Zweig

Memorial Fund News Capsule

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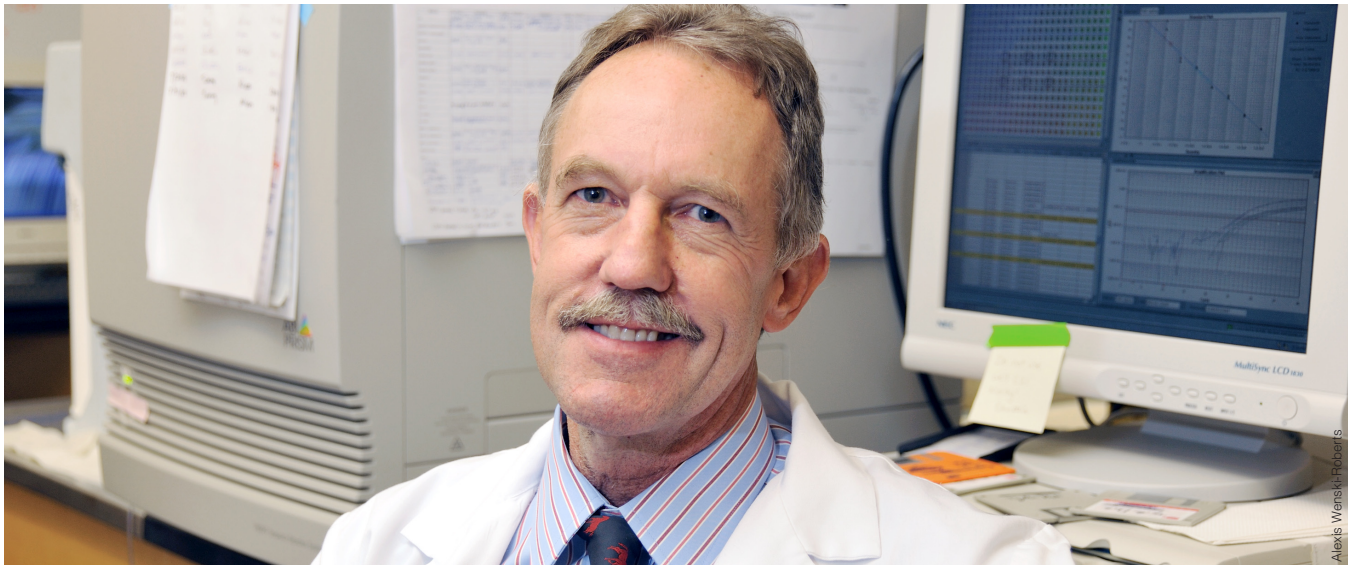
A report from the Harry M. Zweig Memorial Fund for Equine Research at the College of Veterinary Medicine at Cornell University

National Institutes of Health Grant Continues Alan Nixon's Research



Alexis Wenski-Roberts

Alan Nixon has been working to improve musculoskeletal repair systems in horses since coming to Cornell in 1988. Taking frustrating conditions like torn tendons and serious joint injuries to the lab and returning with cell and gene implant systems has been a richly rewarding program both from a scientific and an equine health perspective. "Our new grant allows us to push further toward arresting arthritis before it causes painful loss of joint function," says Nixon.

As a culmination of 18 years of support from the Harry M. Zweig Memorial Fund for Equine Research, Alan J. Nixon, BVSc, MS, has been awarded a five-year, $1.8 million grant from the National Institutes of Health to continue his research in techniques to improve the healing of damaged joints, thus staving off debilitating arthritis.

"We're at a stage where our research is beginning to show appeal to the medical community, which will allow us to go on treating the horses' disabilities while addressing the same issues of cartilage disease we see on the athletic field and in the aging human population," says Nixon, who has received a Zweig grant every year since 1990.

Nixon's focus has always been on cartilage transplant as a technique for repair because the tissue itself has a poor capacity to heal—next only to spinal cord and certain brain cells. Cartilage is composed of only a single cell type, sparsely populating the joint surface, with no supporting blood vessels and little capacity to replicate.

"So the end result," says Nixon, "is if you lose a big chunk of cartilage, the joint surface is virtually unable to heal itself."

In his early Zweig studies, Nixon established surgical procedures to add healthy cartilage cells to a damaged joint along with a matrix to hold those cells in place. Then he watched what happened and, over the years, observed that mitigating factors limited the outcome in simple replenishment approaches. He found that a successful and long-lasting repair depended on effectively addressing two simultaneously occurring activities: on the one hand, enhancing the activity of the transplanted cells and, on the other, mitigating the destructiveness of certain enzymatic activity (promoted by catabolic cytokines) that begin at the time of joint injury and continue to erode the newly reestablished cartilage surface.

INSIDE

Nixon

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Surgical insertion of screws to fuse a joint (here the pastern), should become less necessary as we implement joint-sparing cell therapy, especially when those same cells carry genes that enhance their repair potential and help quiet the reaction of the joint to trauma.

In the mid 1990s, Nixon discovered that an insulin-like growth factor –I (IGF–I) enhanced cartilage repair. At first he introduced the peptide forms of IGF–I into the cartilage cells awaiting transplantation, and later figured out how to clone the gene for IGF–I and insert the cloned gene into the cells. Last year, in a high point of his career, Nixon published a paper in the *Journal of Bone and Joint Surgery* that detailed the sequence of studies which lead to accomplishing a dependable long-term gene expression of IGF–I in the transplanted cells.

Yet as effective as the IGF–I enhanced transplanted cells are, every time they produce molecules to reestablish the cartilage surface, cytokines erode it away. Cytokines also seem to suppress the activity of IGF–I. So it's essential, Nixon concluded, to create an environment that controls their degradatory activity. He's identified interleukin –1 (IL–1) as one of these molecules "high up in the cascade of events" leading to joint erosion and ongoing pain. With this year's Zweig award titled "Pro-Inflammatory Cytokine Targets in Joint Disease as Check-Points for Gene Inhibition," he's searching for other molecules harmful in the new cartilage growth environment, principal among them tumor necrosis factor–α (TNF– α).

The long-term aim of the NIH studies, "Gene Enhanced Cartilage Repair in a Cytokine Permissive Environment," is to package and insert genes that constantly express the stimulatory growth factor and quell the catabolic cytokines.

"We want our cells to carry both of those little armamentaria so they can stimulate tissue around them while con-

With Cartilage Cells Scarce, Researchers Turn to Stem-Cell Transformation

Imagine if veterinarians could take bone marrow from a horse, derive stem cells from it, turn those stem cells into cartilage cells, then inject them into a damaged joint and watch them hone in on the site of cartilage damage and go about repairing the tissue in an effective way. That's the goal of Alan J. Nixon, BVSc, MS. Achieving it has been hampered by the way stem cells behave.

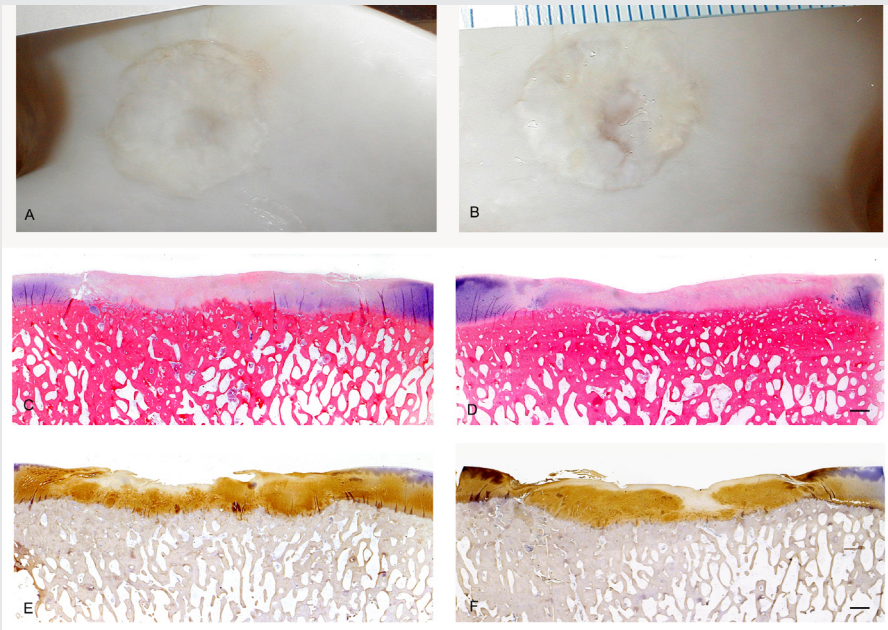
"With all the hype surrounding stem cells, they've turned out to be quite tricky to force down some tissue lines," says Nixon, whose research in stem cell harvest, isolation, and propagation has been supported for eight years by the Grayson–Jockey Club Research Foundation. "Stem cells seem to want to be tendon cells, or bone cells, but making cartilage cells from them is taking more time than we'd expected."

In the early years of Grayson support, Nixon established that harvesting cartilage cells from another animal and inserting them into the damaged joint of a horse was not an effective approach. The cells produced an immune response in the animal and their impact on repair was neither robust nor long-lasting, with the repaired tissue beginning to deteriorate within a year.

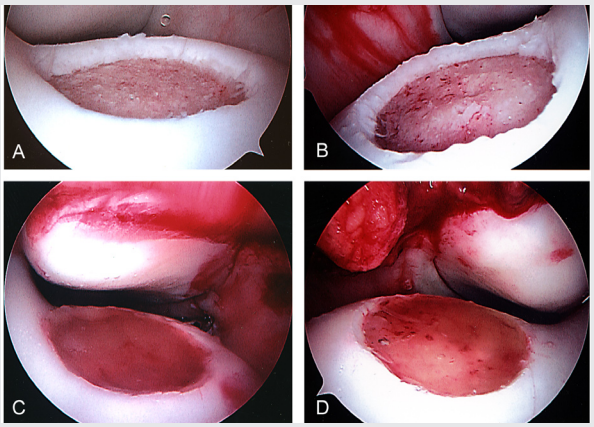
Stem cells are the pluripotent cells that respond to the body's call for repair of musculoskeletal tissues such as muscle, tendon, bone, and cartilage. Previous studies in Nixon's lab have also shown that implanting simple stem-cell cultures has only a transitory effect in improving cartilage repair.

Nixon's current Grayson grant allows him to use equine specific genes (Sox transcription factors and Transforming Growth Factor–β) that have been recombined into non-viral vectors that insert them into the chromosomal DNA of target cells.

"There we expect they will exert cellular and tissue-specific pressure to drive cartilage differentiation in stem-cell pools," Nixon explains.



Bone marrow–derived stem-cell implants manipulated down the cartilage cell lineage hold potential for permanent cartilage resurfacing. Repaired defects (left panels) are thicker and more durable, but not the dense glass-like cartilage we are born with.



Minimally invasive arthroscopic repair has been a must for equine cartilage resurfacing procedures. Stem-cell implants are secured in large defects (B) using clottable vehicles derived from blood, which are injected to set up in the shape of the original cartilage (D).

Since Nixon's Zweig-supported work has shown that IGF–I proteins may drive the synthesis of new cartilage components by these transformed cells, he's also investigating gene-mediated approaches to incorporating the IGF gene directly into stem cells, so that they become their own "microfactory" for IGF–I production. ■

trolling the enzyme activity that would erode the new matrix as it's being synthesized," Nixon explains.

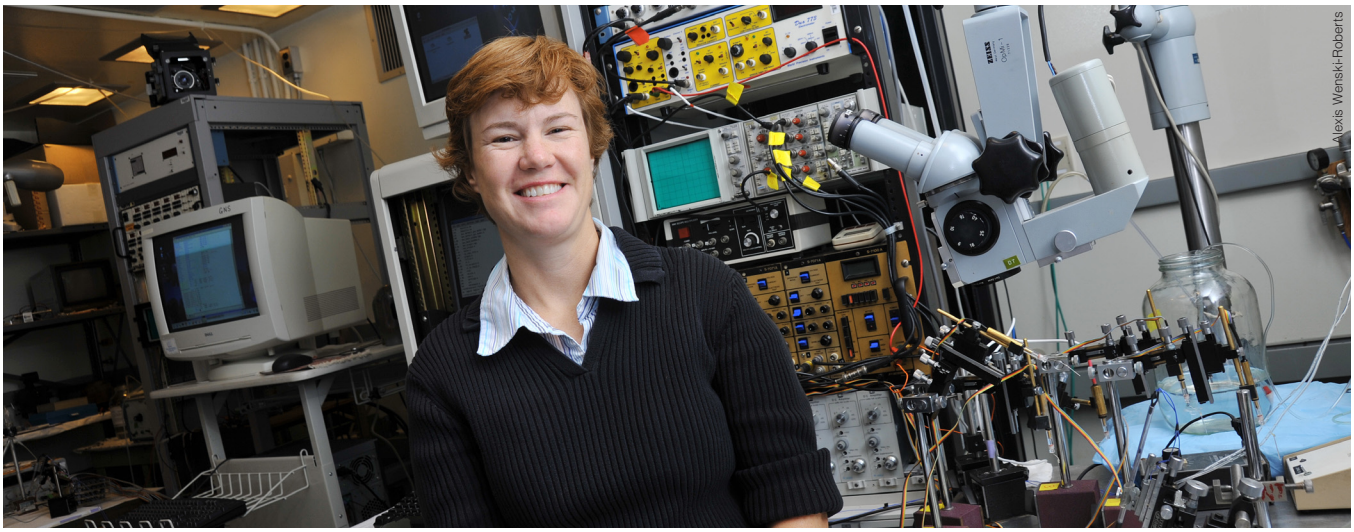
Along the way he'll also be forging new territory in addressing one of the most vexing issues in gene therapy: how to deliver genes without using viruses. Nixon's proposed investigation of how to make a safe delivery system is part of what attracted the NIH, as there have been adverse press reports surrounding several deaths that occurred when using viral vectors, although as vectors they work very well (that is, they are highly infectious, easy to make, and integrate well into the host's cells).

"Safety is an intrinsic part of a non-viral system," Nixon explains, "so we'll be trying to devise a system that is both safe and highly effective in inserting genes into target cells. The latter is the crunch."

In another five to eight years Nixon anticipates integrating the research programs on stem cells (see sidebar above), catabolic cytokine suppression, and gene-induced function. Then, Nixon says:

"We'd have a single program where our stem cells would come from marrow, and would be induced down a cartilage-cell pathway. We'd add a gene to drive the stimulus and another to control the catabolic cytokine environment, and will have gone a long way toward controlling arthritis." ■

Zweig Funds College's First Veterinary Clinical Fellow, Sophy Jesty



Sophy Jesty in front of electrophysiology equipment in Robert Gilmour's lab that is designed to measure and record electric currents traveling through cardiac tissue.

When Sophy Jesty was twenty years old and an animal science major at Cornell, she met N. Sydney Moise, DVM, MS, Dipl ACVIM (internal medicine, cardiology), now professor of medicine and chief of the section of cardiology at Cornell's College of Veterinary Medicine.

Jesty had worked with horses all her life, having been attracted by their gentle nature. She began assisting Moise as a student research assistant and that experience, Jesty says, "put the bug of cardiology in me."

Today, as a member of the first class of the college's innovative Clinical Fellows Program, Jesty BS '94, DVM '01, Dipl ACVIM (large animal internal medicine, cardiology), will spend the next two years tackling the most common cardiovascular cause of poor performance in horses: atrial fibrillation.

"A horse that is working at maximum heart rate cannot do its job while in a-fib. It must be converted to normal sinus rhythm for it to perform up to expected levels," Jesty explains. So her goal—during the Zweig-supported fellowship—is to attempt to identify a new pharmaceutical that's both safe and easy for practitioners to administer and which will do just that.

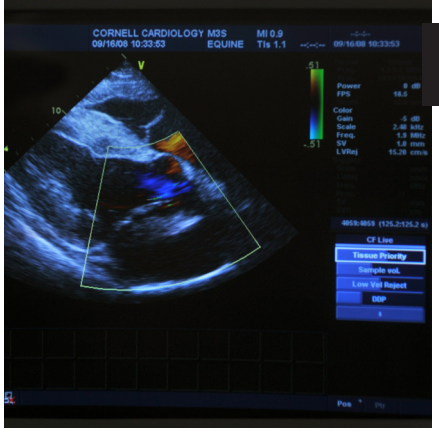
Jesty is the right person for the job, says Robert Gilmour, PhD, the associate dean for research and graduate education and a professor of physiology, whose laboratory specializes in studying heart rhythm disorders.

"The application process was open to everyone across the country who had finished a residency," says Gilmour, the senior administrator for the program. "She was clearly the top candidate."

"Jesty's project has the potential to make significant contributions to our understanding and management of atrial fibrillation in horses, which is consistent with her demonstrated dedication and expertise in equine medicine in general and cardiology in particular," says Bruce Kornreich, DVM, PhD, Dipl ACVIM (cardiology), a senior research associate in the

> "We're hoping to find a drug for conversion of arrhythmias in horses that is as effective as quinidine but has fewer and less harmful side effects."

Sophy Jesty comforts a horse just before a high-speed treadmill test.



An image from an equine stress echocardiogram that shows the structure and function of the horse's heart just after maximal exercise.

Department of Clinical Sciences who is one of Jesty's four faculty mentors during the fellowship.

The opportunity to become a clinical fellow was the logical extension of training Jesty began 11 years ago. Every step of the way was focused on gaining a comprehensive package of experiences that could equip her to move equine cardiology forward, particularly in the arena of performance evaluation and improvement.

Jesty's first step after completing her bachelor's degree was at the Weill Cornell Medical College, in New York City, where she spent two years studying DNA replication in a molecular biology basic science research laboratory. After completing her veterinary degree, she went to the New Bolton Center at the University of Pennsylvania, where, between 2001 and 2003, she completed a fellowship in large animal cardiology and ultrasound with Virginia Reef, DVM, Dipl ACVIM (internal medicine), the chief of the Section of Sports Medicine and Imaging.

"Dr. Reef sees more equine cardiology cases than anyone else in the country," Jesty explains. "I saw more while I was there than I would have anywhere else."

Also at the New Bolton Center, between 2003 and 2005, she completed a residency in large animal internal medicine.

Still, she felt she wanted to learn more cardiology and the very best place to do that was back here at Cornell, with her long-time mentor Sydney Moise. Even though the model animal that Moise uses is the dog, the training in cardiology applies to all species and in quality is unsurpassed. As evidence of that, Jesty was awarded first place (out of approximately 60 submissions) for her resident research abstract presented at the ACVIM annual forum in San Antonio, Texas, in June. The title of the abstract was "Cardiomyocyte Calcium Transients in German Shepherd Dogs with Inherited Ventricular Arrhythmias."

"Intracellular transients of calcium are the basis of excitation contraction coupling—so calcium is a cornerstone for an important aspect of the mechanisms of arrhythmias," Jesty explains. "It was a very in-depth project because the cardiology residency here—compared to other programs—has such a strong research base."

The first of Jesty's tasks, during the clinical fellowship that began in August, will be to perfect a system by which promising anti-arrhythmic pharmaceuticals can be tested. She'll work closely with Gilmour, Kornreich, Moise, and Dean Michael Kotlikoff, VMD, PhD, Austin O. Hooey Dean of Veterinary Medicine and professor of physiology. Gilmour and Kornreich have had some initial success using preparations that avoid the need to conduct testing on whole animals.

Once the system is up and running, Jesty can screen any number of anti-arrhythmics. One has already been identified as promising.

"Any veterinarian who has used quinidine—the drug used for conversion in horses for decades—has come to dislike its side effects, even though it does quite effectively convert atrial fibrillation to sinus rhythm," Jesty says. "We're hoping to find one that is equally or more effective with fewer and less harmful side effects." ■

Making Translational Scientists

"Just like any other aspect of being a scientist, you need training to be a good quality researcher," says Sophy Jesty BS '94, DVM '01, Dipl ACVIM (large animal internal medicine, cardiology). "This is my opportunity to work for the next two years on an important problem alongside people who are phenomenal at research."

Intensive mentoring by top-flight researchers on issues of animal well-being is the idea behind the college's innovative new clinical fellows program. There's none other like it.

"By providing a new model for academic veterinary training, we hope to address the critical shortage of clinicians and scholars who are needed to train the next generation of veterinarians and to continue to make advances in the treatment of disease," says Michael Kotlikoff, VMD, PhD, Austin O. Hooey Dean of Veterinary Medicine and professor of physiology.

The fellowships were created in recognition of the hardships in time and finances the traditional MS/PhD/Post Doctoral Fellowship training route demands, says Robert Gilmour, PhD, associate dean for research and graduate education.

"One of the reasons veterinarians don't go into research is that they come out of vet school with a debt load upwards of $80,000," says Gilmour. "Then to think of another seven years of training [four for a PhD and three more for a post-doc] with pay that averages $35,000 a year is very daunting." The two-year fellowship carries with it a salary of $65,000 a year and an additional $15,000 per year to fund their research project.

There is ample evidence in human medical education that laboratory experience through clinical fellowships, rather than pursuing additional degrees, is just as effective a context in which to learn science. Like Jesty, all of the clinicians chosen for the fellowships—this year there are three—must submit research proposals on a disease or condition with which they're familiar and about which they are intensely curious.

"So it won't be an esoteric, arcane type of experience for them that graduate training can sometimes be," Gilmour says. "Through their clinical experience they're already connected to the whole animal, hence highly motivated to gain the training needed to make new discoveries."

Jesty's goal, as for the other fellows, is to become what are called translational scientists—clinicians trained in the best research techniques, so they can make discoveries in the laboratory and apply those directly to the care of animals.

"We hope she'll combine what we know in the laboratory about the basic science behind atrial fibrillation with she what knows about the actual performance in horses," Gilmour says. "I know a lot about fibrillation, but I can't make horses better."

And in the process she'll gain the techniques and information that will allow her to go on solving other problems in the future.

"This isn't a so-called 'once and done' type of training," Gilmour adds. "It will train clinician/scientists who can meet the challenges of the future." ■

Cornell University
College of Veterinary Medicine
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The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state's equine industry. In 1979, by amendment to the pari-mutuel revenue laws, the New York State legislature created the Harry M. Zweig Memorial Fund to promote equine research at the College of Veterinary Medicine, Cornell University. The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

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