aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 10 (9): 500-508, 2015
ISSN 1683-9919 / DOI: 10.3923/ajava.2015.500.508

Regenerative Therapy for Equine Osteoarthritis: A Concise Review

Ashraf M. Abu-Seida
Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University, Giza, P.O. Box 12211, Egypt

ABSTRACT

Osteoarthritis (OA) is a common cause of lameness in equine and a potential cause of wastage of valuable animals. Furthermore, horse is the ideal large animal model for the preclinical study of cell-based therapy in human joints. In contrast to the drug therapy, regenerative therapy promotes the body's own healing, restoring the structural architecture and biomechanical function of the injured tissue. Therefore, regenerative therapy field in veterinary medicine continues to evolve rapidly both experimentally and clinically. As the field of regenerative therapy continues to advance, equine practitioners need contemporary information regarding the choice of regenerative biologic type and recommendations regarding clinical implementation of regenerative therapies. Meanwhile, clinicians must also be aware of the limitation in the available knowledge regarding regenerative therapy and the impending regulatory laws that may limit its use in clinical joint diseases in equine. Although, preliminary data generated from clinical trials in human patients and experimental studies in equine osteoarthritis are encouraging, preliminary data have been published about very limited clinical application of regenerative therapies in horses suffering from clinical joint disease and the commercialization of these treatments may be premature. Additional studies are needed to determine the optimum conditions for harvest, culture and expansion of these biologics, appropriate dosing, optimal delivery method, short and long term safety. This review describes the three main biologics used for regenerative therapy, namely; stem cells, Platelet Rich Plasma (PRP) and Autologous Conditioned Serum (ACS) and draws together research findings from *in vitro* and *in vivo* studies to give an overview of current regenerative therapies for treatment of osteoarthritis in equine.

Key words: Autologous conditioned serum, horse, osteoarthritis, platelet rich plasma, regenerative therapy, stem cells

INTRODUCTION

Osteoarthritis (OA) is defined as a progressive and permanent destruction of articular cartilage resulting in loss of function and associated changes in the bones and soft tissues of the joint (Pool, 1996). Several predisposing factors of OA, such as repetitive trauma (Senna *et al*., 2015) and synovitis from various causes, such as osteochondrosis (Mostafa *et al*., 2014a) and joint infection (Mostafa *et al*., 2014b) were reported.

Osteoarthritis is a common cause of lameness in equine resulting severe economic losses. Furthermore, horse is the ideal large animal model for the preclinical study of cell based therapy in human joints. The advantage of this animal is the size of the joints, which allows feasible surgical applications in analogy to human surgery. Moreover, the horse is the only animal model with a cartilage thickness in the knee joint comparable to that of humans. Additionally, horses

develop clinical joint disease discerns this species from other large animal models. The therapy of clinical disease in animal model represents the ideal method for preclinical studies of novel therapeutic strategies (Brehm *et al*., 2014).

The articular cartilage is a highly differentiated structure frequently injured and has a limited reparatory potential due to lack of organization, innervation and lymphatic vessels which result in reduced inflow of blood progenitor cells (Luyten, 2004). Therefore, in cartilagenous injuries the formed repair tissue is mainly consisted of fibrocartilage that lacks the functional and morphological recovery of the tissue resulting in progression of osteoarthritis (Yamada *et al*., 2013). Periosteal arthroplasty, perichondral arthroplasty, autologous osteochondral transplantation, autologous chondrocyte transplantation, autologous cancellous grafts and tendons autografts are frequently used to form a new chondral surface but results from animal models reveal inefficiency of these techniques (Fraser *et al*., 2006; Schaffler and Buchler, 2007).

In last decade, regenerative therapy has gained momentum in veterinary medicine because it promotes the body's own healing, restoring the structural architecture and biomechanical function of the injured tissue. Moreover, small and large animal species serve as valuable models for preclinical studies of regenerative therapies in human beings and in veterinary patients (Fortier and Travis, 2011).

In contrast to drug therapy, regenerative therapy do not rely on a single target receptor or pathway for its action. Therefore, the outcome of using the conventional methods for treatment of osteoarthritis is a newly formed weakened tissue, which does not respond to the animal's locomotor needs and is more prone to re-injury. Recently novel therapies aim to enhance healthy tissue regeneration resulting in a better performance and reduction in recurrence rates as compared with those obtained after the conventional methods of treatment.

Regenerative therapy is a broad term including the use of many biologics. In the equine field, there are currently several emerging regenerative therapies, such as stem cells (Csaki *et al*., 2007; Frisbie and Stewart, 2011), Platelet Rich Plasma (PRP) (Pichereau *et al*., 2014; Rios *et al*., 2015a), Autologous Conditioned Serum (ACS) (Fortier *et al*., 2010; Fortier, 2012) and Autologous Protein Solution (APS) (Bertone *et al*., 2014). This review describes the current regenerative therapies for treatment of osteoarthritis in equine as follow:

STEM CELLS

In last decade, stem cells research has attracted the attention of the biomedical research community due to its therapeutic potential in several incurable diseases. In equine practice, stem cells are commonly used to treat musculoskeletal disorders including tendonitis, osteoarthritis and more recently laminitis. The literature is full of studies on experimental animal models and sporadic clinical reports in which stem cell therapy results in promising outcomes, MSCs have not reached the mainstream clinical practice. Undoubtedly, stem cells are promising for regenerative therapies that are currently studied in pre-clinical trials for osteoarthritis in equine.

Stem cells are unspecialized cells having the ability for self-renewal, extensive proliferation and differentiation into one or more cell/tissue types. The self renewal of stem cells is a unique unlimited ability to make identical copy of themselves while differentiation potential is the ability to produce tissue specific cells. These abilities rank the stem cells therapy as the most important regenerative therapeutics.

Stem cells can be classified broadly according to their source as Embryonic Stem Cells (ESC), adult or tissue specific stem cells (ADC) or induced Pluripotent Stem Cells (iPSCs). Adult stem cells are obtained from adult body organs whereas the embryonic stem cells are drawn from embryos (Amarpal *et al*., 2013).

Adult Stem Cells (ASC) are found in various tissues of the body and they can be of endodermal, mesodermal and ectodermal origins giving rise to many types of stem cells. These cells include hematopoeitic stem cells, mesenchymal stem cells, neural stem cells, skin stem cells, retinal stem cells, etc (Hui *et al*., 2011). The embryonic stem cells are pluripotent having the ability to generate all tissue types in mammalians (He *et al*., 2009).

Therapeutic applications using adult stem cells are promising for treating joint and bone diseases (Hui *et al*., 2011). Both local or systemic applications are required for stem cells delivery into the diseased animals for stem cell therapy. Mesenchymal Stem Cells (MSCs) have been commonly used in experimental, preclinical and clinical trials (Lee, 2008). Mesenchymal stem cells have been most widely isolated from fat, bone marrow, umbilical cord, dental pulp, Warton's jelly, amniotic fluid, skeletal muscles, tendons and synovial membrane (Crisan *et al*., 2008; Gade *et al*., 2012; Pratheesh *et al*., 2013; Tawfik *et al*., 2013; Nagy *et al*., 2014). In addition, recent studies documented that MSCs have the ability to differentiate into cells of ectodermal origin as neurons and endodermal origin as hepatocytes as well as producing cytokines and growth factors promoting cell expansion and differentiation (Violini *et al*., 2009). The MSCs can be expanded without differentiation up to 40 generations and have the ability to differentiate into the cells of mesodermal origin as chondrocytes, osteoblasts, adipocytes, visceral stromal cells, skeletal myocytes and tenocytes (Barry and Murphy, 2004). Moreover, MSCs may be immune-privileged cells which avoid allogenic rejection (Jung *et al*., 2005). Owing to these properties, stem cell therapy has obtained enormous popularity in last decade (Guillot *et al*., 2007; Brennand and Cage, 2011).

Growth factors may enhance the cartilage repair techniques through several mechanisms including, chemotaxis (recruitment of chondrogenic cells), mitogenesis (stimulation of chondrogenic cell proliferation) and enhancement of cartilage matrix synthesis. The effect of Insulin-like Growth Factor (IGF) and Platelet Derived Growth Factor (PDGF) are studied. The IGF plays an important role in cartilage homeostasis, balancing proteoglycan synthesis and breakdown. Incorporating IGF into a fibrin clot placed in an equine cartilage defect improved both quality and quantity of repair tissue and decreased synovial inflammation. The PDGF is a potent mitogenic and chemotactic factor for all cells of mesenchymal origin, including chondrocytes and mesenchymal stem cells. Combining tissue growth factors with a biological matrix can provide a physical scaffold for cell adhesion and growth as well as controls the release of these potent molecules. This could result in biological devices that enhance the predictability and quality of current cartilage repair techniques (Schmidt *et al*., 2006).

Adipose Derived-Mesenchymal Stem Cells (AD-MSCs) are preferred than Bone-Marrow Mesenchymal Stem Cells (BM-MSCs) due to easy and repeatable access to subcutaneous adipose tissue, easy isolation, little pain, easy cryopreservation and approximately 500-fold greater numbers of fresh MSCs (Schaffler and Buchler, 2007). However, the bone marrow was vastly superior to adipose tissue as a source of MSCs with osteoblastogenic potential (Ahern *et al*., 2011).

In addition, the activation of AD-MSCs has been a big obstacle in this field. Laser photostimulation is a cheap and safe method for stem cells activation when compared with activation by growth factor and cytokines. In horses, adipose and BM-derived MSCs were capable of producing lipids, glycosaminoglycan and mineral.

The stem cells opened a new area for treatment of osteoarthritis in equine. The goal of using MSCs as a therapy in joint disease is to harness the regenerative nature of these cells focusing on their potential to grow new tissues to replace damaged one (Taylor *et al*., 2007).

There are 3 basic mechanisms explaining the reparative effect of MSCs including creation of a milieu by secreting cytokines that enhance regeneration of endogenous cells, trans-differentiation into the cells of host tissue and fusion with the host cells (Amarpal *et al*., 2013).

Autologous adipose derived mesenchymal stem cells (ADMSCs) have been used to treat joint diseases in horses on a commercial basis since 2003. The ADMSCs are considered ideal stem cells for the regenerative medicinal applications. They are found in abundant quantities, harvested with a minimally invasive procedure, can be differentiated along multiple cell lineage pathways in a regulatable and reproducible manner and can be effectively and safely transplanted to either an autologous or allogenic host. Moreover, there are no moral or ethical issues in harvesting autologous adipose tissue (Black *et al*., 2008; Alderman and Alexander, 2011).

Equine joint diseases have been treated with a non-expanded adipose derived autologous preparation called Stromal Vascular Fraction (SVF). This fat derived mononuclear cell fraction contains MSCs, T regulatory cells, endothelial precursor cells, macrophages and pre adipocytes. Adipose tissue derived cells such as those found in the SVF preparation do not require the 3-4 weeks to expand MSCs, or the laboratory costs and expertise needed to avoid contamination during ex *vivo* expansion (Ricco *et al*., 2012). A greater improvement was reported in experimentally induced osteoarthritis treated with bone marrow derived mesenchymal stem cells when compared to adipose derived stromal vascular fraction (Frisbie *et al*., 2009).

In vitro, equine Mesenchymal Stem Cells (eMSCs) from Bone Marrow (BM) possessed the highest osteogenic potential; eMSCs from adipose tissue also had robust osteogenic potential. The tuber coxae and the sternum were good sources of BM-eMSCs in yearlings and 60 mL of BMaspirate was sufficient for culture and expansion (Toupadakis *et al*., 2010). Recently, Adams *et al*. (2013) reported that the first 5 mL aspirates from the sternum and ilium offer a rich source of bone marrow derived MSCs with similar growth rate properties. Additionally, expanding BM-eMSCs in Autologus Serum (AS) to avoid possible immunologic reactions decreased the total yield because BM-eMSCs grew significantly slower in AS than in fetal bovine serum (Toupadakis *et al*., 2010).

Chondrogenesis was highest for MSCs obtained from ilial aspirates, although it is not known whether chondrogenesis is indicative of activation of other proposed pathways by which MSCs heal tissues (Kisiday *et al*., 2013). The accurate placement of a Jamshidi needle into the medullary cavity of the 4-6th individual sternebrae facilitated by the use of ultrasonography is an optimised safe technique for obtaining bone marrow derived MSCs from the sternum of the Thoroughbred horses (Kasashima *et al*., 2011).

In an equine model, delivery of bone marrow concentrate to cartilage defects has the clinical potential to improve cartilage healing, providing a simple, one-step, autogenous, cost-effective, arthroscopically applicable and clinically effective approach for cartilage repair (Fortier *et al*., 2010).

Recently, genetically engineered MSCs can act as vehicles to deliver gene products into the joint. Intra-articular MSCs injection resulted in a moderate acute inflammatory joint response that was greater for allogeneic and xenogeneic MSCs than autologous MSCs. Clinical management of this response may minimize this effect (Pigott *et al*., 2013; Remacha *et al*., 2015). Tissue engineering is proposed as the future direction of stem cell based therapy for osteoarthritis (Whitworth and Banks, 2014).

PLATELET RICH PLASMA

Notably, PRP could be considered as one of the most worldwide clinical regenerative therapies used in equine. However, there is little information on the basic mechanisms by which PRP relieves

pain and improves the joint function. Platelet Rich Plasma (PRP) preparations are a common treatment in osteoarthritis (OA) and synovitis. However, there is ambiguity concerning the ideal concentration of leukocytes and platelets in these preparations necessary to induce an effective anti-inflammatory and anabolic response in joint tissues.

Platelets are a rich source of growth factors, cytokines and chemokines, all of which are released during the early stages of reparative processes of tissue healing. Platelet Rich Plasma (PRP) is derived from centrifugation of whole blood after red blood cells and the buffy coat have been separated from the plasma, which is rich in platelets. The PRP enhances physiological processes of clotting and wound repair, thereby stimulating intrinsic tissue regeneration in which endogenous platelets are a vital component.

The PRP stimulate angiogenesis, proliferation and migration of fibroblasts, collagen synthesis and chemotaxis of macrophages, which are necessary for tissue healing. A series of growth factors participate in healing including, IGF-I and IGF-II, TGF-β1, FGF, VEGF, PDGF and Platelet Derived Epidermal Growth Factor (PDEGF) are released from the degranulation of alpha granules in the platelet cytoplasm following activation (Ricco *et al*., 2012).

Not limited to stem cells, laser photoactivation is better than the traditional methods ($CaCl_2$ or thrombin) which activate only the platelet derived growth factors.

Some reports indicate the beneficial effect of PRP in horses with naturally occurring OA horses (Carmona *et al*., 2007; Pichereau *et al*., 2014). Recently, the anti-inflammatory and anabolic joint responses of PRP depend on the leukocyte and platelet concentration of the PRP preparation and on the volume of this substance injected. Moreover, it is possible, that leukoreduced PRP preparations are more effective for the medical treatment of patients with OA and inflammatory synovitis (Rios *et al*., 2015b).

The PRP is generally administered either as a sole agent or alternatively with other regenerative therapies. Injection of PRP with ADMSCs helped in proliferation and differentiation of stem cells into chondrocytes during treatment of osteoarthritis (Van Pham *et al*., 2013).

The PRP provides a scaffold and growth factor concentrate that enhance the cellular repair of musculoskeletal lesions. The main advantages of PRP as a regenerative therapy are its autologous nature, rapid preparation and non invasive collection process (Alderman and Alexander, 2011; Textor, 2011).

AUTOLOGOUS CONDITIONED SERUM (INTERLEUKIN RECEPTOR ANTAGONIST PROTEIN)

Autologous Conditioned Serum (ACS), known commercially as Interleukin Receptor Antagonist Protein (IRAP®) is a natural anti-inflammatory product used for treatment of many joint injuries. The ACS is produced from the horse's own blood and injected into the affected joint to treat inflammation and lameness and to help joint healing.

Osteoarthritis is characterized by local release of cytokines triggering the destruction of hyaline cartilage and its matrix. This leads to additional release of intracellular pro-inflammatory and destructive factors that promote further destruction of hyaline cartilage by causing an imbalance in matrix turnover (Wehling *et al*., 2007).

Interleukin-1 (IL-1) is the major mediator of joint disease, play an important role in degenerative musculuskeletal diseases including osteoarthritis. Agents that inhibit the action of such cytokines have a high therapeutic potential in osteoarthritis (Meijer *et al*., 2003).

One hypothesis is that the local IL-1 receptor antagonist (IL-1ra) concentration is too low in degenerative diseases to inhibit the destruction of cartilage and other joint structures. But to date,

it is not clear that a complete blockage of all biologically active IL-1 receptors is necessary to significantly impact such pathologic conditions such as osteoarthritis or nerve inflammation. It is now evident that several natural and recombinant additional anti-inflammatory cytokines and soluble receptors which display differential dissociation rates for IL-1α, IL-1β and IL-1ra can affect IL-1 receptor signaling and inflammatory conditions (Wehling *et al*., 2007).

The use of natural antagonist (Irap®) (aka autologous conditioned serum, ACS) to block IL-1 activity and decrease the progression of joint disease was evaluated (Wehling *et al*., 2007). The injection of ACS into affected tissues has shown clinical effectiveness and safety for treatment of synovitis, capsulitis and mild to moderate osteoarthritis in animal models (Ricco *et al*., 2012).

CONCLUSION

In conclusion, regenerative therapy is a promising future for treatment of osteoarthritis in equine practice and the research should continue in this subject in order to determine the efficacy, short-term and long-term safety, new techniques and regimens of such type of therapy in clinical cases of osteoarthritis in equine.

REFERENCES

Adams, M.K., L.R. Goodrich, S. Rao, F. Olea-Popelka, N. Phillips, J.D. Kisiday and C.W. McIlwraith, 2013. Equine Bone Marrow-Derived Mesenchymal Stromal Cells (BMDMSCs) from the ilium and sternum: Are there differences? Equine Vet. J., 45: 372-375.

Ahern, B.J., T.P. Schaer, S.P. Terkhorn, K.V. Jackson, N.J. Mason and K.D. Hankenson, 2011. Evaluation of equine peripheral blood apheresis product, bone marrow and adipose tissue as sources of mesenchymal stem cells and their differentation potential. Am. J. Vet. Res., 72: 127-133.

Alderman, D. and R.W. Alexander, 2011. Advances in stem cell therapy: Application to veterinary medicine. Today's Vet. Pract., 1: 23-29.

Amarpal, K. Dhama, S. Chakraborty, R. Tiwari and S. Natesan, 2013. Stem cells and their clinical/therapeutic applications in biomedical and veterinary science-the perspectives. Res. Opin. Anim. Vet. Sci., 3: 261-279.

Barry, F.P. and J.M. Murphy, 2004. Mesenchymal stem cells: Clinical applications and biological characterization. Int. J. Biochem. Cell Biol., 36: 568-584.

Bertone, A.L., A. Ishihara, L.J. Zekas, M.L. Wellman and K.B. Lewis *et al*., 2014. Evaluation of a single intra-articular injection of autologous protein solution for treatment of osteoarthritis in horses. Am. J. Vet. Res., 75: 141-151.

Black, L.L., J. Gaynor, C. Adams, S. Dhupa and A.E. Sams *et al*., 2008. Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. Vet. Therapeut., 9: 192-200.

Brehm, W., J. Burk and U. Delling, 2014. Application of stem cells for the treatment of joint disease in horses. Methods Mol. Biol., 1213: 215-228.

Brennand, K.J. and F.H. Gage, 2011. Concise review: The promise of hiPSC-based studies of Schizophrenia. Stem Cells, 29: 1915-1922.

Carmona, J.U., D. Arguelles, F. Climent and M. Prades, 2007. Autologous platelet concentrates as a treatment of horses with osteoarthritis: A preliminary pilot clinical study. J. Equine Vet. Sci., 27: 167-170.

Crisan, M., S. Yap, L. Casteilla, C.W. Chen and M. Corselli *et al*., 2008. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cells, 3: 301-311.

Csaki, C., U. Matis, A. Mobasheri, H. Ye and M. Shakibaei, 2007. Chondrogenesis, osteogenesis and adipogenesis of canine mesenchymal stem cells: A biochemical, morphological and ultrastructural study. Histochm. Cell Biol., 128: 507-520.

Fortier, L.A. and A.J. Travis, 2011. Stem cells in veterinary medicine. Stem Cell Res. Ther., Vol. 2. 10.1186/scrt50

Fortier, L.A., 2012. Making progress in the what, when and where of regenerative medicine for our equine patients. Equine Vet. J., 44: 511-512.

Fortier, L.A., H.G. Potter, E.J. Rickey, L.V. Schnabel and L.F. Foo *et al*., 2010. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. J. Bone Joint Surg., 92: 1927-1937.

Fraser, J.K., I. Wulur, Z. Alfonso and M.H. Hedrick, 2006. Fat tissue: An underappreciated source of stem cells for biotechnology. Trends Biotechnol., 24: 150-154.

Frisbie, D.D. and M.C. Stewart, 2011. Cell-based therapies for equine joint disease. Vet. Clin. North Am.-Equine Pract., 27: 335-349.

Frisbie, D.D., J.D. Kisiday, C.E. Kawcak, N.M. Werpy and C.W. McIlwraith, 2009. Evaluation of adipose-derived stromal vascular fraction or bone marrow-derived mesenchymal stem cells for treatment of osteoarthritis. J. Orthop. Res., 27: 1675-1680.

Gade, N.E., M.D. Pratheesh, A. Nath, P.K. Dubey, Amarpal and G.T. Sharma, 2012. Therapeutic potential of stem cells in veterinary practice. Vet. World, 5: 499-507.

Guillot, P.V., W. Cui, N.M. Fisk and D.J. Polak, 2007. Stem cell differentiation and expansion for clinical applications of tissue engineering. J. Cell. Mol. Med., 11: 935-944.

He, S., D. Nakada and S. Morrison, 2009. Mechanisms of stem cell self-renewal. Annu. Rev. Cell Dev. Biol., 25: 377-406.

Hui, H., Y. Tang, M. Hu and X. Zhao, 2011. Stem Cells: General features and Characteristics. In: Stem Cells in Clinic and Research, Gholamrezanezhad, A. (Ed.). Chapter 1, InTech Publisher, Croatia, ISBN: 978-953-307-797-0, pp: 3-20.

Jung, J.W., I.T. Hwang, J.E. Park, E.H. Lee, K.H. Ryu, S.H. Kim and J.S. Hwang, 2005. Mutation analysis of the MCM gene in Korean patients with MMA. Mol. Genet. Metab., 84: 367-370.

Kasashima, Y., T. Ueno, A. Tomita, A.E. Goodship and R.K.W. Smith, 2011. Optimisation of bone marrow aspiration from the equine sternum for the safe recovery of mesenchymal stem cells. Equine Vet. J., 43: 288-294.

Kisiday, J.D., L.R. Goodrich, C.W. McIlwraith and D.D. Frisbie, 2013. Effects of equine bone marrow aspirate volume on isolation, proliferation and differentiation potential of mesenchymal stem cells. Am. J. Vet. Res., 74: 801-807.

Lee, K.D., 2008. Applications of mesenchymal stem cells: An updated review. Chang Gung Med. J., 31: 228-236.

Luyten, F.P., 2004. Mesenchymal stem cells in osteoarthritis. Curr. Opin. Rheumatol., 16: 599-603.

Meijer, H., J. Reinecke, C. Becker, G. Tholen and P. Wehling, 2003. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. Inflammation Res., 52: 404-407.

Mostafa, M.B., A.M. Abu-Seida and A.I. Abd El-Glil, 2014a. Radiographical, ultrasonographic and arthroscopic findings of osteochondrosis dissecans of the tarsocrural joint in horses. Res. Opin. Anim. Vet. Sci., 4: 318-322.

Mostafa, M.B., A.M. Abu-Seida and A.I. Abd El-Glil, 2014b. Septic tarsitis in horses: Clinical, radiological, ultrasonographic, arthroscopic and bacteriological findings. Res. Opin. Anim. Vet. Sci., 4: 30-34.

Nagy, M.M., H.E. Tawfik, A.A.R. Hashem and A.M. Abu-Seida, 2014. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. J. Endodontics, 40: 192-198.

Pichereau, F., M. Decory and G.C. Ramos, 2014. Autologous platelet concentrate as a treatment for horses with refractory fetlock osteoarthritis. J. Equine Vet. Sci., 34: 489-493.

Pigott, J.H., A. Ishihara, M.L. Wellman, D.S. Russell and A.L. Bertone, 2013. Inflammatory effects of autologous, genetically modified autologous, allogeneic and xenogeneic mesenchymal stem cells after intra-articular injection in horses. Vet. Comp. Orthop Traumatol., 26: 453-460.

Pool, R.R., 1996. Pathological Manifestations of Joint Disease in the Athletic Horse. In: Joint Diseases in the Horse, Mcllwraith, C.W. and G.W. Trotter (Eds.). W.B. Saunders Co., Philadelphia, pp: 98-99.

Pratheesh, M.D., N.E. Gade, A.N. Katiyar, P.K. Dubey and B. Sharma *et al*., 2013. Isolation, culture and characterization of caprine mesenchymal stem cells derived from amniotic fluid. Res. Vet. Sci., 94: 313-319.

Remacha, A.R., L. Barrachina, S. Alvarez-Arguedas, B. Ranera and A. Romero *et al*., 2015. Expression of genes involved in immune response and *in vitro* immunosuppressive effect of equine MSCs. Vet. Immunol. Immunopathol., 165: 107-118.

Ricco, S., L. Boone and J.F. Peroni, 2012. Regenerative Medicine. In: Equine Surgery, Auer, J.A. and J.A. Stick (Eds.). 4th Edn., Chapter 8, Elsevier Inc., USA.

Rios, D.L., C. Lopez and J.U. Carmona, 2015a. Platelet-rich gel supernatants stimulate the release of anti-inflammatory proteins on culture media of normal equine synovial membrane explants. Vet. Med. Int., Vol. 2015. 10.1155/2015/547052

Rios, D.L., C. Lopez, M.E. Alvarez, I.J. Samudio and J.U. Carmona, 2015b. Effects over time of two platelet gel supernatants on growth factor, cytokine and hyaluronan concentrations in normal synovial membrane explants challenged with lipopolysaccharide. BMC Musculoskeletal Disorders, Vol. 16. 10.1186/s12891-015-0605-3

Schaffler, A. and C. Buchler, 2007. Concise review: Adipose tissue-derived stromal cells-basic and clinical implications for novel cell-based therapies. Stem Cells, 25: 818-827.

Schmidt, M.B., E.H. Chen and S.E. Lynch, 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.

Senna, N.A., M.B. Mostafa, A.M. Abu-Seida and Y.M. Elemmawy, 2015. Evaluation of limb conformation in jumping thoroughbred horses. Asian J. Anim. Sci., 9: 208-216.

Tawfik, H., A.M. Abu-Seida, A.A. Hashem and M.M. Nagy, 2013. Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. Int. Endodontic J., 46: 910-922.

Taylor, S.E., R.K.W. Smith and P.D. Clegg, 2007. Mesenchymal stem cell therapy in equine musculoskeletal disease: Scientific fact or clinical fiction? Equine Vet. J., 39: 172-180.

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.

Toupadakis, C.A., A. Wong, D.C. Genetos, W.K. Cheung and D.L. Borjesson *et al*., 2010. Comparison of the osteogenic potential of equine mesenchymal stem cells from bone marrow, adipose tissue, umbilical cord blood and umbilical cord tissue. Am. J. Vet. Res., 71: 1237-1245.

Van Pham, P., K.H. Bui, D.Q. Ngo, N.B. Vu and N.H. Truong *et al*., 2013. Activated platelet-rich plasma improves adipose-derived stem cell transplantation efficiency in injured articular cartilage. Stem Cell Res. Therapy, Vol. 4. 10.1186/scrt277

Violini, S., P. Ramelli, L.F. Pisani, C. Gorni and P. Mariani, 2009. Horse bone marrow mesenchymal stem cells express embryo stem cell markers and show the ability for tenogenic differentiation by *in vitro* exposure to BMP-12. BMC Cell Biol., Vol., 10. 10.1186/1471-2121-10-29

Wehling, P., C. Moser, D. Frisbie, C.W. McIlwraith, C.E. Kawcak, R. Krauspe and J.A. Reinecke, 2007. Autologous conditioned serum in the treatment of orthopedic diseases: The Orthokine® therapy. BioDrugs, 21: 323-332.

Whitworth, D.J. and T.A. Banks, 2014. Stem cell therapies for treating osteoarthritis: Prescient or premature? Vet. J., 202: 416-424.

Yamada, A.L.M., A.D.M. Carvalho, A. Moroz, E. Deffune and M.J. Watanabe *et al*., 2013. Mesenchymal stem cell enhances chondral defects healing in horses. Stem Cell Discovery, 3: 218-225.