Breeding Mares with Frozen Semen in Private Practice

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In the last decade several registries have approved the use of frozen semen commercially. With the recent approval by AQHA, the largest horse registry in the country, to use frozen semen, it is expected that inseminating mares with frozen semen will become a common procedure. There are several advantages in using frozen semen. Among them are the following: 1) transportation of a nitrogen tank is less expensive than transporting the mare, 2) semen is continuously available at the mare site, 3) insemination can be timed more closely to ovulation, 4) the genetic pool can be increased, and 5) semen properly frozen and stored will be available for several decades. Despite advantages, disadvantages include the following: 1) lower pregnancy rates from some stallions, 2) increased breeding costs to the mare owner because of an increase in veterinary examinations, 3) wide variation in semen quality, and 4) lack of standard protocols for breeding mares with frozen thawed semen. Veterinarians using this technology must be aware of the procedures needed to maximize pregnancy rates with frozen semen and understand the factors that affect fertility of frozen semen so that they have realistic expectations for themselves and the stallion and mare owners. The objective of this paper is to describe a veterinary practice that uses frozen semen. The facilities and equipment needed, factors affecting the success of frozen semen, insemination timing and technique, and mare management are topics discussed. Authors’ address: Department of Clinical Sciences, Kansas State University, Manhattan, KS 66506. © 2001 AAEP.

1. Facilities and Equipment

Although it is possible to breed mares with frozen semen in an ambulatory practice, it is preferable to have mares brought to a central location where the veterinarian can examine them regularly and where there is proper equipment for thawing and evaluating the semen. Ultrasound equipment for accurately timing insemination and a long-term liquid nitrogen storage tank (18 or 34 liters) for storing semen are necessities. Equipment needed for thawing semen includes an incubator, an accurate thermometer, a clean water bath, a good quality microscope, clean slides, cover slips, and a watch.

2. Factors Affecting the Success of Artificial Insemination with Frozen Semen

Management of specific factors is vital for achieving a successful breeding program. These factors include the following: 1) proper handling and identification of semen quality after thawing, 2) strict selection of stallion and mare, and 3) reproductive management of the mares.

3. Quality of Frozen Semen

There is a wide variation between stallions in the ability of their semen to tolerate freezing and thawing. Rarely does the individual inseminating a mare with frozen semen have control of semen qual-
ity, and it may be suboptimal. These factors have a negative impact on the use of frozen semen in the U.S.A. Although data are limited, it appears that about 25% of stallions have first-cycle conception rates comparable with those obtained with fresh semen even when frozen semen is inseminated into healthy mares at the proper time. End of the season pregnancy rates in the remaining 75% of stallions may exceed 80%, although first-cycle pregnancy rates are suboptimal. The drawbacks of repeatedly breeding a mare to achieve a pregnancy are expense and the likelihood that the mare may develop persistent mating-induced endometritis.

A. Thawing
Frozen semen is commonly shipped internationally in large quantities to a central storage place and distributed to individual mare owners in dry shippers. Frozen semen is packaged in different types of straws with the size and volume of the straw(s) dependent on laboratory preference. Thawing temperature is dependent on the type of straw in which the semen was frozen. Most commercial laboratories send instructions for thawing semen. Semen that is packaged in 0.25 or 0.5 ml straws is generally thawed at 37°C for a minimum of 30 seconds. When thawing multiple 0.5 ml straws at one time, straws need to be submerged individually in the water bath to avoid sticking together; otherwise the thawing rate of the individual straws will be affected adversely. Some laboratories recommend thawing 0.5 ml straws at 75°C for 7 seconds. If this method is used, it is critical that the thawing time and temperature are accurate. Semen frozen in 2.5, 4, or 5 ml straws is generally thawed at 50°C for 45 seconds.

B. Post-Thaw Evaluation
How to interpret the results of a semen evaluation conducted after semen is thawed is controversial because there are no accepted tests that correlate well with fertility of frozen semen. In addition, there are no standards describing the minimum quantity of progressively motile sperm needed for an insemination dose. Evaluation of sperm motility after thawing is the most common and most frequently performed test after a mare is bred with frozen semen. Unfortunately, motility, besides being a fairly subjective measure of quality, is a poor predictor of fertility. Sperm morphology should also be assessed, particularly when repeated use of semen from a specific stallion results in poor conception rates. Sperm defects that affect oocyte penetration and attachment (but not motility) may be identified. Frozen semen from some stallions may have fairly good motility but poor morphology, which could account for the low pregnancy rates seen in those stallions that have good post-thaw motility. Despite the lack of standardization, most individuals that freeze semen commercially agree that the minimum acceptable criteria for a single insemination dose after it is thawed should include the following: at least 30–35% progressively motile sperm, a minimum of 50% morphologically normal sperm, and greater than 600 million sperm.

There is no consensus on the minimum number of progressively motile sperm needed per dose to maximize fertility. Data between laboratories and between stallions differ, indicating that there may be a stallion-dependent factor which has been reported for the bull. Insemination doses containing 150 million sperm results in fewer pregnancies regardless of motility. The same group also reported that insemination of 300 million sperm every 24 hours when a mare has a >35 mm follicle and is displaying behavioral estrus resulted in higher pregnancy rates compared with inseminations having 150 million sperm per dose (41% vs. 32%). When the total number of sperm per dose is increased to 800 million, there is a linear increase in fertility. However, no further increase was detected when total sperm concentration exceeded 800 million. Colorado investigators reported that stallion semen frozen in 0.5 ml straws containing 320 million progressively motile spermatozoa post-thaw resulted in a 44% pregnancy rate per cycle. Some countries have placed standards on semen quality. For instance, semen frozen in the Netherlands must have a minimum of 300 million progressively motile and morphologically normal sperm per dose after thawing to be sold commercially.

Because of the difficulties in assessing semen quality after thawing and because of the lack of industry standards, it is critical that the mare and stallion be chosen carefully and that reproductive management is optimized.

4. Stallion and Mare Selection
A. Stallion Selection
It is unfortunate that past fertility is rarely a major criteria used by mare or stallion owners when selecting a stallion for breeding or for freezing semen. The food animal industry has shown repeatedly the importance of genetics in improving fertility. Until data are collected on the fertility of frozen semen from many stallions and reproductive parameters compared, it will be difficult to improve first-cycle conception rates. However, there are sire lines known for good quality semen. Semen from some of these stallions and their sons have consistently resulted in excellent post-thaw quality and pregnancy rates of more than 60% per cycle. Unfortunately, fertility rate of stallions using fresh or chilled semen is not a good indicator of its fertility rate with frozen-thawed semen. Therefore, it is advisable to inquire what the first-cycle pregnancy rate was from semen previously frozen from a particular stallion before the stallion is chosen to breed mares.
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B. Mare Selection

First-cycle conception rate tends to be lower in mares bred with frozen semen as compared with those bred with fresh-cooled semen or by natural breeding. It is critical that mare owners are made aware of the potential reduction in fertility. Breeding reproductively normal mares to stallions that have good first-cycle conception rates may still require breeding the mare for 3–4 cycles to achieve a pregnancy. Mares that are the best candidates for breeding with frozen semen are young mares between 3 and 8 years of age that are either nulliparous or are proven producers. Clinical data indicate that mares that are to be bred with frozen semen should be placed in 1 of 4 groups: a) young maiden mares (<7 years old), b) older maiden mares (>8 years of age), c) barren mares, and d) mares with foal at side. In the authors’ practice, the average pregnancy rate per cycle was 67.3%, 34%, 50.7%, and 50.9%, respectively. Data from Vidament et al. on a larger number of mares indicated that although mare status did not affect the foaling rate (52%), pregnancy rate per cycle was affected by mare status and age. Barren mares that had been open for more than 2 years had the poorest fertility.

C. Management of Mares Bred with Frozen Semen

A complete reproductive work-up, including a uterine cytology and culture, should be performed on all mares that are to be bred with frozen semen unless the mare is <6 years of age and has normal perineal conformation. Mares that are to be bred with frozen semen should exhibit normal estrous cycles. Records from past breedings should be examined if any are available to determine length of estrus, size of follicle that the mare tends to ovulate, and if she accumulates fluid after breeding. Mares that accumulate intrauterine fluid before breeding or that have bacteria isolated from their uterus should receive appropriate intrauterine therapy before breeding with frozen semen. Because semen is frequently sold by the dose and owners may buy only 1–2 doses for a particular mare, it may be best to advise the client that another mare should be chosen if semen quantity is limited.

The reproductive tract of mares should be examined every other day or daily during the first days of estrus and more frequently as ovulation approaches. Mares should be bred within 12 hours of ovulation. When the semen is of good quality, every attempt should be made to breed the mares within 12 hours before ovulation. Mares that do not ovulate after the first insemination should be examined q 12 h and inseminated with a second dose immediately after ovulation is detected if semen is available.

5. Insemination Timing and Technique

A. Timing of Insemination

Sperm that have been frozen and thawed have reduced longevity due to membrane changes that occur during cryopreservation. Therefore, to maximize fertility, mares need to be bred as close to ovulation as possible. There are no standards regarding the timing of insemination, as regards to when mares should be bred in relation to ovulation, how many times should a mare be bred each cycle, should she be bred before and after ovulation, and how frequently should the reproductive tract be examined. In a survey conducted by the authors, of 21 laboratories that processed semen for commercial purposes recommended that mares should be bred with one dose of semen per cycle. Of the 14 laboratories, 12 recommended that mares be bred before and preferably within 12 hours of ovulation. Two laboratories recommended that the mare be bred only once within 6 to 12 hours postovulation. Seven of the 21 laboratories recommended that mares be bred before and after ovulation, whereas the remaining 2 laboratories recommended that mares be bred q 24 h beginning on the third day of estrus and continuing until she goes out of estrus. In the authors’ experience, fertility of mares that are bred only once after ovulation is about 40% per cycle compared with 54% for mares bred either before ovulation with one dose or before and after ovulation. Some experienced veterinarians have reported good pregnancy rates when mares are inseminated only once after ovulation. However, it appears that pregnancy rates for mares bred after ovulation are best when mares are bred within 2–4 hours postovulation. To accomplish this goal, mares would have to be examined every 2–4 hrs because there is no clinical or ultrasonographic parameter that determines how recent a mare has ovulated.

Repeated reproductive examinations need to be conducted to identify changes in the ultrasonographic appearance of the uterus and to measure follicular size and changes in echogenicity of the follicular rim. The mare has an average of 7–10 endometrial folds, which when in estrus, will become markedly edematous, displaying a typical ‘cartwheel’ pattern. Clinical observations indicate that during the first few days of estrus the degree of edema increases concomitant with the follicular size until it reaches a maximum level. As ovulation approaches, the degree of edema is reduced in the normal mare. The follicle just before ovulation could become softer, painful, and have hyperechogenic borders.

An ovulatory agent is given in conjunction with the examinations to time ovulation and reduce the number of reproductive examinations. Two ovulatory agents are currently available, human chorionic gonadotropin (hCG) and the gonadotropin-releasing hormone (GnRH) analogue deslorelin (Ovulplant). Both products are effective in inducing ovulation. Typically 2500 iu of hCG are given intravenously when the mare is displaying behavioral estrus and has a follicle that has a diameter of more than 35 mm. The majority of mares will
ovulate between 36 and 48 hours; however, some may ovulate as soon as 24 hours, whereas a few may hang on to their follicle for up to 60 hours. The reproductive tract of mares treated with hCG should be examined manually via the rectum and by ultrasonography every 6–8 hours after injection. Mares treated with Ovuplant™ seem to ovulate in a narrower window (36–42 hours) after treatment.

There have been reports, however, that an unknown percentage of mares do not return to estrus at a normal interval if they do not conceive after the use of Ovuplant™. Regardless of the ovulatory agent used, the ideal breeding time is best determined by routine ultrasound examinations to determine uterine and follicular echo texture. In our experience, breeding just before ovulation has yielded the best results. The proximity to ovulation is determined by the quality of the semen and the fertility of the stallion with frozen semen. However, every attempt should be made to breed the mares less than 12 hours before ovulation or 2–4 hours postovulation. To accomplish this, 2500 iu of hCG is given intravenously when the mare has prominent endometrial folds and a distinct dominant follicle. Mares are examined at 6–12 hours intervals, and most mares will be bred between 24 and 48 hours after hCG. Alternatively, mares can be treated with Ovuplant™ following the same criteria as for hCG and bred at 32–38 hours after treatment. Mares that have not ovulated after the first insemination should be examined at 12 hour intervals and reinseminated immediately after ovulation is detected, provided that the uterus is normal and no fluid is detected. The frequency of palpations and the number of inseminations is determined by the availability of semen and the semen quality.

B. Insemination Technique

The laboratory processing the semen should send information with the semen that includes the name of the stallion, number of straws needed for an insemination dose, thawing instructions, expected motility and morphology after thawing, and microbiological status of the semen. In general, between 4 and 8 0.5 ml or 0.25 ml straws are needed for one dose. When semen is frozen in 2.5, 4, or 5 ml straws, usually one straw is needed per insemination dose.

After the semen is thawed (see above), the straw is removed from the water bath and dried thoroughly. We have found it to be more practical to cut the straw that constitutes an insemination dose at one end and pour the entire volume of semen into a dry, warm, clean tube, or directly into a warm syringe case. The semen should be evaluated microscopically before the mare is inseminated. It is not recommended to mix the entire insemination dose with extender immediately after thawing because it could cause an osmotic shock to the sperm. The semen should be slowly deposited into the uterus using a regular insemination pipette. The pipette is flushed with air a few times to expel any semen that remains in it. After most of the semen is in the uterus, the pipette can be rinsed with 4–6 ml of a skim-milk glucose extender and the pipette again flushed with air once or twice. It is important to perform these procedures slowly to increase the volume of semen deposited into the uterus.

An alternative to emptying all the straws in a tube or syringe before insemination is to use a disposable insemination pipette with a metal plunger (commonly referred to as an insemination gun). The plunger can be removed and a new 0.5 or 0.25 ml straw can be inserted back into the insemination gun without removing the gun from the uterus. The operator should pass an arm through the mare’s vagina only once at each insemination so that secondary bacterial contamination is reduced. When one 0.5 ml straw constitutes an insemination dose, it is critical to place the entire volume of semen in the uterus. Therefore, it is suggested to use an insemination gun for this purpose. It is difficult to evaluate the semen quality when using this system.

Regardless of the type of straw, the standard insemination technique is to place the semen in the uterine body. Recently several groups have reported differences in pregnancy rates when mares were bred with reduced sperm numbers that were placed around the oviductal papilla ipsilateral to the ovary with the preovulatory follicle. Morris et al.9 reported a 64%, 75%, and 60% pregnancy rate when mares were inseminated with 1, 5, or 10 million sperm, respectively, in the uterotubal junction. Squires et al.10 reported a 40% pregnancy rate when mares were inseminated with 5 million fresh, frozen, or fresh-sexed sperm at the uterotubal junction. In another study, Rigby et al.,11 using a 500 million sperm insemination dose, reported that although only less than 0.001% of the insemination dose reached the oviducts, higher numbers of sperm were recovered from the oviductal isthmus of normal or problem mares that were bred deep in the uterine horn ipsilateral to the dominant follicle, compared with those bred in the uterine body. It seems therefore, that breeding deep in the uterine horn or closer to the uterotubal junction maximizes sperm usage, increases the number of sperm in the oviduct, and could result in higher pregnancy rates. Veterinarians using frozen semen should consider inseminating deep in the uterine horn as an alternative to uterine body insemination.

C. Postbreeding Therapies

In addition to depositing the semen at the appropriate time, the postinsemination examination is a crucial component of the insemination process. This examination should be performed not more than 12 hours after insemination. The purpose of this examination is to confirm that the mare has ovulated and determine if the mare is accumulating fluid. The examination is particularly important in old maiden mares, mares with delayed uterine clear-
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ance, and mares susceptible to uterine infections. It is at this time that appropriate therapies such as uterine lavage, oxytocin injections, post-breeding antibiotic infusions, and castration procedures should be performed. In our opinion, it is not necessary to perform blanket therapies on all mares bred with frozen semen. Mares should be treated based on their clinical signs and previous reproductive history.

D. Allergic Reactions to Frozen Semen

Dutch, Finish, and American investigators have recently addressed the perception that some mares accumulate excessive amounts of fluid due to an allergic reaction. Their data indicated that a slight amount of fluid accumulation in the uterus after insemination is a normal process. This reaction appears to be in response to the spermatozoa and is not dependent on the type of extender or any of its components such as egg yolk, milk proteins, or glycerol. All mares experience a physiological inflammatory response within the uterus after being inseminated with semen. This response is modulated by seminal plasma. Cryopreservation of semen involves the removal of most of the seminal plasma; this perhaps exacerbates the transient inflammatory response in the mare.

The number of mares bred with frozen semen is increasing every year. The technique, although far from optimal, results in the birth of thousands of foals every year. Frozen semen does not allow for many errors in the handling of semen and the timing of insemination. This results in an increase labor for the veterinarian and cost for the mare owner that should be clearly discussed before starting the process. Careful selection of the mares and stallions will further increase the chances of success.

References


