How to Breed Mares With Frozen Semen by Deep Horn Insemination

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1. Introduction
Stallion and mare owners are becoming increasingly aware of the number of foals produced by a variety of low-dose insemination techniques and may request that you, the private practitioner, breed their mares with partial doses of frozen semen. Reproductive veterinarians are breeding mares, including the older less fertile mare, using frozen semen with low sperm numbers, low volume, low progressive motility, and low percentage of normal cells. Stallions may be unavailable due to show schedules, unable to provide good-quality semen, frozen or cooled, or may be deceased. Likewise, some of today’s imported semen from popular stallions can be quite expensive and is frequently sold by the “dose,” without a live foal guarantee.

There are a variety of successful strategies available today to help veterinarians use frozen semen. This list includes estrus synchronization, ovulation induction, timed insemination, endoscopic insemination at the ostium (papilla), and deep horn insemination. All of these techniques require effective participation between owners, managers, and the veterinarian to allow access to mares for treatment and examination. Although frozen semen breeding can be successful in any type of facility with adequate personnel, working areas, and laboratory equipment, most mares being bred with frozen semen in equine practices will come into a hospital or clinic for at least 3 to 5 days. Wherever the mares are bred, the overall success of any frozen insemination is dependent on accurate induction of ovulation; proper timing of insemination; and well-developed veterinary skills, including transrectal palpation, ultrasonography, and artificial insemination techniques. Practitioners also must be familiar with the current methods used to properly handle, transfer, store, thaw, and evaluate frozen semen, as it is imperative that semen samples are not damaged before or during the thaw process.1,2

An accurate review of the previous literature, and results from a large number of mares that were bred using low-dose insemination, are currently available.4 It became apparent in our practice over the last 5 to 6 years that the timely use of deep horn insemination (DHI) could result in favorable pregnancy and embryo recovery rates even when fewer straws or lower numbers of sperm were available. It is certainly appropriate to use a full dose of frozen semen from any stallion using standard AI techniques (insemination in the uterine body) and then gradually drop the number of straws used and switch to DHI as pregnancy rate dictates. Splitting the dose, in most cases, will save semen and may
also produce less post-breeding endometritis in susceptible mares. It makes sense to use as little semen as possible, conserving semen for another cycle or another mare, particularly when the sample’s quality characteristics or pregnancy rates are known. Frozen semen has the distinct advantage that when handling and storage are well managed, it will last for decades.

The aim of this report is to provide valuable references and descriptions of a technique, which with practice will help veterinarians become successful breeding mares with lower doses of semen. It describes a step-by-step technique for DHI in mares using a disposable commercially available pipette. This technique allows mares to be inseminated with multiple straws through one pipette and a reusable stainless steel stylet (empties a 0.50-cc straw at the end of the pipette and retrieves it). There is also a similar pipette with an inner tube to deliver semen from a syringe when semen is packaged in 2.5- or 5-cc straws. This system does require a rectal-vaginal procedure and consequently veterinary skills, yet it avoids the use of additional extender or an endoscope while delivering the semen efficiently and quickly.

2. Materials and Methods

The majority of mares bred by DHI in our practice are placed in breeding stocks for the actual insemination. This is ideal for the mare that will tolerate breeding stocks, as the mare remains more confined during the process. It is also helpful to move mares with foals or have them stabled as close to the lab as possible, so that insemination can be completed in an efficient manner after the semen is thawed while allowing the foal to be contained in a safe manner.

As the mare is being prepared, the correct semen should be located in the storage tank and an appropriate water bath set up. Mare preparation consists of restraint similar to what is needed for palpation of that mare, a rectum free of manure, then a standard perineal cleaning. This particular flexible pipette is easiest to use when kept warm (in an incubator if possible), because it will be more pliable and will retain the curl that is placed in it before introducing it through the cervix.

When the package is opened, a 3-cc syringe is attached to the external end of the pipette to avoid excessive air from being introduced into the uterus. The veterinarian places a sterile sleeve over the arm for the insemination and puts a full circle curl in the warm pipette. The pipette is then passed transcervically (should retain about 45 degrees of bend) with the internal tip kept ventrally. As the gloved hand is removed from the cervix and anterior vagina, the other hand should gently guide the pipette forward. The gloved hand can then be passed transrectally to palpate the pipette tip in the uterine body or base of the horn and assist it in a 90 degree rotation, left or right, toward the appropriate horn. Transrectally, the fingers are used to gently lift the uterine horn base and facilitate passage of the pipette to the appropriate horn’s tip. The entire process tends to go fairly easy when things are in the right position. One should feel the pipette slide up the horn, the rounded tip close to the ovary, and thus the shaft of the pipette is palpated inside the chosen horn. Good palpation skills, knowledge of the mare’s uterine position, and some experience using the technique is necessary.

Fig. 1. Placing a curl in the warm pipette.

Fig. 2. Retained curl in the pipette.

Fig. 3. Transrectal placement of the DHI pipette.
At this time, an assistant can hold and fixate the position of the external end with a hand against the mare’s body next to the vulvar lip (Fig. 4) while the semen is thawed. We use a portable water bath (stainless steel cup with handle, 1000 cc), with a clip-on thermometer (Fig. 5), so that the semen can be transferred to the breeding cart next to the mare during the thaw process. Thermometers used in thawing the semen must be accurate. Obviously, most thawing protocol times are short (20–30 seconds) and therefore the mare must be inseminated quickly. Dry the straws, remove the sealed end furthest away from the cotton plug on all straws with a straw cutter, and then place the first straw into the pipette (Fig. 6). The metal stylet should then be inserted behind the straw and pushed all the way through the pipette, seating the open end at the nipple inside the tip and thus pushing the cotton plug through the straw depositing the semen. This stylet tends to work well with most 0.50-cc straws and allows you to completely empty each straw at the pipette tip one at a time. If only 90% of the last straw is inseminated, then the last bit of remaining semen can be placed on a warm slide in the lab to provide an assessment of post-thaw progressive motility (Fig. 7).

3. Results
Per-cycle success rates included in this report are from mares bred 12 to 48 hours after injection of deslorelin or HCG, using frequent ovarian palpation and ultrasonography. All were bred by DHI. Generally they were given an induction medication between 6 and 8 PM and checked a minimum of every 8 hours until ovulation, starting the next morning. Many of these mares were bred once during the period of 36 to 40 hours after the ovulation induction agent was given, either before ovulation or shortly after ovulation. Mares with limited semen were checked every 2 to 4 hours until ovulation, starting at least the morning they reached 36 hours.

Pregnancy rates in our practice over the last 4 years (2007–2010), using DHI and low-dose frozen inseminations, have been encouraging. This group of mares was an average population of fertile and subfertile mares; there was no selection for fertility. Over the four seasons and 629 cycles that frozen semen was used, 58% (364 cycles) of the inseminations were performed with a partial dose of semen. Per-cycle pregnancy rates with frozen semen averaged 46% (291/629) for all cycles and 49% (178/364) for cycles in which less than one dose of semen was inseminated. Low-dose breeding was generally not attempted unless results were acceptable using a full dose of semen and post-thaw progressive motil-
ity was 40% or higher. A recovered embryo at 7 to 8 days after ovulation was counted as a pregnancy.

Frozen semen from many stallions in our practice has produced pregnancies or embryos from cycles in which two 0.50-cc straws (one-quarter dose) were used once. Several pregnancies from multiple stallions have been obtained with a single 0.50-cc straw (8 straws per dose) in fertile mares. It appears that quality and timing of insemination are more important than sperm numbers with some stallions. Many of these samples were processed by Select Breeders Southwest’s mobile lab, which routinely freezes in 0.50-cc straws at 8 straws per dose, providing 250 to 400 million progressively motile sperm (PMS) per dose (31–50% PMS post-thaw). For example, two straws at 40% post-thaw would provide 80 million PMS. It appears that DHI is successful for low-dose and small volumes, but there may be no difference when larger volumes are used.

4. Discussion

In review, most practitioners use one of two protocols for breeding frozen semen mares: timed insemination at 24 and 40 hours after ovulation induction, or frequent palpation and ultrasonography with insemination occurring once or twice within 2 to 8 hours of ovulation.

The latter lends itself to situations in which the semen source is limited, expensive, or of poor quality, yet is labor intensive. A recent report from two breeding seasons and 251 mares shows that two doses of frozen semen using timed insemination can result in pregnancy rates equivalent to those of mares bred with cooled semen. The timed insemination protocol relies on accurate ovulation induction and access to plenty of semen. It could be a disadvantage if semen is in short supply, if the mare does not respond to the ovulation induction agent, or if she is subfertile and/or susceptible to endometritis after multiple inseminations.

Complicating either of these two protocols is the fact that many mares have erratic estrous cycles. They may show variable signs of estrus over 5 to 10 days and ovulate in the last 1 to 2 days with follicles of inconsistent sizes. Consequently, they require routine and frequent palpation and ultrasonography for effective breeding with cooled or frozen semen.

With the frozen semen technology available today, using low-dose and deep horn insemination techniques seems to be the wave of the future. Conserving semen and limiting the amount of semen exposure in difficult mares has proved to be beneficial and rewarding in our practice.

References and Footnotes


*Minitube of America, Inc™, Verona, WI; 57-cm flexible pipette (ref No. 17209/0005).

*Minitube of America, Inc™, Verona, WI; 57-cm stylet (ref No. 17209/1057).

*Minitube of America, Inc™, Verona, WI; 65cm flexible pipette with inner sheath (ref No. 17207/1165).