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**IMPORTANT! PLEASE READ BEFORE THAWING STRAWS!**
**IN ORDER TO ACHIEVE OPTIMUM SUCCESS WITH SGI FROZEN SEMEN, IT IS ESSENTIAL TO FOLLOW THESE STEPS PRECISELY!!**

**EQUIPMENT NEEDED OR RECOMMENDED** Modena powder (purchase from SGI), distilled water (can be purchased from select grocery or department stores), graduated plastic insemination bottles (capacity of 80 ml minimum), sterile glass or plastic container for mixing extender (1-2 Liter capacity), glass or plastic beaker for pouring extender into bottles; OR ready-made extenders purchased from SGI; thawing device OR sink to thaw straw(s); second-hand timer; tongs or large tweezers to remove straws from tank; paper towels; scissors; Celsius or Fahrenheit thermometer

**1. FROZEN SEMEN EXTENDER—MODENA** Please do not use any other company's frozen or fresh semen extenders, as they have been developed for their specific freezing methods. You may purchase modena powder from SGI to make your own extender, or convenient ready-made extenders to place in your freezer for future use.

**READY MADE EXTENDERS** Place extenders in freezer (-20 degrees Celsius) upon arrival for future use. Extenders expire 6 months after production day and should contain the day of expiration.

**MODENA POWDER** Add the contents of the extender package to a 1000 ml (1 Liter) or 2000 ml (2 Liter) clean, sterile flask or container. Add 1000 ml DISTILLED water (not deionized or charcoal filtered) to the flask and mix thoroughly by shaking. Let extender sit at room temperature for approximately 15 to 30 minutes to stabilize. Dispense equally into 12 graduated plastic insemination bottles, for approximately 80 ml in each bottle. Bottles with graduated lines may be purchased from SGI. Store in freezer (-20 degrees Celsius) until ready to use. Remember to mark the bottles in such a way as to remember when they will expire. Rinse the extender container several times with hot water (please do not use soap) followed by 6 distilled water rinses. Sterilize the flask or container by filling with distilled water and placing in a pan deep enough to allow you to fill with hot tap water to the neck of the flask or container. Boil 10 minutes. Allow container to air dry and store tightly covered until next use.

**2. EXTENDER AT 20 DEGREES CELSIUS / 68 DEGREES F** You may wish to make fresh modena up the day of thawing, or use those stored in the freezer that you have previously made or purchased from SGI. Regardless, make adjustments so that the extender you are using is at 20 degrees Celsius, or 68 degrees F. This may include placing bottles of extender in a sink of cool water or the refrigerator for a few minutes (if too warm), or in a sink of warm water (if just taken out of the freezer, or too cold). I have found placing the number of needed frozen extenders out at room temperature over night is more convenient and will be closer to the desired temperature of 20 degrees Celsius (68 degrees F) by morning than thawing the frozen bottle of extender just prior to needing it. Plus or minus one degree is acceptable.

**3. <u>WATER BATH AT 50 DEGREES CELSIUS (122 DEGREES F)</u>** If you have a straw thawing device, or electrical water bath be sure it is set at 50 degrees Celsius & check it with a thermometer prior to using. Otherwise, fill a sink with hot tap water & adjust to 50 degrees Celsius (122 degrees F). You may need to add either boiling or cold water, depending on the temperature your tap water. Plus or minus one degree is acceptable.

**4. <u>THAWING THE STRAW</u>** Place the liquid nitrogen tank close to your thawing sink or thawing device. This will reduce the amount of time the straw is at ambient temperature during transfer from the tank to water bath. Quickly remove one straw (one breeding dose) from the liquid nitrogen tank by lifting the canister up to the top of the neck of the tank, just far enough to allow you to grasp the straw with your tongs. The canister should not be out of the liquid nitrogen environment for more than a few seconds, especially if other straws remain in the canister. REMEMBER—straws exposed to ambient temperature outside the liquid nitrogen environment for more than 5 seconds may result in a reduction in semen quality. Check the name of the boar as you quickly remove the straw from the tank. Place the straw quickly in the 50 degree Celsius water bath. Let the straw remain there for exactly **45 seconds**. DO NOT try to hold on to the straw during thawing. Very rarely a sealing ball may pop out of the end of the straw, causing the straw to break, making a loud noise. If this happens, please discard the straw & contact SGI. Be sure to maintain the 50 degree Celsius temperature in your water bath in between straws if you need to thaw more than one straw.

After removing the straw from the water bath, dry the straw thoroughly with a clean paper towel. Holding upright, snip the upper ball off with a pair of scissors. Place the cut end into the bottle of extender and snip the other end of the straw to allow the semen to drain into the bottle. Since the semen inside the straw is a thick, concentrated solution, it is recommended to "bob" the straw up and down on each end to avoid a large number of sperm being discarded with the straw.

5. **<u>ANALYZING</u>** If you decide to analyze the quality of your frozen semen, there are a few things to keep in mind. First of all, be sure to warm your sample for at least 30 minutes prior to analyzing. This can be done by microwaving a gel pack until it is tolerated by the inside of your wrist. Place the extended sample on top of the gel pack by placing it in a small sample tube, small sealed container or ziplock baggie. Because frozen semen viability decreases over time, we recommend that you inseminate while the sample is warming. Frozen semen produces more favorable results if inseminated immediately after thawing and should not be used after one hour from thawing. Analyze your sample using both regular slides AND caffeine coated slides (available from SGI). This warming time and caffeine environment is needed to warm and wake the cells and to accurately estimate the cells potential as it would be if located in the reproductive tract. If you analyze a sample without warming and without the caffeine coated slides, it will look very bad! Cells will have very little movement and appear weak, thus indicating that the sample has not had enough time to warm up and stabilize in its new environment. Often times if you are accustomed to analyzing fresh semen samples which average around 80% plus live cells, your eye will not be trained per se or use to accurately analyzing frozen samples that average 40% plus live cells. There is indeed a wide margin between the two! Most reactions are that the semen is dead, which in most cases is false, the error due to improper analyzing.

**6. <u>TRANSPORTING TO BREEDING AREA</u>** Place the semen in an insulated, warm container or area (20-27 degrees Celsius or 68-80 degrees F) while transporting thawed semen to the breeding area AND when preparing to inseminate. For maximum conception rates, it is recommended that frozen-thawed semen be inseminated as soon as possible after thawing since the viability of frozen-thawed semen decreases with incubation. DO NOT use more than one hour after thawing.

## OPTIMUM TIME TO INSEMINATE WITH SGI FROZEN SEMEN

| | Single Insemination | Double Insemination |
|---|---|---|
| **Gilts** | 29-32 hours | 1st 24-29 hours<br>2nd 30-34 hours |
| **Sows** | 33-36 hours | 1st 29-32 hours<br>2nd 34-38 hours |

The above times are hours after the sow or gilt first exhibits standing estrus (wait the above number of hours after the sow or gilt will first stand for a boar to mount before insemination). Double insemination is recommended.

**<u>Thawing Summary</u>**
**Water Bath:** 50 Degree C or 122 Degrees F
**80 Ml Modena extender:** 20 Degrees C or 68 Degrees F
**Thaw Time:** 45 Seconds