

MACHO *Trends*

Are you keeping track of the latest findings in stallion reproduction?

By Christine Hamilton

FEED YOUR STALLION FOR FERTILITY. FREEZE-DRY HIS SEMEN. Diagnose the genetic defects that cause a cryptorchid. Do those ideas sound far out? They're not, really.

There's a lot going on in stallion reproduction research that could affect the operation of your breeding farm in the near future. Check out these findings from American Quarter Horse Foundation-funded research.

Fertility and Fatty Acids

WHAT IF YOU COULD IMPROVE A STALLION'S POST-FREEZING fertility with what you fed him? Recent studies show that could be possible for some horses.

Much of a sperm cell's ability to survive and remain fertile after freezing has to do with how well the cell membrane withstands cold shock. The key is in the lipids that make up the cell membrane.

Sperm cell membranes of all species contain high levels of polyunsaturated fatty acids. Researchers have found that two in particular are linked to fertility: the omega-3 fatty acid docosahexaenoic acid (DHA) and the omega-6 fatty acid docosapentaenoic acid (DPA).

High DHA to low DPA ratios in semen have been linked to increased fertility, but low DHA to high DPA ratios have been linked to decreased fertility. Stallion and boar semen happen to be in that second group.

Studies have shown that if you increase a boar's dietary consumption of omega-3 fatty acids, such as DHA, you could improve its semen post-cooling or post-freezing motility (an indication of fertility).

When researchers at Texas A&M University applied the same logic to stallions, the results were similar. Led by Steven Brinsko, D.V.M., Ph.D., researchers fed a DHA-rich nutraceutical to stallions and were able to increase the ratio of DHA to DPA in their semen.

"The most interesting findings of the study were the effects of feeding the nutraceutical on cooled semen," Brinsko said. The biggest improvement in sperm motility was noticed after 48 hours of cooling.

"For four stallions whose initial progressive sperm motility was less than 40 percent after 24 hours of cooling, we observed a dramatic improvement in progressive sperm motility at both 24 and 48 hours of cooling when they were

fed the nutraceutical," he said.

They also found that omega-3 fatty acid consumption seemed to have less effect on the motility of fresh semen or in stallions that had high motility to begin with or with semen that cooled well.

Similar findings have since been observed in a study at the University of Arizona.

Take-Home Message

"SUPPLEMENTING THE DIET OF SOME MARGINAL STALLIONS MAY improve their semen quality enough to make them commercially viable for cooling or freezing," Brinsko said. "Those stallions whose sperm have poor tolerance to cooling and freezing would be horses that might benefit most from being fed the supplement."

To find out whether your stallion is a good candidate for feeding a DHA-rich nutraceutical, have a thorough semen evaluation performed that includes cooling through 24 and 48 hours.

"The supplement we used in the TAMU study was actually a boar supplement that had a very fishy odor and limited shelf life," Brinsko said. "The supplement has since been reformulated specifically for horses to increase shelf life and palatability."

The supplement is being marketed by Bioniche under the name "Magnitude."

Freeze-Dried Semen

RIGHT NOW, THE ONLY WAY TO PRESERVE STALLION SEMEN FOR the long run is to freeze it and store it immersed in liquid nitrogen. The problem is, stallion semen typically doesn't respond well to freezing, and liquid nitrogen evaporates over time and must be constantly replenished.

But there could be another way to go. Researcher Katrin Hinrichs, D.V.M., Ph.D., of Texas A&M University tried freeze-drying stallion semen and tested its fertility.

In freeze-drying, or "lyophilization," a freeze-drying machine both freezes the semen and creates a vacuum to take the liquid out of the material – going directly from solid to gas.

"Once freeze-dried, the semen can be stored in a refrigerator or regular freezer," Hinrichs explained. "We know in mice that it can be stored in a refrigerator for one year with no reduction in fertility, and work is being done to see how long the fertility does last."

To use the semen, Hinrichs took liquid "sperm factor," an extract of sperm cytoplasm, and added it to the freeze-dried sperm.

"(In freeze-drying), the cytoplasmic contents of the sperm

leak out from their damaged membranes,” she explained. “The egg needs to have a cytoplasmic factor from the sperm to know that it is fertilized.

“Once you add fluid back to the semen, the sperm plump up and look as they did before freeze-drying,” she added.

However, the sperm don’t move because of their damaged membranes. Hinrichs and her colleague, Young-Ho Choi, D.V.M., used intracytoplasmic sperm injection (ICSI) to produce embryos from the freeze-dried sperm. ICSI is a method of injecting one sperm directly into one egg (oocyte) via in vitro fertilization.

The embryos were shipped to David Hartman, D.V.M., of Performance Equine Associates in Whitesboro, Texas, for transfer to recipients. The first foal produced using freeze-dried semen was foaled in late spring 2006.

Take-Home Message

HINRICHS PROVED THAT THE FREEZE-DRIED SEMEN WAS FERTILE. The pregnancy rates of embryos produced using ICSI and fresh semen (70 percent) and embryos produced using ICSI and lyophilized semen (71 percent) were statistically the same.

Two additional findings were of particular interest. For one, it seemed that the type of oocytes used in the procedure had more of an influence on pregnancy rates than the semen used.

“In our study, we found that our method worked in some kinds of eggs (those oocytes that came from growing follicles) but not in other kinds (oocytes from follicles that were starting to degenerate), although these eggs work well in ICSI using semen processed in other ways,” she explained.

The second involved the parentage of the resulting foals. Three of the pregnancies from the study were allowed to carry to term, and one mare lost her foal at about 6 months.

“The other two foaled,” Hinrichs said. “One with a filly that was shown by parentage testing to be from the freeze-dried sperm.

“But testing showed that the other foal was by the stallion used to make the sperm factor rather than the stallion whose sperm was freeze-dried.”

In Hinrichs’ opinion, that discovery merits attention. The sperm used to make the sperm factor had been frozen and thawed repeatedly without the cryoprotectants thought to be crucial for protecting sperm from the freezing process and maintaining its fertility.

“We are doing further parentage testing on embryos that were frozen from the project to see how often this happened,” she said.

The Y Chromosome

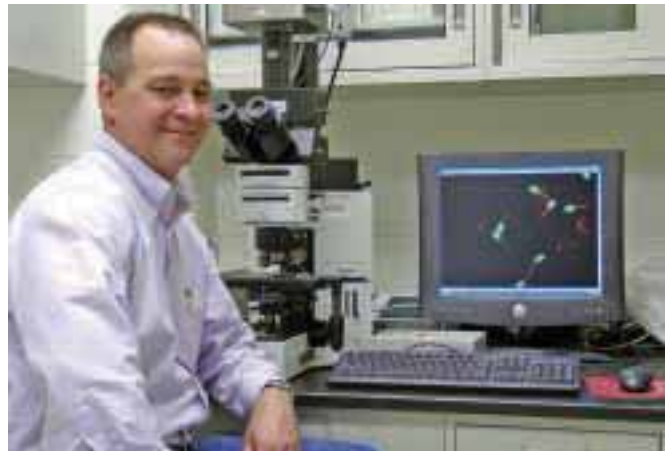
THEY’RE MAPPING IT.

Researchers are making serious headway in their attempts to map out all the genes on the equine Y chromosome. Some of them are critical for testicular development and function.

“Developing a complete map means knowing all the genes and genetic markers on the Y chromosome, understanding their organization and structure, and knowing their relative order or position,” explained Bhanu Chowdhary, Ph.D., of Texas A&M, where the ongoing research is conducted.

Fertility is governed by the combined effect of several genes. Having a genetic map of the equine Y chromosome is the first step toward being able to identify and analyze all the genes that govern different aspects of stallion fertility from testicular development to sperm production.

“Our ambition is to decipher every letter of the genetic message on the horse Y chromosome in the next two to three years, the first among any of the domesticated species,” Chowdhary said. “The findings will serve as a stepping stone for next-gen-



Steven Brinsko, D.V.M., Ph.D., and his research team at Texas A&M University explored what effects feeding omega-3 fatty acids to stallions had on their semen post-thaw motility.

eration approaches for optimizing stallion fertility.

“The first direct application of our research will be the use of the (chromosome map) to detect defects such as rearrangements, deletions and mutations that contribute to a variety of fertility problems,” he added, “including those related to abnormal sexual development.”

In the future, Chowdhary envisions the development of diagnostic tests allowing horse owners to test a weanling or yearling to see whether it could have certain fertility problems.

“It will also lead to novel and targeted treatments,” Chowdhary said, “because we will be able to prescribe treatments keeping in mind the defective gene.”

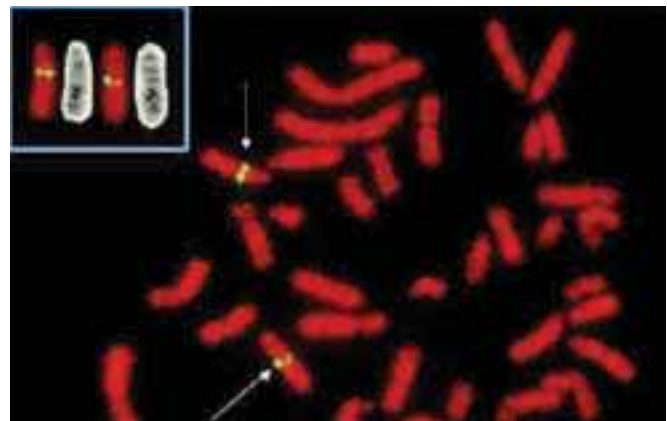
Take-Home Message

CHOWDHARY IS INTERESTED IN EXPANDING TEXAS A&M’S COLLECTION of genetic material from horses with fertility problems.

“We are extremely interested in clinically proven and well defined cases of reduced or complete infertility in stallions,” he said. “We are also interested in cases of horses that are intersex (hermaphroditic) or that have ambiguous phenotypic sex characteristics.”

They would also like DNA from stallions with testicular degeneration or cryptorchidism and from mares with unusually small ovaries or abnormal reproductive systems.

“We would be grateful to receive blood samples (20 – 30 milliliters) from horses with such documented fertility problems, along with a detailed clinical history on the horse,” he said. “We



This is a chromosomal staining of the glucocorticoid receptor gene on equine chromosome No. 14: it shows up in green. Endocrinologists and geneticists at Texas A&M University applied gene-mapping technology to stallion DNA samples to identify the gene’s location on the chromosome.

COURTESY OF STEVEN BRINSKO, D.V.M., PH.D.

TERJE RAUDSEPP, PH.D., AND TINA BRYAN, TEXAS A&M UNIVERSITY

would be willing to pay for the overnight shipment of those samples.”

If you are interested in contributing to this research by sending a sample from your horse, please contact Dr. Chowdhary at (979) 459-0519 or online at: bcchowdhary@cvm.tamu.edu.

The benefits of this research are in the long term. The group at Texas A&M has worked with the equine Y chromosome for more than four years, and it is the only research group worldwide working in this area.

“Dedicated funding for this research will make a huge difference in rapidly achieving (our long-term) goals,” Chowdhary said.



“Lilac”, a filly conceived with freeze-dried semen, runs with her recipient dam. She was foaled in late spring 2006.

JAIME JOHNSON, D.V.M., TEXAS A&M UNIVERSITY

Corticosteroids and Semen Quality

A LOT OF EXTERNAL FACTORS CAN NEGATIVELY AFFECT A STALLION'S reproductive behavior and semen quality, from housing to infection.

Stress can do it, too. When an animal is stressed the adrenal cortex produces the hormone “cortisol,” a natural anti-inflammatory steroid – one of cortisol's effects on the body is to decrease testosterone production.

Does the same thing happen if a stallion is given a *synthetic* form of cortisol such as “dexamethasone,” a synthetic glucocorticosteroid commonly used to combat inflammation? That could be a real problem for a breeding stallion needing regular corticosteroid treatment for a variety of problems such as chronic arthritis or heaves.

A team of physiologists, veterinarians and reproduction experts at Texas A&M set out to see what effects dexamethasone might have on a stallion's production of testosterone and sperm.

The study used 13 healthy, normal Quarter Horse stallions from 5 to 11 years old. Researchers took numerous blood samples prior to, during, and for several days after treatment to see what effects, if any, normal, prescribed doses of dexamethasone had on blood testosterone levels. They also regularly evaluated the stallions' ejaculates to monitor effects on sperm production.

The team expected dexamethasone to significantly reduce testosterone production and semen quality in the stallions, but that's not what happened at the dose level used in this study.

“There were no significant differences in total sperm numbers, motility or morphology due to treatment with dexamethasone,” Welsh said, “based on ejaculates collected daily in the two weeks following treatment, or on ejaculates collected weekly for 60 days after treatment.”

At the study's conclusion, several of the control and dexamethasone-treated stallions were castrated. Their testicular tissue is being examined to determine how the dexamethasone might have adversely affected the expression of the genes that control production of testicular hormones such as testosterone.

Take-Home Message

BASED ON THESE RESULTS, “THERE CAN BE A REDUCED CONCERN about administration of synthetic glucocorticosteroids to stallions,” Welsh said.

However, he pointed out that the study used a moderate dose of dexamethasone over a relatively short period of time.

“Concern with respect to reproductive performance is warranted in the case of administering glucocorticoid therapy to stallions with chronic inflammation,” Welsh said. “Or to stallions that may experience repeated managerial or environmental stressors.”

Christine Hamilton is a field editor for The American Quarter Horse Journal. To comment on this article, write to chamilton@aqha.org.

IT MAKES A DIFFERENCE

When you give to American Quarter Horse Foundation-funded research, your money changes horses' lives.

The Foundation contributed funding to all four of the studies reviewed here; although these were conducted at Texas A&M, the Foundation funds projects at universities nationwide.

Since 1960, the Foundation and AQHA have distributed more than \$6 million in research grants to colleges and universities. The funded research has resulted in discoveries that have internationally changed how humans care for horses: In combating intestinal parasites, preventing musculoskeletal injuries, understanding genetic diseases such as HYPP, managing stallions and mares for breeding, and handling conditions and diseases such as laminitis and equine infectious anemia, just to name a few.

Originally research projects were funded solely through AQHA; in 1975, the Foundation was formed to raise and manage funds for a variety of causes, including research to improve horse care and welfare.

The AQHA Equine Research Committee distributes grant monies each year at the annual AQHA Convention. For a complete list of current and past funded research, follow this link on AQHA's Web site: www.aqha.com/foundation/equine_research/current.html.

For more information on how you can financially support the Foundation's and AQHA's efforts to improve horse welfare through research, call (806) 387-5000, or go online to: www.aqhamembers.com/asp/donations/index.html.