

Considerations for Storing and Handling Frozen Semen

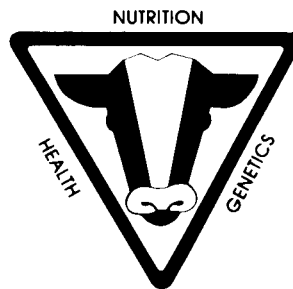
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Considerable change has occurred within the artificial insemination (AI) industry. The most obvious change was the transition to the straw as the predominant semen package. This package system has several advantages compared to the ampule. More units can be stored in bulk at the AI organization and in farm semen tanks. The straw system allows for a more complete delivery of semen during insemination. Probably most important, the straw permits more uniform control of the freezing and thawing process which has led to improved sperm cell recovery. The major disadvantage of the straw system is the vulnerability to mishandling.

In addition to the adoption of the straw there has been an increase in direct sales of semen to owner-inseminators. It is estimated that the average farm semen tank contains semen from two to three breeding organizations. Although the .5 ml French straw is the most popular semen package available today, .5 ml and 1 ml ampules are still present in many tanks. The .25 ml straws may be available in some areas.

Each package system has a different surface to volume ratio which requires unique handling procedures. Recommendations for handling semen also vary among AI organizations using the same package. This whole set of circumstances has resulted in confusion for owner-inseminators and in some cases fostered an attitude that almost any method of handling semen is adequate. Whatever the reason—indifference, ignorance, or confusion—the end result may be lowered conception rate.

This fact sheet will establish some guidelines for semen tank management, handling semen within the tank, proper thawing procedures and preparation of the inseminating device.



IRM-11

Dairy Integrated Reproductive Management

Semen Tank Management

Technical advances have been made on the design and construction of semen tanks. Tanks with six to eight month liquid nitrogen holding times are available. The maintenance of very low liquid nitrogen temperatures in the inner chamber is due to high quality solid insulation material and vacuum in the outer chamber. Although the newer tanks are better insulated they are still susceptible to damage from mishandling.

The inner chamber containing liquid nitrogen is actually suspended from the outer shell by the neck tube. Any abnormal stress on the neck tube caused by sudden jarring or an excessive swinging motion can possibly crack the tube resulting in vacuum loss from the outer chamber. Puncture of the outer shell also will lead to vacuum loss. Since vacuum is the major insulating component of the tank a loss of vacuum causes an increase in temperature within the inner chamber and a rapid evaporation of nitrogen. Accumulation of frost at the top of the tank indicates a rapid evaporation of liquid nitrogen.

Several field studies have indicated that there is no significant damage to semen stored in properly managed farm tanks. However, it should be noted that during a field study in the state of Washington liquid nitrogen completely evaporated in 3 of 60 tanks. This may appear to be a low percentage but it is a significant economic loss to the individual farmer.

To avoid such a tragedy, follow simple management practices:

- Avoid excessive movement or abuse of the tank.
- Routinely monitor nitrogen levels and keep a record of nitrogen usage. Remember even new tanks can have defects and fail.
- Store the semen tank in a well lighted area but out of direct sunlight. Observe the tank daily. Once a tank "goes bad" the nitrogen is lost very rapidly. Develop a plan to have an alternative semen tank available in case your tank is damaged.

- Keep the tank elevated above the concrete floor or other wet and poorly ventilated surfaces. Corrosion of the outer shell shortens the functional life of the tank and possibly causes tank failure.
- Store only the amount of semen needed for one year.

Handling Semen Within the Tank

When extended semen cools during the freezing process microenvironments are created within the semen package. Each chemical component of extended semen freezes or solidifies at a different temperature. Water begins to freeze as temperatures are decreased below 32°F (0°C) forming ice crystals which remain somewhat unstable at temperatures above -112°F (-80°C). This instability is thought to be caused by recrystallization of the ice.

Also as water is converted to ice the sperm are exposed to the remaining concentrated solution of salts and other components of the extender which freeze at temperatures considerably below the freezing point of water. Instability of ice and concentrated solutions are harmful to sperm. Fortunately, incorporation of glycerol as a cryoprotective agent and improved freezing rates helps minimize sperm damage. However, semen must be kept at temperatures well below critical temperatures where the recrystallization of ice begins to occur.

In the typical farm semen tank, dangerous temperatures exist in the upper half of the neck tube (Table 1). Exposure to these temperatures can occur when transferring semen from tank to tank and handling semen within the neck when trying to locate and thaw a specific unit of semen. Remember, the larger surface to volume ratio of the straw makes it very susceptible to temperature changes. Thermal injury to sperm is permanent and cannot be corrected by returning semen to liquid nitrogen. Semen handling practices important to minimizing thermal damage are:

TABLE 1. Temperature Found in the Neck Tube of a Typical Farm Semen Tank

Location in Necktube	Range in Temperature		
Top	+36°F	to	+54°F
1 inch from top	+5°	to	-8°
2 inches from top	-40°	to	-51°
3 inches from top	-103°	to	-116°
4 inches from top	-148°	to	-184°
5 inches from top	-220°	to	-256°
6 inches from top	-292°	to	-313°

Adapted from Saacke 1978, Proc. 12th Conf. on AI of Beef Cattle. Critical danger zone is the upper half of the neck tube or above -112°F.

- Coordinate rapid transfer of semen between tanks. Involve two people and arrange tanks side by side. If possible fill the tanks with nitrogen before transfer. Raise canisters only to a level necessary to locate the rack of semen to be transferred.
- Develop a semen inventory system (Fact Sheet IRM-4) and mount it on the wall above the tank. Try to keep semen from one bull on each rack. Such systems help avoid unnecessary searching and exposure of semen to dangerously high temperatures within the neck region.
- When preparing to thaw semen raise the canister into the lower portion of the neck where the specific rack of semen can be grasped. Lower the canister further into the neck. Secure the rack as low as possible in the neck, thus protecting the other straws from thermal damage. If straws cannot be easily removed from the plastic goblet, bend the top tab of the rack to a 45° angle. This reduces the chance of bending the straw.
- Use tweezers to transfer the straw to the thaw bath. Quickly lower the rack of semen and canister into the body of the tank.

Thawing Semen

When the .5 ml French straw was introduced into this country, there was confusion as to the optimal thawing method. Recommendations varied among the AI organizations. Each organization has a specific method for diluting, cooling, packaging and freezing semen in straws. The total processing system determines the optimal rate of thaw. After considerable research conducted by AI organizations and universities it is generally concluded that warm water thaw (95°F or 35°C) results in improved sperm cell recovery compared to other thaw methods. Warm water thaw exposes sperm to critically dangerous temperatures for only a brief time. The rise in temperature is rapid enough to minimize sperm damage.

A major criticism and concern for the warm water thaw is the danger of cold shock caused by mishandling of the straw following thaw. Cold shock is the permanent injury to sperm caused by a sudden decrease in semen temperature after thawing. It can occur during preparation of the inseminating device or during travel to the cow. If precautions are taken to prevent cold shock, the advantage of warm water thaw will be realized.

Check the temperature of the thaw water immediately before removing the straw from the tank. Use an easy to read, accurate thermometer. Duration of thaw should be between 30 and 60 seconds.

Some organizations recommend the pocket thaw for straws. This method is successful for semen processed and packaged by their system. However, the pocket thaw should not be used for semen packaged in straws from other organizations.

Thaw the standard 1 ml ampules in ice water (41°F or 5°C) for 10 minutes. The smaller .5 ml ampules can be thawed in warm water for 90 seconds or ice water for 3 to 5 minutes. Thaw semen according to the recommendations of the organization supplying that specific unit of semen.

Some Additional Pointers In Handling Semen

- Keep insemination equipment clean and dry at all times.
- Occasionally check the accuracy of your thermometer.
- Do not attempt to thaw semen at temperatures greater than 95°F (35°C).
- Thaw units of semen individually.
- Shake the straw as it is taken from the tank to remove any liquid nitrogen that may be retained in the cotton plug end of the straw.
- Thoroughly dry each straw or ampule of semen. A small drop of water can be lethal to sperm.
- Check the bull identification code on every unit of semen.
- Shake the air bubble from the middle of the straw to the crimped end.
- Cut the tip of the straw squarely and through the air space below the crimp. An angle cut may prevent the straw from fitting securely into the sheath. Check to see that the straw is firmly seated into the plastic adaptor or tip of the sheath depending on the type of inseminating device you use.
- When assembly of the insemination rod is complete, gently depress the syringe to remove the air space at the upper end of the straw.
- Eliminate the chance of cold shock by:
 1. Warming the inseminating rod and sheath to body temperature (do not use water for this purpose).
 2. Handle the thawed semen and prepare the insemination rod in a warm environment.
 3. Wrap the assembled insemination rod in a clean, dry paper towel and tuck it within your clothing for transport to the cow.
- Inseminate the cow within a few minutes after the semen has been thawed.
- Never take shortcuts when handling semen or inseminating a cow. Pay attention to detail.
- Never experiment on your own. The recommendations made are supported by valid research.

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