

Zweig

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

Memorial Fund News Capsule

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CHARLES HARRINGTON

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Studies of the Mechanisms of Fertilization Promise Improved Breeding Success

Conception in the horse and many other species, including humans, is not just the simple act of sperm making their way to an egg. Rather, it is an intricate and dynamic symphony of events, one of which is the change the sperm undergo as they are stored in the oviduct, the tube that joins the uterus and the ovary. ▶

Fertilization *continued from page 1*

"Work from our laboratory and from others indicate that sperm are stored within a reservoir in the oviduct that is formed within two or three hours after insemination," says Barry A. Ball, D.V.M., Ph.D., an associate professor of clinical sciences at the College of Veterinary Medicine at Cornell. Ball has learned that within this reservoir, sperm not only remain viable for several days, allowing mating to precede ovulation by two or three days, they undergo essential biochemical and physical changes without which they could not fertilize the egg.

Funded by the Zweig Memorial Research Fund since 1992, Ball has been studying how sperm form this essential reservoir in mares with the hope that a better understanding of this process might enable veterinarians to better assess the fertility potential of stallions. Such knowledge would be very important to horse owners, potential buyers, breeders, insurance companies, and syndicates.

"In addition to improving our understanding of the basic biology of this process in the mare, we are keenly interested in understanding situations in which formation of an 'adequate' sperm reservoir does not appear to occur," he says. "When this happens, the sperm of the stallion do not remain viable in the mare's reproductive tract. Mating or insemination from these stallions, therefore, requires much more precise timing to ovulation to achieve pregnancy."

Since accessing the oviduct of the mare is too difficult for routine experiments, Ball and his colleagues have developed a laboratory model that combines cells from the lining of the oviduct with sperm in a coculture system. Using this model, Ball has determined exactly where the reservoir forms. He's discovered that sperm that come in contact with the oviduct cells remain viable for up to four days, while those that don't survive only a few hours. Ball has also shown that when the sperm are stored in the reservoir under normal conditions, their

surface proteins undergo the essential changes required for successful fertilization of the egg.

"We have found that the interaction between these cells from the mare and the sperm serves as a selective 'filter' to establish a population of sperm that has a higher motility and a higher percentage of normal sperm compared to the original population of sperm that is inseminated," he explains. "In other words, it appears that the oviduct of the mare plays an important role in selecting an optimal

"Our studies should shed light on the differences in stallion fertility that appear to be related to a shortened life span of sperm in the female."

population of sperm to participate in the process of fertilization."

During this past year, with continued Zweig support, Ball and colleague Dr. Ina Dobrinski have been studying the molecular basis for the sperm adhering to the oviduct cells. The researchers have isolated ten proteins, one of which they have evidence to believe is responsible for the adhesion. "Once we confirm this finding, we may be able to screen stallions to see whether this protein is present or absent on their sperm cells."

Ball and Dobrinski, whose work in this area received the International Embryo Transfer Society Award this past year and who have been chosen for the 1996 American Veterinary Medical Association Excellence in Equine Research Award, have also been studying calcium levels within the sperm cells while in coculture with the oviduct cells.

"Calcium is a very important signaling molecule for sperm, and it seems to be one of the triggers that begins the process of sperm changes which are necessary to prepare for fertilization," says Ball. "Because the lifespan of the sperm is limited once this series of events begins, a careful timing of these events with the presence of the mare's egg during ovulation is critical for successful fertilization."

Through a mechanism Ball does not yet understand, sperm in coculture with the oviduct cells maintain a resting level of calcium compared with sperm that are not in contact with oviduct cells. For this reason, Ball speculates that it is either the process of sperm adhesion to the oviduct cells or some secreted product from these cells that work to maintain low calcium levels, thereby preventing premature changes of the sperm before ovulation. Ball is continuing to examine the relative role of sperm adhesion to oviduct cells and/or secreted products from these cells to determine how the calcium levels are modulated in sperm within the oviduct.

"No information exists regarding the molecular basis for sperm cell adhesion with the oviduct, and our findings regarding the maintenance of a low calcium concentration in sperm during their storage in the oviduct has not been previously described," Ball concludes. "Our studies should shed light on the differences in stallion fertility that appear to be related to a shortened life span of sperm in the female. In addition, although our studies are particularly relevant in the horse, the information gained through these studies may provide important new information regarding the interaction of sperm with the female reproductive tract for other animals and for humans." ■

A Look Back at 18 Years of Zweig-Funded Research

Since the Zweig Fund was established in 1978, many equine researchers at the New York State College of Veterinary Medicine have received support for studies that benefit both equine health and New York State's racing industry. This is the first of a series of articles that will look back at some of their accomplishments.

Zweig-Funded Accomplishments in Equine Reproduction

Horses are selected for breeding based primarily on their performance. As a result, the horse is one of the least fertile domestic animals. All told, about 18 percent of pregnancies fail, and as in humans, the older the mare, the greater her fertility problems. Among older mares, the failure rate can be as high as 80 percent; more than two-thirds of their pregnancies fail within the first two weeks.

At the same time, older mares are often among the most valuable breed mares in the industry. Learning more about the events leading up to conception and early pregnancy loss, therefore, has been a high priority of Zweig-funded research since the fund was established in 1978.

Studying the Role of Hormones in Conception

Joanne Fortune, a professor of veterinary physiology, has used Zweig funding to explore the synthesis of the reproductive hormones in the horse and the cascade of events leading up to ovulation before fertilization ever occurs.

In earlier Zweig-funded work, Fortune, with graduate student Jean



Joanne Fortune

Sirois, conducted basic research into how two types of follicular cells interact to produce the steroid hormones that regulate the entire reproductive cycle. To obtain cells from the ovulatory follicle to culture in vitro, the reproductive physiologists removed an ovary from several horses. After doing so, however, Sirois observed a curious phenomenon: the follicle on the remaining ovary in each horse developed more rapidly than normal and the ovary produced a higher incidence of multiple ovulations.

More recently, Fortune and Sirois followed up on this observation by attempting to superovulate horses without having to remove an ovary. They developed a protocol using hormone injections to produce the same low progesterone environment they had observed in the mares with single ovaries.

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

"Zweig funding has allowed us to develop a treatment that, in the not-too-distant future, could help increase the genetic potential of valuable animals and allow valuable mares to race or be shown while less valuable mares carry their offspring to term."

Researching Enzyme Regulation

Following his work with Fortune, Sirois studied for more than two years at the Baylor College of Medicine to characterize the regulation of an enzyme involved in synthesizing prostaglandins before ovulation. These

hormones play a central role in ovulation.

In the fall of 1995, Sirois returned to Cornell as an assistant professor and has been focusing on prostaglandin synthase, a key enzyme in the synthesis of all prostaglandins. Now at the University of Montreal, Sirois hopes to apply his recent discoveries regarding prostaglandin synthase and



Jean Sirois

follicular development in the rat to that of the horse. He is looking at how and when prostaglandin synthase is expressed prior to ovulation to determine which form of the enzyme is expressed in the equine follicles, and where the synthase is expressed within the follicle.

"Zweig support has been critical in allowing us to investigate the molecular mechanisms involved in the ovulation process of the mare and to clone and characterize the gene encoding the prostaglandin synthase regulated in the preovulatory follicles," says Sirois. "All this information will allow us to provide the equine industry with better protocols to improve and control fertility in mares." *continued on page 6*

Zweig Memorial Trot Highlights the Syracuse Mile

Every year, the Harry M. Zweig Memorial Trot is one of the highlights of the Syracuse Mile's seven-day meet at the New York State Fairgrounds outside Syracuse. This summer's races were held on Sunday, August 18, and featured two divisions, each with a \$144,500 purse. A crowd of 5,000 enjoyed an afternoon of spectacular weather and exciting racing.

On hand for the event were Mrs. Anna Zweig, Dr. Zweig's widow; two of their six children, Brian and Susan; and Mrs. Zweig's sister, Bernie Callender. Mrs. Zweig is the owner of

Middlebrook Farm in Nassau, New York, and both she and Brian are Cornell alums. She graduated in 1952 from the College of Agriculture and Brian is a 1980 graduate of the College of Agriculture and Life Sciences. Also attending the races was Bruce Hamilton, Executive Secretary of the Harness Horse Breeders of New York State and a member of the Zweig Memorial Fund Committee.

Dr. Zweig was the driving force behind the revival of harness racing in New York State. A large- and small-animal veterinarian, he developed an

interest in harness racing when he purchased a race horse named Gypsy Hanover from the actor James Cagney in the 1940s. By the 1960s he was tirelessly lobbying the New York State legislature to help improve racing in the state. He served as a director and chair of the Public Relations Committee of the United States Trotting Association and was president of the Harness Horse Breeders of New York State for 13 years.

"Harry Zweig was instrumental in getting the grandstands built here for harness racing and starting the Syra-

Photography by Charles Harrington



Anna Zweig presents the winner's trophy for the Zweig Memorial Trot open division to Mr. and Mrs. Howard Guggenheim of Boca Raton, Florida, the owners of race winner Running Sea.



Anna Zweig and Bruce Hamilton, Executive Secretary of the Harness Horse Breeders of New York State and a Zweig Fund committee member, visit before the Zweig Memorial Trot.



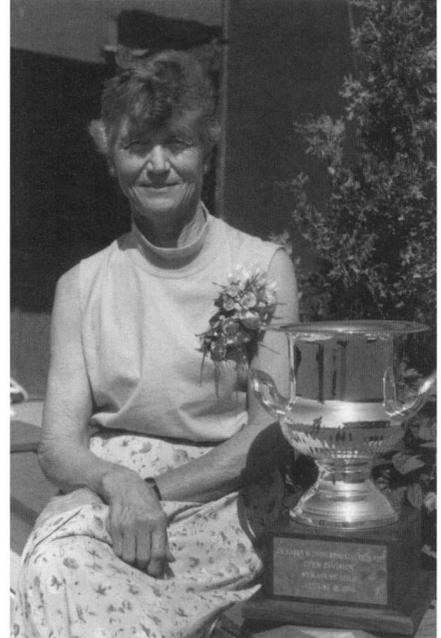
(From left) Brian Zweig, Anna Zweig, Bernie Callender, and Susan Zweig pose with the winner's trophy before the running of the Zweig Memorial Trot open division race.

cuse Mile," said Hamilton. "What we've seen here for the last 25 years or so is a direct result of his work."

Upon Dr. Zweig's untimely death in 1977, the Zweig family asked that donations be made in his name to the New York State College of Veterinary Medicine at Cornell to support equine research. The following year, the Harry M. Zweig Memorial Fund for Equine Research at the College of Veterinary Medicine at Cornell University was officially established by the New York State legislature. Every year, a percentage of the income of both

the thoroughbred and standardbred breeding funds is designated for the Zweig Memorial Fund. To further honor his work, the Syracuse Empire Trot was renamed the Harry M. Zweig Memorial Trot.

"Harry loved the races, but even more than that, he loved the horses," said Mrs. Zweig, who presented trophies to the two division winners in the Zweig Trot. "He grew up with horses and worked with them as a veterinarian. The Zweig Memorial Trot and the Zweig Fund are certainly great honors to his memory." ■



"The Zweig Memorial Trot and the Zweig Fund are certainly great honors to his memory," says Anna Zweig of her husband Harry.



A driver warms up his trotter before a race.



Once the race is over, the real work begins. A horse gets a postrace bath (left). A kindhearted driver gives his horse a break and brings in the sulky himself (right).



A Look Back *continued from page 3*

Understanding Sperm Survival and Embryo Behavior

Once ovulation occurs and a mare is inseminated, how the sperm survive and change before they can fertilize an ovum has been a mystery until recently. Dr. Barry Ball, D.V.M., Ph.D., an associate professor of clinical sciences, has been using Zweig funding over the years to better understand these events and the subsequent signals an embryo gives off to signal the pregnancy to the mare.

Most recently, Ball has developed a laboratory model using cells from the lining of a mare's oviduct to study the biochemical changes sperm undergo in the oviduct. These changes, he found, are critical for the sperm's survival. Contact with the oviduct cells extends the life of sperm from just a few hours to up to four days. The changes that occur in their surface proteins during these few days are necessary for successful fertilization of the egg (see accompanying article on Ball).



Barry Ball

Most pregnancy failure in the horse, however, seem to be due to early pregnancy loss rather than inadequate fertilization. Ball has used Zweig funds to glean vital information about how the embryo triggers essential biochemical signals that help maintain the pregnancy. He has developed a model using embryo fragments known as trophoblastic

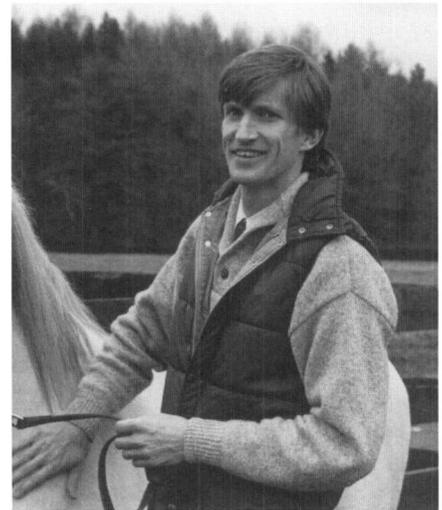
vesicles (TRV) from preplacental tissue. The TRV produce the same biochemical signals as an intact embryo does which trigger chemical and structural changes between the embryo and the mare's uterus. The model helps Ball better understand these biochemical signals that allow the mare to recognize the pregnancy and produce hormones and other changes to support it. Ball's ultimate goal is to enhance that pregnancy signal to prevent early pregnancy loss.

"Zweig funding has been very critical to our research program," he says. "It has provided a base of funding on which we have been able to build with other outside support from individuals involved in horse breeding, from federal funds, and from other horse-related funding organizations."

Preventing Late-Pregnancy Abortion

For breeders, the loss of a pregnancy can represent a major financial setback. Dr. Peter Daels has been studying how to prevent pregnancy loss among horses in mid- and late gestation. His goal has been to better understand the reason for late pregnancy abortion and come up with treatments that can prevent these losses. His work has focused on prostaglandins, which can cause a mare to abort if secreted in inappropriate amounts during pregnancy.

With Zweig grants, Daels confirmed earlier hypotheses that giving a mare prostaglandins during mid- and late-pregnancy can trigger the uterus to secrete prostaglandins. These produce contractions of the uterus that will ultimately result in abortion of the fetus. He later found that nonsteroidal anti-inflammatory drugs (NSAIDs), known to reduce prostaglandin release, were ineffective in suppressing the mare's prostaglandin secretion and preventing the fetus from being aborted. However, these studies indicated that the inappropriate prostaglandin secretion was associated with low progesterone levels during the period prior to



Peter Daels

the miscarriage.

Daels then experimented with different doses of several forms of progesterone to see if they could save the pregnancies. In one study, he found that by using double the recommended dose of ReguMate, a commonly used synthetic progesterone, he was able to save eight pregnancies out of eight cases, thereby strongly suggesting that progesterone supplementation could work.

He has been following up this work by identifying which enzymes are at work at the different sites involved in prostaglandin production. He also has been exploring whether different NSAIDs can prevent prostaglandin production at different sites, particularly in the uterus.

"Zweig funds have been extremely important to my research program by providing a basis to my financial support," says Daels. "In order to successfully compete for national funding in this highly competitive field of equine research, it is essential to remain at the forefront of our area of research. Zweig funds have allowed me and many others at this college to continue to produce innovative, state-of-the-art research. Our collective equine research program remains among the strongest in the nation." ■

Horses are a way of life for Jean Brown, the newest Zweig Committee member. They have been ever since she was born.

With the exception of college and a few years after college, Brown has always lived on a horse farm, and they've been among the biggest and the best. She spent her first eight years on Castleton Farm, the largest standardbred farm in Lexington, Kentucky, where her father trained horses and helped managed the farm. When she was eight, she moved with her family to Blue Chip Farms in Wallkill, New York, the largest commercial standardbred farm in the state. Her father has been the general manager there ever since and Brown is now the administrative manager.

"We've always lived on a breeding farm, but my dad made sure we knew how to drive and train a horse," Brown recalls. When the farm didn't have a track, she and her brothers and father would drive sulkies along the roads and through an old airport in the winter.

"My brothers and I worked part-time on the farm all through middle school and high school," says Brown, now 34. "I started when I was 12, washing windows and slowly working my way up." Her oldest brother still works at Blue Chip in the stud barns. Her other brother manages a horse farm in Pennsylvania.

Upon graduating from high school, Brown left Blue Chip to attend Beloit College in Wisconsin, where she majored in geology. She then went on to Yale University to study paleontology. "But I didn't really enjoy teaching or having to get grants," she says. "I can really sympathize with the researchers who apply to the Zweig fund."

Horses drew Brown away from Yale before she earned her graduate degree when she decided to take a job in the research department of the U.S. Trotting Association in Columbus, Ohio, for a year or so. She then worked as an assistant sales manager for the Garden State Sales Company, a stan-



Jean Brown

COURTESY OF JEAN BROWN

"This horse business is as natural to me as drinking water. Once you're smitten, it's hard to leave."

standardbred sales company in Monticello, New York.

But Blue Chip was beckoning her. An owner retired in 1990 and the farm needed a replacement. Brown was it. She's been back home ever since, managing the office and "doing a little bit of everything," from writing up lease agreements and contracts to proofing pedigrees for sales to reporting to the associations and working with customers.

The 800-acre farm is home to seven stallions that breed some 640 mares a year. As many as 450 horses may be on the premises at any one time during breeding season. Mares stay until their pregnancies are confirmed; fetal heartbeats are detected between 25 and 30 days after insemination. About 150 mares stay at the farm year-round with their foals and yearlings.

In her free time, rather than getting away from it all, Brown is a volunteer with horse-related organizations. She is a member of the auxiliary of the Goshen Historical Track, the oldest harness track in the country, where she helps raise money. She also serves as secretary to the Orange County Horse Council and helps put out their newsletter.

Brown became a member of the Zweig Committee last year. Although illness prevented her from going to her first meeting, she still read through the multitude of proposals and made her recommendations.

"You know, this horse business is as natural to me as drinking water," she says. "Once you're smitten, it's hard to leave it. I also like to do things like read and hike, but I have to admit, most of my life is focused around horses." ■

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College of Veterinary Medicine
Cornell University
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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 parimutuel 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.

1996 Harry M. Zweig Memorial Fund Committee

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