



# Storing and handling frozen semen

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## Topics Include:

- Introduction
- Semen tank management
- Handling semen within the tank
- Thawing semen
- Inseminating a group of synchronized cattle
- Additional pointers in handling semen

## INTRODUCTION

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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 over the ampule. More units can be stored in bulk at the AI organization and in farm semen tanks. The straw system allows 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 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 0.5-ml French straw is the most popular semen package available today, 0.25-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 the notion that almost any method of handling semen is adequate. Whether this attitude comes from indifference, ignorance, or confusion, the end result may be lowered conception rate.

## SEMEN TANK MANAGEMENT

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Technical advances have been made on the design and construction of semen tanks. Tanks with six- to eight-months 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. Abnormal stress on the neck tube caused by sudden jarring or excessive swinging motion could crack the tube and result in vacuum loss. Since vacuum is the major insulation

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 three of 60 tanks. This may appear to be a low percentage but it is a significant economic loss to the individual producer.

## SEMEN TANK MANAGEMENT, CONT.

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To avoid such a tragedy, follow these simple management practices:

1. Avoid excessive movement or abuse of the tank.
2. Routinely monitor nitrogen levels and keep a record of nitrogen loss. Remember, even new tanks can have defects and fail.
3. Store the semen in an area with good light but out of direct sunlight.
4. Observe the tank daily. Once a tank fails, nitrogen is lost very rapidly. Plan to have an alternative semen tank available in case of a failure.
5. Keep the tank elevated above the concrete floor or other wet and poorly ventilated surfaces. Corrosion of the outer shell will shorten the functional life of the tank and possibly cause failure.
6. Store only the amount of semen needed for six months.

## HANDLING SEMEN WITHIN THE TANK

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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 freezes as temperatures drop below 32°F, forming ice crystals which remain somewhat unstable at temperatures above -112°F. This instability may be due to 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 extender which freeze at temperatures considerably below the freezing point of water. Instability of ice and concentrated solutions are harmful to sperm. Fortunately, incorporating glycerol as a cryoprotective agent and improving freezing rates have minimized 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 (see **Table 1**).

Exposure to these temperatures can occur when semen is transferred from tank to tank or when handling semen within the neck while 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 thermal fluctuation.

Thermal injury to sperm is permanent and cannot be corrected by returning semen to liquid nitrogen. The following semen handling practices are recommended to minimize thermal damage:

1. Transfer of semen between tanks must be coordinated and rapid. Two people should be involved, and tanks should be arranged side by side. If possible, fill the tanks with nitrogen before transfer. Raise canisters only to a level necessary to locate the appropriate rack of semen.

## HANDLING SEMEN WITHIN THE TANK, CONT.

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2. Develop a semen inventory system and mount it on the wall above the tank. It is best 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.

further into the neck. Secure the rack as low as possible in the neck to protect the other straws from thermal damage. If the straw cannot be easily removed from the plastic goblet, bend the top tab of the rack to a 45° angle to reduce the chance of bending the straw.
3. Prepare to thaw semen by raising the canister into the lower portion of the neck where the desired rack of semen can be grasped. Lower the canister
4. Use tweezers to transfer the straw to the thaw bath. Quickly lower the rack of semen and canister into the tank body.

**Table 1. Temperatures found in the neck tube of a typical farm semen tank**

<i>Location in Necktube</i>	<i>Range in Temperature (Fahrenheit)</i>	
Top	+36°	to +54°
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°

**The upper half of the neck tube is the critical danger zone**

Adapted from Saacke 1978, Proc. Conf. On AI of Beef Cattle

## THAWING SEMEN

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When the .5 ml French straw was first introduced in this country, there was confusion about the optimal thawing method. Recommendations varied among AI organizations, each of which has a specific method for diluting, cooling, packaging, and freezing semen in straws. The total processing system determines the optimal rate of thaw. As a result of

considerable research, it is generally concluded that warm water thaw (95°F) results in improved sperm cell recovery compared with other methods of thawing. Success of warm water thaw is due to the fact that sperm are exposed to critically dangerous temperatures for only a brief amount of time. The rise in temperature is rapid enough to minimize sperm damage.

## THAWING SEMEN, CONT.

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A major criticism and concern for the warm water thaw is the danger of cold shock caused by mishandling the straw following thawing. 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 transport to the cow. If precautions are taken to prevent cold shock, the advantage of warm water thaw will be realized.

It is important that the temperature of the thaw water be checked immediately before removing the straw from the tank. Use an accurate, easy-to-read thermometer. The length of thaw should be at least 40 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. The standard 1-ml ampules should be thawed in ice water (41°F) for 10 minutes. The smaller 0.5-ml ampules can be thawed in warm water for 90 seconds or ice water for three to five minutes. Semen should be thawed according to the recommendations of the organization supplying that specific unit of semen.

## INSEMINATING A GROUP OF SYNCHRONIZED CATTLE

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Breeding a group of cattle during a short period of time following estrous synchronization may present problems for the herd manager and AI technician. Although artificial insemination of synchronized cows is the final step in a synchronization-breeding program, it is critical to the success of the entire program. It is important that special consideration be given to proper semen handling and insemination technique to ensure optimum conception rate.

### 1. *Personnel*

To move the breeding phase of this program along efficiently, assign each person a job. One individual should be responsible for thawing semen and preparing the inseminating gun.

This relieves the AI technician of additional tasks, allowing concentration on proper AI technique. Additional people could move cattle to and from the breeding chute. Everyone should be thoroughly instructed in his or her specific jobs prior to the breeding.

### 2. *Physical facilities*

Ensure an adequate holding area is available where heifers can be assembled for treatment, heat detection, and breeding. This area should have a breeding chute or similar arrangement where animals can be treated and artificially bred in a safe and efficient manner. The breeding chute area should be covered to protect semen, supplies, records, and personnel from adverse weather.

## INSEMINATING A GROUP OF SYNCHRONIZED CATTLE, CONT.

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### 3. *Procedures*

A. Prepare a list of selected matings if numerous AI sires are to be used for breeding the synchronized cattle. This list could be used in selecting which unit of semen is to be thawed and inseminated for each particular cow. An inventory system describing the location of semen from each bull within the semen tank is also desirable.

B. Follow thaw procedures according to the recommendations of the AI organization supplying the semen. The thaw water must be maintained at the proper temperature for each dose of semen thawed. Research from Washington State has shown that 10 to 20 straws can be thawed simultaneously as long as the thaw bath water remains a constant 95°F and the environmental temperature is not severely cold. Straws thawed in bulk should be agitated slightly to keep them from sticking together. Bulk thawing of semen should only be considered when a large group of synchronized cattle are to be inseminated.

C. Do not prepare the insemination device too far in advance of insemination.

Breed the cow as soon as possible after the semen is properly thawed and the inseminating equipment is assembled.

D. Prepare insemination devices in a warm, clean environment near the breeding chute, but far enough away to avoid excessive dust and debris near the cattle. This will minimize the chance of contaminating the equipment and semen.

### 4. *Other considerations*

A. Handle animals gently to avoid unnecessary excitement before, during, and after breeding. Undue excitement may adversely affect sperm transport within the female reproductive system causing a lower conception rate.

B. Use proper insemination techniques. Several qualified inseminators should be on hand if many cows are to be bred over a short period of time. Consult your AI representative for advice and help in this regard.

The success of a synchronization-breeding program depends on prior planning, teamwork, and attention to detail by everyone involved.

## ADDITIONAL POINTERS IN HANDLING SEMEN

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- ◆ Keep insemination equipment clean and dry at all times.
- ◆ Check the accuracy of your thermometer occasionally.
- ◆ Do not attempt to thaw semen at temperatures greater than 95°F.
- ◆ Shake the straw as it is removed from the tank to remove any liquid nitrogen that may be retained in the cotton plug end of the straw.
- ◆ Dry each straw of semen thoroughly. A small drop of water can be lethal to sperm.

## ADDITIONAL POINTERS IN HANDLING SEMEN, CONT.

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- ◆ 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 that the straw is firmly seated into the plastic adapter or tip of the sheath, depending on the type of insemination device you use.
  - ◆ When assembly of the insemination rod is complete, depress the syringe gently to remove the air space at the upper end of the straw.
2. handling the thawed semen and preparing the insemination rod in a warm environment;
  3. wrapping the assembled insemination rod in a clean, dry paper towel and tucking it into some clothing for transport to the cow.

Inseminate the cow as soon as possible after the semen has been thawed.

Avoid shortcuts when handling semen or inseminating a cow. Pay attention to every detail.

Refrain from experimentation. The recommendations that have been made are supported by valid research

Eliminate the chance of cold shock by:

1. warming the inseminating rod and sheath to body temperature (do not use water for this purpose);

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