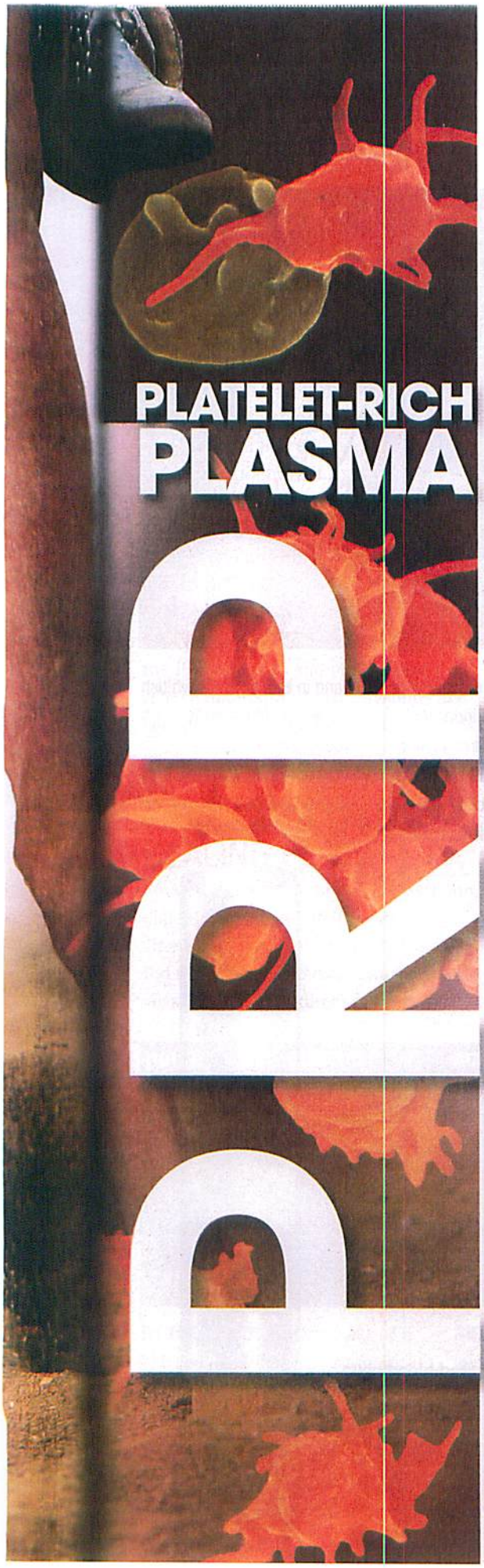


PLATELET-RICH PLASMA

PRP

HORIZONS IN HEALING

The tiniest blood cells carry powerful healing forces that can be harnessed for several therapeutic uses.

By Laurie Bonner

Every time you cut or bruise yourself, you experience the healing power of platelets. The smallest of the blood cells, platelets are well-known for their role in forming clots or scabs to protect injured tissue, both at and below the surface of the skin.

But platelets do more than just build those protective barriers. They release several substances, called growth factors, that stimulate the generation of healthy new cells to hasten healing of the damaged tissue. For decades physicians have harnessed this healing power by creating platelet-rich plasma (PRP)—liquid plasma, minus the red cells, that has four times or more of the normal amount of platelets.

"In human medicine PRP therapy has been around for about 25 years," says Bob Harman, DVM, MPVM, CEO of Vet-Stem, Inc., of Poway, California, which markets a system for creating equine PRP. "It has been used in joint-replacement surgeries, non-healing wounds in diabetic patients, plastic and reconstructive surgeries, dental repairs—anything where there is lots of soft-tissue injury."

More recently, PRP has been making inroads into sports medicine, generating publicity with high-profile cases. Pittsburgh Steeler Hines Ward received the therapy for a sprained knee ligament, then recovered enough in two weeks to play in the Super Bowl in 2009. Tiger Woods received PRP injections after knee surgery in late 2008, then he played in all four pro golf majors in 2009. Pitcher Takashi Saito of the Los Angeles Dodgers received PRP injections for a torn ligament in his elbow before pitching in the 2008 Major League Playoffs.

PRP has also been used in veterinary medicine, especially for wound care, since the early 2000s, but the attention in the sports pages helped spur interest in the therapy as a treatment for tendon and ligament injuries in horses. And, says Harman, "PRP has become widely available in just the past couple of years." No one has counted the horses who have now been treated with PRP, but he says, "the number is certainly in the thousands."

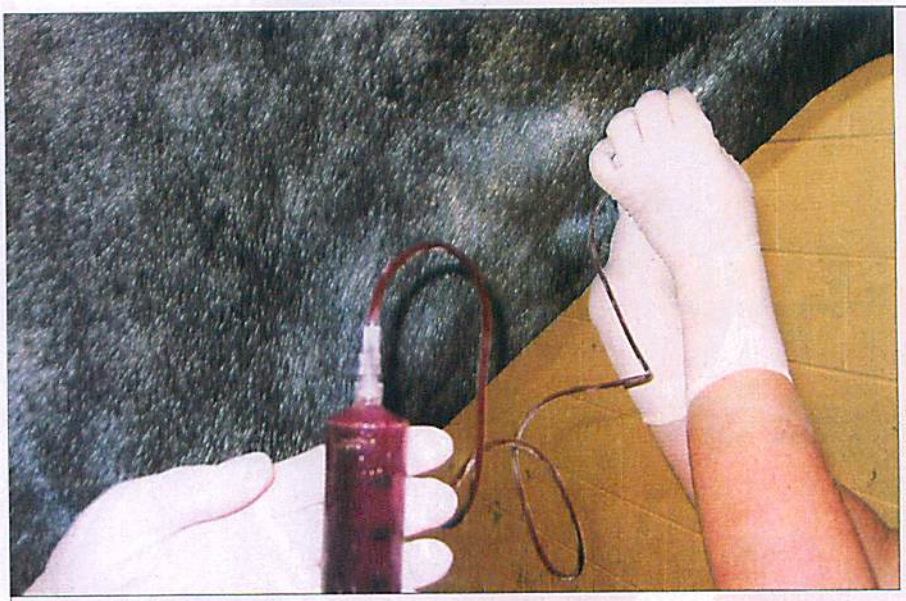
The most effective ways to use PRP therapy in horses—the types of injuries

HORSE: JANIS TREMPER; CELLS: WWW.VISUALSUNLIMITED.COM

that respond to it best and the most effective protocols—are still being explored. Still, the few studies done in horses have been encouraging. And many veterinarians who use it regularly have been pleased with the results.

"We are still on the cusp of learning all the benefits PRP can offer," says Carrie Schlachter, VMD, of Circle Oak Equine Sports Medicine and Rehabilitation in Petaluma, California. "I feel confident in saying that PRP offers a way to jumpstart healing in injuries when they are at certain stages."

Here's a closer look at what PRP is, how it's used and how it might help your horse.



COURTESY: VET-STEM INC.

TO CREATE PRP: First, a horse's blood is drawn into a syringe that has been preloaded with an anticoagulant to prevent clotting. The sample is then spun in a centrifuge to separate the individual components. Three layers result: heavy red cells form the bottom strata, clear yellowish liquid plasma rises to the top, and in between is a whitish layer, called the buffy coat, which contains concentrated platelets. This is the part that is drawn off for use in PRP therapy.

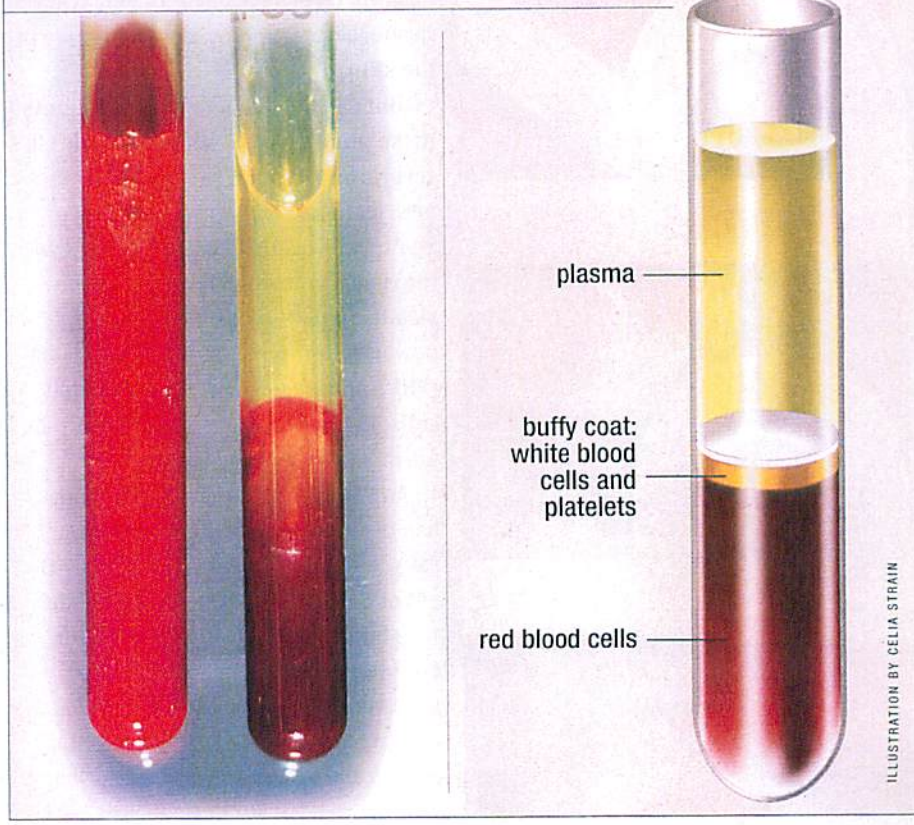


ILLUSTRATION BY CELIA STRAIN

THE **PRP** PROCESS

Normal blood consists of solid cells, which make up about 40 to 45 percent of the total volume, suspended in liquid plasma (55 to 60 percent of the total). Of the solid cells, about 94 percent are red blood cells (RBCs), less than 1 percent are white blood cells and the remaining 5 to 6 percent are platelets. The procedure for isolating those platelets to create PRP is relatively simple:

1. About 50 milliliters of the horse's blood is drawn into a syringe that has been preloaded with an anticoagulant to prevent clotting.

2. The sample is placed in a centrifuge and spun either once or twice, depending on the system, to separate the individual components out of the plasma. If you held up the sample tube after spinning, at the bottom would be a dark layer of the heavier red blood cells; at the top would be clear, yellow serum. At the border between the two is the buffy coat—a thin, almost clear layer where the white blood cells and platelets collect.

3. The buffy coat layer and a small amount of the surrounding plasma and packed RBCs are then drawn off and

COURTESY: VET-STEM INC.

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used in patient treatment. The PRP at this stage may be mixed with an agent to counteract the anticoagulant. For an internal injury, the liquid is injected directly into the tendon or ligament. For a surface wound, the PRP can be loaded into a spray applicator to coat the tissue evenly, or it can be turned into a gel to be packed into an open cavity.

The whole process can be done in one treatment visit, assuming the veterinarian has access to the specialized centrifuge (commercial systems designed to create PRP run at different speeds than standard laboratory centrifuges, and many come with sample containers that automatically separate the PRP, red cells and plasma into separate chambers). But these systems are becoming common, especially in sports medicine practices and larger veterinary clinics.

CONCENTRATED HEALING POWER

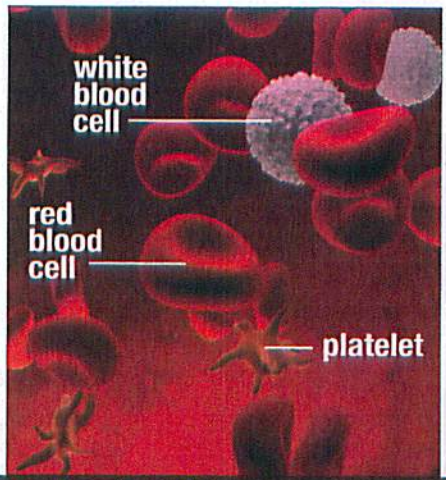
As they circulate in the bloodstream, platelets are rounded, if irregularly shaped—somewhat like sea sponges. But when they encounter injured blood vessels, platelets adhere to the damaged tissue and—as part of a complex cascade of events—are stimulated to convert a protein called fibrinogen, also found in the blood, into fibrin, a protein that forms long, sticky threads.

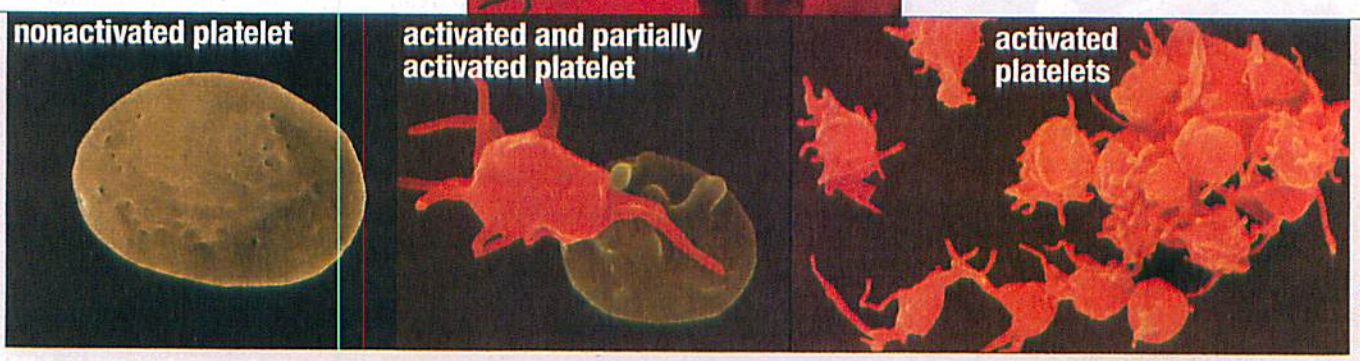
These fibrin tendrils catch and stimulate more platelets, and eventually the threads interlock into a clot; if exposed to air, the clots dry to form scabs, which act like shields to keep the wound clean and free of infection. "This fibrin clot forms a 'scaffold' or 'framework' upon which healing tissues can migrate," says Aimie Doyle, DVM, DACVS, of the University of Prince Edward Island.

But as the platelets undergo the changes that help them form clots, they also release several growth factors—substances that, among other functions, stimulate the generation of new replacement cells—as well as fibrin and other proteins. The growth factors platelets release include:

- *transforming growth factor beta* (TGF-b), which increases the synthesis of collagen, the main component of connective tissue in the tendons, ligaments, skin, blood vessels, cartilage and bone
- *platelet-derived growth factor* (PDGF), which plays a role in the formation of new blood vessels as well as new bone tissue
- *vascular endothelial growth factor* (VEGF), which stimulates the formation of new blood vessels
- *epidermal growth factor* (EGF), which also stimulates cell division and helps form new bone and muscle tissue
- *fibroblast growth factor-2* (FGF-2), which also plays a role in the formation of new blood vessels and other new tissues.

"These growth factors play an important role in all phases of healing," explains Doyle. "The fibrin clot 'scaffold' will have the same effect whether you





AT THE READY: Platelets make up 5 to 6 percent of the total cells in whole blood. They normally circulate in a rounded, inactive form.

INTO ACTION: When platelets encounter injured tissue, they form long, sticky threads that interlock to form clots. They also release bursts of factors that stimulate the growth of replacement tissues.

HEALING POWER: In PRP therapy, concentrated platelets are injected into injured tendons and ligaments in hopes that the growth factors they release will speed healing.

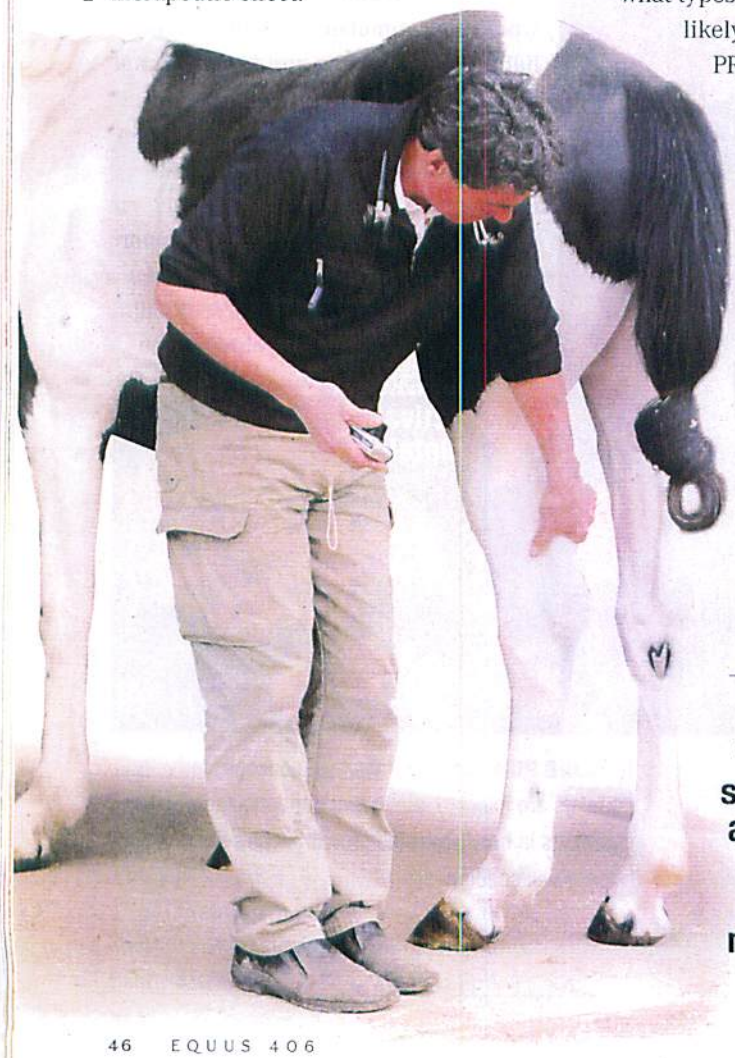
ILLUSTRATION BY CÉLIA STRAIN

DENNIS KUNKEL MICROSCOPY, WWW.VISUALSUNLIMITED.COM

are dealing with a wound in the skin or a tear in other soft-tissue structures such as tendon or ligament. Basically, using PRP promotes faster and better quality healing of soft-tissue injury."

Whether the benefits of PRP therapy arise from the actions of any one or two growth factors or from the synergistic effect of all of them working together is not fully understood. "We have no data to answer that question," says Harman, "but, for sure, no single growth factor has all of the effects that multiple growth factors can offer. So I've got to say we don't really know for sure, but my personal feeling is that it's a synergistic effect." It is also not known whether the presence of the white blood cells in a PRP sample contribute to the therapeutic effect.

DUSTY PERIN



PRACTICAL APPLICATIONS OF PRP

Researchers are still working to identify the best uses for PRP therapy. "PRP is one of those things that took off in horses without a lot of data to support it," says Harman. "Although plenty of research has been done for human medicine, no well-controlled, peer-reviewed papers have been published on the clinical outcomes of PRP in horses, and they are different than people." In other words, no one has yet taken two groups of horses with identical injuries and shown that the ones treated with PRP heal faster and remain sound longer than the ones who aren't. No one has yet determined what types of injuries are most likely to respond well to PRP or when the treatment is most effective.

Nonetheless, several PRP studies done in horses have shown positive results for tendon and ligament injuries. In a 2008 clinical trail conducted at Ohio State University, for example, all nine racing Standardbreds whose severe suspensory ligament lesions were treated with PRP returned to competition within a median time of 32 weeks, and their race records were comparable to those of horses who had never been injured.

Likewise, two 2010 studies from Utrecht University in the Netherlands reported that injured equine tendons treated with PRP for 24 weeks had higher levels of collagen, glycosaminoglycan and more new structural cells and blood vessels than did the tendons receiving a control (placebo) therapy. The researchers also observed that the PRP-treated tendons were stronger and more elastic.

In current practice, PRP is most often used to accelerate the earliest stages of healing—including the generation of new vascular and structural tissues—in tendon and ligament injuries that, because they tend not to get as much blood flow normally, would otherwise heal more slowly. The effects of PRP are short-lived. "If the PRP is clotted (i.e., with thrombin) then the majority of growth factors will be released within 24 hours; if injected unactivated then release is more gradual over several days," says Harman. "After 10 days, they're all gone."

In chronic wounds, the PRP can restart the healing when the natural process has slowed. "An injury that is healing more slowly than normal will often show a marked increase in healing within a 30-day period post-injection," Schlachter says.

As with any other tendon or ligament injury, after receiving PRP a horse will need to progress slowly through rest and rehabilitation to avoid tearing the fragile new tissue as it grows in. "The horse will still require appropriate rest and controlled exercise in a rehabilitation period dictated by ultrasonographic monitoring

PRP is most often used to accelerate the earliest stages of healing in tendon and ligament injuries that, because they tend to not get as much blood flow normally, would otherwise heal more slowly.

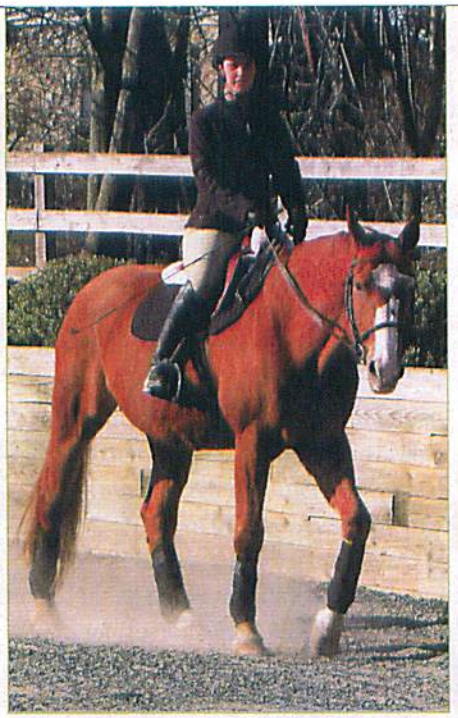
A CLEAR CHOICE

Kelly Haare's plans changed abruptly when she arrived at the barn for a lesson on January 20, 2011. Zimpatico, her 21-year-old Hanoverian gelding, had noticeable swelling on his right front leg, from an inch or two below the knee down to and including the fetlock, and he had a bump on the outside of the leg. Although he wasn't significantly lame—"he was just very slightly off at the trot," she says—Haare called her veterinarian, who recommended a regimen of rest, hand-walking, cold hosing and bute.

But when "Zee" didn't improve after a week, Haare scheduled a more thorough examination, and an ultrasound revealed a two-centimeter tear in his suspensory ligament, a major support structure for the back of the leg, right where it branches over the pastern. Conferring with her veterinarian and a veterinary surgeon, Haare was given four options:

- **Do nothing and let it heal with rest alone.** With that choice, Haare says, "the prognosis was good for a complete recovery, but it would take six to 12 months of stall rest, and the area would most likely remain weakened, with low-quality fibers in the ligament."
- **Extracorporeal shock wave therapy (ESWT),** a noninvasive treatment that stimulates healing by directing a tightly focused beam of acoustic waves at the injured tissue. "This was a good option, and the least aggressive treatment, but with three recommended farm calls over six to 12 weeks, the cost was comparable to PRP," says Haare, and the results would be less certain.
- **PRP treatment.** This choice, Haare was told, "would likely provide a faster, stronger heal. It was cost effective, because it could be done in one day at the clinic, and it was more aggressive than ESWT, but not too terribly invasive."
- **Stem cell therapy.** This option involves harvesting fat or bone marrow cells from within a horse's body, sending them to a lab to produce undifferentiated stem cells, which can then develop into any tissue cell, and injecting them into the injury site to help build healthy new tissue. "The surgeon believes this is a promising treatment," says Haare, "but in Zee's case, because the tear was small, it was unlikely to give significantly better results than PRP, yet the cost would be three to five times more."

To Haare, the choice was obvious: "We chose the PRP treatment because our vet and surgeon both believed it gave Zee the greatest chance for a faster recovery and a stronger suspensory in the long run. When we compared the costs of other treatments, the PRP was the clear winner."



HAPPY BIRTHDAY: Kelly Haare rides her gelding, Zimpatico, on his 22nd birthday, just five weeks after he received PRP treatment for a tear in his suspensory ligament.

Zee received PRP treatment on February 8, and Haare was given instructions for a 30-day regimen of bute, stall rest and steadily increasing amounts of hand-walking. The month went without a hitch, and by mid-March, on a follow-up ultrasound, the tear was down to a half centimeter. Haare was given clearance to ride at a walk, and on March 19—Zee's 22nd birthday—she did just that. He was perfectly sound and ready to go.

Zee was a made hunter in his prime, and Haare had been slowly bringing him back after he'd been out of work for several years but, with her veterinarian's advice, she says, "We've switched to low-level dressage. But his main 'job' is still to be my pleasure horse and best buddy!"

To anyone else considering PRP, Haare says, "Listen to your vet and consulting surgeon. If your medical team recommends it, PRP is a great tool to assist in the healing process. But keep in mind that rehab is still long and tedious. Even after PRP treatment, daily care such as hand-walking, monitored turnout, exercise and keeping him calm were crucial to a healthy recovery."

But, Haare adds, the effort and expense were well worth it: "I've spoken with a lot of people who still believe in the old way of throwing horses out in a pasture for a year to heal. PRP gives us the chance to bring these guys back faster and stronger, at a cost that's manageable for most people. My main concern is Zee's mental and physical well-being. PRP treatment has helped us get him through without losing his physical condition and kept him from getting bored or depressed from too much stall rest."

of the injury," says Doyle. "Too early of a return to exercise will compromise healing and potentially cause a major setback requiring the rehab to start all over from the beginning." Costs vary by the geographic area and the method the veterinarian uses to generate the PRP but may range from $500 to $1,200 per treatment.

Researchers are also exploring the use of PRP to accelerate healing in equine wounds, including surgical incisions. In a 2009 Brazilian study using six horses with identical surgical skin wounds, those treated with PRP formed new skin more quickly and had denser and better organized structural tissue.

RISKS AND CONSIDERATIONS

With PRP, the risk of side effects is low—because the platelets used in the therapy are drawn from the horse's own blood, the body is not likely to recognize them as "foreign" and mount an immune response. However, any injection carries the possibility of infection at the site of the puncture wound. Furthermore, says Doyle, "PRP for tendon and ligament injuries requires accurate placement of the needle directly into the lesion. Therefore, ultrasound guided injection is necessary—but the anatomic location of some injuries may make imaging difficult."

The timing of PRP therapy also appears to be important: Its efficacy tends to diminish if treatment is delayed too long. "I typically recommend treatment after the initial inflammatory period subsides, two to four weeks after injury," says Doyle. "The benefits decrease as the injury becomes more chronic. However, an injury that has become 'static' in healing in the chronic stage can still respond well to PRP."

Another factor that may affect the outcome of treatment is the quality of the PRP itself. Some horses, for example, may have fewer platelets in their blood, and the amount that gets concentrated in the PRP will be correspondingly lower.

Currently, PRP is often recommended for smaller tendon lesions and tears. "PRP can play a useful role in helping minor injuries heal faster, but we do not recommend PRP for a major injury to a tendon or ligament," says Harman. "Platelets do not have a nucleus and thus cannot respond to a stimulus. So when PRP is injected into a wound, regardless of the stage, it will always do the same thing. Larger injuries do better with a cell-based therapy, which is more responsive to the local environment and can create strong new tissue faster."

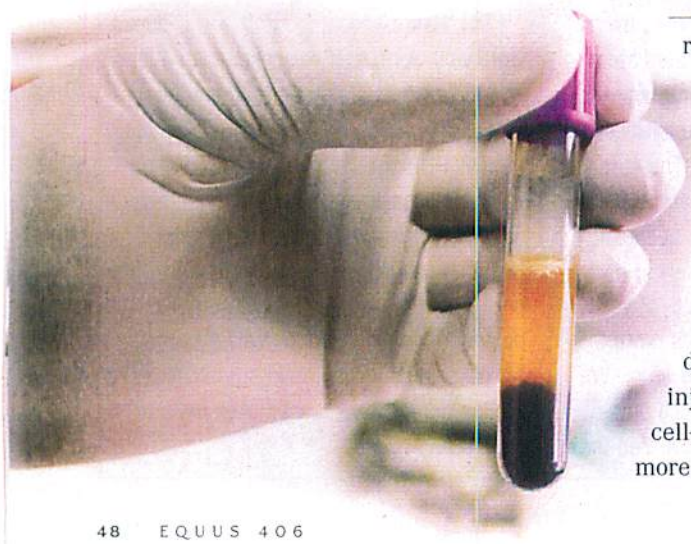
Some horses may have fewer platelets in their blood, and the amount that gets concentrated in the PRP will be correspondingly lower.

On the other hand, Doyle received firsthand experience with the application of PRP to severe tendon injuries when one of her patients, a Thoroughbred mare named Breazy, severed both the superficial and deep digital flexor tendons in one leg. Doyle decided to treat the mare with PRP because the gap between the surgically repaired tendons had not closed weeks after the injury. "I wasn't sure how the response would be because I didn't have a nice lesion to inject it into," she says. With a core lesion you inject the PRP into it and it stays there. With the laceration, I was just injecting it into an open space. I wasn't sure if it would stick around where I put it. However, as we saw, the results were very impressive."

Two weeks after the treatment, new tissue had bridged the gap in her tendon, and after two years of careful rehabilitation, Breazy was sound enough to begin training for dressage. (To read more, see the Case Reports "A Big Bet on Breazy," EQUUS 401, and "Breazy Beats the Odds," EQUUS 402) "So far, I don't have a particular type of injury I don't recommend it for," Doyle adds.

The early history of any therapeutic treatment is both exciting and uncertain, and PRP is no exception. While plenty of experienced veterinarians are confident it helps injured and wounded horses heal faster and better, the data is still emerging to verify and explain those observations. In the coming years, however, continued field experience and research efforts will combine, and a clearer picture of the role of PRP in the veterinary arsenal will emerge.

Coming in the September issue: Stem cell therapy



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