

Performance Equine Vets

3827 Charleston Hwy

Aiken, SC 29801

803.640.9282

888.874.7569

www.PerformanceEquineVets.com



Stallion: _____

Straws per dose: _____

Frozen Equine Semen Handling & Thawing Instructions

This frozen semen has been shipped to you in a special Vapor “Dry” Shipper for Liquid Nitrogen and can only maintain proper storage temperature for a limited period of time. If you do not use the semen in this tank within a few days of arrival, PLEASE transfer it to your own liquid nitrogen storage tank to avoid damage to the semen. This container must be returned using the provided return label within 7 days, or a late fee of \$25.00 per day will apply to the mare owner.

Special Precautions

- Always keep this shipper in the upright position.
- 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 frozen straws for even a few seconds to room temperature may cause partial thawing and permanent damage to the semen.*
- Do Not put Goblet into water bath with straws still contained in it as they will thaw too slowly.
- Do Not pull one straw at a time out of goblet and plunge into water bath, as it will take too long.
- Do Not use less than one liter of water in 37°C bath. 1+ to 2 L of volume is a more stable temperature.
- Do Not pour liquid nitrogen into your bath as it will change your temperature by a few degrees quickly.

Materials needed for semen thawing:

- 37°C constant temperature water bath (Do Not use water temperature that is above 37°C or below 35.5°C.)
- Long handled tweezers or hemostats (pre-cool by holding down inside tank 6 to 10 inches for a few minutes prior to goblet removal.)
- Sterile insemination supplies warmed to 37°C (use only ALL plastic syringes)
- Protective eyewear and cyro-protective or leather gloves.

1. Prepare the mare aseptically prior to opening the container. Do not lift the canister above the frost line at any time. Each dose is packaged in a goblet which is loaded on a cane. Each cane holds two goblets.

2. Lift the tank canister with aluminum canes and /or goblet close to the top of the tank and remove the top goblet allowing cane with other goblet to remain in tank (If the dose you need is the only one remaining on the cane, you may remove the entire cane). BE SURE to pre-cool your hemostats or long tweezers. ((If only the bottom goblet remains, the “AL cane” can be used as a holder to remove and dump out liquid nitrogen from goblet.)) Remove goblet, pour out any liquid nitrogen into sink, then quickly pull **All** straws out of goblet and drop or plunge straws into 37°C bath. Use Cryogenic gloves or leather gloves to handle goblet / cane. Do Not splash or pour liquid nitrogen on your skin as it will burn. **Please Note** - Upon removal of a Goblet, you may notice the small amount of liquid nitrogen that may still be in the goblet after removal. You have a few seconds to dump remaining liquid out into sink, then quickly pull All Straws out of goblet at the same time with your gloved hands, plunging all frozen straws into the 37°C water bath for 20+ seconds. The steel ball sealed straws will bob in bath water with steel ball end down and critoseal straws may sink to bottom. Sink any floaters immediately.
3. The individual straw may remain in the water bath while the remaining straws are removed from the tank and thawed. Once all of the straws for the insemination dose have been thawed, remove them all together and wipe the straws completely with a paper towel in the palm of your hand. Keep straws in your warm hand for stable warm temperature or on 37°C dry heat block. Water leaking into the straw would be spermicidal. Do not lay straws on counter as it could be about 10+° cooler.
4. Holding the straws vertically so that the air bubble is positioned on the top of the straw (the top is crimped or sealed with a sealing ball) cut off the crimped or sealed end with clean uncontaminated scissors.
5. Next, invert all the straw open ends into a sterile, pre-warmed, 37°C glass or plastic container. Now cut off the larger cotton/pvc plug end of the straw at semen/cotton line, allowing semen to drain or flow into the warm tube.
6. **We Do Not recommend extending semen as it may do damage to sperm.** Semen may be deposited in uterine body or deep horn, gently plunge syringe to inseminate the entire amount of semen within minutes of loading pipette using 3cc of your air dam. Hold pipette at a down angle while still in uterus if possible, which will allow the pipette wall coated semen (about one fourth of a straws volume) to drain down into the end of pipette. After a minute or two, plunge the remaining 2cc air dam and remaining drained semen. A few drops of semen will always be left in the AI pipette when held up right for a period after AI and can be examined if desired. Also, the unloaded empty straws can

be taped end down on a warm glass slide after unloading straws to examine the unused small droplet with a cover slip. The semen concentration is about 400 million sperm per ml in concentration with a minimum of 35% or better motility unless specified different on shipment form. Thawed frozen semen microscopic evaluation may look best at about 5 minutes on a 37°C warmed glass slide. Be sure to read frozen semen straw shipment form information.

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Mare Management for Insemination with Frozen Semen

Prior to insemination verify the mare is a suitable candidate for AI with frozen semen by performing a routine reproductive examination that may include culture, cytology, and biopsy when indicated. Data suggests that aged (>15 years) or repeatedly barren mares may have a reduced pregnancy rate and are not the best candidates for AI with frozen semen.

Once the mare comes into estrus, ultrasound daily to monitor follicular activity. Upon detection of a large (35-40mm) pre-ovulatory follicle, administer hCG or deslorelin.

If only one dose is available for insemination:

- Examine the mare via ultrasound at 6 hour intervals starting 12-24 hours after hCG or deslorelin administration.
- 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.

*** In our hospital, mares are palpated every 2 hours once ovulation is pending, to more accurately time insemination and therefore, increase pregnancy rates.

If more than one dose of semen is available during a given heat cycle:

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

****All mares in our hospital are given at least one injection of 1ml Oxytocin 4-6 hours post insemination.*

A general rule for mares inseminated with frozen semen is to inseminate within 12 hours prior to and/or within 6 hours after ovulation. This protocol ensures that viable sperm are in the oviduct during that interval for any mare ovulating within a period of 18 to 52 hours following administration of hCG or deslorelin.

Suggested schedule for insemination of mares 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 deslorelin at approximately 4:00pm

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

Day 2 Inseminate a single dose of frozen semen at 8:00am
(40 hours post injection).

Day 3 Examine to confirm ovulation & inseminate a third dose if the mare has not ovulated at 8:00am

Feel free to call if you have any questions or concerns, we are more than happy to help guide you through the process.

~Thanks, Performance Equine Vets Staff

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