Advancements in Breeding Friesian Horses

by Claudia Gregory Rayner MSN, CNE, PhDc

Breeding your Friesian horse is not for the faint of heart. Before you even begin, you had better research the best stallion qualities that complement your mare, consider inbreeding coefficients, and understand the kinships of each pairing. There are tests now to determine whether or not a particular pairing has a predisposition to genetic issues such as dwarfism and hydrocephaly. Then one must consider fertility issues in the mare as well as pregnancy issues before, during, and after, which can not only exacerbate a breeder's frustration but reflect in high cost with less than optimal success rates.

In an effort to understand what assistance modern reproduction science can offer the breeder, I reached out to Dr. Kristen Loncar of Equine Medical Services in Columbus, MO, a facility known throughout the nation for its advanced equine reproductive services. In my interview with Dr. Loncar, we discussed various types of breeding options as well as topics related to equine reproduction success rates, costs, and the future of breeding. In addition to the discussion with Dr. Loncar , some of the information used in this article has been taken from the Equine Medical Services website.

Dr. Loncar, what are some of the biggest advancements in Equine Reproduction veterinary care?

Repro in general is getting better and better each year. We are seeing advancements in equipment such as ultrasound technology helping us to more clearly visualize anatomy like never before. In addition to technological changes, improvements are being made, especially with the freezing of embryos and semen, as well as narrowing down which mediums for growing and preserving are most efficient. With these advancements, Repro medicine is widening its scope to include successful breeding of mares that had difficulty breeding for various anatomical reasons, or that are in training, showing , have limited time for breeding, or have owners with a desire for multiple foals per year . Further, stallions that are deceased, have limited semen availability, or stallions with less than perfect semen quality, now have the opportunity for offspring as well. This really opens up the possibilities in breeding.

I'd like to have a discussion regarding the mare's role in reproduction, her Oocytes or eggs, and some interesting and helpful facts related to reproduction.

The mare is born with all the oocytes she will ever have, and contrary to popular belief freezing of oocytes is not yet perfected and therefore clinically not done. Hopefully someday technology on that will change, but for now it isn't recommended. That said, oocytes are collected from follicles on the ovaries and can be collected by using transvaginal follicular aspiration (TVA) or a percutaneous method. Dr. Landon explains that in TVA, the mare is properly sedated and placed on pain medication for comfort. A transvaginal ultrasound (U/S) probe is inserted rectally into the mare, while the needle guide is inserted into the vaginal vault. This long needle has a double lumen (similar to a straw beside a straw) allowing for simultaneous flushing and aspiration of the follicle. The needle is guided towards the ovary with the U/S probe to help visualize the follicle. With precise needle placement and clear U/S visualization of the ovary and follicle, the oocyte is aspirated. The benefit of this procedure is that the veterinarian has good control of the anatomy and good visualization to collect the eggs. Some of the potential risks from this may include rectal tears, peritonitis, and hemorrhagic ovary. However, complications are not often seen.

In addition to the above, a breeder can also choose to have the mare's oocytes harvested by a local equine vet, closer to home, and shipped fresh overnight for processing. This can be very beneficial for working mares not able to travel to a Repro clinic for this procedure. That said, a working mare in vigorous training can still produce high quality oocytes. This may be affected in a negative manner for mares that are stressed, at the time of egg harvesting, as they may have higher body temperature or elevated cortisol levels. Also, it is noted that a working mare may have a slightly lower embryo recovery rate, which is subject to the individual mare. Medications such as antibiotics, gastric ulcer treatment, and anti-inflammatory drugs, just to name a few, do not appear to influence obtaining eggs or egg quality.

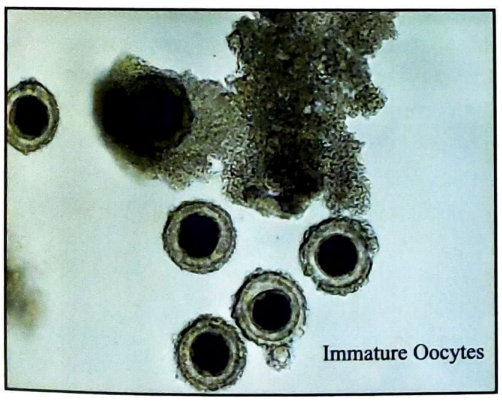
Can harvesting of mares oocytes be obtained post mortem?

Many owners and breeders are unaware that an Oocyte can be taken from a mare that has unexpectedly passed, or that was humanely euthanized. In the unfortunate event that this occurs, a local equine vet can perform a surgical excision of the mare's ovaries and ship them fresh, overnight to one of the many facilities across the country, to process the ovaries to harvest eggs. Once the fresh ovary is received for processing, the reproduction vet will make an

incision over the follicle to attempt to recover oocytes from the follicular wall. The number of eggs successfully harvested will depend on the mare and the number of follicles she has. Once harvested, these eggs can be matured and fertilized similar to a live mare. Since the donor mares eggs cannot be frozen, the fertilization process would need to be done when the egg is ready. Once the harvested egg is fertilized, it can be either frozen or implanted, depending on the breeders' preference.

Is there a certain window of time that the ovaries must be removed and shipped for optimal success?

Yes, the sooner the better. However, it can be acceptable to remove the ovaries and ship to be processed no more than 12-24 hours post-mortem depending on the weather. Also bear in mind that success rates may possibly be up to 2-3 embryos but success rates are usually lower than from traditional egg harvesting, In many cases this method can be considered a last ditch effort. However, with maturation medium improving, so could the success rates using this method.



Immature Oocytes

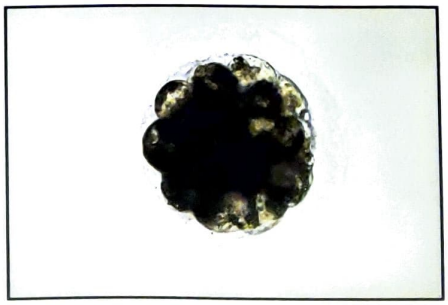
What is Embryo transfer, and the process involved?

Embryo transfer is the process of recovering and flushing an embryo from the donor mare approximately 7 days after conception and transferring it to a recipient mare. The recipient mare then becomes pregnant and carries the foal to term. According to Dr. Loncar ,the freezing of embryos has vastly improved over the past 10 years. Success rates, using two embryos, which is optimal, to breed a recipient or surrogate mare, is approximately 55-70%.

For example: the donor mare has been bred, is in foal and flushed. This is done for 2 cycles, producing 2 embryos ready for freezing. The recipient mare is ready to be bred and has 2 embryos frozen and available. One embryo is thawed and implanted. Four days later, the mare is checked; if she is pregnant, the other embryo is kept frozen; or, if the recipient is not bred, on her next cycle the second embryo will be thawed and implanted.

Donor mares are brought to a reproduction clinic for monitoring in preparation for breeding. The donor mare is checked daily to accurately determine ovulation days and is then bred via artificial insemination using either chilled or frozen semen. Approximately 1 week after the breeding, an ultra sound is obtained to determine if she is confirmed in foal. The donor mare's uterus is then flushed with an electrolyte and nutrient-rich solution, via vaginal catheter insertion. The solution, which is then carefully allowed to drain from the donor mare, is filtered in hopes of harvesting an embryo. (At this juncture the embryo is approximately 0.3-0.5 mm in diameter). The embryo is then carefully washed and placed in a nutrient rich media for transfer, or frozen. If the recipient is to be bred immediately, she is generally checked via ultrasound at four to seven days.

Success rates for ET are measured by the ability to recovery a viable embryo from the donor mare, and the confirmation of pregnancy in the recipient mare. That said, success rates can vary depending on individual mare's fertility, technique of the practitioner, and the grade level of the embryo to be implanted. Embryo recovery rates in young donor mares can be as high as 80% per cycle, or as low as 30% in older mares with infertility issues. In contrast, once the embryo is recovered, the expected rate of pregnancy success in the recipient is approximately 80% at 14 days with an estimated loss of 10 % after 10 days.

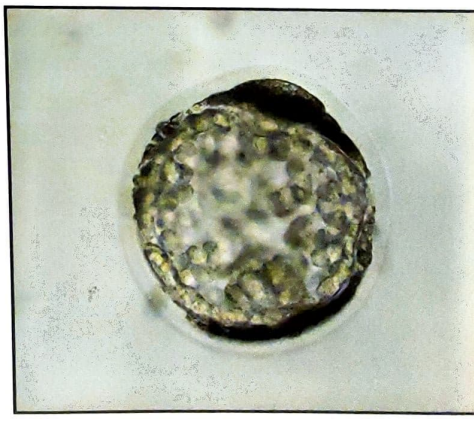


Four Day Old Embryo

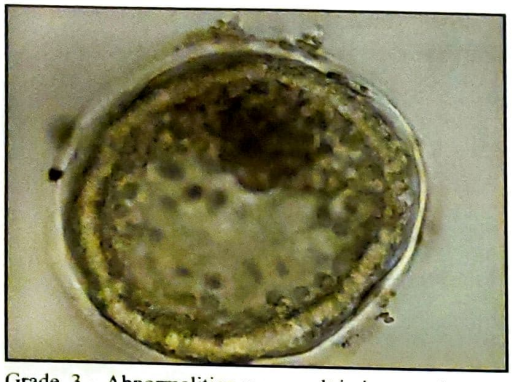
Dr. Loncar, you talked about grading embryos and how it can potentiate breeding success, could you elaborate on that?

Once embryos are flushed from the donor mare, they are evaluated under a microscope to determine their quality. The better the quality of that embryo, the more the likelihood of a successful pregnancy. The embryos are graded 1-4, with 1 being the best quality and 4 being the worst. The majority of embryos, flushed and used for breeding, are grade 1 or 2, however, depending on the circumstances (like a deceased stallion with limited sperm for instance) the breeding of lower grades 3-4 of embryo may be transferred into the recipient mare to give it an attempt. It is important to remember that grading of the embryos are only indicative of normal verses abnormal morphology, and cannot determine genetic abnormalities.

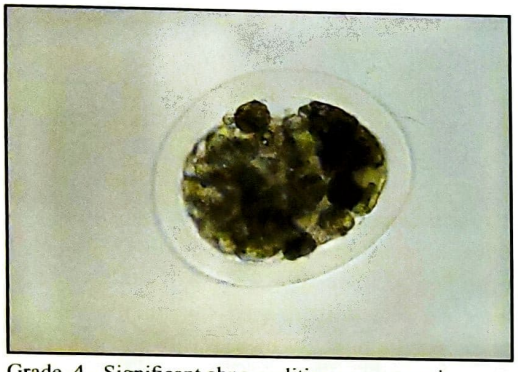
Grade 1 - Few detectable abnormalities, consistent wit potential for breeding success



Grade 2 - Mild abnormalities not expected to negativ impact establishment of pregnancy



Grade 3 - Abnormalities may result in increased chance of pregnancy



Grade 4 - Significant abnormalities, pregnancy is unlikely

There has been a lot of discussion around the Intracytoplasmic Sperm Injection (ICSI) procedure. What is it, and its benefits?

Intracytoplasmic Sperm Injection (ICSI) was first developed for older mares with issues related to reproduction. It is currently being used for various other reasons such as a decrease in stallion semen quantities and quality. Through the use of the ICSI procedure, the need for large doses of semen for breeding a mare is a thing of the past, considering it only takes 1/10th of one straw to fertilize many oocytes. In this procedure, an oocyte is collected from the donor mare's follicle prior to her ovulating, then cultured to maturity using a nutrient rich medium and specialized incubation. Once the egg is mature, it is fertilized in the laboratory via a micro-injector of a single sperm cell. The oocyte - now fertilized - starts to divide to become an embryo with the first cell division occurring 24-30 hours after sperm injection. At about seven to ten days in a specialized incubator, this fertilized egg is in a blastocyst stage. At this point, the newly developed embryo is ready to be transferred into the uterus of a recipient mare, shipped to another facility, or frozen for use in the future.

As stated previously, the mare's eggs can be harvested via transvaginal follicular aspiration (TFA). That said, oocyte quality does tend to decline with the mare's age, so older donors may have lower success rates. On average, a donor mare requires three cycles to establish a pregnancy. The recovery of the dominant follicle can be expected on approximately 75% of attempts. Usually at the time of retrieval, immature smaller follicles are harvested as well. On average oocytes fertilized by ICSI typically divide and grow on 70-80% of attempts, and of those 20-30% will grow in culture to blastocysts stage.

Semen used for ICSI can be fresh, cooled, or frozen; however, frozen is most commonly used. The sperm cells are processed and selected for use in many ways, but most common is to utilize the" swim-up" technique. Here the sperm swim–up the medium and are taken from the top layer, ensuring healthy motility. Individual spermatozoa are then selected under a microscope by immobilizing the tail; this is accomplished by crushing it with a pipette. Once in the oocyte, this damage made to the cell wall by crushing it will facilitate the sperm factor into the oocyte.

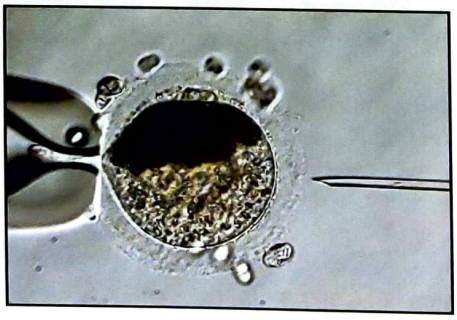
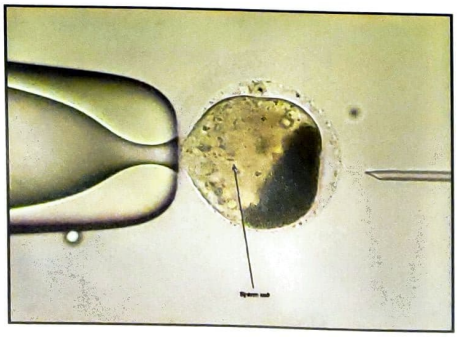


Photo demonstrates ICSI with one single sperm in the pipette.



ICSI Post sperm injection

ICSI 26 hours post sperm injection

What are the costs of breeding using Embryo transfer and Intracytoplasmic Sperm Injection?

The fee structure varies depending on what the breeder's expectations are, and the ability of the mare to accomplish those goals physically. This is a sample fee structure and may vary with other practices. Equine Medical services offers to breeders a yearly nomination fee of usually $1,800, which covers any number of cycles, and ultrasounds. This is a great option for multiple tries at breeding. In addition, a breeder may want to breed using a per-cycle schedule, where certain portions of the breeding are covered, and other portions are a fee for service. This could be very costly depending on the mare and her ability to conceive. The costs for breeding with frozen semen, for artificial insemination, is approximately $800-$1200. As the procedures become more involved, so do the costs. Embryo transfer can run $5,000 and varies with mare age, but covers the cost of leasing a surrogate mare. This is breeding, flushing, and transferring to the recipient. ICSI will run approximately $7,000-$8,000 range and is much more involved. Please bear in mind that these fees are in addition to stallion stud fees, shipping of either eggs or semen, and/ or freezing, as well as transport fees for the mare and recipient mare.

What does the future of breeding hold for us?

Advancements such as the ability to freeze a mare's eggs, better semen freezing techniques, improved supplements for increased semen life, dehydration of semen, and elite embryo

creations, and other more controversial and costly advancements like cloning, are where reproduction medicine may be heading.

In closing, I have a few random questions members have been asking for answers. Could you give us your opinion?
Is there really a way to predict genetic factors to create a perfect foal?

In theory, if you breed a nice mare to a nice stallion, you should get a nice foal. However, sometimes this doesn't happen. Nature is unpredictable. The one thing we do know for sure is when breeding a mare and stallion, you get one strand of DNA from each and typically that's a 50% mare and 50% stallion genetic traits.
Which ones you get just depend on nature.

Do you think a surrogate can influence a foal?

A surrogate will not influence the foal, genetically, as each parent gives 50/50 to this baby. However, the behaviors of the surrogate may influence the foal's size, rate of growth, and certain behaviors like willingness to work, or fear of humans. That's why surrogate mare selection can be important in breeding a healthy, happy baby.

Dr. Kristin Loncar, DVM, MS, DACT

Special Thank You to Dr. Kristin Loncar for making this article possible, and to Dr. Rob Foss for the reproduction photographs and for allowing the use of information from the Equine Medical Services, Inc. web site. For more information on the breeding advancements, please visit www.Equinemedicalservices.com.

Glossary of Terms

Oocyte: The female gamete; commonly called the egg.

Ovulation: The process by which the oocyte is released from the ovary.

Blastocyst: A stage in embryonic development when cells have begun to differentiate. An inner cell mass has formed which will become the embryo proper. The outer layer of cells, called the trophoblast, will form the placenta. When developing embryos using ICSI, the blastocyst is the stage at which the embryos are mature and ready for transfer into a recipient.

Embryo: The earliest stage of development of a fetus. Once the egg has been fertilized by the sperm and cellular division begins they are referred to as embryos.

ICSI: Intra-cytoplasmic sperm injection. The technique whereby a single sperm cells is injected directly into the center of an oocyte for fertilization.

Medium: The fluid that is used to support and nurture developing oocytes and embryos. Each stage has specific requirements regarding pH, temperature, hormones, and nutrients.

Maturation: The process by which eggs become ready for fertilization. The process by which embryos develop.

Morphology: The structure of an embryo. When flushing embryos, morphology is used to give an idea of how well that embryo will do at developing a pregnancy. An embryo with normal morphology can be expected to perform better than an embryo with defects in morphology.

Flushing: The act of infusing the uterus with a sterile solution and then filtering the returning fluid to recover an embryo.

Dominant follicle: The follicle on an ovary that grows and produces estrogen, leading to signs of estrus. Generally horses develop one dominant follicle on each cycle. This follicle will go on to eventually ovulate, release an oocyte and lead to fertilization.

Immature follicle: Horses can have numerous immature follicles on their ovaries at any given time in their cycles. These are follicles that each contain an egg in various stages of development.

Percutaneous: Through the skin.

Transvaginal Follicular Aspiration (TFA): The process by which an ultrasound-guided needle is directed through the vaginal wall and into an ovary to recover oocytes.

In vivo: A process that takes place in a living organism. For example, embryos recovered via embryo flushing are considered in vivo-produced embryos since they develop within the mare.

In vitro: A process that takes place outside of a living organism. For example, embryos that are developed in an incubator after ICSI are considered in vitro-produced embryos.