

# 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*

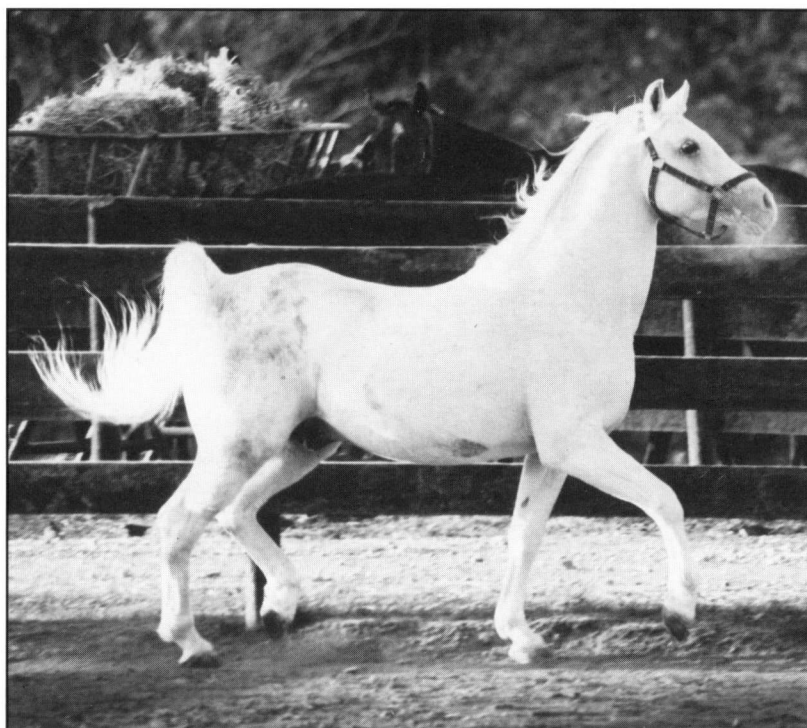
Number 8, 1990

## Reliable Tests For Evaluating Fertility in the Stallion

In the past, the human eye, and subjective human measurement and judgment were the sole means of determining a stallion's semen quality. Today commercial manufacturers are moving to develop computer-assisted technology to do the job more quickly, accurately and with a high degree of repeatability. But just how good are such tests?

With support from the Zweig Memorial Fund, Dr. Donald H. Lein and Dr. David J. Jasko from the Diagnostic Laboratory at Cornell's College of Veterinary Medicine and Dr. Robert Foote, from the Department of Animal Sciences at Cornell's College of Agriculture and Life Sciences, examined two commercial computer-automated semen analyzers (CellSoft Automated Semen Analyzer and Hamilton-Thorn Motion Analyzer) to see how useful these tests were in predicting stallion fertility. They found them to be very reliable indeed, if the tests were conducted repeatedly and at the correct time in the stallion's breeding cycle.

In their study, Drs. Lein, Foote and Jasko used the two tests with 96 stallions bred



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yearly to 20 or more mares over three seasons, for a total of more than 4,000 breedings. The test systems, which consist of a video camera paired with a microcomputer and a semen analysis software package, are designed to provide a variety of information about the sperm cells in an ejaculate. The procedure counts the sperm cells to determine concentration, indicates the shape of the sperm and whether that shape is normal or abnormal, and records sperm motility in terms of a percentage of motility as well as motility type—progressive, erratic, circulating, and oscillating.

Drs. Lein, Foote and Jasko were interested in which of the three factors—morphology, concentration, and

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motility—was the best predictor of fertility. While all three factors are important they found the highest correlation was with progressive sperm motility. Dr. Lein emphasizes, however,

that the tests must be done more than once and at the proper time in the stallion's breeding cycle for the results to be reliable. This is especially true in the horse with poor semen quality. "You can't just take a single ejaculate from any horse and predict fertility from that," he warns. "You have to look at him over a period of time."

For many stallions, semen quality drops as the days get shorter then gradually rises during the six to 12 weeks or so after the winter solstice, Dr. Lein explains. So it's important to test the stallion during the peak of its breeding season. "A lot of people look at stallions in the fall for purchase or syndication. This is the wrong time of the year to predict fertility by semen quality. A more reliable judgment must wait until spring."

*(continued on page 4)*

## Shedding Light On The Normal Events Of Pregnancy

**L** eading up to and during birth, a complex and tightly controlled system of hormonal and genetic signals prepares the mare and the fetus to endure a monumental change. The fetus must be made ready to live outside the uterus; the mare must become physiologically and behaviorally able to deliver the foal in a fashion that allows it to survive.

Just how these signals work is largely a mystery. Although research studies have focused on one or two aspects of parturition, until now none has attempted to investigate what happens to both the mare and the fetus during late gestation and birth. Support from the Zweig Memorial Fund enabled Dr. Claire Card, instructor in the section of theriogenology at Cornell's College of Veterinary Medicine, to undertake just such a ground-breaking study. Her results are expected to provide a vital foundation of knowledge about one of life's most basic, yet least understood processes.

To succeed in such a challenging endeavor, Dr. Card had to find a way to observe the physiological changes taking place simultaneously within the mare and the fetus. For this she needed blood samples from both the mare and the fetal foal, as well as indicators of uterine activity. Yet in order for her results to be accurate, techniques had to be used that would subject the mare and fetus to as little stress as possible. Dr. Card's first challenge was to employ a surgical technique for inserting fine plastic tubing into blood vessels within the placenta that lead to the unborn foal.

"We expected that it would be very difficult to get blood from the foals as this had only been attempted in ponies before," says Dr. Card. "But we were able to draw blood each day, from the 300th



A mare and her newborn foal

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*The final "big picture" question is what happens to the foal as birth approaches.*

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day of gestation through the first day of life, from one fetus." This alone she considers a major accomplishment.

During the surgery, when the catheter was inserted into the placenta, another catheter was placed in the mare's blood supply leading from the uterus. A pair of electrodes—strands of stainless steel about the diameter of fishing line—was also sewn into the muscular wall of the uterus. These devices made it possible to monitor both uterine activity and the hormonal changes occurring within the mare's body.

Dr. Card is currently at the data analysis phase of the study. One of the first questions she expects to answer is what happens to the uterus during late pregnancy and birth. "We performed the surgery toward the end of gestation so that we could look at the transition from the quiet state in late pregnancy to the more forceful contractions of labor," Dr.

Card says. "Data from our almost continuous monitoring of uterine activity will show the progression of changes into labor—whether labor happens rapidly, for example, or whether there are a long series of changes leading up to it."

The results of such electronic monitoring of normal pregnancies will also provide baseline data with which to do subsequent studies on drug effects. "All the drug therapies to date are evaluated anecdotally so there's no way to assess efficacy by clinical means," says Dr. Card. "By using a technique for placing electrodes in the uterus, we'll be able to tell what happens to the uterus when different drugs and dosage levels are administered."

Samples taken from the mare's blood allow Dr. Card to determine which substances are essential to natural delivery. She is looking particularly at three hormones: oxytocin, cortisol and relaxin. "With oxytocin the question is whether the mare can secrete this on demand, and therefore 'time' her own delivery," Dr. Card explains. Cortisol is known to mature fetal lungs in other mammals, and also to alert the mother that it's time for delivery, that there is no going back. Dr. Card wants to know if this is so in the horse. Relaxin is a substance secreted by the placenta which may be responsible for allowing the cervix to soften, the ligaments to relax and the vulva to stretch. Dr. Card wants to know what signals the surge in secretion of relaxin and whether the signal's source is in the mare or the foal.

The final "big picture" question is what happens to the foal as birth approaches. "For years in the veterinary profession, the premature foal was really considered an animal that wasn't meant to survive. There was nothing we could do," Card explains. "This was so because we really had no clues as to the developmental process the foal undergoes before it is born. Blood samples from the fetal foal will give us a very good idea of its hormonal and metabolic preparation for birth."

## Stopping the Spread of Equine Arteritis Virus

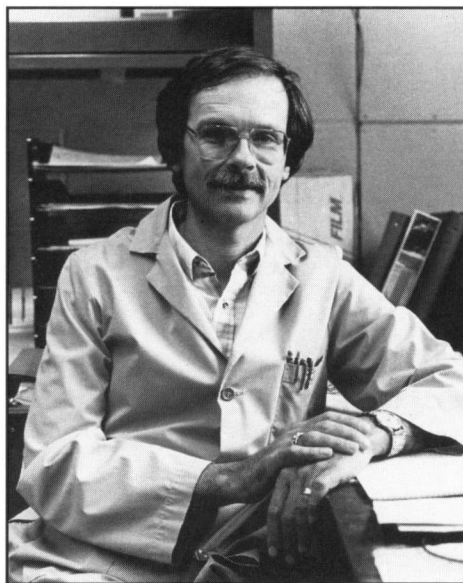
**W**hen researchers at Cornell's College of Veterinary Medicine set out to develop a definitive diagnostic test for equine arteritis virus (EAV), very little was known about the disease. Less than half a dozen laboratories in the world were then—and are now—investigating the nature of this potentially destructive virus.

EAV resides in the reproductive tract of stallions and any semen the animal produces is contaminated with it. Consequently, every mare bred to that stallion, if she doesn't have an antibody to EAV, becomes infected with the disease. It is not uncommon for stallions to shed the virus for years.

Although only recently of concern to thoroughbred breeders, equine arteritis virus has been "a problem for the standardbred industry for a long time. Yet it often goes undetected and unregulated," said Dr. Edward J. Dubovi, associate professor of virology, who headed the research team. "At present EAV-contaminated semen from standardbred stallions can be shipped all over the world."

Once a single mare has been infected through a stallion's semen the virus spreads quickly to other horses, just as an upper respiratory tract infection does. Depending on its strain, EAV can cause disease and death in adult mares and stallions. More critically, it can cause abortion and death in newborns.

Outbreaks of various strains of the virus occur from time to time. The most virulent strain, which kills adult horses, appeared in 1953. Another major outbreak of a less destructive strain hit the thoroughbred breeding farms of Kentucky in 1984. In 1986, there was an out-



Dr. Edward J. Dubovi

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break of the disease in western Canada, one in Arizona and New Jersey in 1987, and another in New York, Florida and Canada in 1988.

With support from the Zweig Memorial Fund, Dr. Dubovi and his team sought to fill a critical gap in diagnosing EAV by developing a rapid and accurate monoclonal antibody test to detect the virus in infected cell cultures and tissues. Hampered by a general lack of knowledge about EAV, the team's initial attempts failed. "For reasons as yet not understood, there is something inherent in this virus that makes developing monoclonal antibodies more difficult than doing so with other viruses," Dr. Dubovi explains. Recent efforts appear to have been successful and work will continue on development of rapid diagnostic aids.

While persisting with attempts at producing the needed antibodies, Dr.

Dubovi's group seized a number of clinical opportunities to learn more about the virus and the spectrum of clinical diseases caused by it. They made two important observations: one confirms the severity of the disease in young animals, and the other could lead to the treatment of stallions who are chronic shedders.

"In a series of studies on the potential of the virus to cause neonatal death we found that if you don't identify and deal with shedding stallions, the virus will cause death in neonates and, if EAV infects the fetus near term, the survival of the foal is unlikely," Dr. Dubovi says. The opportunity to develop an effective treatment came through the team's investigation of the site where EAV multiplies.

When the researchers began their work it was unclear where the virus resided within horse. "We found that the testis proper is not involved, but rather that the virus replicates in the accessory glands of the reproductive tract," Dr. Dubovi says. Knowing this lays the foundation for developing an effective chemical treatment which could cause the virus-producing cells to be expelled from horse's body without impairing its future fertility.

Although Dr. Dubovi continues to work on a monoclonal antibody test for EAV, he says that "what we learned about the natural history of the disease may have a longer lasting impact on controlling it than would simple diagnostics."

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## Fertility *(continued from page 1)*

It's also important to test repeatedly during the late winter and spring for standardbreds and thoroughbreds, and into the fall for other breeds as infertility can occur at any time. "The more often the tests are done the more accurate the results will be," says Dr. Lein.

Dr. Lein cites a number of advantages to the computerized test systems. They provide a record of exact, detailed measurements from an examination which can be repeated over the breeding cycle and from year to year. These numbers can then be graphed to provide a track record of the stallion's fertility over time. It also removes the human error and subjective judgmental errors that may occur in the present microscopic test.

"If breeders have several years' worth of such records, made throughout each season and especially at the stallion's most fertile time, then they can make a good prediction of the animal's fertility," says Dr. Lein.

Dr. Lein and his colleagues are also investigating two other promising avenues for predicting stallion fertility. The first involves microscopic changes in the head of the sperm cell that occur before fertilization. "Work done in bulls and humans suggests that the greater the number of sperm showing the change, (known as the acrosome reaction), the more fertile the sperm," says Dr. Lein. "We want to determine if this is the case with stallions too."

The other potential predictor of fertility is known as the penetration rate. A count is made of the number of sperm from a single ejaculate that penetrate a single ovum. Work done with humans and cattle, and preliminary studies on horses using hamster ova, lead Drs. Lein, Jasko and Foote to believe that a high rate of penetration may be a useful predictor of fertility in stallions too.

As with the computer-assisted semen analyzers already on the market, commercial tests for the acrosome reaction and penetration rate are likely to be developed soon. Then there may be three dependable methods for predicting what every breeder wants to know—the fertility of their stallions.

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 an amendment to the pari-mutuel revenue laws, the New York State legislature created the Harry M. Zweig Memorial Fund for the promotion of 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. Current committee members are Daniel J. Burke, Longford Farm; Donald G. Butcher, former Commissioner of the New York State Department of Agriculture and Markets; Dr. Wendell Cooper, Lana Lobell Farms, Inc.; Richard Corbisiero, Jr., Chairman, New York State Racing and Wagering Board; John L. Hardy, Tucker and Hardy Associates; Charles Knauss, Jr., Executive Director, Agriculture and New York State Horse Breeding Development Fund; Albert W. Miller, DVM; Everett Schoenborn, Climax, New York; Patricia Wehle, Scottsville, New York; William H. Welch, Executive Administrator, New York State Thoroughbred Breeding and Development Fund; Theodore J. Zornow, Avon Farms; Anna Zweig, widow of Dr. Zweig; and Robert D. Phemister, Dean of the College of Veterinary Medicine, Cornell University, who serves as chairman of the Committee. The Zweig Fund receives two percent of all monies accruing to the Agriculture and New York State Horse Breeding Development Fund and the New York State Thoroughbred Breeding and Development Fund from the state's tracks and off-track betting.

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