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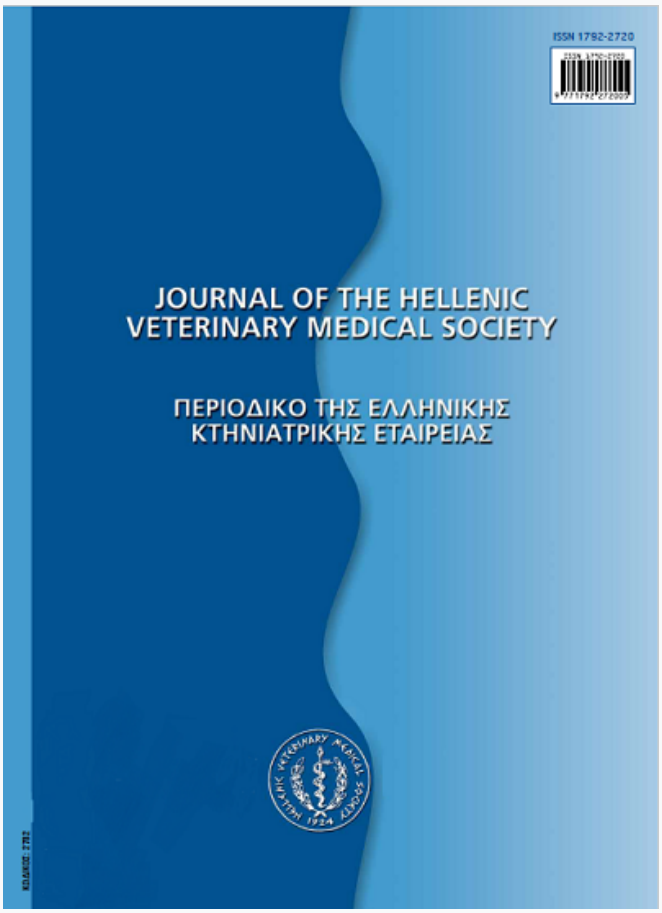

Clinical application of adipose-derived stromal vascular fraction in 3 Thoroughbred horses with superficial digital flexor tendonitis: Case Report

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Case Report
Κλινικό περιστατικό

Clinical application of adipose-derived stromal vascular fraction in 3 Thoroughbred horses with superficial digital flexor tendonitis: Case Report

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Κλινική εφαρμογή κλάσματος βλαστοκυττάρων προερχόμενων από λιπώδη ιστό σε ίππους της Αγγλικής καθαρόαιμης φυλής με τενοντίτιδα του επιπολής καμπτήρα των φαλάγγων μυός: Αναφορά σε 3 περιστατικά

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ABSTRACT. Tendon injuries are considered major causes of wastage in active Thoroughbred racehorses as they are among the most common musculoskeletal injuries in equine athletes. There has been considerable interest in the potential use of concentrated adipose tissue digest, such as stromal vascular fraction, in tendon overstrain injuries. It has been reported that the adipose-derived stem cells, which are present in stromal vascular fraction of adipose tissue, can promote neovascularization and improve healing process. The purpose of this study was to evaluate the potential effects of intralesional use of autologous adipose-derived stromal vascular fraction on tendon healing in three Thoroughbred active racehorses with moderate to severe superficial digital flexor tendonitis. Based on the results of this report it seems that intratendinous injection of autologous adipose-derived stromal vascular fraction leads to marked clinical improvement and reduction of rehabilitation period in racehorses suffering from superficial digital flexor tendonitis.

Keywords: adipose-derived stem cells; horse;stromal vascular fraction;tendonitis

ΠΕΡΙΛΗΨΗ. Οι τενοντίτιδες, μια από τις πιο συχνές μυοσκελετικές παθήσεις των ίππων αθλητικής ιππασίας, αποτελούν σημαντική αιτία πρόωρης αποχώρησης από την αθλητική δραστηριότητα των δρομώνων ίππων. Την τελευταία δεκαετία, υπάρχει έντονο ερευνητικό ενδιαφέρον όσον αφορά στη χρήση βλαστοκυττάρων προερχόμενων από το λιπώδη ιστό σε τραυματισμούς των τενόντων. Έχει αναφερθεί ότι τα βλαστοκύτταρα τα οποία προέρχονται από λιπώδη ιστό προάγουν την νεοαγγείωση και επιταχύνουν τις επουλωτικές διεργασίες. Σκοπός της παρούσας μελέτης ήταν η αξιολόγηση της αποτελεσματικότητας της ενδοτενόντιας έγχυσης αυτόλογου κλάσματος βλαστοκυττάρων προερχόμενων από λιπώδη ιστό σε 3 αθλητικούς ίππους της Αγγλικής καθαρόαιμης φυλής με μέτριου έως σοβαρού βαθμού τενοντίτιδα του τένοντα του επιπολής καμπτήρα των φαλάγγων μυός. Η ενδοτενόντια χορήγηση αυτόλογου κλάσματος βλαστοκυττάρων του λιπώδους ιστού φαίνεται πως βελτιώνει την κλινική εικόνα ίππων με τενοντίτιδα του επιπολής καμπτήρα των φαλάγγων μυός, μειώνοντας σημαντικά τη διάρκεια του χρόνου αποκατάστασης.

Λέξεις ευρετηρίασης: βλαστοκύτταρα, κλάσμα βλαστοκυττάρων προερχόμενων από λιπώδη ιστό, ίππος, τενοντίτιδα

INTRODUCTION

Tendon over-strain injuries are the most common musculoskeletal injuries (46% of limb injuries) in athletic horses. A longitudinal study in United Kingdom reported an incidence rate of tendon or ligament injury of 2.77 cases per 1,000 race starts, in flat racing Thoroughbreds (Williams et al., 2001). In a 12-year epidemiological study conducted in racing Thoroughbreds in Hong Kong, tendon injury has emerged as the most common cause of retirement (Lam et al., 2007).

Superficial digital flexor tendonitis represents a substantial cause of wastage within the horse industry, with an incidence of 8-43% in racing Thoroughbreds (Dowling et al., 2000), whereas most of the published data are related to flat racing horses (Genovese et al., 1996; Gibson et al., 1997). Superficial digital flexor tendon (SDFT) injuries have prolonged healing process and their prognosis is best described as guarded, since only a 20-60% of the affected horses return to racing and up to 80% of them subsequently sustain re-injury (Sawdon et al.,1996; Dowling et al., 2000).

Recently, there has been a considerable interest in the potential use of autologous biological products for the treatment of tendinopathies. Cell based and biologic autologous therapies, such as adipose-derived stem cells (ADSCs), have been rapidly popularized over the past 10 years for the treatment of overstrain tendon injuries (Caplan and Bruder, 2001; Hildebrand et al., 2002; Schnabel et al., 2007). It is currently known that concentrated adipose

tissue digest (e.g. stromal vascular fraction; SVF), derived from manipulation of adipose tissue such as homogenization, enzymatic of mesenchymal stem contains a large number of mesenchymal cells (MSC) that can promote tissue healing (Gimble et al., 2011).
The purpose of this study was to present and evaluate the outcome of the intralesional administration of adipose-derived SVF in three Thoroughbred racehorses suffering from superficial digital flexor tendonitis.

Case description

All 3 Thoroughbred horses were geldings presented with acute onset of severe lameness in a forelimb due to a moderate to severe tendon injury. One horse had competed one day prior to the onset of lameness. The cross-sectional areas of the tendonitis lesions were obtained at the site of maximal injury and were recorded as the percentage of the total tendon area. All horses included in this study, achieved high performance levels during the last 6 months.

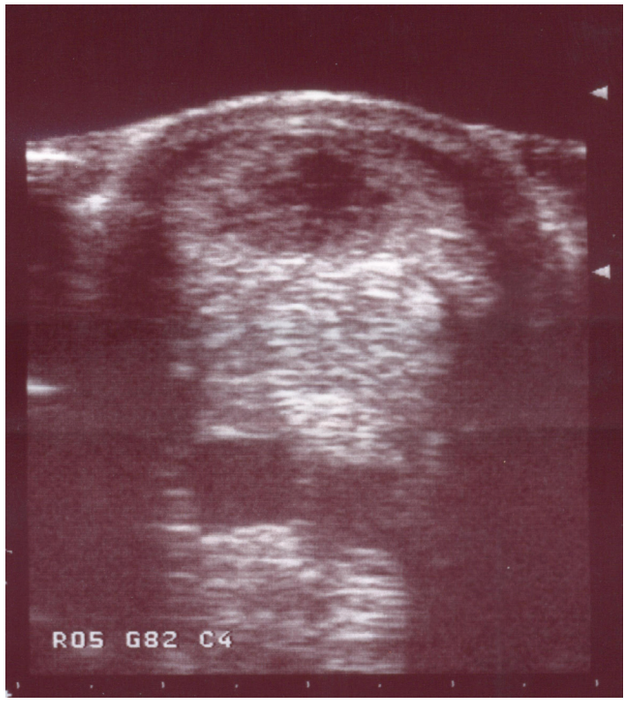

Figure 1.

CLINICAL EVALUATION

Case 1

A 5-year old Thoroughbred gelding was examined for lameness evaluation 5 days after racing. Palpation of the right forelimb revealed an enlarged SDFT over the middle and distal third of the left metacarpal region. In the same region, oedema and pain on palpation were also detected. The horse, first examined at walk and trot on a straight line, revealed severe lameness on the right forelimb, 3/5 according to AAEP scale (Anon, 1991).
The forelimb was prepared in a routine manner and ultrasonographic examination of the palmar metacarpal region with a 7,5MHz linear transducer was performed, including longitunal and transverse views. Ultrasonography of the flexor tendons revealed enlargement of SDFT and a hypoechoic core lesion, covering approximately 60% of the cross-sectional area extending from region 1B to 3A (Genovese 1986), with the widest point in region 1B **(Fig.1)**

Case 2

A 4-year old Thoroughbred gelding was presented the day after a 1400m flat race with severe lameness (3/5 according to AAEP scale) on the left forelimb. Clinical examination revealed swelling of the proximal third of the palmar metacarpal area, localized heat and pain upon palpation. Cross-sectional size of the flexor tendons was evaluated; an extended anechoic core lesion (Zone 2A and B) covering approximately 50% of the cross-sectional area was detected (**Fig.2**).

Case 3

A 5-year old Thoroughbred gelding was presented with severe lameness, 4/5 according to AAEP scale, on the left forelimb. According to history data, a diagnosis of SDFT tendonitis had been made 6 months previously and the horse had returned to training three months prior to presentation. Upon clinical examination there was evidence of a localized chronic swelling on the middle

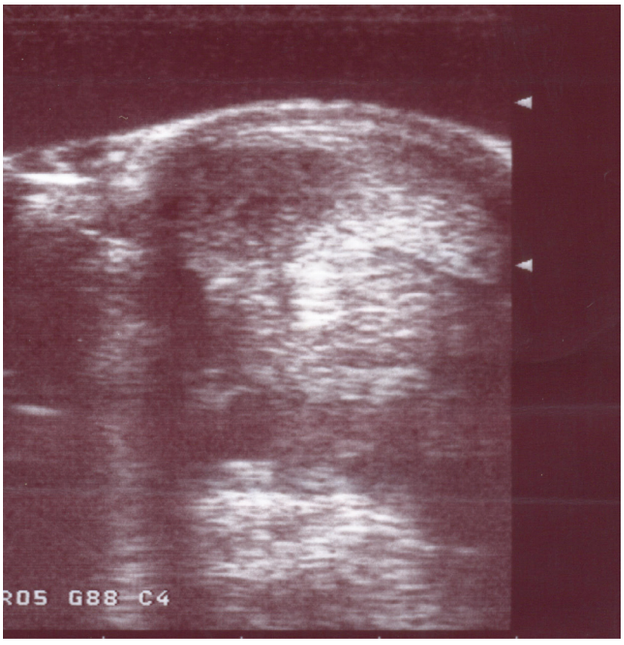

Figure 2.

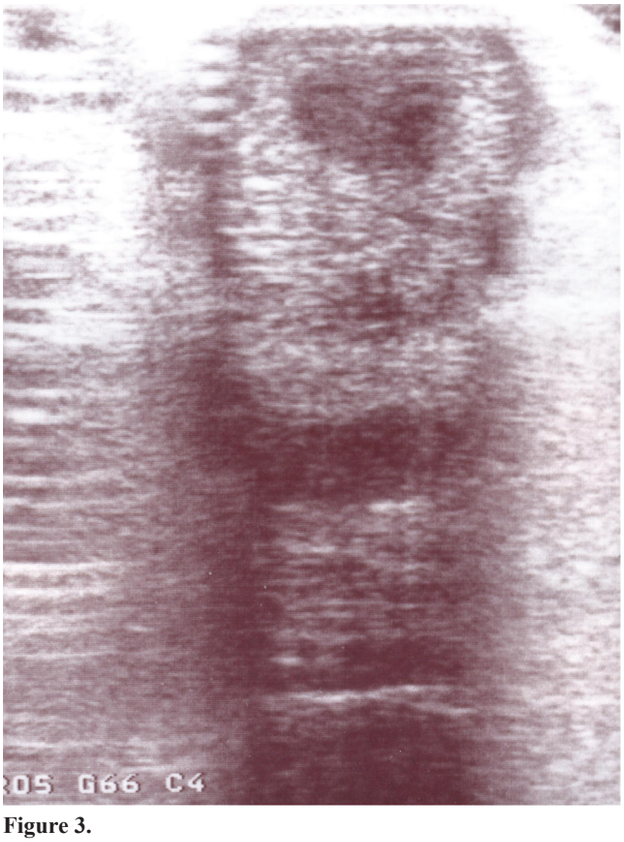

Figure 3.

and distal third aspect of the palmar metacarpal region. Ultrasonographic examination revealed poor definition of tendon margins, enlargement of the cross-sectional area, ectopic fibrosis and a hypoechoic core lesion extending to 70% of the total cross-sectional area (**Fig. 3**).

In all horses, initial treatment included stall confinement, supportive bandaging, cold hosing and NSAIDs medication for 5 days (phenylbutazone 4.4mg kg^{-1} bw iv).

Seven days after each case diagnosis, it was decided to enhance healing by injecting autologous adipose-derived SVF. A routine harvest of adipose tissue from the region above the dorsal gluteal muscles was performed. The horse was sedated using romifidine (40μg kg^{-1} bw iv) in combination with butorphanol (80μg kg^{-1} bw iv). After surgical preparation, the skin and subcutaneous tissues were desensitized by local anesthetic infiltration (lidocaine HCl 2%). A 6-8cm incision, parallel to the spine, was made and 16gr of supragluteal subcutaneous adipose tissue were harvested. A routine closure using nylon suture material was performed.

The isolation of adipose-derived stem cells was performed based on standard reported techniques for human adipose-derived SVF isolation. The adipose tissue was minced with a No11 surgical blade and washed with phosphate-buffered saline solution (PBS) to remove blood. Adipose tissue was treated in equal volume of PBS containing 100U/ml type I collagenase (Biochrom, Berlin, Germany) for one hour, under constant agitation. The digested tissue was then centrifuged at 300g for 30 minutes in order to isolate the SVF in pellet form. The SVF pellet was then re-suspended in PBS and washed twice. The final pellet was re-suspended in a mixture of 10% autologous serum, 10% dimethyl sulfoxide (DMSO) and 2% hydroxyethylstarch (Haes-steril 200®) in PBS. Viable nucleated cells were counted using a Newbauer plate and trypan blue. A mean total of $1x10^6$ viable nucleated cells per gram of adipose tissue were isolated. The cell suspension was placed in a cryovial, gradually cooled at a rate of 1°C per minute up to -80°C and then stored in

liquid nitrogen until use. On the day of adipose-derived SVF application the cryovial was rapidly defrozen for 1 min at 40°C, washed once with PBS and re-suspended in 2ml autologous serum.

Ten days after adipose tissue harvesting the palmar metacarpal region was prepared for aseptic injection. Following standing sedation (romifidine, kg^{-1} bw iv) and local analgesia (lidocaine HCl 2%), 1-2ml of the adipose-derived SVF were implanted into the core lesion of the SDFT under ultrasonographic guidance. A sterile bandage was applied for 4 days, after SVF injection.

A routine standardized rehabilitation program according to Athens racetrack was performed in all three horses. It consisted of an initial period of two months' box rest followed by gradually increasing levels of exercise (trot, canter, gallop) depending on the healing progress of each individual case. Ultrasonographic examinations were performed one and two months post implantation and subsequently at four-month intervals for one year.

FOLLOW-UP

All horses responded positively to the treatment described above and were able to race again within 4 months (Case 1 and Case 2) and 7 months (Case 3) of implantation. Ultrasonographic examination revealed improved density of the fibre pattern, normal fiber alignment, marked improvement in echogenicity and restoration of tendon margins (**Fig. 4-6**). During the recovery period, no complications such as local oedema or pain at the site of injection were recorded. Furthermore, no signs of recurrence were detected during the 1 year follow-up period. All horses were able to race again achieving high performance levels 18 months post treatment.

DISCUSSION

Soft tissue injuries, such as tendonitis of the SDFT, represent an important cause of wastage in racehorses, taking into consideration the prolonged

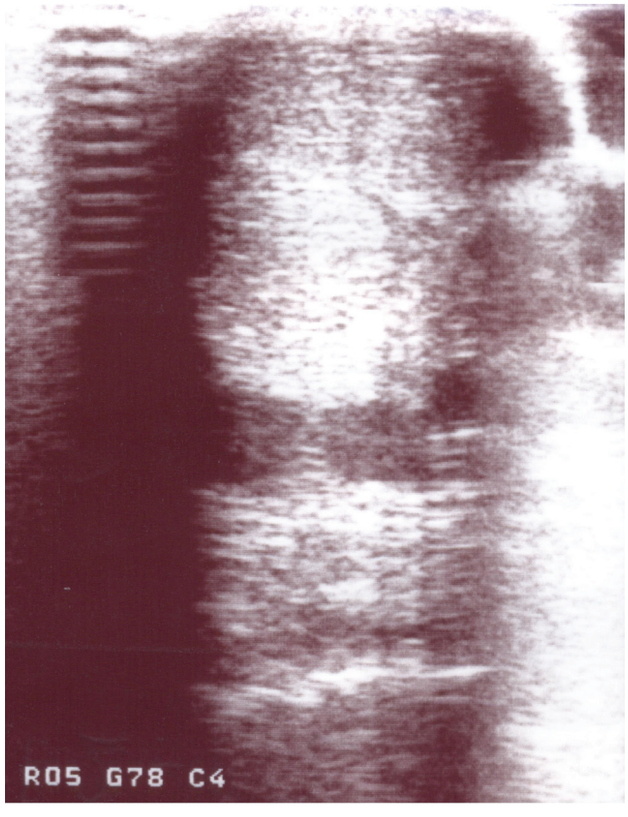

Figure 4.

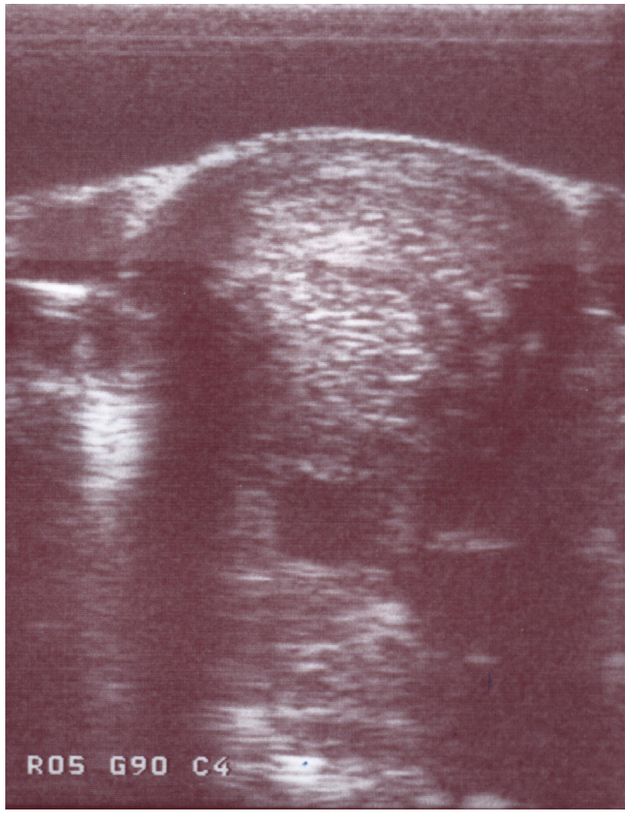

Figure 5.

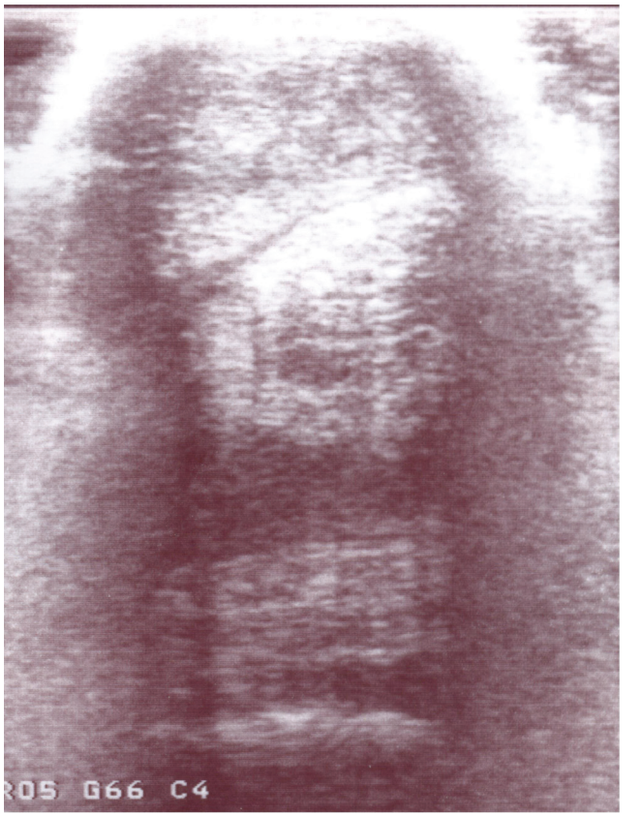

Figure 6.

recovery periods before their return in athletic activity or the possible retirement (Goodship et al., 1994). The cumulative incidence rate of flexor tendonitis among sport horses has been reported up to 66% with recurrent injury rate as high as 56% (Fortier et al., 2008). The majority of tendon injuries occur to the forelimbs (Kasashima et al., 2004; Lam et al., 2007), with the SDFT being more prone to injury in 75-93% of cases.

Tendon elasticity allows the horse to maintain high speed, storing and returning strain energy (McNeill Alexander, 2002). When a tendon is injured, the resultant scar tissue is functionally deficient in comparison to normal tendon and is also prone to re-injury at a rate of 56-66% (Dowling et al., 2000; Dyson, 2004); thus, repair process has important consequences for the affected horse in terms of athletic performance.

This primary need to restore functionality has emboldened research to the development of regenerative therapies. In equine orthopedics, MSCs have been clinically used for bone fracture treatment, subchondral bone cysts, cartilage and tendon repair (Fortier et al., 2008). The crucial role of MSCs in tendon repair is further emphasized by several equine studies where in naturally occurring or collagenase induced core lesions treated with MSCs healing was greatly improved compared to horses treated with conventional methods and having similar rehabilitation protocol (Pacini et al., 2007; Nixon et al., 2008). Ferris and colleagues (2009), in a long term follow-up controlled study on horses with soft tissue injuries treated with MSCs, reported that 85% of the cases returned to full work presenting excellent ultrasonographic image and optimal racing results.

After their initial discovery in bone marrow, MSCs have been found in several adult and fetal tissues including adipose tissue, synovial fluid, dermis, periosteum, umbilical cord blood, placenta and amniotic fluid (Murphy et al., 2013). ADSCs have demonstrated ability to differentiate as the bone marrow-derived cells do, though to a slightly lesser degree (Im et al., 2005). In our study, the rationale of using adipose-derived SVF in order to improve tendon healing and rehabilitation is further supported by the study of Behfar and colleagues (2009) highlighting the improvement in structural properties of tendon repairs after intratendinous injection of SVF in New Zealand white rabbits. The use of MSCs, such as the ADSCs or their digest, in the treatment of tendon injuries is, therefore, well encouraged by the aforementioned studies.

The damage that follows trauma or repetitive loading of a tendon results in a prolonged period of athletic inactivity, whereas it is uncommon for the damaged tendon to restore its normal functional properties (Thorpe et al., 2010). In the present study, in horses suffering severe tendonitis of SDFT, the rehabilitation period was markedly shorter after SVF injection in the damaged tendon, compared to the mean one recorded in the Athens racetrack. The majority of horses with SDFT overstrain injuries require at least 9 months of rehabilitation before

resuming full athletic function, whereas in more severe cases, the rehabilitation is prolonged up to 18 months until the tendon restores its functionality (Davis and Smith, 2006). Apart from the clinical outcome assessed in terms of horses' return to their previous performance, the success of SVF injection in our study is further emphasized by the absence of re-injuries in the 1-year follow-up period.

Although further in vitro studies are necessary to determine the distribution and therapeutic mechanisms of adipose-derived SVF in order to optimize its use as part of regenerative strategies, its effect on large tendon core lesions is clinically emphasized. Based on the results of this case study, despite the limited number of horses and the lack of control group, the beneficial role of SVF on accelerating the healing process is further established; the athletic success rates during the follow-up period reflect the quality of tendon repair and thus position the horse as a good long-term animal model for regenerative therapies.

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