Artificial Insemination in Goats

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Introduction

Artificial insemination (AI) is a technique for placing collected and fractionated semen in the female reproductive tract. Basically, it is used to disseminate desired productive characteristics of males of high genetic value, at any time of the year.

It must be considered that AI is only a tool within a programme of genetic improvement.

Frozen semen technology leads to further multiplication and spread of genes. Also, unlimited conservation of genes is possible.

Using frozen semen can greatly improve genetic improvement globally, by considerably increasing the flow of genetic material from superior flocks to ordinary flocks. In addition, worldwide transport of semen is facilitated, and the expense of moving males is avoided and sanitary risk decreased.

Anatomy and goat reproduction

A basic understanding of the reproductive tract and physiology of the caprine female, will permit a better understanding of what follows.

The reproductive system comprises: ovaries, oviducts, uterus and vagina. Oocytes or eggs originate in the ovaries. Oviducts are small ducts guiding oocytes to join spermatozoa, to produce fecundation. Then, through successive cell division, the embryo is formed and descends along the oviduct to the uterus where gestation develops.

An anatomic differentiation of the uterus, the cervix, connected to the vagina, will require further consideration in relation to AI. The vagina is the female copulating organ and it ends in the vulva. Female goats that have never given birth present a fibrous growth known as hymen, which can be detected by introducing the finger into the vagina. Normally, it ruptures when giving birth and can be used as a sign of the female first kidding.

Goats are seasonal breeders. The beginning and duration of the reproductive period depends on their geographic location, is longer in tropical regions, and decreases with increasing latitude. Other factors affecting reproductive activity are environment, breed, and nutrition.

Estrus behaviour in goats is cyclical and seasonal (autumn-winter). The time interval between estrus is known as the 'sexual or estrous cycle', and it normally lasts 19 to 21 days.

The estrous cycle comprises four periods. Proestrus is the day before the beginning of estrus. During this short period, goats act restlessly and evasively in the presence of repeated male mounts. Externally, a swollen and reddish vulva discharging mucus is evident, though more visible in the adult female than in a young female.

The following period, the estrous period, shows a marked change in sexual behaviour and females accept being mounted several times. At the same time, external signs in the vulva are more evident. This period lasts 18 to 63 hours, though more frequently 24 to 36 hours. It must be considered that estrus in young animals is less visible and of short duration.

Once the estrus is over, a phase known as metaestrus begins, during which ovulation normally occurs. The last stage of the cycle is the diestrus period, that extends until goats start a new sexual cycle, unless already pregnant.

The estrous cycle previously described is regulated by four hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), both generated in a gland in the brain hypophysis (pituitary gland), together with estrogen and progesterone, produced by the ovaries.

The FSH stimulates the development of the ovary follicles and oocytes production, and the LH acts in the final stage of the follicle growth and consequent ovulation.

Estrogen is secreted by mature follicles and its increment triggers estrous behaviour and copula acceptance.

After ovulation, the luteal body is formed, which in turn, produces progesterone to maintain gestation if the female is pregnant. If not, the luteal body gradually loses its biological activity and a new estrus will occur 19 to 21 days after ovulation. This process continues during the reproductive period and is only interrupted by pregnancy, illness or insufficient nutrition.

Finally, there is oxytocin, a hormone produced by the hypophysis, to facilitate sperm transport in the female reproductive tract and stimulate the initial production of milk.

With regards to males, they are capable of fertilizing throughout the entire year, though seasonal variations in sexual activity (libido) and semen quality are observed. During autumn, the reproductive season, sexual capacity increases, although subjected to the same modulating factors mentioned above for the females.

In males, FSH is involved in the generation of sperm, while LH acts on the testicles by stimulating the production of androgens (testosterone), and together, they promote spermatozoa maturation.

Testosterone acts on sexual activity or libido, secondary sexual characters and male sexual behavior.

Knowledge about reproductive activity in goats will permit the operator to correctly manage hand-mating or AI.

Some aspects to consider with respect to hand-mating or AI:

- During the breeding season, estrus appears every 19 to 21 days.
- The majority of female goats remain in estrus (accept copula) for 18 to 36 hours.
- Ovulation usually takes place between 6 hours before, to 12 hours after, estrus is over (the female rejects mounting).

Estrus detection

Identifying estrus can involve pen or field procedures. In the first case, work is carried out with few animals at a time (20 to 25 females with 2 teasers, or harnessed males in a pen), bearing in mind that estrus in young females may pass unnoticed, as it is not so evident. It is good practice to change heat-seeking males if they show little sexual interest. Estrus detection performed in the morning must start when the flock becomes unaffected by cold temperatures.

The field procedure or paddock mating involves the use of teasers with marker jackets, or with chest painted with ferrite. The ratio of males to females must be 1 to 20.

Controlled breeding

To identify progeny sire, two techniques can be performed:

- Hand-mating: once the female is identified in estrus, only one mount would be sufficient to ensure pregnancy.
- AI programme: insemination is carried out 12 hours after estrus is detected. It can be repeated every 12 hours as long as the female accepts being mounted by a teaser or harnessed male.

Handling and examination of semen

Evaluating male reproductive capacity comprises its ability to mount and ejaculate fecund semen.

When starting a semen collection programme, for use as fresh or frozen semen, or for seminal analysis, nutritional and sanitary aspects of the males must be taken into consideration and a clinical examination is required.

The most recommendable procedure to collect semen is the use of an 'artificial vagina'; providing thermal (temperature) and mechanical (pressure) stimulation to provoke ejaculation. It consists of an external rigid part, for example a thermal polypropilene pipe (17 cm x 5.5 cm), with an interior latex lining. This last is folded back and fixed to the ends of the external tube using elastic bands, so as to create a hermetic compartment for the water. A collecting glass is placed at one end of the vagina.

The artificial vagina is two thirds filled with hot water (50°C or more), depending on heat loss while not in use, requiring that the interior vagina temperature during ejaculation be around 38-40°C. Final conditioning of the vagina involves introducing air into the water jacket to produce an interior vaginal diameter of around 1 cm.

Semen collection is performed in a clean and dust-free environment. Prepuce is washed with physiological solution and foreskin hairs shaved off to reduce possible semen contamination.

Male goats generally adapt with ease to ejaculation in the artificial vagina. The technique requires displacing the penis manually when the animal mounts an immobilized female. The collecting glass must be

protected from sharp temperature changes throughout the whole operation and until placed in a water bath at 36°C.

When males are reluctant to mount the female, the latter can receive an intramuscular injection of 0.5 ml of estradiol cipionate (ecp estradiol, Konig, Argentine). Treated females will exhibit estrus 48 hours later. It is necessary to repeat the dose every second day to maintain estrus induction.

When the semen collection season starts, and if the animals have not been sexually active, it is convenient to discard the first ejaculates for freezing. This ensures the removal of old sperm reserves. But if the males have been mating, a sexual rest of one week is recommended before semen collection begins.

Male goats can exhibit sexual activity during the whole year, although their service capacity (number of females mated by day), and also sperm quality, is lower during spring and summer than in autumn.

Frequency of semen collection depends specifically on each animal. When carrying out a frozen semen programme, one or two semen collections per day are recommended and during 4 to 5 consecutive days; then a 2 or 3 day rest should follow.

Once semen is obtained, it must be placed in a water bath at 36°C; volume, density and colour are registered. A small drop on a pre-warmed slide can be used to assess mass movement or sperm motility and thus estimate the percentage of live spermatozoa and vigour. Volume, percentage of live spermatozoa and semen concentration vary between individuals and even between ejaculates from the same animal. Average values for goat ejaculates are: 1 cm^3, 85% and 3,500 million spermatozoa per ml, respectively.

Artificial insemination

AI and genetic improvement programmes are usually assigned to superior genetic animals of the flock or nucleus. Before incorporating animals into an insemination programme, the following nutritional, sanitary and reproductive factors must be taken into account:

- Females must have a body condition (BC) score of at least 2 at insemination. BC is a subjective value that measures fat deposited in lumbar muscles below lumbar vertebra (maximum, 5; minimum, 0).
- Females should be free of illness and parasites.
- Kids must be weaned 6 to 8 weeks before insemination.
- 'Old' animals and those with udder problems (blind nipples, cut udders, mastitis), as also those unable to become pregnant for two consecutive years, should be discarded.

Estrus synchronization

Estrus synchronization methods prove of great utility when hand-mating, paddock mating or AI are carried out. They greatly simplify animal handling as it becomes unnecessary to enclose them daily for 21 days in order to detect normal estrus. These methods can be classified as: 1) natural and 2) pharmacological.

1) Natural methods. In Patagonia, where producers keep males separate from females out of the breeding period, the introduction of males into the herd at the beginning of the mating period produces a high concentration of estrus in females (around 50-60%), between 8 to 12 days following their incorporation. This sexual stimulus is known as the 'male effect' and can be employed as a 'natural and economical estrus synchronization method' both for hand-mating or AI. Estrus which occurs previously, usually prove to be of low fertility, and non-pregnant females repeat heat, now fertile, after 5 to 7 days. Following this period of concentrated estrus, there is 0 to 3% daily heat with good fertility.

The 'male effect' can be used in combination with other estrus synchronization methods at the onset of the breeding season. This ensures that all females will be cycling when hormonal treatment commences.

The introduction of males in the herd must take place around 20 days before estrus synchronization activities are undertaken.

2) Pharmacological methods. These have the advantage of concentrating a high estrus percentage in a short period of time, which facilitates the programming and carrying out of AI tasks.

Intravaginal sponges with progestagens simulate corpus luteum secretion, by slowly liberating progesterone. They are placed in the goat

vagina for 15 to 17 days, a period of time coinciding with the average life of the corpus luteum.

This method produces an intense concentration of estrus and makes possible *timed artificial insemination*, this is to perform AI after a fixed period of time following the end of the hormonal treatment. It also concentrates estrus out of the reproductive season, and consequently allows the production of kids out of season.

As there is a variable percentage of females which do not respond to this treatment or which do not present synchronized ovulation, as well as an altered sperm transport due to the use of progestagens, the utilization of intravaginal sponges in combination with a dose of equine Chorionic Gonadotrophin (eCG), is recommended. It is administered by intramuscular injection at progestagen removal when used during the breeding season; and 48 hours before progestagen withdrawal when used during the anestrus period.

Synchronization of estrus and ovulations results in summary. Doses of eCG used vary from 200 to 400 UI, depending on body weight, breed and time of the year. It is recommended to start with the minimum dose. High doses of eCG risk causing multiple pregnancies, leading to serious loss of animals due to perinatal mortality.

Intravaginal sponges can also be combined with the 'male effect' instead of applying eCG. In this case, males are introduced in the herd 48 hours before the removal of sponges.

Placing and removal of sponges

1. Before introducing sponges, it is advisable to spray them externally with a corticoid-free antibiotic aerosol.
2. Compress the sponge and introduce it in the bevilled edge of the applier, making sure the string hangs outside.
3. The rod is placed in the applier through the free end till it touches the sponge.
4. The applier is moistened externally with vaseline.
5. To make the placing of the sponge easier, it is convenient that the female stands in a natural position. The applier and rod are introduced gently to the bottom of the vagina.

6. The applier is removed 3-4 cm, keeping the rod in place, until the sponge is free.

7. The sponge string should be cut so that it projects 2-3 cm from the female vagina (to avoid loss of sponge).

To remove the sponge, pull the string firmly but gently backwards, with a slight downward inclination. If the string is not visible in any animal, it is advisable to verify that the sponge is not in the interior of the vagina, by using a vaginoscope.

Sponge placing is not recommended when dealing with young goats that have not given birth, as tearing of the hymen when the sponge is introduced will result in sponge laterals adhering to the internal walls of the vagina at sponge removal. An alternative is to break the hymen with the sponge applier and place the sponges one week later.

The cost of estrus synchronization using sponges and eCG is about 1 dollar per goat.

In the case of Angora goats, 60 mg of medroxyprogesterone acetate (MAP) has been used for 17 days, in combination with the male effect (4% teasers for 48 hours), before sponge removal. A total of 80-90% of females exhibited estrus between 24 and 72 hours after sponge withdrawal.

There is an alternative variation to this method which allows reducing the time interval between the insertion and removal of sponges, for up to 11 days. This requires the application of 100 μg of cloprostenol (prostaglandins) and a corresponding dose of eCG, 48 hours before removing pessaries. The application of prostaglandins on day 9 after sponge insertion shortens the biological activity of the corpora lutea which are still functional.

Handling of fresh semen

Artificial insemination with fresh semen means the immediate use of the ejaculate obtained, by depositing it in the female reproductive tract. Once analyzed and found suitable, namely complying with minimum requirements for use, it can be diluted or else split into fractions to obtain 100 million spermatozoa per dose, not surpassing 0.25 cc per dose. Thus,

for example, an ejaculate containing 3,000 million/cc of semen can be used to inseminate 30 females.

Semen can be employed pure, without dilution, using 0.03 cc per goat (1 cc/30), or diluted, by adding 10% cow skimmed milk. Dilution increases conservation (as a reference: approximately 1 hour between ejaculate obtention and last insemination). Furthermore, dosage is made easier. For example, in the case of the values given above, and by adding 2 cc of diluent, 30 insemination doses of 0.1 cc per animal are obtained.

The diluent is preheated to 92-94°C for 10 minutes in order to inactivate a spermicidal factor – lactenin - present in the protein fraction of the milk. Then it is cooled to 28-30°C and added to the ejaculate, pouring along the tube walls and mixing by oscillatory movement.

Determining spermatozoa motility every 5 or 6 inseminations is very important. This ensures that fecundity is conserved and has not suffered alterations as a consequence of undetected technical accidents (contamination with water, thermal stress, etc.).

Fresh diluted goat semen refrigerated at 5°C must not be conserved for more than 8-12 hours, as it loses its fertilizing capacity. Diluent is prepared with 10% skimmed milk, 1% glucose and by adding trace amounts of antibiotics. Reference insemination doses for refrigerated semen contain 150 million spermatozoa per goat.

Handling of frozen semen

Thawing frozen semen is performed at 36°C. Once thawed, it should be employed quickly. If semen was frozen in pellets, thawing can be performed in dry hemolysis tubes warmed at that temperature, in a water bath.

Doses frozen in straws are thawed directly in a water bath. After 30 seconds, the straw is removed from the bath and dried with a discardable paper towel. Both ends are cut off for emptying it into a hemolysis tube. It is important that canisters are not raised above the open end of the liquid nitrogen thermos during the thawing procedure.

It should be considered that AI activities must take place in an air-conditioned environment, at 25°C.

A liquid nitrogen thermos has double walls (external-internal) separated by vacuum, to preserve cold temperatures. Inside the thermos, temperatures are stable, while a minimum 12-14 cm of nitrogen is maintained. To determine the quantity contained, a wooden rule painted black is used. The rule is submerged for 30 seconds, removed and shaken until a white frozen layer appears, and the height of this frozen layer corresponds to the liquid nitrogen level. It must be taken into consideration that as the volume decreases, the loss of nitrogen through evaporation increases.

Great care must be taken when handling liquid nitrogen as direct or indirect contact with such low temperatures can produce serious injury.

Cervical artificial insemination

In caprine species, unlike ovines, cervical AI can be used for either fresh or frozen semen. Owing to difficulties encountered in introducing the insemination sheath through the cervix, besides reduction in sperm viability as a result of freezing and thawing, an increase in the number of spermatozoa per dose is required when using frozen semen in comparison with fresh semen (200 and 100 million spermatozoa, respectively).

The place where insemination is carried out must be clean and free of air currents, at temperatures of 20-25°C. Females are restrained as quickly as possible in a bail or race, in a standing position, to avoid unnecessary stress. The race can be constructed on the floor of the shed or elevated off the floor to a more comfortable height for the inseminator. The method which allows most convenient presentation of goats for cervical insemination is referred to as the 'over the rail' method. Females are presented for cervical AI by an assistant standing in the race and lifting the hindquarters of the animal over the rail.

The vulva is cleaned with a discardable paper towel and a small quantity of vaseline is applied to the vaginoscope, to facilitate its introduction. As one hand holds the tail of the animal, the other slowly introduces the vaginoscope in dorsal direction (of the animal). Once having penetrated a few centimeters, it must be directed horizontally to the bottom of the vagina. The cervix can be located by gentle manipulation of the vaginoscope sideways or downwards. If abundant mucus is present, this can be absorbed and removed by means of a plastic pipette with a syringe.

When this is done, the semen is handed over by an assistant. The tip of the insemination sheath is guided towards the entrance of the uterine orifice and introduced with gentle rotating movements, without hurting membrane mucosa, until resistance is encountered (Figure A).

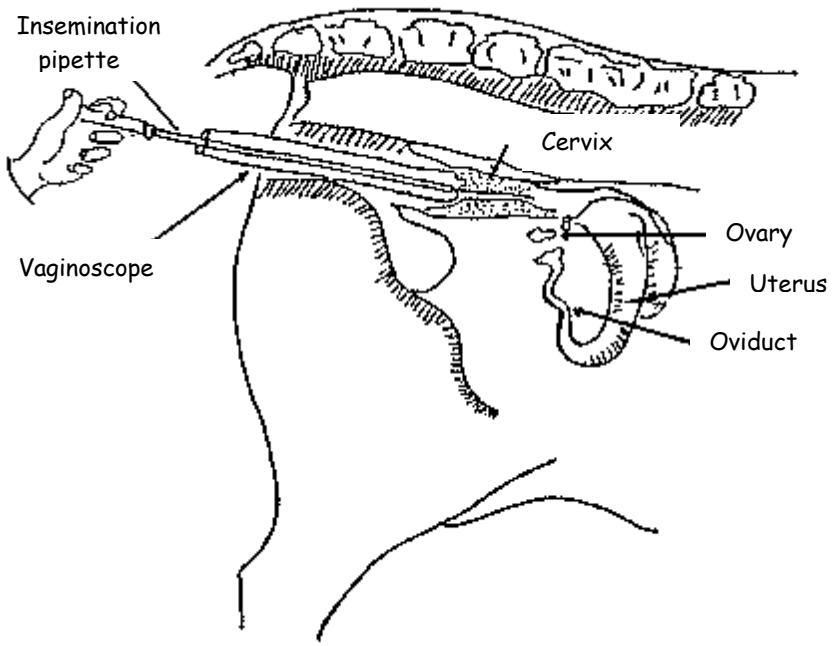

Figure A. Cervical artificial insemination.

Once semen has been discharged, it is advisable that goats remain for 2 or 3 minutes in the insemination position and then be placed in a pen next to males for a few hours.

In the case of young females, placing the vaginoscope is very traumatic. So it is preferable to perform AI by introducing only the insemination pipette in the vulva and discharging semen at the bottom of the vagina.

Laparoscopic artificial insemination

Endoscopy is performed by introducing an optical system into the animal body via abdominal puncture; a flexible optical fiber provides light so that internal organs can be observed without resorting to surgery.

When AI is carried out, this technique is known as laparoscopy, as it permits observation of interior reproductive organs, via the abdominal wall.

A small orifice is made with a trocar close to the mammary gland and the dose of semen is inseminated through it. Insemination consists in injecting half the semen dose in each uterine horn by means of a pipette supplied with a fine needle at one end (Figure B).

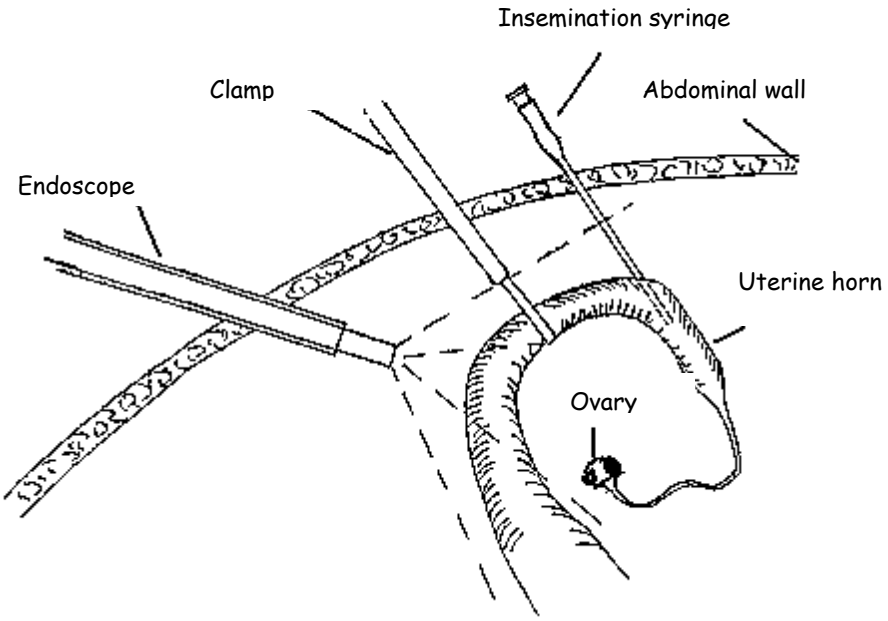

Figure B. Laparoscopic artificial insemination.

This technique of insemination by laparoscopy is widely used in ovine species, because of low pregnancy rates obtained when using frozen semen via cervical insemination (20-35%). This is due to the difficulty in transposing the ovine cervix with an insemination pipette.

In goats, it is used with a double purpose; to increase the pregnancy rate above that obtained via cervical AI, and also to reduce the number of spermatozoa in each insemination dose.

SEMEN	VIA	DOSE	AI	PREGNANCY
FRESH	Cervical	100 mln	With estrus detection	60-70%
FROZEN	Cervical	200 mln	With estrus detection	50%
			Timed AI*	(±10%)
	Laparosc.	50 mln	With estrus detection	55%
			Timed AI**	(±10%)

* 45 hours after removal of progestagen sponges
** 55 hours after removal of progestagen sponges

Frozen storage of semen

Procedure for freezing goat semen is as follows:

a) Preparation of seminal diluent

Skimmed powdered cows milk is diluted in distilled water (10%), for example 50 ml distilled water, 5 g skimmed milk. The diluent is kept at 92-94°C in a water bath for 10 minutes. After this time, it is cooled to 36°C, and 1% glucose (for example, 0.5 g glucose in 50 ml diluent), penicilin and streptomicine are added (1000 UI and 1 mg per ml of diluent, respectively). The resulting volume is divided into two equal parts: DILUENT A without adding glycerol, and DILUENT B adding 12% glycerol. Thus, for example, for 25 ml of diluent B, add 3 ml glycerol. Glycerol is warmed and added to the diluent, still hot, to obtain good homogeneity. Diluent A is maintained at laboratory temperature, while diluent B is placed in a refrigerator at 5°C (Very important: refrigerator temperature must not fall bellow 5 °C).

If work is carried out in hot surroundings, it is convenient to place solution A in the refrigerator to avoid it becoming acid and then warm it shortly before use. The pH of diluent must be between 6.8 and 7.2.

b) Collection and assessment of semen

Semen collected in an artificial vagina is maintained in a water bath at 36°C for evaluation. A drop of semen on a slide is placed on a warm stage on a microscope and examined with 100x magnification. An idea of the semen quality is estimated from the vigour of wave-like motion (spermatozoa motility), as waves form and disappear. Motility is estimated on a subjective scale (minimum: 0; maximum: 5) based on vigour of movement. Semen is frozen only if spermatozoa motility is equal or greater than 4.

Semen must be constantly protected from rapid temperature changes, from contact with water or with direct solar radiation, or from impurities. Therefore all materials must be clean, dry and maintained at semen temperature.

c) Determination of spermatozoa concentration

This is performed in a Neubauer Chamber. In loading the chamber for spermatozoa counting, the following steps must be followed:

- Adhere the slide cover to the chamber by moistening the edges with vaseline or saliva, pressing firmly against chamber. If good adhesion is obtained, 'Newton´s rings' due to light diffraction will appear at the edges of the cover.
- Introduce (aspirate) semen in a red corpuscle model pipette which must have a temperate and perfectly dry tip, until the 0.25 mark is reached.
- Clean tip of pipette, being careful not to change the semen level.
- Aspirate dilution liquid (can be common water) until mark 101.
- Cover both ends of pipette with fingers and shake horizontally gently around 30 times.
- Discard initial drops.
- Place pipette tip on the border of the slide cover and let the chamber fill by capillarity. The liquid must not surpass lateral grooves, and air bubbles or empty spaces must not remain.
- Wait a few minutes before starting the count.

The chamber can also be filled with a micropipette, using 5 microlitres of semen diluted in 2 cc of water.

Place the chamber for observation in a microscope (100x to 200x magnification). If spermatozoa distribution is not homogeneous, repeat the loading process. Number of spermatozoa in a big square (without internal divisions) is counted per quadrant, and the count is repeated in a randomly chosen quadrant, until five squares in all have been counted. The concentration of spermatozoa/cc is calculated by multiplying the total sum in five squares by 12,800,000.

d) Centrifugation of semen with washing solution

Seminal plasma in goats presents certain composition peculiarities. One of them is the presence of a proteic fraction (BUIII), produced by the bulbo-urethral glands, which interacts with the milk diluent and causes inhibition of sperm motility. To avoid the negative effect of this fraction, it is necessary to 'wash' the semen, to separate and eliminate seminal plasma by centrifuging before mixing the semen with diluent.

A 2.8% sodium citrate solution (2.8 g sodium citrate in 100 ml distilled water) can be used as a washing solution. Solution pH must be 6.8

to 7.2. Periodic pH controls of the washing solution and diluent must be performed.

To dilute seminal liquids, add 10 ml of washing solution and centrifuge for 10 minutes at 2000 rpm. Remove supernatant liquid with a pipette, then repeat the operation so that the sperm mass deposited at the bottom of the centrifuge tube is dissolved in the washing solution. Sodium citrate solution must be added at the same temperature as the semen. Therefore the first washing is performed at 36°C and the second, owing to the temperature drop during centrifugation, at 20-25°C (laboratory temperature).

e) First dilution (addition of solution A)

Volume of diluent to add to semen is calculated on the basis of indications in the annex. Owing to removal of seminal plasma after centrifugation, 10% is discounted from total spermatozoa estimated in the ejaculate (loss of spermatozoa when supernatant liquid is removed). Diluent A is added at 20-25°C.

f) Temperature descent and stabilization time at 5°C

Once diluent A has been added, the diluted semen is cooled from the temperature of dilution down to 5°C. Cooling is performed at a rate of 2°C per 3 minutes. The diluted semen must remain at this temperature in a refrigerator for 45-60 minutes before adding diluent B.

g) Second dilution (addition of solution B)

Diluent B is added in three equal fractions, at 10 minute intervals and mixing homogeneously each time. It is advisable to place the pipette in the refrigerator before carrying out the dilution. Ten minutes after adding the last portion of solution B, semen is prepared for freezing.

h) Preparation of semen for freezing

Semen can be frozen in straws with liquid nitrogen vapours (at -196°C) or in dry ice tablets (solid carbon dioxide at -79°C). Semen frozen by either of these two methods is kept in a liquid nitrogen thermos.

Freezing in pellets is easier than in straws, but the latter are easier to tag individually and are easier to manipulate during AI.

It is important to perform freezing in a low temperature environment and also to ensure homogeneous semen doses, to obtain similar seminal quality. Special care is required in identifying different lots.

1. Freezing semen in pellets

To freeze semen in pellets, a block of dry ice with a flat surface is required. Using flame preheated nails, cells are molded in its surface.

Semen is taken out from refrigerator in a water bath at 5°C. Another container with remaining diluent, as a cooling agent, is used to keep the glass pipettes at low temperature.

Once semen is aspirated in the pipette, volumes of 0.25 ml (±4 drops) are deposited in each cell, rapidly and in succession, trying not to delay more than a minute between the first and last pellet. The semen doses remain there for 1-2 minutes until their surface turn opaque. Then they are transferred to the liquid nitrogen thermos.

2. Freezing semen in straws

The straws, polivynilic alcohol, and a syringe provided with a 1.5 cm needle, are maintained in the refrigerator prior to freezing. Straws are filled by aspiration through the triple plug (cotton wool – polivynilic alcohol – cotton wool), placing the plug-free end in the semen. Straws are held from the plugged end (in order to avoid hand warmth affecting semen quality). Polivynilic alcohol in the triple plug jellifies and seals when aspirated semen touches it. Straws are dried with absorbing paper and an air-filled space measuring 1.5 cm is formed at the plug-free end by means of the syringe with appropiate needle. This end is then sealed by soft, vertical tapping on a plaque containing polivynilic alcohol. Then straws are immediately submerged in a container with water bath at 5°C.

A thermally insulated box, with cover, measuring approximately 39 cm long, 34 cm wide and 25 cm high is necessary. Also necessary are wooden blocks, a grid, and an aluminium frame, measuring 11 cm, 7 cm and 2 cm, respectively. The aluminium frame on wooden blocks and grid will reach, with respect to the bottom of the box, 20 cm in height (1st level). When

removing the grid, the height of the aluminium frame on wooden blocks, will reach 9 cm (2nd level).

Liquid nitrogen is poured into the box reaching 6 cm height from the bottom. Cover for a few minutes till 'boiling' is over and the interior is cold. After drying the straws, these are placed horizontally on the aluminium frame, making sure they do not touch each other.

Straws on the frame are placed in the interior of the box in the 1st level, for 2 minutes. Then the box is uncovered and straws are placed 3 minutes in the 2nd level (after removing wooden blocks).

Finally, the straws are poured into the liquid nitrogen and placed in canisters in a liquid nitrogen thermos.

i) Thawing and evaluation of the seminal batch

Semen is thawed as explained previously (see: Handling of frozen semen). By evaluating 10% of the frozen doses, acceptance or not of the batch is decided. Several observations of the same straw or pellet must be performed.

After 5 minutes of incubation at 36°C, sperm motility is evaluated with 100x magnification using a warmed slide over a thermal plate. A drop of semen is placed between the warmed slide and cover, to estimate the percentage of live spermatozoa and progressive individual motility (forward moving velocity of live spermatozoa: 0, minimum; 5, maximum).

For acceptance of the seminal batch, straws or pellets must have:

a) Masal motility post thawing.
b) Percentage of live spermatozoa greater than 30%.
c) Progressive individual motility equal or greater than 2.5.

j) Thawing semen for AI

Thawing is performed in a water bath at 36°C. The content of each straw or pellet is poured into a hemolysis tube and introduced in the insemination syringe.

Annex: Caprine protocol for semen freezing

buck id.	Ejaculate volume	Spz conc	Total spz	Total spz x 0.9	Number doses	Total volume	Diluent volume	Vol solution A	Vol solution B	Vol B/3
1	2	3	4	5	6	7	8	9	10	11

1. Animal identification
2. Ejaculate volume
3. Spermatozoa concentration
4. Total number of spermatozoa: Ejaculate volume x Spermatozoa concentration
5. Total number of spermatozoa x 0.9
6. Number of insemination doses:
   Total number of spermatozoa x 0.9 / Number of spermatozoa per dose
7. Total volume (ejaculate + diluent): Number of insemination doses x Dose volume
8. Diluent volume: Total volume – Ejaculate volume
9. Volume solution A: Diluent volume / 2
10. Volume solution B: Diluent volume / 2
11. Volume solution B / 3