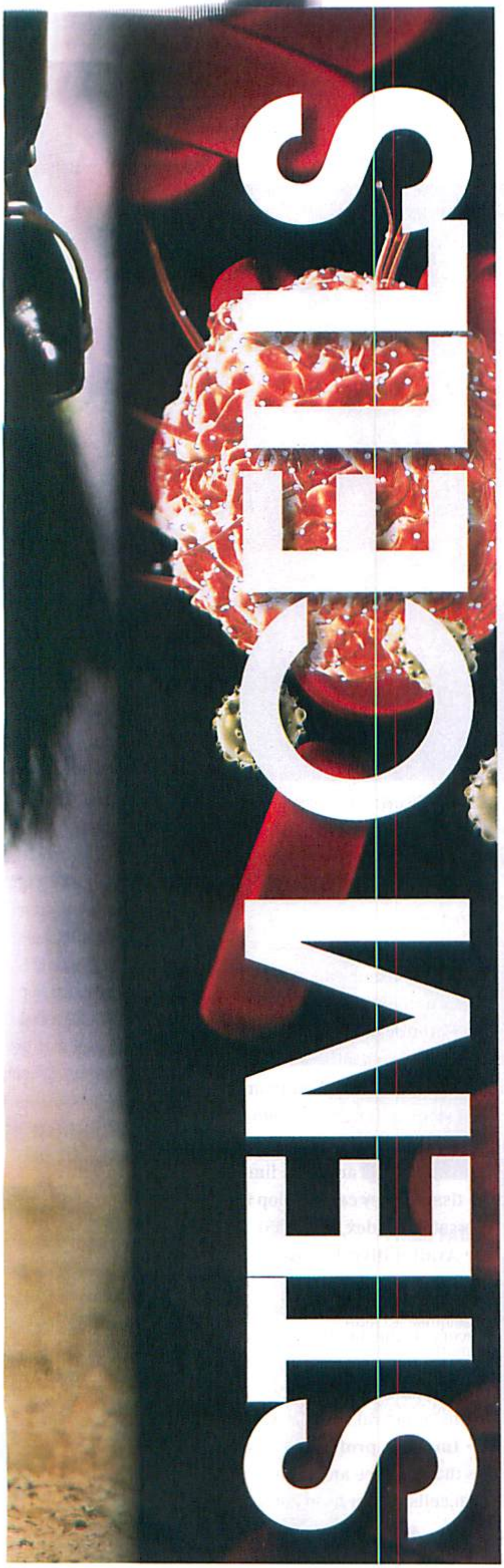


FREDERIC CHEHU/ARND BRONKHRST PHOTOGRAPHY

Stem cell therapy is already restoring soundness in horses with serious orthopedic injuries. Soon this innovative treatment may also offer real cures for dozens of equine diseases.

By Laurie Bonner

Few topics in human medicine have been as controversial in recent decades as stem cell therapy. Nor has any other treatment method shown as much promise for treating a myriad of deadly and debilitating human ailments, including Parkinson's disease, leukemia and multiple sclerosis.

Stem cell therapy has also been making inroads into equine medicine. It has been used to treat serious tendon and ligament injuries, and the door is opening to explore the use of stem cells to treat laminitis°, uveitis°, recurrent° airway obstruction (heaves) and other serious problems.

This relatively new type of therapy offers a promise different from other treatments. Stem cells go beyond simply aiding healing or controlling inflammation to create an environment conducive to tissue regrowth; they may actually help improve tissue repair and decrease scarring.

"It offers the hope and chance to heal injuries more completely than ever before," says Carrie Schlachter, VMD, of Circle Oak Equine Sports Medicine and Rehabilitation in Petaluma, California.

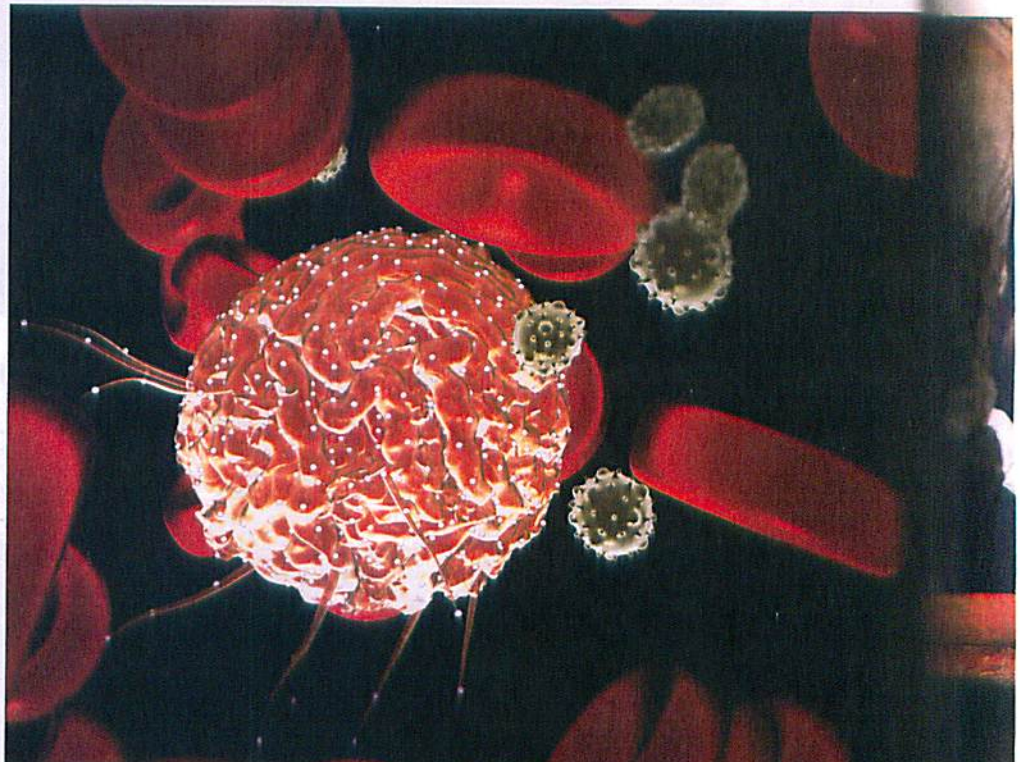
Yet stem cells, like any new therapy, have limitations as well as potential. "Stem cell therapy is not a panacea," says Gregory L. Ferraro, DVM, director of the Center for Equine Health at the University of California-Davis. "Regenerative medicine is still in its infancy, and clinicians and medical scientists have a lot to learn regarding its use."

Here's a closer look at what stem cells are, how they work and how they can be used to help your horse.

WHAT STEM CELLS **DO**

You are probably familiar with the rudiments of what stem cells are and how they work. They are "undifferentiated," which means they haven't yet developed into specialized cells. A stem cell can create either a copy of itself by dividing or a new cell by differentiating into a cell with a specialized function, ready to replace another nerve, organ, muscle, skin or bone cell.

Stem cells play their most significant role in the body during youth: They are how we grow. "When you feed your daughter corn flakes in the morning, and she gets bigger, it's not the food that actually creates new tissue," says Greg McGarrell, chief operating officer of V-Care Biomedical GmbH, based in Leipzig, Germany. "The body keeps a reservoir of stem cells in all its tissues



REPAIR CREW: Stem cells, shown among blood cells in this stylized image, occur naturally throughout the body. They turn into specialized cells when needed to repair injured tissue.

as it grows, and they are like the 'bricks' that build new structures to make our bodies bigger. When the tissue sends out the signal, 'We need bricks,' the stem cells show up and are used in the construction."

As it grows, the body generates new stem cells to meet two demands--to create new tissue and to heal injuries. That is why children recover so quickly and completely from a broken arm, for example. "But," says McGarrell, "once you reach skeletal maturity, the 'brick factory' shuts down. We keep a reservoir of stem cells around to be used in repairs, but there aren't as many bricks left."

And that has implications when an adult is injured. "In nature, a horse might expend a burst of energy to run from a predator, and each time he does it, there might be a little damage, a little inflammation," says McGarrell. "The damaged tissue sends out a signal, and the stem cells naturally migrate to the area and infiltrate the injury and repair it. When the body doesn't have enough bricks, it gives us something rather than nothing, and that is scar tissue."

Scar tissue is less elastic than the normal tendon and ligament structures it replaces. And the more scar tissue a horse accumulates from small inflammatory events, the greater the likelihood that he will eventually experience a major tendon or ligament injury. "Once you have a catastrophic injury, it is so unnatural that we could never get enough stem cells to repair it," says McGarrell. "When the tissue sends out its repair signals, you might have a few dozen stem cells show up, but there might be a need for millions."

The goal of stem cell therapy is to supply the construction materials the body needs to repair itself. "We've learned how to turn off the switch that says 'don't make too many of these cells'; in effect, we can turn the brick factory back on," says McGarrell. "So we go in and get a few of these stem cells, flip the switch, and then we can grow them into the millions. We put them back in the patient, and suddenly the body has got the bricks it needs to repair the damage."

STEM CELL CHARACTERISTICS

Stem cells are derived from various sources. In addition to embryonic or fetal stem cells, there are also somatic cells—those taken from an adult—which are isolated from many body tissues, including skin, blood, bone marrow, umbilical cord tissue or blood, and fat. But even within these two broad categories, cells can differ from each other in many ways, including:

• **Life span:** their ability to replicate themselves for long periods in a laboratory. Cell lines drawn from embryos, for example, can survive and reproduce for over a year, but cell lines isolated from adult tissue can survive only weeks or months.

• **Potency or plasticity:** the number of different specialized cells they are capable of creating. Embryonic and fetal stem cells can turn into more than 200 different adult cell types, whereas adult stem cells are more limited in the tissues they can develop into (see "Versatility Index," page 35).

• **Availability:** how readily stem cells can be isolated in useful quantities. Stem cells may be present in nearly every tissue, but they are far more plentiful and accessible in fat or bone marrow, for example, than in internal organs or neural tissue.

• **Immune profile:** the characteristics that produce an immune response. Stem cells drawn from adult tissues

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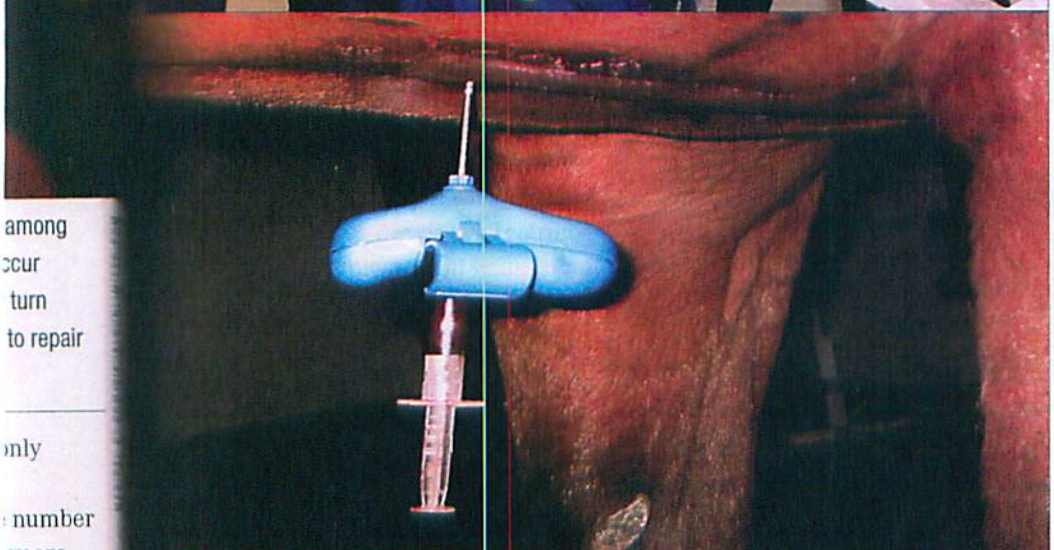
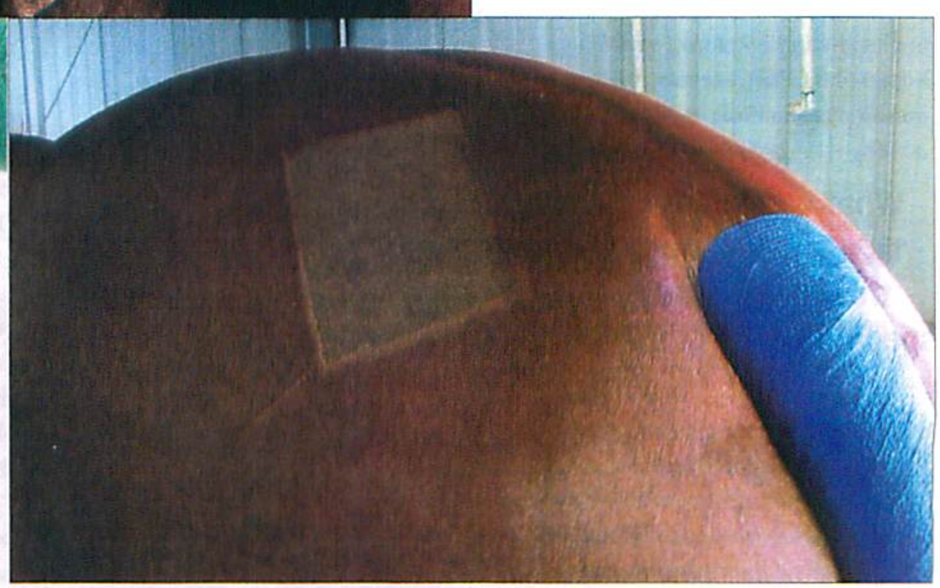


FROM BONE: Stem cells can be derived from bone marrow drawn from the sternum or hip and used to treat injured tendons and ligaments.

COURTESY, ADVANCED REGENERATIVE THERAPIES

retain their immune profile, which raises questions about whether they may trigger a rejection response if injected into an individual other than their donor. In practice, however, adult stem cells have not caused rejection issues in horses or people and in fact may dampen the immune response. Nevertheless, the only forms of stem cell therapy currently approved by the Food and Drug Administration must be derived from the patient's own body. Stem cells derived from embryonic and fetal tissues have a lessened immune profile.

The stem cells currently in most common use in horses are somatic mesenchymal cells, those that are predisposed to turn into tendon, ligament, bone, cartilage, muscle or other connective tissue. And the most common sources of stem cell therapy are either fat or bone marrow samples drawn from the horse who requires treatment. Stem cells derived from umbilical blood are also now available and are often



FROM FAT: Stem cells can also be isolated from fat samples harvested from various sites on a horse's body.

COURTESY, VET-STEM INC

cryopreserved and banked for future use, and equine fetal-derived cells are currently undergoing trials and may become available within the next few years (see "Sources of Therapeutic Stem Cells," page 36).

HOW STEM CELL THERAPY **WORKS**

The stem cell therapy procedure is relatively straightforward: The veterinarian harvests a tissue sample from the horse's bone marrow or fat and sends it to a laboratory, where technicians isolate the stem cells and create a therapeutic dosage, a process that takes a few weeks depending on the number of cells desired. The stem cells are then returned to the veterinarian, who injects them into the injury site. The laboratory's work is relatively complex and specialized, and the service is currently available from only a handful of veterinary hospitals and private companies.

What the stem cells do once they are within the injured tissue sets them apart from other treatments currently available: They respond to the specific needs of the particular injury. "They size up the injury and its real nature, and then they multiply and produce just enough new cells to fill the lesion and no more," says Oleg Kopyov, MD, PhD, executive vice president and chief research officer

What stem cells do within injured tissue sets them apart from other treatments: They respond to the specific needs of the particular injury.

of Celavet in Reading, Pennsylvania.

Stem cells can affect an injury site in other ways as well: "They also have an anti-inflammatory effect, which is much more closely modulated

FULL CIRCLE: Tissue samples taken from the horse are sent to a laboratory, where the stem cells are isolated then sent back to the veterinarian to be injected into the injury.

COURTESY, VET-STEM, INC

toward normalizing the internal environment than any drug therapy," says Kopyov. "There is also an analgesic effect—that doesn't last long, but it's there."

Another important healing property of stem cells, especially for horses, is that they can minimize the formation of scar tissue: The new tissue produced by stem cells tends to look more like the long, parallel fibers of an uninjured tendon. "That," says Bob Harman, DVM, MPVM, CEO of Vet-Stem, Inc., of Poway, California, "is because stem cells produce anti-scarring growth factors that block the formation of scar tissue as they grow into new tendon."

The replacement tissue is "nearly the same as normal tendon, but not quite," says McGarrell, "but it's way off the scale compared to scar tissue."

REALITIES AND **LIMITATIONS**

A single course of stem cell therapy generally costs between $2,000 and $3,000 and often includes the preparation of multiple doses, so that you would not have to have a second tissue sample drawn if your horse requires additional treatments.

Currently, stem cell therapy in horses is most commonly used to treat tendon and ligament injuries and as an aid in fracture repair. Multiple studies with cells drawn from all current sources have shown good success rates for treating horses with injured tendons and ligaments; in general, 70 to 80 percent or more of horses treated are able to return to work, at least 50 percent at their previous levels. Examinations of treated tendons show that the stem cell therapy produces stronger, more flexible tendon tissue with better fiber alignment than can be achieved with other treatments.

"Stem cells work better than standard treatments, including other biological treatments, especially in severe cases," says David Frisbie, DVM, PhD, DACVS, DACVSMR, of Colorado State University's Orthopaedic Research Laboratory. That said, they are usually reserved for severe problems. "Stem cell therapy probably would help in most cases, but it is more expensive, and the cost-benefit ratio needs to be taken into consideration," Frisbie adds. "The more severe cases that won't get better with other standard treatments are most likely the best candidates."

Meanwhile, researchers are still working to answer many more questions. "Right now, we can heal and prevent reinjuries in about 80 percent of the horses that we treat," says McGarrell. "But that means that 20 percent aren't being helped with stem cells, and we still don't know why. So it's important to understand that it's not a cure-all."

WHAT'S **NEXT?**

Stem cell research is really just in its infancy. "While stem cell therapies in the horse and dog are most often used clinically for orthopedic injuries to bones, tendons, ligaments and joints," says Ferraro, "it is rapidly expanding into other areas of organ and metabolic disease. At this point the uses for biologically based medical therapies seem limitless, but we have a lot to learn, and the development of these new treatments will take time."

In the coming years, we may well hear more about the use of stem cell therapy to treat a number of illnesses and injuries:

• **Laminitis.** "The use of stem cells for the treatment of laminitis looks extremely promising," says Ferraro. Research is under way at multiple facilities to help laminitic horses with stem cells, and results so far have been good. For example, at Rood and Riddle Equine Hospital in Lexington, Kentucky, 12 horses with severe laminitis that was unresponsive to other therapies were treated with stem cells; all but one showed significant new growth in the sole and wall.

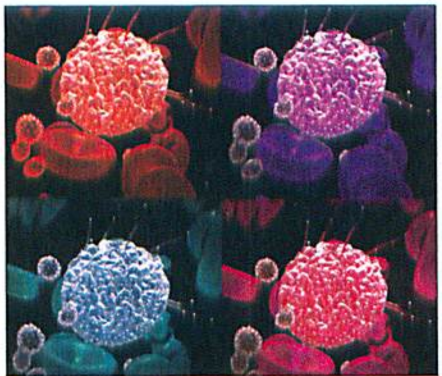
• **Neural injuries.** Stem cell therapy has long offered hope for healing spinal cord injuries as well as other neurological diseases in people and animals. But regenerating neural tissue has proven to be more difficult than orthopedic tissue. "We're having a tough time with nerve regeneration," says Harman.

Still, studies have shown that stem cell treatments improved healing of spinal cord injuries in rats, compared to untreated controls. And in 2010, a donkey named Eli was treated with stem cells after a pasture attack caused a spinal cord injury that left him unable to stand on his own. Eli, who was treated at Alamo Pintado Equine Medical Center in Los Olivos, California, received three injections of stem cells into his spinal canal between late May and June. On July 31, he rose and was found standing on his own in his stall.

• **Recurrent airway obstruction (RAO, heaves).** Researchers around the world are investigating ways to use stem cells to treat various lung diseases. In one recent Korean study, for example, rats with emphysema induced by cigarette smoke showed significant improvement over controls when treated with bone marrow-derived stem cells. The treatment "significantly increased cell proliferation and the number of small pulmonary vessels, reduced apoptotic cell death, attenuated the mean pulmonary arterial pressure, and inhibited muscularization in small pulmonary vessels," the researchers wrote.

The current studies offer hope that one day stem cells may be able to heal horses with allergic inflammatory lung disease, which produces similar effects. "When you put cells in intravenously they migrate to the damaged tissue in the lungs and can repair scar tissue that forms," says Harman.

• **Equine recurrent uveitis (moon blindness).** Work to investigate the use of stem cells in eye diseases is just beginning but, wrote a team of ophthalmologists in a 2010 Canadian paper, mesenchymal stem cell therapy is "poised for significant impact in ocular molecular therapeutics, particularly for



COURTESY, VET-STEM INC

VERSATILITY INDEX

One of the more significant differences among stem cells is the number of different tissues they can develop into. Here are the terms that describe that ability:

• **totipotent**—able to differentiate into all possible cell types, including placental tissue. These stem cells are derived from the first few cells formed after an egg is fertilized but before it would be implanted.

• **pluripotent**—able to differentiate into all cell types, except the placental tissue. These stem cells originate from the embryo at a slightly later stage of development.

• **multipotent**—able to differentiate into several closely related cell types. These are derived from adult tissue within the same family. Stem cells derived from skeletal tissue, for example, are best suited for development into other connective tissues.

• **oligopotent**—able to differentiate into only a few types of cell. These are derived from closely related adult tissue.

• **unipotent**—able to produce only one type of tissue other than themselves. Stem cells derived from muscle tissue, for example, can develop only into muscle cells.

chronic diseases, such as retinal degeneration, glaucoma°, and uveitis."

"Some data suggests that stem cells may help heal damage from uveitis and prevent ongoing damage," says Harman.

• **Chronic or progressive orthopedic injuries.** Currently, stem cells are injected into specific, well-defined lesions in tendons or ligaments. However, some sources of lameness—such as high° suspensory desmitis, which affects the support ligament behind the knee or hock—tend to be vague and more difficult to isolate.

But a technique called regional limb perfusion may help. In regional perfusion, a tourniquet is applied to temporarily halt blood flow into an extremity, and a therapeutic agent is injected to be carried through the vascular system to reach all tissues in the target area. The technique is often used to carry drugs more deeply into a specific part of the body than can be accomplished with systemic administration. And when this method is used to deliver stem cells, it has been demonstrated that they tend to migrate to the source of the injury.

"Regional limb perfusion is proving to be a highly useful avenue for stem cell delivery," says Ferraro. "Recent studies have shown that stem cells delivered with this method will indeed home to the site of injury. I think you can expect this method of stem cell delivery to increase in the near future."

What else does the future hold for stem cell therapies? Only time will tell. "I started doing this research out of curiosity, but now I view it as one of the most promising new health technologies, one that can address an enormous number of diseases in both human and veterinary medicine," says Kopyov. "It will develop into a very potent tool—but it is not just our future. It is also our present."

SOURCES OF THERAPEUTIC STEM CELLS

Fat (adipose) tissue

Procedure: A fat sample, usually about two tablespoons, is collected from under a horse's skin and sent off to a lab. The resulting stem cell solution, which contains a mixture of stem cells and various growth factors, is usually ready within 48 hours and is returned for injection into the treatment site. A portion of this processed material can also be reserved for culturing and the growth of additional stems cells through a process known as expansion.

Considerations: Because they are derived from a mature animal, adipose stem cells are multipotent, meaning that they can differentiate into only a limited number of other types of tissues, and they are also classified as mesenchymal, which means that they are best suited to becoming connective tissue, including tendon and bone. Fat-derived stem cell suspension is called "multinucleated cell fraction."

Research: In a 2008 study from Cornell University, four horses were treated with adipose-derived cells for experimentally induced tendonitis of the superficial digital flexor tendon, and four more were treated with a saline solution as a control. After six weeks, the tendons treated with stem cells were significantly closer to a normal appearance, with less extensive lesions, less inflammation and better formation of new fiber with normal alignment.

Sources: In addition to Vet-Stem, adipose-derived stem cell therapy is available in the United States through MediVet America, a division of an Australian-owned company.

FROM THE HIP: After bone marrow is extracted from a horse's sternum or the pelvis (above), it is sent to a lab where stem cells are isolated and cultured.

COURTESY, ADVANCED REGENERATIVE THERAPIES

Bone marrow

Procedure: A sample of bone marrow tissue is extracted from either the sternum or the pelvis and sent to a lab where the stem cells are isolated and cultured to produce the amount needed for a particular injury. The therapeutic solution is generally ready within two or three weeks.

Considerations: It takes longer to produce stem cell suspension from bone marrow but the resulting product contains more therapeutic cells: "Bone derived culture expanded cells afford the clinician the ability to treat with cell numbers in the millions," wrote David Frisbie, DVM, PhD, of Colorado State University, in a paper published in the *Equine Veterinary Journal* in 2010.

Research: The uses of stem cells from bone marrow have been under investigation since the 1950s, and multiple studies support their effectiveness for treating various equine injuries. In published data on 162 horses treated at Colorado State University or Advanced Regenerative Therapies, 85 percent (52 of 61) of those with soft-tissue injuries returned to work—51 percent at a level that met or exceeded their previous level, and 34 percent at a lesser level. Also, 73 percent (29 of 40) of horses treated for orthopedic injuries returned to work.

In a 2008 British study, 168 racehorses with moderate to severe acute injuries to the superficial digital flexor tendon were treated with bone-derived stem cells and then underwent 48 weeks of rehabilitation. Of those who returned to racing, 18 percent experienced a reinjury during the following two years, compared to the 56 percent reinjury rate reported among horses receiving conventional treatments for these injuries.

Sources: Advanced Regenerative Therapies in Fort Collins, Colorado, formed in 2003 (and of which Frisbie is a founding partner); VetCell, formed in 2002, which is based in the United Kingdom but also has laboratories in the United States and Canada; Rood and Riddle Equine Hospital in Lexington, Kentucky; University of California–Davis Center for Equine Health; Alamo Pintado Equine Medical Center in Los Olivos, California.

Umbilical cord tissue and blood

Procedure: Stem cells are harvested from umbilical cord tissue and/or blood left behind in the placenta after a foal is born and used to grow stem cells that can later be used in that individual, its siblings or its mother.

Considerations: Umbilical cord-derived stem cells are versatile. "Younger cells tend to be more abundant, and they are appropriate for all current therapeutic uses," says Mark Sorrentino, MD, medical director of EquStem. "The advantage of getting stem cells from cord blood and banking them is that you already have them. Cord blood and/or tissue can be drawn from the umbilicus with no pain or inconvenience to the mare or foal, expanded and stored in the laboratory and then when you need them, they can be delivered in a suitable dose within 48 to 72 hours." More stem cells come from umbilical cord tissue than from umbilical cord blood, but the latter is the only current source of hematopoietic stem cells, which are used to treat such things as leukemia in humans.

Research: Although in human medicine cord blood stem cells have been found to produce less of an immune response in unrelated individuals than other adult-derived cells do, EquStem suggests that equine cord blood cells be used only in the dam, full siblings and half siblings as well as the horse himself.

EquStem has only just begun returning stem cells to clients for therapeutic uses, but Sorrentino reports that, despite the small amount of data they have, the success rates shown so far have equaled or exceeded those reported with other types of stem cells. Ownership of the cells transfers if the horse is sold.

Sources: EquStem, in Hackensack, New Jersey; University of California–Davis Center for Equine Health; Rood and Riddle Equine Hospital in Lexington, Kentucky; Alamo Pintado Equine Medical Center in Los Olivos, California.

Fetal tissue

Procedure: A therapeutic solution is produced using stem cells extracted from tissue taken from an equine fetus.

Considerations: One company, Celavet in Reading, Pennsylvania, is currently conducting safety trials with fetal-derived cells with the goal of gaining Food and Drug Administration (FDA) approval for their use in horses. Currently, 49 veterinarians in 40 clinics across the country have the authority to administer the treatment for clients who agree to participate in the study, and so far just over 400 horses have been treated. The cells are cultured and stored in a laboratory and sent out to veterinarians when requested.

Although "embryonic" and "fetal" are often used interchangeably, they have distinct meanings: Embryonic tissues are derived from the fertilized egg, after its first few cell divisions. Fetal cells are derived from a later stage of development. The difference is significant. "Embryonic blastocyst-derived stem cells are totipotent, meaning they can turn into everything," says Oleg Kopyov, MD, PhD, Celavet's executive vice president and chief research officer. "However, there is a price. Because they are so flexible, they are also highly tumorigenic. When you use them in immunodeficient laboratory animals about 30 percent of them develop tumors."

The Celavet stem cells are derived from the equine fetus during a specific window of development when they are still pluripotent, like embryonic cells, but no longer tumorigenic, and have not yet developed an immune profile, so they can be used safely in any adult horse.

Research: In the safety trial, the fetal-derived cells have been used to treat mostly tendon and ligament injuries, both acute and chronic. The results have been promising so far: At 120 days after treatment, 79 percent of horses with acute tendon injuries showed significant improvement in fiber alignment on ultrasounds, and 59 percent of horses with acute ligament injuries improved by at least two points on the lameness scale developed by the American Association of Equine Practitioners. If the results of the current study earn a green light from the FDA, the next stage would be a double-blind placebo-controlled trial to prove efficacy.