

# **HEALTH ZONE**

## Stem Cell Therapy

## Vet Services

## BY HEATHER SMITH THOMAS

STEM CELL THERAPY has been utilized in horses to help heal tendon. ligament, and joint injuries for two decades. At this point there are basically two sources of stem cells for clinical use-from bone marrow and from fat tissue. Cells harvested from bone marrow are usually cultured and expanded, putting them back into the horse some weeks later. Cells harvested from fat can be collected and utilized within a much shorter time.

"We use autologous cells—from the patient's own body," says Dr. Scott Mc-Clure, owner of Midwest Equine Surgery & Sports Medicine in central Iowa.

Allogenic stem cells, from another (donor) horse, and preserved cells, such as from umbilical cord blood from newborn foals, are not commonly used.

"The primary and most common applications for stem cells are in treating ligament and tendon injuries," he said.

## Continues to be an option for treating injured horses

"Stem cells are also used fairly frequently in osteoarthritis cases, but they don't work quite as well as we'd hoped.

"Meniscal injuries in the stifle joint are one area where we often tend to use stem cells. There have definitely been some positive outcomes associated with stem cells in meniscal injuries. This depends on case selection, however; some of these injuries will respond better than others. You need to have a stable joint for good results. If there is meniscal injury and additional ligamentous injuries, stem cells are not going to solve that entire problem."

There has been a lot of discussion regarding the best source of stem cells.

"Some people use the bone marrow cultured stem cells, and others use the adipose (fat) stem cells," McClure said. "There are other things on the market that pop up from time to time, that people are calling stem cells, but there are actually very few to no stem cells in some of these products. Horse owners need to discuss these things with a veterinarian who understands the difference before using them.

"The ways we can use a large number of stem cells are either to utilize harvested bone marrow (and cultured cells) that are sent to a lab to culture and produce a high number of stem cells, or the adipose stem cells that are processed on the spot, to get a large number of stem cells out of the fat," he said. "The cells from fat have the advantage that you can do this on site; you can liposuction fat from the horse, process, and reinject those cells. You don't have to wait for culturing. There are 500-1,000 times the number of stem cells in adipose than there is in bone marrow."

There are some other therapies, such as bone marrow concentrate, that have some stem cells and growth factors but relatively few stem cells. This extract contains a mix of things that might be helpful, but the actual number of stem cells is very low.

"There are also therapies such as amnion that have benefits but are not actually true stem cell therapy," McClure said. "It is important to be working with a veterinarian who understands the differences and has experience with these."

## HISTORY OF STEM CELL USE **IN HORSES**

Dr. Troy Herthel, a surgeon for the Alamo Pintado Equine Medical Center in Los Olivos, Calif., has worked with stem cells for a number of years. His father, Dr.



It is important to work with a veterinarian who understands stem cell therapies



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## **HEALTH** ZONE

Vet Services

Doug Herthel, pioneered the early use of stem cells from bone marrow in treating injured tendons and ligaments. Alamo Pintado has utilized stem cell therapy on more than 4,000 patients over the past 23 years.

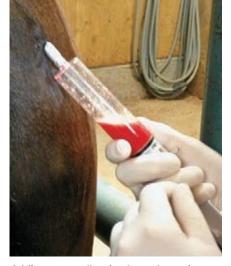
"The first stem cell procedures in equine athletes were done in the mid-1990s with direct bone-marrow injections," Herthel said. "We harvested bone marrow from the sternum of the horse and injected it directly into the lesion we wanted to treat—which at that time was primarily suspensory ligament injuries. That was one of the original uses and beginnings of regenerative medicine for equine athletes.

"Then we progressed to using bone marrow concentrate, a further refinement of using native or unprocessed bone marrow," he continued. "In this procedure we harvest bone marrow and centrifuge it in a special apparatus to

concentrate some of the stem cells as well as a lot of the growth factors and some of the other components we are looking for that can help stimulate healing.

"That use evolved into actually expanding and growing stem cells. When we do the expansion and grow stem cells from the original bone marrow source, it takes about two-to-three weeks to obtain the number of cells we feel would be adequate for the appropriate dose for a typical bowed tendon or suspensory ligament injury. We typically harvest 20-30 million cells, to be injected into the lesion, often via ultrasound guidance.

"We started out primarily treating soft tissue injuries and then progressed to treating joints. Some people started to treat laminitis with stem cells-and it blossomed from there. That's where we are now, and because there's been such an expansion of stem cell therapy in veterinary medicine in the past 15-plus



Adding stem cells stimulates the patient to send more local stem cells into the lesion

years, the Food and Drug Administration is trying to make sure this use is regulated and that this kind of therapy isn't used inappropriately," he explained.

As stem cell therapy was expanding its role in regenerative medicine, other techniques were also becoming popular, such as autologous condition serums, and components such as PRP (platelet rich plasma). These modalities are often vet-

## EQUISUL-SDT<sup>®</sup>

### (Sulfadiazine/Trimethoprim) **Oral Suspension**

NADA 141-360

CAUTION
Federal law (USA) restricts this drug to use by or on the order of a

EUUSU.SDT is a broad-spectrum antimicrobial from the potential suthannial collection and the potential suthannial collection and the potential block different sequential steps in the biosynthesis of nucleic acids. Sufficializarie inhibits bacterial synthesis of dihydrotfolic acid by companies of the superior of tetrahydrotfolic acid by more superior superior of tetrahydrotfolic acid by more superior superior of the superior of tetrahydrotfolic acid by more superior superior of the superior superior of the superior superior of the superior superior of the superior sup itory concentration of each agent (synergism) and to ariostatic action to a bactericidal action. Sulfadiazine is the rvers a pacteriostatic action to a bactericidal action. Sulfadiazine is the sproprietary name for 4-amino-N-2-pyrimidinylbenzenesulfonamide. methoprim is the non-proprietary name for 5-[(3,4,5-trimethoxyphenyl) thyl]-2,4-pyrimidinediamine.

Each mL of EQUISUL-SDT contains 400 mg co (333 mg sulfadiazine and 67 mg trimethoprim)

### DOSAGE AND ADMINISTRATION

inister EQUISUL-SDT orally at the dosage of 24 mg combined active didents per kilogram body weight (10.9 mg/lb) twice daily for 10 days. ISUL-SDT can be administered by volume at 2.7 mL per 45.4 kg mL/100 lb) body weight.

## CONTRAINDICATIONS EQUISUL-SDT is contraindicated in horses with a known allergy to

### Do not use in horses intended for human consumption HIIMAN WARNINGS

nicrobial drugs, including sulfonamides, can cause mild to re allergic reactions in some individuals. Avoid direct contact

The administration of animinicionals, including stilladiazine and unifiering prim, to horses under conditions of stress may be associated with acute diarrhea that can be fatal. If acute diarrhea or persistent changes in fecal consistency are observed, additional doses of EQUISUL-SDT should no tered and appropriate therapy should be initia

The safe use of EQUISUL-SDT has not been evaluated in breeding, pre-

creased hematopoetic activity and blood dyscrasias have been ed with the use of elevated doses and/or prolonged administrat tolentiated sulfonamides. EQUISUL-SDT should be discontinuous longed clotting times, or decreased platelet, white blood cell or "" prolonged clotting times, or dec

Neurologic abnormalities have been reported in several species following administration of potentiated sulfonamides. In horses, potentiated sulfonamides have been associated with gait alterations and behavior changes that resolved after discontinuation of the drug.

The safe use of EQUISUL-SDT has not been evaluated in horses less than 1 year of age.

### ADVERSE REACTIONS

ADVERSE REACTIONS
Adverse reactions reported during a field study of 270 horses of vario breeds, ranging from 1 to 25 years of age, which had been treated wit either ECUISUL-SOT (n = 182) or with a saline control (n = 88) are summarized in Table 1. At least one episode of loose stool of varying severity was observed in 69 of 182 (38%) of the EQUISUL-SDT-treats severify was observed in 69 of 182 (38%) of the EUJISUL-SU1-risk horses, and 29 of 88 (33%) salins control horses. Of those animals experiencing loses stool, 2 of 182 (1,1%) of the EUJISUL-SDT-res horses and 01 68 (60%) placebo-treated horses were removed from study due to diarrhea (defined as at least one episode of watery sto 80th cases of diarrhea in this study were self-limiting and resolved treatment within 5–10 days after discontinuation of EQUISUL-SDT.

Table 1. Number of Horses with Adverse Reactions During the Field Study with EQUISUL-SDT

Adverse Reactions	Equisul-SDT (n=182)	Saline control (n=88)
Loose stool (including diarrhea)	69 (38%)	29 (33%)
Colic	3 (1.6%)	2 (2.2%)
Diarrhea	2 (1.1%)	0 (0%)

CLINICAL PHARMACOLOGY

Calleguing oral administration, EQUISUL-SDT is rapidly absorbed and

reached peak concentration in 0.5 to 12.0 hours. The median plasma elimination half-life was 3 hours, with a range of 2.3 to 4.96 hours. Peak sulfadiazine concentrations were reached within 1.0 to 12.0 hours in the same study. The median plasma elimination half-life for sulfadiazine was approximately 7.80 hours, with a range of 6.78 to 10.39 hours. Only minor accumulation to do thougs was observed following repeat oral administration of EQUISUL-SDT and both drugs reached steady state

Table 2. Median (Range) of sulfadiazine and trimethoprim pharmacokinetics parameters following repeat dosing of 24 mg/kg bid EQUISUL-SDT for 7 days to six horses in fed condition

Drug	Sulfadiazine	Trimethoprim
Tmax (hr)	4.75 (1.00–12.00)	8.50 (0.50–12.00)
Cmax (µg/mL)	17.63 (10.10–31.15)	0.78 (0.60–1.14)
AUC 0-12 (last dose) (hr*µg/mL)	159.35 (73.90–282.54)	5.47 (3.31–10.91)
T 1/2 (hr)	7.80 (6.78–10.39)	3.00 (2.31–4.96)

ECUISUL-SU1 administered as a combined sulfadiazine-timethopi dose of 24 mg/kg body weight twice daily for 7 days provided concer-trations of sulfadiazine and trimethoprim with T-MIC90 (%T) values r00% and 98% respectively. The minimum inhibitory concentration (values for EcUISUL-SDT against indicated pathogens isolated from respiratory tract infections in horses enrolled in a 2010–2011 effectiveness field study are presented in Table 3. All MICs were determined

Table 3. Trimethoprim/sulfadiazine minimum inhibitory conco (MIC) values<sup>a</sup> of isolates recovered from horses with lower re-infection caused by *Streptococcus equi* subsp. zooepidemic treated with EQUISUL-SDT in the U.S. (2010–2011)

Treatment Outcome	Success	Failure			
Number of Isolates	65 <sup>C</sup>	46			
Time of Sample Collection	Pre-Treatment	Pre-Treatment			
MIC 50 <sup>b</sup> (μg/mL)	0.25/4.75	0.25/4.75			
MIC 90b (μg/mL)	0.25/4.75	0.25/4.75			
MIC Range (µg/mL)	0.12/2.4 to 0.5/9.5	0.12/2.4 to 0.5/9.5			

- The correlation between in vitro susceptibility data and clinical effectiveness is unknown.
   The lowest Mic to encompass 50% and 90% of the most susceptible isolates, respectively.
   One isolate of S. equi subsp. zooepidemicus was not tested.

### EFFECTIVENESS

EFFECTIVENESS

A negative control, randomized, masked, field study evaluated the effectiveness of EQUISUL-SQT administered at 24 mg/kg body weight, comply, hotic adily for 10 days for the treatment of lower respiratory tract infections in horses caused by Streptococcus equi subsp. zooepidemicu in this study, a total of 192 horses were treated with EQUISUL-SQT, and 88 horses were treated with Salleu. One hundred seventy-three horses 89 horses were treated with saline. One hundred seventy-three horses (112 EQUISUL-SDT and 61 saline) were included in the statistical analysis Therapeutic success was characterized by absence of fever and no worsening of clinical signs at Day 5 and Day 10, and significant clinical improvement or resolution of clinical signs of lower respiratory tract infectio by Day 17. The observed success rates are \$5.9% (66/112) and 14.8% (961) for the EQUISUL-SDT and saline-treated groups, respectively.

	Equisul-SDT	Saline	P-value*		
Least Square Means	61%	13.1%	0.0123		
* P-value and estimated success rates are based on back-transformed mean estimates from the statistical analysis.					

NIMMAL SAFETY as a target arimal safety study, EQUISUL-SDT was administered orally a target arimal safety study. EQUISUL-SDT was administered orally 3.2 healthy adult horses at 0 (0X), 24 (1X), 72 (2X), or 120 (5X) mg/kg beservation. Observations of loose stood (palets with liquid or unformed) worked to the control of the control

Hinderina via Coulcilu. SDT groups demonstrated statistically significant higher mean serum creatinine concentrations, and those in the 3X and 5X groups demonstrated statistically significantly higher mean serum albumin concentrations. Statistically higher mean neutrophil counts and mean serum graining plutingly transferse (GGT) activity were seen in the 1X and 5X groups. Individual animal creatinine, GGT, and albumin concentrations remained within the reference range. Individual animal elevations in absolute neutrophil counts ranged up to 7.09 x 10<sup>3</sup> micl. Inference range. 164, 95-63.31 x 10<sup>3</sup> micl. elevations in absolute neutrophil counts (reference range: 1.96-5.31 x 10<sup>3</sup>/mcL).

based upof flood concentrations obtained until give audit, was note that the sufficient and trimethoprim plasma concentrations did not increase in proportion to dose. For sulfadiazine, a 3X and 5X dose resulted in an average exposure of 2.0X and 2.6X the concentrations observed following a 1X dose. For trimethoprim, the corresponding value were 2.5X and 3.5X as compared to the 1X dose. Furthermore, marked

STORAGE CONDITIONS
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HOW SUPPLIED
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- Dr. John Bennett **Equine Services, LLC** Shelbyville, Tennessee



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# HEALTH ZONE Vet Services

erinarian-specific, in terms of how each veterinarian prefers to treat certain injuries and which regenerative therapy is utilized.

"There has been a lot of clinical research as well as research at the microscopic level, looking at stem cell therapy, trying to determine what is truly going on in that horse when we use those cells," Herthel explained. "It was once believed that stem cells would develop into whatever tissue type they are injected into. Now we realize it is more likely that these cells are a composite mixture and have many effects other than just the pure cells themselves. Their presence stimulates the patient to send more local stem cells into the lesion. It is a complex biologic process. Stem cells are like paramedics that rush in to give assistance and provide multiple factors that improve the healing process."

## ADVANCES IN HARVESTING STEM CELLS FROM FAT

Dr. Michael Coleman (former president and CEO and now on the scientific advisory board of InGeneron in Houston, Texas) says that in the past dozen years scientists have found that all body tissues have a pool of regenerative cells (stem cells).

"These cells are located on the outside of small blood vessels," he said. "We can obtain these cells from many different tissues, but it's easiest and less traumatic for the patient to harvest them from fat, and there are lots of blood vessels in fat.





Stem cell infusion

"These cells, in the matrix of the tissue, are frequently called stromal cells. They can have a regenerative effect on different tissues in the musculoskeletal system, for example, such as tendons and ligaments, even though we got them from fat. When cultured, they can actually be differentiated into bone, cartilage, or tenocytes (tendon cells)."

These cells are good at becoming whatever tissue they are placed into, to aid the healing of that tissue.

"I liken it to putting a plant in different soils," Coleman said.

"Stem cells, when put into the context of a tendon or a bone, they don't make fat; they secrete soluble factors—mostly proteins—called growth factors. These help the neighboring cells and have a nursing effect on the damaged tissue. On their own they can actually form new tissue. In an injured tendon or bone, these are the two main ways they have an effect. They can have a bystander nursing effect and



Needle for bone marrow aspiration

a direct regenerative effect by forming new tissue.

"In arthritis these cells can actually reprogram the body's immune cells within the inflamed joint to reduce the level of inflammation and slow the progression of the disease," he continued. "This strategy is used in horses, dogs, and humans.

"In the early days of stem cell processing, fat tissue was obtained from the horse by making an incision and dissecting out a piece of fat. This is fine for obtaining good stem cells but leaves a scar, and horse owners didn't like that. Now we use liposuction, just like in humans. We can take a small amount of fat from the horse and leave no visible evidence."

There is only a small incision into the tissue.

"The tube we use is very small in diameter, no larger than a big needle," Coleman said. "This is minimally invasive. The horse is mildly sedated and local anesthesia is used in the area where we harvest the fat."

Once the fat tissue is removed with liposuction, cells from that fat can be isolated in about an hour and then re-administered into the injury. The horse only has to be at the veterinary clinic or hospital for a short time. The veterinarian only has to see the horse once, rather than waiting more than a day for the sample to be sent to a lab, processed, and sent back—necessitating having the horse brought in again for administration of the processed cells.

## PROCESSING THE REGENERATIVE CELLS

"We, as a company, have created a small machine (portable, if desired), comprised of a processing unit that looks and functions such as a centrifuge but also has several other functions," Coleman said. "The spinning force of the centrifuge sends the heavier material to the outside.

"For this processing step, when the inner tubes are fixed in inverted position, the outside is a little higher than the inside. When centrifugal force is applied and everything goes to the outside, it travels upward because the tube is a little inverted. When it stops and there is no more force to push the contents of the tube to the outside, gravity causes the material to fall—and it gently falls back to the center.

"In the processing steps, rather than needing multiple pieces of equipment to accomplish the separation of these cells from the fat tissue, our one piece of equipment has multiple functions, and this is one of the key functions," Coleman said.

"We can accomplish the whole process for a horse in about an hour—collecting and processing the fat to harvest the cells. For a dog it might take a little longer because the fat from a dog is more dense.

"We now have a portable unit. For instance, one of our veterinarian customers sees a lot of horses in the Ocala, Fla., area and travels with this device. We supply a sterile drape and other items that can be used, and the veterinarian can do the procedure at the horse farm."

The portable unit was road tested in the back of an SUV to make sure it wouldn't be damaged by the movement and jostling of travel. Veterinarians can put it in their truck and take it to their patients.

"It weighs about 48 pounds and can be run off a DC converter and powered from the veterinarian's vehicle," Coleman said. "It draws about the same amount of power as a desktop computer, but can be readily operated out of the vehicle."

With some injuries, such as a tendon injury, it's best to treat them at a specific time, such as just after the inflammation subsides. The veterinarian can monitor the patient and then collect and process stem cells to inject them at the most optimum time.

"This process enables the veterinarian to use the cells on demand," Coleman said. "When he/she determines the best time to treat, the fat can be harvested and the cells processed and available. This provides more flexibility in a treatment schedule."

Heather Smith Thomas is a freelance writer based in Idaho.

