

# Adding small ruminant reproduction to your practice: Breaking the myth that you need expensive equipment and extensive training

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## Abstract

Equipment needs for transcervical insemination (TCI) of whitetail deer and goats are essentially the same. Transcervical insemination of sheep can use the same equipment, but pregnancy rates are low in this species with TCI, and the author does not recommend this technique for use in sheep, especially with frozen-thawed semen. Laparoscopic artificial insemination requires more equipment and some practice, but can be mastered with a little practice and patience. Collecting and freezing semen is not difficult, but highly technical and requires attention to detail. Most equipment is readily available at a reasonable cost. A list of the equipment needed and comments concerning the equipment are included in this presentation.

**Key words:** small ruminant, sheep, goat, reproduction, theriogenology

## Résumé

Le matériel nécessaire à l'insémination transcervicale (IT) chez le cerf de Virginie est essentiellement le même que chez les chèvres. Le même matériel peut être utilisé pour l'insémination transcervicale chez les moutons. Toutefois, le taux de gestation est bas chez cette espèce avec l'IT et l'auteur ne recommande pas cette technique avec les moutons surtout si la semence a été congelée puis décongelée. L'insémination artificielle par laparoscopie demande plus d'équipement et d'entraînement mais la technique peut s'acquérir avec un peu de pratique et de patience. La cueillette et la congélation de la semence ne sont pas difficiles mais très techniques et demandent une attention minutieuse. La plupart des équipements sont facilement disponibles et à prix abordable. Une liste de l'équipement requis et des remarques sur l'utilisation du matériel font partie de cette présentation.

## Introduction

There has been an increased interest in advanced reproductive techniques in small ruminants in recent years. Increased demand for these services is driving the market and some veterinarians are taking advantage of this, but there are also numerous lay people doing some of these

procedures. Many veterinarians think the cost of equipment and training and the return on investment may not be worthwhile for their practice. The author will attempt to dispel those myths and show practitioners how they may incorporate these techniques into their practices and make them a profit center. Specific equipment, techniques and practice tips will be discussed.

**Vaginal speculum(s).** A wide range of speculums have been used and are commercially available through a variety of sources. Due to the range in size and the difference between non-parous females and multiparous females, you will need at least 3 different sizes of vaginal speculums to visualize the cervix. Canine speculums will work very well for some occasions and goat speculums are available through many goat specialty catalogs. Deer speculums are available from several sources commercially, although the goat speculums will work just as well in most cases. A human proctoscope works well, but many will find it too long to use with the shorter goat AI equipment. Homemade speculums using syringe cases may also work well for some.

**Light source for transcervical insemination (TCI).** Many of the speculums, especially commercially made ones, come with a light source that may consist of a flashlight to connect to a fiber optic transducer to a simple flashlight adapted to attach to the speculum in some manner. Whatever the source, it needs to be small so that space is available to pass the insemination rod, durable to stand up to the water, lube and abuse it will take, and have batteries or other power source that is easily portable and usable. The author prefers a very small (micro) flashlight clipped to the inside of the speculum. Head lamps are also of value and there are a variety of available models to choose from. You should have several different light sources available to you, spare batteries, and spare bulbs.

**TCI insemination gun.** There are a variety of commercially made insemination guns available for small ruminants. A Cassou gun for cattle AI may also be used. Goat supply catalogs, veterinary reproductive companies, and the internet are all good sources for this equipment. Personal preference and differences in the cervix may dictate what gun works best in the species you are working with.

**Lube.** There are several different companies that make lube appropriate for use on the vaginal speculum to make insertion easier. One should try to use a lube that is sterile, but more importantly, one that is free of spermicidal ingredients. Make sure to check the ingredient list and read the fine print as many of the products on the market contain chlorhexidine or other spermicidal agent.

**Thaw unit.** Commercially available units to thaw semen are available through a variety of sources. You should probably obtain one that will work off of AC or DC or have an adaptor or converter so that you can work in a remote location or on farms that don't have electricity available.

**Generator.** In some remote locations or on farms that don't have electricity, a small generator to run your electrical equipment may be important. The author has also had several experiences where the electricity went out or fuses blew and to complete a job, had to rely on generator power.

**Straw cutter.** Commercially available from a variety of suppliers or one can use a razor blade, sharp knife or scissors to cut the end of the sealed straw. Using a straw cutter will probably distort the end of the straw the least to assure a good seal when loaded in the insemination gun, but the other methods will work, especially if one pays attention to the end of the straw prior to loading and straightens out any imperfections in the straw.

**Extender.** Many producers will want the straw "split" (especially whitetail producers) 2, 3, or even more times to inseminate multiple animals with. You may choose to inseminate using the insemination gun and either calibrating it and using the appropriate fraction of the straw, or you may choose to thaw the straw and extend it and reload straws from the extended semen. This method is probably best in most situations (especially deer), as the does being bred to that particular straw of semen may not be back-to-back in the breeding order, or you may have to hold the semen for a long period of time between does.

**Straws.** Empty straws, for reloading extended semen or in cases where the original straw is either flawed or explodes but the semen can still be recovered and used, are essential. Having both  $\frac{1}{4}$  mL and  $\frac{1}{2}$  mL straws available may save the day.

**Permanent marker.** An ultrafine permanent marker will be useful for identifying straws and other equipment. It is also essential when writing on cuvettes.

**Records.** Keeping track of who gets inseminated with what semen, and when inseminated is essential. Some producers will lose track of animals or change their minds after inseminations have started. Most are now using DNA

to keep track of the sire and dam as well. Giving them the empty straws to confirm DNA and what they have bred with is also valuable.

**Microscope.** A good quality microscope is essential. One with a slide warmer is nice but not essential as one can use heat packs, warm "cool" packs or other creative ways to warm slides for semen evaluation. It will be impossible in most field situations to do anything other than a gross motility evaluation while doing AI. As most whitetails are bred with "split" straws, most of these owners expect the semen to be evaluated prior to insemination for determination of dilution rate. This is especially true when they have paid a very high price for the semen and want some sort of assurance that it is good prior to insemination. With experience, one can usually tell concentration and motility quickly so that the AI process is not slowed down much if any with this quick analysis.

**Cuvettes.** Since most semen for whitetails is split, using cuvettes or other similar tubes to "hold" semen in is essential. Most semen comes in  $\frac{1}{2}$  mL straws and is thawed and then expelled with a plunger or other end cut to allow gravity flow of the semen into the warmed cuvette suspended in a warm water bath. To this, the proper amount of extender is added and a sample of the warmed, extended semen is placed on a warmed slide for the quick evaluation under the microscope.

**Slide warmer.** While one can get around using a slide warmer, I find it essential for warming slides, straws, aspics and cuvettes, especially in colder weather. Holding multiple samples of semen in cuvettes can get confusing yet they can be laid out in good order and labeled on the slide warmer.

**Floating cuvette holder.** This piece of equipment makes it handy for holding cuvettes in the water bath. It is simply a piece of Styrofoam with holes cut in it to hold the cuvettes in the water bath. There may be other methods to accomplish this, but this is cheap, easy, and requires no specialized equipment to make.

**Straw loading equipment.** This can be very easily done by taking a tom cat catheter and cutting it to fit the end of a  $\frac{1}{4}$  or  $\frac{1}{2}$  mL straw and attaching the other end to a syringe. Simply attach to the plug end of the straw and plunge the open end into the extended semen and aspirate the semen up to the plug on the end of the straw. There is commercially available equipment for this that is also very easy to use.

**Insufflation equipment.** Some sort of gas and a delivery system is necessary for laparoscopic insemination (LAI). Some people use room air, filtered room air, or a commercially available gas system using oxygen or carbon dioxide. Medical grade CO<sub>2</sub> is the preferred gas in most insufflation systems, but it may be difficult to obtain and

transport in some situations. There are insufflators that automatically maintain pressure and insufflate the abdomen but are expensive and too slow (in the author's hands) for LAI in the small ruminants, especially in a field situation. In most cases, silastic tubing attached to a CO<sub>2</sub> regulator and a teat cannula is the preferred method, although other people may use another system and feel comfortable with it. Some people use an assistant to turn the gas on and off and others use a foot pedal set up so the surgeon can regulate the amount of insufflation.

**Trocars.** These will depend on the size of scope selected, but 10 mm and 5 mm are very commonly used for most LAI. There are reusable and disposable trocars as well as cutting and non-cutting available as well. The Aspic fits the 5 mm trocar and the author prefers a 10 mm scope so these are what he uses. Other people may prefer other equipment or purchase used equipment that will work equally well for the purpose.

**Laparoscope.** The laparoscope can be either a 5 or 10 mm diameter and 30 cm long rigid scope or other length or size as the surgeon sees fit. A 0 degree head is preferable although some prefer an angled scope of 20 degrees.

**Light source for laparoscope.** Generally a high intensity fiber optic cord is attached to the scope and a high intensity variable power light source is used to illuminate the abdomen. Battery powered sources may work and should be available for backup as well as extra bulbs and a backup lighting system if going on extended trips or in case of equipment failure. If using a flashlight, make sure to have extra batteries or that it is able to be charged from a DC or AC power source or inverter. One needs to make sure the fittings for the light source fit the laparoscope or an adapter is available for it.

**Cradle/surgical table.** A LAI cradle makes the process much easier although animals can be restrained and moved without one. Depending on the number of animals and facilities, it is usually best to have 2 or 3 cradles so animals can be prepped, moved, and inseminated without interruption. The cradle should have fairly large wheels so that it can be rolled on uneven surfaces, and locking wheels are a plus to keep the cradle from rolling on uneven surfaces as they are inseminated or prepped. It should be light enough that it can be moved easily, have adequate restraint of the legs and head, and provide support for the animal at the same time. It is imperative that the rear legs be restrained so that the animal doesn't slip from the restraints when it is inverted for the LAI. There are several designs on the market, but the author has custom aluminum cradles that lengthen and collapse to some degree so they don't take up as much room and can adjust to different animal sizes and species.

**Monitor.** Some people use a monitor with a camera set up to view through the laparoscope. This could be a computer screen or a small TV monitor. This is a personal preference for the surgeon but it will allow all people watching to see what is going on. An excellent teaching tool, it may also be stressful for some to know that everybody is watching their every move and may subject them to even more stress in an already stressful situation. It may also slow the process down as people stop working to watch instead of doing their job.

**Procedure for TCI.** With the doe or ewe restrained either in a chute, standing or anesthetized, the vulvar area is cleaned and dried prior to insertion of the speculum. Most deer are clean enough that they require little or no cleaning and using soap and water or just water or disinfectant can contaminate the speculum and vagina and kill sperm. Some prefer that the rear quarters of the animal be raised to make it easier to visualize the cervix and inseminate. It will depend on the species and restraint method used as to whether this is advantageous. Using a non-spermicidal lube on the vulva or the speculum, the speculum is inserted into the vagina until reaching the cervix. Choosing the proper size speculum will depend on experience and the size and previous birthing status of the doe. After visualizing the cervix, the insemination rod is passed through the speculum and an attempt is made to pass the rod through the cervix into the uterine body. In some animals the cervix will be wide open and the rod readily passed, in others it may be difficult, if not impossible, to pass the rod through any or all of the cervical rings. In either case, once the insemination rod is passed to the point where the inseminator is either satisfied that they can go no further without doing more harm than good, the semen may be deposited in a slow and deliberate manner. The author prefers to withdraw the speculum partially prior to semen deposition to allow the vaginal wall to collapse over the end of the insemination rod. One can also use a "split" speculum so that complete withdrawal of the speculum can be done prior to insemination. Once the semen is deposited, the doe is given any medications necessary and released or reversed after transport to an appropriate place for recovery.

**Procedure for LAI.** The animal is prepared for surgery either with light to heavy sedation (sheep and goats) or with full anesthesia (whitetail deer and other cervids) by clipping the area cranial to the udder and extending almost to the navel and to the flank, and doing a surgical scrub on said area. This is usually done while the animal is restrained in a LAI cradle or other device used for restraint and tilting the animal for the surgery. Two spots approximately 6 to 8 cm (a hands width) cranial to the udder and approximately 6 to 8 cm lateral to the midline are located, and a local block with 2% lidocaine injected subcutaneously and intramuscularly in the abdominal wall. This takes approximately 2 to 4 mL of lidocaine and can be marked with surgical ink or scratched with the needle used for the injection so that the surgeon

may easily see the place that has been blocked. Care must be taken to avoid the major vessels in the area so that they are not cut and bleeding is kept to a minimum. This will lead to blood running down the scope and obscuring the view of the internal organs as well as cause concern to the owners. The animal is tilted on the table and 2 small incisions (10 mm) are made in the skin taking great care to not cut into the abdomen (it will be next to impossible to maintain insufflation if the hole is very large), and at the same time to make sure that the skin is incised completely and underlying fascia has been exposed so that the insufflation needle (teat tube) can easily penetrate the musculature and the peritoneum without excessive pressure being applied to it. As soon as the skin is incised properly, the animal is tilted rear-end up to approximately a 45 degree angle or more depending on surgeon preference. The insufflation needle is then inserted into the abdomen with a sharp thrust taking care to angle away from all internal organs (I angle the needle slightly caudal and lateral so that I miss the bladder and intestines) and gas is pumped into the abdomen till slightly taunt. At this point the trocar for the laparoscope is inserted using the same angle as for the insufflation needle, the dilation or cutting tip pulled, and the laparoscope inserted and visualization of the abdomen, bladder and uterus attempted. Occasionally the omentum will be trapped by the gas, insufflation needle or the trocar and will impair visualization of the abdomen. The omentum must be swept away from the trocar or needle by gentle use of the scope or movement of the needle so that the gas may push the omentum out of the way to allow visualization and manipulation of the uterus for insemination. Once the abdomen has been evaluated and the uterus identified, the insemination trocar is inserted and the Aspic introduced with the needle withdrawn into the Aspic gun. The uterus is gently manipulated with the Aspic gun until the gun and the uterus are at a 90-degree angle and then the inner sheath of the Aspic protruded and the uterus pierced with the needle and the semen deposited into the uterine horn. Usually  $\frac{1}{2}$  of the straw (dose) is injected into one horn and the other half injected into the other horn. Once the semen is deposited, the insemination gun and the laparoscope are withdrawn and some, if not all, the gas is allowed to escape from the abdomen via the trocars' gas valves, and the skin incision is closed in some manner. Staples, suture or glue are all used successfully, and in some instances no attempt to close the incision is made. However, the aesthetics are usually such that this would only be done in a very high-speed sheep breeding situation. The animal is usually given antibiotics and an anti-inflammatory and returned to the recovery area. The scope, trocars, and scalpel are usually stored between animals in a tray with 70% alcohol or rinsed with alcohol and wiped dry with gauze sponges between animals to keep alcohol from running down the scope and trocar, which will impair visualization of the abdomen. Other disinfectants could be used as well, but something that is easy on the equipment and won't irritate the abdominal cavity is essential. Grasping the trocar

and the laparoscope with the same hand and using the thumb to slide the scope up and down and the hand to control the depth of the trocar and scope is perhaps the most difficult to master. There will also be times when the bladder is obscuring access to the uterus and it must be expressed or drained. With practice, the scope can be used to hold the bladder out of the way while manipulation and insemination take place. Some surgeons wear surgical gloves, others use exam gloves or no gloves. The procedure is clean, fast, and has little risk. Most complications are due to handling (capture myopathy), anesthesia, or disease from stress.

Generally speaking, these skills can be mastered with moderate practice. Hand-eye coordination can be developed using such simple tools as a cardboard box with objects inside to visualize and manipulate. Using cull animals to practice on or doing the procedure on owners animals with their full knowledge that you are just starting may be acceptable. Much of the equipment can be purchased new or used through medical suppliers or dealers. When on the road, it is imperative that you have parts for equipment, spare equipment, and a plan to deal with emergency situations that may arise. Electrical cords and power strips, paper towels, scrub, gauze 4x4s, tool boxes to carry and organize equipment, tables to work on, semen tanks, and other miscellaneous items can make or break you. Check-off lists of equipment are helpful and highly recommended.

### Semen Collection and Freezing

In most cases, a large animal or mixed animal practice will already have an electroejaculator (EEJ) on hand that they have been using for bulls or small ruminants. There are also sheep and goat EEJ on the market. Sheep, goat, red stag, and wapiti semen collection can be done with little or no sedation. Whitetail deer and mule deer are generally done under anesthesia, but EEJ may be accomplished under sedation in a chute using haloperidol (Woodbury, personal communication).

The author prefers to exteriorize the penis to ensure a clean sample and provide the ability to fractionate the sample and avoid urine contamination. Exteriorization may be difficult to master, but can be done, especially if one has an able assistant with gauze sponges to help grasp and retract the glans penis. Pushing caudally on the prepuce while simultaneously pushing on the sigmoid flexure usually helps. After exteriorization of the penis, the rectum is cleared of feces and the internal sex glands palpated if possible (digital exam only on smaller species).

The lubed probe is then inserted into the rectum. For sheep and goats, a rhythmic pulse of 2 to 4 seconds on and 2 to 4 seconds off will generally evoke ejaculation within 2 to 5 stimulations using a standard sheep/goat EEJ. Using a variable EEJ will require increasing power using the same pulsing schedule until ejaculation is achieved. For maximum semen volume and sperm numbers, EEJ continues at this level



until clear fluid is obtained. A rest period is then usually allowed and collection is then attempted again to maximize the amount of sperm for freezing. On wapiti, red stag, whitetail deer and mule deer, the author uses a step-up approach to EEJ. Starting at the lowest setting, stimulation is applied for 2 to 4 seconds, 2 to 4 seconds of rest, and then increasing power level slowly until ejaculation occurs. Power is held at this level until collection turns clear, and a rest period allowed before a second EEJ is attempted to maximize sperm output.

Semen should be collected into a sterile, warmed container (author uses 15 mL plastic test tubes in a 50 mL test tube water bath), then evaluated quickly under a microscope on prewarmed slide and cover slip for concentration and motility. If acceptable, it is then extended with prewarmed extender (some are species specific) in roughly a 1:1 or 2:1 ratio of extender:semen. The cooling process is then started by inserting the extended sample in a warm water bath (generally 150 mL of water) into a cooling box (Styrofoam with ice packs) or a refrigerator for usually a minimum of 4 hours until the sample reaches 39.2 to 41°F (4 to 5°C). Following directions for different species is important, as sheep and goat semen can be tricky to freeze with good results. Goat semen generally does best with centrifugation and washing, while certain extenders work better in sheep or cervids.

A sperm count is conducted on the sample to determine the total number of sperm. If it has been fractionated, each sample is evaluated and if determined to be acceptable, all

samples may be pooled and 1 single count done. Extender is generally added to make the final concentration be approximately 50 million per straw for cervidae and 100 million per straw for sheep and goats. These numbers are not hard and fast and may be adjusted depending on insemination technique (LAI vs TCI) or known fertility of the sire or the wishes of the owner or buyer of the semen. Precooled straws are generally loaded in a cold room (inside a refrigerator), sealed (balls, PVC powder, ultrasonic sealer or heat-sealed), and then frozen in either a programmable freezer or over 3 to 5 cm of liquid nitrogen vapor on a rack. If freezing over vapor, the straws are generally plunged into the liquid nitrogen after 12 to 15 minutes in the vapor. Straws are then loaded into goblets in canes and then put into a liquid nitrogen tank for storage. Straws should be labeled (preprinted or hand labeled) with date, species, owner name, animal name or registration number, and facility or person who froze/processed the semen. All handling of frozen straws should be done under liquid nitrogen in a workbox (Styrofoam works great!).

If doing this infrequently, it may be easiest and best to simply collect the semen and ship the extended sample off to a freezing facility for the final stages. Talk with a reputable facility about how they want the semen shipped, what extender they prefer, and how to handle storage, shipping of frozen semen, etc. Semen can routinely be collected, extended, and shipped overnight for processing. This system works well for distant locations or where travel limitations exist.