Platelet rich plasma treatment of superficial digital flexor tendon lesions in racing Thoroughbreds

K. Zuffova, S. Krisova, Z. Zert

University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: The main goal of this study was to evaluate the effectiveness of a modified method for preparing platelet rich plasma for the treatment of superficial digital flexor tendon lesions in race horses and its influence on the future recuperation of the animals. The applied concentrate of plasma was prepared by centrifugation followed by aspiration close above the buffy coat. There were no negative reactions resulting from the application of the biological material into the damaged tendons. The success of the therapy was proven by the shortened rehabilitation time and the higher number of horses returning to racing.

Keywords: tendonitis; growth factors; racing carriers; ultrasonographic examination

Injury of the superficial digital flexor tendon (SDFT) in racing Thoroughbreds is the most frequent musculoskeletal problem causing early retirement in the racing industry (Lam et al. 2007). Once seriously injured, the tendon never returns to the original quality, elasticity and functional ability. Re-injury of the repaired tendon is common (Marr et al. 1993; Dyson 2004). Complete tendon healing is a long process and, dependent upon the severity and size of the lesion, usually takes 6–18 months and more (Goodship et al. 1994; Smith and Schramme 2003).

The SDFT functions to transfer muscular activity to the distant fetlock joint, enhance the effect of muscular contraction in the periphery and works as a source of accumulated elastic energy and absorber of impact energy during movement of the horse (Evans and Barbenel 1975; Alexander 1991). Maturity of the SDFT tendon tissue is achieved at the age of two years when the cross-linking bindings have stabilised and the fibres have crimped (Patterson-Kane et al. 1998). The elasticity and mechanical properties of the tendon decrease from the age of two years due to an increasing number of non-elastic cross-linking bindings and the smaller size of fascicles in older tendons (Gillis et al. 1995, 1997). The disruption of collagen fibres is accompanied by bleeding and the formation of the intra tendon haematoma followed by growth of the granulation tissue (Goodship et al. 1994; Aspenberg 2007). The severity and intensity of the healing inflammatory process directly influence the size and quality of the new scar tissue (McIlwraith 2002). The surrounding tendon tissue of the scar is predisposed to repeated injury (Smith and Schramme 2003; Benjamin et al. 2008). For this reason it is preferable that the healing process should be more regenerative than reparative in nature through the application of growth factors into the healing tendon (Fortier 2011). The first clinical application of platelet rich plasma (PRP) was published by Marx et al. (1998) in the supportive treatment of mandibular defects in humans with bony implants. More recently, PRP was used in human and equine sport medicine for therapy defects in ligaments and tendons (Mishra and Pavelko 2006; Arguelles et al. 2008; Waselau et al. 2008). A great advantage of autologous PRP therapy is the lack of immunological response in the treated organism (Kajikawa et al. 2008).

The main growth factor typical for the α-granules of thrombocytes is platelet-derived growth factor (PDGF) (Anitua et al. 2004; Blair and Flaumenhaft 2009). The following thrombocyte factors are, transforming growth factor beta (TGF-β1, TGF-β2), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and fibroblastic growth factor (FGF) (Pintucci et al. 2002; Anitua et al. 2004; Blair and Flaumenhaft 2009). Insulin-like growth factor (IGF I) is an important plasma factor for musculoskeletal healing (Boswell et. al., 2012).

Bosch et al. (2010, 2011) studied the healing of a mechanically-induced experimental central core lesion in the SDFT of a horse and found, after only one application of PRP into the lesion, a significant positive effect on the biochemical, biomechanical and histological characteristics of the healing process. The healed tissue was rich in collagen fibres and glycosaminoglycans compared to the control group. Based on these results the authors found the application of autologous PRP to be useful in the treatment of acute tendon injury in the horse.

MATERIAL AND METHODS

This study included 22 racing Thoroughbreds (Table 1) treated in the Equine clinic of the University of Veterinary and Pharmaceutical Sciences Brno during the period from 2008–2011. In all patients the SDFT lesion was treated by intralesional application of modified prepared platelet rich plasma (PRP) and the horse was then trained under a prescribed controlled training regime by its own trainer. All the horse owners and trainers in the study were informed about the principles of the therapy and possible complications after its use in their animals. The racing horses were monitored from the time of application until the end of the year 2012.

History and ultrasonographic examination

For the evaluation age, gender and type of use were recorded. Ultrasonographic examination was performed in all the clinical cases to determine the status of the injury in the particular tendon. In cases of doubt and for control reasons tendons were also examined contralaterally.

Ultrasonographic evaluation was performed with the ultrasound machine Honda HS 2100 (Honda Electronics CO., LTD, Japan) with a linear probe of 7.5 MHz. Better visualisation of the superficially located SDFT was achieved by a standoff pad for the linear probe. The horse's hair was clipped, scrubbed with soap and an ultrasonic gel (Topvet,

Table 1. Chronological overview of the horses in the study

Number of patient	Hospitalization number	Age (years)	Gender	Type of racing
1	080766	5	gelding	flat
2	080945	3	stallion	flat
3	080944	3	stallion	flat
4	090352	4	gelding	flat
5	090797	3	mare	flat
6	090593	6	stallion	flat, hurdles, steeplechase – crosscountry
7	100334	2	stallion	in the training for flat
8	100592	5	gelding	flat, hurdles
9	100411	6	gelding	flat, steeplechase
10	100453	4	mare	flat
11	100454	4	gelding	flat, hurdles
12	100688	6	mare	flat, steeplechase – crosscountry
13	100686	5	gelding	flat, steeplechase – crosscountry
14	100727	4	mare	flat, hurdles, steeplechase – crosscountry
15	100533	7	gelding	flat, hurdles, steeplechase
16	100750	5	gelding	flat, hurdles
17	100828	5	gelding	steeplechase – crosscountry
18	101095	4	stallion	in the training for flat
19	110351	3	mare	flat
20	110562	3	stallion	flat
21	110876	3	gelding	flat, hurdles
22	110919	3	stallion	flat, hurdles

Czech Republic) was used for better contact between the probe and the skin.

The palmar metacarpal region was evaluated in seven zones transversally and longitudinally according to Reef (1998) and Rantanen et al. (2003). Evaluation of the changes was made by ultrasound according to Craychee (1995), and the locality in the tendon, length of the lesion, changes in echogenicity, pattern of echogenicity changes (homogenous/heterogeneous, focal/diffuse) and disturbances in the longitudinal orientation of tendon fibres were determined. The length (cm) was measured externally on the skin using an applied paper scale. Grade of echogenicity was evaluated according to Rantanen et al. (2003) and partly with reference to Denoix (1996):

1. Isoechoic**:** echogenicity of the structures is unchanged;
2. Hypoechoic**:** lesion is less echoic than isoechoic and there were two types of these lesions:
   Type 1: lesion is diffuse grey of grey-white
   Type 2: lesion is a mixture of black and white spots;
3. Anechoic: lesion is mostly black in appearance
4. Hyperechoic: Denoix (1996) divided this category into two types:
   Type 1: lesion is brighter than isoechoic (dense scar tissue without acoustic shadows)
   Type 2: lesion is typical for mineralised deposits in the soft tissue with the acoustic shadow; this pattern is rare and is common in older or repeated tendon injuries.

The lesion was examined by ultrasound according to Genovese et al. (1987) to determine if it was acute, mixed or chronic in its echogenicity, which was higher with time. The severity of the tendon lesion was assessed ultrasonographically using the criteria in Table 2.

The percentage of cross sectional area (% of CSA) was measured from the digital ultrasonographic pictures directly in the USG machine or in the case of irregular shape of the lesion in the computer with the programme Paint NET 3.5.10 (Microsoft Corporation, USA).

A modified method for preparing platelet rich plasma (PRP)

Full blood was taken under aseptic conditions into a blood transfusion bag (Blood bag single, content 450 ml, CPDA 63 ml, JMS Singapore PTE LTD, Singapore) from the external jugular vein in each of the studied patients. The bag was stored in the vertical position for one hour. After the sedimentation of erythrocytes the plasma was separated by a pressure device (Plasma Expresor 120, Chis ltd., Brno, Czech Republic) into a 50 ml syringe. These 50 ml of blood were further divided into 24 Eppendorf microtubes (Eppendorf Safe-lock, content 1.5 ml) each of which was centrifuged in a Hermle Z 300 centrifuge (Hermle Labortechnik, Wehingen, Germany) with a rotor Hermle 220.87 V10 (angular rotor, 24 × 1.5/2 ml, max. 20 000 rpm, Hermle Labortechnik, Wehingen, Germany) for 5 min with a rotational speed of 1500 rpm/min. One ml and 300 µl of plasma were removed from each microtube after centrifugation. The remaining 0.2 ml of plasma concentrate close to the layer of the buffy coat were aseptically aspirated into a few sterile Eppendorf microtubes from all 24 tubes. The Eppendorf microtubes were then used to apply the rich plasma into the lesion in the damaged tendon.

Comparison of the concentration of platelets in full blood and PRP

From the finally prepared microtube with PRP for application 0.3 ml plasma were withdrawn for measurement of the platelet concentration. Blood was taken from each horse for the determination of thrombocyte content in a routine manner. Both samples were analysed on a haematological analyser (Celltac alpha MEK 6318, Nihon Kohden, Japan) in the Central Laboratory of the University of Veterinary and Pharmaceutical Sciences, Brno.

Table 2. Assessment the severity of the tendon lesion

Grade of severity	% tendon lesion	% of CSA and length of the lesion
Mild	0–15	% of CSA < 50% and/or length < 100 mm
Middle	16–25	% of CSA 50–75% and/or length 100–160 mm
Severe	> 25	% of CSA > 75% and/or length > 160 mm

CSA = cross sectional area

Application of PRP

The place of application was scrubbed with povidon iodine soap, flushed with saline and wiped with isopropyl alcohol. Patients were sedated with a combination of detomidine hydrochloride 0.012 mg/kg (Cepesedan 1% inj., CP-Pharma Handelsges. GmbH, Germany) and butorphanol tartarate 0.025 mg/kg (Butomidor 1% inj., Richter Pharma AG, Wels, Austria). Local anaesthesia of *n. palmaris lateralis et medialis* on the level of the proximal metacarpus was achieved with injection of 5 ml bupivacaine (Marcaine 0.5% inj., AstraZeneca AB, Sodertalje, Sweden).Platelet rich plasma was aseptically aspirated into the syringe and activated by 0.1 ml calcium for 1.0 ml PRP (calcium chloratum Biotika 10% inj., Hoeschst-Biotika, ltd., Prague, Czech Republic). The intralesional application was performed aseptically under direct ultrasonographic control in a routine manner.

The size of the needle was chosen according to the consistency of the PRP. More dense plasma was applied using a 20 G needle; a more liquid plasma using a 21 G needle. The amount of injected PRP was relative to the size of the lesion to fill the whole anechoic or hypoechoic space in the tendon under ultrasonographic control. After the application the leg was scrubbed with povidon iodine soap and disinfected with isopropyl alcohol. The place of application was covered with Ialugen Plus (IBI Ltd., Prague, Czech Republic) and with a covering bandage. Procaine benzylpeniciline 8 mg/kg *i.m.* and dihydrostreptomycine 10 mg/kg *i.m.* (Norostrep AUV, Norbrook Laboratories Limited, Newry, Northern Ireland) was applied to all the patients once.

Rehabilitation after the application of PRP

All the patients in the study were left for two days in the box with a bandaged extremity from the hoof to the carpal joint. The rehabilitation plan was individually tailored according to the severity of injury of the tendon and results from ultrasonographic monitoring of the healing process. Description of the training program for the horse and post-application monitoring is presented in Table 3.

Evaluation of therapy results

1. The effectiveness of the concentration and application of modified prepared platelet rich plasma was evaluated.

2. The success rate of the particular therapy was evaluated by the time of rehabilitation to the first race start, by the number of racing starts in groups of horses with different severities of the tendon lesion, by the number of racing starts with regard to the stage of healing of the lesion and by the number of racing starts in different age groups of horses.

RESULTS

Table 4 shows the results of laboratory measurements of thrombocyte concentration in the full blood and in the sample from PRP applied into the tendon and patient healing including the number of racing starts after the treatment. A statistical summary of laboratory analysis results is presented in Table 5

Table 3. Suggested training programme after the treatment and scheduled ultrasonographic controls

Week after application	Time and type of exercise	USG control
0–2	15–30 min handwalking	no
3–4	30 min walk under the rider	yes – 3nd week
5–8	60 min walk + 10 min trot	yes – 8th week
9–12	60 min walk + 2 × 10 min trot	no
13–20	20 min walk + 2 × 20 min trot	yes – 20th week
21–28	45 min walk + trot, twice a week 1600 m canter (400 m/min)	yes – 28th week
29–32	45 min walk + trot, twice a week 2400 m canter (400 m/min)	no
33–36	45 min walk + trot, three times a week 600 m canter (600 m/min)	yes – 36th week
37–40	45 min walk + trot, three times a week 1200 m canter (600 m/min), jump	yes – 40th week
41 and more	increasing level of the exercise to the race	

USG = ultrasonographic

Table 4. Laboratory results of full blood and PRP examination of individual horses, patient continuation and number of starts after the therapy

Number of patient	Blood – platelet content ($\times 10^9$/l)	PRP – platelet content ($\times 10^9$/l)	Time to the first race (months)	Number of starts
1	70	363	excluded	0
2	108	307	excluded	0
3	93	184	excluded	0
4	105	509	23	4
5	74	467	11	8
6	84	271	11	10
7	135	286	13	3
8	82	447	2,5	6
9	86	425	excluded	0
10	103	430	11	9
11	99	431	22	2
12	101	466	11	4
13	78	529	10	3
14	85	561	23	4
15	85	558	excluded	0
16	55	606	excluded	0
17	53	271	11	1
18	89	517	7	4
19	88	1035	excluded	0
20	95	821	2	3
21	99	640	10	3
22	65	661	excluded	0

PRP = platelet rich plasma

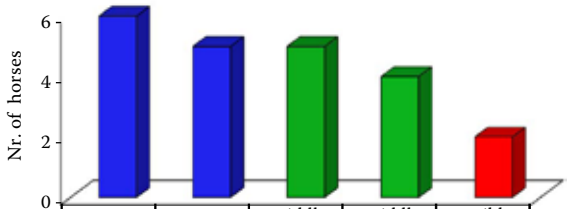
The multiplication of the platelet concentration in the sample of modified prepared PRP was on average 5.6 times higher than in the plasma of the individual horses. There was an obvious variability in the success of the thrombocyte concentration (Table 5).

The whole preparation of the plasma concentrate lasted about two hours. After the activation of the thrombocytes with added calcium, the PRP was applied before the expiration of 30 min to avoid gel formation in the sample. There were no serious complications registered after the instillation of the platelet rich plasma into the tendon. In two patients the application of plasma into the full length of the tendon in the metacarpal region an increased sensitivity of the tendon was registered, but this subsided within one week without any treatment. In four

Table 5. Statistical evaluation of the modified method of concentration of the thrombocyte count in blood and PRP of all horses (n = 22)

	Blood	PRP
	platelets content ($\times 10^9$/l)	
Range of values (minimal/maximal)	53–135	184–1035
Mean ± standard deviation (ä)	87.8 ± 18	490 ± 188
Median	87	466.5

PRP = platelet rich plasma



	severe R	severe N	middle R	middle N	mild R
Nr. of horses	6	5	5	4	2
Nr. of starts	37	0	19	0	8

Figure 1. Number of racing starts of horses with different grade of tendon injury (Nr. = number, R = raced, N = not raced)

	acute R	acute N	chronic R	chronic N	mixed R	mixed N
Nr. of horses	6	4	5	1	3	3
Nr. of starts	36	0	19	0	9	0

Figure 2. Number of racing starts after the treatment according to the duration of the injury (Nr. = number, R = raced, N = not raced)

patients an anechoic tunnel was discovered during the ultrasonographic control examination after the needle passage; those were filled with echoic healing mass with no need of any further attention. In one patient a thickening of the subcutaneous tissue due to the tight covering bandage was observed.

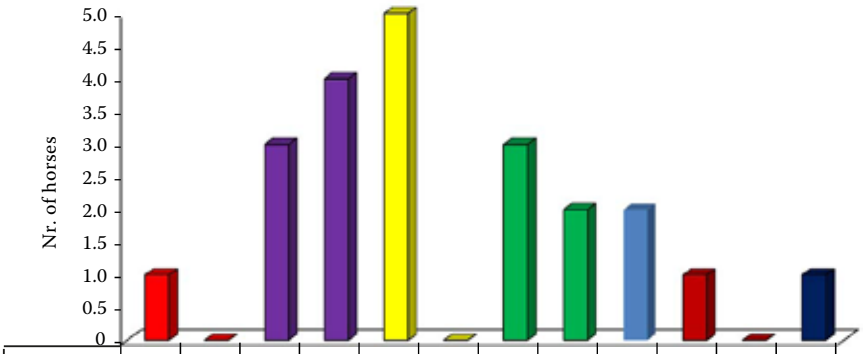
After the tendon treatment with modified prepared PRP the monitored patients raced up to 64 races in the follow-up period. From 22 managed horses 13 (59%) were able to race at least one race; from these 13 horses 9 raced their first race within 12 months.

Figure 1 shows the patients divided into groups regarding the grade of severity of tendon lesion and number of racing starts in the follow-up period. In the study were included 11/22 horses (50%) with severe, 9/22 horses (41%) with moderate and 2/22 horses (9%) with mild SDFT injury. The horses with the severe lesions ran 37/64 races (58%), those with moderate lesions 19/64 (30%) and horses with mild lesions ran 8/64 (12%) races during the follow-up period. Sixty-four percent horses with severe, 56% horses with moderate and 100% horses with mild tendon injuries ran more than one race after the PRP therapy.

Figure 2 shows the number of racing horses after the therapy with regard to the duration of the lesion. 10/22 horses (45%) presented with acute, 6/22 horses (27.5%) with chronic and 6/22 horses (27.5%) with mixed lesions of SDFT. Horses with acute lesions were able to run 36/64 races (56%), with chronic lesions 19/64 races (30%) and horses with mixed injury ran 9/64 races (14%).

Figure 3 shows the age structure of the patients and the relationship to the number of racing starts of treated horses during the follow-up period. The highest number of starts was registered by the four year old horses which ran 23/64 races (36%), three and six year old horses ran 14/64 races (22%), five year old horses ran 10/64 races (15.5%), one two year old horse ran 3/64 races (4.5%) and one seven year old horse did not run after the therapy.

From the non-running horses after the treatment with PRP we registered a re-injury of the same SDFT in horses 1, 2, 3 because of a premature start of intensive training; in horse 9 an SDFT injury of



Years	2 R	2 N	3 R	3 N	4 R	4 N	5 R	5 N	6 R	6 N	7 R	7 N
Nr. of horses	1	0	3	4	5	0	3	2	2	1	0	1
Nr. of starts	3	0	14	0	23	0	10	0	14	0	0	0

Figure 3. Number of racing starts of horses after the treatment in relation to their age (Nr. = number, R = raced, N = not raced)

the contralateral front limb was found; horses 15 and 16 had so serious an injury of the tendon that the owners decided to retire the horses before the treatment which resulted in a lower exercise routine; mare 19 was retired for breeding purposes and horse 22 was sold for lower level show jumping.

DISCUSSION

Injury of the superficial digital flexor tendon in racing Thoroughbreds is a common orthopaedic problem which has challenged equine veterinarians for many years. Long in duration and demanding individual care, therapy does not promise full recovery of the athletic performance of the horse and re-injury of the healed tendon is frequent. Dahlgren et al. (2005) and Bosch et al. (2010) published studies evaluating the positive healing abilities of PRP on SDFT lesions in an artificial collagenase and surgical model. However, no artificial model simulates the character of the lesion which attacks the tendon under natural conditions after training or racing.

The main task of our study was the evaluation of platelet rich plasma prepared in our laboratory for the treatment of exercise-induced lesions in the superficial digital flexor tendon of racing Thoroughbreds by assessment of the patient after treatment. A very important factor was the financial feasibility of the therapy for the owners. The method of preparation was chosen with regard to literature sources, and the materials and resources available to the Equine Clinic, University of Veterinary and Pharmaceutical Sciences, Brno. The laboratory process was adapted to achieve maximal concentration of thrombocytes for the enhancement of the healing.

PRP is prepared either using commercial sets specially developed for this purpose or in a protocol involving two centrifugations. The first centrifugation is performed to separate plasma and erythrocytes and the second is carried out to concentrate the plasma (Textor 2011). The sedimentation of erythrocytes for 60 minutes in our method substituted the first centrifugation. The final concentration of thrombocytes was in our method higher than in the double centrifugation. This runs counter to the study of Feige et al. (2003) who determined sedimentation to be less effective for the concentration of platelets in plasma and recommended plasmapheresis for the separation of erythrocytes. This method was not available in our clinic for technical and financial reasons.

McLellan (2011) found differences in the concentration of thrombocytes and leucocytes using different methods of preparation of PRP, discovered that some methods could cause premature activation of platelets and recommended different methods of application. The collection of the plasma just above the buffy coat as in our study confirms the high efficiency of the author's proposed method (McLellan 2011).

Marx (2001) described a clinical influence of PRP already after a four times increased concentration of thrombocytes compared with the full blood. In our study an average concentration of thrombocytes 5.6 times higher than the concentration in full blood was achieved. The successful concentration of platelets was very variable. The variability in our study was caused by different numbers of thrombocytes in the original full blood and the final sample concentration was influenced by the necessity of using a different amount of fluid to fill the whole space of the lesion. The concentration of platelets was inversely proportional to the amount of plasma necessary. The effect of the applied PRP is not only dependent on the concentration of thrombocytes but also on the fact that the plasma contains bioactive factors. Controling the amount of platelets in the PRP sample for application is important (Boswell et al. 2012). In our study, 4/22 patients (18%) of PRP samples had less than a four times concentration relative to blood. Before the injection the plasma was activated by the addition of calcium in the amount recommended by Eby (2002). The activation of thrombocytes is accompanied by a higher release of growth factors (Textor 2011).

In our study only racing and intensively trained Thoroughbreds were evaluated. The risk of tendon injury grows with the intensity of exercise (Williams et al. 2001). In our study most horses were flat racing Thoroughbreds; three horses that were treated were only in training; one horse was running steeplechase without previous flat racing experience. The possibility of injury of the SDFT during intensive training preparation was noted by Ely et al. (2004).

Marr et al. (1993) evaluated the effectiveness of different types of tendon treatment without PRP or stem cells and reported successful returns to the racetrack in 63% of the horses with mild tendon injury, 30 % of horses with moderate tendon injury and 23% horses with severe tendon injury of SDFT. In our study 50% of the horses

suffered with severe SDFT injury and only 9% of our patients were injured slightly. The proportion of horses returning to racing in our patients was 100% in horses (only 2) with mild injury, 56% in the group of Thoroughbreds with moderate injury and 64% in horses with severe injury. This was better than the above mentioned work in which a traditional conservative approach was utilised. In the follow-up period all patients ran a combined total of 64 races and horses with severe injury started in 58% races, moderately-injured horses ran in 30% races and mildly injured animals ran in 12%, respectively.

Bosch et al. (2011) recommended only one intralesional application of PRP in the acute stage of the injury but Textor (2011) who also reported management of SDFT injuries in the acute stage recommended repeated application. In our study we applied PRP in all cases only once because the lesion was ultrasonographically not visible during the control examination.

The positive influence of PRP treatment of the lesion in the acute stage of injury was confirmed in our study because horses with acute lesions ran 56% of races in the follow-up period and horses with a lesion in the chronic stage recorded 30% of starts. According to our results the positive effect of the application of PRP was registered not only in cases with acute injury but also in the chronic as well, which was similar to the results of Sutter et al. (2004) who recommended application 30 days after the initial injury.

Kasashima et al. (2004) showed that there was an increase in the incidence of SDFT injury in racing horses from the age of two. They found that the most risky category is the five year old horses and that the probability of injury is three times higher than in two year old racing Thoroughbreds. In conclusion, our group of horses was too small to assess the influence of age on the effectiveness of PRP treatment. However, we achieved the best results in four year old horses which ran 36% of races after the treatment and in the categories of three and six year old horses.

In 13 horses running more than one race 69% (9/13) the first race took place within a recovery time shorter than 12 months. This illustrates a possible improved effect of PRP in comparison with the more than 18 months which is the obligatory interval recommended for similar lesions treated conservatively (Goodship et al. 1994, Smith and Schramme 2003).

The procedure for the preparation of autologous PRP for the treatment of tendon lesions is simple and financially feasible. Where necessary it is possible to repeat the application of PRP according to the ultrasound control findings. With respect to the asepsis rules, we did not find any negative reaction of the tissue or whole organism of the patients in the study. Our results show that our modified method of preparation of platelet rich plasma had a positive influence on the healing of the superficial digital flexor tendon lesions in acute and chronic cases when compared with conservative treatment using controlled exercise. The time of rehabilitation was shortened and the number of horses returning to racing was higher.

A distinct disadvantage of our study was the heterogeneity of our group of patients. The size and character of the lesions was not uniform; the horses came from different training conditions and were rehabilitated in the original training centre without strict supervision of the training regime. It was not possible for practical reasons to design the study with a more defined stage, size and character of the lesion and standard post-therapeutic rehabilitation regime. Moreover, the duration of the follow-up therapeutic effects should be evaluated in the future over a longer period of time.

REFERENCES

Alexander RM (1991): Energy-saving mechanisms in walking and running. Journal of Experimental Biology 160, 55–69.

Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT (2004): Autologous platelets as a source of proteins for healing and tissue regeneration. Thrombosis and Haemostasis 91, 4–15

Arguelles D, Carmona JU, Climent F (2008): Autologous platelet concentrates as a treatment for musculoskeletal lesions in five horses. Veterinary Record 162, 208–211.

Aspenberg P (2007): Stimulation of tendon repair: mechanical loading, GDFs and platelets. A minireview. International Orthopaedics 31, 146–149.

Benjamin M, Kaiser E, Milz S (2008): Structure-function relationships in tendons: a review. Journal of Anatomy 212, S211.

Blair P, Flaumenhaft R (2009): Platelet alpha-granules: basic biology and clinical correlates. Blood reviews 23, 177–189.

Bosch G, van Schie HTM, de Groo MW, Cadby JA, van de Lest CHHA, Barneveld A, van Weeren PR (2010):

Effect of platelet-rich plasma on the quality of repair of mechanically induced core lesions in equine superficial digital flexor tendons: A placebo-controlled experimental study. Journal of Orthopaedic Research 28, 211–217.

Bosch G, Moleman M, Barneveld A, van Weeren PR, van Schie HTM (2011): The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions. Scandinavian Journal of Medicine and Science in Sports 21, 554–561.

Boswell GS, Cole BJ, Sundman EA, Karas V, Fortier LA (2012): Platelet-rich plasma: A milieu of bioactive factors. Journal of Arthroscopic and Related Surgery 28, 429–439.

Craychee TJ (1995): Ultrasonographic evaluation of equine musculoskeletal injury. In: Nyland TG, Mattoon JS (eds.): Veterinary Diagnostic Ultrasound. WB Saunders, Philadelphia. 348–352.

Dahlgren LA, Mohamed HO, Nixon AJ (2005): Temporal expresion of growth factors and matrix molecules in healing tendon lesions. Journal of Orthopaedic Research 23, 84–92.

Denoix JM (1996): Ultrasonographic examination in the diagnosis of joint disease. In: McIlwraith CW, Trotter GW (eds.): Joint Disease in the Horse. WB Saunders, Philadelphia. 165–202.

Dyson SJ (2004): Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992–2000). Equine Veterinary Journal 36, 415–419.

Eby BW (2002): Platelet-rich plasma: Harvesting with a single-spin centrifuge. Journal of Oral Implantology 28, 297–301.

Ely ER, Verheyen KL, Wood JL (2004): Fractures and tendon injuries in National Hunt horses in training in UK: A pilot study. Equine Veterinary Journal 36, 365–367.

Evans JH, Barbenel JC (1975): Structural and mechanical properties of tendon related to function. Equine Veterinary Journal 7, S1.

Feige K, Ehrat FB, Kästner SBR, Schwarzwald CC (2003): Automated plasmapheresis compared with other plasma collection methods in the horse. Journal of Veterinary Medicine 50, 185–189.

Fortier L (2011): Clinical use of stem cells, marrow components, and other growth factors. In: Ross MW, Dyson SJ (eds.): Diagnosis and Management of Lameness in the Horse. 2nd ed. WB Saunders, Philadelphia. 761–764.

Genovese RL, Rantanen NW, Simpson BS (1987): The use of ultrasonography in the diagnosis and management of injuries to the equine limb. Compendium on Continuing Education for the Practicing Veterinarian 9, 945–955.

Gillis C, Sharkey N, Stover S, Pool RR, Meagher DM, Willits N (1995): Effect of maturation and aging on material and ultrasonographic properties of equine superficial digital flexor tendon. American Journal of Veterinary Research 56, 1345–1350.

Gillis C, Pool RR, Meagher DM, Stover SM, Reiser K, Willits N (1997): Effects of maturation and aging on the histomorphometric and biochemical characteristics of the equine superficial digital flexor tendon. American Journal of Veterinary Research 58, 425–430.

Goodship AE, Birch HL, Wilson AM (1994): The pathobiology and repair of tendon and ligament injury. Veterinary Clinics of North America: Equine Practice 10, 323–349.

Kajikawa Y, Morihara T, Sakamoto H, Matsuda KI, Oshima Y, Yoshida A, Nagae M, Arai Y, Kawata M, Kubo T (2008): Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. Journal Cellular Physiology 215, 837–845.

Kasashima Y, Takahashi T, Smith RK, Goodship AE, Kuwano A, Ueno T, Hirano S (2004): Prevalence of superficial digital flexor tendonitis and suspensory desmitis in Japanese Thoroughbred flat racehorses in 1999. Equine veterinary Journal 36, 346–350.

Lam KH, Parkin TD, Riggs CM, Morgan KL (2007): Descriptive analysis of retirement of Thoroughbred racehorses due to tendon injuries at the Hong Kong Jockey Club (1992–2004). Equine Veterinary Journal 39, 143–148.

Marr CM, Love S, Boyd JS, McKellar Q (1993): Factors affecting the clinical outcome of injuries to the superficial digital flexor tendon in National Hunt and point-to-point racehorses. Veterinary Record 132, 476–479.

Marx RE (2001): Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dentistry 10, 225–228.

Marx RE, Carlson ER, Eichstaedt RM (1998): Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surgery 85, 638–646.

McIlwraith CW (2002): Diseases of joints, tendons, ligaments, and related structures. In: Stashak TS (ed.): Adam's Lameness in Horses. 5th ed. Williams & Wilkins, Philadelphia. 459–644.

McLellan J (2011): Does it matter which platelet-rich plasma we use? Equine Veterinary Education 23, 101–104.

Mishra A, Pavelko T (2006): Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. American Journal of Sports Medicine 34, 1774–1778.

Patterson-Kane JC, Firth EC, Goodship AE, Parry DAD (1998): Effects of training on collagen fibril popula-

tions in the suspensory ligament and deep digital flexor tendon of young Thoroughbreds. American Journal of Veterinary Research 59, 64–68.

Pintucci G, Froum S, Pinnell J (2002): Trophic effects of platelets on endothelial cells are mediated by platelet-associated fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF). Thrombosis and Haemostasis 88, 834–842.

Rantanen NW, Jorgensen JS, Genovese RL (2003): Ultrasonographic evaluation of the equine limb: Technique. In: Ross MW, Dyson SJ (eds.): Diagnosis and Management of Lameness in the Horse. WB Saunders, Philadelphia. 166–188.

Reef VB (1998): Physics and instrumentation. In: Reef VB (ed.): Equine Diagnostic Ultrasound. WB Saunders, Philadelphia. 1–23.

Smith RKW, Schramme M (2003): Tendon injury in the horse: current theories and therapies. In Practice 25, 529–539.

Sutter WW, Kaneps AJ, Bertone AL (2004): Comparison of hematologic values and transforming growth factor-β and insulin-like growth factor concentrations in platelet concentrates obtained by use of buffy coat and apheresis methods from equine blood. American Journal of Veterinary Research 65, 924–930.

Textor J (2011): Autologous biologic treatment for equine musculoskeletal injuries: Platelet-rich plasma and IL-1 receptor antagonist protein. Veterinary Clinics of North America: Equine Practice 27, 275–298.

Waselau M, Sutter WW, Genovese RL (2008): Intralesional injection of platelet-rich plasma followed by controlled exercise for treatment of midbody suspensory ligament desmitis in standardbred racehorses. Journal of the American Veterinary Medical Association 232, 1515–1520.

Williams RB, Harkins LS, Hammond CJ, Wood LJN (2001): Racehorse injuries, clinical problems and fatalities recorded on British racecourses from flat racing and National Hunt racing during 1996, 1997, and 1998. Equine Veterinary Journal 33, 478–486.

Received: 2013–03–25
Accepted after corrections: 2013–04–09

Corresponding Author:

Kristina Zuffova, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, Brno, Czech Republic
Tel. +420 777 742 485, E-mail: zuffovak@vfu.cz