Zweig

Memorial Fund News Capsule

A report on equine research at the College of Veterinary Medicine at Cornell sponsored by the Harry M. Zweig Memorial Fund

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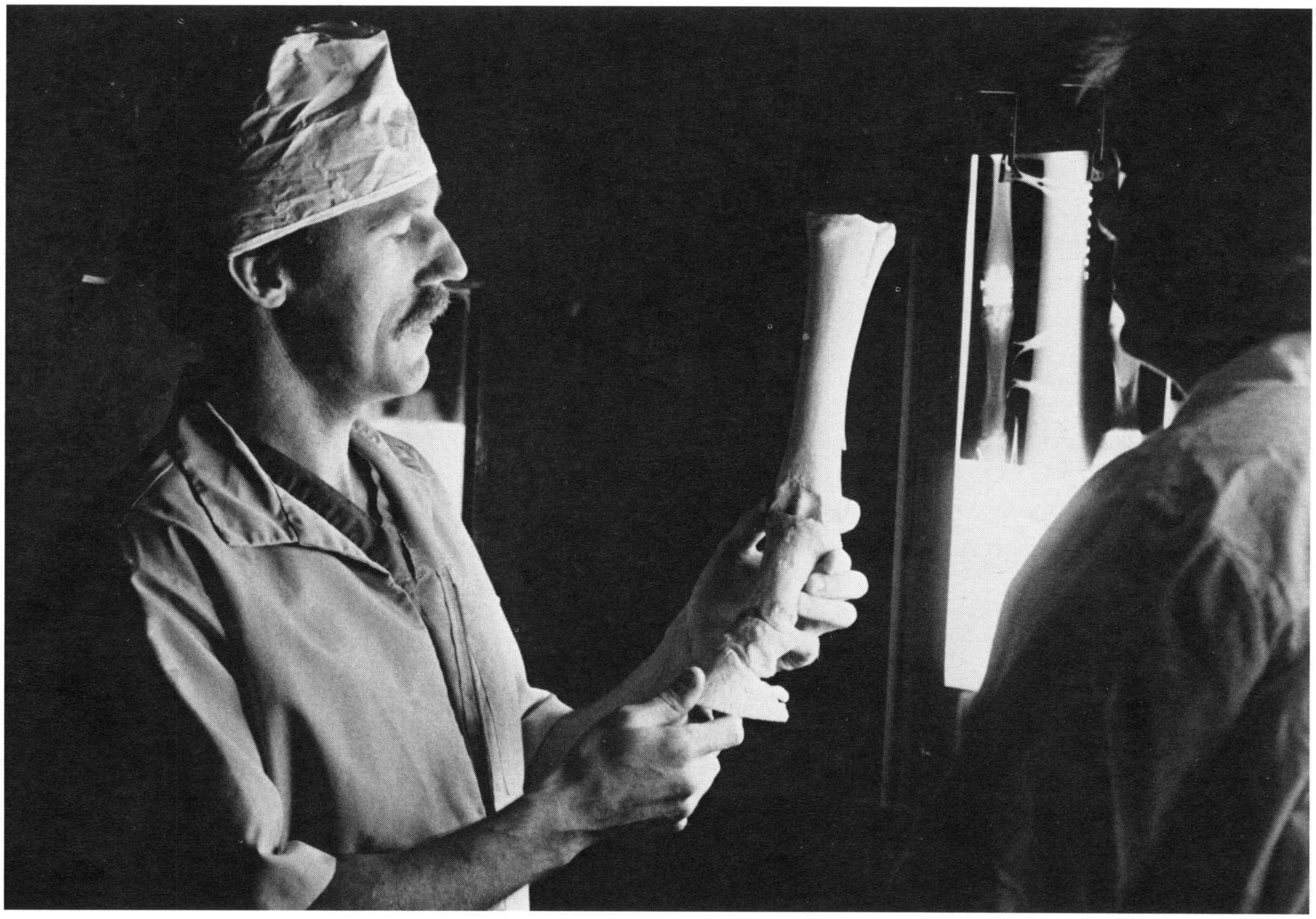


DAVID GRUNFELD

INSIDE

Cartilage Implants to Prevent Arthritis Progressing Well

Almost every athletic horse will experience some arthritis in some joint before its career is over, said Dr. Alan Nixon, an equine orthopedist at the College of Veterinary Medicine. A new resurfacing technique is providing fresh hope that cartilage could be repaired before the irreversible and debilitating disease of arthritis sets in. The technique uses an artificial implant consisting of collagen fibers and live equine cells to fill in the spaces. ▶

Until now, veterinarians have had poor results trying to repair or resurface damaged cartilage caused by fractures, knee and fetlock chips, infection, or osteochondritis dissecans (OCD)—a disease in which the cartilage develops poorly—or simple wear and tear on the joint. Since cartilage has little ability to repair itself, once it erodes or flakes off, the smooth, lubricated action cartilage gives the joint is lost; pain and swelling result.

Normal cartilage consists of a matrix of collagen fibers that gives cartilage its resiliency and cartilage-producing cells, known as chondrocytes, filling in the gaps. Some chondrocytes produce a wet and slimy substance called proteoglycan that gives cartilage its water-absorbing quality.

"If our technique proves effective, we could have a routine clinical method suitable for horses and humans, allowing early operations on cartilage-damaged joints to resurface them."

As previously reported (Number 6, 1990), Nixon's team had successfully developed a novel technique for harvesting immature chondrocytes from foals and culturing them to increase their number, size, and productivity. With support from the Harry M. Zweig Memorial Fund, Nixon and collaborators then preserved the cells and successfully embedded several million of them into one-inch disks of collagen, which are commercially manufactured from bovine tendons.

"Since then, we've found that the cells not only survived well in the matrix, but they also showed a long phase of producing proteoglycan," said Nixon, an associate professor of clinical sciences. "The cells populated the disks very nicely, especially at their base where extra cells are most needed."

Next, Nixon's team put 12 horses under anesthesia and, using a motorized bur, produced a 1.5 cm (about one-half inch) circular cartilage defect in both a left and right stifle. Each horse then received one cultured-disk implant. The defect in the other stifle was left to serve as a control. In the past, embedding cartilage cells has been unsuccessful because they were simply washed away in the joint fluid. Nixon and his colleagues, however, not only use a collagen matrix to support the chondrocytes, but also use two resorbable tacks to firmly attach the implant to the bone.

"Since cartilage cells are held in place by collagen fibers in normal cartilage, our cartilage cell-collagen composite for transplant is the closest thing to artificial cartilage yet devised," Nixon points out.

Another important aspect of these implants is that the surgery can be performed through a small, quarter-inch incision by using an arthroscope. "Our mission all along has focused on finding a resurfacing technique that could be done through an arthroscope because open-joint surgery is now considered obsolete—it causes much more pain and swelling and delays the return to athletic activity," Nixon said.

Although the artificial implants have only been in place for about half of the eight-month experimental period, signs of progress so far are good: "There's significantly less fluid, which means there's less of a reaction in the joints of the implanted sides," Nixon said.

In early 1992 the team will perform intense assessments of the implants to see whether the joint surfaces have deteriorated or improved. For example, the team will determine how well the cells have established themselves and created a matrix environment; what percentage of each area has filled in with new cartilage; how active the chondrocytes continue to be; how much proteoglycan they produce; whether they are producing the type of collagen needed (collagen type 2); and how closely the cells and collagen resemble normal cartilage.

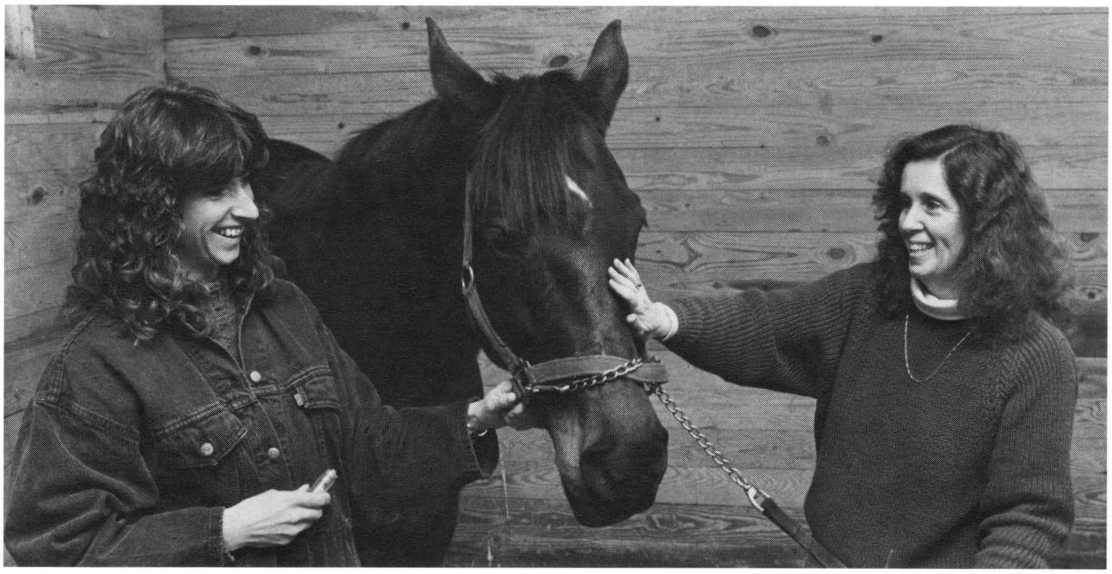
Using recent technology, Nixon's team also will conduct genetic "fingerprinting" tests to identify whether the active chondrocytes are from the chondrocytes transplanted from the original foal donors or are cells from the recipient horse that grew in as a consequence of the meager healing response normally seen in cartilage defects. "This is important to confirm that our implants are actually surviving," Nixon said.

In the meantime, Nixon's team plans to treat cultured chondrocytes in the laboratory with growth hormones to see if the cells can be stimulated to synthesize proteoglycan and collagen more aggressively before implantation.

"Most of our work is also highly applicable to humans," Nixon said. "If our technique proves effective, we could have a routine clinical method suitable for horses and humans, allowing early operations on cartilage-damaged joints to resurface them. Hopefully, the implants would go a long way in preventing the development of crippling arthritis later on." ■

New Technique Can Triple the Rate Of Double Ovulations

BRUCE WANG



Associate Professor Joanne Fortune (right) and technician Tammie Kimmich soothe Roshinka after injecting the mare with a hormone that stimulates follicles.

In contrast to other domestic animals and to humans, horses have always been resistant to treatments meant to boost the number of eggs that ovulate in a given cycle. By using a new protocol that stemmed by serendipity from unplanned observations during a basic research project, reproductive physiologist Joanne Fortune and collaborators have developed a reliable way to induce mares to double ovulate about 65 percent of the time.

Normally, only one follicle ovulates per cycle in mares; two follicles ovulate in about 15 to 20 percent of the cycles. Unlike cows or humans, however, twin births in horses are very dangerous and quite rare. Usually, one of the eggs is resorbed by the body, or the embryo fails to develop.

Fortune, an associate professor of veterinary physiology in the College of Veterinary Medicine, and Ph.D. student Jean Sirois were conducting basic research on how two types of follicular cells interact to produce the steroid hormones that regulate the entire reproductive cycle (a project funded by the Harry M. Zweig Memorial Fund and reported in issue Number 4, 1989). The researchers had removed one ovary from several horses to obtain cells from the ovulatory follicle for cultures in vitro. Sirois decided to follow what happened in the single ovary that remained in the mares.

"He observed two curious things," Fortune said. "The follicles on the remaining ovary developed more rapidly than normal, and there was a higher incidence of multiple ovulations."

"This work is important because it would make embryo transfer to foster mothers more practical."

The reproductive physiologist wondered if these developments were linked to the lower-than-normal level of progesterone induced by the removal of the ovulatory follicle that produces progesterone after ovulation. Without that ovary, progesterone stayed low rather than rising as normally happens during the luteal phase of the estrous cycle. With funds from the Harry M. Zweig Memorial Fund, Fortune and Sirois set out to see if they could use this information to stimulate multiple ovulations without having to perform a unilateral ovariectomy (removal of an ovary).

Previous attempts to superovulate horses had been made using hormone injections beginning at day 14 of the 21-to-22 day estrous cycle, but recent data obtained by modern ultrasound techniques indicate that by day 14 the selection of the follicle destined to ovulate has already occurred.

Fortune and Sirois decided to begin injections of follicle-stimulating hormone (FSH) much earlier in the cycle, before a wave of follicular development began. Rather than using extracts of hormones from equine pituitary glands, which are not commercially available, Fortune decided to work with a commercially-prepared version of FSH called Folltropin, which is extracted from pig pituitary glands and was generously donated by the Canadian firm Vetrepharm, Inc.

Fortune, Sirois, and technician Tammie Kimmich then injected forty mares (twenty horses during each of two seasons) with Lutalyse, a drug to trigger luteal regression on day 5 or 6, to duplicate the low-progesterone environment Fortune and Sirois had observed in the mares with single ovaries. They also injected varying doses of Folltropin beginning on Day 6 when most mares have not yet developed any large follicles. Injections continued every 12 hours until at least

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Dorothy Ainsworth

CHRIS HILDRETH

Dr. Dorothy Ainsworth gathers data at the treadmill on how a healthy horse breathes during vigorous exercise.

After a childhood of riding and caring for horses, Dr. Dorothy Ainsworth pursues her love of horses in her professional life. She earned a D.V.M. at Washington State University and pursued a two-year residency in equine medicine at Michigan State University.

During that residency, Ainsworth became perplexed. Horse after horse came into the clinic because they were failing to perform up to their potential. Why were certain horses having respiratory problems? What was limiting their athletic capabilities? Why weren't there better answers about the prospects for horses with respiratory problems and the efficacy of the recommended treatments?

To find out, Ainsworth, now 38, decided to immerse herself in the mysteries of respiratory physiology by pursuing a Ph.D. in veterinary science at the University of Wisconsin. Specifically, she studied the mechanisms and muscles that control breathing and how certain factors inhibit optimal performance, using dogs as her model.

"When I was offered this job as an assistant professor in the Large Animal Clinic here at Cornell last year, it was like heaven to me," Ainsworth said. "I could combine my knowledge of pulmonary functions with my love of horses."

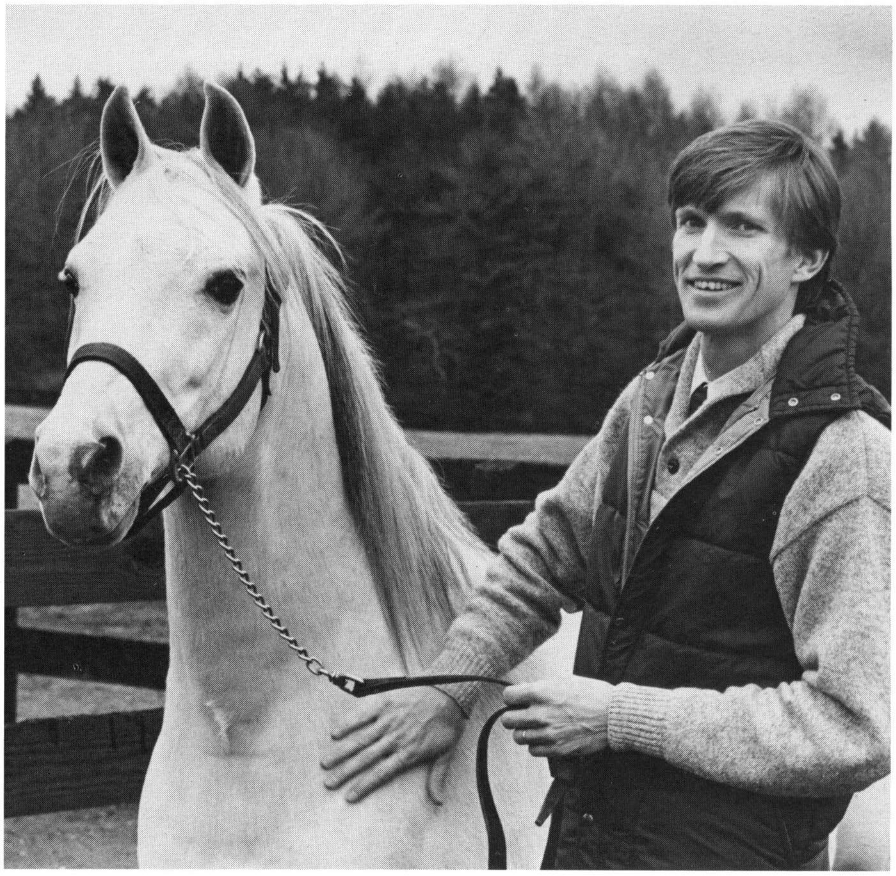
Ainsworth and her husband, Steve Eicker, a University of Wisconsin professor of veterinary anesthesiology, switched roles when they came to Cornell. Ainsworth became a faculty member and Eicker became a student, a doctoral student in epidemiology.

In addition to teaching clinical skills related to internal medicine to students in the College of Veterinary Medicine, Ainsworth is developing programs that assess pulmonary disease in large animals. Specifically, the Cornell large-animal pulmonologist seeks to develop tests that will identify horses with inflammation or infection in their lungs; examine how those conditions might affect athletic performance; and determine the usefulness of currently used therapeutic regimens.

In her research funded by the Harry M. Zweig Memorial Fund, Ainsworth is studying how the diaphragm—the major muscle of respiration—and the abdominal expiratory muscles work in healthy horses during exercise and under other conditions.

"For example, as exercise becomes more intense, does the activity of the diaphragm increase proportionately?" Ainsworth asks. "Exactly what is the role of the abdominal muscles during breathing? Do they assist breathing or do they primarily help the horse to run?"

Once normal patterns of these muscle activities are better understood, Ainsworth will look at whether diaphragm fatigue compromises athletic performance as has been suggested in the literature on humans. "We believe these studies will provide important clues to the sources of potential limitations to exercise." ■



BRUCE WANG

Stallion Hawk's Decoy is part of the teaching herd that Dr. Peter Daels oversees at Cornell's Equine Research Center.

After receiving his D.V.M. degree from the Dutch-speaking State University of Ghent in Belgium, Dr. Peter Daels was curious about American veterinary medicine. So he planned a year's internship to observe three universities in this country before returning to Belgium to practice veterinary medicine.

Daels got no further than his second veterinary college, the University of California at Davis. Daels said he was watching "the famous Dr. John Hughes" tackle problems in equine reproduction when he was unexpectedly offered a resident position there.

During the two-year residency, Daels plunged into the research problem at hand: how does endotoxemia affect the outcome of pregnancy in mares? When a pregnant mare develops an intestinal disorder such as colic or severe diarrhea early in pregnancy, poisons from intestinal bacteria called endotoxins may escape from the gut into the bloodstream. When that occurs, most mares fall ill and lose their pregnancies.

After the residency, Daels stayed at Davis (where he met his Mexican-born wife, also a veterinarian and now a Ph.D. student in physiology of reproduction at Cornell) to pursue his research interest and to work toward his Ph.D. degree.

"We found that endotoxemia didn't harm the fetus directly but threatened it by compromising the corpus luteum, a temporary ovarian gland formed after ovulation that provides the hormone progesterone, which is necessary for pregnancy maintenance," said the 32-year old Cornell theriogenologist. "We also discovered that the oral progesterone, ReguMate, could prevent pregnancy loss following endotoxemia by compensating for the damaged corpus luteum."

As an assistant professor of theriogenology in the College of Veterinary Medicine, Daels is now studying the mechanisms that cause the corpus luteum to regress in non-pregnant mares. He hopes to better understand why some mares who have never been pregnant do not return to estrus when there are no other clinical signs present.

With funds from the Harry M. Zweig Memorial Fund, Daels is following up his other discovery that estrogen in early pregnancy is secreted mainly by the corpus luteum, not the fetus, as previously believed. In humans and horses, the fetus itself secretes hormones—known as human chorion gonadotropin (hCG) and equine chorion gonadotropin (eCG), respectively—that stimulate progesterone and estrogen production in the corpus luteum.

Daels's current challenge is to confirm that the main effect of eCG is stimulation of the corpus luteum to secrete progesterone and estrogen in early pregnancy. "I hope to show that when cells from the corpus luteum of pregnant mares are incubated in the laboratory and stimulated with eCG, they will produce both progesterone and estrogen, and that the production of these hormones is the result of a two-cell mechanism, similar to follicular hormone production." [See News Capsule # 4.]

In other research, Daels is working to suppress the libido of stallions during their peak performance years without compromising their future fertility.

Daels's other responsibilities at Cornell include teaching clinical skills involving bovine and equine reproduction, supervising the teaching horse herd, and conducting graduate seminars on the endocrinology of pregnancy. ■

Double Ovulations
(continued from page 3)

one ovulatory-sized follicle was present. Blood samples confirmed diminishing progesterone levels, and daily ultrasound exams of the ovaries monitored follicle growth and number.

"We think we've now found the optimal dose that can reliably trigger double ovulations three times more often than normal," Fortune said. "This work is important because it would make embryo transfer to foster mothers more practical."

A reliable superovulatory protocol would also boost the genetic potential of valuable animals and allow mares to be raced or shown while less valuable mares carried their offspring to term.

"Also, a method for reliably superovulating equids would aid greatly in the reproduction of endangered equine species, and could be used to generate multiple embryos for research on various aspects of embryonic development and maternal-fetal interactions," Fortune said.

Next, Fortune plans to fine-tune the doses and timing of the injections to trigger more reliable hormone-induced ovulations and to test the ovulated eggs to assess whether they are viable. ■

In the next Zweig News Capsule

A complete listing of research projects awarded by the Zweig Fund for 1992.

The Harry M. Zweig Memorial Fund 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 funds and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

The Harry M. Zweig Memorial Fund Committee

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