

Regenerative Therapies in the Horse

Part 1 - What Exactly Are We Talking About?

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Photography by Dr. Sarah Peters©

Regeneration can be defined as renewal or restoration of a body, bodily part, or biological system after injury or as a normal process. Regenerative therapy has found a place in both human and veterinary medicine as a way to promote the body's own healing of injured tissues in an attempt to restore both structure and function. The horse is a particularly good model to assess both the methods and success of these regenerative therapies due to the fact that horses both exercise and get injured, much like humans.

In the field of regenerative therapies, there are several different types of these therapies that are currently in use in equine veterinary medicine today. These include stem cells, Platelet-Rich Plasma (PRP), Autologous Conditioned Serum (ACS) and Autologous Protein Solution (APS).

STEM CELLS

Stem cell therapy has been of interest in veterinary medicine for the past 20 years. It is the stem cell's potential ability to repair or regenerate almost all of the tissues in the animal's body that has led to an attempt to better understand the biology of these cells so that their therapeutic potential can be realized. 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 copies of themselves while differentiation potential is the ability to produce tissue specific cells." Stem cells can be broadly categorized as to their source, being referred to as Embryonic Stem Cells (ESC), Adult or Tissue Specific Stem Cells (ASC) or Induced Pluripotent Stem Cells (iPSC). ESC are unique in that they have the ability to generate all tissue types in mammals. ASC are the type used most commonly today in treating joint and soft tissues disease in the horse and it is Mesenchymal Stem Cells (MSC) that we are most familiar with. MSC have the ability to both proliferate and differentiate into cells such as chondrocytes (which produce cartilage), osteoblasts (form

new bone), adipocytes (form fat tissue), as well as cells that can form skeletal tissue and tendons. MSC can also produce cytokines (substances secreted by certain cells of the immune system that can exert effects on other cells) and growth factors that help with cell proliferation and differentiation. MSC are the type isolated from adult fat tissue and bone marrow (from the sternum or tuber coxae), but they can also be recovered from other sources as well, such as the amnion, the umbilical cord (cord blood), or dental pulp (PulpCyte®, for example, manufactured by VetGraft). Once harvested, the bone marrow is cultured sterily in a lab so that the MSC are isolated and expanded (increased in number). This process can take 2-4 weeks. A typical "dose" of MSC is between 10-20 million stem cells per joint or per site. "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 ones."

PLATELET-RICH PLASMA (PRP)

PRP is "a concentration of platelets used to provide growth factors secreted by the platelets to a site of injury. These growth factors act to enhance healing by improving access of



Equipment set-up for APS processing



Injection of a stifle with APS.

healthy inflammatory cells to the tissue through formation of new blood vessels and connective tissue, and regeneration of skin.”² Blood is drawn from the patient, centrifuged to separate the platelets contained within the plasma (red blood and white blood cells are removed), which then release the growth factors once activated. It takes approximately 30 minutes from the time the blood is drawn to the time it is ready for injection. PRP kits are available commercially, with the number of platelets obtained and the purity of these cells dependent on the processing method that is used. The PRP is then injected into the injured site, either by itself or with stem cells, and is thought to provide a scaffold for the repair process as well as providing growth factors that help to increase the cell’s ability to repair muscle and bone lesions.

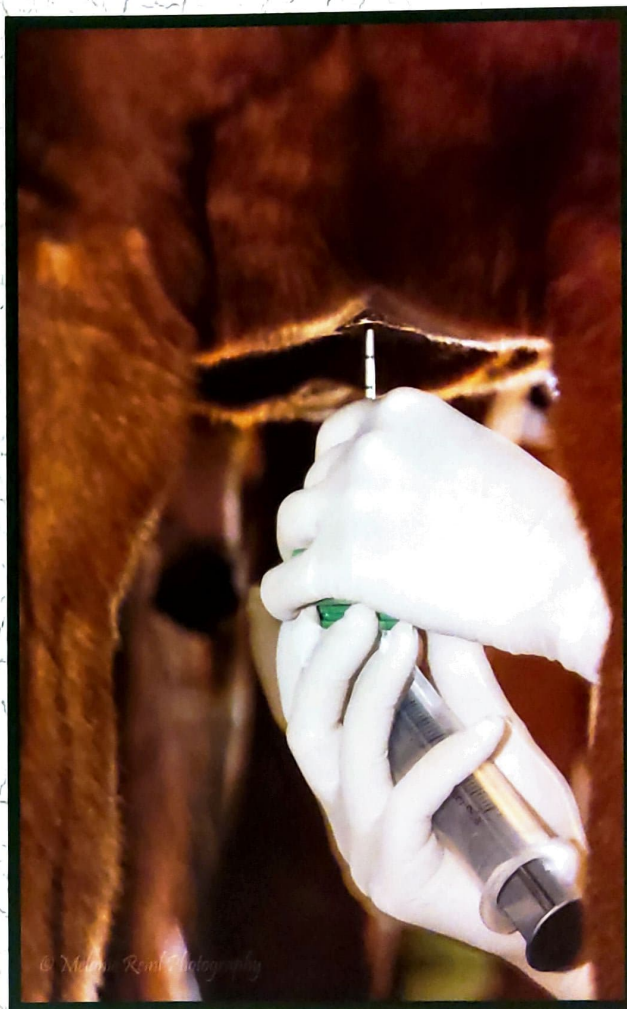


APS prepared and ready for injection.

AUTOLOGOUS CONDITIONED SERUM (ACS)

ACS, known more commonly by its commercial name Interleukin-1 Receptor Antagonist Protein (IRAP), is a product produced from the horse’s own blood. ACS has natural anti-inflammatory properties as well as the ability to block the effects of certain chemicals that are released during injury or disease causing inflammation, more specifically that involving joints, that can be highly detrimental to the healing process. One such cytokine, Interleukin-1 (IL-1), is a major negative

mediator in joint disease, causing further joint damage and progression of joint disease. “Interleukin-1 receptor antagonist protein works to counteract the destructive effects of IL-1 in sites of inflammation by blocking IL-1 receptor in the joint to break the cycle of inflammation.”² The processing of IRAP involves taking a blood sample from the patient with a special syringe, that is then incubated for 24 hours to stimulate the production of the antagonist protein. After incubation, the blood is centrifuged and the plasma containing the IRAP is separated from the blood cells. This plasma with the IRAP can then be divided into multiple doses and frozen in a special freezer. The doses can then be thawed and injected into the affected joint according to the treatment protocol.



Bone marrow collection from the sternum.

AUTOLOGOUS PROTEIN SOLUTION (APS)

Known most commonly by the trade name Pro-Stride, APS is a variation of ACS (the makers of Pro-Stride point out that “Pro-Stride is not an autologous conditioned serum”) and has gained popularity because the product does not require an incubation period and is often used as a single injection treatment. A blood sample is taken from the patient via a syringe whose contents are then transferred into special separator devices. “A bench-top centrifuge firstly isolates white blood cells, platelets, and plasma proteins, then they are further concentrated in a second

centrifugation step.” It has been shown that the resulting product is higher in both white blood cells and platelets than whole blood. This increased white blood cell level results in an increase of the IL-1 receptor antagonist protein (IRAP) as well as 2 other important anti-inflammatory proteins, all of which are key in fighting the inflammation that leads to joint damage and disease progression. The resulting product (Pro-Stride) is a “concentrated solution of cells, platelets, growth factors, and anti-inflammatory proteins (including IL-1ra and other anabolic proteins)”⁴.

Much research has been done on regenerative therapy in the horse and its use in equine veterinary practice today is widespread. In Part 2, we will look more specifically into how each of these therapies is used to treat our equine partners and what the expectations might be for success. We will also take a look at where this exciting field is expecting to go as continued research finds new uses for regenerative therapy.



1. Abu-Seida, A.M. Regenerative therapy for Equine Osteoarthritis: A Concise Review. Asian J. of An. and Vet. Advances 10 (9): 500-508. 2015, ISSN 1683-9919 / DOI: 10.3923/ajava.2015.500.508. (open access).

2. From the ACVS website (acvs.org): Regenerative Medicine in Equine Surgery.

3. Bogers, S.H. Cell-Based Therapies for Joint Disease in Veterinary Medicine: What We Have Learned and What We Need to Know. Front Vet Sci. 2018; 5:50. DOI: 10.3389/fvets.2018.00070. (open access).

4. omiveterinary.com (website for the makers of Pro-Stride)

Table 1

Regenerative medicine products used in the dog and horse for OA.

Category	Description	Examples of US based veterinary suppliers/products	Effects in OA
Autologous-conditioned serum	Autologous blood product that increases anti-inflammatory cytokines including interleukin-1 receptor antagonist	IRAP (Dochra/Orthokine); IRAP II (Arthrex); MediVet; Biologics; EC-ACS (Vetlinebio)	Improved lameness, synovial thickness, and cartilage fibrillation (21, 22)
Platelet-rich plasma (PRP)	Autologous blood product that contains growth factors including IGF-1 and PDGF	MediVet; VetStem; Osteokine (Dochra); Arthrex ACP; V-Pet (Fall Life Sciences); PRPKits.com; DrPRP USA; RegenKit-BCT (RegenLab); E-Pet (V-Care); V-PET (Nupsala)	Variable response to intra-articular injection in horses, some show reduction in lameness and joint effusion (23-25). In dogs has a pain-relieving effect that is slower onset but similar effect compared with corticosteroid injection (26, 27)
Autologous protein solution	Autologous blood product that contains both growth factors and anti-inflammatory cytokines via a 2 step process	Pro-Stride; N-Stride	Reduced clinical signs of pain and lameness in dogs at 12 weeks (28) and horses at 14 days and 12 weeks via client assessment (29)
Adipose-derived stromal vascular fraction	Digest of autologous adipose tissue that contains ~1-2% of CFU-fibroblasts	VetStem (Biopharma)	Subjectively less effective than cultured bone marrow-derived stem cells when compared with placebo for experimental OA in horses (30). Functional improvements in naturally occurring and induced canine OA, with some evidence of improvement when paired with PRP (31, 32)
Mesenchymal stem cells (MSCs)	Autologous or allogeneic plastic adherent cells that are commonly isolated from bone marrow or fat. Capable of differentiating into osteogenic, chondrogenic, or adipogenic cell lines	Variable—stem cell therapy may be offered by comparative orthopedic research laboratories	Bone marrow-derived MSCs showed no significant effects for naturally occurring OA; however, it can improve return to work of horses with intra-articular soft tissue injury (33). Canine studies using adipose-derived MSCs show improved functional outcomes, their effect may be complemented when PRP is used as a vehicle (34)

5. O'Shaughnessy et al. Autologous protein solution prepared from the blood of osteoarthritic patients contains an enhanced profile of anti-inflammatory cytokines and anabolic growth factors. JOR 2014, 32, 1349-1355.

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