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Breeding with Frozen Semen

EqCS Thaw Instructions and Handling Instructions for Equine Frozen Semen .5 ml Straws Sealed with Steel Ball or Critoseal Sealants.

Product Description and Instructions are specific to .5 ml (1/2 ml) straws processed at Equine Cryogenic Service. YOUR SUCCESS in getting a pregnancy DEPENDS UPON FOLLOWING THE INSTRUCTIONS CAREFULLY! Please read product description with instructions. Be sure to have the proper equipment first, and follow our recommendations for thawing the semen, as frozen semen is not all the same.

EqCS Frozen Semen Product:

The EqCS Stallion Numbered, Aluminum Cane (if Goblets on canes) generally holds 2 “Goblets” with One Breeding Dose each; one top goblet/dose and one bottom goblet/dose. Each “Goblet” consists of “One Insemination Dose”, holding 5 straws (.5ml each with 200 Million sperm per straw) or one billion total spermatozoa in approximately 2.5 mls total volume. We package 5 straws in each goblet, having a one billion total count, for ease of use and retrieval from tank. Most stallions processed are 40 to 60%+ progressive motile sperm upon thawing. Breeding your mare twice within 24 hours of ovulation will put approximately one billion or more (motile sperm) in the uterus at ovulation. See our recommended breeding timing. When removing the dose, remember to always keep the top of the cane/goblet Two inches Below The Top of Tank Opening until removal for thawing. Goblets may contain Liquid Nitrogen upon removal.

Straws Sealed with Steel Balls processed at EqCS – 2007 +. Our Preferred sealing method.

Steel Ball (and/or glass ball) sealing of straws are used to seal a variety of biological products in straws and widely accepted for sealing biological storage straws of semen. This straw seal product differs from other sealing methods in that it is a “Hard” Seal (solid rigid type material for plug) that generally does not become dislodged upon thawing, reassuring that semen is contained within straw. EqCS prefers this method of sealing having different benefits of straw handling. The negative aspect of the “Hard” seal is that a possibly damaged or cracked straw can explode or rocket out of thaw bath, all of which is not uncommon when thawing hard sealed straws. It is why we wear and recommend protective gloves and eye/face wear plus always use pre cooled hemostats or tweezers for handling frozen semen. Do not be surprised if a straw explodes with a pop sound or jumps out of bath at thawing, during the dramatic temperature change in the straw at thawing. Poor handling techniques can crack frozen straws that will most likely “pop” or jump out of water bath. We prefer the steel ball sealing as the negative aspects are rarely observed with proper frozen straw handling technique. The thawed straws will stand up, bobbing up and down in the water bath with steel ball end down, having uniform thawing and easy removal, (not laying on bottom) as with other seals. Be sure to read Un-Loading Thawed Semen. Unloading the straws is basically the same procedure for all .5 ml straws.

The bubble should be at the steel ball end. We require that your First Straw cut is at the small steel ball sealed Semen / Bubble line, which will remove all sealant material and any possible bath water at the smaller plug straw end. Some straws may need to be held/tapped with steel ball plug end up to get bubble back to the sealed end while drying straws off. The opposite end has a cotton/pvc powder plug.

Straws Sealed with Critoseal used on all straws processed at EqCS – Prior to 12-2006.

Critoseal is a high quality human product used to seal a variety of biological straw products and widely accepted in human and animal reproduction for sealing biological storage straws of semen and embryos. This product differs from other sealing methods in that it is a “Soft” Seal (white clay type material for plug) that sometimes becomes dislodged upon thawing. Do not be surprised if a plug or two pop out at thawing, during the dramatic temperature change in the straw bubble and volume pressure at thawing. The bubble should be at the Critoseal end. Be sure to read Un-Loading Thawed Semen. Unloading the straws is basically the same procedure for all .5 ml straws. We require that your First Straw cut is at the Semen / Bubble line with smaller clay plug, which will remove all sealant material and any possible bath water at the small plug straw end. This Critoseal sealant and sealant removal procedure allows for a slower straw equalization in pressure at thawing and maintains semen integrity, even if plug dislodges. Straws should not explode or rocket out of bath with soft seals. Plug dislodging may vary depending on your elevation vs. ours at 1800 feet and variation in water bath temperature. Remember to cut at the straw semen bubble line even

if clay plug is dislodged. Some straws may need to be held/tapped with smaller white plug end up to get bubble back to the sealed end while drying straws off. The opposite end has a cotton/pvc powder plug.

Semen Straws that are Pre-Printed.

Pre-printed straws have the name of the stallion, his registry and id number, owner name, the USDA EqCS id #, freezing extender and 200 Mill written on the outside of the straw. All USDA processed straws are pre printed and ship with a processing sheet signed by the veterinarian inspecting stallion on that collection day as part of the semen freeze processing tracking log. A Cryogenic *Processing Date with id*, Label will be on 1 of the 5 straws inside goblet. This "Straw label" can be peeled off and removed from straw to be used for accurate records keeping of the breeding with frozen semen for each dose.

Semen Straws that are Generic or Non Printed plain clear.

Generic or plain non printed clear straws have a Cryogenic label on one of the 5 straws for id, that show the name of the stallion, his registry with his id number, owner name, freezing extender and date processed by EqCS. The other 4 straws will be blank generic straws having the same identical frozen semen, all contained in the goblet, without id. The "Straw label" can be removed and re-used for accurate records keeping of the breeding with frozen semen for each dose.

THAW Procedure for Straws in Goblet and Removal from Liquid Nitrogen Tank

Prior to removal of any semen, prepare a breeding pipette with all plastic 10 cc syringe for insemination. After preparation of all AI equipment needed in 37 degree centigrade incubator, washing the mare in preparation to AI, confirming 37 degree water bath temperature; 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 or one of the loose/bulk goblets. BE SURE to Pre-cool your hemostat or long tweezers by holding down inside tank 6 to 10 inches for a few minutes prior to goblet removal. ((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 in to 37 degree C bath. Use Cryogenic gloves or leather gloves to handle goblet / cane and always wear protective eye glasses. 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. This will give you 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 degree centigrade 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.

Things not to do!!

- 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 water temperature that is above 37 degrees or below 35.5 degrees C.
- 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.
- >>>>> Be sure your thermometer is accurate! A few degrees can make a big difference! Check it!
- >>>>> Be sure to pre cool hemostat or tweezers so as not to damage or crack straws if touched.

Un-Loading Thawed Semen Straws for Mare Insemination and A.I. breeding timing with medications

All straws will be thawed at 20 seconds + in 37C. At this temperature, the time factor of a few additional seconds or so in water bath is not a problem, however the straws should all be removed and dried quickly so as to keep the same temp. Remove all straws from bath together after 20+ seconds and dry off in the palm of your warm hand with paper towel. Keep straws in your warm hand for stable warm temperature or on 37 C dry heat block. Keep small plug end up and tap straws to get bubble position next to seal. Do not lay straws on counter as it could be about 10+ degrees cooler.

After straws are padded dry with a paper towel, cut off the Steel Ball or Critoseal End (small end plug) at the top Semen/Bubble line with clean uncontaminated scissors. Be sure to cut on semen bubble line as the bath water at straw end would be spermicidal, should plug be dislodged. (The semen should not come out with only this end cut off.) All five straws, can be held in line together in your fingers and cut at one time on bubble semen line. Next, invert all the straw open ends into a 5 to 15 ml sterile glass or plastic test tube maintained at 37 degrees C. 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. A few taps on the side of the tubes with the scissors can be helpful. We use a 15 ml conical plastic centrifuge tube that can be warmed in the water bath or dry heat block prior to putting semen in tube. Using your pre warmed insemination breeding pipette, draw a 5 ml air dam and then the 2.5 mls of thawed semen. This is only 2.5 mls (just in the insemination pipette) that probably holds 4 mls, which needs to get into the back of the uterus quickly to avoid any large temperature change. The Mare should already be washed and prepped, standing ready to be inseminated within a minute or two, once the pipette is loaded. We Do Not recommend extending semen as it may do damage to sperm. Less highly concentrated semen is better than more diluted semen. With pipette through cervix to the back of the uterus, (allowing a one inch pull back from pipette tip in uterus), gently plunge

syringe to inseminate the entire amount of semen within minutes of loading pipette, (to the 2 cc syringe line). Plunge all but the 2 cc's of remaining 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 un-loading straws to examine the unused small droplet with a cover slip. The Semen Concentration is about 400 million sperm per ml in concentration with 40% 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.

Our frozen semen extender versions used do not seem to create a uterine inflammation reaction as do some extenders however we always administer 1cc of Oxytocine at approximately 4 to 6 hours post insemination as a good breeding practice.

Breeding with EqCS processed frozen semen, We generally breed one dose per day starting at a mid heat cycle 3.5cm to 4cm follicle, 24 hrs after HCG or Deslorelan injection, checking for ovulation at 48 hrs or mare teasing out. IF Possible, breed mare at 24 hrs – day 1, (36 hrs-optional) and next morning 40 to 48 hours (After) HCG or Deslorelan injection given at noon, day 0 – mid heat cycle. This protocol should stimulate Mare to ovulate 36 to 48 hrs post injection, at night or early am on day 2, plus allot mares will generally ovulate in the night or early mornings when ovulating normally on their own without meds. We try to Coordinate meds with Mother Nature! If mare did not ovulate day 2 am, breed next dose in PM and re check day 3 am for ovulation and so on. Beyond day 3 without an ovulation, the mare probably has issues that will require a closer analysis of heat cycle and time of breeding.

Quick Reference on Frozen Semen Breeding.

1. Water Bath and breeding supplies are at 37 degrees C, not higher than 37.
2. Mare should be washed and ready to AI >> prior to thawing semen or washed while you thaw.
3. Pre-cool hemostat or tweezers and remove one goblet or remaining goblet on cane.
4. Pour off any liquid nitrogen, Pull all straws from goblet and plunge into water bath for 20 seconds+.
5. Remove all straws, pad dry in palm with paper towel and cut off small plug at bubble/semen line.
6. Invert all straws into a pre-warmed tube and cut of cotton plug end at semen line.
7. Tap straws to remove balance of drained semen straws into tube.
8. Draw semen into pipette having a 5 ml air block.
9. AI mare ASAP. This entire procedure should take a few minutes once mare is ready.
10. Repeat procedure daily until mare ovulates.
11. Give one cc of Oxytocine 4 to 6 hours after each insemination.
12. Check Mare Pregnant 14 to 16 days after ovulation!! = Good Job for all to celebrate.

Materials needed list:

1. 37 C Water Bath – electric temp bath or thick styrofoam box or Semen shipping box
2. Accurate Thermometer to also check electric water bath temperature or Styrofoam box temp.
3. Gloves and eye protection for sure
4. 10 inch+ Hemostat or long tweezers
5. Paper towels for drying straws
6. Clean Scissors for straw cutting – cleaned with alcohol-not soap
7. Sterile AI pipette + All plastic 10cc syringe (no silicone rubber plungers), sterile glove & lube or AI Kit
8. Sterile test tube for unloading straws. 5cc min > 15cc conical max. – do not use Red top w silicone
9. Dry Heat block or use water bath and extra person with warm hand to hold warm semen tube.
10. Semen tank or storage tank with frozen semen placed near water bath.
11. Microscope and glass slides – always good to look at semen, after AI, if possible.
12. Mare washing supplies and extra person.

WARNING: *BREEDING WITH FROZEN SEMEN IS EASY AND CONVIENENT*, PROVIDED YOU ARE KNOWLEDGABLE OF THE EQUIPMENT REQUIRED AND PROCEDURES USED. PLEASE RE-READ THE PROCEDURE. AVOID MISTAKES AND ERRORS AS IT MAY CAUSE DAMAGE TO YOU AND / OR THE SPERM. ALWAYS WEAR PROTECTIVE GLASSES AND GLOVES WHEN WORKING WITH LIQUID NITROGEN, WHICH IS AS COLD At -197 C, AS WATER IS BOILING HOT ! IT CAN BURN YOU !! THAWING SEMEN STRAWS CHANGES THE STRAW TEMPETURE ABOUT 230 DEGREES IN SECONDS THAT CAN SOMETIMES EXPLODE OR ROCKET PAST YOUR FACE. >> BE SAFE!