Detailed methods for Goat Assisted Reproduction and Artificial Insemination

Two-Day Seminar

Canton, New York
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to obtain a link to
digital copies of these methods,
and to view YouTube-type
instructional videos of these
techniques and procedures

Synchronization of Does during the Fall Breeding Season

Does that are within the physiological breeding season may be synchronized for insemination during a concentrated 2-day period using a

12-day CIDR protocol combined with

Intramuscular injection of 1 mL of Estrumate at the time of CIDR removal.
(Estrumate treatment potentially increases the %age of does that come into heat)

While CIDR's may be purchased directly by producers,
Estrumate is a prescription item that must be ordered from your veterinarian.

Video instructions on how to insert CIDR's into does are available on the Web.

Hints for Success:

- Higher pregnancy rates will occur when does are given an increasing plane of nutrition for one month prior to the synchronization ("called "Flushing").
- This CIDR protocol is not effective for does bred outside of the Fall breeding season.

Out-of-Season Synchronization of Estrus in Does

Requires additional "stimulation to re-activate reproductive cyclicity

Out-of-Season synchronization may be achieved in several ways, none of which is perfect:

1. Administer a gonadotropin-like drug at CIDR removal to stimulate small follicles to mature and ovulate.

   PG-600 works, but the only available gonadotropin (PG-600) is not approved for use in small ruminants, so its Extra-Label use must be authorized by a licensed veterinarian.

   No withholding period has been established for PG-600 in goats, which makes it problematic to sell meat, milk or even offspring from treated does.

2. Manipulate photoperiod so that does are experiencing fall-length days (<12 hour photoperiod) during the summer, then administer CIDR treatments.

   Four to six weeks of decreased photoperiod should start estrous cycles, but you need a light-tight barn where your does would be locked in the dark each day!

3. Continuously expose does to a mature buck for several days. This will cause many to come into heat during the non-breeding season ("Buck Effect").

Combining a 12-day CIDR synchronization with the introduction of a buck at the time of CIDR removal should cause out-of-season does to come into heat 3 to 4 days after buck introduction.

Choosing the Optimum Timing for Insemination

To maximize fertility, one dose (Straw) of semen should be placed within the doe's cervix within an hour or two of ovulation.

The CIDR synch gives us a 24-hour period where insemination should happen.

Two additional techniques are needed to fine-tune
the timing of insemination
to coincide with peak fertility.

The timing of insemination in CIDR-treated does should be determined based on;

1) detection of behavioral estrus in individual does starting about 24 hours after the CIDR is removed,

and

2) changes in vaginal mucus **clarity** and **viscosity** that occur during estrus

- Does should be checked for estrous behavior at least twice daily, starting at 12 hours post-CIDR removal.
- During each heat-check, buck should be brought across the fence from a group of post-CIDR does, and animal interactions should be observed for 10+ minutes.
- Does that approach the buck and remain in his vicinity, that vocalize, and that wiggle their tails are showing signs of heat.

Does should not be distracted by food or people in their pen during heat detection.

- Once a doe has displayed heat after exposure to a buck, she needs to be restrained in a stock at least every 4 to 8 hours for examinations of her cervix / vaginal mucus.

(more frequent exams will be necessary as doe gets closer to optimum time for breeding)

Evaluation of Vaginal Mucus to Identify the "Best" Time for Insemination

During estrus, does produce copious amounts of colorless mucus that spills from their cervix into the vaginal cavity.

Vaginal mucus may be evaluated repeatedly during estrus using a clear polycarbonate speculum with an integrated light source.

Most of the visible mucus will pool just below the external opening of the cervix in at the far end of the vaginal cavity

The speculum should be lightly coated with sterile, non-spermicidal lubricant, and then gently inserted into the vagina at an upward angle.

1. At the start of estrus, the mucus is clear and colorless, and will appear nearly watery.

   Clear, thin mucus indicates that it is
   too early for insemination.

2. As estrus progresses, the mucus will gradually become cloudy, and will increase in viscosity and "thickness". This type of mucus is often observed around 48 hours after CIDR removal.

   Cloudy, viscous mucus indicates that
   the doe should be inseminated,
   because ovulation is imminent.

3. Toward the end of a 24 to 36 hour behavioral estrus, the mucus will become very cloudy, and will take on a stringy, sticky consistency.

   Cloudy, sticky mucus indicates that
   the optimum time for insemination has passed,
   and the doe should not be bred.

Collection of Semen from Bucks

Just prior to collecting semen from the buck;

1. Use the buck to identify a doe who is displaying strong signs of heat / estrus.
2. Make sure that the laboratory incubator / water bath are at the correct temperature (one should be at about 30 degrees C and the other at 48 to 50 degrees C)
3. Thaw an aliquot of diluted semen extender (see below), pre-warm in water bath set at 30 degree C.
4. Assemble and fill the artificial vagina (instructions follow). Bag the AV, then place in 48 degree water bath.
5. Make sure to have microscope slides / cover slips available on pre-warmed surface (fill a square-sided bottle with warm water, cover with paper towel)

Tips for Success:

- Bucks trained to collection should be mature adults.
- Ideally, AI bucks should have had previous experience with natural breeding.
- The buck should be evaluated for fertility by a veterinarian using a Breeding Soundness Examination (more on this later)
- Bucks should initially be trained for collection using does that are displaying estrus. Estrus may be induced artificially (see procedure below) so that estrous does are available during practice sessions.
- Ideally, practice semen collection multiple times **before** you are counting on the semen for insemination / cryopreservation!!! Most bucks can be artificially collected after two or three practice sessions with an estrous doe.
- Both the buck and the doe should be acclimated to the semen collection area and to leading with a halter.

Evaluating a Buck for Use as a Frozen Semen Donor

Fertility of bucks should be evaluated with a Breeding Soundness Exam
prior to collecting and processing semen!

(a BSE should be completed
early in the breeding season by a veterinarian)

- The buck should be healthy, in good body condition and not show any lameness.
- The buck should have two descended testicles of approximately equal size and shape.
- Total scrotal circumference in an adult buck should be at least 32 centimeters.
- Scrotal palpation should reveal normal testicular tone, and no epididymal abnormalities.
- The buck should rapidly display interest in an estrous doe, and should attempt to mount her within a few minutes.
- The ejaculate (collected naturally or using an **electroejaculator**) should contain a minimum of 2 billion sperm cells.

Two billion sperm cells is not a strict requirement for passing a BSE,
but less than this minimum reduces the number of "doses per collection
and make the buck less valuable as an artificial insemination prospect.

- Sperm should possess strong forward "swirling" motility when viewed at low magnification
- Percentage of sperm with normal morphology should be >70%.
- Bucks should be test-free from reproductively transmissible diseases.

Disease testing should be completed so that diagnostic results are available

well before the start of the breeding / collecting season.

Evaluation and Processing of Buck Semen

Warning!

We are now getting into technique-heavy protocols!

While collection of semen from a buck is fairly straightforward, since it takes advantage of his natural desire to mate with the doe, preservation of sperm cells in the lab, and after cryopreservation, requires familiarity with a variety of laboratory skills, and access to "laboratory-grade" ingredients such as ultra-high purity water, buffered formalin and liquid nitrogen.

Unless you already have the basic skills, and are willing to invest in a laboratory / equipment / supplies, it might be best to pay a veterinarian to collect and process your buck's semen for frozen semen insemination.

Preparation of Semen Extender

Semen from many bucks and rams has been handled and preserved using a commercially available small ruminant semen extender called Andromed one-step with antibiotics, made by Minitube International.

(*Andromed extender has been shown to preserve ram and buck sperm motility during room temperature and chilled storage, and also after cryopreservation of semen for artificial insemination. Andromed uses soybean lecithin to preserve cells, Lecithin has a number of advantages over older products for sperm cryopreservation, as it preserves cell viability without the use of cryopreservatives that are toxic (i.e., glycerol, propylene glycol) or that use animal products (i.e., egg yolk, serum albumin).*

Note that many other semen extenders are available,

and individual bucks

may "freeze" better using another

Extender / semen freezing protocol.

Preparation of the Artificial Vagina

- We use an artificial vagina (AV) that is manufactured by MiniTube. The AV is available as a "kit" from Reproduction Provisions, and comes with a rigid rubber cylinder, inner latex liners, a filling valve and a tulip-shaped graduated glass semen collection vial.

(we will practice assembling and preparing the AV during the Sunday session)

Plan to have spare parts (one or two extra inner liners and glass vials) on hand during breeding season in case one gets damaged.

- Before its first use, and after each collection attempt, the AV should be cleaned by rinsing well with very hot tap water (do not use soap!!! It can be absorbed by the latex liner and cause damage to sperm).
- Shake the AV dry and rinse with distilled water, and then rinse about 1 ounce of 70% isopropyl (rubbing) alcohol over the latex rubber parts. Allow the AV to dry thoroughly before use, as water and alcohol are both toxic to sperm cells.
- Use sterile **non-spermicidal** lubricating gel to coat the inner liner of the AV (we use a product that is sold to equine breeding farms)

  Remove the AV valve stem, and then attach a funnel to the valve opening. Fill the AV with **50 degree C water**. **Be careful not to spill water all over the outside of the AV**.
- Water temperature can be closely controlled by pouring heated and cold water into an insulated thermos until the correct temperature is reached. This water can then be used to fill the AV.

If you have invested in a thermostatically controlled water bath, you can simply use water that has been previously been warmed to the correct temperature by the water bath.

Before use, make sure that the water bath has reached the correct temperature!

- During cold weather, pre-warming the AV by filling, draining and re-filling might help it to maintain the correct temperature. If you are collecting semen in a cold barn, you will need to be extremely careful to avoid cold-shocking the sperm cells.
- Reinsert the valve into the AV, then push the glass collection vial into one end of the AV.
- Blow a small amount of air into the AV through the valve (there is a threaded cap that can be loosened to do this) to adjust the size of the water jacket opening.
- The AV can be used immediately, but it often it needs to sit for a few minutes before use.

To maintain the optimum AV temperature;
Seal the prepared AV in a gallon Ziploc bag with most of the air pressed out. The bag is then immersed in 50 degree C water in an insulated cooler until just before use. This works especially well when ambient temperatures start to drop in the Fall!

Invest in an accurate dial-type thermometer that measures in degrees C for use during all water measurements.

Semen Collection Procedure

(*requires two animal handlers*)

- The first person (animal handler) leads the doe on to a raised platform and secures her lead rope to a wall-mounted eye-bolt, or post, or locks her into a head-catch.

An inexpensive platform may be constructed from seven wooden pallets –

two stacks of three stacked side by side
with an additional pallet on one side as a ramp.
Set up the raised platform against a wall,
then cover all horizontal surfaces with rubber stall mats.

- Once the doe is restrained, the animal handler leads the buck up the ramp until the buck is behind the doe.
- A second person will then remove the AV from the water bath, then signalfor the animal handler to lead the buck up to the doe's hindquarters. The timing must be fast so that the AV temperature does not drop.
- The buck handler should allow the buck to attempt tomount the doe, and then should stand next to the doe to stabilize her. The buck will extend his penis almost immediately after starting to mount the doe.
- As soon as the buck mounts, the person with the AV should insert the open end of the AV on to buck's penis, making sure that the glass collection vial is held firmly.
- If everything is works, the buck will thrust once or twice into the AV, after which he will dismount. At this point, tilt the AV downward so any semen will flow into the glass collection vial.
- If 0.5 to >2 mL of cloudy fluid is collected, you probably have an ejaculate. If the fluid is clear, try again.

Correct AV water temperature is critical for getting a collection!

If the buck does not seem to be on-board,
empty and RE-FILL THE AV with water at 48 to 50 degrees C,
re-lubricate the AV, and try again

- After a successful collection has occurred, lead the buck and doe back to their pens.

Working with the Semen Collection in the Laboratory

- After a successful collection, carefully remove the glass vial from the AV.
- Wipe off any lubricant from the vial.
- Immediately place the vial / semen in a clean temperature-controlled environment at room temperature or above.
- If you need to leave the semen for more than a few seconds (for example, to put the buck back in his pen), place the glass vial in the 30 degree C water bath, being careful not to introduce any water into the semen.
- Estimate the initial semen volume using the gradations in the glass collection vessel, and then immediately dilute the raw semen at least 1:2 with warm extender (i.e., one mL of semen would need the addition of two mL of extender).
- After you complete multiple collections from a particular buck, you will become familiar with his typical semen characteristics (for example, semen concentration and total volume tend to be fairly repeatable over multiple days).

Some bucks will require a greater initial dilution because they have concentrated semen, and others may only require the 1:2 dilution.
Your objective is to make an initial dilution that causes your hemocytometer counts to fall between 100 and 200 cells (this protocol will be explained later).

- Transfer extended semen into a new, sterile 15 mL centrifuge tube, making sure that the semen remains at room temperature or above while its concentration is being determined.

Procedure for Cryopreserving Semen

Evaluating and processing semen requires a controlled environment, with clean surfaces, controlled temperature and good lighting.

A water bath (or small insulated cooler) filled with 30 degree C water, and an un-insulated glass or metal container should be available. A large rectangular cooler made of hard plastic (not Styrofoam) will also be needed to hold liquid nitrogen used for final freezing of semen straws.

It is vital that semen not be cold-shocked during storage and assessment of motility. The semen processing lab should be clean, well lit, with lots of benchtop space, and should maintain a comfortable "room" temperature (at least 20 degrees C).

Only semen samples that are graded at **Good** to **Excellent** "Wave" motility at 40x magnification, and that are at an initial concentration of **>2 Billion cells per mL** should be used for artificial insemination.

- Immediately following collection, estimate the volume of semen in the collection vial.
- The entire semen sample should be diluted and gently mixed with the freezing extender immediately after collection.

Note that Andromed Extender comes as a concentrate, and must be diluted before use according to label instructions. This dilution must be accurate, done using sterile technique, and only **laboratory-grade water** should be used as a diluent.

Fresh working stocks of extender should be prepared daily, and should be pre-warmed in a 30 degree C water bath before use.
Unused extender in the water bath should be discarded.

- The initial extender dilution will vary based on buck's average sperm output over multiple collections, but is often in the range of 1:2 (1 part semen + 2 parts extender).
- The tube containing the extended semen should then be allowed to cool in room air to room temperature during the counting and diluting process.
- A small sample of he 1:2 mixture of extender to semen will now be assessed for;

  1) Overall motility

  2) Sperm concentration (the entire semen sample will eventually be diluted to a final concentration for freezing based on its initial concentration).

Calculating Sperm Concentration in the Sample

The sperm concentration of the sample should be enumerated using a Hemocytometer, a glass slide with a central well that contains a known volume when filled with liquid. The floor of the countable area is divided by numerous small grid lines.

If you plan to process large numbers of semen samples, think about investing in an electronic sperm cell counter that will calculate the concentration automatically.

A video of the sperm cell counting process will be made available after this conference, and will show you exactly what you would see during a hemocytometer counting session.

- Countable area of hemocytometer (25 large squares, each bounded by double lines) = 0.1 cm wide x 0.1 cm high x 0.01 cm deep = 0.0001 cc = 1/10,000 mL of liquid.
- To determine the sperm concentration of a sample, count only the large squares in the four corners and the one center square (each large square is bounded by double lines, and contains 16 small squares) out of 25 total large squares. This counting covers an area that is $1/5^{th}$ of the total countable area of the hemocytometer.

  Total area counted in 5 large squares = 0.001 mL x 1/5 = 0.0002 mL = 1/50,000 mL.
- To prepare a sample for counting, dilute an aliquot of the extended semen sample 1:100 with counting diluent made from 9 parts of 0.9% saline plus 1 part of 10% buffered formalin

  (use stock formalin that is made for tissue preservation, i.e., a 10% solution of saturated formaldehyde in a buffered saline solution).

- Typically add 0.01 mL of mixed semen to 0.99 mL of counting diluent

(you might need to increase the dilution rate when counting samples with very high sperm concentrations – if so, make sure to use the new dilution rate in subsequent calculations!)

- Mix well and use to fill a hemocytometer chamber using a yellow-tipped micropipettor.

Accurate dilution is critical to obtaining repeatable doses of semen for insemination.

You will need to invest in a set of highly accurate micro-pipettors / disposable pipette tips for your lab.

Determining Sperm Concentration of the Undiluted Ejaculate

(we will eventually need to fill a number of 0.5 mL straws with 200 million sperm cells apiece)

EXAMPLE: We collect a semen sample with a volume of 1.5 mL, and immediately increase its volume to 6 mL through the addition of 4.5 mL of warm extender.

In this example, each sperm cell counted would represent

<u>20 million cells / mL</u>
in the original ejaculate,

(1/4 extender dilution x 1/100 counting dilution x 1/50,000 mL counting volume in the hemocytometer = 0.25 x 0.01 x 0.00002 = 0.00000005 = 1/20 million total dilution factor)

5 million cells / mL
in the extended sample that was sitting in the water bath
(taking into account the 1:4 extender dilution)

- A 0.01 mL sample of the extended semen is then mixed with 0.99 mL of counting diluent, and an aliquot is pipetted on to a hemocytometer chamber.
- We count a total of 100 sperm cells within the large 4 corner squares and the one center square of the hemocytometer. This count of 100 sperm cells for this extended sample would equal 100 cells x the dilution factor of 20 million cells / mL
  = **2 billion cells / mL in the original ejaculate**.
- Since the 1.5 mL of undiluted semen was initially diluted with 3 parts of extender, the sample that we just measured has a concentration of 20 billion / 4
- = **0.5 billion cells / mL in the extender-diluted semen sample.**
- The entire 6 mL of extended sample contains a total of 3 billion cells, so it would produce 15 semen straws, each of which contains 200 million cells in 0.5 mL of volume (3 billion / 15 = 200 million cells per straw).
- In order to package 200 million sperm cells in a 1/2 mL straw (400 million cells per mL), the extender-diluted semen now needs to to be diluted one more time,
- **from 500 million cells / mL to 400 million cells / mL (1:1.25 dilution factor).**
- Therefore, the 6 mL volume of extended semen should be multiplied by 125% (from 6 mL to 7.5 mL) by adding an additional 1.25 mL of extender.
- Another way to calculate final concentration would be to determine the amount of dilution necessary to dilute the original 2 billion cells per mL in the undiluted sample to the final concentration of 0.4 billion cells per mL (5x dilution factor). Since the original volume was 1.5 mL, the final volume should be 7.5 mL (1.5 mL x 5x dilution factor = 7.5 mL).
- Since the sample that we measured with the hemocytometer was 6 mL, we would need to add an additional 1.5 mL of extender to raise the volume to 7.5 mL.
- 7.5 mL final volume x 0.5 mL per straw = 15 semen straws.

Packaging and Storage of Extended Semen

- The extended semen at the final concentration of 400 million cells per mL has now cooled from body temperature to room temperature (about 20 degrees C).
- Load semen into ½ cc wick-and-powder semen straws by drawing it into the straw with suction from a 3 mL syringe with a Luer-Lock / ½ cc straw adapter (these adapters, as well as the semen packaging supplies, are available from Reproduction Provisions).
- For identification purposes, each buck's information can also be printed on his straws.
- Seal the open end of the semen-filled straw by packing polyethylene powder into it (tap the open straw end into a small pile of powder). The packed powder will harden and seal the straw as soon as it makes contact with liquid in the straw.

From this point on, the semen needs to be slowly cooled to 4 degrees C during a 90 minute to 2-hour period.

- Place semen-filled straws into a cooling chamber that will cool them from 20 degrees C to 5 degrees C over 90 minutes (0.25 degrees C per minute). Semen cooled at faster rates will exhibit "cold shock" and reduced fertility.
- The cooling apparatus may be as simple as a refrigerator with straws wrapped in paper towels and placed in a plastic bag, or you could invest in a programmable embryo freezer. In either case, ensure that the cooling rate is within limits by doing several trial runs where you repeatedly monitor the temperature of the straws with an accurate thermometer.
- Once the straws reach 5 degrees C, they should be quickly loaded on to a commercial straw holder that allows air to circulate between them.
- The straw holder is then placed into a rack that is elevated 5 cm above a layer of liquid nitrogen within an insulated cooler.

The semen in the straws will freeze at a controlled rate within the "vapor phase" above the liquid nitrogen.

- Allow straws to cool within the liquid nitrogen vapor for ten minutes, and then use a set of plastic straw tweezers to take each straw off the rack and plunge it into the liquid nitrogen.
- Once all of the straws have been frozen, they need to be loaded into pre-labeled "goblets" that are attached to "canes".

All of this manipulation needs to be done while the straws and canes are submerged in the liquid nitrogen!

- The canes are stored long-term in "Canisters" within tanks filled with liquid nitrogen. These tanks will keep semen frozen for weeks to over one month on a "fill".
- Producers who store frozen semen should devise a "foolproof" scheme to ensure that tanks do not go dry!
- Semen can also be stored at central facilities that use safeguards such as alarm systems, and that offer insurance against loss of semen.

Intra-Cervical Insemination of Does

The ultimate goal of the previous instruction is;
"…to place a substantial dose of healthy, previously frozen sperm cells into a part of the doe's reproductive tract that allows one or more spermatozoa to fertilize one or more freshly ovulated ova."

Research has shown that frozen-thawed sperm will reach the oviduct if a minimum number are placed in the uterus (**Intra-Uterine**) or within the cervix (**Intra-Cervical**).

***** Non-surgical uterine insemination is not achievable in most goats *****

We teach producers how to pass an insemination pipette past one or more cervical rings, and deposit the contents of a semen straw into the central portion of the cervix.

Use of a speculum to pass an insemination pipette into the external cervical os:

Just prior to insemination, a straw of cryopreserved buck semen should be thawed according to the instructions of the company that sold the semen to you.

The thawed straw is then loaded into a **small ruminant insemination pipette**, and the combination is covered with a clean, disposable **insemination pipette sleeve**.

Since the assembly process for the insemination pipette is best conveyed visually, a video of this process will be made available to participants.

- The sleeve is locked into place on the pipette, and the pipette is wrapped in paper towels and guarded inside a coat or shirt to prevent cold shock during transport.
- Goat does that are ready to be inseminated should be restrained in a head-catch that restricts side-to-side movement. Ideally, the head catch should be on an elevated platform so that the doe's hind end is at about shoulder height of the inseminator.
- Clear, transparent Lucite (polycarbonate) speculums are available from many small ruminant repro supply businesses. They are basically a clear tube with a long hole drilled down the length of one wall.
- Coat the speculum with sterile, non-spermicidal gel, and gently insert into the vagina at a slight upward angle until the operator feels slight resistance to forward progress.
- The speculum should then be retracted about an inch.
- A light wand with a LED light source is then passed into the drilled hole of the speculum until it seats near he far end of the tube. When the light is turned on, it causes most of the speculum to "glow, and throws light on the external cervical os.
- While watching through the speculum, operator should pass the pipette into the opening of the cervix. Once inside, Carefully manipulate the pipette so that it passes forward through at least two cervical rings.
- Once pipette is in place, depress plunger to eject the semen.
- Remove pipette and speculum from doe.
- Check straw / pipette to ensure that the semen was properly ejected, and did not run "retrograde" back up the pipette barrel.
- Record who was inseminated with what!

Methods for Determining Pregnancy after Insemination

Once a synchronization / artificial insemination is completed, producers will often introduce a buck for natural "Clean-Up" breeding. To differentiate between the artificially and naturally produced kids, a waiting period of at least two weeks is recommended prior to buck introduction.

Knowing when a doe is due dramatically increases your preparedness during kidding season, so pregnancy checks are a good investment.

Two approaches to testing follow:

1. **Use Ultrasound to identify pregnancy-related structures in the abdomen of does.**

   Requires a veterinarian.

   Accurate as early as four weeks after insemination, but it may be more practical to "Preg-Check" about 6 weeks after the end of a synchronized round of inseminations.

   Advantages – Veterinarians can often determine the approximate kidding date from an ultrasound examination, and can identify individual kids. This allows you to plan for singles, twins or triplets.

2. **Chemical detection of pregnancy-associated proteins in blood / milk from does.**

   Accurate as early as four weeks after insemination

   Dairy goat breeders can submit "strip" milk samples to their milk testing lab.

   No information on number of kids in a pregnancy.

   Dairy One Cooperative in NY sells kits for taking and sending milk or blood samples for pregnancy detection. Kits can be ordered from their website:

   https://dairyone.com/product-category/kits/animal-health-diagnostic/.

   Detailed information on Dairy One pregnancy tests is available here:

   https://dairyone.com/services/other-laboratory-services/animal-health-diagnostic-laboratory/