

Using the New Reproductive Technologies in your Breeding Program

M.M. LeBlanc

Rood and Riddle Equine Hospital
Lexington, KY

Advanced reproductive technologies such as cooled semen, frozen semen, embryo transfer and gamete inter fallopian tube transfer (GIFT) have given horse owners choices and freedom. Mares can be bred at home with semen collected from stallions that live anywhere in North America, Europe or Australasia. Stallions can compete during the breeding season while mares are bred with previously frozen semen. Embryos can be collected from performance mares between competitions. The genetics of valuable infertile mares can be maintained through GIFT, a procedure that involves the aspiration of eggs from ovarian follicles from the donor mare. The eggs are then placed in a fertile recipient mare's oviduct after which she is inseminated with semen. But as with all freedoms, these technologies come with a price. To be successful, a higher level of veterinary expertise is needed, mares need to be examined more often, pregnancy rates are lower and the costs are higher. Equine semen does not tolerate cooling, freezing and the manipulation needed for processing as well as other species. So, more money will be spent to obtain somewhat lower pregnancy rates than that seen with natural breeding or breeding with fresh semen by artificial insemination. The following article discusses the logistics of using cooled semen, frozen semen and embryo transfer in order to provide a better understanding of each procedure.

The Ins and Outs of Breeding Mares With Cooled Semen

To breed mares successfully with cooled semen all parties involved, mare owner, stallion manager and veterinarians, need to cooperate when coordinating the semen shipments with the timing of the mare's ovulation. Before shipping semen, the attending veterinarian or a representative for the veterinarian should clarify several points with the stallion manager.

- The cost of stallion collection
- The cost of preparing the semen for shipment, the number of collections provided gratis (if any), the cost of shipping semen tanks by air, and when and how the semen tanks must be returned
- The days of the week the stallion is collected
- Times during the breeding season when the stallion will not be available
- The number of days notice that the stallion manager needs before the semen shipment
- The latest time one can call to obtain semen (for example-one must call by 9am to receive semen by the next day)
- The longevity of the semen-does it live in the tank for 12, 24 or 36 hours
- First-cycle conception rate of the stallion
- The method of air transport used (same-day air or overnight shipment)
- Number of times the mare can be bred if she does not conceive (is the contract limited to 1, 2 or 3 years)

- The breed registry requirements, and the number and timing of post-insemination clinical (pregnancy) examinations must be established

First cycle conception rates tend to be slightly lower with shipped semen than with natural breeding or when using artificial insemination with a stallion housed at the same facility as the mare. Also, breeding management is more intensive and veterinary cost are higher. Mares need to be examined daily when in heat and bred within 24 hours of ovulation. Stabling a mare at a facility, such as a veterinary clinic or farm where the veterinarian visits daily, saves money on veterinary travel fees. Furthermore, these facilities have a stallion to tease the mare to determine when she is in heat, thereby, limiting the number of times that she will need to be examined.

Pregnancy rates are highest when mares are bred within the 24 hours before ovulation using semen of high fertility. The quality of the semen is of paramount importance: stallions of low fertility usually have much lower conception rates than those with high inherent fertility. In addition, the handling of the semen is critical; failure to prepare it correctly as well as poor subsequent handling at the mare end can make the process very disappointing. Timing of the breeding with the ovulation can be difficult especially if the stallion is collected only 3 times a week. Ovulation can be induced with drugs such as hCG or Ovuplant, however the window from injection of the drug to ovulation varies. Mares may ovulate as quickly as 24 hours, as late as 48 hours after administration of hCG or they may not respond at all. The window from injection of Ovuplant to ovulation is tighter than that of hCG with most mares ovulating between 42 and 48 hours, however, it costs about 2.5

times more than hCG. In either case it is extremely helpful if you know the specific idiosyncrasies of your mare's estrous cycle, especially the number of days she is in heat and the size of the follicle that she ovulates.

There are standards that the semen needs to meet to be considered of adequate quality. A dose of semen should contain a minimum of 500 million progressively motile sperm with at least 30% of the sperm being progressively motile. Each time the mare is bred with cooled semen, it should be examined carefully after it has been warmed for a minimum of 3 minutes. If it is of poor quality the stallion manager or veterinarian for the stallion should be notified.

After insemination, the reproductive tract of the mare should be examined daily until she ovulates. If she does not ovulate within 24 hours she should be bred a second time.

Trials and Tribulations of Breeding With Frozen Semen

Breeding mares with frozen/thawed semen often results in a significantly lower first-cycle pregnancy rate than when mares are bred with either fresh or fresh, cooled semen. Pregnancy rates of 0 to 70% /estrous cycle have been reported with an average pregnancy rate/cycle of 35 to 40%. The highest conception rates are achieved by using a full dose of frozen semen from stallions of known fertility in young mares of known fertility. Mares are examined every 6 to 8 hours, given either hCG or Ovuplant and inseminated during the period from 12 hours before ovulation until 6 hours after ovulation.

Breeding with frozen semen can be confusing for the horse owner because there

are no standards concerning size of the straw semen, thawing rate and temperature, number of straws/dose, and how the semen is sold, ie by the dose or by the straw. If a half milliliter straw is used, a dose consists of 4 to 8 straws. However, semen may be sold by the straw and not by the dose. A mare owner may attempt to breed the mare with less than what is considered a complete dose of semen. This often times leads to the mare owner being disappointed and the veterinarian frustrated as the mare does not conceive. Points that must be clarified before a mare is attempted to be bred with frozen semen are as follows:

- What is the first cycle conception rate of the stallion when breeding with the frozen semen
- What is the cost of semen
- What constitutes a dose- number of straws
- What is the size of the straws
- Will there be directions with the semen on how to thaw the straws
- Does your veterinarian have nitrogen tanks to store the semen
- What is the rental fee for the dry shipper and how long can it be rented
- How frequently can your veterinarian examine the mare
- What paperwork will be included and what does the breed registry require

Mares can be managed in a variety of ways during estrus depending on the facilities, semen quality and availability of the veterinarian. One management method is to examine the reproductive tract every 24 hours during estrus until the dominant follicle reaches 30 to 35 mm in diameter (35 mm for a warmblood mare; 30 mm for an Arabian, Morgan or Paso Fino mare). Either hCG or Ovuplant is given at that time. The reproductive tract is then examined by ultrasonography at 24, 30, and 36 hours after

hormone treatment. If, at any of these times, the mare has ovulated, she should be inseminated immediately. If the follicle is still present 36 hours after hormone treatment, the mare should be inseminated at that time. Twelve hours after insemination, the reproductive tract should be re-examined. If the mare has not ovulated, she should continue to be examined until ovulation at which time she is inseminated again. This regime reduces the period of intensive monitoring and, with a maximum of 2 inseminations, it should be effective for most mares. The system may be modified to accommodate an individual mare's estrous cycle and the rate of follicle development.

If only one dose of semen is available or the owner wishes to use only one dose of semen per cycle, the management must be more intensive. In this scenario the mare must be examined every 6 to 8 hours beginning 24 hours after she is given hormone treatment until she has ovulated. Once ovulation is detected, the mare is bred immediately. Pregnancy rates may be lower and the costs higher when only one dose is used for a cycle.

Some mares may develop a prolonged inflammatory reaction to the semen because there is no seminal plasma in the frozen semen (if the seminal plasma is included, motility of sperm is poor semen post thaw). Therefore, many veterinarians routinely perform a uterine lavage twelve hours after breeding in an attempt to clear any remaining inflammatory by-products induced from the breeding. If a mare develops a prolonged inflammatory reaction after she is bred twice with frozen semen from the same stallion and she does not conceive, it is advised that she be bred with either fresh semen or fresh cooled semen as both contain seminal plasma.

There are no clear cut recommendations on what is the recommended dose. Doses vary from stallion to stallion, on the method of freezing the semen, on the country of origin and on the individual freezing the semen. Doses range from 100 to 500 million progressively motile sperm with a dose of 200 to 250 million being the average. Most investigators suggest that there be a minimum of 25% progressively motile sperm after thawing and warming to 37C for at least 3 minutes. Motility of the semen after thawing is not always correlated with pregnancy rates and reliance on it as an indicator of fertility must be cautioned.

The Joys of Embryo Transfer

Embryo transfer has become a relatively commonplace procedure in the equine industry, especially with the establishment of larger recipient herds throughout the country. Success rates vary depending on the fertility of the stallion and mare and on the synchronization of the recipient mare. Many individuals in the business feel that the quality of the recipient mare and how she is managed is the most important factor in achieving high pregnancy rates. Mean pregnancy rates/cycle if both the mare and stallion are fertile are 50-60%. Rates drop precipitously from there if either horse is subfertile or if the recipient quality is poor. The average cost of a foal produced from an embryo transfer if both the stallion and mare are fertile, will range from \$5500 - \$7500. Therefore, if a mare owner is to break even, the foal needs to be worth a minimum of \$10,000 to \$12,000 as a weanling.

There are a number of large well managed recipient herds through the country. The mare owner needs to discuss options with their veterinarian and then contact the recipient facility that they wish to work with in the future. A contract will

be sent detailing the specific costs, what will be covered such as number of attempts, when the pregnant recipient needs to be picked up, if the recipient can be returned and the price that will be paid to the mare owner for returning the recipient. Some facilities include 3 embryo flush attempts in their fee after which time the mare owner pays for each attempt. Once a contract is signed a holding fee, usually \$1500, needs to be paid. The remainder of the fee is paid (\$2500-\$3500) when the mare is declared pregnant.

The technique is relatively simple, however, one must strictly adhere to management guidelines. The donor is bred using proper veterinarian management. The mare should be palpated every 12 hours until ovulation after she is bred to accurately determine the time of ovulation. If the mare owner prefers to use their own recipient mares, it is best (highest success) to monitor ovulation in recipient three mares for every transfer attempt. Three mares are needed because there is wide variation in the length of estrus, in the rate of follicular development and in the timing of ovulation after either hCG or Ovuplant is given. It is preferred that the recipient ovulates 12 to 48 hours after the donor. Embryos are collected from the mare by uterine lavage using specialized fluids eight days after the mare has ovulated. The fluids are collected from the mare through tubing that is attached to a special cup containing a filter. The holes in the filter are very small and will not allow passage of the embryo through it. The fluids that remain in the cup are searched using a dissecting microscope. Once an embryo is identified, it is aged and graded for quality and stage of development. It is then removed from the wash fluids, placed in a dish containing transfer media and washed a number of times. The recipient is then brought up, and readied for the transfer. The

majority of embryos are now placed into the recipient by a non-surgical technique. The embryo is put into an embryo insemination gun which the operator carefully inserts into the cervix and then discharges the embryo into the uterus.

Conclusions

Reproductive technologies have given horse owners and veterinarians many

options. These techniques have improved and widened genetic pools. Using them is exciting for all involved. However, methodologies must be strictly adhered to and owners must do their homework before they begin if they are to be successful.