

VETERINARY REGENERATIVE MEDICINE (PART 1)

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REGENERATIVE MEDICINE, THE MEDICAL SCIENCE OF REPLACING OR REGENERATING CELLS, TISSUES OR ORGANS TO RESTORE OR ESTABLISH NORMAL FUNCTIONS IN THE BODY, IS A RAPIDLY GROWING FIELD IN EQUINE VETERINARY MEDICINE AND WITH GREAT POTENTIAL. IT REQUIRES A DEEPER INVESTIGATION TO SELECT THE BEST TREATMENTS AND BETTER EVALUATE THEIR RESULTS SINCE NOT ALL PRODUCTS ARE EQUAL AND SOME OF ITS MECHANISMS OF ACTION ARE NOT YET FULLY UNDERSTOOD.

In recent years, equine sports medicine has made very significant advances: diagnostic methods such as digital ultrasound, digital radiography and magnetic resonance imaging and the development of new therapeutic options. The advances in the field of tissue regeneration especially opened a new opportunity for treating problems which in the past were considered the end of a sports career.

To understand this field, it is important to distinguish three concepts:

1. Healing: A natural process in which the body repairs itself.
2. Repair: Re-establishment of health and function after injury or damage.



▲ Figure 1 Obtaining APC (PRP)

3. Regenerate: Replacement of a tissue (or organ) lost or damaged by the formation of new tissue identical to the original.

To avoid misunderstanding, it should be clear that only the regeneration of a tissue restores it to its original structure and function. Repair with scar tissue formation, especially in tendons and cartilages, although providing structural support is always of lower quality and causes lower tissue performance (weakness), which often leads to repeated injury.

Tissues need three components to regenerate properly:

1. A support structure, which provides the matrix on which cells can grow.

2. Growth factors, to provide the necessary stimulus for the cells to grow (Autologous Blood Concentrates).

3. Cells, able to produce a suitable tissue matrix (Cell Products).

AUTOLOGOUS BLOOD CONCENTRATES

Autologous Platelet Concentrate (APC):

The APC commonly known as Platelet Rich Plasma (PRP) is an important therapeutic tool to naturally accelerate regeneration of ligaments, tendons and cartilage. Platelets contain more than 20 growth factors and cytokines in their cytoplasmic granules. The most important ones are platelet-derived growth factor (PDGF), transforming

growth factor beta (TGF-), the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and the insulin-like growth factor (IGF-I and II).

The purpose of the method is to obtain a high concentration of platelets in a small volume of plasma (Figure 1) separated from other cellular components of blood (red and white cells).

There are several commercial systems and kits to produce PRP from peripheral blood of the horse.

In our Equine Clinic (Portugal) we have been using PRP to treat orthopaedic injuries in sport horses for many years and verify the best results with the double tube centrifugation method and ultrasound guided injection directly inside the lesion (Figure 2). It should be performed in a hospital environment and under strict surgical asepsis to prevent contaminations during the preparation.

Although the PRP is not a cell therapy, there is evidence of recruitment of mesenchymal cells (adult stem cells) that migrate to the site of injury due to the chemotactic effect of the growth factors.

Autologous Conditioned Serum (ACS):

ACS, or IRAP as is commonly known, has an analgesic and anti-inflammatory effect, based on the inhibition of interleukin-1, an important inflammatory mediator.

The method is based on the selective increase of interleukin-1 receptor antagonist protein (IRAP) from the patient's own blood.

Producing ACS requires incubation at 37° C for 24 hours of venous blood in the presence of glass beads. Blood leukocytes produce high amounts of anti-inflammatory cytokines that accumulate in the serum.

The IRAP is used primarily for the treatment of osteoarthritis (OA) in horses, but due to its complexity and



▲ *Figure 2 ACP (PRP) ultrasound-guided injection*

elevated cost it is becoming less interesting in light of the growing evidence of the efficacy of PRP in the treatment of equine OA.

Bone Marrow Aspirates:

Bone marrow contains mesenchymal cells, other types of cells and platelets. Concentration of growth factors is similar to that of the PRP. Some veterinarians still use bone marrow obtained by sternal or iliac puncture as direct injection (or after centrifugation) for the treatment of tendon injuries.

But treatment with raw bone marrow should not be confused with stem cell therapy because the concentration of these cells in the bone marrow is extremely low (0.01% to 0.001% of mononuclear cells). It is a very invasive technique without significant comparative advantages and tends to become obsolete.

CELL PRODUCTS Stem Cells:

Stem cells are undifferentiated cells that have the ability to divide or self-renew indefinitely. They can differentiate resulting in various types of cell lines and turn into

mature cells with specialized characteristics and functions, like nerve, heart, skin, blood, bone and cartilage cells.

Mesenchymal Stem Cells (MSC):

MSCs obtained from adult tissues are a population of undifferentiated multipotent cells, capable of differentiating into various tissues of mesodermal origin such as bone, cartilage, ligaments, tendons and muscles. MSCs can be isolated, expanded by replication and induced to differentiate into various cell lines.

Almost all tissues contain MSC including bone marrow, fat tissue or umbilical cord tissue. However, the content of MSCs varies significantly among different tissues.

In Veterinary Medicine, MSCs obtained from the patient's own tissues (bone marrow or fat tissue), are sent to a specialised laboratory (Figure 3) where they are expanded *in vitro*. Once it reaches the adequate cell number to the extent of the lesion (usually 10-20 million), these are sent to the veterinarian for implantation by ultrasound-guided injection inside the lesion (tendon or ligament) or joint.

In our Equine Clinic we have been using fat tissue derived MSCs (Figure 4) from the horse itself (with or without combination with PRP) to treat tendon and joint diseases in sport horses. These therapies have given us very satisfactory results. Unfortunately, the invasive nature of the cell collection methods and its elevated cost, limit its application to a few high-performance, high-value horses.

After being treated with growth factors (PRP) or MSC implantation, the horses should be subjected to a rigorous rehabilitation program with levels of exercise increasing slowly and progressively.

Clinical recovery of horses treated with regenerative therapies in our clinic depends largely on a thorough case selection, rigorous standardized protocols and close supervision during the execution of the rehabilitation plan, specific to each individual.

In the second part of this article we will present some of our case studies and future prospects of Veterinary Regenerative Medicine. 🏠

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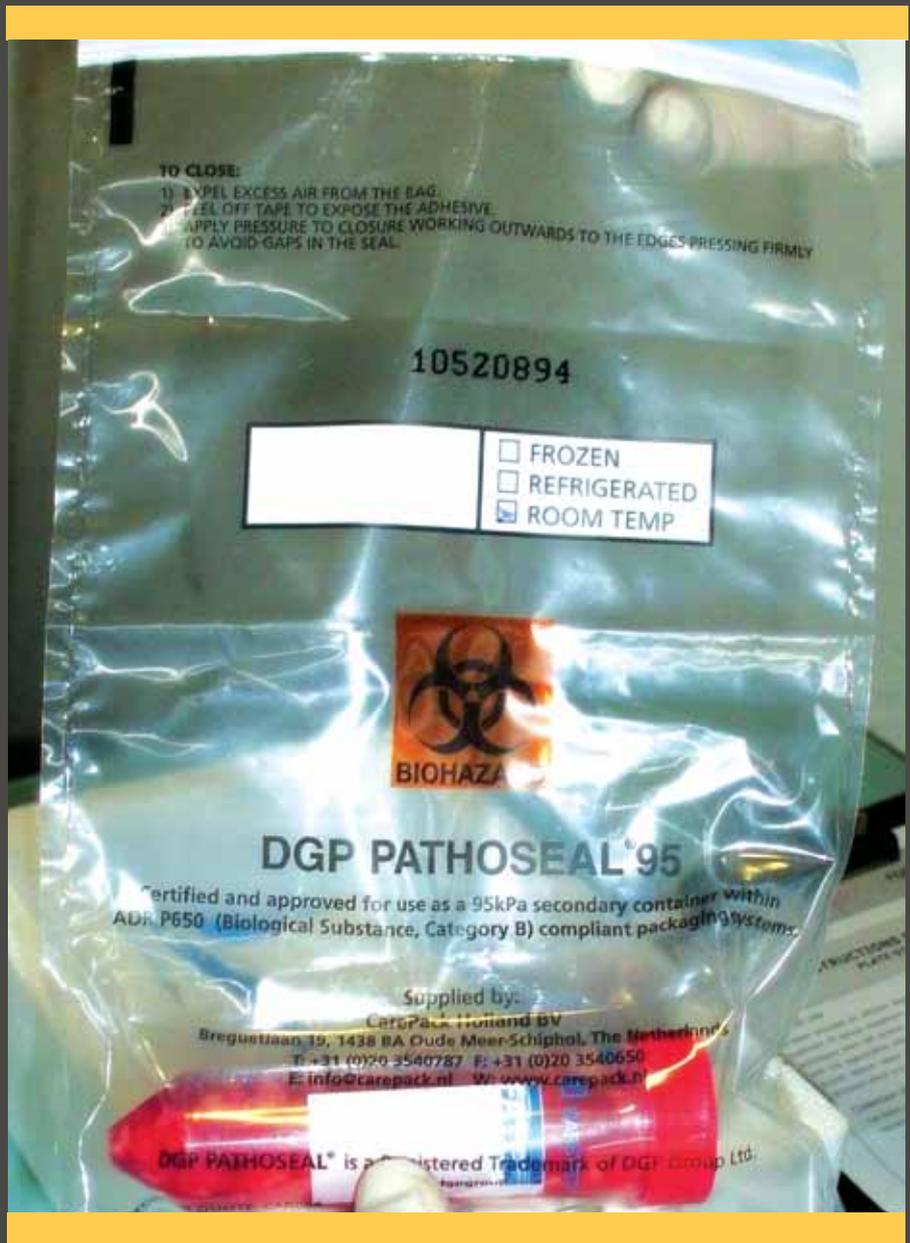
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▲ Figure 3 - Sample ready for shipment

▼ Figure 4 - Fat tissue collection

