Single injection of autologous platelet rich plasma (PRP) in suspensory ligament lesions in horses: a clinical trial

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INTRODUCTION
Suspensory ligament injuries in horses are a major problem for any kind of discipline and attitude, often demanding long periods of rest, introduction in lower classes of competition and sometimes retirement from the sport activity (Smith, 2008). The site of the lesion, the affected limb and the use of the horse are the main concerns in assessing the possibility of recovery and the prognosis (Ross & Dyson, 2006). Many are the possible therapies experimented throughout the years, but none of them ensures to completely restore the anatomical and functional integrity of the injured tissue. Recently regenerative medicine has risen great expectations and lesions have been treated with autologous blood derivates and MSCs (Fortier & Smith, 2008). Platelets concentrates have been in the last decades addressed as possible proper solutions to the problem, being a substantial source of growth factors potentially boosting the reparative process and improving the quality of the heal (Borzini & Mazzucco, 2005). Nevertheless there is a general lack of knowledge about the precise indications, timing, number of applications and minimum number of platelets for the treatment with PRP and its effectiveness is yet to be clinically investigated. Too many are the possible interactions of platelets’ growth factors in the site of lesion and each one can affect the outcome of the healing process, not forgetting that the age, the rehabilitation protocol and the characteristic of the lesion itself play a role too (Weibich et al., 2002). All of these variables are hard to investigate for importance and far to be decoded at the moment.

Clinical trials on large numbers of subjects could better outline the guidelines for the effective and actual use of blood derivatives. The aim of this study was to verify the effectiveness of a single PRP application in suspensory ligament spontaneous lesions in sport horses.

MATERIALS AND METHODS
Ten adult horses different for age (mean 7.7 y), sex, breed and sport discipline (4 trotters, 5 jumpers, and 1 galloper) were referred for lameness and assessed to be affected with a lesion of the suspensory ligament after a complete lameness investigation and an ultrasonographic exam. To include the horse in the study, the lesion had to be echographically evident, less than 1 month old, and treated with a single ultrasound-assisted intra-lesional injection of fresh PRP. Fore and hind limbs lesions were included not considering the different sites of lesion (proximal, midbody, distal, branches). The cross sectional area (CSA) of the lesions varied from 10 to 75% of the ligament, and the average alignment of fibres was 1.6.

Four-hundred-fifty ml of whole blood were collected from each horse in CPDA-1 and PRP was obtained with a double centrifugation in a closed, sterile, blood transfusion double-bag system. The first centrifugation was performed at 500 g for 20 minutes. The PCV was manually separated from the supernatant which was then centrifuged at 3000 g for 10 minutes. Eighty% of the supernatant was then eliminated and the platelets re-suspended in the remaining plasma. Platelets were concentrated 7 to 11 times. Horses were sedated with acepromazine (20 microg/kg IV), detomidine (10 microg/kg IV) and butorphanol (20 microg/kg IV) and a proximal truncular anaesthesia of the palmar/plantar nerve was performed (lidocaine 2%) for the ultrasound-assisted intra-lesional injection of PRP. A soft simple bandage was applied and horses were hospitalized for two days. A standardized rehabilitation/training protocol, suitable to the activity of the horse, was recommended to reintroduce the horse in competition after 6 months. The outcome of the treatment was evaluated with a lameness investigation and an ultrasonographic control at six months, and on the basis of the return to the activity.

RESULTS
None of the horses showed any adverse reaction to the treatment. Six horses returned to competition after the scheduled period of rehabilitation (6 months), whereas other 3 needed an average of 6 more weeks to get back to the activity. One horse was unable to get back to contests without further treatment due to a relapse of the pathology during rehabilitation. Of the 9 horses that returned to the activity 2 showed a clinical relapse of the pathology within 1 year after treatment. The ultrasound control showed a reduction of the CSA of the lesion in 9 horses with an average score of alignment of fibres of 1.
CONCLUSION
The results obtained in this clinical trial are encouraging. Seven of the 10 treated horses have had a positive outcome with a 12 months follow up. This datum is consistent with the literature (Arguelles et al., 2008) but a longer follow-up is needed to understand whether if the result is long lasting.
The 3 horses with a negative outcome were all trotters, affected in hind limbs. This result might have a correlation both with the discipline itself and with the management and training of such horses, which are often put back into competition with an urgency potentially undermining a complete recovery.
In this study were considered only horses treated with a single application of PRP, which was demonstrated to be effective on rats (Virchenko & Aspenberg, 2006) and that has here shown a satisfactory outcome.
In this report we included only the horses in which it was considered, based on an ultrasonographic evidence, that multiple injections might even cause a damage to the fibrils in shaping. Until more consistent data become available, it is possible to state that the treatment of suspensory ligament lesion with PRP seems to be effective, uneventful, cheap and easy, and able to accelerate and improve the quality of the heal, thus it should strongly be considered for these injuries in sport horses.

REFERENCES
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