Stem Cell Therapy for Repairing Tendons and Ligaments

by HEATHER SMITH THOMAS

Stem cells are primitive cells located in various parts of our bodies. They are very versatile, able to transform into bone, muscle, tendon or other kinds of tissue to replace damaged cells in those tissues. Research the past few years has concentrated on stem cell therapy for human diseases, but now this new technology is available for horses.

Vet-Stem Inc. (at Poway, Calif.) now offers this technology to the equine industry. Robert Harman, DVM, MPVM formed Vet-Stem Inc. in 2002 to provide laboratory service to veterinarians. He and his colleagues were also earlier involved in human cell-based therapies (founding HTI Bio-Services and HTI Bio-Products in 1990).

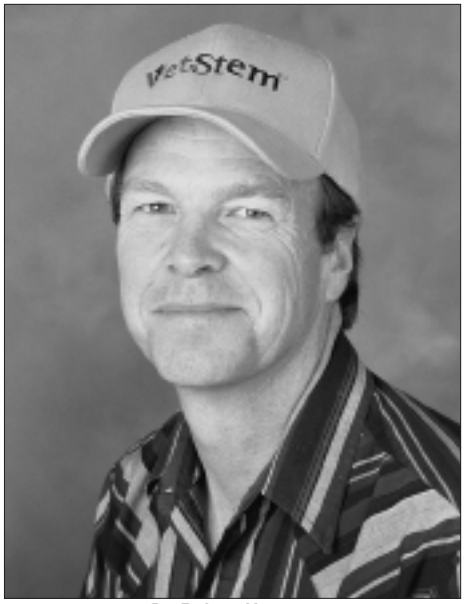
Equine veterinarians send a small sample of an injured horse's fat to the lab, and the stem cells are isolated and concentrated. The harvested cells are sent back to the veterinarian for injection into the injured tissue to aid healing of tendons, ligaments or fractures. Most of the horses being treated are racehorses or high level performance horses, where correct healing (to regain full use and soundness at peak competitive levels) is very important.

The public is becoming aware of stem cell therapy and what it can do. "They read the story in *Readers Digest* about the kid who survived a nail gun injury through the heart; people are aware of what stem cells are. They still get confused about embryonic stem cells versus adult stem cells, however," says Harman.

Advantages of Using Fat-Derived Stem Cells

During early studies, the only known source of stem cells were embryos. Research was controversial because embryos were killed to obtain these cells. Now we've found that there are many tissues in the patient's own body that contain stem cells capable of transforming themselves into most of the major tissues during the healing process. Stem cells from bone marrow or fat tissue, for instance, can help heal a torn ligament or a damaged heart muscle.

"A number of veterinarians have used bone marrow and cultured bone marrow to treat suspensory ligament and tendon injuries in the horse with some success, and most horsemen and veterinarians think of bone marrow as a good source of stem cells. But there's mostly growth factors in bone marrow and very few stem cells. They are not the same. Allan Nixon at Cornell showed that only one in 100,000 of those cells are actually stem cells. Bone marrow is very rich in growth factor, which also helps the healing process. A lot of people call us up to ask us about our bone marrow work, and we have to tell them that we don't do bone marrow—we use fat-derived stem cells," explains Harman.



Dr. Robert Harman

There is a higher concentration of stem cells in fat tissue (about one in 50, or two percent of the cells, are stem cells, as compared with one in 100,000 in bone marrow). "The stem cells are easier to obtain from fat, as well. Most veterinarians don't want to stick a needle into the sternum of a horse (where bone marrow is usually obtained). If you poke it in too far, you end up in the chest cavity, which can be quite serious," he says.

Early work with bone marrow by Dr. Doug Herthel showed it was effective in helping heal tendon and ligament injuries. "Doug's pioneer work with bone marrow made people aware of what you could do with cell-based therapy and growth factor therapy. He's one of the people who got me interested in looking at this," says Harman.

"I'd been in the pre-clinical testing business, and one of the human stem cell companies was my client. I'd seen what cell-based therapy did in fracture repair. It was dramatic—twice as fast, twice as strong. So, four years ago, I started researching this. All the models for humans are veterinary species; the research is done on goats, dogs and sometimes horses, to test various therapies for humans. With all this data there had to be a way to figure out how to bring this to veterinary medicine as well as human medicine, and make it affordable for the horse owner," says Harman.

"So I was pondering this, and then I read Doug's articles and realized that someone was actually already doing this. I thought about doing bone marrow therapy as a business, then started looking at fat-derived stem cells because this was easier for a veterinarian to do safely," he explains.

It was an unusual path that led him to fat cells instead of working with bone marrow. "A decade ago, university researchers were studying obesity in humans—looking at cells (preadipocytes) and wondering why those cells turn on, and why people gain weight. They kept looking at these cells and realized these are very primitive cells. They do turn into fat, but when the researchers developed culture techniques to study them, they found that

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these same cells can turn into bone, liver, nerve, muscle, etc., as well as fat. With their new techniques and cell surface markers, the researchers realized that these were true mesenchymal stem cells—and there is a huge reservoir of these in fat tissue," he says.

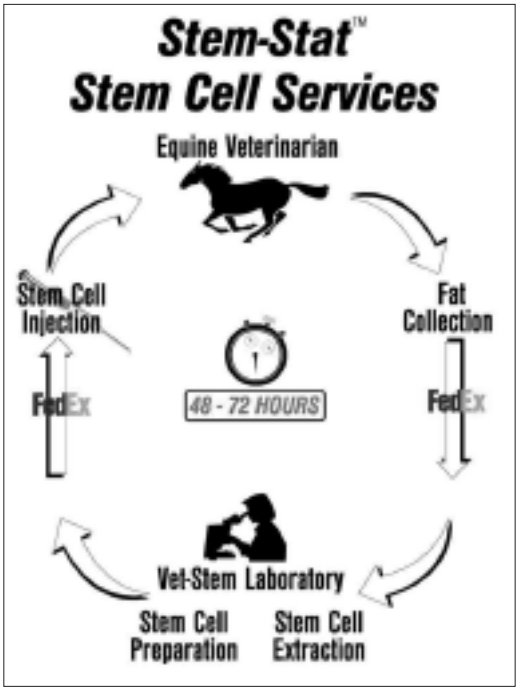
To date, nobody knows why so many of these are found in fat, except that it may be a convenient storage place. They don't get mixed up with the stem cells that make blood. "There are two very different kinds of stem cells. Most people have heard of bone marrow transplants in children or in people who have cancer of the blood. There are lots of blood-forming stem cells in bone marrow. Those only turn into red cells and white cells. That's why you can take bone marrow and transplant it into a child who's had chemotherapy, and replenish those cells—so his white cell count comes back up and he's not anemic anymore."

But the blood cells are not mesenchymal stem cells, which turn into other tissues. There are many blood- forming cells in the bone marrow, but not as many true stem cells for making new tissues. "Those are stored in fat—in high numbers. There are also some in muscle and other tissues. But fat is the only tissue we have an excess of, and can harvest. You can take some fat out, and the animal doesn't miss it. You could also get stem cells from a piece of muscle, but that's not so good for the animal," explains Harman.

"We take a little piece of fat out surgically, because it's easier to do. In humans this is done a lot, in liposuction. If you went to a plastic surgeon for liposuction, you could have the cells harvested under sterile conditions and frozen in liquid nitrogen and held for future use, if you wanted—to treat some later condition," he says.

Right now, the main therapy done with stem cells is on injured ligament and tendon tissues—primarily because it is difficult to bring these types of injuries back to peak strength after healing. They don't heal well, and if there is not good natural healing you get scarring in tendon and ligament injuries in the horse.

"In pre-clinical models tested in rabbits and goats (where tendons were cut and stem cells put in to see how they'd heal) the data was very compelling. These healed really well with a more elastic tendon. Our process is designed to provide a more natural regeneration of the tendon tissue, without the scarring. If you're just using a horse for slow work, a tendon injury that scars won't hinder him much. But if you want an athletic horse to return to peak performance, he can't do it if he has a scarred tendon, and if he tries, he will tear that tendon again," says Harman.



A Description of the Stem Cell Therapy Process

"Stem cells can heal fractures very well, but bone heals well on its own. Tendons don't, so we picked this as the primary injury to put our resources into and show that tendon and ligament disease is very amenable to treatment," he says.

Harman recently attended an Orthopedic Research Foundation meeting in San Francisco, where more than 100 papers were presented on stem cell use in orthopedics. "There were many animal models—from rats to goats to horses. There were 10 papers just on fat-derived stem cells. So people know those cells are useful. We will start doing small animals later this year, with similar kinds of applications," he says.

How the Cells Are Prepared In the Horses

The fat sample is taken from the pad of fat next to the horse's tail head. Dr. Harman chose this spot because of ease and suitability. There are few blood vessels, nerves or bones in that location. "The veterinarian can make an incision about two inches long, to the left or right of the tail head, then go in with a pair of surgical scissors and collect about two tablespoons of fat," he explains.

The stem cells can be put back into the horse within 48 hours. "The veterinarian puts the fat in our special transport media, and sends it to our lab via FedEx, on ice. It takes about six hours to process it in the lab. If we get it by FedEx in the morning we can send it out in the afternoon," says Harman. The cells are collected and put into syringes, ready to inject into the horse.

"These concentrated cells are in their natural state, and when they go into a tissue, the local tissue damage tells those cells what to do. If we put them in a tendon, they make new tendon. We can put them in a fracture site and they turn into osteoblasts and make bone. If you inject them into a heart, they make new heart muscle. They know exactly what to do," he says.

"They find their way around in the body and are attracted to where the injury or inflammation is located. You can put them into the blood stream intravenously and they go wherever they are needed." This is how mother nature heals body tissues. The stem cells are in the fat or bone marrow, waiting. If the body suffers a wound, torn tendon or fracture, signals go out through the blood stream. The cells hear that signal, and come. They are the body's little EMTs.

"The reason not enough of them get attracted to a tendon when it's injured is

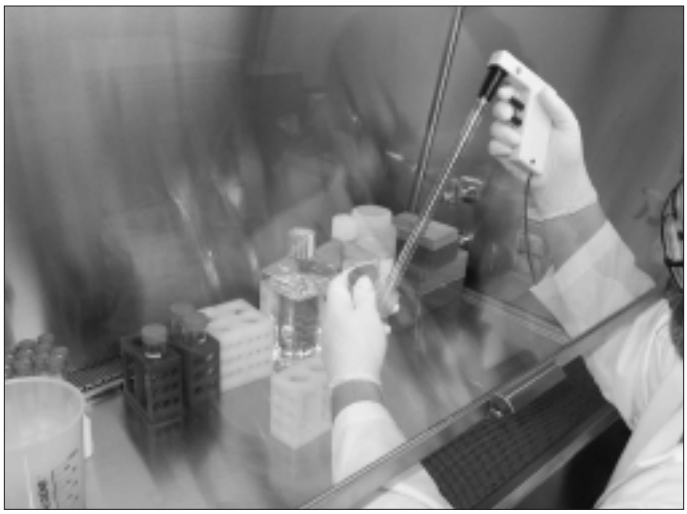
partly because tendons do not have a good blood supply. Muscle has a huge blood supply, and that's why it heals so well. Skin has a great blood supply, and heals fast. But a tendon is not very vascular, especially in the horse. He has long tendons in the lower legs, with no muscle around them. So injecting these cells into the tendon directly can aid healing," he says.

The Phases of Healing

"The body, when injured, goes through three phases. The first phase is inflammatory—swelling, redness, heat and soreness. That lasts a couple of days and this is when all the signals are going out. The body says, let's regenerate this tissue that got damaged," says Harman. For regeneration, the injury needs growth factors, blood supply, and stem cells coming in—so the body can make new tissue to replace the damage. This is phase two.

"If the body doesn't get it repaired quickly enough, and decides it can't regenerate new tissue, it creates a scar to stabilize the injury. This is a natural repair mechanism at a fracture or around a joint, where there is a lot of instability. The body makes a huge scar around it—to wall it off and fibrose it in, so the body can still function. This is phase three," he says.

"We try to intervene early, putting cells in before that phase, so we can get good regeneration—before the body turns off the signal, gives up on the repair and starts making scar tissue. If we put enough cells there, they attract other cells. They make new blood vessels, and new true tendon; they make normal elastic tendon, the way it's supposed to be. That's our goal—trying to get cells in there before the body decides to make a scar. This happens everywhere in the body; if it can't fix it right, it makes scar tissue to wall off the problem and get on with life," he says.



Sterile Cell Processing

If the horse has an older injury, starting to scar, it may or may not be too late to "fix" it. "We have some data now to show that in a three- to five-month-old tendon tear or suspensory ligament tear, you may still be able to get some healing. There will be a black hole on the ultrasound in the area where cells have not infiltrated and it hasn't made new tissue. It's mostly fluid and edema in those spots. If a tendon doesn't heal properly, some of those areas just become static," says Harman.

"We've found that if you put some primitive stem cells into those areas, some of the places that have been sitting static for several months will start the healing process again. It recruits the new cells, forms new blood vessels, and fills in. You may still have some scar tissue, but there's not an open hole that will tear so easily if the leg is stressed again," he explains.

A damaged tendon will often retear; it needs to heal as well as possible. "We've been able to get some regeneration in slightly older injuries using stem cells. We had a veterinarian in Missouri who recently did two horses with suspensory ligament tears that were three or four months old and static. The owners didn't know what else to try. So the veterinarian did stem cell transplants, using fat, and within 30 days those injuries were healing, as evidenced by ultrasound, filling in the defect. They were not fully healed, but the ultrasound showed the holes were filled, and the horses were back in rehab. That's what we want to see," he says.

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Future Use: Preventing Injuries

"Someday we'll move to the next phase—preventing injures with stem cells. When you start to put a horse into heavy work, that's when he is at highest risk for injury, when he's first getting fit. You could treat the horse with stem cells, preventatively. It's a little too expensive right now and we don't have any data yet, but in a year we might have the data to say a horseman could treat once a month or every other week and repair that little bit of microdamage that might become cumulative and result in injury," says Harman.

It's cumulative damage that results in catastrophic injury. Researchers at racetracks have found that breakdown during a race actually started much earlier—with weeks or months of small microfractures in the bone, small tears in the ligaments, etc. The work was too heavy and the body couldn't keep up with it (in tissue repair) and finally just reached a breaking/tearing point.

Knowing that, we might be able to prevent the big injuries by keeping the body ahead of the game—by helping heal the ongoing wear and tear. "This is exactly what those cells are doing in the body anyway, and we could help them by putting supplemental doses into strategic spots," he says. "This is not unlike what we are already doing with the oral supplements of glucosamine or the Adequan injections, to help the joints heal as they go," he adds.

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If a horse has a brand new injury, it's generally recommended that stem cell therapy be done between day seven and day 28 after the injury. "You can actually put cells in on day one, however, and it wouldn't matter. That's what the body does; these cells are in the middle of the battle, in the early inflammatory stage. But many veterinarians feel it might be best to let the injury 'cool down' a little. People use ice and anti-inflammatory treatments to get rid of the early inflammatory reaction. None of that will hurt these cells; you could inject them the very next day. But by the time a horse is brought for treatment, the veterinarian talks to us, gets the okay from the owner, and ships the cells back and forth, it's generally a few days after the injury. But there is really not a lower limit on how soon you could inject these," he says.

Collection Site Example

"On the upper limit, day 28 gets you in that optimum window when the body is still in the repair phase, but it doesn't bother me to treat horses that are farther out. If you want the optimum chance for good healing, however, get cells in there during that first month; don't wait. You don't want scar tissue to start, and have to deal with it on the rehab. You are better off to use stem cells early, while the body is still in its highest signaling phase for creating new tissue—the best time for regeneration to happen."

Anti-inflammatory drugs will not harm the stem cells, but steroids are detrimental because they retard healing. Reducing swelling is important and you can use ice, compression bandages, DMSO (dimethyl sulfoxide), or anti-inflammatory drugs; the swelling causes more tissue damage and tearing. "But don't use steroids. Ice and cold water therapy are probably the best, to bring swelling down. Some people use short-acting steroids the first day or two, and that won't hurt, because the affects of those will usually be gone by the time our cells are put in, after the swelling phase. But once we put the cells in, steroids can interfere with the stem cells' work."

"You want the horse doing some normal walking to put a load on the limb—which tells those cells that they are supposed to be tendons, so they will make good, strong tissue," says Harman.

The Importance of Early Rehabilitation

"Rehab is hugely important in getting these tendons back to normal, to be strong again. The body has to exercise in order to strengthen something. When you put the little cells (repairing the tendon injury) under some kind of load, they form the proper kind of collagen and make strong tendons. If you don't, they won't heal in an organized manner," he explains.

"Early rehab is probably the most important thing for getting these horses back into function. Immediately after the stem cell injection, we tell people to start walking the horse. There's quite a bit of data that shows how important early rehab is to proper healing. You can't just take the horse out and run him right away, but you need to be walking every day for the first 30 days, then do some jogging. Tension on the tendon makes the cells line up and make normal elastic tendon tissue. If you don't put the stem cells in there (and don't have the building blocks), it will scar. But if you put the stem cells in and stand the horse in a stall for six months, you won't get good healing either," he says.

Years ago, when someone had a broken leg or surgery, they were kept in the hospital for weeks—and could hardly do anything when they got out. "Now they get you out of the bed and make you walk! We're doing the same thing

Banked Stem Cells

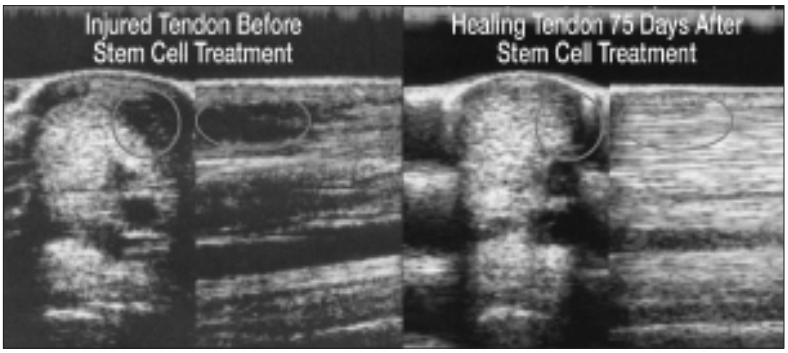
"We've also developed a technique to freeze and bank cells. We can take part of them or all of them, and freeze them in liquid nitrogen, like stored semen or embryos. They freeze and thaw very well. We can hold part of the cells, if a client wishes, for future treatment. There is also talk about banking cells from foals, in case they need them later in life. In all species, younger animals have more total (and more viable, more active) stem cells. An older person or animal does not heal as well—because they don't have as many stem cells. So older animals benefit more from stem cell therapy, but their stem cells aren't as active. You may have to take more fat or try to get more cells. If you save some cells when the horse is young, and he later has a fracture or torn tendon or ligament, you can use those nice little fresh cells. This is happening already in humans—people are banking their own stem cells. Later on if a person has arthritis or a heart attack, those cells can be implanted," says Harman.

with horses now—horsemen are starting to realize how to rehab a horse. It has to be controlled, but within 30 days you should be starting to jog those horses and give them light work—and the tendons heal very well," he says.

"The loading tells the body the leg is still working to build tissue. If you take the weight off, or put a limb in a sling or a cast, then the body says 'now I don't need it' and the limb starts losing strength." You use it or lose it. You need to be careful how you do it, but you must start that horse walking, immediately, to put a load on. Then the tissue realizes it has to go back to work and regenerates. If you don't rehab a tendon while it heals, it will scar. Later, when you try to use it (especially a horse, with so much body weight), it will tear that scar tissue. There's no stretch to it at all, says Harman.

At present he cautions horsemen not to put horses put back to work any quicker than they would under traditional therapies. The advantage of using stem cell therapy is that you end up with much better healing and a normal, elastic tendon. The horse will be able to go back to his career, with less risk of re-injury.

People shouldn't jump to the conclusion that they can start working the horse again sooner just because they used stem cells. "Everyone wants a shortcut! All the work with Bapten® for tendon healing was a disaster, partly because it slowed the healing to try to let the cells come in before it scarred—and horsemen didn't understand the program and tried to put horses back to work early. This tore the tendons and created huge inflammatory reactions."



Differences Between the Equine Superficial Digital Flexor Tendon Before and After Treatment

Trying for a short cut doesn't give the horse a chance. "We want the horse to heal, but we want him to be able to win races, and not just go back with a scarred tendon," he says.

"It's expensive enough that most folks listen. If something is cheap and you can just stick an injection into a horse, people sometimes don't pay attention very well." But with the cost of stem cell therapy, a horseman doesn't want to waste the investment by rushing things and damaging the horse again. The cost of using stem cell therapy is about $2,000 to $3,000 depending on what other work is being done by the veterinarian. "The veterinarians at the track put it all together with a follow-up ultrasound once a month for a couple of months to see how the healing is doing—to know when those horses can go back into training—so they might charge on the high end," explains Harman.

"You should have a veterinarian follow up with ultrasound when you start working the horse again, to measure the cross section of the tendon, and the effects of exercise. If you put the stem cells in and four months later you have the horse jogging in his ground work and are ready to start working under saddle, you can then measure the tendon diameter once a month with ultrasound. You can see if it starts to tear again, and can back off. If it looks good, you can go up to the next level. An ultrasound check every 30 to 45 days will tell you how that tendon is coming along. If you wait until you see a problem clinically, you've already got a tear."

Advantages to Using Uncultured Cells

"All of the first work with stem cells, especially for cartilage healing, people tried to grow and make cartilage cells from stem cells and then implant them. You can heal rabbits this way, but it doesn't work very well in horses or goats. With anything that's really heavy load-bearing, those cells don't integrate very well. Part of the reason is because you've grown them already in culture, and they change. They are not as strong—they've lost some of their early signaling. But if you put the very primitive cells right back into the body, and let them figure it out themselves, they do better. The researchers right now are saying that's where the industry went wrong in our early work—we tried to make end tissues instead of letting the cells themselves figure it out," says Harman.

"The cells 'talk' to each other and interact, sending signals. The tissues are very complex; it's not just one tissue type. In a tendon, there are about five different kinds of tendon cells. So how do you know how to signal which one? It works best to let them figure it out and do it themselves. Later on, when researchers at academic institutions figure it out, we might be able to intervene and do some other things, but for now this works best. That's why we are very pragmatic; it has to be easy and it has to be cheap, or it won't be used in horses. If we had to grow and culture cells for a month, it would be a more expensive procedure (and we'd lose some of our time advantage), and only the owners of elite horses would do it. Maybe later they'll figure out how to genetically engineer these cells and make them be super tendon cells, but we are a long way from that. So right now we just want to treat enough horses that we can find out all we can about how these cells work clinically, how to optimize the rehab program, and how to get the most horses back to their normal performance level."