

Select Breeders Services

MANAGEMENT OF MARES FOR FROZEN SEMEN INSEMINATION

- Verify that the mare is a suitable candidate for AI with frozen semen by performing a routine reproductive exam that may include culture, cytology and biopsy when indicated. Data suggests that aged (>15 years) or repeatedly barren mares will have a significantly reduced pregnancy rate and should not be selected for use with frozen semen.
- Once the mare comes into estrus, palpate and / or scan daily to monitor follicular activity.
- Upon detection of a large (35 – 40 mm) pre-ovulatory follicle, administer HCG or Ovuplant.

1. If more than one dose of semen is available for insemination on a given heat cycle:

- a. Continue to examine the mare via ultrasound once daily and inseminate a single dose of frozen semen approximately 24 hours after HCG administration.
- b. Examine the mare approximately 16 hours after insemination and inseminate a second dose of frozen semen even if the mare has already ovulated.
- c. Examine the mare the following day to confirm ovulation. Insemination of a third Dose may be required if the mare has still not ovulated.

Note: *A general goal for mares inseminated with frozen semen is to inseminate within 12 hours prior to or within 6 hours after ovulation. This protocol insures that viable sperm are in the oviduct during that interval for any mare that ovulates within a period of 18 to 52 hours following administration of HCG or Ovuplant.*

2. If only one dose of semen is available for insemination:

- a. Examine the mare via ultrasound at 6 hour intervals starting 12 – 24 hours after HCG or Ovuplant administration.
- b. Inseminate the single dose of frozen semen as soon as ovulation is detected. **It is extremely important that mares being inseminated post-ovulation are inseminated within 6 hours of ovulation. A significant reduction in fertility will occur if mares are inseminated more than 6 hours post ovulation.**

Suggested schedule for insemination of mares with frozen semen when *more than one dose is available*:

Daily examinations during estrus (any time)

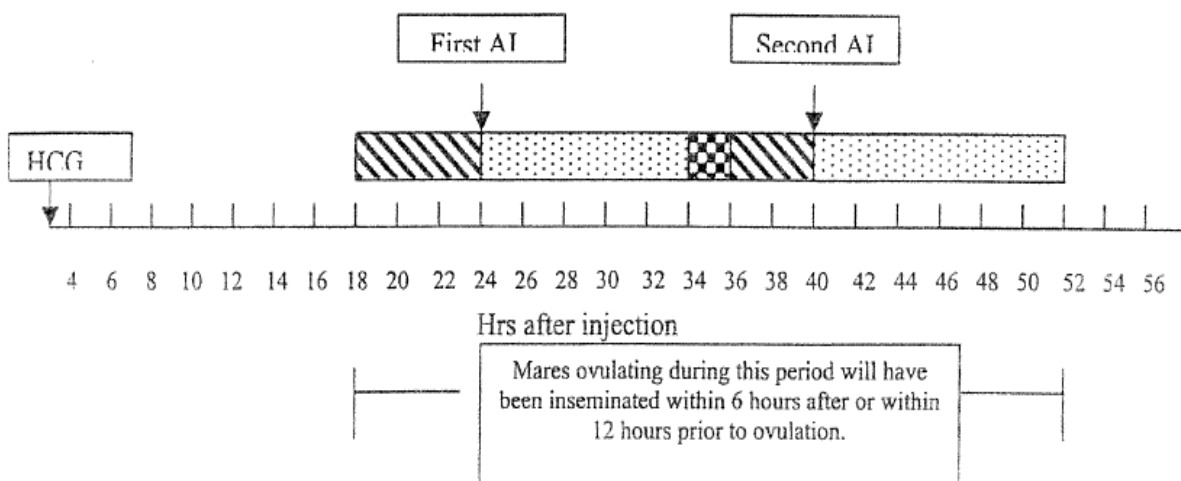
Day 0 – Day of 35 to 40 mm follicle detection.

Administer HCG or Ovuplant (deslorelin) at approximately 4:00 PM

Day 1 – Inseminate a single dose of frozen semen at 4:00 PM (24 hours post-injection).

Day 2 – Inseminate a second dose of frozen semen at 8:00 AM (40 hours post-Injection).

Day 3 – Examine to confirm ovulation and inseminate a third dose if the mare has not ovulated at 8:00 AM.



Simple and Effective Management Scheme for Insemination of Mare with Frozen Semen

One of the most common objections to the use of frozen semen is the increased cost to the mare owner associated with frequent ultrasound exams, often including “midnight exams” in an attempt to inseminate mares as close to ovulation as possible. It is generally accepted that optimum conception rates with frozen semen are obtained when viable sperm are inseminated in the period of 12 hours prior to 6 hours after ovulation. When frozen semen is marketed without guarantee of pregnancy, sold “by the dose”, or as only a few doses per breeding fee, veterinarians conserve doses by inseminating only a single dose of semen within the period. Since predicting ovulation within such a short time frame is very difficult for even the most experienced clinicians, frequent examination of the growing follicle (often every 6 hours) are performed so that a single insemination can be made as soon as ovulation is detected. While this scheme minimizes the number of doses used, it also unfortunately requires frequent examinations, which are expensive for the mare owner and time consuming and impractical for the veterinarian.

Hilltop Farm frozen semen breeding contracts provide ample doses of frozen semen to employ a new, simpler insemination scheme developed by Select Breeders Service. The SBS program is much more cost effective for mare owners and allows veterinarians to manage mares for insemination with frozen semen using a protocol similar to that used for cooled semen. The protocol involves single daily examinations, use of the ovulation-inducing agents HCG or Ovuplant, and 2 inseminations timed to occur at specific intervals from administration of the ovulatory agent. This protocol has been tested in both clinical and laboratory fertility trials and has proven to be as effective as more expensive and labor-intensive protocols. All shipments of frozen semen distributed through Select Breeders Service (including all Hilltop Farm stallions) will include the management recommendation sheets enclosed with this letter.

Results obtained using the SBS insemination scheme

Select Breeders Service Europe: Clinical Trial 2000 & 2001

A comparison was made between fertility obtained when two insemination schemes were used to inseminate mares with frozen semen. Frozen semen from each of 12 stallions was used to inseminate mares either once per cycle, in the period within 6 hours immediately after ovulation, or twice per cycle, 24 and 40 hours post – HCG treatment. The single AI scheme required ultrasound examinations every 6 hrs during the periovulatory period and resulted in first cycle and seasonal pregnancy rates of 70.6% and 82.8% (24/29), respectively. The two-insemination protocol required only once daily ultrasound examinations and resulted in first cycle and seasonal pregnancy rates of 78.3% and 92.3% (36/29), respectively. These pregnancy rates are very high and reflect the fact that the stallions were selected based on proven fertility with frozen semen.

Colorado State University Study 2001

In this controlled study, semen from 3 stallions whose fertility with frozen semen was unknown was used to compare two insemination schemes. Two groups of 20 mares each were inseminated with frozen semen. The first group was inseminated with one dose (800 million total sperm) within 6 hours post-ovulation. The second group was inseminated twice, 24 and 40 hours after HCG treatment, with a half dose (400 million total sperm) per insemination. There was no difference in fertility between the two groups. First cycle pregnancy rates of 55% were obtained using both insemination schemes.

These data, along with reports of very good results from Select Breeders Service's commercial distribution program, illustrate the effectiveness of SBS's new and simpler scheme for management of mares using frozen semen.

If you have any questions regarding the use of this protocol or would like assistance, please feel free to contact Select Breeders Service, toll free at 877-658-3328.

READ CAREFULLY BEFORE OPENING CONTAINER

Instructions for Handling and Thawing Frozen Semen
Packaged in 0.5 ml Straws
(straw dimensions: length = 13.2 cm (5 3/16"))



Always keep this nitrogen container upright. Tipping will cause the liquid nitrogen to spill out of the container. If this occurs, return the container to its upright position as quickly as possible contact the sender. (The liquid nitrogen that has spilled will evaporate within a matter of seconds)



Always wear gloves while handling this liquid nitrogen container.



The transferring of straws to another liquid storage container should only be performed by a technician experienced in the handling of frozen semen. **Exposure of the straws to room temperature for even more than a couple of seconds will initiate thawing and cause permanent damage to the semen.**



Materials needed for semen thawing:

37°C constant temp. water bath
long handled tweezers or hemostats for grasping the straws
sterile insemination supplies warmed to 37°C



When ready to inseminate:

Prepare the mare aseptically prior to opening the nitrogen container.

Prepare a constant temperature water bath of 37°C

Using a pre-cooled hemostat, or tweezers, remove one straw from the nitrogen container and immediately place it into the 37°C water bath. *It is important to make this transfer as quickly as possible, exposing the straw to room temperature for no more than 2 to 3 seconds.*

Leave the straw in the water bath for *at least* 20 seconds to insure proper thawing.

5. The individual straw may remain in the water bath while the remaining straws are removed from the tank and thawed. The number of straws that comprise a single insemination dose may vary according to the freezing procedure employed. Consult the Frozen Semen Transaction Report included in this information packet for this information.
6. Once all of the straws for the insemination dose have been thawed, remove them one by one and wipe the straws completely. Water leaking into the straw would be spermicidal.
7. Holding the straw vertically so that the air bubble is positioned on the top of the straw (the top is crimped or sealed with a steel sealing ball) cut off the crimped or sealed end.
8. Place the open end of the straw over a sterile, pre-warmed, 37°C container (a centrifuge tube or all plastic syringe case is suitable) and cut off the plug on the other end of the straw. Allow the semen to empty into the sterile container. Tap the end of the straw to insure that all of the semen is obtained.
9. Repeat this procedure for all of the thawed straws, combining the contents into a single container.
10. If not already in a syringe, the semen should be immediately drawn up into a pre-warmed sterile syringe and insemination pipette and inseminated just as would be done with fresh semen.

Note: Each insemination dose contains 600 – 1000 X 10⁶ total sperm concentrated into the breeding dose. A minimum of 30% progressive motility is expected upon proper thawing. It is Important to deposit the entire insemination dose into the uterus for optimum results.

PLEASE DO NOT HESITATE TO CONTACT SELECT BREEDERS SERVICE at (877) 658-3328
IF THERE ARE ANY QUESTIONS CONCERNING THE PROCEDURE FOR USING THIS FROZEN SEMEN