, Hopes R, Wingfield-Digby NJ, Offord K. Epidemiological study of wastage among racehorses in 1982 and 1983. Vet Rec 1985;116:66–9.

Smith RKW. Ultrasonographic evaluation of the digital sheath and its abnormalities. UK Vet 2001;6:5-12.

Smith RK. Autogenous stem cell implantation. Proceedings of ACVS Veterinary Symposium, October 7–9, Denver published in Vet Surg, 33. ; 2004. p. 199–201.

Smith RK, Webbon PM. Harnessing the stem cell for the treatment of tendon injuries: heralding a new dawn? Br J Sports Med 2005;39:582–4.

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. Equine Vet J 2003;35:99-102.

Spaas JH, Gambacurta A, Polettini M, Broeckx S, Van Hoeck F, De Schauwer C, et al. Purification and expansion of stem cells from equine peripheral blood, with clinical applications. Flem Vet J 2011;80:129–36.

Stashak TS. Management of wounds associated with tendons, paratendons, and tendon sheaths. In: Stashak TS, editor. Equine wound management. Philadelphia: Lea & Febiger; 1991. p. 238.

Strem BM, Hedrick MH. The growing importance of fat in regenerative medicine. Trends Biotechnol 2005;23:64–6.

Watts AE, Yeager E, 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(1). [article 4].

Wilke MM, Nydam D, Nixon AJ. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. J Orthop Res 2007;25:913–25.

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279–95.