Regenerative Therapies in Elite Equine Athletes: Optimizing Recovery and Performance in Sport Horses by PRP and Stem Cell Therapy

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Abstract

Elite equine athletes always remain under massive physical stress, which results in a high prevalence of musculoskeletal injuries affecting their joints, tendons, ligaments, and bones. Traditional treatments like anti-inflammatory drugs and surgeries fall short in complete recovery from these intense injuries, so it is necessary to develop advanced therapeutic strategies. Regenerative therapies, especially platelet-rich plasma (PRP) and stem cell treatments (SCTs), offer an innovative approach by healing damaged tissues at the cellular and molecular levels. They help in enhancing the body's innate healing capabilities. PRP therapy uses concentrated platelets to release growth factors that facilitate in reduction of inflammation, provide stimulation to tissue regeneration, and speed up recovery. Stem cell therapy, mostly used mesenchymal stem cells (MSCs), supports tissue repair via direct differentiation into damaged cells, promoting new blood vessel formation, immunomodulation, and extracellular matrix production. Techniques for preparation, administration, and mechanisms of action for these therapies are continually being refined to improve results. Future directions point toward cell-free regenerative approaches, genetically enhanced stem cells, and the integration of bioengineered supports to further augment healing. As research advances, regenerative therapies are poised to become the cornerstone of equine sports medicine, improving performance longevity and welfare of athletic horses while reshaping the standard of care.

Keywords: Equines, Injuries, Chemical Drugs, Surgeries, Alternatives, Regenerative therapies

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Introduction

Equines, especially horses, play an important role in the economy of the nation. Athlete Horses, like human athletes, put too much physical stress on their bodies, especially on joints, bones, tendons, and ligaments (Bartolomé & Cockram, 2016). The possibilities of acute and chronic injuries are also extreme in athletes. Rigorous training and continuous movement make the recovery very difficult.

Conventional medicines like anti-inflammatory medicines and surgeries have constraints in complete recovery (Paoloni et al., 2009). Retrieval from these injuries is very significant not only for the welfare of horses but also for the restoration of competitive potential. These horses have superior genetics and the best stamina in their sport, but injuries can ruin their capability. Traditional medicines are mostly used for symptomatic relief, but they work at the cellular level, remold the repair processes at the molecular level, and restore the exact structure of the cell or tissue for fully functional working by introducing bioagents like growth factors, platelets, and stem cells (Mahla, 2016).

So, it is foremost to figure out modern techniques that can help them to recover completely (Upadhyay, 2015). Regenerative therapies are procedures that are used to reinstate or restore the damaged cells or tissue of the body (Sánchez et al., 2012). They pace up recovery by restoring the damaged tissue or cell via using the normal healing process of the body (Upadhyay, 2015). They increase the capacity of body to heal quickly. This chapter explores various modern therapies that are being used for the welfare of equines to enhance their efficiency and make their muscles and bones strong.

Key Regenerative Therapies in Equine Practice

Platelet-Rich Plasma (PRP)

PRP was developed only for the use of humans in the 1970s (Alves & Grimalt, 2018), in which only platelets are transfused to those patients with low platelet numbers, but with further progressions, it was used as a regenerative therapy agent in the 1990s, initially in maxillofacial and dental surgery. The transition to veterinary medicine ensued in the 2000s, especially used in equine orthopedic (Camargo Garbin et al., 2021).

Doctor Alan Nixon and the team were pioneers of equine PRP at Cornell University College of Veterinary Medicine. They were first used for the tendon and ligament repair, which showed wonderful results. They work on evaluating the ability of PRP for the reduction of inflammation, enhancing cellular proliferation, and stimulating collagen synthesis in damaged tendons, especially in the superficial digital flexor tendon (Schnabel 2023). Through in vitro (lab-based) and in vivo (live animal) research models, Nixon's team demonstrated the benefits of PRP in equine rehabilitation therapies as compared to traditional medicines. This research recognized the credibility of PRP, which leads towards the practice of PRP to treat soft tissue injury in sport horses (Specchio, 2013; Schnabel, 2023). PRP is an autologous concentration of platelets in a small volume of plasma. It is prepared by the centrifugation of the horse's own blood to isolate the platelets (Miranda et al., 2019). It is categorized into three types: pure PRP in which low leukocyte contents are present with minimal RBCs (Yin et al., 2016), leukocyte PRP which has higher leukocyte number and inflammatory cytokines (Di Martino et al., 2022), and platelet-rich fibrin (PRF) is prepared without anticoagulants and forms a gel matrix (Singh et al., 2012).

Preparation of PRP is a crucial procedure in which whole blood (50-60 ml) is collected from the jugular vein of the horse by sterile methods in a syringe or collection tube (ACD-A) for the prevention of clotting during the process. Strict aseptic techniques are mandatory to avoid contamination because PRP will be injected directly into sterile organs like tendons and joints (Dashore et al., 2021; Segabinazzi et al., 2021). After collecting, the next process is centrifugation, in which two spin centrifugation protocol is applied. First spin is a soft spin at the slow speed of 1000-1500rpm for 10-15 minutes. After the first spin, the blood divided into three layers. The top layer is plasma, the middle layer is a buffy coat, which is rich in platelets and white blood cells, and the bottom layer is RBCs. This spin separates the plasma and buffy coat from heavy red blood cells (Fukuda et al., 2020; Xue et al., 2025).

The second spin is called hard spin, in which the centrifugation happens at 3000- 3500 rpm for 10-15 minutes. Platelets are forced to the bottom to form pellets. The top portion is removed while plasma and pellets are mixed to form platelet-rich plasma (Seidel et al., 2019; Fantini et al., 2021). Then this platelet-rich portion is carefully extracted by using a syringe or a pipette. Commercial PRP kits are generally used to standardize the preparation of PRP, such as ACP, Equine Pure PRP, or GPS III (Xue et al., 2025).

Mechanism of Action

If an immediate application is necessary, then use it in natural form, but it can be enhanced into activated form by adding Calcium chloride, thrombin, etc. This activation leads toward degranulation of platelets, forming a fibrin shell and releasing growth factor (Camargo Garbin et al., 2021). Growth factors, including PDGF, TGF-B, VEGF, and IGF-1, are released for various functions (Roberts & Halper, 2021). Platelet-derived growth factor (PDGF) stimulates fibroblast and smooth muscle cell proliferation (Sánchez et al., 2025). Transforming growth factor (TGF-B) promotes the synthesis of collagen and tissue regeneration (Huang et al., 2024). Vascular Endothelial growth factor (VEGF) enhances angiogenesis (process of forming new blood vessels) (Chávez et al., 2025). Insulin-like growth factor 1 (IGF-1) promotes tissue remodeling and cell differentiation (Zhang et al., 2024).

PRP also has anti-inflammatory effects (Oneto & Etulain, 2021). Leukocyte-rich PRP contains IL-β, IL-6, and TNF-α, which are inflammatory cytokines, and can have pro- and anti-inflammatory effects according to the environment (Tramś et al., 2022). Timely administration can reduce the inflammation of tendons due to injuries. PRP provides chemotactic stimulation, which supports tenocytes proliferation, ligament fibroblasts, and recruits progenitor cells that help in tissue repair and scar formation (Tramś et al., 2022). The route of administration for PRP is very important. The following are some routes for PRP in equine medicine.

Intralesional injection is generally used in tendon and ligament injury, especially injuries of the superficial digital flexor and suspensory ligaments. It is performed under an ultrasound guide for exact placement at the injury. A 22G or 20G needle is used to inject PRP under sedation or limb restraint. Volume of PRP varies with the size of the lesion, usually injected between 2- 5 mL. Sometimes this is repeated after several weeks if needed. It helps to remodel collagen in the lesion (Hijazi et al., 2022).

Intra-articular injection is used to inject directly into the joint space in the condition of joint inflammation or mild osteoarthritis. For this type of PRP joint is aseptically prepared, and PRP is injected into the landmark under sedation. 3 - 6 mL PRP is injected according to joint size. It helps in modulating inflammation and promoting cartilage repair (Rodríguez-Merchán, 2022a). Subcutaneous or perilesional injection is used for the soft tissue swelling around the tendons, as mentioned in Table 1. PRP is injected around the injury site rather than directly into it. Its result becomes best by adding fibrin matrix or PRP gel. It helps in stimulating local healing and reducing external inflammation (Rani et al., 2023).

Intradermal or topical application of PRP is used to heal chronic wounds in horses. PRP is applied directly to the wound or injected into the wound margin. It may be used with bandages or dressings. It helps in improving granulation tissue and reducing infection (Abd Elraouf et al., 2023).

Stem Cell Therapy in Horses

Stem cell Therapy is a revolutionary regenerative therapy used in equine sports medicine, particularly in the cure of musculoskeletal injuries that are common in sports horses (Cequier et al., 2021). This therapy benefits from augmenting tissue regeneration, reducing reinjury rates, and promoting faster recovery as compared to traditional medicines. Stem cells are the undifferentiated cells with the ability to self-renew and differentiate into specific and specialized cell types, e.g. tendons cells, cartilage cells, bone cells (Chowdhury et al., 2021). Their regenerative potential makes them highly desirable for the treatment of injuries that heal poorly in normal conditions (Zeng, 2023).

Several types of stem cells have been isolated in equines: mesenchymal Stem Cells (MSCs) are the specialized type of adult stem cell that originates from the mesodermal layer during embryonic development. They are multipotent, meaning they can be differentiated into various cells, particularly those forming connective tissues, including osteocytes, chondrocytes, tenocytes, fibroblasts, and myocytes (Zhang et al., 2024). Due to these properties, they are highly able to treat musculoskeletal injuries in horses. In horses these stem cells are harvested from bone marrow and adipose near tailhead or cutaneous fat stores (Yildirim et al., 2020).

Table 1: Mechanism of PRP action and immune modulation based on origin and activation

Step	Key Elements	Biological Effects	References
Bone Marrow	Hematopoietic stem cells	Differentiate into megakaryocytes	(Stone et al., 2022)
Megakaryocytes	Platelet production	Platelets enter blood circulation.	(Carminita et al., 2024)
Platelets (Resting)	Platelet production	Inactive until activated by stimulants.	(Veninga et al., 2022)
Activation Stimuli	Thrombin / Calcium chloride ($CaCl_2$)	Converts resting platelets into an active form in PRP.	(Ahmed, 2023)
Activated Platelets in PRP	Release of: - PDGF (Platelet-Derived Growth Factor) - HGF (Hepatocyte Growth Factor) - FGF (Fibroblast Growth Factor) - Histamine	Promote angiogenesis, endothelial migration, and proliferation.	(Cecerska-Heryć et al., 2022)
Inflammatory Signaling	Release of: RANTES, IL-8, Platelet microparticles	Recruit immune cells, then monocyte polarization. and trigger cytokine and chemokine production	(Scopelliti et al., 2022)
Response of Immune Cell	Influx of immune cells	Improves healing, reduces inflammation	(Caballero-Sánchez et al., 2024)
Interaction with MSC (Mesenchymal Stem Cell)	Stimulated by PRP factors (e.g., SDF-1, HGF)	Promote stromal cell proliferation, cytokine production, and angiogenesis.	(Bouland et al., 2021)
Tissue Anabolism	Growth factors (IGF, TGF-β, MMP)	Supports extracellular matrix production and tissue regeneration.	(Klein, 2022)

Embryonic Stem Cells **(**ESCs) are pluripotent cells taken from the inner cell mass of blastocyte which is an early stage of preimplantation embryo about 6-7 days post fertilization in mammals (Irie et al., 2020). They can differentiate into three germ layers (ectoderm, mesoderm and endoderm) which can make any cell type in the body. Their self-renewing ability can proliferate in dedifferentiation states under proper culture conditions by which large number of cells can be made (Efthymiou et al., 2014). These properties make ESCs extremely powerful for regenerative medication, at least in theory. They are garnered from early embryos via breeding programs e.g. embryo flushing (Kingham & Oreffo, 2013). Then isolate the inner cells mass under sterile laboratory conditions, culture the isolated cells in specific media that preserve their pluripotent state. However, this process is rare in equine compared to human and mice but has extensive ability to use in regeneration therapies. This therapy is very beneficial because they can be used to treat any type of injury including tendon, bone and nerve injury. Their growing ability in culture media makes it more favorable without losing their characteristics (Górska et al., 2024). But they are used rarely in horses because it is unethical to destroy the early embryo. Moreover, the risk of tumor formation is higher in these ESCs than in other stem cells, and they can form teratomas if injected without proper differentiation.

Mechanism of Action

In the initial study, scientists believe that stem cells directly replace the damaged tissue by differentiating into specific cells. But with modern research, a different mechanism has been found. The stem cells create the paracrine effect by releasing growth factors and cytokines that help in the reduction of inflammation, as shown in Figure 1 (Smith et al., 2024). They stimulate resident cells to proliferate and repair the damaged tissues, promote the new blood vessels (Angiogenesis), and modulate the immune response as mentioned in Table 2. They act as biological stimulators for healing rather than simple building blocks (Reis et al., 2024). In the case of MSCs, they can act as building blocks by differentiating into specific cell types and replacing the damaged cells when they get signals from certain environments(Salsbury). In injury, they can detect local biological signals like growth factors and cytokines. As the right conditions arrive, they differentiate to replace damaged cells, like as tenocytes in case of tendon injury or into chondrocytes in case of damaged joints (Augustin et al., 2024). Beyond simply replacing damaged cells, MSCs apply immunomodulatory effects that make them precious in mastering inflammatory and immune-mediated conditions in horses (Feng et al., 2025).

Collection, Culture and Administration

Stem cells used in equine regenerative therapies are mostly mesenchymal stem cells (MSCs). They are collected from two main sources: Bone marrow or adipose tissue (Siddiqui et al., 2024). Bone marrow-derived MSCs are obtained from the sternum or the tuber coxae. For this process, horses are sedated, and local anesthesia is administered. A sterile bone marrow biopsy needle is injected into the bone marrow space. 10-30 mL of bone marrow aspirated is removed into a heparinized syringe, which prevents clotting (Ferris et al., 2014; Al Naem et al., 2020).

Adipose-derived MSCs are collected from the tail head or abdominal fat of the horse. Under sedation and local anesthesia small incision is made to collect 10-20g subcutaneous fat (Cequier et al., 2021).

Strict aseptic techniques are crucial to avoid contamination, which can lead to severe infection in the body of horse. After collection, bone marrow or adipose fat is processed to isolate and expand the MSCs (Al Naem et al., 2020). For isolation density gradient centrifuge is used to separate MSCs from RBCS and other unwanted components (Al Naem et al., 2020). Cells are then plated into a cell culture flask with media such as DMEM or alpha-MEM supplements with antibiotics and fetal bovine serum (Pezzanite et al., 2021). Then, cells are cultured in an incubator with a temperature of 37°C, 5 % CO_2, and high humidity. MSCs stick to the plastic surface and start forming colonies. Media should change every 2-3 days to remove non-sticky cells (Espina et al., 2016). As confluency reached 80-90%, cells were passaged to new flasks to continue the expansion. Expansion takes around 2-4 weeks, depending on the source and horse-specific cell growth rate. MSCs makers are confirmed by using a flow cytometer, while trilineage differentiation assays are performed to confirm multipotency (De Schauwer et al., 2011).

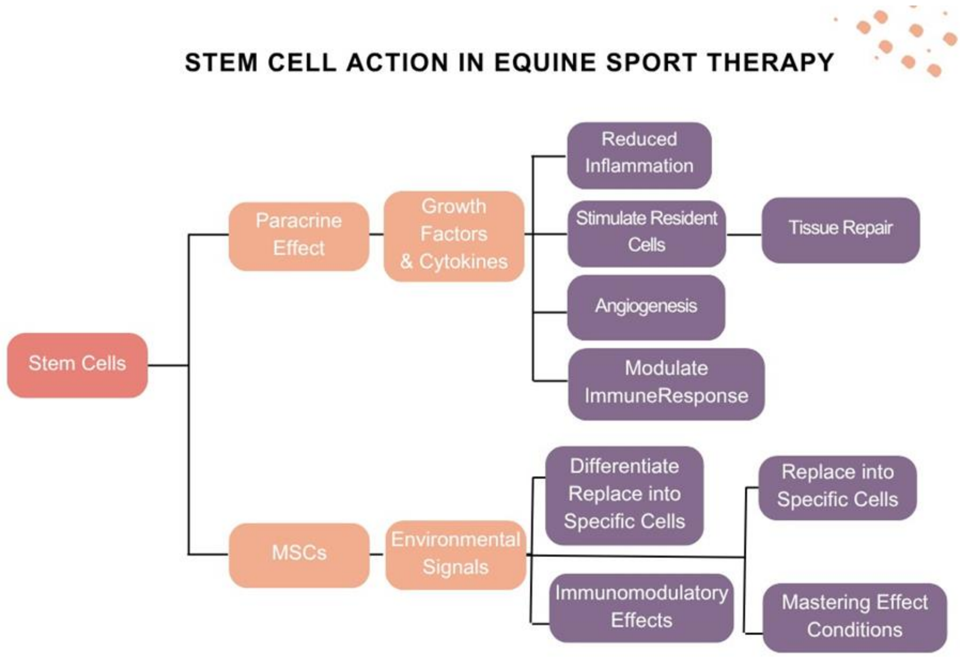

Fig. 1: Mechanism of action of stem cells in equine sport therapy

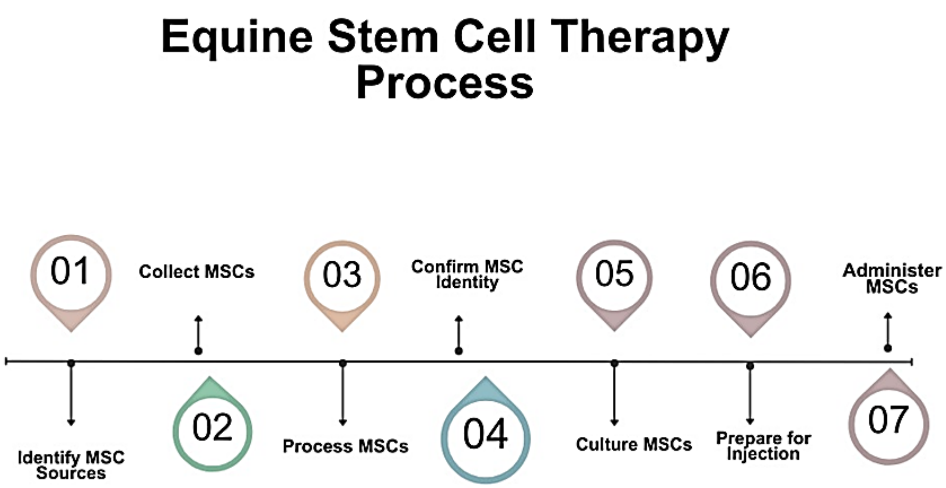

Fig. 2: The process of equine stem cell therapy

As a large enough number of MSCs is obtained, the cells are prepared to be injected into the body. They are harvested by using enzymes that detach them from plates. After detachment, cells are washed and resuspended in saline or plasma for injection, as shown in the therapy process, Figure 2. The final product contains 10-50 million MSCs (Broeckx et al., 2019). Several routes are adapted for the administration of stem cells according to the injury site: Intralesional injections are used to inject directly into the lesion site under ultrasound guidance. About 1- 5 mL volume stem cells is injected. This administration is used in case of tendon or ligament injury (Geburek et al., 2016; Geburek et al., 2017). Intra-articular injections are directly injected into the joint space in case of joint injury or osteoarthritis at a dose of 3-6 million MSCs per joint (Joswig et al., 2017; Magri et al., 2019). Intravenous routes are rare, but they are used in cases of systemic inflammatory conditions. MSCs are administered intravenously for generalized actions (Broeckx et al., 2014). The perilesional route of administration is used if intralesional injection is not possible. MSCs are injected around the damaged structure (Wilke et al., 2007; Barrachina et al., 2018).

Future Directions of Regenerative Therapies

The field of regenerative therapy is expanding steadily with the progression of medical science. In the future, it will be the most used veterinary practice to treat the acute and chronic injuries of sport horses and other animals. The regenerative therapies that we are using nowadays are at their first stage, studies are paving their way to enhance the therapeutic uses of these procedures. Advancements in genetic and molecular biology are thriving on gene therapy, which is a futuristic approach to personalized therapies according to a horse's genetic makeup and characteristics of injury. This technique helps in healing injuries and other problems at the gene and molecular levels. In regenerative medicines, researchers are using whole cells like stem cells and plates, but there is raised curiosity in using secretory components besides whole cells, such as exosomes, micro vesicles, and growth factors. These cell-free therapies reduce the risk of immune response, formation of tumors, and regulatory complications, although they still give regenerative benefits.

Table 2: Mechanism of Stem Cell Therapy in Equine Medicine

Step	Key Elements	Biological Effects	References
Stem Cell Origin	Mesenchymal Stem Cells (MSCs)	Bone marrow, adipose tissue multipotent	(Soliman et al., 2021)
Isolation and Expansion	Collection by bone marrow aspiration or fat harvest lab expansion under sterile conditions	Increase the number of viable therapeutic cells before injection.	(Pakzad et al., 2022)
Delivery to Target Site	Injection into injured tendon, ligament, or joint (intralesional or Intra-articular routes)	MSCs are home to the injury site	(Rodríguez-Merchán, 2022b)
Cellular Action	Direct differentiation into local cell types (e.g., tenocytes, chondrocytes)	Replace damaged cells and regenerate tissue structure.	(Donderwinkel et al., 2022)
Paracrine Action	Secretion of: growth factors (e.g., TGF-β, IGF-1) cytokines exosomes	Stimulate local progenitor cells, enhance tissue repair, and modulate immune response	(Xue et al., 2024)
Immunomodulation	Suppression of T-cells, B-cells, and macrophages Reduction of pro-inflammatory cytokines	Reduces chronic inflammation and supports tissue healing (e.g., in osteoarthritis).	(Babu et al., 2021)
Tissue Regeneration	ECM (extracellular matrix) production, angiogenesis, reduced fibrosis	Enhances biomechanical strength and functional recovery	(Potekaev et al., 2021)

In mesenchymal stem cells therapy, we use native cells that we extract from the body, but researchers are investigating the modification of MSCs for the extra extraction of beneficial proteins like growth factors. The modified MSCs have more potential to repair the targeted injured tissue with more precision and faster as compared to native cells. Many futuristic materials can be combined with stem cells and PRP to enhance their abilities, such as hydrogel, fibrin matrices and nanoparticles, which lead to tissue support and promote healing of joints, tendons, and ligaments. One of the future goals is to establish a standardized protocol for the preparation and use of PRP and stem cell therapy. This can lead to improving the treatment strategies, research facilities, and increasing clinical confidence in regenerative therapies. Regenerative therapies should be used on a regular basis as sophisticated equine sport therapies to enhance the healing process and prevent reinjuries. This will increase the confidence of the veterinarians, and they will do more research on it to enhance its benefits.

Conclusion

Equines, especially horses, play an important role in the economy of the nation. Athlete Horses, like human athletes, put too much physical stress on their bodies, especially on joints, bones, tendons, and ligaments. Various traditional drugs and surgeries were used, but they may cause complications. Regenerative medicine has opened new ways for treating musculoskeletal injuries in horses, especially elite equine athletes. The benefits of PRP and Stem cell therapy are undeniable. They help in promoting natural healing as well as stopping reinjuries. Due to working at the cellular level, healing becomes fast and precise, helping equines a lot in returning to their peak performance. Progress can be made in this therapy by continuous research and clinical use of these therapies. They promise care and a long career to athletic horses.

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