A DISEASE FREE FUTURE?

The ability to genetically test embryos may mean horses no longer have to pass disease genes to their foals.

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Promising new embryo transfer research may hold the key to eradicating genetic disease in future generations of horses. By Michael Mahaffey

WHEN IT COMES TO BREEDING YOUR HORSE TO GET THAT FOAL YOU'VE ALWAYS WANTED, many factors come into play. Regardless of how good the breeding of both the sire and the dam is, there is always mystery and some apprehension when it comes to how the foal will ultimately turn out. Will it be the color or sex you want? Or more importantly, will it be born healthy and free of any genetic diseases?

Recent breakthroughs in research into screening the genetic material of an embryo prior to its being transferred to a recipient mare may soon give breeders and owners much more peace of mind, and in some cases control over how their foals turn out—a scientific breakthrough that may revolutionize the embryo transfer procedure in the process.

The New Research

This new line of embryo testing research began with the completion of the sequencing of the equine genome in 2008-2009, according to Dr. Mats Troedsson, a professor and researcher at the Gluck Equine Research Center at the University of Kentucky and Director of Research for the Minitube International Center for Biotechnology. Sequencing of the equine genome was an international effort that he says gives researchers a tremendous tool for the future when it comes to identifying disease-associated genes, as well as desirable genes that may enhance performance or be related to a phenotype, like coat color.

Until recently, the only way to determine if a potential foal would be at risk for contracting a genetic disease was to test the parents to see if they were carriers, and then breed those carriers only to non-carriers.

"The problem with that approach is your selection of sires and dams or combinations are limited," Troedsson says. "Also, if a non-carrier is bred to a carrier of a recessive trait, you will end up with about half of the offspring being carriers, so you don't really eradicate the disease from a population."

In fact, he says says one can argue that by breeding in this way, we are actually making choices that will result in most horses eventually becoming carriers of the bad genes.

"That's what triggered my interest in developing this method," Troedsson says. "Rather than predicting the likelihood of a foal to carry a gene or not, to instead determine if a gene is present or not in an embryo."

Troedsson and researchers at the Gluck Center and Minitube International reached the first step in making such an advance on Jan. 27, 2010, with the birth of the genetically healthy "Biopsita," the first foal to be born from an embryo that had first been biopsied, so genetic material could be tested, and then vitrified, or frozen, prior to transfer to a recipient mare.

The Process

For Troedsson, developing a technique that allowed researchers to biopsy and take 2-4 cells from an early embryo, at about 6 to 6.5 days, was not hard. It has been done in other species, including humans, for many years. The trick was developing a plan for handling the embryo while genetic testing was taking place on the cells that had been removed.

The solution came when Minitube International developed its vitrification kit, which presented a way to freeze an embryo after it was biopsied, much like semen is frozen after it is collected from a stallion.

"We vitrified the embryos while waiting for the results of the genetic testing," Troedsson says, "and if the embryo has undesirable genes, it is easy to just discard that embryo. If it has desirable genes, then we put it into a surrogate mare, or potentially back into the uterus of its biological mother."

While the term "selecting desirable genetics" may sound to some as if the process involves Frankenstein-ing material from multiple embryos to create a 'perfect' embryo, that is absolutely not the case.

Dr. Mats Troedsson

Dr. Mats Troedsson, DVM, PhD, DACT, DECAR, currently serves as director and department chair of the Gluck Equine Research Center at the University of Kentucky, as well as the Director of Research for Minitube International. He was an equine practitioner for 12 years before earning his PhD in reproductive immunology at the University of California Davis. He has held faculty positions at the University of Minnesota and the University of Florida and has been at the Gluck Center at the University of Kentucky since 2008. His research interests are reproductive biology and reproductive health.

"This research is definitely not aiming towards that at all," Troedsson says. "As a horse person, I don't think we really want to go there. I think that genetics are too complicated to even try to custom design. If you tried to custom-design something, I'm sure there would be some unpredicted negative consequence of that.

"Genetic engineering, where you transfer genes from one embryo to another or manipulate specific genes of an embryo, that has nothing to do with this technology. That is totally different."

Instead, the process Troedsson is developing relies on the science of genetics, which can predict that out of a number of embryos produced by a stallion and mare combination, there will be a percentage that have the traits one would hope to be present.

"The breeding process is exactly the same as in any other situation," Troedsson says. "The important thing is to know exactly when ovulation occurs because, at least with the technique we have today, we need to work with an embryo about 6 to 6.5 days after ovulation. The timing of ovulation is very important, and the best way to do that is to use an ovulatory agent that has a pretty narrow window, so we can accurately predict the time when ovulation will occur."

Then researchers simply count the time from ovulation to between 6 and 6.5 days and do an embryo flush exactly as they would for a traditional embryo transfer.

"Once that embryo is recovered, it will go into the laboratory and into the very skilled hands of a technician who can do micro-manipulation of embryos," Troedsson says. "They will fix the embryo under the microscope with one device and exactly go

Biopsita, the now yearling Lippizan who was the first foal born from a biopsied and vitrified (frozen) embryo.

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into the embryo with a needle and aspirate out 2-4 cells and then save those cells for genetic testing."

Immediately after that process, the embryo is vitrified, or frozen—which is also done in the laboratory, but can be done anywhere—using cryoprotectant chemicals that help prevent ice crystals from forming in the embryo. It is then placed in liquid nitrogen, where it can be stored indefinitely. As soon as the results from the genetic testing of the aspirated cells returns, which takes only a day or two, the scientists and the embryo's owners can make the decision of whether to transfer the embryo or discard it.

In addition to Biopsita, two more foals were born from biopsied and vitrified embryos in October 2010.

According to Troedsson, the only risk involved throughout the entire procedure is the potential loss of the embryo itself.

"We cannot do more harm than losing that embryo," he says. "We have three foals on the ground, and they are all in excellent health. The only risk is to lose the embryo."

There is also no risk that removing the cells will prevent a foal from developing normally or damage the foal that may be born.

"When we talk about the 2-4 cells that we take out, the question comes up, 'What if those are the wrong kind of cells [to be taking]?" Troedsson says. "In this situation, you are working with undifferentiated stem cells. They differentiate into whatever they should."

He credits the expertise at the Minitube International Center for Biotechnology under the leadership of Dr. John Dobrinksy for helping the process develop more smoothly than he thought it would. However, more testing needs to be done before they can predict how successful the procedure will be when implemented on a larger scale.

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Above: The EquiPro Vit Kit, developed by Minitube International, gives any veterinarian the ability to flush and prepare an embryo for vitrification. **Left:** Ozzie, one of two foals born in October 2010 from embryos that were biopsied, vitrified, thawed and then implanted into a recipient mare.

"Of the eight embryos that we did biopsy and use genetic testing on and transferred, six of those eight embryos actually developed into a pregnancy, which was very, very high, and I thought that was very encouraging," Troedsson says. "We did lose three of those embryos, so we need to do more research to find out whether that was bad luck or whether we can count on losing half of the embryos. That [loss of half of the developing embryos] has been reported in the human literature, but I'm not sure if it is the same for horses. Two of the embryos were lost in the same mare, who later was determined to not be a very good recipient mare, but there are still many questions."

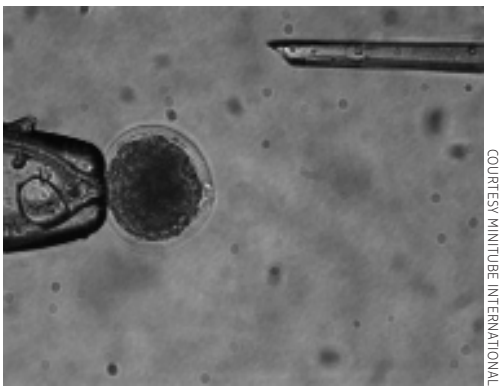
The Potential Benefits

According to Troedsson, numerous research groups around the world are using the equine genome to discover both desirable and undesirable genes. Some are working on finding disease genes, while others are working on finding genes related to performance and appearance.

"I think we have to wait and see what these research groups come up with," he says. "We have one group here at the Gluck Center in Kentucky that is looking at disease resistance for specific diseases. So we're not talking about a genetic disease, but resistance to infectious diseases."

Currently, there are about 10 genetic diseases that have been identified through the equine genome project, including Hyperkalemic Periodic Paralysis (HYPP), an inherited disease in Quarter Horses that causes the muscles to be overly excitable and contract involuntarily, and Lethal White, in which foals appear normal externally, though they have all-white or nearly all-white coats and blue eyes, yet internally, they have a nonfunctioning colon.

"I see that list growing exponentially in



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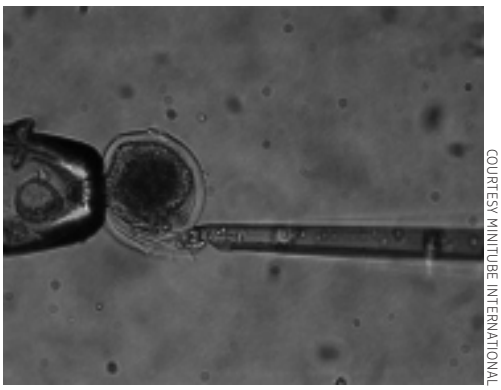

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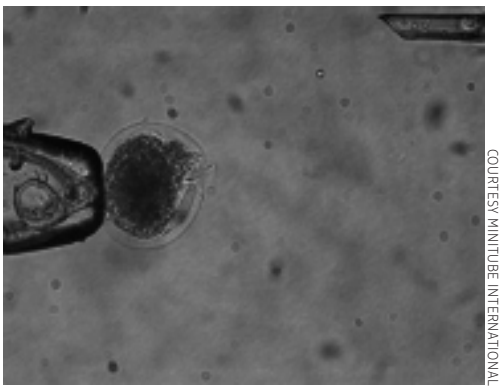

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To biopsy the embryo, skilled technicians must first fix the embryo in place with one tool (left). They then carefully insert a needle to collect 2-4 of the embryos undifferentiated stem cells (middle), before withdrawing the cells to prepare them for genetic testing (right).

the next four or five years as people are using the equine genome research-wise to identify genetic diseases," Troedsson says. "There are several genetic diseases that have not been coupled to a specific gene yet, and once that is done, they can also be used for testing."

Having these genes identified makes it possible to take the guesswork out of the potential outcomes of crossing a specific stallion with a specific mare.

In the case of inherited recessive traits, using conventional tests that only take genetic material from the parents, you can only determine if one or both carries the genes associated with a specific disease. If only one parent is a carrier of the disease gene that is not wanted, and it is bred to a non-carrier, 50 percent of the couple's offspring are guaranteed to be carriers of the gene, while 50 percent will not.

But if both parents are carriers of a specific disease gene, like Lethal White, 50 percent will become carriers of the gene and not get sick, 25 percent will be born completely disease free, and 25 percent will be born with the disease and have to be put down.

However, with this new ability to test the genetic material of the embryo itself before it is transferred, a whole new era of possibilities opens up.

"In this case, we can select and only use the [embryos shown to be] non-carriers," Troedsson says, "and therefore we can, if we want to, eliminate the carriers from an entire breed or the entire population of horses."

With this technique, it is even possible to breed a stallion and a mare that are known carriers of a disease gene, like Lethal White, and guarantee that the foal will be born healthy and completely disease free.

"We can breed two carriers, and we will still have 25 percent of the embryos come out completely non-carriers," Troedsson explains. "So we can use those embryos even in [what is currently considered] an impossible combination of parents."

However, the biggest positive for this new technique is its potential to help veterinarians and horse owners eradicate diseases like Lethal White and HYPP altogether.

"We have the tools to do that," Troedsson says. "Then it is, of course the decision and the desire of the breed associations to decide if they want to eliminate the disease. If it is just a problem of getting rid of [disease causing genes], then we have the tools to effectively eradicate the disease from the horse population.

"Then again, it is not a decision for a veterinarian or a researcher. We can only provide the information to the breed associations."

Looking Ahead

Troedsson says there is still work that needs to be done before this new process becomes practical for the mass market; however, he believes there is potential for it to become fully available within the next year.

The first step involves developing a clear methodology for collecting and delivering the embryos to one of the few laboratories in the world that are skilled at working with the micro-manipulators needed to biopsy the embryos, which requires about the same technical ability as cloning and ICSI. Once these methods are set, the procedure would allow your personal veterinarian to perform all of the hands-on work with the mare whose embryos are being sent for testing.

For example, if you're in Texas, you can have your veterinarian come out and flush an embryo from your mare, put it in one of these shipping containers, as is done routinely, but instead of going to a recipient herd, it goes back to the laboratory at the Minitube International Center of Biotechnology in Wisconsin. That afternoon or the next day, they receive the embryo and take a biopsy, and they send the material to a genetic testing lab, such as the one at the University of Kentucky, and then they vitrify the embryo.

"When you have the results, if you want to use that embryo, Minitube ships it back in a container just like we do frozen semen, to your farm in Texas, and you have your veterinarian put it into your mare," Troedsson says. "That's what we need to test. I think today what we can say is this is a possibility and can be successful, but I cannot say if this has a 60-70 percent or 20 percent success rate, and we have to figure that out before we can market the technology."

If tests of this shipping procedure can be fully vetted this year, then Troedsson hopes the service can be offered next year. His only concern is making sure it is affordable.

One way he believes the added costs of the testing and vitrification procedures may be defrayed is that the procedure may make it possible to greatly reduce the expense of keeping a large herd of recipient mares.

"The reason we have a large number of recipient mares during normal embryo transfer is to synchronize the ovulation of the normal mare and the recipient mare," Troedsson says. "But in the case of the frozen embryo, if you know that it was recovered at day 6 or 6.5, it will still be at day 6 or 6.5 a day from now, a week from now, a month from now, a year from now, so you really only need one mare. It could even be the owner's mare or any mare that they want to be a recipient mare, or even the mare the embryo was originally flushed from. Just see when she ovulates and wait for 5-6 days before you put in the embryo."

Troedsson believes if the procedure becomes too expensive, then the scientific community will lose the opportunity to do something special for the horse industry.

"We have to make it affordable," he says, "particularly if we're talking about a breed association deciding to eradicate a disease, that can't be done if it is more expensive than the owners can afford. So it has to be affordable, but at the same time recognize the tremendous expertise that goes into doing this right in the laboratory."

The potential that this new procedure and the information that continues to be discovered in the equine genome provides has Troedsson excited for the health and well-being of horses in the future.

"As a veterinarian, I have been thinking about this technology for over 10 years," Troedsson says. "What I have been interested in is to be able to avoid having a foal being born with a debilitating disease in its genetic makeup. If we can prevent that mare becoming pregnant when it comes to lethal diseases—to go through a pregnancy and a foal to be born just to be put down at some point—that is not really what you as a veterinarian like to see. So if we can eliminate some of these diseases through Pre-implantation Genetic Testing, then I think we will have contributed to the industry and the health of the horses."

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