Regenerative Therapies in the Horse Part 2 - How do we use this amazing technology?

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I n part 1 of this article, I went over the different types of regenerative therapy in use in equine veterinary medicine today, namely mesenchymal stem cells (MSCs), interleukin receptor antagonist protein, (IRAP), platelet-rich plasma (PRP), autologous conditioned serum (ACS) and autologous protein solution (APS). In part 2, I would like to discuss how we are using some these different therapies to help and ultimately heal (if possible) our equine partners in all phases of their life and use.

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EQUINE LAMENESS (JOINT/SOFT TISSUE):

Most of us are familiar with the most common use of virtually any of these regenerative therapies and that is to treat lameness in the equine athlete. The most common joint issue addressed is osteoarthritis (OA), "an irreversible, complex disease that involves all tissues of the joint in a cycle of inflammation and tissue degradation. OA affects over 80% of horses >15 years of age and up, to 2/3 of thoroughbred racehorses, making it one of the highest causes of wastage and loss of use in this population."¹

Traditionally, OA has been treated by joint injection of corticosteroids to which has been added compounds such as polysulfated glycosaminogylcans, glucosamine, chondroitin sulfate or hyaluronic acid. While these therapies have been successful in reducing inflammation, improving the joint environment and alleviating discomfort for a period of time. they do little to modify the disease itself. While steroids are useful for decreasing joint inflammation (thus decreasing joint pain), they can have a negative impact on the cartilage within the joint. The use of intra-articular (within the joint) steroids must be carefully considered depending on the joint needing treatment and the number of times a particular joint would require treatment for the horse to remain sound. Any therapy that can either alter the inflammatory cycle of OA or regenerate damaged tissues that result from OA, or ideally do both, would be a huge step in the treatment of OA. These cell-based, regenerative therapies have shown potential in both clinical research and actual clinical use to provide just the results that we have been searching for. There has been much research done on the use of these therapies in the treatment of equine



Figure 1: Diagrammatic representation of undifferentiated stemcell sources in equines. Courtesy Journal of Equine Veterinary Science.

lameness, but many of these studies, due to funding issues, have included only a low number of animals, have not had a control group to compare results with or have not been able to progress beyond the pilot stage of the study. The widespread use of regenerative therapies for equine lameness continues; our ability to share our clinical experiences as well as use what data is available to guide this type of therapy has allowed the successful treatment of many equine athletes.

While the use of regenerative therapies for the treatment of equine lameness is a very important and intriguing topic, I have also found other uses of these therapies to not only be interesting but also very practical and involving real "outside of the box" thinking. Here are some of the projects that researchers are working on that will be of real benefit to our horses:



Figure 2: Venogram of a horse with laminitis. Courtesy of American Farriers Journal.

LAMINITIS:

While not all horse owners have had first hand experience with laminitis, also referred to as "founder," the vast majority of them understand what a devastating issue this can be for a horse. Just to review, laminitis (inflammation of the lamina of the hoof) is a multifactorial disease that affects the hoof, more specifically the laminar structures that make up the very strong bond between the hoof wall and the coffin bone. Laminitis has several primary causes, such as grain overload or severe intestinal disease, as well as issues that can predispose a horse to laminitis, such as equine metabolic syndrome, stress or even poor hoof conformation. One of the end results of laminitis can be destruction of the blood supply within the hoof, which can be seen radiographically using a technique called a venogram. This technique uses contrast material to outline the individual blood vessels with the hoof revealing areas where the blood vessels are reduced or even absent usually on the front of the foot. A group of researchers hypothesized that a "local administration of mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP) might contribute to establishing an anti-inflammatory and pro-angiogenic environment and could stimulate the injured tissue in order to restore its functional integrity."2 Nine horses with severe laminitis that had not been successfully treated with conventional therapies were injected locally (in the vein of the lower limb) with MSCs and PRP three times at one week intervals. There were no adverse reactions noted. In all nine horses, there was evidence of revascularization of the foot as well as an improvement in the structure and function of the hoof and these horses returned to a comfortable quality of life. The authors note that the number of horses in this study was small but their results suggest, "that regenerative therapies in chronic laminitis could be useful, and are worthy of further investigation."³

EQUINE METABOLIC SYNDROME (EMS):

This is a condition that has been described in domestic horses and has become increasingly more prevalent. EMS is an endocrinopathy (a disease caused by the dysfunction of an endocrine gland, a gland that secretes its products or hormones directly into the blood) that is characterized by obesity, laminitis and insulin resistance (a failure of the body to respond correctly to insulin, a hormone that regulates movement of sugar from the bloodstream into tissues). Nutritional management and exercise have been the mainstays of therapy for this disease. It has recently been recognized that EMS can negatively affect the body's stem cells, in particular their ability to self-renew and differentiate. A group of researchers found that stem cells harvested from horses with EMS were unable to differentiate as expected into bone cells and that these cells also appeared to be in the process of apoptosis (programmed cell death). ⁴ Additional research looked at treating EMS horses with stem cells along with nutritional management and the results were promising. Stem cell possess the ability to modify the immune response or the functioning of the immune system and can act to improve the horse's response to insulin to help better control the blood sugar level.⁵ In human studies utilizing stem cells to treat diabetes, a specific type of cell, called a beta cell, was cultured from adipose-derived stem cells and implanted into a human patient. Beta cells are found in the pancreas and release insulin to encourage cells in the body to take up glucose from the blood. Researchers are taking this information gained from human studies and applying it to the horse with EMS. There are some questions to be answered, such as if the EMS horse's own stem cells should be used in its treatment or even which type of stem cell should be used. The fact remains that the use of stem cells, along with nutritional management and exercise, to treat a condition like EMS is exciting and definitely warrants further investigation.

ENDOMETRITIS:

Endometritis, by definition, is inflammation of the endometrium, which is the lining of the uterus. One of the most common causes of endometritis in the mare is the process of breeding the mare, termed persistent post-breeding endometritis (PPBEM). Endometritis is a common cause of infertility in the mare. In a normal, healthy mare, the deposition of semen, bacteria and debris into the uterus, whether by live cover or artificial insemination, causes a short, self-limiting endometritis that is usually cleared by 48 hours. Mares

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that are more susceptible to PPBEM may have less immune defenses and uterine clearance ability, thus making these mares unable to resolve the inflammation and leading to the accumulation of fluid within the uterus. Current therapies to treat this condition, such as uterine lavage and oxytocin, help to remove the products of inflammation but can do little to decrease or influence the inflammatory response itself. A group of researchers looked at using autologous conditioned serum (ACS) or mesenchymal stem cells (MSCs) to positively impact the uterus' inflammatory response to spermatozoa after breeding. Two separate experiments were performed using 6 normal mares. Experiment 1 looked at the effects of treating the uterus (intrauterine) with ACS, dexamethasone or a placebo after breeding. Experiment 2 investigated the use of MSCs or a placebo as an intrauterine treatment post-breeding. Treatment with either the ACS or the MSCs resulted in less neutrophils (a white blood cell that helps to heal damaged tissue and fight infection) in the uterus 6 hours after breeding. The researchers speculated as to how the ACS and MSCs were able to exert this positive effect but concluded that "Autologous conditioned serum and bone-derived culture expanded MSCs were able to modulate the uterine inflammatory response to spermatozoa in normal mares."⁶ This is exciting information for owners of mares that have chronic endometritis that hasn't responded to more traditional therapy. ACS and MSCs provide another "tool in the toolbox" to assist in the successful breeding of this type of mare.



Ultrasound image of excessive edema in the uterus of a mare.

OTHER POTENTIAL USES FOR REGENERATIVE THERAPIES:

Peripheral nerve damage – MSCs are being investigated as a way to repair damaged nerves.

Corneal injury and healing – MSCs are being evaluated as a method to promote corneal wound healing. Initial results are promising and MSCs would provide veterinarians and owners an immediate and fairly cost-effective treatment.

Skin wounds – it has been found that factors secreted by equine MSC do act on cell types found in horse skin wounds in ways that may lead to improved wound healing. These initial results are promising and warrant future studies to test the efficacy of using MSCs, and thus these factors that they secrete, as a

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treatment for horse skin wounds in a practical "every day" setting.

Equine Asthma – research into the use of MSCs in the treatment of human asthma has led researchers to look at the horse as a potential model to evaluate MSCs ability to repair the damage and lessen the response of lung tissue to allergens. This is due to the fact that asthma is a naturally occurring condition in the horse, much like the human. "Cell therapy may prove advantageous over conventional therapy in that it may repair or regenerate the site of injury and reduce the reaction to allergens, "ather than simply modulating the inflammatory process."

Muscle Repair/Regeneration – It was found that an additional "spin" of PRP to remove more of the platelets, leading to a platelet-poor plasma, may be more useful to stimulate myoblasts, the cells needed to repair and regenerate muscle tissue.

It sure seemed that the more I looked for novel ways to use regenerative therapies, the more I came to realize that the possibilities seem almost endless, at least at this point in time. While we know and understand quite a lot already about these therapies, there is so very much yet to learn. It is almost inevitable that some of these experiments will fail or at least not pan out to be as effective as we might hope, but I have no doubt that regenerative therapies are here to stay and will find their place in advancing a wide variety of treatments of our equine partners.

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