



## Evaluation of intra-articular injection of autologous platelet lysate (PL) in horses with osteoarthritis of the distal interphalangeal joint

Panagiota Tyrnenopoulou, Nikolaos Diakakis, Maria Karayannopoulou, Ioannis Savvas & Georgios Koliakos

To cite this article: Panagiota Tyrnenopoulou, Nikolaos Diakakis, Maria Karayannopoulou, Ioannis Savvas & Georgios Koliakos (2016) Evaluation of intra-articular injection of autologous platelet lysate (PL) in horses with osteoarthritis of the distal interphalangeal joint, Veterinary Quarterly, 36:2, 56-62, DOI: [10.1080/01652176.2016.1141257](https://doi.org/10.1080/01652176.2016.1141257)

To link to this article: <http://dx.doi.org/10.1080/01652176.2016.1141257>



Accepted author version posted online: 12 Jan 2016.  
Published online: 01 Feb 2016.



Submit your article to this journal [↗](#)



Article views: 67



View related articles [↗](#)



View Crossmark data [↗](#)

SHORT COMMUNICATION

## Evaluation of intra-articular injection of autologous platelet lysate (PL) in horses with osteoarthritis of the distal interphalangeal joint

Panagiota Tyrnenopoulou<sup>a</sup>, Nikolaos Diakakis<sup>a</sup>, Maria Karayannopoulou<sup>b</sup>, Ioannis Savvas<sup>c</sup> and Georgios Koliakos<sup>d</sup>

<sup>a</sup>Equine Unit, Companion Animal Clinic, Department of Clinical Studies, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece; <sup>b</sup>Surgery and Obstetrics Unit, Companion Animal Clinic, Department of Clinical Studies, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece; <sup>c</sup>Anaesthesiology and Intensive Care Unit, Companion Animal Clinic, Department of Clinical Studies, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece; <sup>d</sup>Department of Biochemistry, Medical School, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

### ABSTRACT

**Background:** Regenerative medicine has become one of the most promising therapies of equine osteoarthritis. Platelet lysate (PL) is rich in bioactive proteins and growth factors that play a crucial role in tissue healing.

**Objective:** To evaluate the efficacy of intra-articularly injected autologous PL in equine athletes with naturally occurring osteoarthritis.

**Animals and methods:** Fifteen warmblood geldings aged 8–19 years with osteoarthritis of the distal interphalangeal joint were included in this study. They were randomly divided into two groups; 10 horses received intra-articular injections of PL and 5 of normal saline (controls). Before treatment, platelet-derived growth factor (PDGF) levels in basal plasma and prepared PL were estimated. Each joint was injected twice within a three-week period. Lameness was evaluated using the American Association of Equine Practitioners grading system, before treatment and 10 days after each intra-articular injection. Horses were examined fortnightly for one year. Radiographic examination was performed six months post-treatment. The generalized estimating equation test was used for statistical analysis.

**Results:** Acceptable levels of PDGF were detected in PLs (mean  $\pm$  SD: 258.0  $\pm$  52.3 pg/ml). The majority of horses (9/10) responded positively to PL treatment presenting lower lameness grades ( $p < 0.0005$ ) compared to controls 10 days after the second injection, and returned to normal athletic activity. Radiographs revealed no changes in osteoarthritis lesions six months after treatment. One year post-injections, however, all horses relapsed to their initial degree of lameness.

**Conclusion:** Intra-articularly injected autologous PL is an efficient method for temporarily managing osteoarthritis of the distal interphalangeal joint in athletic horses.

### ARTICLE HISTORY

Received 23 May 2015  
Accepted 8 January 2016

### KEYWORDS

Horse; equine; autologous platelet lysate; platelet-derived growth factor; distal interphalangeal joint; osteoarthritis

## 1. Introduction

Degenerative joint disease or osteoarthritis represents one of the most common problems encountered in equine athletes, accounting a large proportion of equine orthopaedics (Pool 1996). It is commonly characterized by deterioration of articular cartilage, accompanied by changes in bone and soft tissues of the joint including subchondral bone sclerosis and osteophyte formation (McIlwraith & Vachon 1998).

Although a number of treatment strategies based mainly on the control of pain and inflammation, as well as on the inhibition of progressive erosion of articular cartilage, have been proposed focusing on the improvement of lameness (Goodrich & Nixon 2006; Monteiro et al. 2015), the need for further clinical proof of efficacy or new therapeutic approaches is currently evident.

During last decades, regenerative medicine has been considered as one of the most promising therapies of osteoarthritis (Filardo et al. 2011; Monteiro et al. 2015). Although the existence of growth factors has been well known since many years, the application of platelet-derived therapies in musculoskeletal injuries and especially osteoarthritis, in both human and veterinary medicine, has been popularized the last decades (Carmona et al. 2007; Sánchez et al. 2008; Kon et al. 2010; Nguyen et al. 2011; Textor 2011). Growth factors released mainly from platelet  $\alpha$ -granules are a group of biologically active polypeptides with anabolic and anti-catabolic effects that may regulate neo-chondrogenesis, as well as chondrocyte metabolism and differentiation, potentially improving cartilage repair (Van Den Berg et al. 2001; Sun et al. 2010; Fortier et al. 2011; Xie et al. 2014). In addition, Anitua et al. (2007)

found that platelet-released growth factors enhanced hyaluronic acid secretion and might be useful in joint homeostasis. Platelet lysate (PL), among other autologous platelet-derived preparations as sources of growth factors and cytokines, has been evaluated for its usefulness in bone and cartilage engineering, mainly in humans, *in vitro* and in clinical studies (Zaky et al. 2008; Pereira et al. 2013; Ruggiu et al. 2013; Zhu et al. 2013; Xie et al. 2014).

The purpose of this study was to evaluate the efficacy of an intra-articularly injected preparation of autologous PL in 10 athletic horses suffering from osteoarthritis of the distal interphalangeal joint and compare treatment with controls.

## 2. Materials and methods

### 2.1. Animals

In this study, all the applicable guidelines of the Hellenic State Veterinary Authorities on Animal Care and Use were followed. Fifteen horses with moderate to severe naturally occurring osteoarthritis of the distal interphalangeal joint of one forelimb were selected for this study from equine athletes with lameness. The selection was based on physical examination, perineural and intra-articular anaesthesia, radiographic and ultrasonographic examination. Specifically, the horses were evaluated at walk, trot in hand on a straight line and lunged on soft ground. Lameness was graded on a 0–5 scale according to the American Association of Equine Practitioners (AAEP) grading system (AAEP 1991). Full and distal limb flexion tests were also performed. Subsequently, perineural anaesthesia with mepivacaine hydrochloride was performed on the lame limb in order to localize the site of pain. Palmar digital nerve blocks followed by abaxial sesamoid nerve blocks were performed in all horses, with the limb bearing weight. One day after perineural anaesthesia, intra-articular injection of mepivacaine hydrochloride to the distal interphalangeal joint was performed to identify the source of lameness. In order to localize and assess the degree of joint damage, a radiographic examination of the foot was carried out including lateromedial views of the distal interphalangeal joint (Figure 1). Furthermore, ultrasonographic examination was performed to exclude any possible lesions in the collateral ligaments of the specific joint. Generally, diagnostic evaluation was performed independently by two veterinarians.

The 15 selected horses were randomly allocated into two groups; 10 horses received intra-articular injections of the PL protocol described below (PL group), whereas 5 horses received normal saline (control group).



**Figure 1.** Lateromedial view of a distal interphalangeal joint of a horse. Note the presence of osteophytes at the extensor process of the distal phalanx.

### 2.2. Preparation of platelet lysate (PL)

Fifty millilitre of whole blood was collected aseptically from the left jugular vein of each horse of the PL group, via an 18G needle, and was placed in vials containing about 6 ml of an anticoagulant solution (citrate phosphate dextrose). The samples were centrifuged, initially at  $270 \times g$  for 7 min and then at  $1000 \times g$  for 5 min, the supernatant plasma just above the buffy coat was separated carefully to avoid leukocyte aspiration, and a mechanical activation of platelets by a freeze-and-thaw process (frozen in  $-80^\circ\text{C}$  for 30 min and then thawed at room temperature) was performed. The final volume of PL obtained from each horse ranged from 3 to 7 ml.

Platelet and leukocyte (WBC) counts were performed in whole blood and in the supernatant plasma before activation, by an automated haematology analyzer (Advia 120, Siemens, Erlangen, Germany).

Aliquots of basal plasma and PL from each horse were also assayed in order to measure the AB isomer of platelet-derived growth factor (PDGF-AB) by using a specific enzyme-linked immunosorbent assay kit (Nor-iTM PDGF-AB ELISA Kit, Genorise Scientific, Glen Mills, PA, USA). The level of PDGF-AB was expressed in pg/ml.

### 2.3. Treatment protocol and post-treatment evaluation

In all cases of the PL group, 3 ml of PL preparation was injected into the distal interphalangeal joint in a routine sterile manner at the horse's premises, within 90 min after platelet activation. Control animals received

intra-articular injection of 3 ml of sterile normal saline. If it was necessary, horses were sedated using romifidine intravenously (40  $\mu\text{g}/\text{kg}$  of body weight), otherwise they were restrained with a lip twitch. Following PL or normal saline injection the limb was bandaged for two days, whereas the horse received box rest and was gradually put back to normal exercise levels within 5–10 days. Every horse received a second injection after a three-week interval. In all horses, lameness evaluation was performed 10 days after each intra-articular injection by using the AAEP grading system. Furthermore, horses were examined fortnightly for the subsequent year. In addition, radiographic examination was performed six months post-treatment.

### 3. Statistical analysis

For statistical analysis the generalized estimating equation test was used, with one between-subjects factor (group) and one within-subjects factor (time). Differences were considered significant at  $p \leq 0.05$ . For all the statistical calculations commercial software (SPSS; version 21.0, IBM-SPSS Science, Chicago, IL, USA) was used.

## 4. Results

### 4.1. Animals

All horses were warmblood geldings, aged 8–19 years (median 15 years) in the PL group and 9–18 years (median 14 years) in the control group. According to selection criteria, as it was revealed from clinical examination, plain radiography and ultrasonography, the 15 horses selected for this study suffered from osteoarthritis of one distal interphalangeal joint. More specifically, the grade of lameness ranged from 2 to 4 in both groups, whereas distal limb flexion tests were found positive in all horses.

### 4.2. Haematological and growth factor measurements

The mean platelet number  $\pm$  standard deviation (SD) in the supernatant plasma was  $338.7 \pm 76.2$  G/L (range: 224–458 G/L) compared to  $125.1 \pm 20.9$  G/L (range: 95–157 G/L) in blood equivalent to a twofold to threefold increase in platelet concentration in the supernatant plasmas before activation compared with whole blood. On the other hand, leukocyte counts revealed the presence of just a few leukocytes in the supernatant plasma ( $0.4 \pm 0.2$  G/L) ranging from 0.2–0.6 G/L, whereas the mean WBC number in blood was  $7.5 \pm 1.5$  G/L (range: 5.2–9.8 G/L).

The level of PDGF-AB in activated plasma (in PL) of each horse in the PL group ranged from 182.5 to

335.5 pg/ml (mean  $\pm$  SD:  $258.0 \pm 52.3$  pg/ml). No detectable levels of PDGF-AB were found in the basal plasma aliquots.

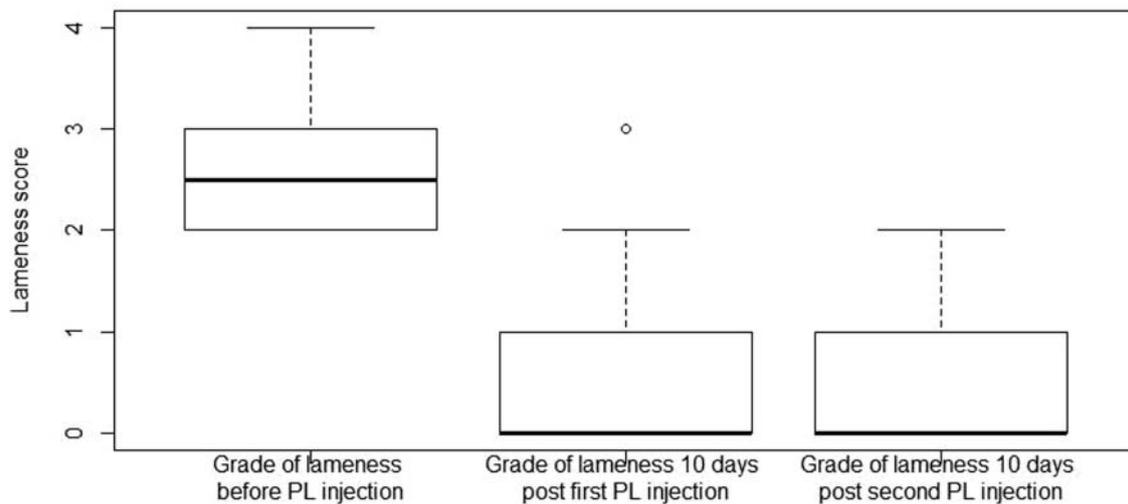
### 4.3. Clinical outcome

The majority of horses in the PL group responded positively to the aforementioned protocol with either elimination of lameness (7/10 horses) or a distinct improvement (2/10 horses), 10 days after the last PL injection. Only one horse showed a slight improvement. In total, 9 out of 10 horses returned to normal athletic activity. During the recovery period, no side-effects were reported in any horse. Radiographic examination six months post-treatment did not reveal any changes in osteoarthritis lesions. Lameness, however, started relapsing gradually from the seventh month post-treatment and at the end of the observation period (one year post-PL injections) all horses returned to their initial degree of lameness. In control group, no changes in the grade of lameness were detected after the intra-articular injections of normal saline.

The pooled median (minimum–maximum) lameness grade, for all measurement times, was 1 (0–4) in the PL group and 2 (2–4) in the control group. There was no significant difference in the pre-treatment grade between the two groups ( $p = 0.268$ ). The lameness grade, however, was lower 10 days after the first intra-articular injection, marginally not reaching statistical significance ( $p = 0.083$ ), but significantly lower ( $p < 0.0005$ ) 10 days after the second injection, in the PL group compared to control group. Within groups, the lameness grade after the first and second injections was significantly lower compared to the pre-treatment grade ( $p = 0.003$  and  $p = 0.001$ , respectively) only in the PL group (Figure 2).

## 5. Discussion

Last decades, there have been several experimental and clinical reports, mainly in humans, supporting the beneficial effect of platelet-rich preparations in the stimulation and acceleration of soft tissue healing and in bone and cartilage regeneration (Amable et al. 2013; Andia & Maffulli 2013; Zhu et al. 2013; Xie et al. 2014). PL is considered a perfect candidate for the management of osteoarthritis, mainly due to its ability to promote ex vivo expansion of mesenchymal stem cells (MSCs) used for bone and cartilage engineering (Zaky et al. 2008; Chevallier et al. 2010; Xia et al. 2011; Sofat & Kuttapitiya 2014; Tan et al. 2015). MSCs are found in multiple tissues including synovial joints and have shown the potential to differentiate into cells of several tissues including bone and cartilage (Sofat & Kuttapitiya 2014). The intra-articular injection of MSCs, autologous PL and dexamethasone in a human patient with



**Figure 2.** Changes in lameness grade of 10 horses (PL group) after the first and the second intra-articular injections of platelet lysate (PL). The dark horizontal lines represent median values and the boxes the 25th and 75th percentiles.

osteoarthritis of the knee resulted in a significant decrease in cartilage defect size (Centeno et al. 2008). Furthermore, in human patients with knee osteoarthritis, the use of intra-articular injections of various platelet-rich preparations as sources of growth factors resulted in significant clinical improvement and pain reduction up to 6 or 12 months post-treatment (Kon et al. 2010; Filardo et al. 2011). Similar results were reported when such treatment was compared with hyaluronal (Sánchez et al. 2008) or normal saline injections (Patel et al. 2013). In an adolescent soccer player with a large avulsion of the articular cartilage of the knee, arthroscopic treatment with an autologous preparation rich in growth factors accelerated cartilage healing and resulted in an excellent clinical outcome and rapid resumption in athletic activity (Sánchez et al. 2003).

As Monteiro et al. (2015) reported, laboratory animal and human studies have shown that platelet-released growth factors have the ability to stimulate chondrocyte proliferation and the synthesis of collagen and proteoglycans, which are major components of cartilage extracellular matrix and their content and composition loss is directly linked to osteoarthritis process (Pearle et al. 2005; Roughley 2006). In the catabolic and inflammatory joint environment of osteoarthritis, growth factors from platelets may diminish inflammatory effects on chondrocytes by inhibiting activation of inflammatory factors, such as the nuclear factor-kappa B (NF $\kappa$ B) (Imagawa et al. 2011; Van Buul et al. 2011; Xie et al. 2014; Monteiro et al. 2015). In cultures of synovial cells isolated from osteoarthritic patients, platelet-rich plasma (PRP) induced balance in angiogenesis and increase in cell proliferation and hyaluronic acid production, suggesting a chondroprotecting effect in case of intra-articular use (Anitua et al. 2007). Finally, pain control mechanisms have been attributed to autologous platelet products, as far as decreased markers

associated with pain have been found in human patients with osteoarthritis treated with PRP (Monteiro et al. 2015).

In equines, PL has also been used for MSCs expansion, as in humans (Seo et al. 2013). Autologous equine PL, however, evaluated *in vitro* among other cellular therapy products for facilitating healing of acute orthopaedic lesions, did not appear to stimulate the proliferation and migration of endogenous MSCs, but seemed rather to contribute to healing by the growth factors and matrix proteins that it contains (Kol et al. 2013). In an experimental study in horses with extensive loss of cartilage, treatment with chondrocyte grafts supplemented with insulin-like growth factor (IGF), a growth factor that is released from platelet-rich preparations, increased the production of type II collagen and improved gross filling of defects, perhaps due to IGF anabolic effect on chondrocytes (Fortier et al. 2002). In cultures of normal equine synovial membrane explants, platelet-rich preparations stimulated the release of anti-inflammatory proteins suggesting an anti-inflammatory and anabolic effect in equine osteoarthritic joints (Ríos et al. 2015). In another study, however, measurement of growth factor and cytokine concentrations in the synovial fluid of normal equine fetlock joints after intra-articular injection of a platelet-rich product, suggested minimal catabolic effect of cytokines on cartilage degradation but uncertain anabolic contribution of main growth factors (Textor et al. 2013).

In this study, the lack of progression of osteoarthritis lesions as revealed radiographically and perhaps the control of pain resulted in a significant lameness improvement with horses returning to athletic activity that lasted at least six months after treatment. Similarly, in four horses with osteoarthritis of different joints including the distal interphalangeal joint, intra-articular injections of autologous platelet concentrate induced

a significant improvement in lameness that persisted for 2–8 months over a one-year follow-up period (Carmona et al. 2007). Furthermore, the use of an autologous platelet concentrate in 20 athletic horses with refractory fetlock osteoarthritis gave better results; one year post-treatment the 80% of treated horses returned to work (Pichereau et al. 2014). Similarly, in horses with naturally occurring degenerative joint disease in fetlock joint, the combined use of PRP and MSCs significantly improved the functionality and sustainability of damaged joints up to 12 months post-treatment, maybe due to enhanced MSC proliferation and chondrogenic differentiation induced by PRP (Broeckx et al. 2014).

Among growth factors such as PDGF, transforming growth factor-beta (TGF- $\beta$ ) and IGF that are known to affect osteogenic cells, PDGF is considered the major growth factor in clotted blood and a potent mitogen for cells of mesenchymal origin, particularly of osteoblastic cells (Graves et al. 1989). Therefore, in order to ensure the quality of the injected PLs, we decided to determine the PDGF level in the activated plasmas. The PDGF, which is originated mainly from platelets but also from smooth muscle cells, fibroblasts, endothelial cells and macrophages, is a dimeric molecule that may exist as a homodimer (PDGF-AA or PDGF-BB) or a heterodimer (PDGF-AB) (Roughley 2006). Recent studies extrapolate evidence to support the significant role of PDGF as a potent regulator in cartilage repair; PDGF-AB was found to stimulate DNA synthesis in cells from all three regions of meniscus, in a dose-dependent manner (Schmidt et al. 2006). In our study, the increased level of PDGF-AB in the PLs was in concordance with the reduced lameness grades evaluated post-treatment. The fact that no PDGF-AB was detected in the basal plasma of horses, as in similar studies (Yoshioka et al. 2013), could be perhaps attributed to non-detectable quantities existed in non-activated plasma. The level of PDGF in the prepared PLs, however, was found within acceptable limits compared to studies where different activation methods of platelet-rich preparations were used (Torricelli et al. 2011).

Regarding the duration of clinical improvement, in this study, as revealed from lameness and radiographic evaluation, the majority of horses (9/10), which responded positively in the aforementioned treatment protocol, showed no adverse effects or relapses at least six months after the last intra-articular injection. Only one horse showed a slight lameness improvement and this outcome could be perhaps attributed to a higher degree of articular degeneration. It is important to bear in mind that, during the six-month period, the positively responded horses returned to their regular athletic activities with the majority of them maintaining high performance levels. As aforementioned, in a similar study in equine patients, the lameness improvement was most significant two months post-therapy

and persisted for eight months (Carmona et al. 2007). In most human studies, especially in young patients and in low-severity osteoarthritis, function improvement and control of pain were also stable for a short-term period (usually six months) (Kon et al. 2010; Patel et al. 2013; Monteiro et al. 2015). Since no side-effects were noticed in our study, one could postulate that repetitive treatments could only be to the best benefit of the equine patient. The rationale of using intra-articularly injected autologous blood products for managing equine osteoarthritis is further emphasized by the potential catabolic effects on articular cartilage of the commonly used corticosteroids (Goodrich & Nixon 2006).

## 6. Conclusion

This report suggests that, in spite of definite limitations such as the small number of animals, the non-blind evaluation of lameness post-treatment and the lack of synovial fluid analysis, the effectiveness of intra-articularly injected autologous PL in athletic horses with osteoarthritis of the distal interphalangeal joint is clinically significant. Moreover, horses in this study remained sound for a period of at least six months while being in training. This positive outcome suggests that the aforementioned treatment can be an efficient, minimally invasive and cost effective way to control the debilitating effects of equine joint disease.

## Acknowledgments

The work was supported by the Research Committee of Aristotle University of Thessaloniki under grant (no. 89652). The authors would like to acknowledge Serafeim Chaintoutis for his valuable contribution in ELISA measurements.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

Research Committee of Aristotle University of Thessaloniki [grant number 89652].

## References

- Amable PR, Caris RBV, Teixeira MVT, da Cruz Pacheco I, Corrêa do Amaral RJF, Granjeiro JM, Borojevic R. 2013. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. *Stem Cell Res Ther.* 4:67.
- American Association of Equine Practitioners. 1991. Guide for veterinary service and judging of equestrian events. 4th ed. Definition and classification of lameness. Lexington (KY): American Association of Equine Practitioners; p. 19.

- Andia I, Maffulli N. 2013. Platelet-rich plasma for managing pain and inflammation in osteoarthritis. *Nat Rev Rheumatol.* 9:721–730.
- Anitua E, Sánchez M, Nurden AT, Zalduendo MM, de la Fuente M, Azofra J, Andia I. 2007. Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. *Rheumatology.* 46:1769–1772.
- Broeckx S, Zimmerman M, Crosetti S, Suls M, Mariën T, Ferguson SJ, Chiers K, Duchateau L, Franco-Obregón A, Wuertz K, et al. 2014. Regenerative therapies for equine degenerative joint disease: a preliminary study. *PLoS One.* 9:e85917. doi:10.1371/journal.pone.0085917
- Carmona JU, Argüelles D, Climent F, Prades M. 2007. Autologous platelet concentrates as a treatment of horses with osteoarthritis: a preliminary pilot clinical study. *J Equine Vet Sci.* 27:167–170.
- Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M. 2008. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells, platelet lysate and dexamethasone. *Am J Case Rep.* 9:201–206.
- Chevallier N, Anagnostou F, Zilber S, Bodivit G, Maurin S, Barault A, Bierling P, Hernigou P, Layrolle P, Rouard H. 2010. Osteoblastic differentiation of human mesenchymal stem cells with platelet lysate. *Biomaterials.* 31:270–278.
- Filardo G, Kon E, Buda R, Timoncini A, Di Martino A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. 2011. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. *Knee Surg Sports Traumatol Arthrosc.* 19:528–535.
- Fortier LA, Mohammed HO, Lust G, Nixon AJ. 2002. Insulin-like growth factor-1 enhances cell-based repair of articular cartilage. *J Bone Joint Surg Br.* 84:276–288.
- Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. 2011. The role of growth factors in cartilage repair. *Clin Orthop Relat Res.* 469:2706–2715.
- Goodrich LR, Nixon AJ. 2006. Medical treatment of osteoarthritis in the horse – a review. *Vet J.* 171:51–69.
- Graves DT, Valentin-Opran A, Delgado R, Valente AJ, Mundy G, Piche J. 1989. The potential role of platelet-derived growth factor as an autocrine or paracrine factor for human bone cells. *Connect Tissue Res.* 23:209–218.
- Imagawa K, De Andrés MC, Hashimoto K, Pitt D, Itoi E, Goldring MB, Roach HI, Oreffo ROC. 2011. The epigenetic effect of glucosamine and a nuclear factor-kappa B (NF- $\kappa$ B) inhibitor on primary human chondrocytes – implications for osteoarthritis. *Biochem Biophys Res Commun.* 405:362–367.
- Kol A, Walker NJ, Galuppo LD, Clark KC, Buerchler S, Bernanke A, Borjesson DL. 2013. Autologous point-of-care cellular therapies variably induce equine mesenchymal stem cell migration, proliferation and cytokine expression. *Equine Vet J.* 45:193–198.
- Kon E, Buda R, Filardo G, Di Martino A, Timoncini A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. 2010. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. *Knee Surg Sport Traumatol Arthrosc.* 18:472–479.
- McIlwraith CW, Vachon A. 1998. Review of pathogenesis and treatment of degenerative joint disease. *Equine Vet J.* 20:3–11.
- Monteiro SO, Bettencourt EV, Lepage OM. 2015. Biologic strategies for intra-articular treatment and cartilage repair. *J Equine Vet Sci.* 35:175–190.
- Nguyen RT, Borg-Stein J, McInnis K. 2011. Applications of platelet-rich plasma in musculoskeletal and sports medicine: an evidence-based approach. *PM&R.* 3:226–250. doi:10.1016/j.pmrj.2010.11.007
- Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. 2013. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med.* 41:356–364.
- Pearle AD, Warren RF, Rodeo SA. 2005. Basic science of articular cartilage and osteoarthritis. *Clin Sports Med.* 24:1–12.
- Pereira RC, Scaranari M, Benelli R, Strada P, Reis RL, Cancedda R, Gentili C. 2013. Dual effect of platelet lysate on human articular cartilage: a maintenance of chondrogenic potential and a transient proinflammatory activity followed by an inflammation resolution. *Tissue Eng Part A.* 19:1476–1488.
- Pichereau F, Décory M, Ramos GC. 2014. Autologous platelet concentrate as a treatment for horses with refractory fetlock osteoarthritis. *J Equine Vet Sci.* 34:489–493.
- Pool RR. 1996. Pathologic manifestations of joint disease in the athletic horse. In: McIlwraith CW, Trotter GW, editors. *Joint disease in the horse.* Philadelphia (PA): Saunders; p. 40–70.
- Ríos DL, López C, Carmona JU. 2015. Platelet-rich gel supernatants stimulate the release of anti-inflammatory proteins on culture media of normal equine synovial membrane explants. *Vet Med Int.* 2015:547052. Available from: <http://dx.doi.org/10.1155/2015/547052>
- Roughley PJ. 2006. The structure and function of cartilage proteoglycans. *Eur Cell Mater.* 12:92–101.
- Ruggiu A, Ulivi V, Sanguineti F, Cancedda R, Descalzi F. 2013. The effect of platelet lysate on osteoblast proliferation associated with a transient increase of the inflammatory response in bone regeneration. *Biomaterials.* 34:9318–9330.
- Sánchez M, Azofra J, Anitua E, Andia I, Padilla S, Santisteban J, Mujika I. 2003. Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. *Med Sci Sports Exercise.* 35:1648–1652.
- Sánchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. 2008. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee AO: a retrospective cohort study. *Clin Exp Rheumatol.* 26:910–913.
- Schmidt MB, Chen EH, Lynch SE. 2006. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. *Osteoarthritis Cartilage.* 14:403–412.
- Seo J, Tsuzuki N, Haneda S, Yamada K, Furuoka H, Tabata Y, Sasaki N. 2013. Comparison of allogeneic platelet lysate and fetal bovine serum for *in vitro* expansion of equine bone marrow-derived mesenchymal stem cells. *Res Vet Sci.* 95:693–698.
- Sofat N, Kuttapitiya A. 2014. Future directions for the management of pain in osteoarthritis. *Int J Clin Rheumatol.* 9:197–216.
- Sun Y, Feng Y, Zhang CQ, Chen SB, Cheng XG. 2010. The regenerative effect of platelet-rich plasma on healing in large osteochondral defects. *Int Orthop.* 34:589–597.
- Tan HB, Giannoudis PV, Boxall SA, McGonagle D, Jones E. 2015. The systemic influence of platelet-derived growth factors on bone marrow mesenchymal stem cells in fracture patients. *BMC Med.* 13:6.
- Textor J. 2011. Autologous biologic treatment for equine musculoskeletal injuries: platelet-rich plasma and IL-1 receptor antagonist protein. *Vet Clin North Am Equine Pract.* 27:275–298.

- Textor JA, Willits NH, Tablin F. 2013. Synovial fluid growth factor and cytokine concentrations after intra-articular injection of a platelet-rich product in horses. *Vet J.* 198:217–223.
- Toricelli P, Fini M, Filardo G, Tschon M, Pischedda M, Pacorini A, Kon E, Giardino R. 2011. Regenerative medicine for the treatment of musculoskeletal overuse injuries in competition horses. *Int Orthop.* 35:1569–1576.
- Van Buul GM, Koevoet WLM, Kops N, Bos PK, Verhaar JAN, Weinans H, Bernsen MR, van Osch GJVM. 2011. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. *Am J Sports Med.* 39:2362–2370.
- Van Den Berg WB, Van Der Kraan PM, Scharstuhl A, Van Beuningen HM. 2001. Growth factors and cartilage repair. *Clin Orthop Relat Res.* 391:244–250.
- Xia W, Li H, Wang Z, Xu R, Fu Y, Zhang X, Ye X, Huang Y, Xiang AP, Yu W. 2011. Human platelet lysate supports ex vivo expansion and enhances osteogenic differentiation of human bone marrow–derived mesenchymal stem cells. *Cell Biol Int.* 35:639–643.
- Xie X, Zhang C, Tuan R. 2014. Biology of platelet-rich plasma and its clinical application in cartilage repair. *Arthrit Res Ther.* 16:204.
- Yoshioka T, Kanamori A, Washio T, Aoto K, Uemura K, Sakane M, Ochiai N. 2013. The effects of plasma rich in growth factors (PRGF-Endoret) on healing of medial collateral ligament of the knee. *Knee Surg Sports Traumatol Arthrosc.* 21:1763–1769. doi:10.1007/s00167-012-2002-x
- Zaky SH, Ottonello A, Strada P, Cancedda R, Mastrogiacomo M. 2008. Platelet lysate favours in vitro expansion of human bone marrow stromal cells for bone and cartilage engineering. *J Tissue Eng Regen Med.* 2:472–481.
- Zhu Y, Yuan M, Meng HY, Wang AY, Guo QY, Wang Y, Peng J. 2013. Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: A review. *Osteoarthr Cartilage.* 21:1627–1637.