ALL ABOUT A.I

1.Is AI For You?

If you have a few backyard does that you enjoy as a hobby, with little concern for genetic improvements of their offspring, then artificial insemination (AI) is probably not for you, assuming a suitable buck can be located for servicing the does. The expense of purchasing the necessary equipment and learning to do AI are likely not worthwhile. However, if there is an experienced inseminator in the area who is willing to work with your goats, then this may prove to be a viable alternative and certainly is much simpler than hauling your does in heat to the buck's home.

2 .AI has some key advantages over natural breeding.

1) It eliminates the necessity of keeping one or several bucks on the farm (depending on herd size). Costs of feeding, housing separate fencing and labor are eliminated. However, heat detection may be more difficult in the absence of a buck.
2) AI can increase the rate of genetic improvement in a herd, as long as superior bucks are consistently selected. In natural service, the prospective breeder has only the buck's pedigree to rely on, whereas AI bucks should be progeny tested for their transmitting ability of milk and fat percentage, weight gain, type conformation, etc.
3) AI allows breeding of different portions of the herd to different bucks. Young does may be bred to not yet proven but high potential bucks, while the majority of the herd can be bred to proven high quality bucks.
4) AI permits breeding of many does on one day when synchronization is practiced. No long drives to top bucks are involved.
5) The danger of transmission of diseases or parasites is greatly reduced. (The transmission of diseases through frozen semen needs further study.)
6) The time of breeding can be more carefully regulated, and the owner knows exactly when the doe was bred, as opposed to pasture servicing by a buck that is allowed to run with the herd.
7) AI induces good record keeping of dates of heat, breeding, pedigrees, etc. This will aid in herd improvements and enable the owner to make better culling decisions.

Some anatomical differences in the reproductive organ of cattle and buffalo are presented below.

Organ	Animal	
	Cattle	Buffalo
Oviduct		
Length (cm)	25	28.2
Uterus		
Type	Bipartite	
Length of horn (cm)	35-40	34.5
Length of body (cm)	2-4	6.2
Surface of lining of endometrium	70-120 caruncles	
Cervix		
Length (cm)	8-10	6.75
Outside diameter (cm)	3-4	
Vagina		
Length (cm)	25-30	20.25

Dimension of Reproductive Organs of Female Buffalo and Cow

ITEM	Buffalo	Cow
Adult Body Weight (kg)	350 - 500	700
Adult Wither Height (cm)	125	135
Ovary Length (cm)	2.2 - 2.9	2.8 - 3.8
Ovary Weight (gm)	3.0 - 4.0	5.0 - 9.4
Size of biggest follicle (cm)	1.4 - 1.7	1.9 - 2.2
Size of Mature CL (cm)	1.3 - 1.6	1.7 - 3.0
Weight of Mature CL (gm)	0.7 - 1.5	1.2 - 2.5
Size of Ovum (um)	169	169

Data from : Aboule - Fadle, Fahni and Shaftz 1974, 1, b, Luhtuke & RAO1960/ Mobarak 1969

Graph1.

3.Once the decision to use AI has been made, the next step is to determine whether to do the inseminating yourself or pay someone else to do it. If there are only a few does in your herd, and an experienced inseminator of goats is available, then it may be more practical to pay to have the service done. However, if the number of does in the herd is rather large, or an experienced inseminator is nowhere to be found, then it's probably time to learn how to practice AI techniques yourself.

4. AI technicians of the cattle industry may not necessarily be of much help when it comes to inseminating goats, for the modern method of inseminating cattle (rectal palpation) differs from that of breeding goats (speculum method) considerably. The speculum was used on cattle early in AI history, and some cattle inseminators may be capable of teaching goat insemination.

5. The cost of getting started in AI, not including semen purchases, will generally run around $500, of which $400 to $450 is tied up in the liquid nitrogen tank, which is necessary for storing semen any length of time. Temperatures must be kept at -320F (-196C) for sperm survival to be maximized at breeding time. It may be possible to share the cost of the tank with neighboring goat owners or dairy farmers, thus alleviating some initial costs of an AI program.

6. If AI is to be used with any hope of achieving a good level of success must be known and well understood by the prospective inseminator.

1) Basic knowledge of the doe's reproductive organs and their functions;
2) Understanding of storage and handling of semen;
3) Ability to use, in a proper and sanitary manner, the equipment required for inseminating goats;
4) Ability to accurately detect heat at an early stage;
5) Necessity of keeping accurate, up to date records of heat Cycles, breeding, kidding, reproductive problems, treatments, and any other pertinent information that may reflect on the goat's reproductive patterns.

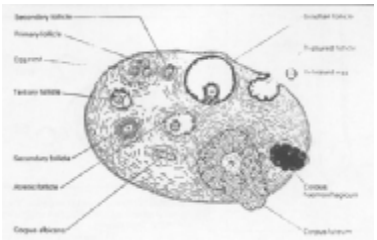


Figure1. Ovary.

7. Reproductive Organs and Functions the two ovaries are the sites of egg formation. They produce estrogens and progesterone, and as such are determining factors of heat cycle, ovulation and pregnancy. Basically the estrus (heat) cycle in goats operates as follows:

1) Postures are the time of follicle growth. As an egg (ovum) begins to mature in an ovary, it becomes surrounded by a fluid filled sac on the outside of the ovary, much like a blister forms on the skin. This growth is accompanied by increasing levels of estrogen in the blood.
2) Estrus - As estrogen levels peak, the doe will come into heat. This can be observed by changes in behavior (increased bleating and restlessness), willingness to be bred, and the swelling of the external genital area. The period of ''standing heat'' (acceptance of the buck) will generally last for 24 to 36 hours.

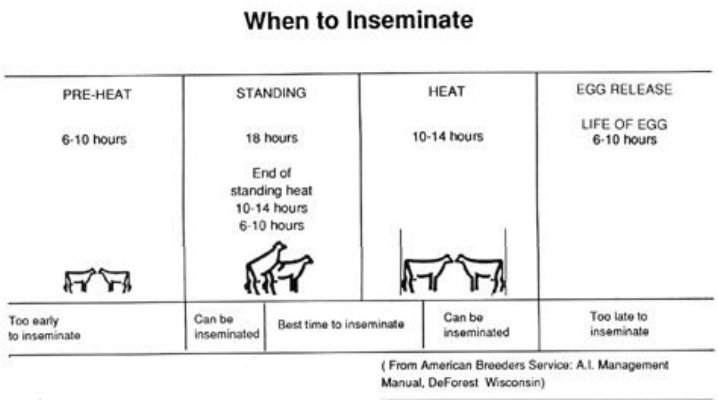


Figure2.

3) Ovulation, or the release of the egg, is accomplished by the rupturing of the follicle, expelling the egg from the ovary, and receiving it into the oviduct via the fimbria funnel. This occurs very near, or soon after, the end of standing heat (6 hours before to 12 hours after). Egg life is 12 to 24 hours, while the sperm lasts 24 to 48 hours.

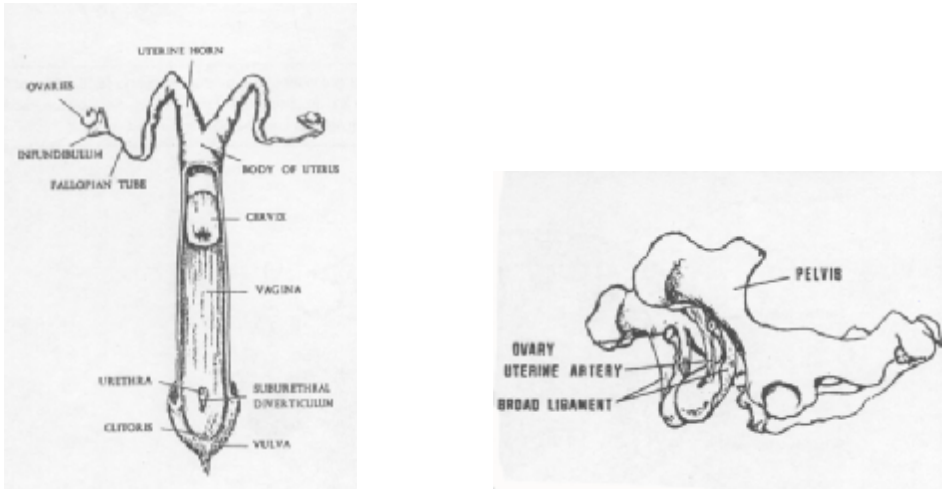


Figure3.Female organs system

4) Met estrus - in this stage, the ruptured follicle is undergoing cellular differentiation to form a functionally important tissue mass, the corpus lustrum (yellow body). This structure is responsible for the secretion of progesterone, a hormone that prevents the development of another follicle and prepares the uterus to receive a fertilized egg.

5) Diestrus - is the longest period of the estrous cycle in does. During this period of corpus luteum influence, two events may happen:

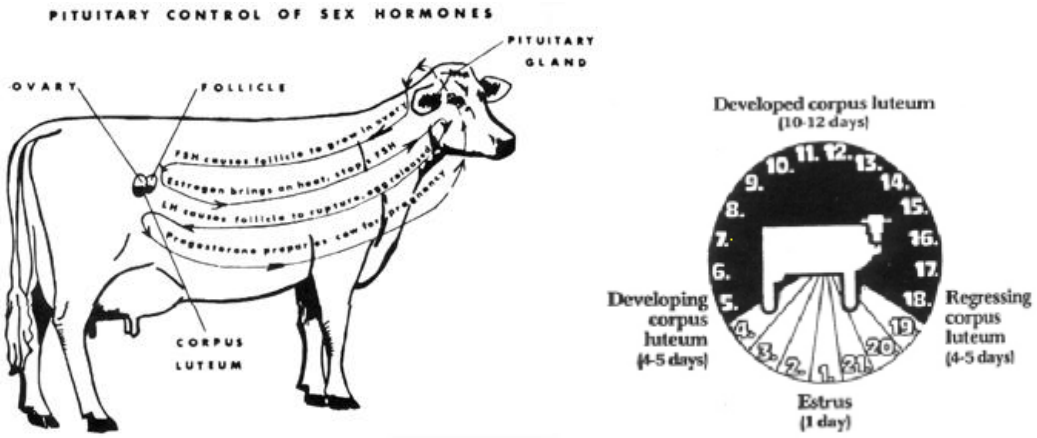


Figure4. Developed corups luteum

a) If fertilization of the egg occurred, the corpus luteum will persist for the entire gestation period, preventing follicular development and keeping estrogen levels low.

b) If no fertilization took place, the progesterone secretions of the corpus luteum gradually lessen, allowing a new cycle of follicular development to begin, with a corresponding increase in estrogen levels. The length of time required for one estrous cycle without fertilization, ranges from 17 to 24 days in goats, with the majority taking 21 days. Shorter cycles are not uncommon (5-10 days).

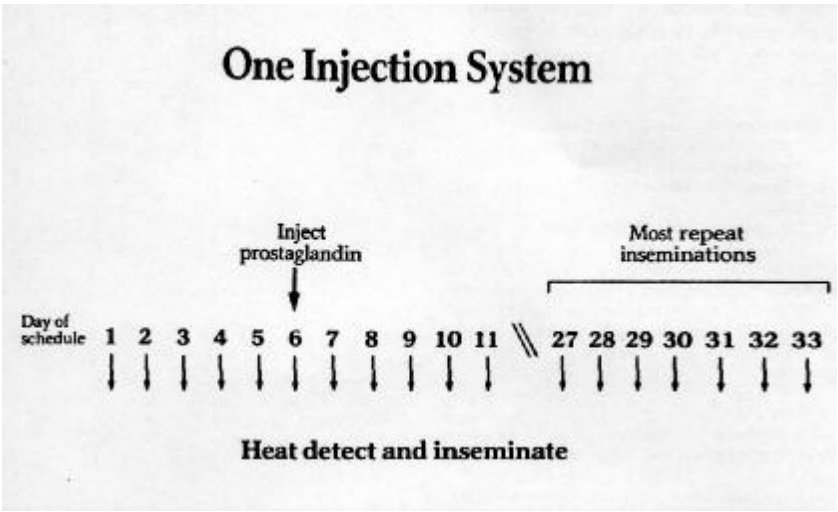


Figure5. Synchronized hormone – one injection system

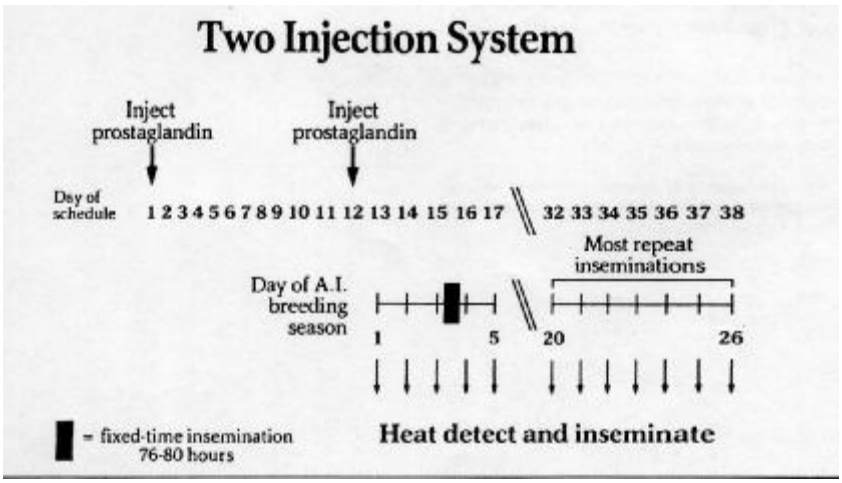


Figure6. Synchronized hormone – two-injection system

8. The egg, after being expelled from the ovary, passes into the oviduct via the infundibulum, and toward the cornua (horns) of the uterus. This movement is produced by wave-like motions of the ciliated (hair-like projections) cells of the oviduct. Sperm and eggs meet in the oviduct and fertilization occurs in the middle to upper one third of the duct.

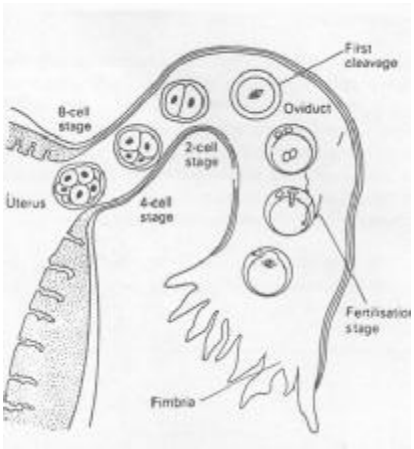


Figure7. Ovulation, and the release of the egg system.

9. The egg continues into the horn of the uterus, where, if it has been fertilized and undergone several cellular divisions, it will become attached to the uterine wall. If no fertilization has occurred, the egg will degenerate and the cycle goes on.

10.The cervix of the uterus plays a key role in artificial insemination, as it is the external entrance to the uterus, which must be located and penetrated with the inseminating instrument. The cervix is normally tightly closed, except during periods of heat or kidding. Semen is deposited on the vaginal side of the cervix in natural services, but AI requires the deposition of semen in the uterine side of the cervix. This is because of the greatly reduced volume of semen that is used in AI. If the 0.5 to 1 cc of semen in AI were deposited on the vaginal side of the cervix, there is a good chance that none of the sperm would reach the egg.

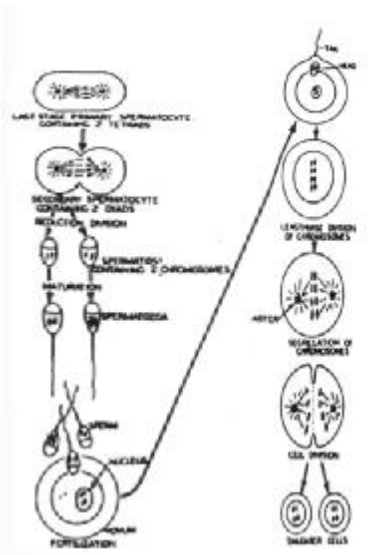


Figure8. Fertilization system

11.The vagina serves as the connecting tube between the uterus and the outside opening, the vulva. It is part of the birth canal, and also contains the urethral opening, from which urine will pass during emptying of the bladder.

12. Purchase and Preparation of Semen In most cases, the inseminator will acquire the semen needed by direct purchase from a commercial operation, in which case it will be shipped to the inseminator. It is of the greatest importance that the semen be transferred to permanent storage (the liquid nitrogen tank) without exposing it to anything approaching air temperature. Generally, this means transferring the container element, which houses the semen directly to the liquid nitrogen tank. Here it can be safely stored for long periods of time, since biological activity practically stops at liquid nitrogen temperatures (-320F). Semen is generally to be used within 6 months, but conceptions have resulted from semen stored for several years, although sperm survival is decreased, resulting in lower conception rates.

13. Semen Collection Bucks are handled basically the same way as bulls for semen collection. Three basic methods may be employed, but all three require an artificial vagina, a double walled device with an opening at one end and collection tube at the other. The inner lining holding warm water should be coated with a light application of water-soluble lubricating jelly. The three methods are:

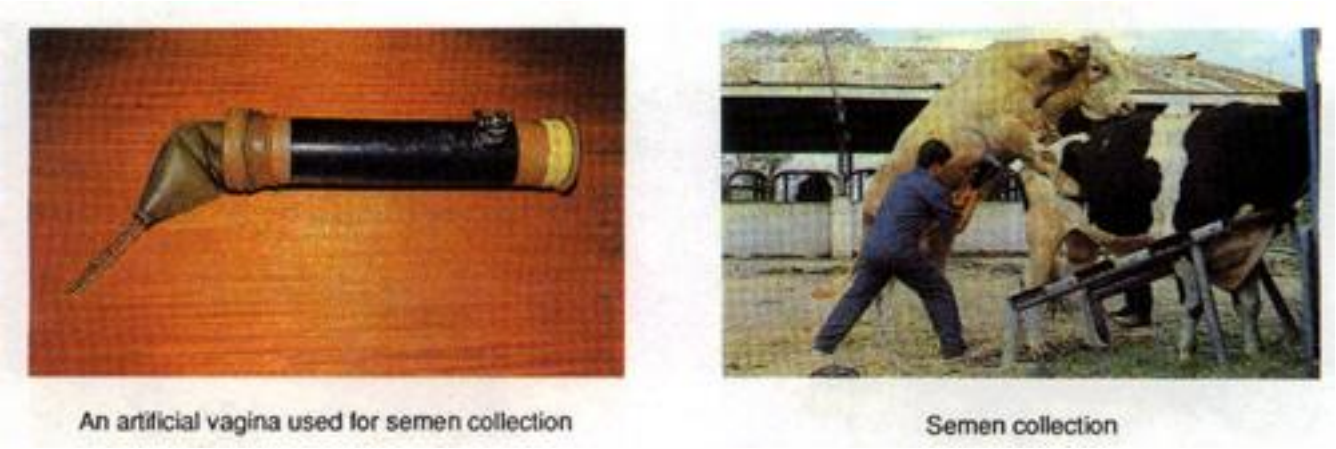


Figure9.

1) A buck may be allowed to mount a doe, with the semen collector manually diverting the buck's penis into the artificial vagina (ram or dog size). Don't touch the penis directly; instead direct the penis into the artificial vagina by grasping the buck's sheath. After ejaculation (usually 0.5 to 1.0 cc) has occurred, remove the artificial vagina and tip it so that the semen will all run into the collection tube. This method may require practice and adjustment by both the buck and the collector before good samples are collected.
2) A buck is trained to mount a dummy instead of a live doe. The same procedures are followed for sample collection. Applying vaginal mucus scrapings of a doe that is in heat to the dummy, at least during the training process may facilitate mounting.
3) Use of electro-ejaculation. The buck is not required to mount an object, although an artificial vagina should still be used for semen collection. An electrode unit, which has a number of contact rings, is inserted into the buck's rectum. Slight electric stimulation brings on ejaculation. This technique generally results in good samples in quantity and quality. However, the sperm concentration of the sample will be lower. This method does not require extensive training, and will allow collections from bucks that may refuse or are unable to mount and serve an artificial vagina.

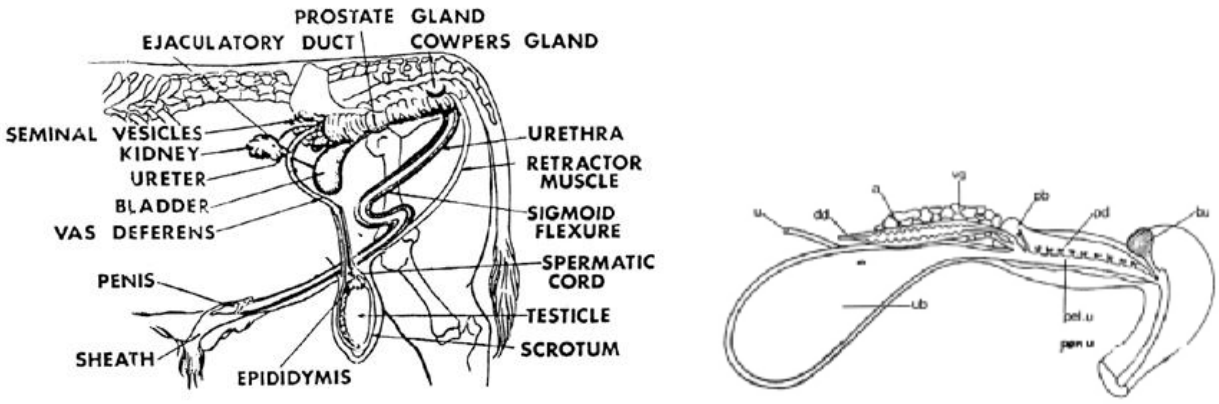
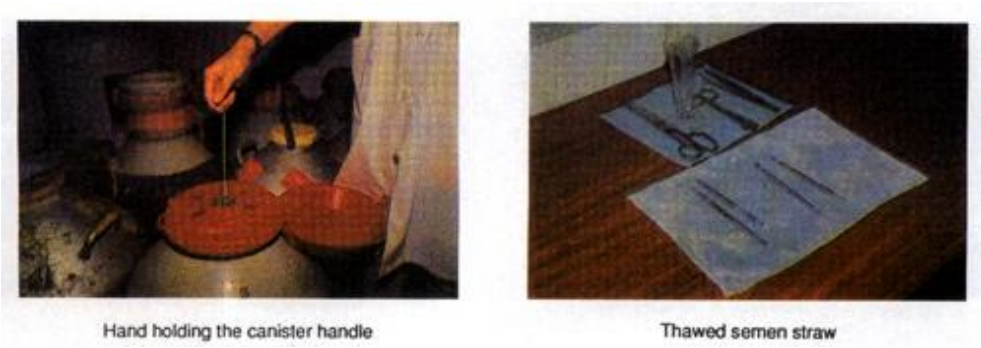


Figure10. Male reproduction system

14. Semen, once collected, may be used in one of three different ways:
1) As liquid semen, directly or on the same day one ejaculate can serve 3 to 5 does. If kept at body temperature, the semen may be good for three hours.
2) Semen may be stored 24 to 48 hours by placing the collection tube in a container of water and putting this unit in a refrigerator. No diluter is needed, although plain egg yolk can serve as simple extender to double the number of does that can be served.



Hand holding the canister handle

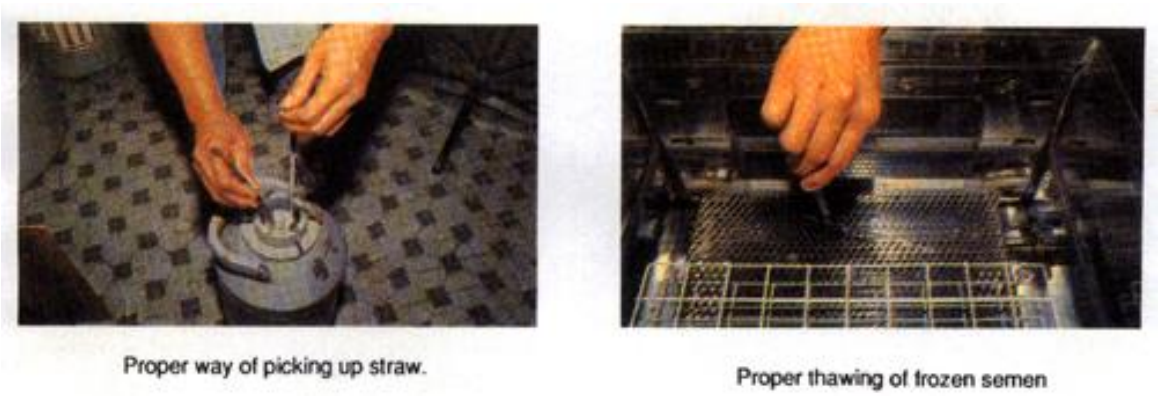
Thawed semen straw

Figure11

3) Semen that is to be stored for longer periods of time must be mixed with a diluter and very carefully frozen. A commercially prepared diluter extender, such as Ortho Semen Diluter is desirable, although plain milk can be used successfully also. Following are steps in semen extending:

a) With a commercial preparation, use a diluter to semen ratio of 19:1, adding the semen to the diluter, and rolling the bottle gently to achieve a thorough mixing. The semen and diluter should be at the same temperature. This mixture can be stored in the refrigerator and used for a week, or slowly cooled and stepwise frozen for storage in a liquid nitrogen tank for later insemination.

b) For a homemade milk diluter, it is best to use fresh 3.5 pasteurized, homogenized whole milk. It must be heated and held at 210F for 10 minutes in a glass boiler, keep the lid in place so that no moisture is lost. Next, the milk is cooled in a water bath with the lid on. When the milk is in equilibrium temperature with the water bath, the water condensation on the inside of the lid is shaken back into the milk. To every 400 cc of milk, add 100,000 units of potassium G crystalline penicillin and 500 mg crystallin di-hydrostreptomycin sulfate, mixing well. Warm this diluter to about body temperature before adding the fresh semen at 19:1 ratio. Place the diluted semen in a water bath at body temperature of 101F and allow cooling slowly. Semen may be frozen, if the extender contains an antifreeze compound, slowly, stepwise for storage on dry ice or in liquid nitrogen.



Proper way of picking up straw.

Proper thawing of frozen semen

Figure12.

15. A microscope, capable of 900x magnification is an essential tool when doing your own semen collection in order to determine semen quantity and quality. First, place a semen sample on a clean slide and cover with a cover slip or another slide. Set the magnification to 400x and observe the appearance of dark patches or spots thru the scope; four dark areas or more per microscope field represent high concentrations of sperm, a really good sample. Three dark areas are somewhat chancy for use at a diluted service, but are good enough for natural service. Two dark areas should be used only for natural services and one dark area means that the concentration of sperm is too low for even natural service.

Equilibration

Figure13.

16. Switching to 900x, the sperm cells can be individually observed for normal structures. Diluting in warm saline is helpful. Coiled tails, broken tails, absence of tails and abnormal shapes all constitute deficient sperm cells. Sixty to 70 2.256835e+199od motility before freezin should be observed in a good sample, with a minimum of 30motility after freezing and thawing. Any insemination program, no matter how carefully carried out, will yield poor results if the concentration and quality of the collected sperm is not of high standards. Sophisticated techniques of washing the sperm free of seminal plasma before extending and freezing will improve post-thaw viability.

Filling and Sealing

Figure14.

17. The concentration of a buck semen ejaculate can be determined accurately by using a red blood cell diluting pipette and standard hemocytometer techniques. Typical results during the breeding season are 3 to 5 billion sperm per cc. Optical density can also be used to estimate sperm concentration if the photometer has been calibrated for buck semen. A simpler technique involves the determination of a spermatocrit using microhematocrit pipettes. The aliquot of semen is centrifuged for 10 minutes; for each percentage point of packed sperm, approximately 200 million sperm cells per cc are present. Correction is made for the percent motile sperm, after which the ejaculate can be diluted appropriately to supply a minimum of 125 million motile sperm in each breeding dose. It is often difficult to introduce more than 0.2 ml of semen into the cervix, so dilution to a final concentration of 600 million to 1.2 billion live sperm per cc has been recommended. When no laboratory support is available, fresh semen for immediate use may be diluted up to 5 times in extender if it is yellowish and 10 times if the ejaculate is white. A straw holding 0.5 cc of this diluted semen will provide adequate sperm if excessive reflux does not occur.

18. Storage and Removal of Semen from the Liquid Nitrogen Tank A liquid nitrogen tank is basically a very large thermos-bottle in which liquid nitrogen is placed to keep the inner temperature near -320F (-196C). The spacing between the inner and outer walls is insulated and under vacuum. The temperature in the tank is maintained uniformly at -320F up to the bottom of the tank neck until the liquid nitrogen level gets down to around 5''. To measure liquid nitrogen, use a piece of black metal rod that is long enough to hold and touch the bottom of the tank. Dip the rod to the tank bottom and remove after 30 seconds. By waving it in the air, a white frost line will appear on the rod. This line indicates the liquid nitrogen depth of the tank. Levels nearing 5'' require a refill. The only real differences between tanks are their storage capacity (number of ampules or straws that they will hold) and their length of holding time (liquid nitrogen evaporation rate). The neck diameter varies somewhat also, with wider openings being easier to work with, but an increased evaporation rate usually results.

19. When working with semen in the liquid nitrogen tank, it is important to keep the racks below the frost line in the neck of the tank. Removal of semen from the tank for periods as brief as 10 seconds, such as for identification, before replacing it to the tank will often result in lowered fertility levels. If the right rack can't be located in 5 seconds, lower the canister back to the bottom of the tank for at least 30 seconds before trying again. Also, when handling semen, try to stay out of any direct sunlight, as ultraviolet light has a spermicidal effect.

20. The semen comes in two basic types of packaging: ampules (1 ml) and straws (0.5 or 0.25 ml). The ampule is the most common type of packaging for buck sperm. Both ampules and straws are stored in racks (canes), which are aluminum pieces that hold a vertical row of ampules, usually six, or two g

21. A few key reminders concerning semen storage:

1) Always keep the liquid nitrogen level above 5’’.

2) Never lift a canister above the frost line of the tank.
3) When the semen is removed with a forceps from the tank it should be placed immediately in the thaw box.
4) Never expose semen to direct ultraviolet light.
5) Never refreeze semen that has been thawed, as it will be destroyed.
6) Check for proper identification on ampule or straw.
7) A defective ampule may blow up after it is removed from the tank. This is due to a small leak that allows nitrogen to enter the ampule. When removed from the tank, the gas expands too rapidly to vent back out the hole and it explodes the glass. A hissing sound is usually audible when it is removed. Keep your hand between the ampule and your face when putting it into thaw box.
8) Always wear gloves and goggles for your own protection when working inside a liquid nitrogen tank.

Transfer of straws from mother tank to field tank.

Figure15.

22. Thawing Procedures

Methods for semen thawing vary among manufacturers, and it is best to follow their recommendation. The thawing procedure for 1cc ampules, the most common for goat semen, is generally the ice water bath:

1) Ice water (38-42F) is placed in a Styrofoam box long enough beforehand to allow temperature to equilibrate.
2) Remove the ampule from tank and place immediately into thaw box. Ample may be placed in a small plastic cup with holes in the bottom. This prevents ice from coming into direct contact with ample.
3) Ample should thaw in 3 to 5 minutes. Check for slushiness and allow more time if needed.
4) Ample may sit in ice water for as long as 30 minutes with no damage. Once removed, the semen must be used right away.
5) The layer of ice on the ampule must be peeled off before opening to avoid possible contamination.

23. The ice water thaw method is especially good during winter breeding of does because of low risk of cold shock to thawed and exposed semen. Thawing of semen can be done from -320F rapidly, but any subsequent exposure to lower temperatures after thawing will kill many or all of the sperm.

24. The warm water method of thawing is more exact than the ice water method, but probably will not work in cold weather, although it may give somewhat better results the rest of the year. The procedure is basically the same as for the ice water thaw except that:

1) The water must be maintained at 92 to 98F. This requires a source of warm water and an accurate thermometer.
2) Thawing will be complete in about 1 minute with no ice layer formation of the ampule.
3) Ampules thawed with the warm water method should be used within 5 minutes.

25. Straws (0.5 or 0.25 ml) can be thawed by either of the previous two methods. A given amount of semen in a straw will take about one half as long to thaw as an equal amount in an ampule. Many inseminators simply thaw straws by placing them into their shirt or pants pocket.

26. Inseminating Procedures

All the care in handling, storage and preparation of semen will be useless if the inseminating process is not done carefully and cleanly. Hygienic practices at this point cannot be over-emphasized. All reusable items such as inseminating guns (for straws), scissors for cutting straws, scribe for cutting ampules, etc. must be wiped clean with 70 0sopropyl alcohol and allowed to dry before reuse. Disposable items should be kept in their sealed packages until they are to be used. The speculum should be sterilized after each use (this is one reason why the cattle industry discontinued the speculum method; the

inseminator would have to carry a few dozen specula on his daily rounds, sterilizing them each night). This is best accomplished by boiling for 10 minutes, allowing to air dry. Then place inside a sterile container or wrapping, such as a new plastic AI glove. Disposable plastic type specula for goats can be obtained from mail order companies, eliminating the need for constant resterilization.

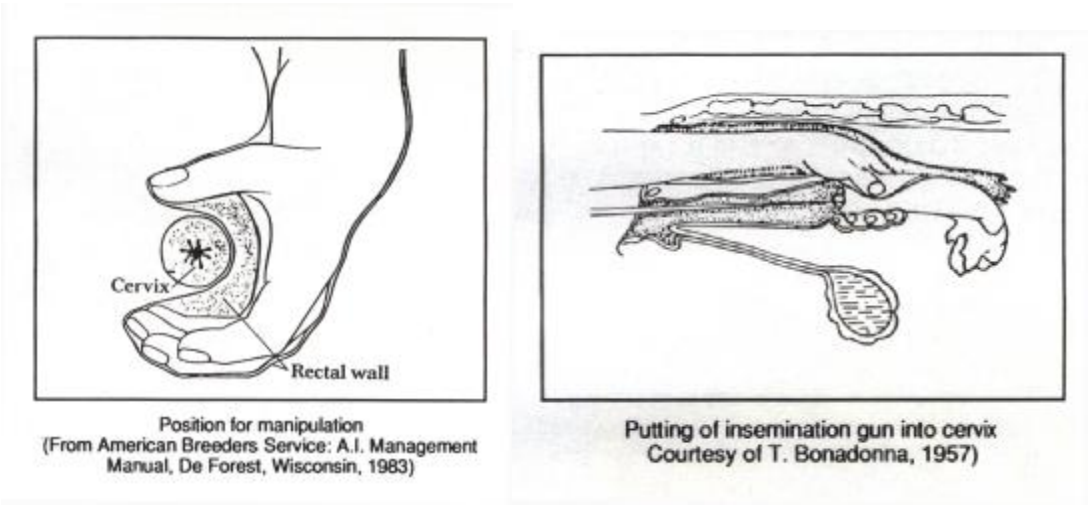


Figure16.

27. Materials needed for artificial insemination:

1) Speculum, Pyrex 22 x 175 mm for doelings; 25 x 200 mm for adult does; or stainless steel human vaginal speculum; or plastic disposables; with a small clip-on flashlight.
2) Sterile lubricating jelly (K-Y)
3) Thaw box
4) A. Inseminating pipette with bulb or syringe (ampules only) or b. Inseminating gun (straws only)
5) Paper towels
6) Facility for securing doe (stanchion, fence, rope hoist)
7) Recording journal for breeding dates, buck's name, etc.

Figure17.

28. Preparing Ampules:

1) Partially remove an inseminating pipette from its plastic bag.
2) Place bulb or syringe on exposed end.
3) Thaw ampule according to the described methods.
4) Dry ampule after thawing, hold in paper towel and scribe (with proper tool) one side of ampule collar. Some ampule types do not need to be scribed, but can be snapped open.
5) Pull syringe back 1/2 cc on plunger or squeeze bulb closed before placing pipette into ampule.
6) Tip ampule to slight angle and maintain constant suction on pipette while it is slowly inserted into the ampule. Try to get all the semen into the pipette, keeping the semen column down near the end of the pipette.
7) When filled, the pipette should have a semen column with no air spaces, with the bottom of the column being 1 to 2'' from the pipette tip. Do not draw semen into the syringe or bulb.
8) Keep the ampule for information to complete breeding records.
9) Keep the pipette away from sunlight or cover with paper towels.

10) The semen is now ready to be placed into the doe in estrus.

29. Preparing Straws:

1) An inseminating gun, designed for your type of straw is needed, obtainable thru farm supply houses or the local cattle AI technician. Have cover sheath available, sealed until needed.

2) Place straw in thaw box.

3) Remove when thawed, wipe dry. Check buck information.

4) Pull plunger on gun back 4 to 6'' and insert straw into gun, cotton plug end first (towards plunger).

5) Hold gun in upright position, allowing air bubble to rise to the sealed end.

6) Cut sealed end of straw with scissors. Take care to cut straw squarely for proper seating.

7) Install the sheath over the gun, fastening it down with the provided O-ring. Install it so that the wider side of the ring faces the straw, with the narrower side facing the syringe end.

30. Insemination:

Assuming that the doe has been observed in heat has been suitably restrained (i.e. in stanchion) and the steps for preparing the ampule or straw have been followed. The next steps are:

1) Position doe on milk stand. The inseminator places his left foot on the stand and drapes the hindquarters of the goat across his horizontally positioned thigh. The goat is allowed to stand as long as she does not struggle or collapse. The vulva is cleaned.

2) Hold pipette or inseminating gun, wrapped in a paper towl, in your mouth; or let someone else hold it if extra hands are available.

3) Turn head light on and insert lubricated speculum in a slow and gentle manner. Begin entrance at a somewhat upward angle for the first several inches. This is to prevent the speculum from scraping across the vaginal floor, possibly doing damage to the urethral opening.

4) Complete insertion of speculum and locate cervix. Center the end of the speculum over the os uteri (entrance to cervical canal).

5) Cervix should be of a red-purple coloration with a viscous whitish mucus present if doe is truly in heat.

6) Insert pipette or inseminating gun into speculum to the cervix. Gently manipulate the instrument through the cervical canal (cervix is 1 to 2'' long) to the 4th or 5th annular ring.

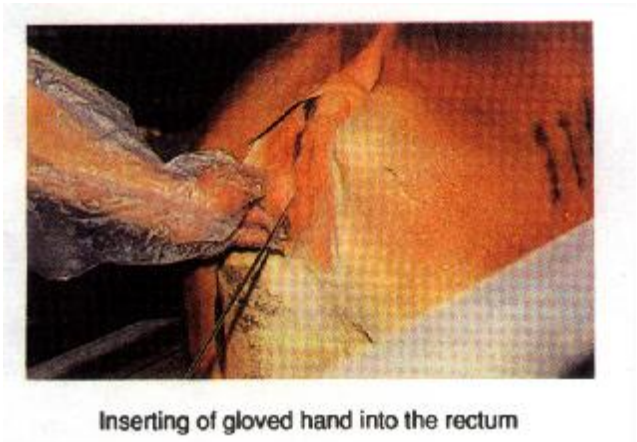
7) Deposit semen near the uterine end of the cervix or just inside the uterus. Do not enter too far into the uterus, as the semen will then tend to be dumped into one horn or the other. If the semen is pushed into the wrong horn (i.e. egg produced in left ovary, semen dumped into right horn) then fertilization may not occur.

8) Deposit semen slowly, taking at least five seconds.

9) Slowly withdraw instrument without release of syringe or depressed bulb, then carefully remove the speculum.

10) Record all pertinent breeding information.

11) Carefully discard all disposable materials. Arrange to sanitize reuse able items and sterilize the speculum (if it is a non-disposable type).



Inserting of gloved hand into the rectum

Figure18.

Figure19.

31. Frequently, the pipette cannot be passed all the way through the cervix even though the doe is in heat. If it has penetrated deeply into the cervix (3 to 4 cm, as determined by laying another pipette alongside the first and observing the distance by which the outer ends are offset), cervical insemination will provide a conception rate almost equal to that of intrauterine semen deposition. The conception rate expected from intra-vaginal insemination, however, is less than 30 if semen is very valuable, it may be advisable to pass a trial pipette to Determine potency of the cervix before thawing the semen unit.

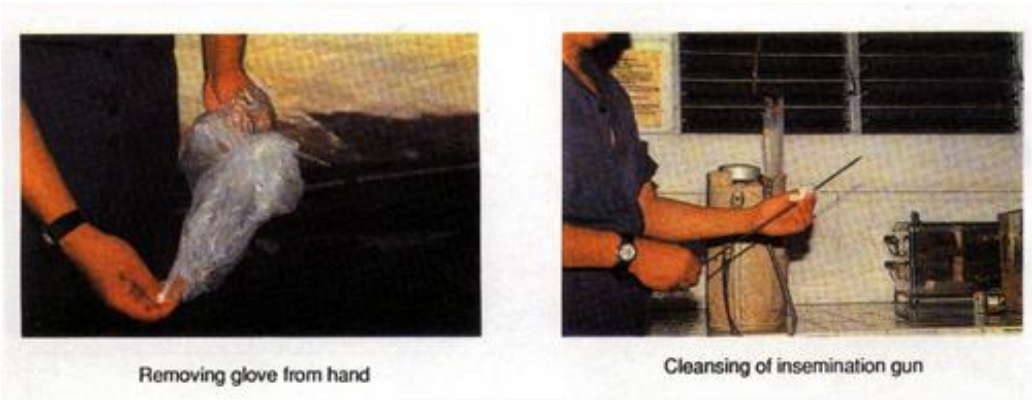


Figure20.

32. In France, a doe is usually restrained by a second person that straddles the doe's neck and elevates the hindquarters to a vertical position while holding the hind limbs tightly flexed. The inseminator is free to stand in a comfortable position. He holds the speculum and the goat's tail in one hand and the pipette or straw gun in the other hand. If excess mucus is a problem, the assistant lowers the goat's hindquarters almost to the ground; if the mucus does not run out of the speculum, the latter is removed and shaken to clear it. The goat is then lifted to its former position. If many goats are to be bred, the assistant may tire using this technique. If the doe is not held in a vertical position, it is often impossible to adequately visualize and penetrate the cervix. Various slings have been devised to suspend the goat in the appropriate position.

Reference:

HANDBOOK MANUAL OF ARTIFICIAL INSEMINATION IN LARGE RUMINANTS: DA-BAI JICA/JOCV National Artificial Insemination Program
THE MERCK VETERINARY MANUAL Veterinary Medicine: OMRADOSTITS DC BLOOD CCGAY.