Production and storage of goat semen for artificial insemination

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Abstract

Environmental influences on reproduction and semen production in the buck, the problem of interaction between seminal plasma and egg yolk or milk constituents in diluent, liquid storage and processing of semen for freezing are discussed. A review is given on the use of frozen-thawed semen for artificial insemination (AI) in spontaneous and induced oestrus and factors influencing the fertility. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Artificial insemination (AI) has an important role in goat breeding, especially in intensive systems of production, to control reproduction and, in conjunction with accurate progeny testing, to improve the production of milk, hair and meat. At the farm level the control of reproduction in particular populations of goats allows kidding at a precise season of the year, a synchronization of kidding over a limited period of time, and facilitates supplementary feeding to meet the demands of lactation. Other advantages of AI include more efficient genetic selection schemes, and the manipulation and storage of the genetic material. Compared with natural mating, AI gives an increase in
the numbers of offspring per sire, and allows a spatial and temporal (in the case of frozen-thawed semen) dissociation between collection of spermatozoa and fertilisation. These advantages have consequences in genetic improvement programs, first to evaluate and select sires, and secondly to compare the genetic value of animals in different flocks by linkage through reference sires. AI allows rapid and widespread diffusion of improved genotypes and the exchange of genotypes without transmitting diseases.

The success of an AI program depends on the proper management of semen collection, storage and use. The problem of the detrimental interaction between seminal plasma and the preservation media has been solved and efficient processing methods and diluents have been elaborated for storage of goat semen.

2. SEMEN PRODUCTION OF THE BUCK

2.1. Reproductive performance

2.1.1. Sexual maturity and sexual behavior

The initiation of sexual behavior in young bucks (sniffing, flehmen, nudging, mountings) appears at variable ages, from a few weeks after birth (European breeds) to 1 year old (Damascus breed). There are extreme interbreed differences in the age at which sexual maturity is acquired: from 4 to 8 months to 1 to 4 years of age, depending on whether the animal belongs to an early- or late-maturing breed (Lall, 1947; Elwishy and Elsawaf, 1971).

In the adult male, sexual motivation and efficiency depends directly on hormonal secretions and social events. The beginning of the sexual season is preceded by the secretion of testicular androgens (Hoffman et al., 1972), as revealed by a spectacular increase in plasma testosterone concentration (Saumande and Rouger, 1972). Triggering of the sexual act involves interaction between androgen secretion and social events, the latter being the initiator. Motivation and sexual efficiency of the buck can be modulated by competition and the dominance hierarchy within a group. The social environment has an important role in facilitating the full expression of sexual behavior in males and stimulates the production of semen. Estrous females play an important role in facilitating the expression of full sexual behavior in males (Rouger, 1974) interacting with factors such as nutrition or season (Walkden-Brown et al., 1994a).

2.1.2. Sperm production

Few investigations have been carried out in the buck to assess sperm production. The evaluation of daily sperm production (DSP) per testis, varied from \((2.76 \times 10^9) \) to \((7.23 \times 10^9)\) spermatozoa per testis, with a slight seasonal and breed variations (Derashri et al., 1992; Walkden-Brown et al., 1994b). In mature Australian cashmere bucks the testis weight and total testicular sperm were closely associated with scrotal circumference \((r = 0.88\) and \(0.72\) respectively). The scrotal circumference provided the simplest and most accurate indirect estimate of testis size and testicular sperm content. Ritar et al. (1992) estimated the DSP in Angora males, in the breeding season, to be \((4.0 \times 6.4) \times 10^9\)
spermatozoa. In this breed epididymal reserves of spermatozoa were correlated with testicular weight \((r = 0.50, P = 0.01)\), and number of spermatozoa in the testes \((r = 0.42, P = 0.07)\), but not with epididymal weight.

The daily sperm output (DSO) estimated from intensive collections is always 40–80% of the DSP. In Alpine and Saanen bucks, the DSO was 2.96 ± 0.36 (Degadillo et al., 1993). The considerable variation in studies of DSP in bucks may be due in part to the different techniques used to estimate testicular and epididymal sperm. Nevertheless, the buck appears to have sufficient daily output of spermatozoa to support the demands of most AI programs, especially if the semen is extended and stored.

2.1.3. Seasonal variations in sexual activity and sperm production

The length of the breeding season varies inversely with the latitude, increasing when the latitude is decreasing, with goat breeds from mid and high latitudes (higher than 35°) showing marked seasonality. The timing of parturition is not uniform throughout the year and most local breeds give birth at the end of winter or beginning of spring. The mating period of these seasonal breeders begins at the end of summer and continues through autumn and winter. In contrast, breeds in the tropics or subtropics may breed at all times of the year or show trends in kidding related to environmental factors other than photoperiod (Chemineau and Xande, 1982; Restall, 1991).

In the seasonally breeding buck, sexual behavior, testicular size and sperm production are influenced by photoperiodic changes (Ortavant, 1977; Laubser et al., 1982; Branca and Cappai, 1989) with decreased sexual activity observed during the spring in opposition to feeding activity (Rouger, 1974). However, the decrease was attenuated if the animals were trained to copulate regularly (Corteel, 1981). The seasonal resumption of sexual activity in the late summer and the autumn periods is related to changes in live weight, growth, scrotal circumference, testis growth, odor score, testosterone concentration and voluntary feed intake in the Australian cashmere buck (Walkden-Brown et al., 1994c,d).

The volume of the ejaculate of Alpine and Poitevine breeds is high in autumn and winter, during the breeding season and decreases to a minimum in the spring and summer, the nonbreeding season. The sperm concentration of the ejaculate follows an opposite trend (Corteel, 1977; Fig. 1) reflecting seasonal variations in the secretion and release of the seminal plasma from the accessory glands, which are active when the concentration of testosterone is high during the breeding season and quiescent when testosterone is low during the nonbreeding season.

The quality of the spermatozoa is affected by season. The percentage of motile spermatozoa was found to be high during the sexual season and low outside this period (Degadillo, 1990). In the nonbreeding season, sperm motility in diluted semen decreased to a low level for several weeks (Fig. 2), but at a variable time depending on the particular male or year (Corteel, 1981). When Alpine goats were inseminated with diluted semen outside the breeding season, the variation in fertility followed the variation in motility (Corteel, 1976). Little seasonal variation in the percentage of spermatozoa with morphological anomalies has been observed, with levels of 5–8% during the sexual season, and 10–18% at other times (Corteel, 1977; Delgadillo, 1990; Tuli and Holtz, 1992).
2.2. Control of sperm production

2.2.1. Photoperiodic treatments

The use of artificial photoperiodic cycles, initially developed in rams (Pelletier et al., 1988), was shown to control reproductive activity in bucks from seasonal breeds (Delgadillo et al., 1992). Rapid alternation between 1 or 2 months of long days (16 h light:8 h darkness; 16 L:8 D) and 1 or 2 months short days (8 L:16 D) decreased seasonal variation in sexual activity of Alpine and Saanen bucks compared to a control group (Figs. 3 and 4). In animals exposed to these photoperiodic treatments during three consecutive years, with semen collected twice weekly, the total number of sperm produced was 61% greater than in control bucks with no loss of fertility (Delgadillo et al., 1992). Furthermore, the quality of semen after freeze-thawing did not exhibit the seasonal changes observed in untreated bucks. The advantages of these manipulations are considerable, as the total number of AI doses produced during the first two years of the treatments was 62% higher than for the control bucks (Delgadillo et al., 1992). Such photoperiod manipulations in seasonal breeds allow semen collection all the year round instead of only during the 6-month breeding season, and the stock of semen doses for AI can be greatly increased in the buck’s first year of life.

2.2.2. Thermal environment

In mid and high latitudes, the thermal environment does not appear to be a regulator of sexual activity in the buck. However, in subtropical and tropical climates, temperature may limit reproductive ability. The volume of semen and concentration of spermatozoa...
were found to be decreased by high temperature (Hiroe and Tomizuka, 1966; Masaki and Masuda, 1968, Yokoki and Ogasa, 1977; Murugaiyah, 1992), and by high relative humidity and rainfall in Indian goats (Mukherjee et al., 1953; Murugaiyah, 1992).

2.2.3. Social environment and managerial conditions

The different interactions between males and females play an important role in the triggering and maintenance of sexual behaviour in both sexes. Permanent presence (or absence) of other animals, from the same or from the opposite sex, can modify medium- or long-term male reproductive ability (Signoret et al., 1990).

Young bucks used for semen production in intensive production systems, are usually reared in individual pens from weaning for the rest of their reproductive life (De Montigny and Lequenne, 1975). The rearing of bucks in unisex groups during the pre-puberty period is detrimental for the future sexual behavior, particularly for young males required to serve an artificial vagina (AV) at a later age. However, Orgeur et al., (1990) showed that rearing bucks in heterosexual groups had a favorable effect on
sperm production, while rearing in unisex groups had no detrimental effect on semen production and semen quality when grouping took place as early as possible.

In short, rearing male goats in individual pens appears the better solution. However, from an economic viewpoint it would be interesting to isolate males in groups as early as possible in life. The ultimate management of adult bucks is less important if the males have had previous sexual experience.

2.2.4. Method and frequency of semen collection

The semen is generally collected by AV in the presence of an oestrous doe, or less frequently by electrical stimulation. The latter method yields ejaculates of larger volumes and lower sperm concentration than those obtained by AV, but does not affect
motility of spermatozoa (Akusu et al., 1984). As seminal plasma has been found to be
generally detrimental to the storage of spermatozoa (Nunes, 1982), electro-ejaculation is
not a preferred method of semen collection.

During the sexual season in high latitudes, an increase from 2 to 7 semen collections
(AV) per week gave a threefold increase in the number of spermatozoa obtained per
week (Corteel et al., 1978). However, as the duration of sexual rest between collections
has a positive effect on sperm freezability, it is recommended to increase its duration
from 2 days in the first half of the breeding season to 3 days during the latter half (Boue
and Corteel, 1992). During the breeding season, repeated semen collections, two within
5 min, can be made by AV.

2.2.5. Selection on reproductive performances

Other methods which could maximise the amount and quality of semen are the testing
and selection of young bucks on their ability to serve an AV, to produce good ejaculates
and to give spermatozoa able to survive freeze-thawing. When adult performances of
bucks from Alpine and Saanen dairy breeds, previously selected at a young age, were
compared with the performances of unselected bucks, the behavioural efficiency to serve
the AV, the number of ejaculates suitable for processing, the ability to survive
deep-freezing and the number of doses useful for AI were lower for unselected than for
selected bucks (Corteel et al., 1987). In a population of 107 young males, 63% were
selected on the following criteria: (a) 80% successful attempts to serve an AV, (b)
production of a satisfactory ejaculate with volume greater than 0.2 ml and sperm
concentration greater than 1 billion, in at least 80% of attempts, (c) more than 60% of
the ejaculates surviving freeze-thawing with more than 30% live cells and a motility
score of 3.0, 5 min after thawing.

3. Storage of semen

3.1. General considerations

The storage of semen, particularly in frozen state, causes ultrastructural, biochemical
and functional damage to the spermatozoa resulting in a reduction of motility, viability,
impaired transport and fertility. Much research has been devoted to these problems, but
fertility of stored semen is generally lower than that of fresh semen. A specific problem
in the preservation of goat semen has been the detrimental effect of seminal plasma on
the viability of the spermatozoa in diluents containing egg yolk or in milk-based media.
The problem regarding egg yolk diluents has been attributed to an enzyme originating
from the bulbourethral gland secretion (BUS) in seminal plasma, named egg yolk
coagulating enzyme (FYCE) (Roy, 1957; Iritani et al., 1961). The depressed survival of
spermatozoa during storage in milk diluents is due to a protein fraction also from the
goat BUS (called SBUIII) which, interacting with the milk constituents in the diluent,
strongly inhibits the motility of buck spermatozoa. The bulbourethral component
responsible for this effect has been recently purified, characterized and identified as a
tricylglycerol lipase (Pellicer, 1995).
3.1.1. Egg yolk coagulating enzyme (EYCE)

The toxicity of EYCE differs with the quantity of the hydrolysates, which vary with pH, temperature, seminal plasma concentration, season of semen production and breed of fowl providing the egg yolk. When ejaculated goat spermatozoa were washed once before dilution, sperm survival after storage at 4°C was better than for nonwashed spermatozoa (Roy, 1957; Iritani et al., 1961). These authors also found that the EYCE activity was destroyed by heating at 60°C for 2–5 min. A linear relationship was found between the coagulating activity and the concentration of the EYCE, within a limited range, either in seminal plasma or bulbourethral gland extracts.

The EYCE was identified as phospholipase A which catalyses the hydrolysis of egg yolk lecithin into fatty acids and lysolecithin (Iritani and Nishikawa, 1961, 1963). The latter component is toxic for goat spermatozoa. Phospholipid A activity in seminal plasma or BUS was evidenced by using egg yolk as the substrate and measuring the amount of fatty acids released. However, egg yolk contains both phospholipids and triglycerides and examination of the reports on the measurement of phospholipase activity reveals that the liberation of fatty acids from egg yolk triglycerides by a lipase cannot be excluded. Therefore, more precise structural and enzymatic properties of EYCE remain to be investigated in order to confirm its identification as a specific phospholipase A.

3.1.2. Proteins interacting with milk

A protein fraction named SBUIII from goat BUS, interacting with the constituents of a milk-based diluent, was found to be responsible for the depression of in vitro survival of spermatozoa in this medium (Nunes, 1982). The addition of the SBUIII to washed sperm cells was detrimental to sperm viability in the milk diluent, whereas SBUIII had no effect on spermatozoa diluted in Krebs–Ringer-Phosphate-Glucose (KRP) solution. These results suggested that either an enzyme from BUS would act on a milk component, or conversely an enzyme from milk would act on an SBU component to give a product toxic to the spermatozoa. Moreover, observations with the electron microscope revealed that 95% of caudal epididymal sperm had an acrosome reaction after exposure to the BUS in the presence of milk diluent. The presence of vesicular gland secretions (VGS) caused an acrosome reaction in only 5% of sperm, a mixture of the two (VGS = 2.5 μl/ml plus BUS = 2.5 μl/ml) caused 26.7% reactions, indicating the protective action of the vesicular gland secretions (Courtens et al., 1984).

Recently, the SBUIII component responsible for deterioration of spermatozoa diluted in skim milk, has been purified, characterized and identified as a monomeric 55–60 kDa N-glycosyl-protein (BUSgp60) that exhibits heparin affinity (Pellicer, 1995). It was shown to promote a decrease in the percentage of motile spermatozoa, deterioration in the quality of movement, breakage of acrosomes and cellular death of goat epididymal spermatozoa diluted in skim milk. BUSgp60 displays triacylglycerol hydrolase activity, and the N-terminal (21 amino acid residues) and an internal peptide sequence (17 amino acid residues) of BUSgp60 exhibited 50–70% homology with sequences of various types of pancreatic lipases. The pancreatic lipases have been classified into three subgroups based on primary structure differences: classical (PL), pancreatic lipase-related proteins 1 (PLRP1) and related proteins 2 (PLRP2) (Giller et al., 1992). The highest homology in the sequences of BUSgp60 was found with PLRP2 sequences,
supporting its identification as a novel pancreatic lipase, most probably belonging to the PLRP2 subfamily. However, a more precise structural and enzymatic characterization of BUSgp60 is needed to confirm its definitive classification as a genuine member of the PLRP2 subfamily (Pellicer, 1996). This author also identified the milk components interacting with BUSgp60 to cause sperm deterioration. On one hand, milk proteins, especially caseins and lactoglobulin, were found to enhance the enzymatic activity of the BUS lipase. On the other hand, the sperm-deteriorating effect of BUS was only observed when triglycerides (triolein or residual triglycerides from skim milk) were present in the media. This strongly suggested that the sperm-deteriorating effect of BUS in milk diluents was mediated by the hydrolysis of milk triglycerides. Indeed, SBU lipase was found highly effective in hydrolyzing milk triglycerides and the major cis-unsaturated fatty acid from milk (oleic acid), and not the major saturated fatty acid (palmitic acid) promoted sperm deterioration. Therefore, the release of sperm-toxic fatty acids, such as oleic acid, from residual skim milk triglycerides by BUS lipase appears to be responsible for the deterioration of goat spermatozoa diluted in skim milk.

The investigations suggest that EYCE identified as a phospholipase A and BUSgp60 lipase could be identical compounds. Indeed, examination of the report on measurement of phospholipase A activity in BUS (Iritani and Nishikawa, 1961, 1963) revealed that the involvement of a lipase cannot be excluded. Moreover, as BUSgp60 probably belongs to the PLRP2 subfamily and it could display both lipase and phospholipase A1 activity (Thirstrup et al., 1994).

3.1.3. Seminal plasma and its removal

In the buck, removal of seminal plasma by washing the spermatozoa immediately after collection increases the percentage of live cells and their motility during storage in egg yolk or milk diluents, but ejaculated and washed semen is unable to attain the quality found for epididymal semen. This means that some detrimental compounds for in vitro storage are present in the seminal plasma.

Ejaculates collected in the breeding season contain more seminal plasma than those collected in the nonbreeding season, and a high negative correlation between the volume of seminal plasma in the ejaculate and the percentage of motile cells after thawing was found on a full-year basis (Corteel, 1977; Fig. 5). The percentage of motile cells after freeze-thawing was proportional \( r = 0.9 \) to the concentration of sperm cells and conversely proportional \( r = -0.9 \) to the initial volume of the ejaculate.

When the in vitro survival of ejaculated and epididymal goat sperm were examined in a milk diluent, the percentage of live spermatozoa and their motility after cooling to 4°C, freeze-thawing and incubation at +37°C was found to be higher for epididymal than ejaculated sperm (Chemineau, 1978). The seminal plasma produced in the nonbreeding season was more detrimental on motility and survival rate of epididymal spermatozoa in skim milk extender than seminal plasma from the breeding season. It was suggested that the negative effect of bulbourethral gland secretion was partially inhibited by the presence of vesicular gland secretions during the breeding season, which does not occur in the nonbreeding season (Nunes, 1982; Fig. 6).

Washing of spermatozoa in a physiological solution increased the percentage of motile spermatozoa and their motility before and after freezing (Corteel, 1974; Fig. 7).
The improvement in the capacity of washed spermatozoa to withstand freeze-thawing was observed not only in milk diluent (Corteel, 1974), but also in egg-yolk-containing media (Fougner, 1974; Ritar and Salamon, 1982). The latter workers found that the concentration of egg yolk in diluted semen that allowed an acceptable sperm survival rates during 6 h post-thawing incubation varied from 0% to 12%, depending on the month of collection. Moreover the recovery after thawing of washed spermatozoa was similar to nonwashed cells, but washing markedly improved survival during incubation at 37°C for 6 h, especially when egg yolk (1.5% and 6%) was present (Ritar and Salamon, 1982). In spite of the favourable effect of sperm washing, a difference between season of semen collection was observed. Thus, the aptitude to withstand freeze-thawing conditions was better for spermatozoa obtained during the sexual season than outside the sexual season (Corteel et al., 1980).
Fig. 6. Effect of vesicular and bulbourethral gland secretions on the survival of epididymal spermatozoa from sexual season (adapted from Nunes, 1982).

Regarding the washing, it should be noted that it is a complex and time-consuming process, and also causes some loss of spermatozoa (Corteel, 1981). Further, it did not appear to improve fertility of spermatozoa in dairy goats (Corteel, 1975b) and fibre-producing goats (Ritar and Salamon, 1983). The latter authors recommended the use of 1.5% egg yolk in a tris-based diluent, without removal of seminal plasma, as a practical alternative.

3.2. Liquid storage of semen

Goat semen has been stored at temperatures ranging from 2 to 15°C, mostly at 4–5°C. No systematic investigations have been carried out on the composition, tonicity and other characteristics of the diluents required for liquid storage of goat semen. A number of diluents have been used in laboratory and fertility tests, such as saline, sodium citrate–yolk, sodium citrate–fructose–yolk, saccharose–EDTA, CaNa₂–yolk,
whole, skim, or reconstituted milk with or without egg yolk, CUE (Cornell University Extender), Spermasol, Dilopten, Neoseminan, IVT (Illini Variable Temperature) diluent (the latter four media with egg yolk).

The in vitro survival and fertility of spermatozoa chilled-stored in the above diluents for 5–72 h showed great variability, except for Neoseminan (containing acetone precipitated egg yolk). In the latter diluent the spermatozoa survived during storage at 4°C for 8–15 days and maintained their fertilising capacity for 6 days (Nishikawa et al., 1961). However, Neoseminan, due to its complexity, did not gain popularity and the maintenance of fertilising capacity in the presence of acetone precipitated egg yolk in citrate diluent also could not be confirmed (Corteel, 1967). The variability in the performance of the other listed diluting media is not surprising in the light of the interactions of seminal plasma with egg yolk in diluents and with the constituents of milk media. Most investigators used nonwashed semen. While most of the reported results on the viability and fertility of nonwashed spermatozoa stored in chilled state for 5–8 h were satisfactory, longer storage periods (beyond 12–24 h) reduced the fertility. The successes obtained with egg-yolk-containing diluents were attributed by Corteel (1973) to two factors: (a) seasonal and individual (buck) variation of egg yolk coagulating enzyme concentration in seminal plasma (observed by Japanese workers), (b) variation in the egg yolk composition, depending on the breed of fowl (noted by German workers). The egg yolk was more favoured when originating from heavier rather than light breeds. Nowadays the generally used media for liquid storage (4–5°C) are skim milk, sodium citrate- and tris-based diluents.
Table 1

Percentage of goats pregnant at day 50 after insemination with nonwashed semen stored at 5°C (adapted from Eppleston et al., 1994)

<table>
<thead>
<tr>
<th>Method of Insemination</th>
<th>Days of storage</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Cervical</td>
<td>64(39)</td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>65(37)</td>
</tr>
</tbody>
</table>

( ) Number of females inseminated.

Storage of goat semen at ambient temperature and reversible inactivation of spermatozoa by incorporating organic (acetic and caproic) acids in citrate–yolk (or other) diluent, may be a promising method, as it maintained the viability of spermatozoa for 14 days (Nimbulkaret et al., 1992). Fertility data were not provided.

There seem to be no studies on the causes of depressed fertility after cervical insemination with chilled-stored goat spermatozoa. The causes are most likely the same as in sheep: an impaired pattern of transport and poor viability of spermatozoa in the female reproductive tract (Maxwell and Salamon, 1993). However the situation may be complicated by seasonal influences on motility and fertility of spermatozoa which have been found to be decreased during 2–3 months (nonbreeding season) of the year (Corteel, 1976). The concept that poor transport and survival of spermatozoa in the female tract could be responsible, at least in part, for the depressed fertility is supported by the studies of Eppleston et al. (1994). These workers bypassed the cervix and by using intrauterine (laparoscopic) insemination have demonstrated that goat spermatozoa have retained their fertilising capacity for at least 8 days of storage at 5°C in tris–fructose–citric acid–yolk (2%) diluent, as shown in Table 1. Pomares et al. (1994) reported an improvement in viability of spermatozoa during 12 days storage (5°C) when ‘glutathione peroxidase (1 unit/ml) was included in the tris-based diluent. Incorporation of other antioxidants into the tris-based diluent such as catalase, superoxide dismutase, cytochrome c, beside glutathione peroxidase improved the viability of spermatozoa during 6 days of storage (5°C), but this was not reflected in better in vitro (oocyte) fertilisation rates (Stojanov et al. 1994; Pomares et al., 1995).

### 3.3. Frozen storage of semen

Since goat spermatozoa were first frozen (−79°C) by Smith and Polge (1950), and Barker (1957) stated that fertility of frozen-thawed goat semen ‘‘was too low to be of practical value’’ many investigators have been engaged in freezing caprine semen. The method of freezing and most of the diluents used by early workers were those that proved successful in the bull. The reported fertility results (reviewed by Corteel, 1973) varied from poor (3–15%), moderate (20–48%) to satisfactory (up to 70%). The great variation in fertility is not surprising, as in ‘early days’ semen containing seminal plasma was diluted and processed for freezing, despite the finding in the late 50’s to early 60’s that goat seminal plasma is detrimental to survival of spermatozoa (see previous
section). Since Corteel (1974) showed that elimination of the plasma shortly after ejaculation is beneficial for the survival of spermatozoa and proposed washing of semen, most investigators have adopted this method. The notable variation in fertility rates before introduction of Corteel’s washing method, apart from other factors, could have been partly attributed to the different equilibration periods which varied from 1 to 24 h. The most acceptable equilibration time was between 1 and 3 h (Corteel, 1974; Das and Rajconvar, 1995; Singh et al., 1995). Washing of goat semen consists of dilution of freshly collected ejaculates with a washing solution (1:5–1:10) and centrifugation for 10–15 min at 600–1000 g. Different washing solutions were used such as Krebs–Ringer phosphate buffer (with or without glucose) or the freezing diluent (skim milk, tris or other media) without glycerol.

The efficiency of seminal plasma elimination depends on the intensity of the washing procedure, i.e., dilution ratio of semen before washing and number of washings (centrifugations). After 1:5 or 1:10 dilution double washing may be more effective than single washing, and the efficiency of each method could be increased by a higher dilution ratio. Thus, the number of washings can be reduced by extension of semen at a higher rate (1:20) before single centrifugation (Ritar and Salamon, 1982). In the method of (Corteel 1974, 1975a, 1990) the washed spermatozoa are resuspended with nonglycerated diluent to half the final cell concentration, cooled from 30°C to 4°C in 1 h and further diluted with glycerol-containing diluent portion (14%) to a cell concentration of (400 to 500) × 10⁶; the final glycerol concentration is 7%. Then follows a 1 to 3 h equilibration period at 4°C before freezing. The washed spermatozoa can be resuspended also with the glycerol-containing diluent to a final cell concentration or dilution rate, cooled in 1 h to 4–5°C, further equilibrated (if included) and then frozen.

When the semen is frozen without elimination of the seminal plasma (nonwashed), the freshly collected ejaculates can be diluted by a single addition of the glycerol-containing diluent at 30°C (one-step), cooled in 1–1.5 h to 4–5°C and frozen without equilibration (Ritar and Salamon, 1983, Ritar et al., 1990a). Alternatively, dilution of nonwashed semen is done in two steps, first after collection with nonglycerated diluent at 30°C and second after cooling in 1–1.5 h to 4–5°C with the glycerated diluent portion, followed by an equilibration period of 1.5 h before freezing. The two-step method has no advantage in comparison with the one-step dilution with regard to post-thaw viability (Ritar and Salamon, 1982).

3.3.1. Diluents and cryoprotectives

A number of diluents have been examined for freezing goat semen such as reconstituted skim cow milk, sodium citrate–glucose–yolk, lactose–yolk, saccharose–EDTA.CaNa₂–yolk, raffinose–yolk, Spermasol–yolk, IVT–yolk, tris–yolk and test–yolk. Based on a limited number of studies on comparisons of diluents in which post-thawing viability, GOT release and fertility were the criteria for evaluation, no clear preference can be established among the diluents examined. The most widely used media seem to be reconstituted skim cow milk–glucose (0.5 M) (Corteel, 1974, 1975a) and tris–glucose–citric acid–yolk (Salamon and Ritar, 1982), in the latter the diluent components being adjusted for one-step dilution rates of the nonwashed semen (Evans and Maxwell, 1987).
Ethylene glycol and propylene glycol gave less protection to goat spermatozoa than glycerol (Waide et al., 1977), which seems to be the only cryoprotective used to freeze goat semen. The glycerol concentrations used by different investigators varied from 3\% to 9\%, with the optimum of 4\%–7\% in the diluted semen.

In the method of (Corteel 1974, 1975a, 1990) the glycerol containing skim milk (14\%) is added at 4°C in three steps at 10-min intervals, resulting in 7\% final concentration and (400 to 500) × 10^6 spermatozoa per ml. In the method of Ritar and Salamon (1982) the glycerolated tris-based diluent is added to the nonwashed semen in one step at 30°C and the final glycerol and egg yolk concentrations are adjusted for rates of dilution, being 4\% and 2\% respectively in the diluted semen (Evans and Maxwell, 1987). Addition of glycerol at 5°C had no advantage over addition at 30°C (Salamon and Ritar, 1982; Tuli and Holtz, 1994).

### 3.3.2. Methods of freezing

After cooling the diluted semen or the resuspended spermatozoa to 4–5°C and subsequent inclusion or avoidance of equilibration, freezing is done in straws or in pellet form. In the former case the straws containing the semen are suspended horizontally in liquid nitrogen vapour 4–5 cm above the liquid nitrogen (LN) level for 4–5 min, then are immersed into the LN. In France, it is recommended to suspend the straws above the LN in 2 steps, first at 16 cm for 2 min, then at 4 cm for 3 min before immersion into LN. The velocity of cooling can be regulated by the distance of the straws from the level of LN and the size of the straw: fine (0.25 ml) or medium (0.50 ml).

Pellet freezing on dry ice at −79°C and immersion of pellets into LN at −196°C is a rapid and simple method. The velocity of cooling to −79°C can be regulated by the volume of the pellet. The volume of semen in the straw and pellet had a marked effect on the cooling curve but not on the post-thawing survival of spermatozoa within the packaging method. However, freezing of straws on a precooled 1-cm wire mesh or perforated plate gave better viability results than freezing on a rack giving only two contact points with the straws at their ends. This has been attributed to earlier seeding by the multiple contacts with the racks which most probably minimised or eliminated supercooling (below the freezing point) and thus the temperature fluctuation (Ritar et al., 1990a). Although the post-thawing viability (Ritar et al., 1990a) and fertility (Ritar and Ball 1991) were better after pellet than straw freezing, and the latter method is more time-consuming (Corteel, 1981), freezing in straws is preferred by most commercial traders, as it allows more accurate identification of semen doses.

### 3.3.3. Thawing of semen

For most mammalian species, several thawing regimes have been recommended in order to achieve good post-thaw fertility. Goat semen frozen in straws or in pellet form and thawed at 37°C (Salamon and Ritar, 1982; Corteel, 1974) gave acceptable fertility results. Deka and Rao (1987) observed higher motility rates and more intact acrosomes after thawing goat semen at 37°C than at 5°C. Earlier work (Andersen, 1969) with a Norwegian goat breed, found that thawing at 75°C for 10 s was better than at 35°C in 30 s. Tuli et al. (1991), using the semen of Boer goat, found that thawing of straws at 70°C for 7 s was superior to either at 37°C for 2 min, or at 40°C for 20 s. However, it should
be noted that thawing semen at 37°C is more suitable under practical conditions of AI and the risk of overheating the thawed semen is also excluded. Semen frozen in pellet form is usually thawed (37°C) in dry test tubes without a thawing solution.

3.4. Comparison between methods of storage

Comparisons between methods and protocols for freeze-thawing of semen are difficult because of the different parameters involved and the lack of uniformity in the methodology. The various procedures may differ by the following points:

(a) Use or not of males selected on the freezability of their semen.
(b) Use of ejaculates devoid of or containing seminal plasma, washing method, composition of washing solution, intensity of washing: dilution rate and centrifugation.
(c) Composition of freezing diluent and presence or absence of egg yolk.
(d) Method of dilution, glycerol addition and its concentration.
(e) Inclusion or avoidance of equilibration time and its duration before freezing.
(f) Packaging methods for semen in mini (0.20 ml), medium (0.5 ml) straws, or in pellets.

3.5. Long-term frozen storage of semen

As in other species, in the goat arises the important consideration of whether or not there is any decrease in fertility during frozen storage. Corteel (1975a) observed a decline in the percentage of motile spermatozoa in skim milk–glucose diluent which for periods of 3–90 and 91–180 days were 16.6% and 22.0% for nonwashed ejaculates and 0% and 1.3% for washed ejaculates, respectively. Ritar and Salamon (1991) also found a deterioration in the viability of spermatozoa during 6 months storage in tris–glucose–citric acid–yolk (4% final glycerol), and the rate of decline was similar in the presence or absence of egg yolk and whether the spermatozoa were washed or not before freezing.

There are also favourable reports on prolonged storage of goat semen. Thus, the conception rates obtained by Waide et al. (1977) after deep cervical insemination (using forceps) with nonwashed semen stored for 1–1022 days in sodium citrate–glucose–glycerol–yolk diluent with a final 7% glycerol concentration were 63.3%, 79.4%, and 77.7% for semen stored 1–30, 31–102, and 210–1022 days, respectively. Fougner (1979) reported 63.4% kidding rate after intrauterine inseminations (via the cervix) with washed spermatozoa stored for 1–3 years in tris–fructose–citrate–yolk medium (8% final glycerol concentration). Further research is needed to examine the efficiency of long-term storage of goat semen.

3.6. New directions

Theories of cryoinjury have been reviewed by Watson (1995) who concluded that sperm cells are particularly sensitive to osmotic change, and that bulk water movement
into the cells creates membrane damage. As well, lipid reorganisation because of thermal changes alters membrane sensitivity to various stresses. Watson (1995) proposed some intriguing theories on cryoinjury with significant consequences for future studies. He assumed that either sperm recovering after freeze-thawing are a subpopulation of spermatozoa, which are viable but relatively infertile, or that freezing generally modifies cell membranes, the survivors being affected in some deleterious way. The destabilisation of membranes leads to premature acrosome reactions, shortens the life span and reduces fertility. Thus, frozen-thawed spermatozoa are partially capacitated and capable of achieving fertilisation over a short period of time. This concept fits well with the practical methods of insemination developed for the use frozen-thawed semen (see later).

The above theories represent a change in direction of research for semen storage. Future work may focus on modifying membrane structures before freeze-thawing to render them resistant to cryoinjury. A reversible membrane change that increased resistance would undoubtedly lead to higher fertility.

4. Use of stored goat semen and factors influencing fertility after insemination

Chilled-stored and frozen-thawed semen is used for insemination in natural, synchronised or induced oestrus in which several factors can influence the fertility.

4.1. Natural oestrus

Successful AI at natural oestrus depends in the first instance on an efficient method of oestrous detection. Sexual receptivity of the female is evidenced by mounting by the male, or more particularly by the postural immobilisation of the female in response to mounting by the male. Different practical methods of oestrus detection are used, including entire males provided with an apron, vasectomized males or androgenized females fitted with a harness and marking crayon. These ‘teasers’ may be run with the flock and marked does removed once or twice per day, or they may be taken to the females at intervals and their behaviour observed directly.

In order to control the time of oestrus in the normal breeding season, simple methods make use of the ovulation inducing effect of bucks (‘buck effect’) and oestrous females (‘female effect’) to synchronise oestrus and ovulation (Chemineau, 1987; Restall, 1988, 1992).

4.2. Synchronised and induced oestrus

In large commercial herds economic considerations necessitate the synchronisation of oestrus in females which are cycling naturally, or its induction during the non-breeding season, with insemination performed at a fixed time either in relation to the beginning of oestrus or the end of a hormonal treatment, PMSG.
The hormonal treatment generally used to induce oestrus and ovulation is based on combinations of progesterone or a progestagen treatment followed by eCG, and may include treatment with commercial prostaglandin analogues. Hormonal treatments based on a double prostaglandin injection in cycling females have also been used (Bretzlauff et al., 1981). A synchronisation treatment used on a large scale in France for dairy goats consists of: insertion of a vaginal sponge impregnated with a synthetic analogue of progesterone (45 mg fluorogestone acetate: FGA) for 11 days, intramuscular injection of eCG (400 to 600 IU according to the milk production and season of treatment) and 50 mg of cloprostenol, both administered 48 h before sponge removal. Treated goats are inseminated once with 100 million frozen-thawed spermatozoa, at 43–45 h after sponge removal (Corteel et al. 1988; Corteel and Leboeuf, 1990). The fertility rate in dairy goats after one insemination was 60–65% (Boué and Sigwald, 1995). In the Australian cashmere goat, Ritar et al., (1990b) indicated that controlled internal drug release (CIDR) devices containing progesterone were equally effective as intravaginal sponges for the control of ovulation, when combined with an injection of eCG. Greyling and Van Nickerk (1991), in the noncyclic Boer goat, have successfully used vaginal sponges impregnated with medroxy-progesterone acetate (MAP) for 14 days.

Photoperiodic treatments have been used to induce oestrous cyclicity in goats outside the breeding season. Such treatments involve application of extra artificial light, with a fixed dawn and a nocturnal lighting, for 2 months, before administration of a melatonin implant for 40–50 days (Chemineau et al., 1996). After this treatment, AI can be performed at the natural oestrus. Association of photoperiodic and hormonal treatments, synchronisation of oestrus and AI is under current investigation.

When oestrus was induced by treatments with progestagen (FGA) sponges for 18–21 days and eCG administration 48 h before sponge removal, the kidding rates after two intracervical inseminations with frozen-thawed spermatozoa were dependent upon motility of spermatozoa (Corteel et al., 1988) (Table 2), and probably involved impaired sperm transport through the cervix due to prolonged administration of FGA, as it has been shown in sheep (Quinlivan and Robinson, 1969). When FGA treatment was shortened to 11 days, followed by eCG and Cloprostenol administration, the kidding rate, measured over several years, was increased from 56.7% \( (n = 6210) \) to 61.1% \( (n = 6970); \) Corteel et al., 1988). After deposition of the semen into the cervix, fertility

### Table 2

<table>
<thead>
<tr>
<th>Duration of FGA treatment (days)</th>
<th>Motility scores</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 1.0</td>
<td>1.5</td>
<td>≥ 2</td>
</tr>
<tr>
<td>18–21</td>
<td>46.3% (1222) **</td>
<td>51.6% (533) **</td>
<td>57.9% (1344)</td>
</tr>
<tr>
<td>11</td>
<td>53.9% (349) **</td>
<td>60.9% (220)</td>
<td>61.0% (264)</td>
</tr>
</tbody>
</table>

\(^{(*)} \) Number of females;

\(^{*}\) Females inseminated twice with 150–200 million spermatozoa.

\(^{**} P < 0.05.

\(^{**} P < 0.01.\)
was influenced by sperm motility (Table 2). In females hormonally treated for 18–21 days the fertility was lower after semen deposition into the cervix than into the uterus (Table 3). Similar results have been obtained in Angora goats, with fertility increasing with the depth of insemination and little difference between fresh-diluted or frozen-thawed semen after intrauterine insemination (Table 4) (Ritar and Salamon, 1983).

4.2.1. Antibodies against eCG (PMSG)

The techniques using FGA/eCG treatments with AI at a predetermined time after sponge removal can result in variable fertility rates due to variation in the time of oestrus and ovulation. The fertility of goats which came into oestrus more than 30 h after sponge removal was significantly lower than those which were first observed in oestrus 24 or 30 h after sponge removal (33% vs. 65% respectively, Baril et al., 1993). The distribution of the time of onset of oestrus after sponge removal was affected by the number of hormonal treatments previously received by the females (Baril et al., 1996). The delay in the onset of oestrus behavior was associated with a delay in preovulatory surge of LH (Maurel et al., 1992) and a delay in the time of ovulation (Leboeuf et al., 1996).

In another French survey, eCG binding in plasma before the onset of treatment was found to be significantly lower in flocks never treated, compared to flocks with a history of eCG treatments, and was not dependent on the age of females. eCG binding was higher in females that had previously received two to five treatments compared to females that had no or only one treatment. Fertility decreased (51% vs. 66%, 166 vs. 353 females), when eCG binding was higher than 5%. (Baril et al., 1996).

Table 3
The effect of site of deposition of frozen-thawed semen in kidding rate in adult goats after progestagen treatment (adapted from Corteel et al., 1988)

<table>
<thead>
<tr>
<th>Duration of FGA treatment (days)</th>
<th>Site of semen deposition</th>
<th>Cervix</th>
<th>Uterus</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–21</td>
<td>51.7% (3392) * *</td>
<td>62.6% (2848)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>59.3% (3970) * *</td>
<td>64.3% (2156)</td>
<td></td>
</tr>
</tbody>
</table>

( ) Number of females inseminated.
* * P < 0.01.

Table 4
Effect of depth of insemination with fresh and frozen-thawed semen on kidding rate (%) in Angora goats (adapted from Ritar and Salamon, 1983)

<table>
<thead>
<tr>
<th>Depth of cervical insemination</th>
<th>Fresh diluted semen</th>
<th>Frozen-thawed semen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 1 cm</td>
<td>40.9 (88)</td>
<td>27.0 (63)</td>
</tr>
<tr>
<td>1.0 to 3.0 cm</td>
<td>53.3 (127)</td>
<td>45.9 (85)</td>
</tr>
<tr>
<td>Into the uterus</td>
<td>69.1 (81)</td>
<td>68.6 (102)</td>
</tr>
</tbody>
</table>

( ) Number of females.
4.3. Other factors influencing fertility

4.3.1. Characteristics of individual animal

Irrespective of the process used for freezing, differences have been observed between males regarding the freezability and fertility of semen, so they could be classified as ‘good freezers’ or ‘bad freezers’. This variability is relatively independent of prior semen quality, and the semen of certain individuals consistently freezes with less cryoinjury than that of others (Corteel et al., 1987). Spermatozoa acquire cold shock sensitivity as they traverse the epididymal tubules (Watson, 1981), and this is believed to be related to changes in membrane lipids during the epididymal transit. Differences in either ejaculation frequency, previously indicated by Boué and Corteel (1992), or in epididymal transit time and sperm mixing in the epididymis may provide a potential mechanism for variability in response to subsequent temperature fluctuation, explaining why ejaculates within individuals can vary in their responses to freeze-thawing (Watson, 1995).

4.3.2. Season of semen production

Reports on the freezability and fertility of semen collected in the breeding and nonbreeding season are contradictory. Some investigators found that freezability of semen (Muhuyi et al., 1992) and fertility of frozen-thawed spermatozoa (Corteel et al., 1978) was better when collected in the breeding than in the nonbreeding season. Other workers (Peskovatskov et al., 1974; Summerrmatter and Flukiger 1982) observed no seasonal difference in fertility. According to Aamdal (1982) in Norway for frozen storage the semen is collected before the breeding season. Corteel et al. (1980) have shown that the kidding rate of dairy goats inseminated with frozen-thawed spermatozoa after hormonal induction of oestrus and ovulation, was significantly lower with semen obtained outside the breeding season (53%, n = 549) than with semen processed in the breeding season (61%, n = 998). When semen stored at 4°C for less than 12 h was used for insemination of dairy goats outside the breeding season, the kidding rate was significantly lower than in goats inseminated with frozen-thawed semen obtained during the previous breeding season, (52.1%, n = 4505, and 56.7%, n = 6240, respectively (Corteel et al., 1988). These observation suggest that the semen of valuable sires intended for long-term storage should be collected and processed during the normal breeding season. There has been little research to date aiming to overcome the seasonal differences in semen quality.

4.3.3. Physiological condition of females

The physiological condition of the doe varies with parity, season, level of production and age, and all these factors have been found to influence the success of hormonal treatments to induce synchronisation of oestrus and ovulation for the subsequent AI.

Fertility after oestrus synchronisation and AI is low during postpartum and lactational anoestrus. The latter increases significantly with time elapsed from parturition and fertility is only fully restored if the hormonal treatment is delayed until 4 full months after parturition (Corteel, 1975b). The time between kidding and re-breeding affects the fertility of goats. Increasing the length of time between weaning and laparoscopic
Insemination with frozen-thawed semen from 3 weeks to 3 months improved the proportion of Cashmere does kidding from 47% to 65% (Ritar et al., 1989).

In the high latitudes, the percentage of anoestrous females is highest in the middle of the nonbreeding season, well in advance of the normal restoration of cyclicity. To minimize this handicap in large-scale commercial AI programs in France, higher doses of eCG have been administered before 15 June (midsummer, 500 IU instead of 400 IU after June). This has resulted in small fertility differences between treatments in the breeding and nonbreeding seasons (Leboeuf, 1989).

To reduce the depressive effect of high levels of milk production on fertility, Leboeuf (1989) injected 600 IU eCG before 15 June and 500 IU of eCG after 15 of June in goats producing higher than 3.5 kg of milk per day during the month preceding the hormonal treatment. This has resulted in small fertility differences between treatments in the breeding and nonbreeding seasons (Leboeuf, 1989).

To reduce the depressive effect of high levels of milk production on fertility, Leboeuf (1989) injected 600 IU eCG before 15 June and 500 IU of eCG after 15 of June in goats producing higher than 3.5 kg of milk per day during the month preceding the hormonal treatment. The increase in the dose of eCG tended to improve the kidding rate of very high milkers: 47.8% (n = 138) vs. 43.9% (n = 139).

4.3.4. Age and parity

After hormonal induction of oestrus, nulliparous goats were less fertile than primiparous or multiparous goats when inseminated intracervically (Leboeuf, 1989). Ritar and Ball (1991) indicated that maiden does should be inseminated laparoscopically rather than cervically because of the difficulty in opening the vagina with a speculum. Little difference in fertility was observed between 18-month-old maiden does and mature does when they were inseminated laparoscopically (Ritar and Ball, 1993).

4.3.5. Time of insemination

4.3.5.1. Natural oestrus. Successful fertilisation depends on the time of insemination relative to ovulation. Dauzier (1966) showed that the fertility of goats inseminated with fresh semen at different time intervals from the onset of natural oestrus (observed by the farmer) was highest after insemination at the beginning of the oestrus period (Table 5). This is in accordance with the known time of ovulation (Harrison, 1948; Lopyrin, 1953; Gonzalez-Stagnaro et al., 1984; Rao and Bhattacharya, 1980), duration of sperm transport in the female genital tract (Ajello, 1958; Lopyrin, 1953) and time of survival of male and female gametes (Lopyrin, 1953). Females in natural oestrus are generally inseminated, after once daily detection, at the first, or highly fertile, stage of the oestrus period.

Table 5

<table>
<thead>
<tr>
<th>Time of insemination after oestrus detection</th>
<th>Method of insemination</th>
<th>Cervical and intrauterine (via the cervix)</th>
<th>Intravaginal</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 12 h</td>
<td>74.8 (298)</td>
<td>66.9 (444)</td>
<td></td>
</tr>
<tr>
<td>12–24 h</td>
<td>66.2 (320)</td>
<td>60.7 (326)</td>
<td></td>
</tr>
<tr>
<td>Later than 24 h</td>
<td>51.0 (100)</td>
<td>44.6 (148)</td>
<td></td>
</tr>
</tbody>
</table>

( ) Number of females inseminated.
4.3.5.2. Hormonally synchronized oestrus. When intravaginal progestagen treatment is used out of breeding season, administration of eCG is imperative to ensure ovulation, and insemination should be carried out in relation to the time of sponge removal. In French protocols, cervical inseminations are performed 43 h (Alpine) or 45 h (Saanen) after sponge removal (Corteel and Leboeuf, 1990). In programmes for fibre-producing goats, cervical inseminations occurred 48–59 h after FGA sponge removal and eCG injection (Ritar and Salamon, 1983). 40–48 h after sponge removal when eCG was injected 48 h before removal (Ritar et al., 1989), and 10–15 h earlier if CIDRs rather than sponges were used (Ritar et al., 1990b).

4.3.6. Volume of inseminate and number of spermatozoa

The total number of spermatozoa inseminated per female is one of the main factors affecting fertility, and it is necessary to know the threshold at which the required fertility is reached for a given breed with females synchronised by particular methods for various conditions of semen storage.

Corteel et al., (1988) reported that fertility after one insemination with 200 million frozen-thawed spermatozoa in a 0.5-ml straw was similar to that obtained after two inseminations with $2 \times 200$ million spermatozoa in hormonally synchronised dairy goats. Further work (Corteel and Leboeuf, 1990) demonstrated that acceptable fertility (around 60%) may be obtained with a reduction in the number of spermatozoa inseminated from 200 to 100 million in Alpine goats and to 60 million in Saanen goats, with a concomitant reduction in inseminate volume from 0.5 to 0.2 ml. Ritar and Ball (1993) found that one and two inseminations were equally effective in natural or hormonally synchronised oestrus in Angora goats, provided the dose of spermatozoa exceeded 120 million.

4.3.7. Site of semen deposition

In goats in which oestrus and ovulation was induced, laparoscopic intrauterine insemination resulted in improved fertility rates and allowed a reduction in numbers of frozen-thawed spermatozoa inseminated (5–20 million motile spermatozoa; Ritar et al.,

<table>
<thead>
<tr>
<th>Main effect</th>
<th>Cervical</th>
<th>Laparoscopic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intravaginal treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIDR</td>
<td>40.7 (204)</td>
<td>64.5 (290)</td>
</tr>
<tr>
<td>Sponge</td>
<td>37.4 (198)</td>
<td>62.7 (279)</td>
</tr>
<tr>
<td><strong>Time of insemination after treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before ovulation</td>
<td>44.0 (200)</td>
<td>69.6 (283)</td>
</tr>
<tr>
<td>After ovulation</td>
<td>34.2 (202)</td>
<td>56.7 (286)</td>
</tr>
</tbody>
</table>

( ) Number of females.

*Does were inseminated cervically either before (45 h after CIDR removal; 55 h after sponge removal) or after (55 h after CIDR removal; 65 h after sponge removal) the estimated time of ovulation.
Table 7
Percentage of cleaved eggs and number of good embryos related to the interval between the onset of oestrus and the insemination time (adapted from Vallet and Baril, 1990) (laparoscopic insemination with 100 million frozen-thawed spermatozoa)

<table>
<thead>
<tr>
<th>Interval between onset of oestrus and insemination (h)</th>
<th>&lt; 12</th>
<th>14–18</th>
<th>20–24</th>
<th>26–30</th>
<th>&gt; 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of cleaved eggs</td>
<td>22.0</td>
<td>57.2</td>
<td>78.3</td>
<td>57.2</td>
<td>29.6</td>
</tr>
<tr>
<td>Number of embryos per goat treated</td>
<td>0.6</td>
<td>3.0</td>
<td>6.4</td>
<td>4.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Number of females</td>
<td>8</td>
<td>22</td>
<td>61</td>
<td>33</td>
<td>11</td>
</tr>
</tbody>
</table>

1990b). Comparisons between laparoscopic and cervical inseminations in dairy goats have shown significant differences in fertility rates (62.6% vs. 49.3%; $P < 0.05$, respectively, Vallet et al., 1992), regardless of the low numbers of total spermatozoa deposited: 10 million sperm, 61.5% vs. 20 million sperm, 63.7%. Fertility was also better after laparoscopic than cervical insemination with frozen-thawed semen in Australian feral goats (Moore et al., 1988) and Australian cashmere goats (Ritar et al., 1990b; Table 6).

4.3.8. Superovulation

The transport and survival of spermatozoa in the female tract are disrupted after progestagen treatment associated with the hormonal induction of superovulation (Evans and Armstrong, 1984). The detrimental effect of the altered uterine environment on spermatozoa was not reduced by increasing the number of inseminations and the number of spermatozoa per female (2 vs. 3 million), and only 48.6% of cleaved eggs were obtained after cervical insemination with frozen-thawed spermatozoa (Vallet et al., 1991). However, an increased percentage of cleaved eggs resulted from laparoscopic insemination with 100 million frozen-thawed spermatozoa, provided the insemination was performed 20–24 h after the onset of oestrus (Vallet and Baril, 1990, Table 7).

5. Conclusion and perspectives

This review has mainly addressed the methods and the techniques that have provided improvements to routine practical AI programs. Satisfactory fertility to artificial insemination requires attention to each step of the procedures for semen storage. The careful management of males that produce semen is the first step toward achieving acceptable fertility after artificial insemination. The second important consideration is the choice of short- or long-term in vitro storage of the spermatozoa. The sperm cells are easily damaged after ejaculation, and the seminal plasma modifies their in vitro viability. However, opinions differ regarding the elimination of seminal plasma before processing of semen for storage, and in practice ejaculates are used either devoid of or containing seminal plasma. The third important consideration is the organisation of the artificial insemination, which should be performed close to the time of ovulation in the females. Accurate and careful detection of oestrus and control of oestrus and ovulation are
necessary to reach a satisfactory fertility level. Hormonal treatments appear to be efficient techniques to permit insemination at a fixed time after the end of the treatment, although fertility levels may be below those attained after insemination at naturally synchronised oestrus.

Major advances have been made in modulating the effect of season on bucks under high latitudes in order to collect satisfactory ejaculates all the year. Notable studies have also identified the secretions of the bulbourethral gland and the interactions between these secretions and the components of milk diluents implicated in the deterioration of sperm cells. More investigations are needed on the inhibition of the bulbourethral gland lipase to avoid washing spermatozoa before frozen storage. Another promising area of investigation is the pretreatment of spermatozoa to protect or preserve membrane integrity during the freeze-thawing process.

One of the unresolved problems is the in vitro evaluation of sperm quality such that fertility can be predicted. Despite many studies on the relationship between the characteristics and fertility of spermatozoa, we are not able to evaluate the fertilising capacity of buck ejaculates. The methods developed for other species and based on morphology, functional integrity of spermatozoa, or in vitro fertilisation, should be used also for goat semen.

In order to achieve satisfactory fertility after deposition of low numbers of spermatozoa into the reproductive tract, it is necessary to inseminate close to the time of ovulation. Despite the improvement in the efficiency of hormonal synchronisation of oestrus, variability in the time of ovulation after treatment is an important impediment to attain acceptable fertility after only one insemination at a fixed time. Further work is also needed to impede any deterioration in the quality of semen during processing for either short- or long-term storage.

For economical and practical reasons, it is desirable to develop a more efficient method for liquid storage. The use of liquid-stored semen, as an alternative to frozen storage would have obvious advantages in widespread selection schemes or in extensive production systems. For this, more investigations are necessary to increase the time of storage in association with the maintenance of fertilising capacity of spermatozoa.

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