REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 3 (2023)
http://www.veterinaria.org
Article Received: 10 September 2023; Revised: 20 October 2023; Accepted: 09 November 2023

A Preliminary Study of Regenerative Treatments for Horse Orthopaedic Disease

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Abstract

Horses with orthopedic problems, especially those with joint abnormalities and osteoarthritis (OA), face significant obstacles to their overall health and athletic ability. The majority of current equine orthopedic disease therapies are soothing, providing momentary respite without addressing the contributing causes. Innovative techniques that support long-term functional rehabilitation and tissue regeneration are required for horse orthopedic diseases. The aim of this research is to assess the efficiency of regenerative treatments, particularly autologous platelet concentrates (PC), in treating orthopedic conditions in horses. To determine how the medicine affects joint effusion, lameness and general joint function. The study included seven horses with osteochondritis dissecans (OCD) a disease and osteoarthritis (OA). An autologous PC was prepared using a reliable and cost-effective technique. The horses received PC intra articular injections every two weeks and prior to each injection, as well as for two months following the last infusion, clinical assessments, lameness scores and joint evaluations were performed. Lameness ratings (p=0.048) and joint effusion (p=0.00043) showed significant improvements with the regeneration treatment. The horses, particularly those with OA, maintained improved joint function for eight months post-treatment. The upgrades in lameness and joint effusion that have been observed suggest possible advantages in fostering tissue regeneration as well as reducing inflammation.

Keywords: Horse, Osteoarthritis (OA), Osteochondritis Dissecans (OCD), Treatments, Disease.

INTRODUCTION

Horses are known for their athleticism and agility, but they can suffer from serious orthopaedic problems that can impact their performance and overall well-being (1). For the horse business, orthopedic disorders provide significant issues, ranging from degenerative joint diseases to tendon and ligament injuries (2). The discipline of regenerative medicine has developed potential in treating orthopedic problems in horses due to its recognition of the necessity for sophisticated therapeutic techniques (3).

The Historical Background of Orthopedic Diseases in Horses

Horses have played a crucial role in transportation, agriculture and sports, all of which have benefited human civilization (4). However, straining these magnificent creatures endurance leads to orthopedic problems that impair their usefulness. Osteoarthritis, ligament, tendon injuries along with other musculoskeletal issues are frequent and impair the horse's ability to function at its best. While anti-inflammatory drugs, surgery and rest have been part of traditional therapy regimens, regenerative medicine (5) presents a new way of thinking by using the body's healing processes.

Regenerative Medicine for the Health of Horses

A paradigm change in the management of orthopedic illnesses was represented by regenerative medicine, which seeks to encourage tissue regeneration and repair in addition to symptom relief. The numerous regeneration

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techniques have shown promise in treating equine patients (6). These consist of bone marrow concentrate, platelet-rich plasma, stromal vascular fraction from adipose tissue, culture-expanded stem cells and autologous conditioned serum. Because each method has unique qualities that help to restore normal tissue function, regenerative medicine provides a flexible and all-encompassing choice for orthopedic care for horses. The developing field of translational medicine known as "regenerative medicine" (RM) aims to replace or rejuvenate organs, tissues and cells that have been lost to disease, aging, or injury to restore normal function. In the last ten years, regenerative treatments for musculoskeletal disorders in horse therapy have acquired clinical momentum. These treatments include mesenchymal stem cells, platelet-rich plasma, autologous conditioned serum and autologous protein solution. Figure (1) represents the overview of equine musculoskeletal diseases.

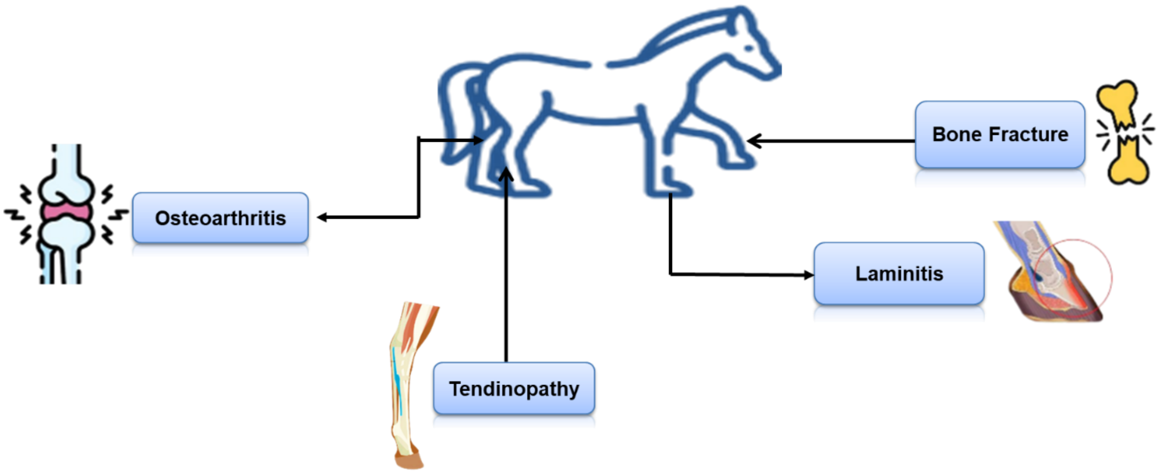

Figure (1): Overview of equine musculoskeletal diseases

(Source: Author)

Scientific Bases: Tenogenic Differentiation and Stem Cell Characterization

A comprehensive awareness of stem cells, their characteristics and their capacity for tenogenic differentiation, a process essential to the regeneration of tendons and ligaments, was crucial to the effectiveness of regenerative medicine (7). Because they are multipotent, stem cells and mesenchymal stem cells (MSCs), in particular, have enormous potential. The exploratory study explores the scientific underpinnings of regenerative therapies and how to maximize MSC tenogenic differentiation for improved therapeutic effects in horses.

Environmental Factors and Cellular Interactions

Beyond stem cell properties (8), the work explores the complex interactions that occur in the wounded tissue between stem cells of mesenchymal origin, other cellular constituents and the surrounding milieu. Comprehending these dynamic interplays was essential for customizing regenerative treatments to the distinct milieu of equine orthopedic diseases. Growth hormones and signaling molecules are examples of elements that affect cell activity and should be taken into account while creating successful regeneration techniques.

Structures and Packing for Cells

The use of scaffolds is a technique used to direct and facilitate tissue regeneration. Scaffolds influence (9) the temporal and geographical aspects of tissue healing by offering a framework for cell attachment, proliferation and differentiation. Developments in cell packing techniques are explored, tackling the difficulties of precisely delivering regenerative cells to designated target locations under regulated conditions.

Therapies Based on Bone Marrow and Blood

Regenerative medicine based on blood and bone marrow was an innovative method that uses the body's healing resources. The therapeutic potential of bone marrow concentrates and platelet-rich plasma (10), which are obtained from the horse's own blood and bone marrow, respectively, was investigated in relation to orthopedic disorders.

Potential for Translation between Humans and Horses

The potential for translating regenerative medicines from horses to humans is explored. Orthopedic problems in horses and humans can benefit from advances in therapy brought by the information obtained in equine regenerative medicine (11). It was a fascinating scientific endeavour, but bridging the gap between veterinary and human care might lead to revolutionary developments in regenerative medicine. The preliminary research was to estimate the effectiveness of regenerative medicine, particularly autologous platelet concentrates (PC), in treating orthopedic conditions in horses. To assess how the therapy affects joint effusion, lameness and general joint function.

The research (12) evaluated the effectiveness of regenerative medicine in treating chronic degenerative disorders and musculoskeletal injuries in horses that affect both active and inactive riders. The process includes providing an overview of the various therapy options and outcomes that are used to treat tendon injuries, tendinopathies and joint diseases. The results are encouraging, but there are gaps in understanding of the spatiotemporal needs for bioactive substances, which makes more studies necessary to realize the potential of regenerative medicine in horse clinical practice. The necessity of more research to improve and maximize these therapies was emphasized in the findings. Article (13) correctly assessed the application of mesenchymal stromal cells (MSCs) in veterinary regenerative medicine, with an emphasis on clinical results concerning musculoskeletal disorders in horses and dogs. The methodology entails examining the data that exists in the literature and investigating the possibility of switching from employing MSCs to secreted and extracellular vehicles (EVs). The pharmaceutical zing EVs to produce Good Manufacturing Practice (GMP) goods for clinical trials was emphasized in the result, which acknowledges the present constraints in the developing sector.

The study (14) investigated the application of MSCs in horse orthopedics, with particular attention to tendinopathies, osteoarthritis, osteochondritis dissecans and abnormalities of the metabolism. The process compares bone marrow, adipose tissue and umbilical cord blood MSC harvesting, emphasizing how crucial it was to characterize stem cells to validate their desired characteristics. Specialized procedures are required for the separation and investigation of EVs and their relevance in therapeutic processes was emphasized. The knowledge and methodological gaps that exist highlight the many applications of MSCs and EVs in horse regenerative medicine. The research (15) investigated the role that regenerative medicine therapies can play in treating soft tissue and joint diseases in both people and animals. These treatments, such as platelet-rich plasma, use the body's resources, such as cells coupled with hemo-derivatives, to try and repair the structure as well as function of the tissue. Examining applications for joint problems, the approach highlights advantages over corticosteroid therapy in terms of anti-inflammatory and healing effects. The result recognizes the limits in the current research and understanding while underscoring the potential benefits, particularly in situations when standard therapies are ineffective.

The retrospective study (16) evaluated the therapeutic results of using MSCs to treat tendinous, ligamentous and articular injuries in 65 horses between 2016 and 2019. As the significant technique, the findings showed a 59.1% to 76.9% improvement, previous therapeutic procedures proved to be more successful than MSCs. Treatments using autologous and allogeneic materials did not vary. The lack of uniformity was one of the limitations, highlighting the necessity of prospective research to improve MSC therapy procedures in horse orthopedics. The article (17) evaluated how well 24 Arabian horses' lameness-causing lesions might be found using thermal imaging. Higher temperatures were found in suspicious locations than in symmetric sections using a thermal camera. There was a substantial difference in the average temperature between the suspicious regions

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(34.7±1.7 °C) and the symmetric areas (31.8±2.1 °C). They indicate that, although there could be some drawbacks in some situations, infrared thermography is an efficient and precise method to locate orthopedic lesions in horses.

The article (18) presented the results of a workshop on translational regenerative medicine for human and horse athletes. Characterization of stem cells, cellular interactions, scaffolds, treatments based on blood and bone marrow as well as clinical applications is among the major subjects. To enhance regenerative medicines for both species by bridging the knowledge gained from horse investigations. Differences in species can provide limitations, highlighting the need for continued study to ensure successful translational applications. The study (19) examined the clinical advantages of PRP in the treatment of OA in horses. Standardization in horse OA therapy was necessary since convincing efficacious assessments are hampered by inconsistent manufacturing processes and the use of PRP, despite the fact that PRP, with developing factors, shows potential in moderating inflammation and OA development. The effect of autologous neonatal cord-derived MSCs on metacarpophalangeal joint OA in horses was evaluated in the prospective investigation (20). Split into groups for single (MSC1) and recurrent (MSC2) injections, there was no difference in the substantial improvement in lameness between the two groups. The radiographic ratings did not change and 18% of owners reported negative impacts. The long-term consequences are yet unknown and there was no placebo control group.

METHODS AND MATERIALS

The 7 horses in the research, ages 1.2 to 15 years, had significant indications of joint diseases. The ten joints that underwent valuation, three had bilateral involvement. To select patients for additional assessment, a comprehensive musculoskeletal assessment and testing techniques such as, ultrasonography, radiography, arthroscopy and regional anaesthesia were employed as indicated in Table (1).

Table (1): Description of Data set

(Source: Author)

Horse Number	Canine	Age Group	Performance Type	Gender	Medical Assessment	Affected Joints	Platelet Concentrate Volume per Joint	Prior Interventions
1	Holsteiner	3.5	Jumping	Male	OCD	Left tibiotarsal joint	15	No previous therapy
2	Spanish Sport Horse	1.2	Intended use, jumping	Filly	OCD	Stifles	20	Arthroscopic Surgery
3	Hanoverian	1.4	Intended use, jumping	Filly	OCD	Stifles	20	Arthroscopic Surgery, IA steroids and HA
4	French Mountain Horse	8	Carriage	Female	OA	Proximal intertarsal and Left tibiotarsal joints	15	NSAIDs

5	Warmblood	6	Jumping	Male	OA	Lesion in the medial meniscal region of the right lateral femorotibial joint	20	NSAIDs
6	Haflinger	15	Dressage	Male	OA	Coffin joint on the left	10	HA and IA steroids
7	Arabian	7	Endurance Racing	Male	OA	Fetlocks	10	IA steroids

For assuring the validity and uniformity of the study, case selection criteria are crucial. To ensure a consistent and similar cohort, they set precise criteria for participant admission, such as the length of the condition and confirmation of the diagnosis. High standards contribute to the removal of confounding factors, improving the validity of research findings. The results of this meticulous screening procedure will be used to determine the safety and effectiveness of the regeneration therapy under investigation.

Inclusion criteria

A comprehensive musculoskeletal examination, as well as diagnostic techniques, including radiography, ultrasonography and regional aesthetics was required for the case selection criteria in this investigation. The horses in the research showed clinical symptoms, such as increasing synovial effusion, discomfort, thickening of the joint capsule and gradual loss of joint function that persisted for at least a year. A criterion was a diagnosis of osteoarthritis (OA) verified radio-graphically, with signs of marginal osteophytosis and subchondral bone remodelling. Participants should not have received any other medical treatments for at least two months prior to study inclusion to maintain the study's integrity. Hyaluronan, glycosaminoglycans, intra-articular corticosteroids, non-steroidal anti-inflammatory drugs and other anti-arthritic medicines are among the treatments. The kind of treatment given to the enrolled horses during the study's duration was intra-articular platelet concentrates (PCs); no additional medical treatments were administered until the study on a regular basis.

Exclusion Criteria

Horses with non-osteoarthritis joint problems, horses with a history of systemic or infectious illnesses and horses undergoing concurrent medical treatments in two months prior to participation were excluded from the study. Additionally disqualified horses with a history of intra-articular corticosteroid administration, surgical procedures performed on the afflicted joints, or systemic illnesses impacting the health of their musculoskeletal system. Horses that showed evidence of lameness for reasons other than osteoarthritis were excluded from consideration, allowing for a targeted assessment of the regeneration potential of intra-articular platelet concentrates.

Comprehensive Equine Lameness Assessment Study

The American Association of Equine Practitioners (AAEP) criteria were used in the study by a single veterinarian (MP) to evaluate patients' lameness. Lameness degrees were scored on a scale of 0 to 5, with half-grade assignments as an option. The ability to trot on a firm surface in a straight line was used for evaluation. Combining clinical and ultra sono-graphic data, synovial effusion was semi-qualitatively assessed by taking into account the joint surface, the amount of synovial fluid, membrane and villi. Clinical evaluation included a

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comparison of the injured joint with typical contra-lateral joint, joint capsule appearance, range of motion, warmth, discomfort during passive activity and pain during probing. Joint effusion was assessed visually and palpably, with a score ranging from 0 to 3. Using Acuson-Aspen ultrasound equipment with a 10-MHz linear probe, the same physician (MP) regularly took ultra sono-graphic measurements at preset sites indicated on the skin overlaying the joints.

When synovial fluid was gathered prior joint injection, participants performed comparative synovial fluid cytology. A single physician evaluated every patient during the trial at the time of one-year clinical follow-up period. The comprehensive technique included both clinical and ultra sono-graphic tests to achieve a complete and accurate evaluation to provide extensive knowledge of lameness and synovial effusion in horse patients. Throughout the inquiry, the study's use of standardized procedures and regular follow-up improved the dependability of the results.

Analysing Cellular Components and TGF-β1 Levels

Using 23G Butterflier's catheters (Terumo, Belgium), aseptic jugular vein blood draws were performed. Blood was collected in 5.0 ml capacity BD Vacutainer^{TM} 3.2% sodium citrate tubes. The first plasma fraction of the supernatant (50%) was separated by centrifugation at 120 g for 5 minutes close to the buffy coat. The 25% subfraction was obtained by centrifuging this fraction one more time for five minutes at 240 g. This subfraction was stimulated with calcium chloride ($4.5\, mEq/5\, ml$) at a ratio of $50\, \mu l$ per ml of platelet concentrate (PC) following transfer to sterile syringes. Each patient's PC was examined in two millilitres to measure $TGF-\beta 1$ levels, leukocyte counts and platelet counts. The haematology system used flow cytometry to count cells and a commercial ELISA kit for humans was used to measure TGF-β1 levels. Aseptic conditions were maintained throughout the painstaking processing stages, from blood collection to analysis, guaranteeing the integrity of the samples obtained for the later investigation of platelet concentrate features in every patient.

Joint Treatment Protocol with Platelet Concentrate

Each horse's injured joints were injected after aseptic preparation. Before the injection, horses were given an intravenous bolus of butorphanol tartrate and detomidine to induce unconsciousness. The injection schedule called for three doses spaced two weeks apart. Thorough clinical tests were performed before each platelet concentrate (PC) delivery and again two months following the final injection of joint. The quantity of PC given to each visitor was assessed based on subjective parameters, which included the weight of the individual horse, the kind and size of the joint. Horses were reserved at a lower level of activity during therapy and for a further two weeks after the last injection. The treatment plan was to protect the horses' health before and after the intervention, all the while maximizing the therapeutic benefits of platelet concentrate.

Statistical Evaluation

The degrees of lassitude and effusion in the joints were displayed with their corresponding ranges as medians. A Kruskal-Walli’s test was used in the statistical analysis and if statistical importance were found, a Wilcoxon paired assessment would be performed. $P \leq 0.05$ was used as the significance criterion for both assessments. The characteristics of synovial fluid and the values of PC were presented descriptively.

RESULT AND DISCUSSION

Seven horses suffering from severe joint disorders were included in the research. Of them, four horses showed signs of osteoarthritis (OA), including front fetlocks on both sides show indications of bilateral OA, as provides the left grave joint. Furthermore, one horse developed osteochondritis dissecans (OCD) affecting the left tibiotarsal joint, while two fillies had bilateral OCD of the stifle joints. The OA horses had received a variety of therapies, such as corticosteroids and hyaluronic acid, as well as rest at intervals of two months to a year. The bilateral arthroscopic operations that involved the trimming of 40% of the lateral trochlear ridges of the femur were performed on the two fillies that were diagnosed with stifle OCD. These varied examples provide a

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thorough picture of severe joint disorders and the related treatment approaches used in the investigation. Figure (2) indicates the Ultrasonography of bilateral cooperation.

Figure (2): Ultrasonography of bilateral joint compromise

(**Source:** https://horsesidevetguide.com/drv/Media/919/radiograph-fetlock-normal-lateral-view-from-side/)

Economic constraints precluded a second surgery to address residual fragments in the joint, which is what motivated the inclusion of fillies in the study. Prior to receiving PC treatment, one filly had undergone surgery three months earlier and had been set out in the paddock one month earlier. Surgery was performed on the second filly six months prior to PC therapy. The fillies were kept turned out, as it was the colt suffering from OCD. While two lightly ridden horses and one in endurance exercises continued their workload despite advice to decrease it during treatment, a carriage training mare lowered her intensity. Following intra-articular PC injection, lameness ratings considerably improved ($p = 0.048$). The significant improvement was shown two periods after the third therapy, as shown in Table (2). Flexibility tests showed a general trend of progress, even if the less handled fillies could not initially undergo them. The joint effusion (JE) decreased during therapy and two months following the last injection (p=0.00043).

Table (2): Lameness and Effusion Evaluation over Time

(Source: Author)

Factors	**Rates**			
	Primary	**Preceding Second Injection**	**Preceding Third Injection**	**Assessment**
Degree of joint effusion	2.25^{b}	1.75^{a}	1.5^{d}	0.875^{c}
	Rate 2-3	Rate 1-2.5	Rate 1-2.5	Rate 0-1
Degree of lameness	1.1^{b}	$0.8^{b,a}$	$0.6^{a,d}$	$0.35^{d,c}$
	Rate 0.5-2	Rate 0-1.5	Rate 0-1.5	Rate 0-1

When feasible, synovial fluid (SF) cytology showed 98% mononuclear cell preponderance. It was not possible to do numerical analysis on SF samples depicted in Table (3). An average of $252 \pm 72.9\, x\, 10^{6}$ blood cells,

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$8.69 \pm 3.79\, x\, 10^6$ leukocytes and 12525 ± 2434 pg of $TGF - \beta 1$ per millilitre of PC were found in the platelet concentrates. With the exception of a tiny, temporary synovial effusion that developed following the first two PC injections in patient No. 6 (stifle osteoarthritis) and a filly with stifle OCD (horse No. 4), the therapy showed no unfavourable clinical symptoms.

Table (3): Inflammatory Parameters Before and After Treatment

(Source: Author)

Factors	**Rates**		
	Primary (n:3)	**Preceding Second Injection (n:4)**	**Preceding Third Injection (n:3)**
Leukocytes ($x10^3$ $cel/\mu lL$)	0.57± 0.06	0.78 ± 0.2	0.53± 0.06
Protein levels (g/dL)	1.74± 0.6	1.5 ± 038	1.74 ± 0.5

Before their lameness gradually increased after the last PC injection, osteoarthritis horses maintained their ultimate lameness score for around eight months. The high-intensity training had yet to begin at the time of the report. The OCD ridden colt continued to be lame.

DISCUSSION

The use of autologous PC is being utilized for the treatment of intraarticular horse joint disease. It is reported that the method for acquiring an equine PC is simple, dependable and economical. The suggested treatment plan is empirical and it is based on a protocol for treating severe inflammation arthropathies in people. The decision to start this pilot clinical trial was based on anecdotal and scientific information that showed PC to have beneficial effects on chondrocytes and human cartilage. The study shows that JE and improvements in lameness (DL) can have been caused by some of the growth factors (GFs) in PC, which can reduce inflammation and promote tissue healing. A number of variables, including the injected volume, the osmotic effects of administered proteins, biochemical reactions in the joint environment, or leukocytes in the prepared PC, can have an impact on the temporary rise in joint distension following PC injections. It was a transient event, with joint fluid leakage decreasing before each further treatment and the trial. The platelet concentration found in this investigation was lower than that found using buffy coat or apheresis techniques. TGF-β1 levels, however, were comparable to those described for the buffy coat technique and more significant than those reported for apheresis. The method's decreased leukocyte count begs the issue of whether PC's effects are mediated by inflammatory cells, a matter that is minimally understood.

CONCLUSION

The preliminary investigation evaluated the effectiveness of regenerative medicine, such as autologous platelet concentrates (PC), for treating orthopedic disorders in horses with an emphasis on anomalies of the joints and osteoarthritis (OA). Every two weeks, seven horses suffering from severe joint disease, such as osteochondritis dissecans (OCD) and OA, underwent intraarticular injections of autologous PC. Standard examinations, clinical assessments and lameness ratings were performed prior to each infusion and for two months following the last injection**.** Joint tests, clinical reviews and lameness ratings were performed prior to each information and for two months following the previous injection. In the PC treatment, they showed significant improvements in lameness scores (p=0.048) and joint effusion (p=0.00043). The significance was the eight-month-long sustained improvement in joint function in OA horses. These results highlight the promise of autologous PC as a novel and successful strategy for long-term rehabilitation in horse orthopedic disorders, with encouraging results in terms of tissue regeneration and reduced inflammation. The lack of age as well as disease-matched controls, a small and non-representative size of sample, a potential, double-masked, controlled research investigation are some of the drawbacks of the study. The results indicate a promising future for intraarticular PC injection for horse joint disease, more in vitro research and clinical evaluations are necessary before endorsing this course of treatment. The calcium chloride activation technique used in this work was thought to be a less expensive and safer substitute for processes involving pure bovine thrombin, which might cause allergic responses. Despite their high concentrations, GFs in PC can have supraphysiological effects, perhaps affecting the inflammatory and catabolic processes linked to joint disorders.

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