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

The Harry M. Zweig Memorial Fund for Equine Research

A Nassau, N.Y., veterinarian, Dr. Harry M. Zweig was a strong supporter of the equine industry in his lifetime. He served as a member of the Equine Advisory Council at the College of Veterinary Medicine at Cornell, was director and chairman of the Public Relations Committee of the United States Trotting Association and, for the last thirteen years of his life, was president of the Harness Horse Breeders of New York State. He helped to revitalize harness racing at the New York State Fair, and worked to pass the 1965 law creating the New York Sire Stakes program.

After his death in 1977, the family and friends of Harry M. Zweig established a trust fund in his name to support equine research at Cornell. 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 New York State Veterinary College at Cornell. By legislation, 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. The statute established a committee to administer the fund; its members include the chairman of the New York State Racing and Wagering Board or his designee, the dean of the College of Veterinary Medicine at Cornell or his designee, a member or the executive director of the Agriculture and New York State Horse Breeding Development Fund, a member or the executive director of the New York State Thoroughbred Breeding and Development Fund, and at least five New York State breeders, owners, trainers or veterinarians in equine practice.

Individuals submit to the committee their proposals for the conduct of research projects to be supported by Zweig grants. Proposals are reviewed by two or more experts in the field. The Committee is responsible for considering need, priority and potential success in determining those bearing the most potential benefit to the equine industry in New York State.

The Zweig Memorial Fund newsletter is intended to inform the equine industry of the progress and potential of those equine research projects supported by Zweig funds at the College of Veterinary Medicine. Published periodically throughout the year, the newsletter will occasionally focus on particular types of research. This inaugural issue highlights projects dealing with reproduction, an appropriate and timely topic.

Dr. Robert D. Phemister, Chairman
Harry M. Zweig Memorial Fund Committee

Evaluating the Stallions

How can a computer assess your stallion's potential as a competent stud? Dr. Donald Lein, director of Cornell's Diagnostic Laboratory, and his colleagues hope that by utilizing the computer's capabilities they can perfect a program to accurately predict what every breeder wants to know. . the fertility of their stallion.

With financial backing from the Zweig Memorial Fund, Dr. Donald Lein, Dr. David Jasko, and their associates from the College of Agriculture and Life Sciences, Dr. Robert Foote and Karen Smith, are utilizing computer-assisted technology to measure, analyze and correlate an accurate assessment of a stallion's semen analysis. No one test can definitely predict fertility from a single semen sample. Rather, the assessment combines measurements from different parts of a semen evaluation to better predict fertility. Using this information, stallion owners can better predict a workable plan that allows for sensible distribution of mares per sire.

In a conventional semen analysis carried out today, semen is judged on gel free volume, percentage of motile cells and progressive motility, concentration of sperm cells, normal spermatozoa and percentage of morphologically normal spermatozoa. Total sperm output and scrotal width are also measured. Using computer programs and two objective methods of analysis outlined below, a more accurate prediction of fertility may be possible. An image-analysis system utilizes a microcomputer and a computer-assisted semen analysis software package. The percentage of motile sperm cells, mean velocity, mean linearity and mean angular head displacement can be measured for groups of sperm cells from an ejaculate of stallion semen. With these additional measurements, Lein's team can determine not just motility, but how fast a sample of sperm can swim, a good indicator of how strong they are. Researchers can assess from this the penetrating power of sperm, an important quality in sperm which must overcome natural barriers enroute to fertilizing an egg in the mare.

Spectrophotometric Swimup Procedure

Spectrophotometric swimup procedure is one of two new methods of objective quality analysis adapted for use with stallions semen. Through use of a spectrophotometer, (measures the density of particles in a solution), a temperature controlled sample holder and a chart recorder that notes changes, movement characteristics of a suspension of spermatozoa can be recorded. The technique relies upon the ability of highly motile spermatozoan performance in this swimup procedure correlated with fertility and the technique provides separated sperm for further characterization and fertility testing.

Dr. Donald Lein

CASA: Analyzing Semen By Computer

The second method of objective analysis adapted for use with stallion semen was CASA, a computer-assisted semen analysis system (Cellsoft, Cryo Resources, Ltd.). This system is capable of determining values of percentage of motile cells, mean velocity of motile cells, mean linearity of motile cells (progressive motility), and mean angular head displacement of motile cells (shift of sperm head during movement). This system tracks motile spermatozoa utilizing a computer integrated with a phase contrast microscope and video camera. Measurements can be made directly from the video image or from high resolution recordings on videotape. Printouts of means and standard deviations for such measurements of spermatozoal motility, velocity, linearity, concentration, head movement and circular patterns are produced. These measurements of human and bovine spermatozoa are highly correlated with fertility. Our research this past year with 55 stallions booked to approximately 2500 mares on commercial breeding farms indicates that the objective analysis accounted for 2 times as much of the variation in stallion fertility compared with conventional semen analysis. In CASA, sperm can be described, for example, as hyperactive, especially if they have been flushed from a mare's uterus and have undergone what the scientists term, "capacitation". Capacitation within the reproductive tract of the mare is a poorly understood phenomenon, although it is known to involve microscopic changes in the head of the sperm (acrosome reaction). Good capacitation means an energetic demonstration in the "swim-up" procedure and, therefore, good penetrating ability of sperm. By designing experiments based on accurate time frames, the research team at Cornell will collect sperm at regular intervals after they have been placed in the uterus. They will look for the location where changes in the sperm occur, the speed at which capacitation happens, and just what's missing from a capacitated sperm.

When subjective methods (conventional analysis) were compared with objective methods (computer-assisted analysis) in field tests, Dr. Lein and co-researchers found over half of the measurements recorded had a direct correlation to a stallion's fertility with CASA's measurements proving slightly better than conventional measurements. Based on analysis of only one ejaculate from most stallions, the percentage of motile cells as determine by CASA gave the highest correlation with fertility.

Even though Dr. Lein and his co-researchers have demonstrated strong correlations between semen analyses and fertility, they are not at a point where they can predict fertility accurately - at least not from a single ejaculate. Further study is planned analyzing multiple ejaculates from the same stallion over a breeding season. Eventually, for an overall picture of a stallion's fertility, Lein hopes to tie the stallion's breeding performance of the previous year to a computer-assisted semen analysis done in advance of the next breeding season to better predict the future fertility and breeding management of the stallion for that year.

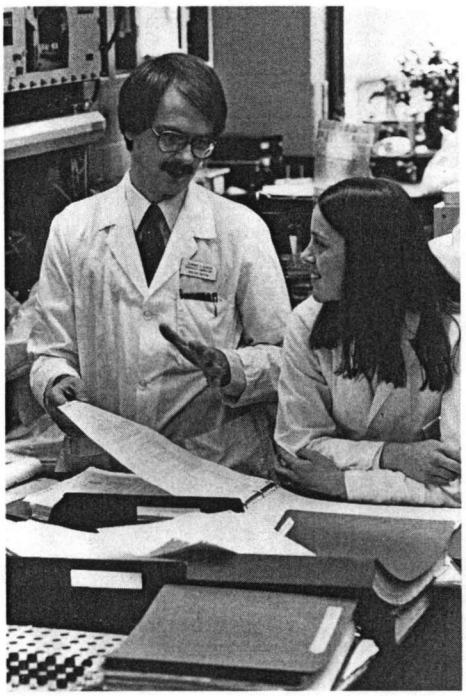
Equine Viral Arteritis . . . the Quiet Disease

Dr. Edward J. Dubovi and his associates at the Diagnostic Laboratory, and the Department of Clinical Sciences, College of Veterinary Medicine at Cornell, are working to try and unravel some of the puzzling questions of equine viral arteritis (EVA). The experience with EVA in Kentucky in 1984 and the Canadian outbreak in Alberta in 1986 brought home the realization that this virus was a real danger, able to infect and spread quickly to susceptible animals especially where proper management and vaccination programs are absent. As far as testing and vaccination programs are concerned, the Thoroughbred industry has responded to the challenge whereas other segments of the equine industry appear less concerned. Elimination of the threat of this viral infection will require a concerted effort by all horse owners. To assist in this effort, Dr. Dubovi's research is pushing in several directions in order to provide the equine industry with a better understanding of EVA.

One aspect of the Cornell effort consists of developing monoclonal antibodies to equine arteritis virus. These antibodies will aid in the development of better diagnostic tests as well as provide some much needed information on the strain differences which exist in nature. Initial efforts on this project were concerned with generating sufficient quantities of virus for use in immunizing mice which are the host animal for use in monoclonal antibody production. This challenge was met with the development of a new equine cell line which has also aided in the isolation of virus from clinical cases. Attempts to produce stable hybridomas secreting antibodies specific for EVA have not been successful as yet, but there are no technical barriers to the success of this project.

For the eventual control of EVA, a clear understanding of the natural history of the virus is essential. From work in Kentucky, it was established that the stallion might be the major reservoir of the virus with spread of the infection through virus-containing semen. The results of a surgical castration of a persistently infected stallion suggested that the virus resided in the reproductive tract and not in the testes proper. To confirm these findings, a vasectomy was performed on a shedding stallion. Periodic semen collections were analyzed for sperm count and for the presence of virus. This animal continues to shed virus six months after the vasectomy even though viable sperm are no longer present in the ejaculate. These data clearly indicate that the virus resides in the accessory regions of the reproductive tract. These findings provide the basis for studies to attempt to eliminate the virus without impairing the reproductive performance of the stallion.

The outcome of an infection is controlledby the genetic potential of the agent, the genetic potential of the host, and by usually undefined environmental factors. Several aspects of this equation are being examined by the Cornell team. The genetic potential of the virus was examined by infecting pregnant mares (approximately 100 days gestation) with an isolate of EVA from a shedding stallion. Two mares were infected intravenously with the virus, while two others served as contact controls. By four days after infection of the mares, the contact mares also became infected with EVA, indicating that the infected mares were shedding virus. Neither the infected mares nor the contact mares showed any clinical signs even though diagnostic tests confirmed the virus infection and none of the four mares aborted. This is in sharp contrast to the Bucyrus strain of EVA and also different from isolates from the 1984 outbreak in Kentucky. Of the four foals from these mares, none showed any evidence of infections in utero either through the production of antibody to the virus or by becoming persistently infected with the virus. These data are in sharp contrast to the findings of Dr. Dubovi involving a cluster of fatal pneumonia in neonatal foals. EVA was the only infectious agent isolated in four such cases. Clearly the EVA infection produced a severe clinical disease. Work is now in progress to determine whether the virus was different or whether the age of the animal is a critical determinant in the disease process.



Dr. Edward Dubovi

As another breeding season begins, Dr. Dubovi and his team hope to monitor breeding farms and to investigate neonatal foal deaths. With this information, they can more precisely direct their efforts in the development of improved diagnostic procedures and can provide more concrete information on the threat of EVA to the equine industry.

Embryo to Uterus

The interaction between the equine uterus and the embryo is critical to the establishment of pregnancy in mares; yet this delicate occurrence is fraught with obstacles, biological errors, and disappointments if the pregnancy fails. Much of the success in establishing a pregnancy, according to Dr. Barry Ball, is related to the communication skills of the embryo with the uterine lining, or endometrium. Failure of the embryo to signal that it is present in the uterus can result in the mare returning to heat with loss of the embryo.

Barry Ball is a veterinarian who earned his Ph.D. at Cornell while investigating problems in equine reproduction. His current interest is the artificial culture of a portion of the early equine embryo known as the trophoblast. He hopes to initiate studies with the resultant tissue, known as trophoblastic vesicles (TRV), that could eventually increase the pregnancy rates of mares.

Dr. Ball with his associates, Dr. Thomas Little and Dr. Robert Hillman, hope to use TRV to study the signal that tells the endometrium that an embryo is present within the uterus. Such signals have been

continued

Dr. Barry Ball

identified in experiments in cattle in which trophoblastic vesicles were found to signal the uterus to maintain production of progesterone, the hormone of pregnancy. Progesterone, in turn, is important to establish a uterine environment that will nourish the developing embryo. Unlike the cow and other ruminants, the equine embryo does not elongate quickly within the uterus to make maximum contact with the uterine wall. Instead, it moves throughout the uterus, and as it travels, provides the signal that will continue production of progesterone.

If the TRV behave as Dr. Ball suspects, they will prove to be the source of the signal to the endometrium to continue to support the growing embryo. Again, experiments from cattle show that TRV transferred to the uterus along with whole embryos can increase the chances of survival of the intact embryos. This may be due to an increase in the intensity of the signal present within the uterus.

How will the Cornell investigators know what is happening inside the uterus after the TRV transfer? Transrectal ultrasonography is a non-invasive and painless method of looking at any fluid filled structure such as the early embryo or the developing TRV. Ultrasound is particularly useful for pregnancy detection in both animals and humans. Researchers, using ultrasound equipment, can follow the movement of the embryo or TRV within the uterus on frequent basis.

This research by Dr. Ball and associates has far reaching implications for horse breeding. By the end of 1988, it will have added significantly to current knowledge on equine reproduction by supplying information on the trophoblastic signals that tell the uterus when an embryo is present within the uterus.

Zwieg News Capsule
College of Veterinary Medicine
Cornell University
Ithaca, NY 14853

BULK RATE
U.S. POSTAGE
PAID
PERMIT 780
ITHACA, N.Y.