THE UNIVERSITY OF ARIZONA
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# Semen Handling 101

*Katie J. Hazlewood Spanyers and Ashley L. Hall*

## Introduction

Using livestock Artificial Insemination (AI) has been shown to improve genetic herd quality, increase consistency and value of calf crops, eliminate the need to purchase, house, and feed a full herd of bulls, shorten breeding and calving season, and generally improve the profitability of a livestock production operation (Baruselli et al., 2018, Lardner et al., 2020; Rodgers et al., 2012; Walker et al., 2021). In order to have a successful AI program within an operation, there are many important factors to take into consideration, and proper planning is needed. Understanding the steps involved in the proper handling of semen and maintenance of equipment in good working order is crucial. To complete the AI process, items needed, include a liquid nitrogen tank for storage (A), thaw bath and water (B), AI sheath (C), AI gun (D), paper towels (E), an electric thermometer or temperature card (F), forceps (G), scissors (H) or straw cutter (J), and a semen straw (K; Figures 1 and 2).


Figure 1. Items for artificial insemination. Items labeled: liquid nitrogen tank (A), thaw bath and water (B). Image credit: Ben Downer.

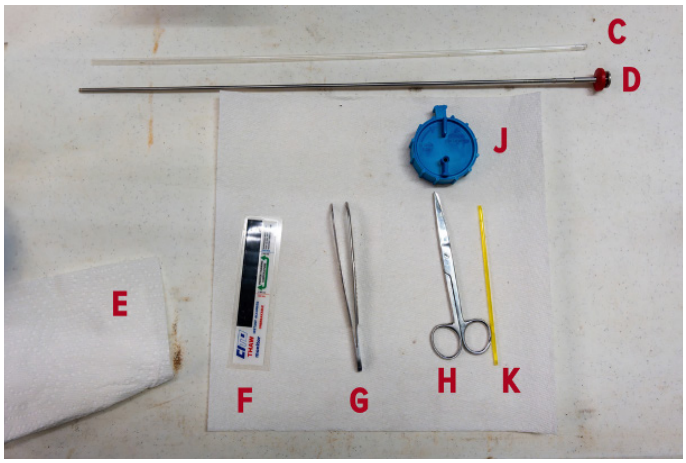


Figure 2. Artificial insemination equipment. Items labeled: AI sheath (C), AI gun (D), paper towels (E), an electric thermometer or temperature card (F), forceps (G), scissors (H), straw cutter (J), and a semen straw (K). Image credit: Ben Downer.

## Semen Storage

Liquid nitrogen tanks are aluminum cryogenic containers essential for storing semen (Figure 3 and 4). Liquid nitrogen preserves semen through extreme cold (-321 °F) by slowing down cellular activity, maintaining sperm viability. Proper maintenance and storage of nitrogen tanks are important for protecting the investment made in purchasing high-quality semen. Tanks come in a variety of sizes, ranging from 3 to 165 liters. The size of the tank determines how many canisters it can hold and should be labeled accordingly. Each tank has a daily evaporation rate and a static holding time, both of which are based on the tank size. The static holding time is the length of days a tank retains the supplied volume of liquid nitrogen and can range from 13 days to 24 weeks.

Within each double-chambered vacuum-sealed tank (Figure 3) are a canister, cane, goblet, and cane tab (Figure 4). The canes and goblets are held within the canister. Each canister can hold multiple canes. The cane, cane tab, and goblet are the three main pieces of the tank that hold semen straws in place. The cane is a metal holder that is inside the tank. At the top of the cane is the cane tab, which is typically

Figure 3. Split liquid nitrogen tank showing interior of the double chamber. Image credit: Katie H. Spanyers.

labeled and visible when the tank is opened. Labeling the cane tab can be useful for organizing semen straws for quick access during the insemination process. The goblet, which is attached to the cane, is a plastic cylinder with an open top that stores the straws, usually of a single bull. Semen straws come in ½ cubic centimeter (CC) volume or ¼ CC. Tanks can hold from 120 ½ CC straws up to 4500 straws in the larger volume tanks.

Nitrogen levels need to be regularly monitored and will need to be refilled periodically. Depending on the manufacturer and tank, a specific level of nitrogen must be maintained in the container. This is often 25 percent of the container capacity. Some manufacturers make sensors (available as an additional cost) that will alert the tank owner if nitrogen drops below the minimum level. The rate of nitrogen evaporation depends on the type of tank, how often it is opened, and the condition of the tank. If nitrogen begins to evaporate at a higher rate than expected, inspect the tank for damage. If you see condensation or frost on the outside of the container, that is a sign the tank has been

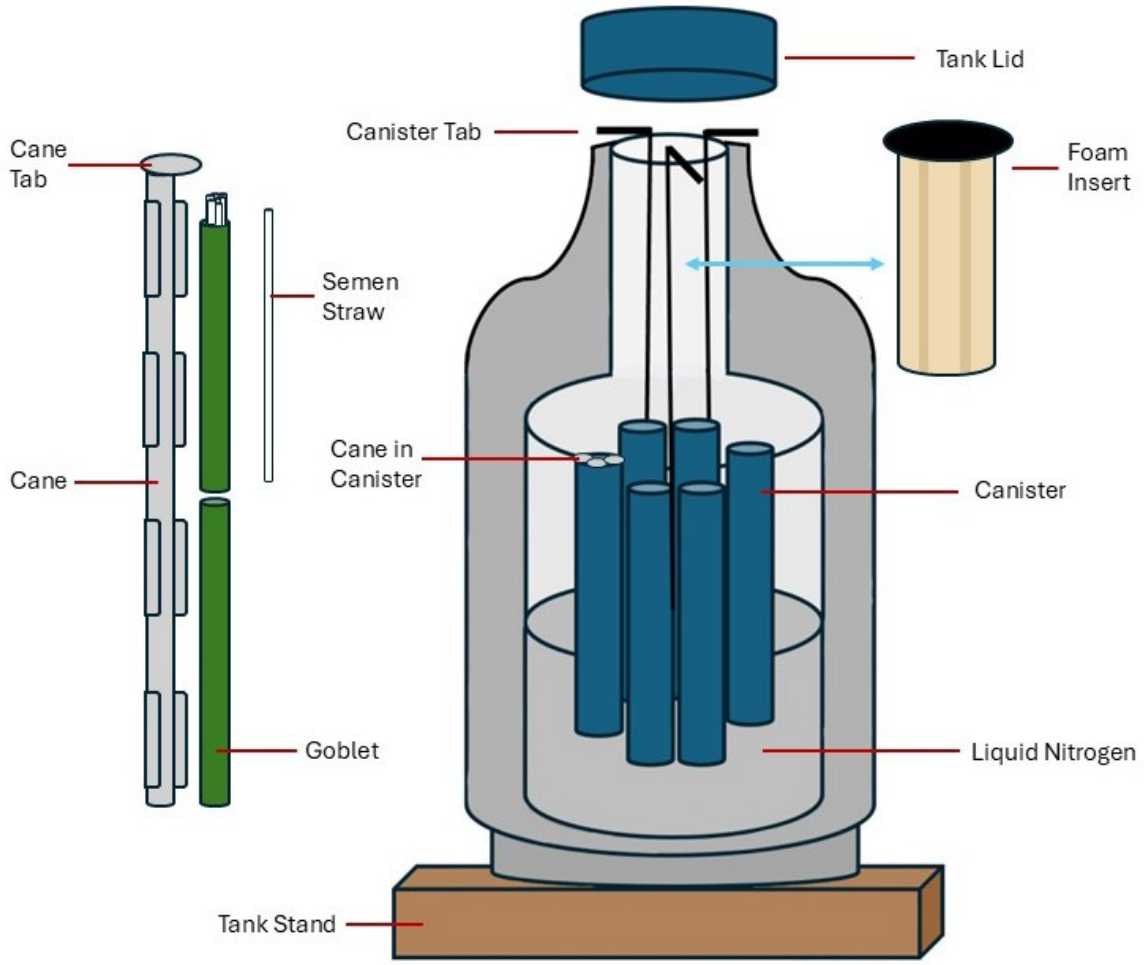


Figure 4. Liquid nitrogen tank and its contents. Image credit: Ashley L. Hall.

damaged and is losing nitrogen. To refill the tank, contact the manufacturer, a semen company, or a local retailer, like a welding company. If the tank needs to be transported, ensure that it is always kept in an upright position, secured, and handled with care.

Liquid nitrogen tanks should be stored in a cool, dry, clean, well-ventilated area, as it is constantly venting nitrogen. It is important to note that nitrogen tanks cannot be directly stored on concrete because of a chemical reaction between aluminum and alloys in concrete (ABS Global, 2011). This reaction will cause the bottom of the tank to degrade and lose its vacuum. Tanks should be stored on a rolling stand, cardboard, pallet, or something that will prevent accumulation of moisture under the tank. With proper care, tanks can last many years but will eventually fail due to loss of the internal vacuum.

It is valuable to keep an inventory of straws in the tank and their location in the canes so that they can be quickly accessed once the tank is open. Labels can be added to the neck of the tank or the cane tab to serve as a reminder of which bulls are in which cane. It is recommended that straws need to be located and removed within 10 seconds of opening the tank lid so other straws in the tank are not negatively impacted. If the needed straw is not located within 10 seconds, replace the cane back in nitrogen for at least 10 seconds.

Liquid nitrogen can cause frostbite-like symptoms and should never be directly touched. When removing semen straws from the tank, be sure to wear proper personal protective equipment (PPE) and/or pick up the straw with tweezers or forceps.

## Thaw Bath

To warm the semen, a thaw bath is used to gradually increase the temperature of the straws (Figure 5). Water is added to an electric warmer. In some models, a green light will indicate when it is ready. The temperature must be checked with a reliable thermometer or a temperature card to be 96°F. If hot water is added, it could be warmer than desired and will then have to be cooled. Semen straws need to be warmed for at least 45 seconds. They can stay in the thaw bath longer; however, straws should be used in the insemination process within 10 minutes of retrieving them from the nitrogen tank and thawing. Only warm as many straws as you can use within that time frame.

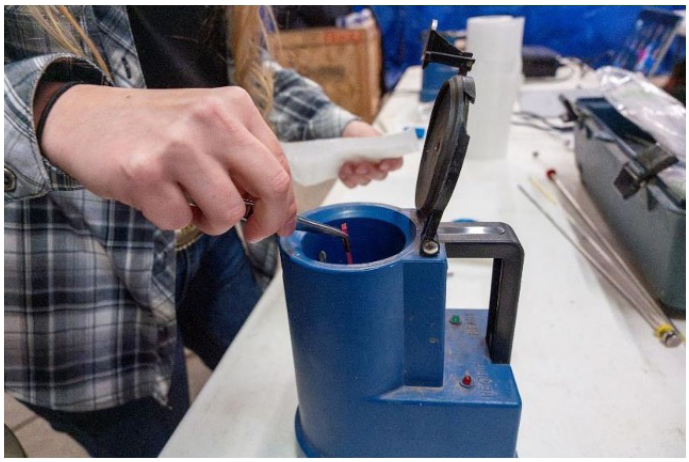

Figure 5. Electric thaw bath. Image credit: Ben Downer.

## Protecting the Straws

Semen contains very fragile genetic material that can easily be damaged by a variety of things. The straws will need to be protected to ensure there is not a decrease in semen quality. Rapid changes in temperature can result in a decrease in sperm mobility. Make sure the water in the thaw bath is the correct temperature before placing the straw in the bath. Once the straw is thawed and loaded, make sure to keep it in a warm place, like tucked into a shirt or next to your body, especially if insemination is taking place on a cold day. Semen can be damaged when going from the thaw bath to cold temperatures. Direct exposure to sunlight can cause sperm damage. It is best to thaw out straws in a tack room or under a canopy. Water is harmful to semen; make sure the straw is completely dry before loading it in the AI gun. It is recommended to use a doubled-over paper towel to dry the straw (Figure 9); this also provides coverage from the sun when transferring from the water bath to the AI gun. Blood, urine, and manure can impact the semen sample; make sure the cow has been wiped off before insemination; however, do not use soap, detergent, or cleaner to wipe off the area, as those can kill semen. When the insemination process is complete, use only hot or boiling water to clean the AI gun, as soap residues left in the gun can kill semen.

## Preparing the AI Gun

An AI gun (Figure 6) is used to deposit semen into the animal beyond the cervix. It consists of a metal catheter that holds and protects the straw with a sheath added over the top and a plunger that pushes the semen out when deposited into the animal. There are different types of AI guns: Conical (O-ring), which uses a hard O-ring to lock the sheath into place (Figure 7A), and Self-Lock, which has a flared base that holds the sheath on (Figure 7B). The sheath needed will depend on the type of AI gun. The size of semen straw (½ CC or ¼ CC) will also determine the AI gun used. There are both universal and size-specific AI guns.

To prepare the gun, the plunger needs to be pulled back far enough for a straw to be loaded. The gun needs to be kept in a warm place while the straws are being warmed and prepared. AI warmers can be used, or simply placing the end of the gun against your body in a jacket or pants will keep it

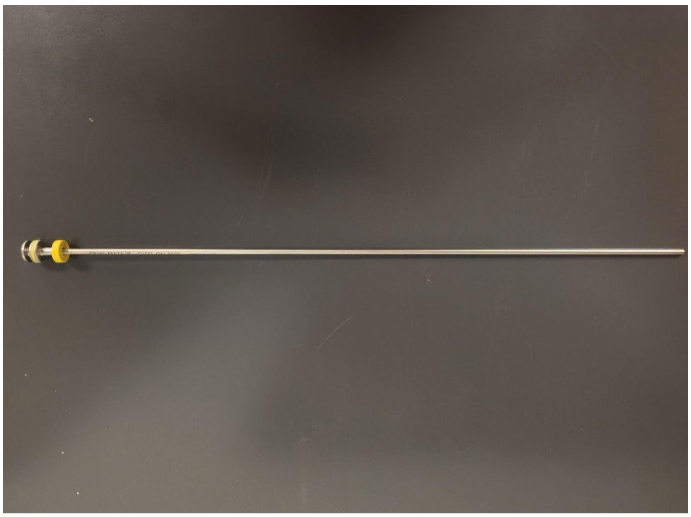

Figure 6. Artificial insemination gun. Image credit: Katie H. Spanyers.

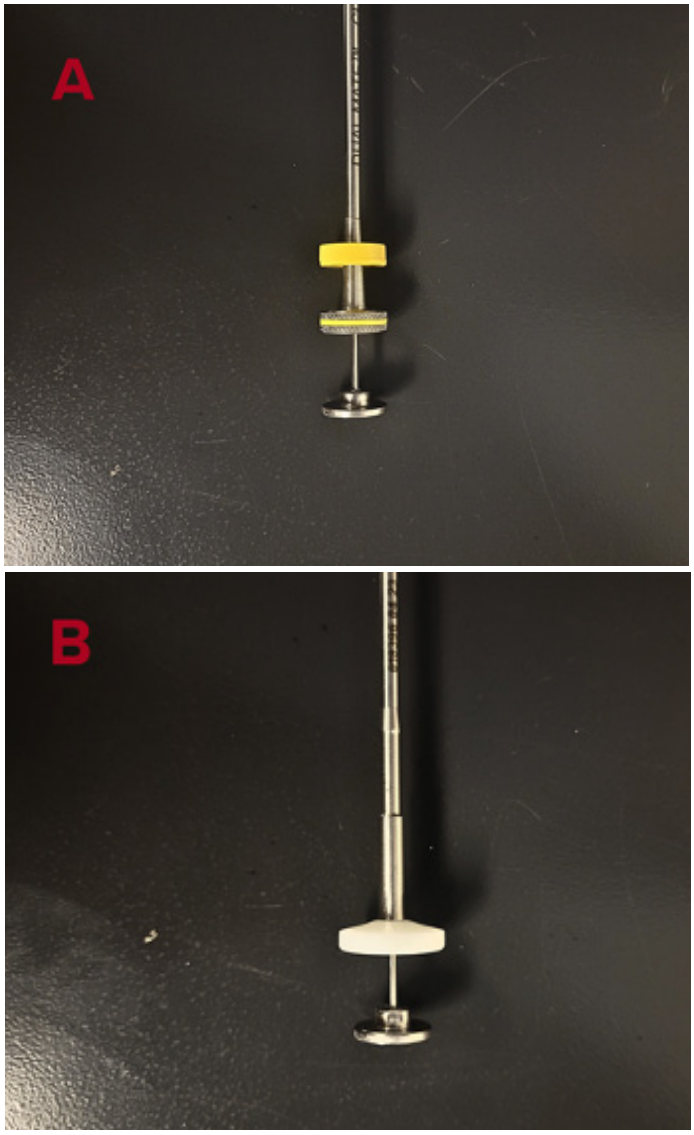


Figure 7. Conical/O-ring (A) and Self-Lock (B) types of artificial insemination guns. Image credit: Katie H. Spanyers.

at body temperature. If the temperature outside is cold, the sheath also needs to be warmed similarly to the gun while preparing the semen. Warming the gun and sheath prevents cold shock, which could damage the semen when the straw is loaded into the AI gun. After the semen straw is warmed and dried, the cotton plug-end is inserted into the gun first. The end of the straw is cut straight across, either with scissors or a straw cutter (Figure 10). Then a sheath is slid over the AI gun and straw. The AI gun then needs to be placed back into a warm place until inserted into the animal.

## AI Steps

1. Prepare and start the thaw bath. Do not start preparing semen until this has reached 96°F for 45 seconds.
2. Prepare AI gun
   a. Pull the plunger out far enough for a straw.
   b. Place the open end of the gun in a warm place (AI gun warmer, against your body in a jacket or pants, etc.).
3. Take the lid off the tank/pull out the foam insert (Figure 4).
4. Pull the canister up by hooking 2 fingers under the handle. Don't pull the canister farther up than the frost line in the tank.
5. Grab the desired cane with the opposite hand and place in the hand holding the canister to keep it at or below the frost line (Figure 8).
6. Pull a single straw out with now free hand or forceps and put it directly into the thaw bath, start the timer (Figure 8).
7. Replace cane into canister and canister into the tank, replace the foam insert.
8. When time is reached, dry the straw off by placing it in a doubled-up paper towel. This also protects from sunlight, wind, etc. (Figure 9).
9. While in the paper towel, insert the cotton plug-end of the straw first into the AI gun.
10. Snip the end of the straw with scissors or a straw cutter by cutting straight across (Figure 10).
11. Slide a sheath over the end of the AI gun.
    a. Conical (O-ring) guns: slide the sheath up below the O-ring, slide the O-ring down over the sheath toward the open end, then slide the sheath all the way up, then slide the O-ring back up until snug.
    b. Self-lock guns: slide the sheath all the way up until it fits snugly over the widened part of the barrel.
12. Place the ready gun back in the warm place it was.

You are now ready to AI!

Figure 8. Retrieving a straw from the tank. Image credit: Ben Downer.

Figure 9. Protecting and drying off the straw with a folded paper towel. Image credit: Ben Downer.

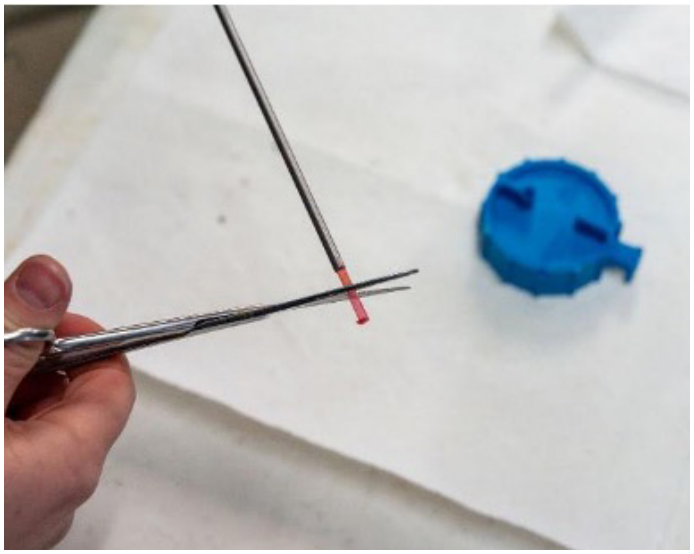

Figure 10. Cutting the end off the straw. Image credit: Ben Downer.

## Planning Considerations

Artificial insemination can only be successful when performed during a specific time frame after the animal is in "standing heat". Standing heat is when the cow/heifer allows other animals to mount her. This observation is indicative of estrus, the fertile period. Semen must be deposited within 8-12 hours of estrus detection to ensure time for sperm to undergo a process of capacitation and the oocyte (egg) to be ovulated. Several fixed-time hormonal synchronization protocols can be followed, and each have their advantages and disadvantages (ABS Global, 2011). A protocol should be chosen based on time available, cow vs. heifer, and preferred labor inputs. Detection of natural heat can also be used to plan AI. This requires spending long periods of time, multiple times a day observing the animals for signs of heat (standing for mounting, becoming more vocal, head butting, mounting other animals, etc.). Natural heat detection, such as estrous observation and heat patches, is a low-cost option as no hormones need to be purchased; however, it can be ineffective if the signs of heat are not well-known or the herd is too large to easily observe every animal. Using a synchronization protocol allows for easy heat detection and the insemination of multiple animals within a short timeframe, making it more efficient for large-scale operations and for having a more uniform calf crop (Oosthuizen et al., 2017). Creating a timeline and allocating time needed for heat observation and/or hormonal synchronization helps ensure effective planning and precise scheduling needed for AI.

## Conclusion

Conception rates will be at their highest when the AI steps are properly followed while paying close attention to detail. It is essential that all equipment, like the nitrogen tank, is well-maintained so that semen straws are not damaged. It is key to limit the amount of time semen is exposed to temperature fluctuations and prevent exposure to direct sunlight, water, manure, and detergents. AI will not be successful if the semen is deposited in the cow outside of the correct window after thawing the sample. If these factors are taken into consideration, a successful AI program can be implemented on any livestock operation.

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THE UNIVERSITY OF ARIZONA
Cooperative Extension

**AUTHORS**

**Katie J. Hazlewood Spanyers**
*Assistant Agent, Yavapai County*

**Ashley L. Hall**
*Associate Area Agent, Gila and Pinal Counties*

**CONTACT**

**Katie J. Hazlewood Spanyers**
khaz@arizona.edu

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