Regenerative Medicine (Stem Cell) Conference

Research on how stem cells can heal injuries in horses and other animals, from the first North American Veterinary Regenerative Medicine Conference

BY ERIN RYDER

ERIN RYDER PHOTOS

Stem cell therapy was one of the regenerative medicine methods discussed at the first North American Veterinary Regenerative Medicine Conference.

Regenerative medicine—approaches that utilize the body's own ability to heal, including methods such as stem cell therapy—might make the idea of "Horse, heal thyself," a real possibility. Nearly 300 practicing veterinarians, university researchers, cell biologists, biomedical engineers, veterinary technicians, students, mainstream medical doctors, and orthopedic specialists/surgeons gathered in California's Santa Ynez Valley for the first North American Veterinary Regenerative Medicine Conference, held March 5-6, 2010, in Buellton, Calif.

Regenerative Medicine

The meeting was coordinated by the University of California, Davis, Center for Equine Health; the Alamo Pintado Equine Medical Center of Los Olivos, Calif.; and Rood & Riddle Equine Hospital of Lexington, Ky.

As much a networking opportunity as a conference, the meeting produced a new association, the North American Veterinary Regenerative Medicine Association, to enable researchers and others to continue the momentum started at the event. Membership in this independent group is open to all regenerative medicine researchers, stem cell biologists, biomedical engineers, clinicians, and health technicians.

Doug Herthel, DVM, of Alamo Pintado, one of the group's founders, has been interested in regenerative medicine for some time. He first tried injecting regenerative cells (bone marrow) containing growth factors and fibrin into the torn ligaments of two chronically lame horses in 1995, which resulted in two sound animals. Since then, as well as using stem cells as a first defense against tendon/ligament woes, he's increasingly turned to these cells for various cases that didn't respond to traditional approaches, such as those with osteoarthritis, lymphangitis, and laminitis.

Bone marrow contains growth factors (proteins that signal cells to rejuvenate, heal, and reproduce).

Herthel gave the opening address and reviewed some of the lessons he's learned through treating more than 5,000 horses with autologous bone marrow injections (tissues collected from the specific patient). He said that while not all of these have been successes, he's seen a good success rate and "virtually no adverse side effects."

He explained that bone marrow contains many types of growth factors—proteins that signal cells to rejuvenate, heal, and reproduce. They can also home in on injured cells and focus their trophic (nutritional) effects on nearby tissue cells such as those in tendons, ligaments, blood vessels, or cartilage. Targeted cells seem to benefit and possibly differentiate as a result of the local environment and the stem cell-laden growth factors, cytokines, and other biological proteins. It's not yet clear how this *in vivo* (in the body) differentiation occurs.

Herthel also reviewed a couple of his "celebrity" patients, including racehorses Lava Man and Thorn Song.

Lava Man, a 7-year-old gelding with more than $5 million in earnings, retired in July 2008 after radiographs (often called X rays) showed marked changes in his front fetlocks from the previous spring. Herthel treated him with stem cells, "just to clean up the joint," but after six to eight months the horse had become sound, he had a clear bone scan, and his amount of joint cartilage had returned to normal. He trained sound, raced sound, and remains sound as of press time after his stem cell therapy. Herthel also highlighted the rehabilitation process used with this horse, including nutrition and careful training.

Another racehorse, Thorn Song, developed laminitis in late summer of 2009 after a training injury. His disease progressed such that the decision was made to euthanize him, when Herthel decided to try stem cells as a last-ditch effort to save the horse's life. He injected 100 million stem cells into the horse's coronary bands. Within 48 hours he saw pain relief, reduced swelling, and the horse was up and walking. In the months since, he's grown a "tremendous amount of hoof," Herthel said. While Thorn Song is not out of the woods, there have been no setbacks since treatment was initiated.

In the Lab

Several of the 25 presenters discussed their theories and findings on the mechanics of stem cell therapies sourced from bone marrow, umbilical cord blood, platelet-rich plasma, and other sources.

Among them was Allison Stewart, DVM, MS, Dipl. ACVS, an equine surgeon at the University of Illinois Veterinary Teaching Hospital in Urbana, Ill., who presented a study in which researchers used dye to stain and track tendon progenitor cells (parent cells intended to reproduce and generate more tendon cells) injected into an injured tendon to see where they went during the four months after treatment.

There was a big cluster of cells at the lesion at one week, but by four weeks most were gone. Four months later, the cells had completely dissipated. (Stewart even found one in an inguinal lymph node [in the groin area].)

While this particular study didn't yield clear results on efficacy of treatment, it did show researchers can customize the type of cell they create, and it verified that the progenitor cells stay around during the initial healing phase.

Another interesting aspect to this study, Stewart noted, was that individuals varied dramatically in their response to the injected collagenase and treatment. One didn't respond at all to the collagenase or treatment, while another had a drastic response to collagenase and responded very well to stem cell therapy. This indicates a need for larger study groups, as individuals can skew results dramatically in small studies.

"There is huge 'effect of animal' (how individuals respond) that we need to take into consideration," Stewart reiterated.

Laurie Goodrich, DVM, PhD, Dipl. ACVS, assistant professor in equine lameness and surgery at Colorado State University, discussed the use of gene therapy in regenerative medicine. Growth factors are important to push these stem cells to become what we need them to be, she noted. Her presentation focused on cartilage and bone healing—more specifically, the production of chondrocytes (cartilage) and osteocytes (bone) from mesenchymal stem cells (stem cells harvested from bone marrow).

"Gene therapy might be one technique to push these cells down the lineage of cells that we want them to become," Goodrich noted.

To explain this further: Tissues naturally produce proteins that enhance healing, but not at high enough levels to complete the process. If a scientist can deliver genes to increase production of these proteins, he/she can turn cells into protein factories. Damaged joints could "produce their own medicine" (proteins important in cartilage healing and minimizing inflammation), and as stem cells are encouraged to mineralize through genetic modification, bone healing could be accelerated.

To do this, researchers place the relevant gene sequence onto a viral or nonviral "carrier" with the genetic replication code deleted (they deactivate the virus so it can't reproduce). The carrier can then invade the cells and deliver the proteins without damaging the cells.

In the case of cartilage and bone, long-term expression is required, meaning the cell needs to produce more of these proteins for a long time. In trials, adeno-associated virus (AAV) seems to be a good candidate for long-term effects. Goodrich reported that AAV is nonimmunogenic (doesn't cause an immune response), efficient, doesn't aggravate cells, is easy to construct, and is safe to work with. When cells were genetically modified with AAV-BMP2 and also treated with dexamethasone, they mineralized (hardened into bone) in a lab environment within two weeks, and the virus' effect persisted as long as the cells survived (six to eight weeks in culture).

In one study of horses treated with stromal vascular fractions, 64.5% of tendon/ligament injury cases and 46.4% of joint disease cases returned to full work.

Goodrich is ready to start putting this vector (AAVBMP2) into horses to test its efficacy with the end goal of treating clinical cases in horses that have cartilage damage and joint inflammation. Additionally, her team intends to genetically modify mesenchymal stem cells with the hope of increasing the speed of fracture repair and, thus, avoiding the devastating complications of implant loosening, cast sores, and supporting limb laminitis.

In the Real World

One of several presenters to focus on the clinical applications of stem cell therapy, Robert J. Harman, DVM, MPVM, chief executive officer of Vet-Stem, presented results of treating 3,553 animals with stromal vascular fractions, small pellets made up of concentrated cells harvested from adipose (fat) tissue. Harman's review included 1,000 tendon injuries, 1,500 ligament injuries, 541 joint problems, 72 fractures, and 398 "other" issues.

To create the pellets, practitioners collect an adipose (fat) tissue sample and send it to the company, which produces the treatment and returns it in a ready-to-use syringe.

When it comes to production, scientists can measure indicators of "stemness," letting them produce a treatment that can be driven down specific tissue lines. This means researchers can measure how likely certain cells are to morph into what they need to become, such as bone, muscle, tendon, etc. They can also add specific growth factors to push stem cells down a desired path, whether the stem cells were derived from fat, bone marrow, or any other tissue.

Harman agreed with Stewart, saying "Animal to animal variation is huge." In some cases a horse will be totally sound after stem cell therapy, but it will have terrible-looking radiographs. In other cases the horse will X ray clean, but it will be lame.

After two years, among horses with tendon/ligament complaints treated with Vet-Stem, the company reported that 64.5% had returned fully to work, 22.9% returned to work at a lower level, and 12% had treatment failure.

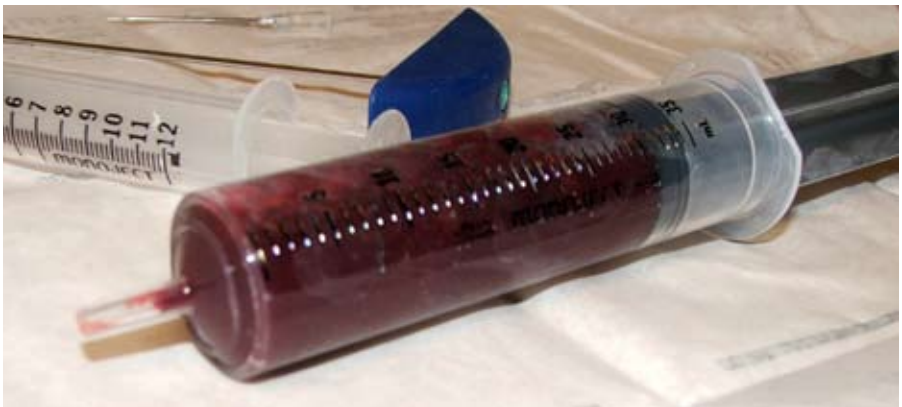
After two years, 46.4% of joint disease cases had returned fully to work, Harman said.

He also noted that Vet-Stem investigates adverse event reports in a manner similar to pharmaceutical companies. No systemic adverse effects have been seen in treated horses, although practitioners have reported local swelling in 0.2% of horses and transient inflammation in 0.4%.

David Frisbie, DVM, PhD, Dipl. ACVS, associate professor of clinical sciences at Colorado State University and president of Advanced Regenerative Therapies (ART), also presented on the issue of joint disease. He reviewed his and his colleagues' clinical intra-articular (within the joint) use of stem cells.

For the research Frisbie conducted, participating veterinarians were asked to treat only severe cases—specifically those in which traditional treatment had failed. Frisbie instructed the veterinarians to use stem cells and hyaluronic acid (which some say encourages the cells to "stick around" the injury) and avoid antibiotics.

Sixty-one cases qualified for long-term



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follow-up, and the investigators had complete information for 40 of them. Injuries included problems in the femorotibial joint (a joint in the stifle), in other locations in both stifles, and medial meniscal damage (also in the stifle). Other synovial structures treated included the navicular bursa, shoulder, and knee.

Of these 40 horses, 73% returned to work, including 35% to full function and 38% at lower levels of work or with ongoing maintenance; 27% did not return to work. There was no significant difference in treatment success rates based on age, breed, or sex.

Frisbie noted that horses with Grade 4 lameness on the American Association of Equine Practitioners (AAEP) scale (obvious lameness with a marked head nod, hip hike, and/or shortened stride) returned to work at similar levels as those judged as Grade 2 (lameness that's difficult to detect at a walk or trot in a straight line, but is consistently apparent under particular circumstances, such as under saddle or on a hard surface or incline).

Some success variation was noted based on injury severity—severe injuries had a 54% success rate, while mild and moderate injuries responded at 100%. Frisbie said he was "surprised" by the level of success seen in severe cases. Similar results were seen in tendon and ligament cases treated by ART, a company that produces autologous stem cell treatments from bone marrow, although these data were not presented.

STEM CELL WORKSHOPS

More than 150 equine practitioners met in roundtable groups to discuss regenerative medicine in veterinary medicine, including case experiences, clinical trials, and research.

Stem Cell Biology and Basic Research

Protocol standardization—the way researchers and practitioners grow, expand, and characterize cells—was a major topic of discussion during the research roundtable, moderated by Dori Borjesson, DVM, PhD, Dipl. ACVP. Attendees discussed efficient means of communication between labs and considered creating a working group to develop a review article on standards in mesenchymal stem cell isolation, culture, and characterization.

"We also discussed the most critical areas for research and discussed possible multicenter collaborations and funding agencies," Borjesson reported. "There were a few representatives from industry there that encouraged our collaborations with them to develop animal models of tissue injury."

Clinical Trials

The clinical trials workshop was focused on developing collaborative arrangements between academic institutions, private practice, and industry "to support and advance stem cell research and regenerative medicine under the highest ethical and medical standards for the discovery and development of therapies and cures for acute and chronic disease and injury," reported moderator Larry Galuppo, DVM, Dipl. ACVS. This blanket statement and the focus of the workshop was based on a summary put forth from the Medical Accountability Standards Working Group to the California Institute of Regenerative Medicine at a previous clinical trials workshop, which took place Feb.17-18, 2009, in Los Angeles, Calif.

Meeting Extras

"For me, stem cells are one of the most interesting, promising tools," said Bill Casner, co-owner of WinStar Farm, who spoke on the horse owner's perspective of stem cell therapy during the Conference Dinner Celebration. WinStar Farm, based in Versailles, Ky., is a top-level Thoroughbred breeding and racing operation.

Casner has used stem cell therapy in his horses—including Distorted Humor (sire of multiple champions) and Dubai World Cup winner Well Armed—since he heard Herthel discuss using it for superficial digital flexor tendon problems at the 2001 AAEP Convention.

Conference attendees could also observe, and in some cases participate in, live demonstrations of stem cell collection techniques—both for bone marrow and fat tissue—at Alamo Pintado. They were also able to see laboratory processing of bone marrow samples at Alamo Pintado's biological medicine laboratory.

Future Plans

Rood & Riddle Equine Hospital of Lexington, Ky., and Woodford Equine Hospital of Versailles, Ky., will host the 2011 North American Veterinary Regenerative Medicine Conference. Organizers plan to add sessions for horse owners and managers.

Take-Home Message

While veterinarians and human orthopedic surgeons are seeing promising results with regenerative medicine, laboratory research has not yet equally reflected this success. This meeting and others will help researchers and practitioners network to create consistent study formats and develop goals to for using equine regenerative medicine to treat injuries.

ABOUT THE AUTHOR

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