

Ultrasound is used to precisely guide the injection of adipose-derived stem cells into the suspensory ligament.

DR. LINDA A. DAHLGREN

Stem cell therapy is helping horses with tendon and ligament injuries.

By Andrea Caudill

Stemming the Tide

A BOWED TENDON IS USUALLY A DEATH BLOW TO A HORSE'S RACE career. The injury, tricky to treat, makes it nearly impossible to bring a horse back to the track, and the likelihood of the horse returning to its previous level of ability is even slimmer.

The still-developing technology of stem cell therapy, which uses unspecified cells from the horse's body, has the potential to help racehorses heal sounder than ever before.

A tendon is a bundle of elastic fibers, mostly made of collagen, that attaches muscle to bone, and help move the skeleton. Ligaments are similar but attach bone to bone and provide stability. When a horse bows a tendon, it tears the fibers at a certain point of the tendon (the location results in a name, such as high or low bow), weakening it significantly. When the tendon begins to knit back together, it is significantly hampered by lack of blood flow. Blood provides several healing mechanisms, including adult stem cells, which are able to convert themselves into specific types of cells the body needs to heal itself (in this case, tendon cells). If the tendon does not get enough help, it eventually develops scar tissue, which weakens the tendon because it is nonelastic and haphazardly knitted together.

The injury takes a long time to heal – a typical racetrack cure was pinfiring or blistering, followed by six months to a year of turnout. If a horse was brought back to the track and

the tendon had mostly healed with scar tissue, the weakened tendon could give way and the injury recur.

What is it?

STEM CELLS ARE IMMATURE CELLS THAT DO NOT YET HAVE A specific job in the body but can form into a particular type of cell. The two main type of stem cells are embryonic and adult stem cells. Embryonic stem cells are able to become any type of cell in the body. Adult stem cells are more limited in what they can become. The first of two types of adult stem cells, known as hematopoietic stem cells, turns into various types of blood components such as red blood cells. The second type is mesenchymal stem cells, which can turn into connective tissue (bone, muscle, tendon, etc.) to heal and regenerate healthy tissue and is being used in stem cell therapy.

Embryonic stem cells were the first to be used in research. Harvesting from embryos, which are destroyed in the process, caused much media attention and controversy. Because of this, active research has driven the development of less controversial sources of stem cells. Two of the most common sources of adult stem cells are bone marrow and adipose tissue (fat).

Bone marrow is composed of a modified form of blood, stem cells, growth factors and other miscellaneous components.



The process of acquiring adipose-derived stem cells: The harvesting site is prepared (left), then about two tablespoons of fat are removed (center). Finally the stem cells are prepared (right) and reinjected into the horse.

support the use of these treatments and suggest that they have the potential to improve the healing process.

Useful Fat

VET-STEM INC. BEGAN DECADES AGO WHEN HARMAN WAS WORKING in the product-testing business. One client, a human orthopedics firm, had purchased stem cell technology that used fat as a source of the cells. In 2002, after negotiation and testing, Vet-Stem became the exclusive licensee of the technology for veterinary purposes. The therapy is in human clinical trials, but is not yet approved for use in humans in the U.S.. It is already available to humans in Europe and Asia.

The treatment is done by the horse's attending veterinarian. The company sends the vet a package with detailed instructions on the procedure. The veterinarian collects fat from the animal while it is under local anesthesia. The usual collection area is the tailhead, where there is a large fat pad. The veterinarian cuts the skin open about two inches and collects approximately two tablespoons of fat. Some veterinarians, however, had problems collecting enough fat from ultra-lean racehorses.

OTHER OPTIONS

A treatment for tendons that does not involve stem cells is a substance known as urinary bladder matrix (UBM). Derived from the wall of a pig's bladder, it contains a mix of proteins, including collagen. It does not contain cells, which are a major source of stimulation to the immune response. The absence of cells should decrease or eliminate the body's autoimmune (allergic) reaction to the substance.

Originally sold as a human treatment, UBM is sold in sheets. For the treatment of tendons and ligaments, the sheets are ground to a fine powder and suspended in sterile saline. The mixture is then injected into the affected tendon or ligament. The proteins form a three-dimensional scaffold onto which cells that create tendon and ligament tissue can adhere.

Marketed by A-Cell Vet, Inc., it is commonly known to the veterinary industry as A-Cell Vet Powder. This product is not currently on the market in the United States due to patent infringement claims by Cook Biotech, manufacturer of an identical UBM sheet for wound repair. A clinical case series performed by Dr. Rick Mitchell of Fairfield Equine Associates at Newtown, Connecticut, reported on IOI tendon and ligament injuries treated with UBM. Released in 2004, the study found greater than 80 percent of these horses that were at least six months post-treatment were sound and in work. These numbers, however, are a preliminary report and do not represent a controlled clinical trial.

"When you have an animal that's really in fit condition, they don't have very much body fat," Harman said. "But stem cells don't go away when you get thin. The amount of fat we can get off the tail head of a horse right off the track is still adequate because it's highly concentrated."

An obese animal, Harman says, might have 50,000 cells in one gram of fat. A really fit racehorse might have 5 million in the same gram.

The company consults with veterinarians, which allows both sides to know the size and amount of doses a horse's injury will require. Once the appropriate amount of fat is collected, the tissue is shipped to the laboratory, purified and concentrated, placed in syringes for injection, then shipped back to the veterinarian. The processing time in the lab is a matter of hours, so horses are treated in a matter of days.

Once the veterinarian receives the doses, the horse can be injected. Depending on the size of the injury, the stem cells can be spread with one to four injections, using ultrasound for guidance. Because the cells are derived from the horse, there is no risk of allergic reaction to the cells.

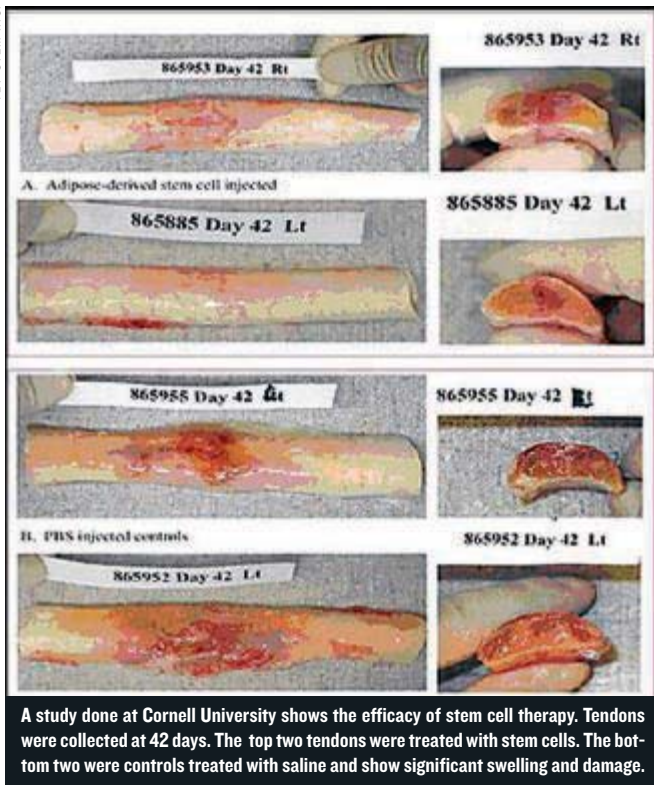
"It is their own tissue," Harman said. "It is just moved around and concentrated where we want. It's about as natural as you can get for the horse, so they usually have the opposite of a reaction in an inflamed area. Generally, those tendons, ligaments and joints are already kind of inflamed, so it helps reduce the inflammation because the cells are actually anti-inflammatory."

Linda Dahlgren, D.V.M., an expert on tendon and ligament healing, expressed her concern for the potential for a damaging immune response due to the porcine (pig) origin of the product.

"The concept of using this scaffold to help regenerate tendon is a good one," she said. "The rationale is sound. I would just rather it be horse protein rather than pig protein. To produce an identical product of equine origin would be a challenge on several levels. The nature of the tissue response following A-Cell injection has not been documented."

Another human process that might prove useful in healing equine tendon and ligament injuries is platelet-rich plasma. Blood is collected in an anti-coagulant and is spun down. The red blood cells are removed, and the remaining plasma can be injected into the injured tendon or ligament. There are a number of growth factors and other material in the plasma that could be beneficial. This, too, can be injected into the tendon. There are not yet any published studies proving its benefits.

A drug called BAPN-f (beta-amino propionitrile fumarate, pronounced "Bapten"), was introduced to the veterinary market in the mid-1990s as a method to improve tendon healing. Made from the seeds of the wild sweet pea, it was FDA-approved after a clinical trial showed initial success based on ultrasound results and race results. It was available to veterinarians for a limited time, however, the product failed to be cost-effective for the manufacturer and has been removed from the market.



Originally thought to be a fountainhead for stem cells, it turned out to contain less than originally expected. The marrow has stem cells but is mostly made up of a modification of blood. Research showed that fat has a greater concentration of stem cells than bone marrow – one of 50 cells in fat, versus one in 100,000 in bone marrow. Stem cells are also found nearly everywhere in the body, but fat was chosen as the most accessible source.

Robert Harman, a veterinarian who founded and runs Vet-Stem Inc., a company that does stem cell therapy in horses, explained that stem cells do more than repair tissue.

“It’s a very big misconception both in the medical community and the lay community that a stem cell just makes tissue,” Harman said. “These cells also make a number of very positive growth factors and they block some of the negative factors that cause degradation. Stem cells do something in managing the whole healing process which goes beyond just making new tissue. They recruit new cells into the area, they produce these growth factors and they manage the healing process in a very different manner than we thought five or 10 years ago.”

From the Bone

BONE MARROW IS TYPICALLY HARVESTED FROM A HORSE’S sternum, about where a girth would be placed. The procedure is done with the horse standing and locally anesthetized. The marrow is aspirated with a special needle and can either be injected directly into the tendon or processed to isolate the stem cells.

The first method is directly injecting the marrow into the tendon or ligament. It is available in the United States and is fairly easily done by a veterinarian experienced in the collection and injection technique. It costs about \$1,000, or about half of other methods.

It was initially performed with the belief that stem cells were numerous in the bone marrow. However, researchers

do not know which marrow ingredients help the tendons. Bone marrow contains a good amount of growth factors, which are thought to help heal tendons. The most-studied type of growth factor is insulin-like growth factor-I (IGF-I). Once injected, it has an anti-inflammatory and growth-encouraging effect.

“Bone marrow is composed of a small number of stem cells plus a fluid component that is basically a modification of blood,” said Linda A. Dahlgren, D.V.M., Ph.D, assistant professor at Virginia Tech University and an expert in stem cell research and tendon and ligament healing. “Proponents of bone marrow therapy speculate that there are proteins contained in the fluid portion of the aspirate that might be beneficial to tendon or ligament healing. What is contained in this ‘soup’ is likely a mixture of beneficial growth factors that are likely candidates to promote tendon healing.”

The problem, she says, is that there is only a small, possibly insignificant, number of stem cells that get injected. In addition, there is the possibility of injecting unwanted tissue, such as tiny bone fragments. Another problem is the large volume of the injection (approximately 30 milliliters, or about six teaspoons). Other treatments available inject significantly less fluid (1 mL). The concern is the additional damage that may occur within the tendon as a result of increased pressure.

“There is not a very large volume of potential space within the injured portion of the tendon,” she said. “There is no way for the tendon to expand as the bone marrow aspirate is injected.”

The second method using bone marrow requires processing of the bone marrow to get the stem cells. This method is not available in the United States, but is performed by VetCell Bioscience Limited in the United Kingdom, Europe, Japan and Australia. The process involves removing bone marrow and separating the fluid (serum) from the cells. The fluid is frozen and scientists culture the cells in the laboratory to increase their numbers, which averages two to four weeks. Once there are enough cells, they are added back to the fluid and injected into the tendon.

Controlled scientific studies to prove the effectiveness of either of the bone marrow methods are lacking, but a number of case studies



Stem Cells: Quick Facts

- **Mesenchymal stem cells are primitive cells with the ability to be any type of specialty connective tissue cell – bone, tendon, muscle, etc.**
- **Stem cell therapy can help many injuries but is most commonly used to help heal tendon, ligament, bone and joint injuries.**
- **The therapy involves gathering stem cells from a source (bone marrow or fat), processing it to concentrate the stem cells and injecting it into an injury.**
- **The procedure requires a veterinarian and is done with the horse standing under local anesthesia.**
- **The costs of the procedures vary between approximately \$1,000 to \$3,000.**
- **The treatment is most effective when done within a month of the injury before scar tissue becomes established, lessening the ability to heal correctly.**
- **Healing time is not necessarily faster with stem cell therapy. Tendons take a long time to heal to full strength. If not given that time, reinjury can occur. However, if adequate time is taken, the tendon will be better healed, and the horse will be less likely to reinjure.**

Each treatment regimen is estimated to cost \$2,000-\$3,000, depending on individual veterinarians and associated procedures.

Vet-Stem specializes in tendon and ligament treatments because those are the injuries most difficult to repair. Stem cell therapy is also helpful in fracture repair and intra-articular joint therapy, arthritis and OCD (osteochondrosis dissecans).

“These cells are particularly good at homing in on inflammation,” Harman said. “When you put them into the joint, they will find the areas of both cartilage and bone injury. We’re only about a year into treating horses with joint disease, but we’ve had some fairly dramatic cases. You get a dramatic reduction in pain, better mobility and repair of both cartilage and bone injury in the joint.”

Treatment and Recovery

VET-STEM CLAIMS IMPROVEMENT CAN BE SEEN IN DEGENERATIVE joint disease within days to a few weeks, the procedure heals fractures twice as fast, and tendon and ligament injuries show improvement within 45 days. Harman pointed out that the window of opportunity to treat an injury is usually relatively small, and horses must be treated quickly for best results.

Vet-Stem recommends all treatments for tendon and ligament injuries happen within a month of the injury. Delayed treatment can have beneficial effects; however, at that stage of healing, the body has already started to mend itself and treatment might not be as effective.

Once the horse has been treated, it must be put on stall rest and a regular exercise program to help strengthen the tendon and ligaments. Anti-inflammatory drugs, such as phenylbutazone, can be given to help pain, but steroids will hinder the healing process. The healing time varies, and Harman emphasized that handlers must respect the need for time to heal.

“(Stem) cells are not only stimulated by the injury, they are mechanically stimulated,” Harman said. “So if you stretch the cells or tissue, it signals them to become tendon.

“Walking every day is not just because your mother said it’s a good idea to exercise,” he continued. “It really does signal the cells to line up, and it reduces scarring.”

He also emphasized the need to work closely with your

veterinarian, using monthly ultrasounds to monitor the progress of healing and determine an exercise regimen and how fast the horse can come back.

“Tendons take a long time to mature into the tensile strength that’s needed,” Harman said. Every horse is an individual. The tendon itself will be the best indicator of how fast it is healing.

“If you just take your horse and turn it out in the pasture, it’s going to take a long time and it isn’t going to heal as well,” he continued. “I can’t predict for any particular horse, but with a regimented program, in general, you are going to be in that six- to 12-month range. I can’t stress enough how important a controlled rehab program is.”

While there aren’t yet published scientific studies supporting the effectiveness of bone marrow-derived stem cell therapy, there is one such study for adipose-derived stem cells. A double-blind study done at Cornell University demonstrated that the stem cell-treated group of horses showed improved tendon healing compared to control horses. The findings were based primarily on key characteristics of the tendons when looked at under a microscope (better healing, less inflammation). Vet-Stem recently reviewed the outcome of clinical cases using an informal poll of its racehorse and performance horse clients. The company found that racehorses treated for superficial digital flexor tendon and suspensory ligament injuries returned to one or more races at a rate of 69 percent. The average time of recovery was 11 months. They also found their nonracehorse clients showed 78 percent of horses returning to their prior level of performance, with another 11 percent returning to a lesser level of competition.

There are several other new treatment options looming on the horizon (see sidebar “Other Options”) but they are either not available in the United States or are lacking clinical research. No matter what method is chosen for helping the horse to heal, the main ingredient will always be the old standbys: time and a controlled exercise program.

Looking Ahead

In the near future, horse owners could get a jump on such injuries by having a horse’s stem cells on file – preserved long before the horse needs them. The cells would be frozen and banked in liquid nitrogen, like semen or embryos are currently stored.

Once research catches up, veterinarians and owners might have a better chance to stay ahead of an injury with preventive injections of stem cells. Similar to injections of steroids or glucosamine, the stem cells might be able to help strengthen the body, thus preventing injury.

“Giving the horse adequate time for the tendon to heal is essential,” Dahlgren said. “None of these treatments that *are* available, *were* available or *might be* available are going to shorten the amount of time it takes for a tendon to heal. I still tell people recovery time is about a year. Some horses can come back in race training as early as nine months. To bring them back sooner puts them at a pretty high risk of reinjury. So what we’re hoping to do with any of these new treatments is that, rather than speeding the process, if we can just improve the healing process, we’re further ahead. If we can make the end product better, it will be better able to hold up to the training process like a normal tendon.” □