

Semen handling for insemination in the field: splitting straws, loading the equipment and juggling eight straws at a time

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Thawing semen in the field for cervid work presents unique challenges due to the time of year that breeding takes place. The months of October to December can range from a comfortable chilly range to whipping winds with very low temperatures. The ideal situation for performing surgical inseminations is an indoor facility with a heat source, cement floors and a semen breeding list that is filled out with the buck to be used and the order that the does will be presented. The reality is each farm is different and nothing is ever the same.

The basic equipment for thawing semen is the same as with any species: thaw unit, slide warmer, microscope, slides, cover slips, and scissors or a straw cutting unit. In addition, you will need aspics (sheath with an attached needle) and two to three insemination guns (both from IMV, L'Aigle, France), 0.25 ml straws, microcentrifuge tubes (1 ml) with snap lids, extra extender for splitting straws, a stylet to empty straws, a device to hand fill the straws (a TB syringe with a tom cat catheter cut down to fit in the end of the 0.25 ml straw works well) and a fine tip marker. It is also helpful to have a thin piece of polystyrene foam that has two holes cut in it to fit the microcentrifuge tubes and allows them to float in the water bath. The slide warmer is set up and slides, cover slips, straws, aspics, and insemination guns are placed on it to help prevent cold shock to the sperm cells.

As the does are brought in and prepared, each ear tag is checked against the breeding list. Since most owners want to split straws, it is best to have all does being mated to the same buck come in together. This does not always happen due to poor planning by the owner or problems with anesthesia. When the doe is brought over to the insemination area, the ear tag is checked again. I find it helpful to have the breeding list arranged so that each doe is listed under the name of the buck being used for insemination. This will help you know if straws from a particular buck need to be split more than one time. It is also beneficial in cases where straws from a buck are unable to be split the way the owner originally intended and you need to see if a straw from another buck can be split one more way.

Once the incisions are made in the abdomen of the doe, the straw of semen is placed in the water bath for the recommended time (generally 45-60 seconds). The abdomen is inflated with CO₂ as the semen straw is thawing. The straw of semen is wiped off and the semen is placed in the warmed microcentrifuge tube in the water bath. A tiny drop of semen is placed on a warmed slide with a cover slip and evaluated on the microscope for movement and concentration. A judgement call based on that drop of semen is made about dividing the straws. The industry has been freezing at 50 million cells per 0.5 ml straw and most owners are using one straw for two does. If the owner wants to divide the straw and inseminate more than two doses, extra extender is added depending on the number of times the straw will be split. I find it easier to draw extra extender into 0.25 ml straws and have them on the slide warmer ready to use. If the owner wishes to divide it between three does, one straw of extender can be added and if four does are to be inseminated, two straws of extender are added. The semen is drawn into the 0.25 ml straw using the TB syringe and tom cat catheter. The lid of the microcentrifuge is snapped closed to prevent water from getting in the sample.

The insemination gun and aspic are loaded with the straw. To prevent cooling, a hand can be cupped around the straw. The straw is placed on the pink plunger and slid halfway into the gun. The handle of the gun must be loosened to release pressure against the O-ring. The aspic is placed over the straw and inside the gun and slid down into the handle. Once the aspic is seated in the handle, the gun is tightened down so the O-ring holds the aspic in place. The plunger is then used to push the straw up and seat it in the end of the aspic near the needle. Sometimes the straws do not seat correctly and when the plunger is rolled the semen does not come out the needle but is dispensed into the aspic. If this happens, move the end of the aspic gently back and forth to re-seat the straw. The needle guard is removed and the guide is placed over the gun to cover the needle for insertion into the trocar. The semen is deposited into

the uterine horns and the gun is removed from the trocar. The guide is removed and the gun is loosened from the handle which allows the O-ring to release the aspic. The aspic is removed and the gun and guide are placed back on the slide warmer. The empty microcentrifuge tubes are thrown out so semen from different bucks is not accidentally mixed. Write down the insemination time on the breeding list for the owner. Make any notes about the sample or the condition of the uterine horns on this paper as well.

Many times the does are not presented in any particular order. When this happens, you may end up with semen from five or more bucks thawed at the same time. Our thaw unit will allow us to have semen from four bucks thawed and in microcentrifuge tubes at one time. The name of each buck is written on the lid of the tube with permanent marker and the lid securely shut. Remember that the water bath will still be used to thaw semen straws and you don't want to inadvertently drop water into any samples. At this time, you need to look at the breeding list and see which does need to be brought in to use the rest of the thawed semen. Many times this cannot be done and you will need to remove a tube from the water bath. If I know a doe is going to be brought in soon I will draw the semen into the 0.25 ml straw and put it on the slide warmer with a 4 x 4 gauze square over it to prevent it from cooling down and being exposed to light. I still have to open the slide warmer to get other straws off the slide warmer so I put this straw towards the back of the warmer. If it is going to be longer than a few minutes I leave the sample in the tube and put it on the slide warmer.

You will need to be able to multitask when you have semen from multiple bucks thawed. Remember to check each ear tag as the doe is presented for insemination and keep track of how long each sample has been thawed. At 10 minutes after thawing I start reminding the manager of which doe is needed and at 20 minutes after thawing I start demanding those does. If you are doing a large number of does, it may not be possible to control the situation and you just do the best you can. I check a very small drop of semen if it has been sitting in the water bath or on the slide warmer more than 15 minutes. The longest I have had a sample be on the warmer or in the water bath is 50 minutes. This sample was still motile and the doe did conceive. This is in no way an ideal situation but it was unavoidable at this particular farm.

In addition to the semen thawing, you will also need to be aware of what is going on in the preparation area and be able to troubleshoot problems that may occur. Many times you have the owner and their assistants preparing the does and they will not have any idea how to care for clippers or how to change the blades. You also need to be watching as does come in to the preparation area so you can differentiate between the does that need more drugs and those that need more time to go down. The owners do not always remember in which order does were darted. Also, make sure that the anesthetized does have their heads extended and their tongues are pulled to the side.

Most of all, have a great time and start writing a book with the first farm you visit!