

Advanced Caprine Reproduction Methods & Techniques
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Unit Objective

After completion of this module of instruction the producer should be able to apply reproductive terminology while communicating with reproduction specialists. The producer should be able to identify signs of estrus and factors that can stimulate a doe to come into estrus. The producer should be able to evaluate the advances of reproduction technology and select and use the ones that are appropriate for their goat production program. The producer should be able to score a minimum of 85% on the module test.

Specific Objectives

After completion of this instructional module the producer should be able to:

1. Match reproductive terms with the correct definition.
2. Identify methods a producer may use to bring a doe into estrus.
3. Select the months of the year when the buck's semen is of optimum quality.
4. Explain how bucks semen is evaluated.
5. Identify the volume (in cc) of semen contained in a straw of semen.
6. Select items that may be imprinted on a straw of buck semen when processed.
7. Identify products that are added to semen during processing.
8. Identify the temperature of the liquid nitrogen bath in which semen is stored.
9. Identify the percentage of semen viability loss during the frozen process.
10. Select the appropriate water bath temperature for thawing semen.
11. State the meaning of seasonally polyestrous.
12. Identify factors that affect a doe's sensory stimulation.
13. Identify how often a doe will cycle during estrus.
14. Select the number of hours a doe will remain in estrus.
15. Identify correct statements regarding light therapy for inducing off-season estrus.
16. Identify correct statements regarding progestagen treatments.
17. Identify correct statements regarding using prostaglandin treatments.
18. Identify correct statements regarding identifying estrus in the doe for purposes of artificial insemination.
19. Identify the three primary factors that make a successful artificial insemination program.
20. Distinguish between cervical versus trans-cervical artificial insemination.
21. Identify correct statements regarding proper semen deposition in the doe's reproductive tract.
22. Match artificial insemination equipment with a description and/or use of the equipment.
23. Distinguish between correct and incorrect statements regarding laparoscopic/intra-uterine artificial insemination.
24. Distinguish between correct and incorrect statements related to liquid nitrogen storage tanks.

25. Distinguish between correct and incorrect statements related to super ovulation and embryo transfer.
26. Distinguish between correct and incorrect statements related to embryo flushing and embryo transfer.
27. Identify ways/means of determining if a doe is pregnant.
28. Evaluate advancements in reproduction technology and select the advancement for use within the individual goat farm.

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Introduction

This module is designed for the producer who is well familiar with basic caprine anatomy and reproductive processes of both the male and female goat. It is suggested that before continuing, the reader complete the “Goat Reproduction” module by Stephan Wildeus.

Assisted animal reproductive methods provide the producer with a variety of avenues to increase the rate of genetic improvement within the herd and to maximize the number of progeny from selected donors. These methods enable the use of genetics otherwise limited by geographic location or untimely death of the desired donor. Increased use and advancing technical expertise are making assisted animal reproduction less cost prohibitive for the caprine producer. Most common methods used are cryogenic preservation of semen, artificial insemination (AI) and embryo transfer (ET).

Semen Collection and Cryogenic Preservation

The use of fresh cooled and cryogenically frozen semen in AI and ET programs is widely used in the caprine industry by the showman, hobbyist and commercial producer. Most buck semen collection occurs on location, either at the producer’s farm or a nearby fellow producer’s location. Some bucks are transported long distances to a buck stud facility where they are housed, managed, fed, and groomed for the sole purpose of improving the quality and quantity of semen produced. Traditionally, semen collected under this type of management offers the greatest viability of any product used by an AI technician. Unfortunately, the amount of semen collected under this type of management is limited due to lack of facilities that offer this type of service.

The quality of semen used in both processed fresh cooled and cryogenically frozen form is largely dependent on the overall management of the buck prior to collection. Semen viability can be greatly influenced by how frequently and recently the buck has been used for service. To increase the likelihood of obtaining semen of optimum viability for preservation, the producer should provide ideal conditions in overall management of the buck for months prior to the actual day of semen harvest.

Care of the buck

The serious producer should prepare his or her animals well in advance of the date of collection. Optimum quality in both viability and morphology of cells produced is at its highest during the cooler fall and early winter months when the buck is in the prime of “rut,” while actively and routinely servicing does. Bucks should be heavily servicing does up to five days prior to their scheduled collection date and then allowed to rest. This ensures that healthiest semen is stored and waiting in the caudal (tail) portion of the epididymis in sufficient quantities for a profitable collection. However, it is important that a producer not house the buck with a herd of does. Whether or not the does have exhibited evidence of estrous (heat) during this time is irrelevant. Bucks that have continual exposure to does may be unwilling to offer service the day of collection. When allowed to service does, bucks scheduled for an upcoming collection should be

controlled on a lead rope by the producer during copulation. This helps to build confidence in the buck and prepares him for the process of collection.

Nutrition is another factor that should be taken into consideration. Breeding bucks should have a satisfactory body condition score and be put on an improved plane of nutrition as the breeding season approaches. Breeding activities and the condition of rut require tremendous amounts of energy. Bucks that are too lean often cannot produce semen of optimum quality or quantity. Overweight bucks are often lazy and less virile. Continuous, year-round, daily access to adequate volumes of water, salt, and trace minerals are vital to the buck's fertility. These minerals are of prime importance to the donor's production of semen with healthy motility and a high sperm count. If the producer is feeding hay from a selenium deficient area, a veterinarian should be consulted to assist in the proper method of boosting the buck's selenium intake at least ten weeks prior to his collection. Daily supplementation or Bo-Se injections are the most common methods chosen by producers.

Semen collection and evaluation

The collection of buck semen is accomplished, most commonly, by the use of an artificial vagina (AV). To assist in the buck's collection, a "teaser doe" (doe showing evidence of estrus) is restrained so that the donor buck becomes aroused and can mount her. Once he mounts the teaser doe, an AV is placed over the penis as it is extended. The stimulation provided by the AV's warm water bladder, lubrication, and pressure produce an ejaculation.

Collection using a teaser doe



Use of an artificial vagina

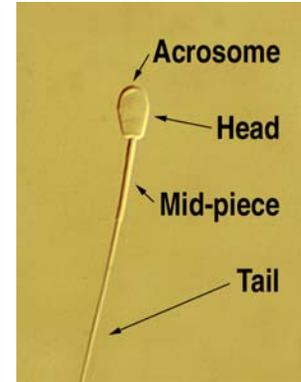


On occasion, if a buck should refuse to mount or ejaculate into the AV, it may be necessary to harvest his semen by way of electroejaculation. However, this is not the preferred method employed by most caprine semen processors. If not performed with proper technique, expertise, and the use of quality equipment, an ill-performed procedure can be extremely painful for the donor buck and the results of such methods can be less than desirable.

Once the semen sample is harvested, it is immediately and thoroughly evaluated for spermatazoa viability (live vs dead), motility (movement), and morphology (normal vs abnormal cell

structure). Any abnormality is categorized as a primary, secondary, or tertiary abnormality. An overall assessment is then made as to the spermatozoa's predicted ability to fertilize.

Additionally, the sample is scrutinized for the presence of excessive quantities of bacteria, interfering microorganisms, and leukocytes (white blood cells). Such latter findings could indicate possible infection or disease. The presence of excessive microorganisms and/or white blood cells within the sample should eliminate it from further processing, thereby avoiding any possibility of infection or disease transfer from the donor to the intended recipient.



Freezing

Once these evaluations are completed, the sample is analyzed for sperm concentration so that a proper dilution of cells can be made for use in the straw. Processors in the United States most commonly use a 0.5 cc straw for packaging, although 0.25 cc straws are more common in the international marketplace. Properly marked straws indicate the donor buck's permanent identification, i.e., donor name, registration number, processing company identification, date of collection, and the animal index number assigned by the individual processing company.

The semen is then subjected to the dilution process using one of several possible mediums. These mediums traditionally consist of milk or egg yolk, sugars, antibiotics, and buffers to provide a stable environment for the semen, particularly during the transport of the fresh cooled semen. Mediums used for the purpose of protecting cells intended to be frozen generally have glycerol added as a cryoprotectant. To further complicate matters, buck seminal plasma contains an enzyme which can cause coagulation when introduced to an egg-based extender. To overcome this potential problem, semen may be "washed" via centrifugation to separate the seminal plasma from the spermatozoa before further processing, egg yolk levels in the extender may be quantitatively reduced, or in some cases a diluent that utilizes skimmed milk as opposed to egg yolk may be used.

The number of spermatozoa used per dose varies according to the intended use of the straw. It is important for producers to be aware that frozen semen straws packaged for use in laparoscopic or intra-uterine AI can consist of as few as 20 million or less cells whereas straws intended for fresh vaginal AI can contain as many as 300 million spermatozoa. The recommended and US industry standard for spermatozoa packaged for frozen storage and cervical or trans-cervical AI mandates 120 million live cells in a 0.5 cc straw, pre-freeze.

Once properly diluted and packaged in straws, the cells are further subjected to a liquid nitrogen vapor and eventually submerged into a liquid nitrogen bath at -320°F (-196°C). The entire cryogenic freezing process, including preparation and collection, can take as long as 18-20 hours to produce a quality product.

Thawing and post-thaw evaluation

Proper thawing technique of frozen semen is critical to the resulting post-thaw motility assessment and to the spermatozoa's ultimate fertilization capabilities. Since correct protocols for semen thawing are determined by the methods used in the freezing process, the producer should consult with the processor for suggested instruction on their prescribed thaw method. In the unfortunate circumstance that the processor is unavailable, a 93-95 ° F (35° C) water bath is widely used by many AI technicians with good success.

It is strongly recommended that each AI technician invest in an inexpensive, good quality microscope capable of magnifying semen cells to such a degree as to ascertain their viability (live vs dead). Depending on the quality of microscope, a minimum magnification of 100x is necessary for even the most basic observations. More experienced technicians should also assess cell motility (the way the spermatozoa move/swim) and morphology (normal or abnormal cell appearance). Technicians and producers should understand that no semen sample is entirely void of abnormalities and that freezing semen results in a 5 to 20% loss of viability due to the freezing process.

Needless to say, a processor's scrupulous practice of sterilization and bio-security, as well as proper semen identification, is of primary importance to the producer and purchaser of any resulting semen. Bacterial contamination can be a primary factor in low conception rates and reproductive problems. In some cases, reduction of sperm motility, acrosome integrity, and sperm cell viability can all be directly related to bacterial contamination that occurred during the processing of semen for fresh or cryogenic storage. The producer is cautioned in their selection of semen purchased for his or her use. Frozen semen production is not a regulated practice in the United States. Any level of bio-security and/or quality assurance is at the sole discretion of the processor. Any judgment made in regard to semen quality is a personal opinion made by those who have handled and observed the semen. Although more common and readily available, on-farm collection can sometimes result in the processing of sub-quality semen. This can then result in a lower quality frozen product available for the producer's use.

Lists of [mobile semen processors](#) equipped for on-site farm collection and processing, [limited area processors](#) that conduct on-farm collection, and [USDA-approved isolation facilities](#) and collection centers can be found at the end of this module. (*Mention of any business or product name does not imply endorsement of those companies or products or non-endorsement of similar companies or products. Lists are intended to be a starting point for producers in their search for desired services or products.*)

Postmortem semen extraction

In the event of expected or unexpected death of a buck, cells in the body can remain viable for several hours. If properly handled and prepared for shipment, the producer can often have semen extracted, postmortem, from the animal's properly harvested testicles. The most successful recoveries are made from buck's whose cause of death was not related to a chronic and/or debilitating disease, nor from an animal that had been routinely receiving steroids.

During spermatogenesis, spermatozoa produced in the testes are ultimately transported through the epididymis where they continue to mature, develop motility and, at least in part, their ability to fertilize oocytes. It is in the caudal portion or tail of the epididymis that immature spermatozoa are stored until the time of ejaculation. Cells found in this storehouse can be recovered, processed, and cryogenically frozen for later use in AI.

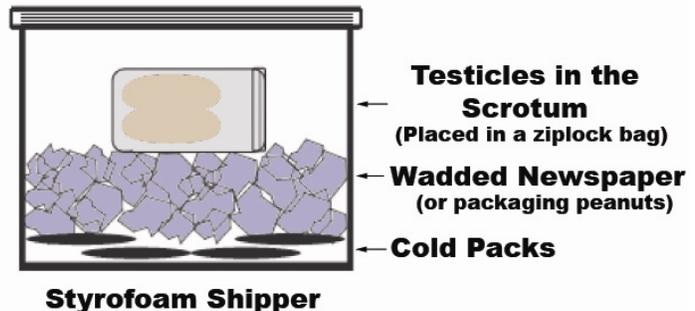
To harvest the testicles for semen extraction purposes, the following protocol has been used widely in the field with good success:

1. Leave the testicles inside the scrotum whenever possible.
2. Tie off as much of the ductus deferens as possible.
3. Once the ductus deferens are securely tied, cut the testicles above the tie, and remove them from the body.
4. The testicles should be allowed to cool slowly, if cooled too quickly the cells could be severely damaged.
5. The scrotum, with the testicles inside, should be placed inside a sealed plastic bag.
6. Prepare a Styrofoam or other cooler type container by first placing a layer of ice packs, on the bottom.
7. A thick layer of wadded newspaper, Styrofoam peanuts, bubble wrap or other such material should then be placed over the ice packs.
8. Place the sealed bag containing the scrotum and testicles in the cooler and tape it closed. Refrain from the temptation to prepare the cooling box prior to its immediate use. The time it takes to cool the cooling box containing the testicles also allows the testicles to cool slowly, preventing potential spermatozoa damage.
9. Place the cooler inside a cardboard box and prepare it for delivery.
10. For semen extraction, delivery should be made to the laboratory as soon as possible, either the same day or by overnight delivery.

Where to tie off the testicles for post-mortem semen extraction



How to package the scrotum containing the testicles



A list of [companies offering postmortem semen extraction](#) services is found at the end of the module.

Bringing a Doe into Estrus

The goat is recognized as a “short-day breeder” and in the United States is usually “seasonally polyestrous.” Meaning, she shows evidence of the desire to breed when day length grows shorter; in the late summer, fall, and winter months. She thereby conceives during the time of year when the ambient temperature is most moderate. She then kids or freshens (gives birth) in the spring when nutritional conditions are optimal for neonatal growth, lactation, and browsing as the kids are weaned. The doe will then exhibit a state of anestrous until the end of summer or early fall when she will again show signs of estrus activity. This transition from estrous to anestrous, in seasonal breeders like the goat, occurs on an annual basis. Some factors affecting this transition on individual farms and in individual animals can be as elementary as the number of bucks within a doe’s view, the farm’s physical location with regard to the equator, and even weather.

The initiation of behavioral estrus in the doe is triggered by sensory stimulation, i.e., what information her brain receives with regard to her surroundings. One or more of her senses receive stimulus, the most likely being:

What she sees:	A change in daylight hours (photoperiod).
What she hears:	The exuberant vocal expressions of the buck.
What she smells:	The buck’s odor when in rut.

Sensory stimulus traditionally aids in the kick-start of hormonal activity in the doe. Such activity eventually results in bringing her from a state of seasonal anestrous (not in heat) to estrus (in heat) on a “regular” 18-21 day cycle. This is often preceded by a silent ovulation. This silent ovulation acts to “prime” the brain through a rise in progesterone levels that increases sensitivity to estrogen in the blood stream. This can result in a strong expression of behavioral estrus.

To help determine the “regular” cycle for each AI candidate, the producer should maintain a “breeding diary” of sorts. Good records are a key element and are of primary importance in any breeding program, especially one involving assisted reproduction by artificial means. If the producer is diligent in documenting any changes in the doe’s behavior, a pattern will soon emerge. Details should be kept regarding not only the first signs of estrus, but also the last signs observed as her estrous cycle comes to an end. Over time, the producer can make a fairly accurate determination as to how long the doe can be anticipated to remain in not just a standing heat, but the entire length of her estrous cycle. The producer should be reminded that does remain in estrus for approximately 30 hours; although this can vary from breed to breed. Many producers have witnessed that some Saanen does exhibit estrus for as briefly as 18 hours, whereas it is not uncommon for the Nubian doe exhibit estrus signs for days on end. Each breed will often be different, as can each doe within the breed. For this reason, all observations made by the producer are worthy of note. The only thing that can be relied on, at least to some degree, is that whatever length of estrus is “regular” for an individual doe will likely be repeated year after year.

One should also not fall into the poor habit of making such observations only at feeding or milking time. The producer should make a point of observing the herd’s behavior twice daily for a 15-20 minute period. This practice should be done when the herd is unaware of the producer’s

presence. The goal is to witness what the herd is doing when the distractions of food and equipment are not interfering with their “normal” behaviors. Is the doe to be inseminated standing at the fence, as near to the bucks as she can get? Is she flagging or hollering for no good reason? Is she mounting her pen mates or allowing them to mount her? These behaviors are strong indicators that she is in some stage of her estrous cycle and should be noted.

The time in which the doe will stand for a pen mate or buck to mount her is commonly referred to as a time of “standing heat” and is the focus of most producers’ attentions. However, for the purposes of AI, inseminating the doe based on this observation alone would be premature and unlikely to offer even average rates of conception.

Light therapy for inducing off-season estrus

Light therapy is a very cost effective, common practice that has proven useful for commercial goat dairies as a means of bringing a large number of does into estrus. This protocol is often used when the desired result is to facilitate off-season freshening for a year round milk supply. Therapy is accomplished by transitioning the doe’s photoperiod in a controlled environment. Does are housed in a building with adequate ventilation and regular cleaning to maintain good health. However, the design of the building allows no outside light to penetrate the interior. Exposure to sunlight can either be partially or totally eliminated by the herdsman. The producer may choose to release the does to enjoy outside, natural light for a controlled period of time, or he may choose to control the photo stimulus by simply turning on and off the interior lighting. The same results can be accomplished using either regime.

The theory is to mimic the decreasing daylight hours that naturally occurs during the fall and winter months. When beginning the program, does could be exposed to perhaps 20 hours of light per day. Over a succession of weeks the hours of light exposure are gradually decreased until an estrous response is induced and breeding can begin.

Therapy should begin between the months of December through February depending on the desired date of freshening. For optimum response to the therapy, an ideal completion date of extended photoperiods should be March 1. The following protocol is suitable for employing light therapy based on current research and field experience:

1. Photo stimulation should be provided using incandescent light intensity equal to 12-15 foot candles at the animal’s eye level for 18-22 hours per day over a 45-60 day period. This light intensity can be achieved by using bulbs giving 400 watts of incandescent light for each 12 x 12 square foot block of the barn. This provision of light should bring about an anestrous period in the subjected herd of does.
2. Following the 45-60 day period of light stimulus, the does should be exposed to decreasing length of the daily photoperiods. If done correctly, this will mimic the onset of fall and winter daylight hours. Six to eight weeks following the termination of the extended light period, the does should be exposed to bucks in rut. Once the bucks are introduced, fertile estrus can be expected 10-20 days later.

If natural service is the desired means of mating, it can be quite beneficial to subject the donor bucks to the same light therapy protocol. This will stimulate the bucks to begin their own breeding cycle, evidenced by their condition of rut.

Utilizing the buck effect for the induction of estrous

Utilizing the buck effect is a very simple and cost effective means of inducing estrous in the doe earlier than would naturally occur. It is, however, not 100% reliable in its results and is not nearly as effective as light therapy in initiating off-season estrous. This protocol is more useful when the goal is to bring one or several does out of seasonal anestrous and into estrous as many as several weeks earlier than would normally be expected. This can be accomplished with reasonable success by first removing buck(s) of any age from the doe's line of sight and sense of smell for an extended period of time, perhaps as long as several months. Three to seven days prior to the time of desired estrus in the does, a buck(s) should be brought within immediate fence line contact. A young, virile buck, in his prime, will facilitate sensory stimulation by his odor, behavior, and vocal expressions. Within a few days behavioral estrus should begin to be seen in many, if not all, of the exposed does.



Progestagen treatments

Progestagen treatment is commonly accomplished by using some type of implant designed for the controlled breeding of goats. Progesterone implants are the most commonly used device and fool the doe's system into thinking it is pregnant, thereby preventing hormonal activity that would induce a state of estrous. Once removed, the decrease in progesterone in the doe's system stimulates the production of a variety of hormones and behavioral estrus can be expected within 24-36 hours. Although not yet approved for use with goats in the United States, progesterone implants are commonly used in many countries and come in a variety of forms.

1. Previously, many producers utilized an implant labeled for cattle, known as Syncro-Mate B™, with good success. Although at the time of this writing Syncro-Mate B™ is not currently available, its future use is still worthy of consideration in the event the implant again becomes available.

This norgestomet implant resembles a small pellet and is "injected" using an applicator made specifically for this purpose. The implant is often cut in half to provide a 3 mg dose (half the bovine dose), and is deposited just under the surface of the loose skin found at the side of the tail web of the goat. Though less recommended, it may also be implanted at the base of the ear as is done with cattle. An experienced technician should be employed for both the insertion and the removal of the cylindrical shaped implant. For easy removal it is important that the implant be deposited just under the first layers of tissue, and not deep in the fat that may be present in the tail. If not deposited properly, the implant can migrate deeper into the fatty tissues making removal difficult. Prior to the implant's removal, a small injection of anesthetic just under the skin is necessary to deaden pain in the surrounding tissue. The implant can then be easily and painlessly

removed by way of a small incision made with a scalpel at one end of the implant. Using a forcep or tweezer the implant should be extracted, and once removed, an antibiotic ointment applied to the small incision.

2. A progesterone-containing vaginal sponge or pessary, such as Veramix®, inserted into the vaginal cavity is a very easy means for the goat producer to maintain progesterone levels in the doe. The vaginal sponge is a porous sponge-like material that is comfortably retained by the vagina until its time of scheduled removal. The “sponge” can be properly inserted deep into the doe’s vaginal cavity using a well lubricated, large sized speculum or oversized syringe that has been cut off and sanded to smoothness. For removal, depending on the manufacturer, a fine piece of “fish line” type material or string is attached to the sponge and protrudes from the doe’s vulva while implanted. The sponge is removed by pulling gently on the string.

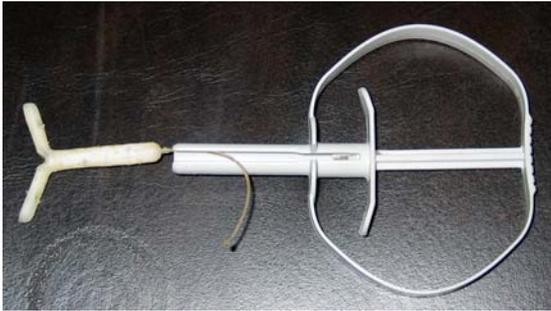
One disadvantage of the vaginal sponge is the porous material of which it is constructed. Although the texture affords good comfort for the doe, it also acts as a host to bacteria and trap to other debris that may enter the vaginal cavity. Bacterial growth can occur that may cause a potential vaginal or uterine infection. In some cases, fetal abnormalities have been reported when vaginal sponges have been used.

3. Controlled Intravaginal Drug Release (CIDR) devices are another progesterone delivering, intravaginal vehicle and a favorite method for most ET programs. Unlike the porous material of the sponge, the CIDR is made of an inert silicone elastomer that is non-porous and does not readily absorb bodily fluids. The producer is cautioned to only use CIDR’s designed and sized for use in goats, not bovines. Depending on the manufacturer, the CIDR made for use in goats delivers 0.3 g of progesterone; a CIDR for use in bovines delivers a substantially greater level and is physically much too large for the goat’s small vaginal cavity. Once inserted, a cattle size CIDR will readily and almost immediately be expunged by the doe. Nor can such bovine devices be carved or cut down to accommodate the smaller size of the doe’s vagina. First, because there is no absolute means to determine the amount of progesterone being delivered by the remaining “piece” of the device, and second, because the resulting rough edges would grossly irritate the interior of the vagina and lay potential for open sores in the vaginal walls.

A CIDR designed for goat use, when used with full-sized goats and according to manufacturer’s instruction, can be used both safely and effectively with no vaginal trauma or other negative results. The CIDR has been found to be the most cost effective device providing ease of use for the producer. Like the sponge, it can be inserted using a cut off and sanded syringe with lubricant, a large sized speculum or, although slightly more costly, an applicator designed specifically for this use can be purchased. Specialized caprine reproduction services can assist you in locating such an applicator. Once properly inserted deep into the doe’s vagina, the CIDR unfolds into a “T” like formation that aids in retention. Be aware that it is not uncommon for pen mates to grasp the clear plastic line that protrudes from the doe’s vulva and remove the device. Some producer’s have found that cutting off the bulbous tip at the end of the line, helps to avoid

pen mates taking notice of the device's existence in the doe. Daily monitoring of the device is advisable to confirm that it has not been inadvertently removed. Some producers running large herds choose to color the clear plastic line with a brightly colored paint or enamel. Although this may cause more notice by pen mates, it enables easy monitoring of the device during routine feeding and as a daily management protocol.

CIDR and applicator



Loaded applicator



Whatever form of implant selected by the producer, it is best to follow the manufacturer's labeled instruction on its proper use and application. No device of any kind should be considered for re-use. All implants are designed by their manufacturer and labeled for single use. Some manufacturer's recommend leaving the device inserted for as long as 18-21 days. However, producers have found in goats 9-14 days are sufficient to induce the desired result. Once the implant is removed, a majority of does will be in estrus within 24-48 hours. However, insemination should not occur until proper timing is achieved for the technique the producer wishes to use. It should also be noted that intravaginal progesterone delivering devices could lend some additional "color" to vaginal mucus. This can be deceiving to an inexperienced technician unfamiliar with working with such a device and who is using mucus color and consistency to gauge a doe's stage of estrus.

For AI with frozen semen, some manufacturers recommend a 200-400 iu dose of PMSG be given up to 48 hours prior to the device's removal. Some manufacturers further recommend that insemination using a laparoscope or cervical technique be performed within 48 hours of the device's removal.

In field trials, producers have gained the highest rate of success when practicing the following protocol involving a progesterone device that delivers 0.3 g of Progesterone.

1. Day one; insert progesterone implant.
2. Day thirteen; 2 cc of prostaglandin administered intramuscular (IM) with dosage dependent on product label and doe body weight.
3. Day fourteen; 1.5 cc dose of PG600 administered IM and device removed.
4. Day fifteen; doe shows sign of estrus.
5. Day sixteen; doe is carefully monitored and inseminated.

Protocols for the same implants may differ when intended for use in an ET program rather than simple insemination methods. Some commercial ET services offer a regimen which involves

the repeated daily use of FSH for a period of four days, accompanied by a single dose of prostaglandin for the donor doe, and a single dose of PMSG for the recipient does. Some ET technicians report that superior results are obtained if the implants are removed from the recipients 12 hours before they are removed from the donor does. The producer is advised to consult with the intended ET service provider for their preferred methods of preparation of any animal intended for use in an ET program. Each ET technician's own techniques and schedules, often based on personal experience, may affect their individual requirements.

MGA feed supplement

Sheep producers and researchers are currently involved in field trials and controlled studies that have returned favorable preliminary results using a progesterone feed supplement, melengestrol acetate (MGA). This supplement is currently used with reported success in cattle and horses. However, at the time of this writing, adequate data to suggest a reliable protocol for goats has not yet been established. Such a protocol is on the horizon and MGA feed supplementation, as a means for herd estrous synchronization, could be of value to the producer in the near future.

As with any feed supplement, constant monitoring of each animal's intake is necessary and can prove labor intensive for the large producer. Certainly electronic and mechanized apparatuses can assist in feeding and intake regulation and are being used by some large producers. In group-fed animals given unrestricted feed access, dominant animals will invariably disrupt each animal from ingesting a proper dose. Individual feeding or other methods of solving this problem may prove impractical for the average producer. However, with proper management and equipment design, a feeding program utilizing a progesterone feed supplement is worthy of consideration.

Prostaglandin treatment

Although not affording the same reliability and consistency of results as progestagen therapies, prostaglandin injections have also proven a cost effective means of producing a synchronized heat for the producer. It is good to note that protocols as labeled and described for cattle use have proven unreliable for goats when using some forms of this product. There are a large variety of opinions for off-label use of this veterinarian prescribed, controlled product in goats. The dosage prescribed seems to range from 0.5 cc to 3 cc IM, depending upon purpose of use, product label, and intended technique and protocol. The length of time in which the producer can expect an apparent estrus response, if indeed one is even achieved, also varies considerably according to dosage administered, breed of goat, geographic location of the animal, and time of year. Time of year is important to the producer because prostaglandin is only effective if a corpus luteum is present on the doe's ovary. If no corpus luteum exists, the prostaglandin injection is useless in stimulating estrus. Another discouraging result of prostaglandin use can be a showing of behavioral estrus, but no ultimate ovulation. This may be due to a variety of reasons including the lack of sufficient LH in the doe's system to elicit such a response.

In field trials by producers across the United States the most favorable results have occurred with the following protocol:

- a. Day one; 2-3 cc of prostaglandin administered IM according to the product label and doe's body weight (2 cc for does under 100 lbs and 3 cc for does over 100 lbs).
- b. Day eleven, hour one; 2-3 cc of prostaglandin administered IM according to the product label and doe's body weight (2 cc for does under 100 lbs and 3 cc for does over 100 lbs).
- c. Hour 48-52; doe shows signs of estrus.
- d. Hour 48-72; doe is carefully monitored for stage of estrus.
- e. When evidence that proper stage of estrus is observed, doe is inseminated.

It is good for the producer to recognize that prostaglandin in any dosage could initiate a fetal abortion in a pregnant animal. Great care and caution should be taken in the exposure of such a product to animals at any stage of gestation if a termination of pregnancy is not the desired result.

Estrus in the Doe for Purposes of Artificial Insemination

The assessment of estrus in the doe is a key factor in the successful outcome of any form of insemination. Times of "standing heat" are targeted in the case of natural service. When utilizing fresh cooled semen, the earliest sign of estrus triggers the producer's response to immediately order its overnight shipment. However, when frozen semen is used for AI, your window of time offering the greatest degree of success is far more limited.

As most of us know, does come into estrus on average every 18-21 days. To be a successful AI technician, careful attention must be paid to the AI candidate and her telltale signs of estrus. Traversing the cervix is the most difficult step in the AI procedure. Timing, coupled with the overall relaxed state of the doe and technician, can play a major role in the ability to slide past one or more of the cervical rings to allow proper deposition of the semen.

Most does exhibit easily recognized signs of estrus such as tail flagging, restlessness and head butting, mounting or allowing pen mates to mount, frequent urination in the presence of a buck, increased vocalization, and vaginal swelling and mucus discharge. These signs, most commonly associated with goats in heat, lack precision for determining the doe's exact stage of estrus. Proper determination of the stage of estrus for AI purposes is done by examining the mucus located in the anterior portion of the vaginal canal. A speculum and light source are the best tools for this purpose. When upon careful examination, the doe's internal mucus appears opaque showing evidence of good elasticity with a viscous consistency and form, the producer should begin preparations to perform the procedure. Mucus found to be clear, thin, and very liquid in its consistency is indicative of a doe far too early in estrus for a properly timed insemination. Mucus found to be white or pale yellow in color and thick, lacking elasticity, would indicate a doe past her proper time.



Many factors come into play to be successful at AI. Correct timing is key for semen deposition to allow the deposited spermatozoa to undergo “capacitation” and the oocyte to mature. These events must be timed properly for fertilization to occur.

During natural service by a buck, fresh semen is deposited in the fornix portion of the doe’s vagina, an area more commonly referred to as the “over-shoot.” The semen then travels to the opening of the cervical canal, known as the “os,” where it begins its journey to the uterus and oviducts. With the use of assisted animal reproduction, other methods to deposit of semen are used.

Artificial Insemination (AI)

Artificial insemination is a learned technique that, when performed with skill properly founded on knowledge, offers ease of use and a good level of success. However, results can be discouragingly poor for a technician lacking knowledge and the necessary attention to details needed for a successful outcome. Producers wishing to perform AI should seek out and attend a class instructing them on proper caprine insemination technique. A clinic offered by a reputable and knowledgeable company, organization, or university specializing in caprine artificial insemination should be attended. Classes that offer hands-on training with live animals, accompanied by in-depth lecture, will offer the maximum benefit for the beginning AI technician. Taking classes focusing on other species, although using similar techniques, often lack the details that determine future degrees of success when working specifically with goats.

A list of some [companies specializing in caprine AI that offer on-location class instruction](#) is at the end of this module.

Because AI techniques and methods are highly dependent on the variety of equipment employed, it is difficult to give a safe, step-by-step instruction to the producer without hands-on activity. However, for the purposes of this module a basic narrative is given on the procedures involved.

It is good to keep in mind that the success of any AI program is largely dependent on three primary factors:

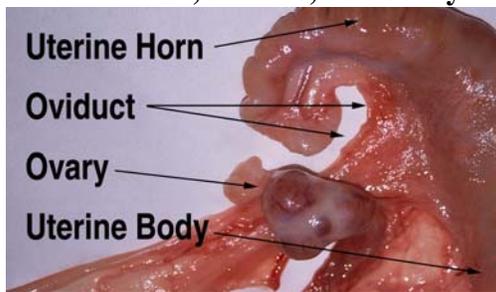
1. The use of live/viable fresh cooled or frozen semen.
2. The appropriate timing of insemination in relation to estrus and ovulation.
3. The proper deposition of semen in the doe.

Not every doe is a good AI candidate. Does who do not cycle normally every 17-24 days with regularity or who are difficult to determine when and if they are in estrus should be lesser candidates in a producer’s AI program.

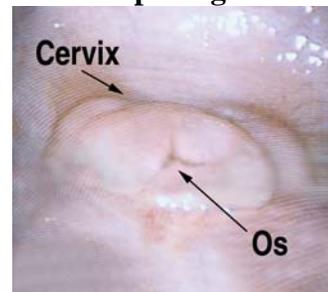
A basic understanding of a doe’s reproductive anatomy is essential before beginning any attempt at AI. Externally, the doe’s anus and vulvar region are easily located. The vulva is the door to the vaginal cavity and consists of two vertical lips, or labia, located just below the anus. The vagina is a smooth-walled, soft, collapsed cavity. It acts as the connecting tunnel between the vulva and the cervix. A lubricated, narrow, and comfortably designed speculum can be used to

open the smooth, muscular walls of the vagina. If inserted properly, following the slope of the doe's rump, the speculum will naturally pass with ease into the fornix portion of the vagina. This area is where the buck would normally deposit semen during live copulation (natural service). Below this area and at the posterior end of the vaginal cavity, the os can be located with the use of a bright light. The light source selected by the technician is often a primary element in the success or failure of an AI attempt. The best lights are bright and ideally generate little to no heat. If the semen straw is exposed to any heat, cells in the vicinity of the heat source can be severely compromised. The light source should also attach securely to the vaginal speculum and be unobtrusive in design due to the limited viewing area. The os is the opening to the cervix and is the ultimate target for the AI gun's entrance to the cervical rings. The cervix itself acts as the gateway to the uterus and is approximately one and one-half inches long. The cervix contains five cervical rings or folds with the os being the first.

The uterus, oviduct, and ovary



The os is the opening to the cervix.



Cervical vs trans-cervical artificial insemination

Cervical and trans-cervical AI are cost-effective, convenient, and less invasive methods of AI than are laparoscopic or intra-uterine procedures. When using a cervical technique for AI, semen is ultimately deposited in the cervix of the goat. With trans-cervical AI the techniques are similar, but the semen is deposited directly into the uterus. Both techniques eliminate the need to penetrate the abdominal cavity for access, or near access, to the uterus as is necessary with laparoscopic or intra-uterine AI. This then lessens the opportunity for any resulting infection.

Unlike AI in larger species, the goat due to its small frame cannot accommodate rectal entry to assist in traversing the cervix. In larger species such as the bovine (cow), equine (horse), etc., an arm can be inserted rectally to assist in manipulating the cervix as it is penetrated with an artificial insemination gun. In the goat, a lubricated speculum (accompanied by a light source) is used to open the vaginal cavity and thereby expose the cervix and os. The os, as mentioned, is the gateway to the uterus and is where the gun is ultimately inserted to deposit the semen.



Proper semen deposition in the doe's reproductive tract

Knowing and determining the proper time to AI the doe is not only critical with regard to the condition of the spermatozoa and oocyte (egg) when they come in contact with one another, but also to facilitate proper placement of semen in the reproductive tract. Proper timing is necessary to be achieved to allow the artificial insemination gun to partially penetrate and traverse the cervix prior to semen deposition. Any deposit of semen in the vestibule, anterior or fornix of the vagina is premature and incorrect. Additionally, depositing semen directly into the uterus is viewed by some to be incorrect and more invasive than necessary for the successful outcome of this procedure. Entering the uterus increases the potential for introduction of foreign microorganisms which could lead to infection. For this reason using sterile, individually wrapped sheaths as opposed to "bulk packs" (although slightly more expensive) is strongly recommended. Conducting a clean, and as near sterile a procedure as possible should be every technician's habit.

A properly timed procedure should allow for relative ease in manipulating through the cervical rings. However, young or maiden does will prove markedly more difficult and are not advised for the beginning technician. Even well seasoned does, if stressed or made uncomfortable due to rough handling or poorly designed or ill-used equipment, can become so tense as to constrict the muscular canal of the cervix rendering its penetration past the os nearly, if not totally, impossible. It cannot be overstressed that AI should be performed with a slow, determined, but gentle approach with adequate time allowed to follow proper protocols.

Once the semen is properly deposited, it is believed that fresh semen can remain viable for over 12 hours in the doe's reproductive tract. Processed and frozen semen is compromised to some degree and can be expected to have a somewhat shorter time of viability. Ovulation occurs just before or shortly following the end of the doe's standing heat. It is also believed that the caprine oocyte will remain viable for approximately 12-24 hours post-ovulation. During natural service, this allows time for both the oocyte and the spermatozoa to fully mature. This maturation is needed to enable the zona pellucida, surrounding the ovum, to be penetrated by the spermatozoon.

Artificial insemination equipment and supplies

Some basic equipment is required for the producer to perform cervical and/or trans-cervical AI effectively. Ultimately the doe's comfort should be in the forefront of the producer's mind in the selection of tools to use.

Items can be purchased in the form of a kit with a substantial cost savings versus purchasing items individually. Kits differ in price between suppliers and in the quality of equipment they contain, and typically range from \$115.00 to as high as \$185.00. When comparing one company's offering to another, keep in mind you often get what you pay for. It is good to become familiar with what should be found in a "complete" kit prior to making a purchase.

Look for the following when reviewing the various kits available:

- *Carrying case*
 - A compact metal or plastic case for the safe and clean storage of AI equipment.
- *Kit warmer*
 - While contained within the carrying case, pre-warms all AI equipment prior to its use.
- *Artificial insemination (AI) gun*
 - A goat length (usually 12 inch) device used for the depositing of semen via a 0.25 or 0.5 cc straw; available in a variety of styles. Can be constructed for disposable use or constructed of metal for multiple applications.
- *AI gun sheaths*
 - Disposable, sterile, individually wrapped outer plastic shells which fit over the gun providing a secure “seat” for the straw. Each AI gun requires a specific style of sheath to accommodate the guns specific design. Although not recommended, sheaths are available in “bulk packs” of 20-25 pieces.
- *AI light*
 - A compact light source which should attach securely to the vaginal speculum. The most easily used light sources are independent of a battery pack, generate little to no heat, and are unobtrusive in design.
- *Vaginal speculum*
 - When used in conjunction with a light source, enables the clear view of the cervical “os.”
- *Speculum brush*
 - A soft brush, sized to provide thorough cleaning of the vaginal speculum.
- *Straw tweezers*
 - Used for the retrieval of straws from both the liquid nitrogen tank, cane, and ultimately the goblet as well as the semen thaw unit’s water bath. Available in both 0.25 and 0.5 cc sizes.
- *Straw cutter*
 - For proper seating in the AI gun sheath, this device delivers the critical square cut to the end of the semen straw.
- *Non-spermicidal, sterile lubricant*
 - Used for the lubrication of the vaginal speculum prior to its insertion.
- *Semen thaw unit*
 - A device designed for the proper control of the semen straw’s thawing process. The unit should be compact in design, providing optimal thermal protection, complete with a thermometer, and water and dry bath compartments.
- *Instruction booklet*
 - A simple guide providing the technician with basic, introductory knowledge of AI concepts and techniques.
- *Insemination reports*
 - Designed to assist in the maintenance of a “breeding diary” and the recording of pertinent data for each performed AI procedure.

Other items needed:

- *Fresh Cooled or Frozen Semen*
 - Packaged in 0.25 or 0.5 cc straws and may occasionally be provided in glass ampules.
- *Vaginal Swabs*
 - Used for the removal of excess mucus from the vaginal cavity.
- *Microscope*
 - Of mid-grade or better quality with a tungsten or halogen light source and capable of examining specimens at a minimum of 100x and 400x magnifications; used for basic thawed semen observations and analysis.
- *Microscope Slides*
 - The platform on which the thawed semen sample is dispensed for viewing with the microscope.
- *Microscope Cover Slips*
 - A small piece of plastic or glass used to cover the semen sample, allowing its proper viewing.
- *Liquid Nitrogen Storage Tank*
 - Available in a variety of sizes, storage capacities, and duration of hold times; an over-sized thermos of a sort, to be filled with liquid nitrogen, for the long term cryogenic storage of both semen and embryos.
- *Liquid Nitrogen Tank Measure Stick*
 - For the measuring and accurate monitoring of the volume of liquid nitrogen contained within the storage tank.

Laparoscopic/intra-uterine artificial insemination

The use of a laparoscope for the purpose of AI facilitates intra-uterine insemination. Because of its invasive nature, a licensed and trained veterinarian familiar with the technique and use of the laparoscope must perform this procedure. Despite its high cost, there are advantages of intra-uterine insemination versus cervical or trans-cervical AI methods; namely, above average rates of resulting fertilization and ultimate conception when proper technique, timing, equipment, and high quality semen are used. With this technique, the veterinarian is able to see the uterus through the abdominal cavity and deposits semen directly into the uterine lumen with an insemination pipette guided by the use of the laparoscope.

For 24-36 hours prior to the scheduled procedure, the doe must be denied food and water. The animal is then sedated and restrained in a ventral position, by use of a cradle, with the head pointed down. This positioning allows for the urinary bladder to fall away from the uterus. The doe's abdomen is surgically clipped and prepared for aseptic surgery. Local anesthetics are administered in two locales where small incisions will be made. In one incision, the laparoscope is inserted via a cannula to see the uterus. In the second incision, another cannula is used to insert an insemination pipette with a special needle attached to the end, or an insemination gun fitted with an injection tip or aseptic needle. Each uterine horn receives a specified volume of spermatozoa. Once the semen is deposited, all equipment is removed and the puncture sites are

stapled or sutured and dressed with an antibiotic ointment. The doe should then experience one to two hours of recovery in a quiet locale, undisturbed.

In vitro fertilization (IVF) and in vitro maturation (IVM)

Another form of fertilization can be accomplished with neither donor animal being present. This protocol involves the collection of spermatozoa from the buck and oocytes from the doe. Only recent achievements in goats have realized the fertilization of an oocyte in a culture dish housed in a controlled laboratory environment. Although new to the caprine industry, the use of in vitro fertilization has been explored with some success as an alternative to conventional cryogenic freezing of embryos. As practitioner techniques and levels of expertise become more accomplished, the use of fresh or frozen semen and oocytes for this procedure should produce regular, favorable, and successful results.

New technologies now afford some successful maturing of oocytes during both the breeding and non-breeding season of goats. Oocytes utilized in an IVF program are most often obtained from superovulated does via laparoscopic methods. The harvested, immature oocyte is washed in a Petri dish and incubated for over 24 hours in a tissue culture medium. Now an in vitro matured (IVM) oocyte, it is further incubated to prepare it for the fertilization process. Frozen semen is prepared for use by means of a process referred to as “sperm swim-up.” This process aids in the spermatozoa undergoing capacitation and in ultimate sperm selection. Once the spermatozoa and oocyte are properly prepared, they are incubated together for a 24-hour period during which time the spermatozoa set about the normal fertilization process. Resulting fertilized ova are then identified and closely monitored for their proper development over an extended period of time.

Liquid Nitrogen Storage Tanks

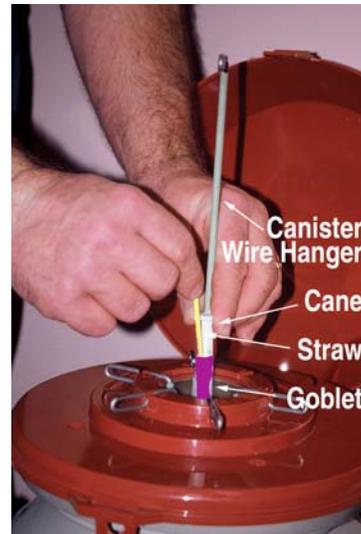
Cryogenic storage containers for semen and embryos come in a number of designs. The most popular are liquid nitrogen storage tanks, vapor shippers, and even dual-purpose tanks that can provide both storage and a means for the shipping of semen. The tanks are essentially a large vacuum container with insulation inside the vacuum chamber, much like an oversized thermos. The working parts are a lid, styrofoam cork, canisters, the inner chamber that holds the liquid nitrogen, and a “spider” to keep the canisters from moving around. The cork is generally 4-6 inches in length with grooves down the sides for the canister hangers. The cork is designed to fit loosely in the neck of the tank, allowing for the evaporating nitrogen gas to escape. If capped too tightly, the gas would build pressure in the tank and cause it to eventually explode.

Hanging from the top/neck of the tank are the canisters, usually six in number, although tanks may contain as few as one or as many as ten. These canisters consist of a long wire hanger/handle used to bring the cylindrical portion of the canister in and out of the tank. The function of the canister is to hold the canes of semen or embryos down in the tank for storage and retrieval when necessary.

Cutout view of a liquid nitrogen semen storage tank showing insulation and canisters

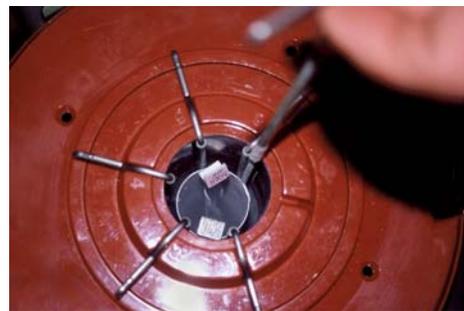


Parts of a semen storage tank



Liquid nitrogen is at a temperature of -320°F (-196°C) and is a hazardous material. Great caution should be practiced when handling a liquid nitrogen tank and its contents. It is good to get in the habit of wearing safety glasses for eye protection and clothing covering exposed body extremities such as legs, feet, arms, and hands when working in your nitrogen tank. Gloves made of any cloth like material should not be worn as they can absorb and trap liquid nitrogen causing a severe burn to the hands and fingers. Rather, gloves should be made of rubber, latex, vinyl, nitrile, or some other tight-fitting, non-absorbent protective material.

Cryogenic tanks come in a variety of configurations to meet the needs of the producer. Some have a long static hold and working time, generally sacrificing straw capacity; while others have large straw capacities, but sacrifice static hold and working time due to their increased neck size. This increased neck size is necessary to accommodate the larger canister size needed for the additional canes of inventory. A rule of thumb is the larger the neck opening, the easier it is to work in the tank, the more straws it can hold, and the faster the liquid nitrogen will evaporate thereby decreasing the static hold/working time between “charges” (liquid nitrogen refills) of the tank.



Things to take into consideration when purchasing a liquid nitrogen storage tank are straw capacity, initial price, liquid nitrogen availability in your area, static/hold time, and intended use of the tank. For example, a tank that holds 540 straws only has the capacity to hold 54 “canes” of inventory, with ten straws to the cane (an industry standard for 0.5 cc straws in the United States). Each cane holds two “goblets,” each goblet holds five 0.5 cc straws, totaling the ten straws per cane. If any of the canes has less than ten straws, capacity drops by the number of

straws each cane is short of ten. For instance, if one cane is holding only two straws, eight straws of the tank's total storage capacity is lost.

It also costs more to maintain four 540 straw capacity tanks than it does to maintain one 2,100 straw capacity tank. This is in addition to the higher initial cost involved in purchasing four smaller liquid nitrogen tanks as opposed to one larger tank. Good quality, new, liquid nitrogen storage tanks can vary in cost from about \$650 to upwards of \$1,000 depending on the make and model.

A person should check availability and cost of liquid nitrogen to assist in the decision of what model tank to purchase that best suits their use and maintenance budget.

A liquid nitrogen tank measuring stick should also be purchased. This is used to monitor the liquid nitrogen level in the tank on a regular basis to ensuring the tank's continuous viability and the safe storage of its contents.

Liquid nitrogen tanks are somewhat fragile and care should be taken in their handling. Close attention should be paid to the vacuum port. Do not spill nitrogen on it or loss of vacuum may occur rendering the tank useless. Tanks should not be set unprotected on gravel, dirt, or concrete. They should be stored unboxed, in plain view, on surfaces such as clean carpet, wood, cardboard, a rubber mat, etc., to protect the bottom from dents and scratches.

It is essential for the producer to recognize the importance of proper product handling, storage, and cataloging of inventory whether it be semen or embryos. The top of each cane should be marked, clearly identifying the cane's contents by the index code assigned to the donor animal by the processor. It is very poor practice to store more than one buck's semen or doe's embryos on a single cane. The producer should maintain a current catalog or "map" of the tank's contents and identifying index codes at all times. Any change in inventory within the tank should be noted in the catalog or on the map. Searching for and locating semen by exposing individual straws of inventory to the air, while attempting to read them, is the poorest of practices and will render your inventory ineffective and severely compromised in short order.

When re-caning (dividing or combining canes of semen), a producer or technician should be in the habit of pouring liquid nitrogen into a small, dense Styrofoam box known as a "transfer box," designed specifically for this purpose. The re-caning should be done in the box with straw tweezers while the goblets and the straws they contain are submersed in the liquid nitrogen. The most detrimental element to the long-term viability of straws frozen inside a storage tank is temperature change and fluctuation. If properly stored, frozen spermatozoa can be expected to remain viable for many, many years. In a perfect world, the frozen inventory would not leave the nitrogen until it is thawed for use. However, this is impractical for the producer's purposes, so the goal is to move straws as quickly as possible, i.e., the "three second rule."

When semen is being transferred or pulled for thawing and insemination use, the canister must be raised to facilitate the handling of the cane and eventual removal of the desired straw. Once removed above the neck of the tank, the cane and canister should be lowered back into the tank or placed in a transfer box within three seconds. This "three second rule" helps to avoid

excessive temperature fluctuation within the straws that may compromise spermatozoa viability. Noise and boiling heard in the tank when canisters are raised and lowered indicates a temperature change has taken place; the more violent the boiling the greater the temperature change and the greater the likelihood of viability loss.

When attempting to locate a cane in a specific canister, the canister should remain at the bottom of the tank. To see into the canister simply position it centrally so that an AI light or flashlight shining through the tank's neck will illuminate the tops of the canes. If properly positioned, there should be no need to raise the canister into the neck of the tank until you have identified the location of the cane where the desired straw is located. If the canister must be raised into the neck of the tank, remember the "three second rule." After lowering the canister back into the tank because the three seconds have expired, allow it to remain in the nitrogen for 10-30 seconds, depending on the nitrogen level in the tank. When pulling straws for insemination, attention should be on the inventory going back into the tank, not the straw you intend to thaw. It is better to pull the canister up three times for three seconds, than one time for nine seconds.

Vapor/dry shippers

Vapor shippers are sometimes referred to as "dry shippers." Their design provides safe transportation of frozen semen and embryos. Newer models are made of lightweight aluminum and most often have a single canister inside as opposed to the usual six in a "wet" storage model. These newer shipping units contain hydrophobic absorbent material which repels water but absorbs liquid nitrogen. Because of the absorbent nature of the material, should the unit tip during transit the liquid nitrogen will remain absorbed in the "sponge-like" material preventing any spillage. This unique design allows for an exempt status with normal carriers who would otherwise render the container hazardous in nature, further allowing a cost effective means of transport for the shipper and its frozen contents.

When semen or embryo inventory is received by way of a vapor shipper, it becomes the receiver's responsibility to verify the contents. When making the transfer from the vapor shipper to the producer's private inventory tank, it is a good idea to quickly glance (remembering the "three second rule") at the goblet and confirm that what has been ordered has been delivered. It is the buyer's responsibility to notify the supplier immediately if there is some discrepancy in the quantity or identification of the inventory received.

Lists of companies featuring [caprine artificial insemination equipment, liquid nitrogen tanks, and supplies](#) as well as [companies that sell frozen buck semen](#) are found at the end of this module.

Superovulation and Embryo Transfer

Embryo transfer (ET) is an invasive procedure performed by a licensed and trained veterinarian that is becoming widely used in the meat goat industry by the showman, hobbyist, and commercial producer. Like laparoscopic AI, this technique is fairly cost prohibitive due to its commonly practiced methods involving surgical collection and transfer of embryos. Although success rates are increasing as techniques are mastered and practitioners gain experience and knowledge, ET programs have many limitations. Still, more and more producers are finding that

a well-planned ET program involving the use of a knowledgeable and well-experienced technician who offers careful attention to detail with regard to sterility, synchronization, superovulation, and the appropriate selection of both donor and recipient does, can often achieve an acceptable rate of recovery and transfer of high-quality embryos.

Embryo transfer can be performed successfully both in and out of the normal breeding season. However, it is best performed when the does are actively cycling and exhibiting normal behavioral estrus. Furthermore, the most successful results occur when the participants are two to five years of age (the prime of their reproductive years), are in good health, and have a body condition score of 3 to 3.5. By observing all of these conditions, an ET program can offer the highest rate of success for both the ET technician and the producer.

A major deterrent to some producers is the requirement for many recipient does. A suitable recipient doe is one who has proven her reproductive abilities. Such abilities include not only trouble-free kidding, but natural mothering characteristics and an adequate milk supply for rearing kids. Once recipient candidates are selected, they should be separately housed and managed for several weeks prior to and after their scheduled transfers. Careful attention to management must be paid to the recipient herd insuring that high levels of nutrition, excellent health, and a low stress environment are maintained for successful implantation and pregnancy rates.

Prior to transfer, each of the recipient does must be synchronized to be at the same stage of their estrous cycle as the donor doe. Does can be synchronized using a variety of methods; the most common choice of most ET technicians being progestagen therapies of one sort or another.

Superovulation methods

Donor does are predominantly does of high value to the herdsman. These does have genetic potential, or proof of such, which warrants the cost and inconvenience of the flushing procedure. Traditional protocol for the donor doe involves a menu of hormonal treatments directed at the superovulation of her ovaries. Follicular stimulation is accomplished by way of the doe's FSH and LH levels, and is of primary importance in achieving a satisfactory superovulation response. Superovulation is commonly induced through administration of decreasing doses of follicle stimulating hormone (FSH), 1 to 5 mg, injected in 12-hour intervals over a period of 3-5 days around the time of termination of the progestagen treatment. Improvements in the consistency and predictability of the superovulatory response have been achieved through corresponding protocols involving prostaglandin (PG) and luteinizing hormone (LH) treatments.

Other methods of superovulation utilize either an FSH product alone or in combination with PMSG (eCG). Some practitioners have concluded that FSH offers superior results when compared with PMSG protocols, in that PMSG can cause overstimulation of the ovaries causing the release of larger numbers of oocytes. These greater numbers can result in higher ratios of unfertilized oocytes and lesser quality embryos. This may be attributed to insufficient numbers of spermatozoa for adequate numbers of "accessory sperm," especially when AI is employed as the means of fertilization. Bucks who are exposed to more than one or two superovulated does

may also fail to have adequate numbers of spermatozoa to properly fertilize the large numbers of oocytes.

It is recommended that insemination of the donor doe be performed 12-24 hours following the beginning of behavioral estrus by either natural service or, although not always as successful, via cervical or trans-cervical AI with the use of 300 million live, motile spermatozoa as would be expected to be found in five 0.5cc straws. To increase the overall success of artificial methods of fertilization, the doe should be monitored to determine the proper time of servicing. These recommendations are based on ensuring that sufficient numbers of “accessory sperm” are present for the successful fertilization of multiple ova resulting in high grade, viable embryos.

Use of laparoscopic/intra-uterine AI is usually not performed in this procedure in an effort to avoid additional manipulation of the uterus and oviducts. However, when performed 24-hours following first evidence of estrus in the donor doe, laparoscopic AI has been reported to offer some success.

Acceptable resulting ovulation rates from the methods described range from 10 to 25 ova produced, but the number of viable embryos for ET use may be significantly lower.

Embryo flushing

Harvesting of the donor doe’s resulting embryos (fertilized ova) traditionally involves a licensed veterinarian and is performed 5-6 days following applied fertilization methods. The donor doe is denied food or water for 24-36 hours before surgery as she will be anesthetized during the procedure. Her abdominal area is clipped and prepared for aseptic surgery. Once under anesthesia, a midventral or flank laparotomy protocol is used to expose the uterus and the ovaries are examined to determine the response to the superovulation therapy. When a positive determination is made, the uterine wall is penetrated and a catheter is inserted into the uterine horn. A flushing media is infused through the catheter which facilitates the flushing of the embryos into a Petri, culture, or collection dish. Once completed, the same procedure is followed to flush the opposite uterine horn. The puncture sites are then left unsutured.

The disadvantage of such an invasive procedure on repeated occasions is the likelihood of resulting adhesions that may interfere in subsequent collections. Recently, newly practiced techniques involving laparoscopy to accomplish the same goal have met with good success in goats. However, this technique is more difficult and requires a technician who is well practiced in such a procedure.

Vaginal flushing/trans-cervical embryo collection methods are in their infancy with regard to goats. Nevertheless, there are many published reports of successful nonsurgical collections. This practice is on the horizon for the producer and should be considered as it offers a lower chance of postsurgical adhesions and allows for a much greater number of collections over the doe’s lifetime. Flushing by this method is performed similarly to the techniques of trans-cervical AI in sheep. A duck billed speculum is used to see the external os of the cervix and grasp it with forceps. The speculum is removed and the os is carefully brought to almost reach the vulvar opening. A catheter is passed through the cervix and positioned to flush either the right or left

uterine horn. Multiple flushings of each horn are performed along with massaging techniques. Reported results are mixed as many technicians feel that the entire uterus is not always able to be flushed. Harvested embryos differ considerably in number according to the flushing method used. It has been reported that in vaginal flushing often no embryos are harvested whereas three to four can be expected. Still other findings report that embryo recovery can be comparable to collections by surgical means.

Once collected, the flushing medium is searched for its expected number (based on the doe's number of corpora lutea) of ova. Once recovered, ova are scrupulously examined for quality and very distinct handling procedures should be practiced to reduce the risk of disease transmission. The ova are then washed and examined to assess the number that have cleaved (fertilized), and their stage of development. Once this is determined, each ovum is drawn into the tip of a small-bore catheter attached to a small syringe and either transferred to the recipient doe or processed for freezing.

For the purpose of cryogenically freezing and storing embryos, only those of the highest quality and grade should be processed. Limited progress has been made in the cryopreservation of goat embryos and success is highly related to the type of cryoprotectant used. Pregnancy rates from the transfer of frozen embryos are substantially less than rates obtained with freshly harvested embryos.

Embryo transfer

The practice of transferring harvested embryos to recipient does is again an invasive technique, surgically performed. Potential recipient does are anesthetized, their abdominal area is clipped and aseptically prepared for surgery. A small, midline incision is made and the ovaries are examined for corpora lutea. If determined to be at a stage suitable to that of the embryo, the embryo is either transferred into the oviduct via a catheter or placed directly into the uterine horn by way of a needle, depending on the stage of the embryo's development.

When the transfer is "laparoscope-assisted" or a "laparoscopic method of transfer" is utilized, the doe is similarly prepared for the surgical method described above except the recipient doe is positioned and two cannulas are used as would be done for laparoscopic AI. The ovaries are examined by way of the laparoscope and a determination is made as to the condition of the corpus luteum. If a positive determination is made, in a "laparoscope-assisted" method the tip of the uterus is punctured and the embryos are transferred as in a surgical method. Once transfer is complete, the technician sutures the small incision. A "laparoscopic method of transfer" requires the embryos be loaded in a 0.25 or 0.5 cc straw that is inserted into an AI gun fitted with an injection tip. Protocol similar to that of laparoscopic/intra-uterine AI is followed for the deposit of the embryo.

In the fresh transfer of embryos to synchronized recipient does, although lesser quality and grade embryos can be used, only #1 grade embryos are recommended for a high success rate and outcome. In some cases where donor does have produced a limited number of #1 grade embryos, recipient does with multiple corpora lutea may be given a matching number of embryos involving the transfer of one #1 grade embryo and additional #2 or lesser grade embryos, in

hopes that one or more of these sub-standard embryos might survive. The use of sub-standard embryos in combination with the #1 grade embryo(s) appears to have no negative influence on the latter, although there is also no additional guarantee of success. The recipient doe's stage of ovulation being as similar as possible to the donor doe's is believed to have the greatest influence on the success of the procedure. By way of any method, the donor doe's embryos are placed in the recipient doe on the side that has a corpus luteum present. The number of corpora lutea on the ovary determines the number of embryos the recipient doe may receive.

Immediately following the procedure, all does are brought through recovery in a carefully controlled, low stress environment with great attention paid to their management and daily conditions for several weeks.

A list of some [companies offering embryo transfer services](#) is located at the end of the module.

Pregnancy Detection

Once any method of assisted animal reproduction is administered, the producer will want to make some attempt at pregnancy confirmation. There are a number of means in which this can be accomplished.

Bumping

Bumping is a method practiced by some producers with a marginal amount of success. The theory behind the technique is to attempt to detect the fetus by way of its "firmness" within the abdomen of the doe. The likelihood of success using this method largely depends on the stage of pregnancy of the doe when the technique is performed, the number of fetus(es) present, and what position the fetus is in at the time the attempt. This method is one of the least reliable for an accurate determination of pregnancy.

Vulva examination

Vulva examination is a method widely used by producers with long-term experience working with both pregnant and open (not pregnant) does. Although not easily recognized until late in the doe's pregnancy, the skin in and around the vulva becomes more placid, stretchy, and loose in late gestation. When compared to does of the same general age and size that are not pregnant, it can be very apparent that the vulvar region of the pregnant doe is undergoing changes that will allow for successful delivery of the kid. These vulvar changes are usually coupled with noticeable signs of mammary development.

Although certainly not used with any proven reliability until late in gestation, this method is widely practiced and, when asked, experienced producers seem to be of the opinion that the method does work more often than not.

Cervical examination

Producers make some claim that when the cervix of a pregnant doe is examined, a “gray plug” can be seen. This observation has reportedly been made as early as 30 days following conception. An explanation of such an observation may be attributed to the following. The cervix is the “gateway” to the uterus and during pregnancy it has the job of blocking entrance to the uterine body. The claim of a “gray plug” is supported by research proving that during pregnancy while under the influence of progesterone the mucus of the pregnant female becomes quite viscous and thick. The mucus can become so thick that during gestation it acts like “glue” holding the folds of the cervix together so that foreign material cannot enter the uterus. This barrier is commonly referred to as the “cervical seal of pregnancy.” Any disruption of this barrier or seal can, and often will, result in abortion. The cause of the abortion is directly related to microorganisms gaining entry to the uterus and causing infection that leads to subsequent embryonic death.

Ultrasonography

For the diagnosis of pregnancy in goats there are three commonly used ultrasound techniques, each with its own specifically designed piece of equipment. The first and most reliable method is referred to as B-Mode, followed by A-Mode, and finally the Doppler machine. All three methods require that the doe be shaved high on the right side of the abdomen just in front of the mammary gland to remove all hair that may interfere with data received by the ultrasound probe. A coupling gel is also used to ensure a clear projection of information to and from the probe.

If the equipment is not owned and operated by the producer, the cost of ultrasound can vary to a large degree depending on the practitioner, distance traveled, and number of animals to be examined. All three ultrasound methods are highly dependent on the experience and technical knowledge of the operator/technician for a correct diagnosis of pregnancy, i.e., openness (not pregnant), or pseudo pregnancy with hydrometra or muco metra (false pregnancy, e.g., uterine development lacking a fetus).

The most popular method and that offering the most accuracy is the use of B-mode or real-time transabdominal ultrasonography. This method is commonly performed in some states by licensed technicians and often by licensed veterinarians. Most practitioners find that detection is easiest made when the doe is between 45 and 90 days of gestation. Some well-experienced practitioners can determine identification of a fetus as early as 27-30 days, and if examined slightly later, the number of fetuses in the uterine body. Pregnancy can still be identified by means of the caruncles very late in gestation, although actual evidence of the fetus itself may be disrupted by the obstacles in and around the abdominal region of the doe. It is not unusual to see a fetus move about due to the stimulus created by the ultrasonic sound waves. Producers should be aware that there is some minimal risk of fetal abortion if an inexperienced practitioner uses an annular array (transrectal) probe for this purpose. External sector scanners or linear array (transabdominal) probes offer little to no risk of abortion and are preferred by most technicians.

Transabdominal ultrasonography



Ultrasound image



A-Mode ultrasound, although not nearly as accurate as the linear interpretations used in B-Mode ultrasonography, is an inexpensive means of pregnancy diagnosis with greatest accuracy achieved at 30-40 days gestation. The A-Mode ultrasound is designed to detect a large body of fluid within the doe's abdominal cavity. This method can often produce a misdiagnosis by mistaking a full bladder or fluid filled uterus for a pregnant uterus.

The Doppler ultrasound is also used. This machine is designed to detect blood flow by way of a fetal heartbeat. Although cost effective, its degree of accuracy is sometimes less than desirable.

A list of some [companies offering ultrasound equipment](#) is listed at the end of this module.

Blood sampling/assay

Although not the least expensive (about \$10-15.00 per sample), blood sampling for hormone or protein assay offers by far the greatest degree of safety to the fetus and is one of the most reliable pregnancy detection methods. Results can most often be expected within 5-7 days of the sample's receipt by the laboratory.

The protocol is very simple, a blood draw of at least 5 cc is made into a sterile, gray or red-topped blood tube as early as 26 days after breeding. Once the laboratory receives the sample, it is screened for a protein in the blood that is only produced by the placenta. This protein, Pregnancy-Specific Protein B (PSPB), can only be present if the doe has, or has very recently, been supporting a viable placenta.

Producers should be aware that a possible false positive could be generated in the event of recent embryonic death enabling residual PSPB to still be detected. False negatives occur most commonly when samples are drawn too early for PSPB detection. Enough time must be given,

once the doe becomes pregnant, for the body to build sufficient levels of PSPB in the blood for laboratory detection (26 days from the breeding date).

The screening for fetal protein (PSPB) is a highly reliable test and is most probably only compromised by poor timing in the collection or handling of samples and their cross-contamination. The producer should take care in the harvesting of the whole blood so that no mislabeling or cross-contamination between animals occurs.

A test of non-pregnancy through progesterone analysis can also be made. The corpus luteum of the goat produces measurable levels of progesterone throughout gestation. Milk, serum, and plasma may be analyzed for progesterone concentration. If none is detected, the presence of a functional corpus luteum is unlikely thereby diagnosing the doe as not pregnant. Blood serum and plasma are superior to milk for progesterone analysis. It has been reported that commercial on-farm cattle progesterone test kits have been used in goats with good accuracy.

A list of [companies offering laboratory detection of PSPB](#) can be found at the end of this module.

Glossary

Accessory sperm - spermatozoa who are supplementary to each other for the purpose of successful ovum fertilization.

Acrosome – the caplike structure covering the anterior portion of the head of a spermatozoon; contains enzymes necessary for the penetration of the ovum.

Anesthesia/anesthetic – a substance that produces physical insensibility to pain or other sensation.

Anterior – situated at or directed toward the front; opposite of posterior.

Aseptic – sterile.

Artificial insemination - the implanting of live spermatozoa into the genital tract of the female.

Capacitation - the process by which spermatozoa become capable of penetrating and ultimately fertilizing an ovum.

Caruncles – fleshy masses by which the placenta attaches to the uterine wall.

Caudal - situated more toward the end or tail.

Cervix - the narrow caudal end of the uterus that opens into the vagina.

Cleavage - the early successive splitting of a fertilized ovum into smaller cells.

Corpa lutea/corpus luteum - a progesterone secreting yellow mass formed from the wall of an ovarian follicle that has matured and discharged its ovum.

Embryo - no longer an ovum or zygote; the cleaved, fertilized ovum in early stages of development prior to becoming a fetus.

Embryo transfer - collection of fertilized ova from one female before they become implanted and transfer to another female to complete the gestation.

Estrus - period of sexual receptivity of the doe (also called 'heat').

Estrous – the time from the beginning of one estrus period to another, normally 18-21 days in goats.

Fertility - the capacity to conceive or to induce conception.

Fertilization - or conception; when the spermatozoa unite or fuse with the ovum creating a zygote.

Follicle - the ovum and its encasing cells.

Fornix – the overshoot; the annular recess around the outside of the cervix.

Gestation - from the time of fertilization of the ovum until birth.

Implantation - the attachment and embedding of the fertilized ovum within the uterus.

Intra uterine - within the uterus.

Laparoscope – an instrument used for the visual inspection of the female reproductive organs and sex glands.

Lumen – the cavity or channel within a tube or tubular organ.

Opaque - neither solid in color nor translucent or transparent.

Hormone - a chemical transmitter substance produced by cells of the body and transported by the bloodstream to the cells and organs on which it has a specific regulatory effect.

Morphology - the normal anatomical structure of the spermatozoon.

Motility - the ability to move spontaneously; used to predict the probable fertility of the ejaculate, subjective estimates based on the degree of wave motion observed under the microscope.

Os - the mouth or opening of the cervix.

Ova/ovum - an egg; the female reproductive cell.

Ovary - the sex glands of the female where the ova are formed.

Oviduct - a passage through which the ova leave the maternal body.

Ovulation - the discharge of the ovum from the ovary.

Plasma – the fluid portion of the blood.

Posterior – directed toward or situated at the back; opposite of anterior.

Postmortem - performed or occurring after death.

Prepubescent - the period before puberty.

Progesterone - a hormone secreted by the corpus luteum which fosters uterine preparation for pregnancy, placental growth and ultimately is required to sustain pregnancy.

Prostaglandin - a fatty acid which affects the action of certain hormones, causes regression of the corpus luteum, stimulates uterine contraction and ultimately is a major contributor to fetal abortion.

Puberty - the time when the capability of sexual reproduction is attained.

Rut - the period of increased sexual and testicular activity, especially spermatogenesis, in the male.

Spermatogenesis - the development of mature spermatozoa.

Spermatozoa/spermatozoon - a semen cell; the male reproductive cell.

Superovulation - production of more than one ovum at ovulation.

Serum – the clear portion of the blood plasma which remains after the solid elements have been separated out by clotting.

Testes/testicle/testis - the egg shaped sex gland, normally situated in the scrotum of the male which produce spermatozoa.

Traverse – to pass or move over, along, or through.

Uterine horn – one of the pair of tubular extensions from the uterine body.

Uterus – the hollow muscular organ of the female where fertilized ova become embedded and are nourished during gestation.

Ultrasonography – an imaging technique in which deep structures of the body are visualized by recording the reflections of ultrasonic waves directed into the tissues.

Vagina – the canal in the female from the external genitalia (vulva) to the cervix.

Vaginal speculum – an instrument for opening the vaginal cavity to permit visual inspection.

Vestibule – a space or cavity at the entrance to another structure.

Viable – live vs dead; to maintain an independent existence.

Viscous – sticky or gummy.

Zona pellucida – the transparent, secreted layer surrounding the ovum.

Zygote – the fertilized ovum until first cleavage, prior to becoming an embryo.

Mobile semen processors equipped for on-site farm collection and processing:

BIO-Genics, LTD

1538 Hwy 28

Salmon, ID 83467

(208) 756-6500

Toll Free: 1 (877) 246-4282

Website: www.biogenicsltd.com

Email: contactus@biogenicsltd.com

Frozen Assets

PO Box 493133

Redding, CA 96049

(530) 549-3825

Email: froznasets@aol.com

Superior Semen Works

1185 White Mountain Highway

Milton, NH 03851

(603) 652-7577

Website: www.superiorsemenworks.com

Email: Superiorsemenworks@yahoo.com

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Limited area semen processors equipped for on-site farm collection:

Hawkeye Breeders Service Inc.
3257 Old Portland Rd
Adel, IA 50003
(515) 993-4711

Reproduction Enterprises, Inc.
908 N Prairie Rd
Stillwater, OK 74075
Toll Free: 1 (877) 525-8037
Website: www.reproductionenterprises.com
Email: info@reproductionenterprises.com

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USDA approved isolation facilities and collection centers:

BIO-Genics, LTD
1538 Hwy 28
Salmon, ID 83467
(208) 756-6500
Toll Free: 1 (877) 246-4282
Website: www.biogenicsltd.com
Email: contactus@biogenicsltd.com

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Companies offering postmortem semen extraction services:

Global Genetics
4011 SH 47
Bryan, TX 77807
(979) 822-4000
Fax: (979) 822-4022
Website: www.globalgeneticsandbiologicals.com
Email: global@globalgenetics.tv

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Companies specializing in caprine AI, offering on-location class instruction:

BIO-Genics, LTD
1538 Hwy 28
Salmon, ID 83467
(208) 756-6500
Toll Free: 1 (877) 246-4282
Website: www.biogenicsltd.com
Email: contactus@biogenicsltd.com

Frozen Assets
PO Box 493133
Redding, CA 96049
(530) 549-3825
Email: froznaset@aol.com

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Companies featuring caprine artificial insemination equipment, liquid nitrogen tanks, and supplies:

BIO-Genics, LTD
1538 Hwy 28
Salmon, ID 83467
(208) 756-6500
Toll Free: 1 (877) 246-4282
Website: www.biogenicsltd.com
Email: contactus@biogenicsltd.com

Superior Semen Works
1185 White Mountain Highway
Milton, NH 03851
(603) 652-7577
Website: www.superiorsemenworks.com
Email: Superiorsemenworks@yahoo.com

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Companies offering a catalog of frozen buck semen available for purchase:

BIO-Genics, LTD
1538 Hwy 28
Salmon, ID 83467
(208) 756-6500
Toll Free: 1 (877) 246-4282
Website: www.biogenicsltd.com
Email: contactus@biogenicsltd.com

Frozen Assets
PO Box 493133
Redding, CA 96049
(530) 549-3825
Email: froznaset@aol.com

Superior Semen Works
1185 White Mountain Highway
Milton, NH 03851
(603) 652-7577
Website: www.superiorsemenworks.com
Email: Superiorsemenworks@yahoo.com

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Embryo transfer reproduction services:

Agrimark Genetics
PO Box 648
Martindale, TX 78655
(512) 357-6931

Reproduction Enterprises, Inc.
908 N Prairie Rd
Stillwater, OK 74075
Toll Free: 1 (877) 525-8037
Website: www.reproductionenterprises.com
Email: info@reproductionenterprises.com

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Companies offering ultrasound equipment:

Renco Corporation
116 Third Ave N
Minneapolis, MN 55401
Toll Free: 1 (800) 359-8181
Website: www.rencocorp.com
Email: sales@rencocorp.com

Classic Ultrasound Equipment/PharVision
19900 Mona Rd #105
Tequesta, FL 33469
(561) 746-9527

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Laboratories offering PSPB detection in whole blood:

Bio Tracking
105 E Second, Suite 2
Moscow, ID 83843
(208)882-9736
Website: www.biotracking.com

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Information contained in this document is part of a web-based training and certification program for meat goat producers (<http://www2.luresext.edu/goats/training/qa.html>) that was developed with funding received by Langston University from USDA/FSIS/OPHS project #FSIS-C-10-2004 entitled "Development of a Web-based Training and Certification Program for Meat Goat Producers."

Collaborating institutions/organizations include Alcorn State University, American Boer Goat Association, American Kiko Goat Association, American Meat Goat Association, Florida A&M University, Fort Valley State University, Kentucky State University, Langston University, Prairie View A&M University, Southern University, Tennessee Goat Producers Association, Tennessee State University, Tuskegee University, United States Boer Goat Association, University of Arkansas Pine Bluff, and Virginia State University.