Arch Med Vet **44, 291-295 (2012)** **DOI: 10.4067/s0301-732X2012000300013**

COMMUNICATION

Autologous implant of bone marrow mononuclear stem-cells as treatment for equine bicipital tendonitis: case report

Implante autólogo de células mononucleares de médula ósea como tratamiento de tendinitis bicipital equina: reporte de caso clínico

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RESUMEN

La bursitis bicipital es la principal causa de cojera asociada al hombro equino, sin embargo representa un pequeño porcentaje de las causas de claudicaciones en caballos. Debido a la alta recurrencia observada en casos de tendinitis, tratamientos en base a medicina regenerativa se han tornado en un importante foco de investigación. El objetivo de este estudio es reportar el implante de células madre mononucleares como tratamiento de bursitis bicipital en un caballo. Un caballo carretonero, castrado, de 7 años de edad, fue presentado al Hospital Veterinario - UACh, exhibiendo claudicación del miembro anterior izquierdo y sensibilidad a la palpación de la región del hombro. Los exámenes clínico y ultrasonográfico revelaron fluido sinovial hemorrágico, disminución de la cojera posterior a anestesia intrasinovial del hombro e inflamación y ruptura de fibras del tendón bíceps braquial. El caballo fue exitosamente tratado a través de inyección yuxtatendínea de triamcinolona y posteriormente, implante intralesional de células mesenquimales. Reposo y ejercicio controlado fueron considerados. Se realizaron evaluaciones clínicas y ultrasonográficas en los días 2, 15, 35, 75 y 120 posteriores al tratamiento. Después del día 120, el caballo fue reintroducido a actividades laborales y a pesar de los reportes de alta recidiva para este tipo de lesión, no se observaron alteraciones clínicas ni ultrasonográficas por 15 meses posterior al tratamiento. Estos resultados sugieren que la aplicación de medicina regenerativa, reposo y un protocolo de ejercicio controlado, aceleraron la reparación tendínea, redujeron el período de recuperación y permitieron el regreso exitoso del caballo a sus actividades de trabajo.

Key words: tendon injuries, cell therapy, horses, shoulder, lameness.

Palabras clave: lesiones tendinosas, terapia celular, caballo, hombro, cojera.

INTRODUCTION

Lameness associated to the shoulder has been reported to be of difficult diagnosis, even for the experienced veterinarian and has been under diagnosed (Tnibar *et al* 1999, Whitcomb 2003). Soft tissues are considered the main cause of lameness referable to this region and the inflammation of the bicipital tendon and its adjacent bursa the most observed injury (Tnibar *et al* 1999, Reef *et al* 2004). The presentation of different types of equine tendonitis can be very high, eventually affecting up to 30% of some equine populations (Oikawa and Kasashima 2002, Oliveira 2008), but specially bicipital tendonitis is of lower incidence (Bassage and Ross 2011).

Exhausting exercise, trauma and working conditions have been considered as predisposing factors to equine tendonitis (Stashak 2002). Clinical and ultrasonographic signs generally include: lameness, increased volume and temperature, associated to an inflammatory infiltrate which generates hypoechoic areas in the tendon and bursal effusion. Regardless of the affected tendon, recurrence rates of equine tendonitis are high (close to 40%) even when anti-inflammatory and rehabilitation therapies are adequately performed. This phenomenon has been associated to functional characteristics of the new formed scar tissue (Dahlgren 2009, Smith 2010).

The development of new approaches to treat tendonitis has become an important area of research due to the impact such injury has on equine industry. Recently, the use of mesenchymal stem cells for the treatment of tendonitis has shown different results from those obtained with conventional therapies (Barreira *et al* 2008), characterized by higher rates to successful return to sports and working activities (Herthel 2001, Smith 2010).

This article reports the diagnosis of a case of equine biceps brachiii tendonitis and its treatment using the application of an autologous mononuclear stem cells implant.

MATERIAL AND METHODS

A 7 year old crossbred gelding, weighting 410 kg, was presented to the Veterinary Teaching Hospital of the

Accepted: 15.05.2012.

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Universidad Austral de Chile for clinical attention. Clinical history included a car accident about a year before, when the horse was hit in the shoulder region of the left forelimb and showed nonweightbearing lameness . The horse rested for several months before going back to work. A week before the current attention, while on heavy draught work, the horse slipped off and presented a lameness onset, but was kept working due to owner's needs, increasing the severity of lameness primarily observed.

Clinical examination evidenced severe left forelimb lameness (5/5) (Ross 2011[a]), intense painful reaction to palpation of the shoulder region and caudal extension of the limb. No crepitation was observed. The horse was submitted for radiographic evaluation in which no abnormalities were identified. An arthrocentesis of the referred joint was performed using a 16 G catheter, producing spontaneous drainage of a dark brown synovial fluid without viscosity. Through the same approach, intra synovial diagnostic anesthesia was performed using 30 ml of a 2% sterile solution of mepivacaine. Since no improvement was evident after 15 minutes, a similar needle was introduced through the distal pouch of the bicipital bursa (Moyer *et al* 2007) and a translucent fluid was obtained which the authors considered to be anesthetic solution injected before. As previously described, 15 ml of the same mepivacaine solution were injected in this area. After 15 minutes of this procedure, slight improvement of lameness was observed (4/5, Ross 2011[a]).

Ultrasonographic evaluation described by Tnibar *et al* 1999 using a linear 7,5 MHz transducer, revealed the presence of an irregular anechoic area between zones B and C of the biceps tendon, characterizing grade 2/4 of fibers rupture [1 = slightly (25%) less echogenic than normal, 2 = half echogenic and half anechoic, 3 = mostly anechoic (75%) 4 = completely anechoic, Genovese *et al* 1986] (Figures 1a and 1c). Areas of mineralization, which can occur in chronic cases, were not observed (Boys Smith *et al* 2007). The same region of the contralateral limb was evaluated for comparison and no similar changes were identified (Figure 1d).

Primary treatment of the inflammatory reaction included 4.4 mg/kg of oral phenylbutazone BID for 4 days but no clinical improvement was observed. A suspension including triamcinolone (12 mg/2 ml) plus 2 ml of a 99% of dimethysulfoxide solution was administered adjacently to the tendon injury using a sterile ultrasound guided injection. Two days later, lameness decreased from 5/5 to 3/5. Seven days after this treatment, the horse was injected with an intralesional autologous implant of bone marrow mononuclear cells. For the collection procedure, the horse

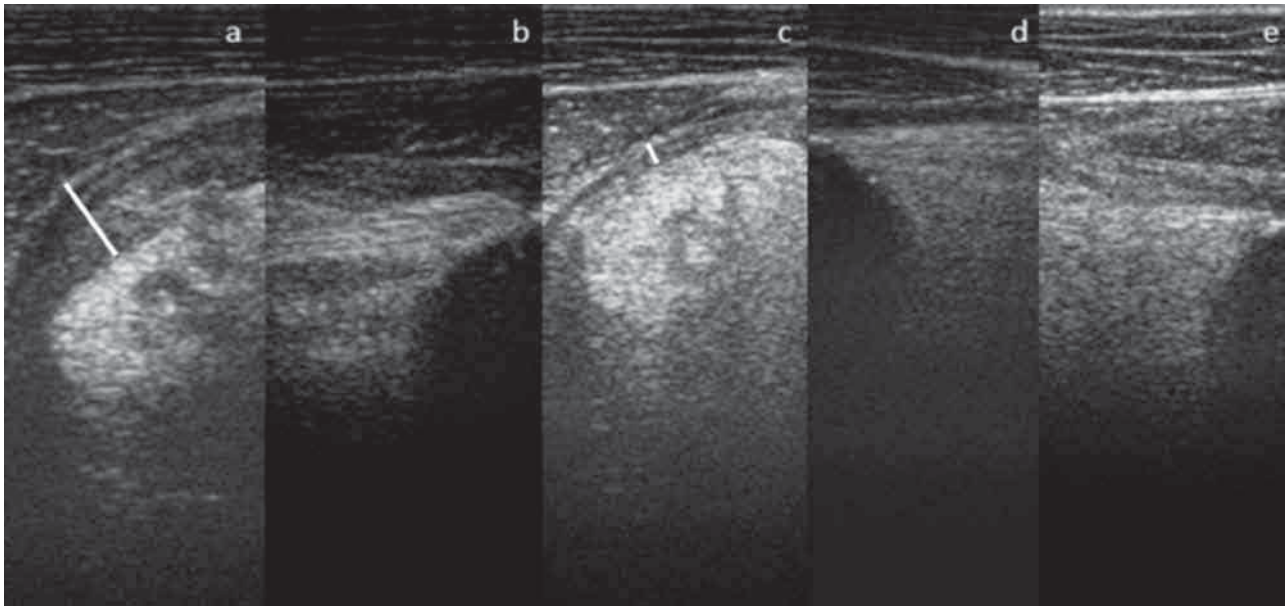

Figure 1. a) Transversal image of the biceps tendon at zone B of the left forelimb exhibiting hypo-echoic central area and fibers disruption at day 0. Note increased volume of biceps brachii muscle fibers (white marker); b) Longitudinal image of zone A and B of the left forelimb exhibiting hypoechogenicity at day 0. At the right side of the image the biceps brachii tendon attachment to the supraglenoid tubercle can be observed; c) Transversal image of bicipital tendon at zone B of the left forelimb presenting decreased size of hypo echoic area at day 32. Note marked reduction of biceps brachii muscle fibers inflammation (white marker); d) Longitudinal image of zones A and B of the normal right forelimb; e) Longitudinal image of zones A and B of the left forelimb at day 75.

Imagen transversal del tendón bíceps braquial en zona B del miembro anterior izquierdo exhibiendo hipo-ecoica central y ruptura de fibras al día 0. Se observa aumento de volumen en las fibras musculares del bíceps braquial (marcador blanco); b) Imagen transversal del tendón bicipital en zona B del miembro anterior izquierdo presentando disminución del área hipo-ecoica al día 32. Se observa reducción de la inflamación de las fibras musculares (marcador blanco); c) Imagen longitudinal de las zonas A y B del miembro anterior izquierdo exhibiendo áreas de hipo-ecogenicidad al día 0. Al lado derecho de la imagen se puede apreciar la unión del tendón bicipital a la tuberosidad supraglenoide; d) Imagen longitudinal de las zonas A y B del miembro contra lateral normal; e) Imagen longitudinal de las zonas A y B del miembro anterior izquierdo al día 75.

was sedated using 0.7 mg/kg of IV xylazine. The region of sternal vertebras was clipped and surgically prepared for aseptic procedure using clorhexidine 2% solution, and local anesthesia along the 5th sternebra area. For bone marrow aspiration an 8 G Komiashiki needle[1] was connected to 20 ml syringes including 5.000 IU of heparin. A 30 ml sample from the referred sternebra was obtained and its origin was confirmed by macroscopic observation of spikes and fat on Petri dishes and by means of a Romanowski staining (Barreira 2005).

Samples were filtered in transfusion sets in order to eliminate cell aggregates, gently transferred to sterile 15 ml Falcon tubes with 2.5 ml of Fycol[2]. Density centrifugation for isolation of mononuclear cells performed at 500 *g* for 30 min. The mononuclear rich cells fraction was rinsed twice with Dulbecco's phosphate buffered saline (DPBS) to remove excess of Fycol.

The mononuclear cells fraction was evaluated to determine its concentration through a Newbauer chamber (73 x 10^6 total cells). After staining with a non penetrating DNA marker[3] it was yielded 89% of viable cells. Autologous blood serum was used to prepare the mononuclear cells suspension (1:1) obtaining 2 ml as total volume, which was injected into the tendon injury by means of ultrasound guidance.

RESULTS AND DISCUSSION

The horse was stall rested for 15 days and in a 50 m^2 outdoor pen for more 30 days. After 32 days of implant the horse was submitted to another evaluation. Despite no evident lameness, a mild restriction on the elevation phase of the stride was observed in the left forelimb. Ultrasonographic evaluation revealed improved in tendon echogenicity (1/4, Genovese *et al* 1986) and absence of surrounding tissue inflammation, however, the shape injury looked similar (Figure 1b).

A second evaluation was performed at day 75. Throughout clinical examination at walk and trot neither lameness nor painful reaction to local pressure was observed, and the horse was able to spontaneously lope without restrictions. Ultrasonographic evaluation revealed normal echogenicity but lack of parallelism of tendon fibers in some locations. Due to owners' necessities, at this time the horse was submitted to controlled exercise (15 minutes of hand walk BID). At day 120 the horse was reevaluated exhibiting no signs of lameness and completely returned to draught activities.

Diagnosing bicipital bursitis is a simple procedure when a detailed clinical examination is performed. A fundamental concept in lameness diagnosis is the application of diagnostic anesthesia in order to localize the source of pain (Ross 2011[b]). The shoulder region has been described to be of difficult assessment for synovial anesthesia (Stashak 2002). However, the injection of the shoulder joint and the bicipital bursa according to Moyer *et al* (2007), could be easily performed. Despite minimal improvement after diagnostic anesthesia, the arthrocentesis performed for this procedure was useful to evaluate synovial fluid characteristics. Although communication of the shoulder joint and bicipital bursa is uncommonly observed, its likelihood to occur increases with inadvertent injection (Bassage and Ross 2011) or trauma. However, independently of communication, diagnostic analgesia of these structures can take up to half an hour or in cases of severe injury both compartments can be refractory to anesthesia (Bassage and Ross 2011, Dyson 2011).

Ultrasonography is a primordial and sensitive diagnostic tool for shoulder injuries (Witcomb *et al* 2006, Dyson 2007), which were previously considered of difficult diagnosis. However, detailed knowledge of the region's anatomy is required. In this case, the application of the ultrasonographic examination protocol according to Tnibar *et al* (1999) and Whitcomb (2003), using a 7,5 MHZ transducer, allowed clear diagnosis and treatment support. Sequenced ultrasonographic follow up associated to clinical examination, provided additional information for the decision to reintroduce the patient to controlled exercise (Whitcomb 2003) earlier than usually recommended by literature.

Despite bone marrow aspiration requires previous training, it is safe and easy to perform. Bone marrow mononuclear cells fraction separation is a simple procedure and requires the same conventional facilities of an hematology laboratory and general techniques for cell separation (Barreira *et al* 2008). Care should be taken in order to avoid contamination of the sample and further septic complications. Cell viability should be ensured, mainly when cells have been manipulated by a non experienced practitioner or when the previously described procedure has not been completely followed. Considering the fact that this was a working draught horse whose owner could not afford ideal conditions for rest and controlled exercise as recommended for tendon injuries (Gillis 2004), this protocol could not be strictly accomplished but was adapted as much as possible. Moreover, this horse represented its owner's main financial intake and needed to be introduced to working activities 120 days after the implant application.

Conventional treatment for tendonitis, such as stall rest and anti-inflammatory drugs generally provide conditions for a healing process, which leads to scar tissue formation and major deposition of type 3 instead of normal type 1 collagen fibers, (Dahlgren 2009, Smith *et al* 2011). Type 3 collagen fibers are longer but thinner, which results in less resistance to stretching forces leading to recurrent rupture of fibers and tendonitis onsets (Oliveira 2008). This has been considered the primary reason why this type of injury has

1 FREMIQSUR Ltda., Temuco-Chile.
2 Ficoll Paque™ Plus [< 0.12EU/ml (1 EU = 0.1ng. Ec5], GE Healthcare Bio-Sciences AB.
3 DAPI 100 ng/ml [4',6-diamidino-2-phenylindole dihydrochloride], Sigma, Saint Louis, MO, USA.

reserved prognosis to return to working and sports activities and explain its high rates of recurrence and degenerative characteristics (Barreira *et al* 2008). In cases o bicipital tendonitis recovery time for acute lesions require, at least 6 to 9 months of rest and the prognosis varies from fair to poor (Dyson 2011). Severe cases demand steroids and/or hialuronan injection for clinical improvement (Ridgway 2006, Dyson 2007) and any condition that affects the smooth movement of the tendon lead to chronic lameness (Hughes 2008). Surgical treatment including bursoscopy and arthroscopy are only recommended for septic and/or chronic cases in which proliferative synovitis are present. Bicipital tenotomy has been reported in dogs (Bergenhuizen *et al* 2008), however, its favorable outcome in horses cannot be guaranteed (Boys Smith *et al* 2007). The application of regenerative medicine as a treatment of tendon and ligament injuries provides higher deposition of type 1 collagen, better collagen fiber pattern organization and overall better prognosis for athletic activities (Dahlgren 2009, Maia *et al* 2009, Smith 2010). Considering previously described aspects, facilities to provide this procedure and make it affordable for this case, the use of regenerative medicine was considered.

It is important to consider that although this case was conducted differently when compared to animals under experimental conditions (Barreira *et al* 2008, Oliveira 2008) and also the rehabilitation protocol (Gillis 2004) was not entirely followed, clinical and ultrasonographic findings were considerably satisfactory allowing the horse to return to working activities before the recommended recovery period (6 to 9 months), without lameness recurrence in a follow up period of 15 months as reported by Gutierrez-Nibeyro (2011) and Smith (2010). These results suggest the use of juxtatendinous steroid injection followed by autologous implant of bone marrow mononuclear cells associated to rest and controlled exercise, accelerated tendinous repair, reduced recovery time and allowed successful return to complete working activity of this horse.

SUMMARY

Bicipital bursitis in the horse, the inflammation of the bicipital tendon and its surrounding bursa, has been reported to represent a low percentage of lameness cause. However, it is the main cause of lameness associated to the shoulder region and it has been under diagnosed. Due to high recurrence in different types of tendon injuries, treatments aiming to re-establish tendon functionality have been a focus of research. The aim of this study is to report the implant of a bone marrow mononuclear cell fraction as treatment for bicipital bursitis in a horse. A 7 year old crossbred draught gelding was presented with severe lameness of the left forelimb and pain in the shoulder region. Clinical and ultrasonographic evaluation revealed hemorrhagic synovial fluid, decrease of lameness after shoulder joint anesthesia and bicipital tendon fibers rupture and inflammation. The patient was successfully treated by triamcinolone injection adjacent to the tendon lesion followed by intralesional injection of bone marrow mononuclear cells seven days after the first treatment. Also, rest and controlled exercise were performed. Further clinical and ultrasound evaluations were executed at days 2, 15, 35, 75 and 120. After day 120 the horse started working and despite bicipital bursitis has been reported to exhibit high recurrence, neither clinical nor ultrasonographic signs of recurrence were reported for 15 months after lameness onset. These results suggest that use of regenerative medicine associated to rest and a controlled exercise protocol, accelerated tendon repair, reduced recovery period and allowed successful return to working activities without recurrence.

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