ASPECTS OF ARTIFICIAL

BREEDING IN GOATS.

A thesis submitted for the degree of Master of Agricultural Science,
University of Tasmania.

by

Peter Douglas Ball B.Agr.Sci.

July 1995

Department of Primary Industry and Fisheries,

Hobart

Tasmania

graduated 1996

Declaration.

The material presented in this study was completed by the author under the supervision of Dr. A.J. Ritar and Dr. A.C. Bray, whilst employed as a Technical Officer with the Department of Primary Industry and Fisheries.

I declare that the material in this thesis has not been submitted for any other degree or diploma and is original work except where otherwise acknowledged.

Peter Ball.

Aspects of Artificial Breeding in Goats.

Summary.

This study examines some aspects of the application of artificial breeding technology to the breeding of Cashmere goats. The development of young male Cashmere and Angora goats was also studied.

Experiments were undertaken to develop a simple procedure for freezing caprine semen in polyvinyl straws suspended in liquid nitrogen vapour. Such a methodology was determined and assessed in the laboratory against a more conventional method of freezing semen in pellets on solid carbon dioxide (dry ice). Acceptable rates of sperm survival after freeze-thawing were achieved for the straw freezing procedure. However, sperm survival was greater after freezing semen as pellets.

Artificial insemination programs were conducted with the aim of improving fertility from both laparoscopic and cervical insemination procedures in Cashmere goats. The factors examined included the hormonal control of ovulation, the time of insemination relative to ovulation, the number of motile sperm inseminated, the depth of cervical insemination, and the age and parity of the females. Fertility was improved by insemination prior to the estimated time of ovulation, and with increasing depth of cervical insemination. Fertility was reduced in kid maidens compared with both hogget maidens and multiparous adult does.

Fertility resulting from the laparoscopic insemination of semen processed and frozen as pellets and straws was also assessed. The fertility achieved with straw frozen semen was not significantly less than from semen frozen in pellets.

A method was investigated for production of inseminate containing high densities of motile spermatozoa for use in cervical insemination. This semen was frozen at dilution rates of 1:0.5, and 1:1 (Semen:diluent). Whilst the percentage of spermatozoa surviving this process was lower than for conventional dilution rates (1:2), the concentration of motile spermatozoa was up to 1.8 times greater in semen frozen at

1:0.5. There was no benefit to fertility from this technique, although fertility was not significantly reduced. Fertility was not significantly affected by timing of insemination, double insemination or the number of motile sperm inseminated.

In addition, a small study was also conducted to examine the affects of breed (Cashmere and Angora), early weaning and foster rearing on the early growth and development of young goat bucks. There was no significant affect of rearing strategy on live weight or testis weight. Testis weight was more closely related to live weight than age in the young buck, and was highly predictable from measures of scrotal circumference. The growth and development of the smaller group of Angoras studied was significantly slower than that of the Cashmeres. Late spermatids and released spermatozoa were observed in 48% of the seminiferous tubules of Cashmeres at mean age 19 weeks and mean live weight 16.8 Kg.

Acknowledgments.

I would like to thank my supervisors Dr. A.J. Ritar and A. C. Bray for their supervision and encouragement of this work, particularly the research supervision and critical advice of Dr. A.J. Ritar. I wish to also thank Mr T. Koen and Mr. B. Graham for statistical advice.

I gratefully acknowledge the considerable technical assistance of Mr P.J. O'May, the management and staff of the Cressy Research Station, Van Dieman Company's property "Woolnorth" and the Tasmanian Dairy Industry Authority at Hadspen Park. In particular the assistance and advice of Mr. C.F. Barker and Mr M.A. Fenton are acknowledged. The help and advice of Ms K. Clarke in the preparation of testes sections for histological study is also acknowledged with thanks.

I would also like to thank the staff of the Live-stock Development Branch for their friendship, encouragement and advice, particularly that of Mr. C.F. King and Ms J.A. Robinson.

My greatest thanks go to my wife, Sandy for her unstinting encouragement and understanding over the long duration of these trials.

Dedication.

I dedicate this thesis to my father, Michael J. Ball, who should have seen it's completion.

Introduction.

Artificial breeding technology offers considerable benefits to the development of a goat industry within Australia. The use of such technology provides producers with access to a wider genetic base than that available to natural breeding programs, and increases the flexibility with which the available genetic resource can be utilised. Selected sires can also be more widely used within a breeding program, contributing to a more rapid infusion of desired genetics and potentially increasing the rate of genetic gain available to the producer.

Such features make artificial breeding technology particularly attractive to developing animal enterprises such as the Australian cashmere and mohair industries. Limitations of the technology are the cost and complexity of the methodologies employed, their efficacy and factors that limit access to as wide a genetic resource as possible.

The efficacy of the artificial breeding program is determined by factors that effect the functional survival of spermatozoa during dissemination of the semen, and those that effect fertility at insemination. Here a series of trials examined some of these factors, with regard to the use of frozen semen.

Frozen semen offers greater flexibility than fresh semen within an artificial breeding program, but generally results in lower fertility. In addition, the preferred method of packaging frozen semen is the polyvinyl straw, although this incurs a further cost in terms of both fertility and the complexity of semen processing. These trials concentrated on the development and application of a simple straw freezing process for caprine semen.

A further limit to the widespread application of artificial breeding technology has been the poor performance of cervical insemination with frozen semen, and the greater cost and complexity involved with the alternative of laparoscopic insemination. Two studies were undertaken to examine means of improving fertility after cervical insemination.

Finally data is presented characterising growth and testicular development in kid goats. Such information can be useful in determining normal developmental parameters that may be useful in the early selection and use of artificial breeding sires.

Table of Contents.

CHAPTER 1.

THE PRESERVATION OF SEMEN FOR USE IN ARTIFICIAL BREEDING.

1. THE PRESERVATION OF SEMEN FOR USE IN ARTIFICIAL BREEDING

1.1 Introduction.

In domestic species, interest in the storage and preservation of semen has developed in conjunction with the development and use of artificial insemination technology. Artificial insemination practices can offer advantages over natural mating. Ritar (1987) states that the use of stored semen considerably enhances the potential use of individual males. The stored semen may be used to inseminate a large number of females and is not necessarily restricted by geographical area, seasonality of breeding or the life-span of the male. Additionally the successful storage and artificial insemination of semen has the potential to provide a rapid spread of improved genotypes within an existing population (Moore, 1985).

Artificial insemination also offers a means of reducing the necessity to keep breeding males in both large and small flocks or herds (Shelton, 1978; Doney et al., 1982). Artificial insemination with off-farm semen may completely replace or reduce the requirement to maintain breeding males. The use of artificial insemination may also make more effective use of the on-farm males available, also reducing the number of breeding males that must be maintained.

The degree to which these benefits are realised depends partially upon the method of semen storage. This is because the conditions of semen storage determine how effectively the population of sperm cells within the semen are preserved and over what time-span. In this review, a brief history of the development of semen processing technology will be given, the seminal milieu will be examined, the methodologies of semen preservation will be introduced and the factors affecting the functional preservation of the sperm cell population will be discussed.

1.2 Development of Semen Preservation Technology.

The widest application of artificial insemination and semen preservation technology has historically been with dairy cattle. The intensive management of dairy cows, the relative ease of oestrus detection and the frequency of handling makes them well suited to artificial insemination programmes. Betteridge (1986) states that up to 100%

of dairy cows are artificially inseminated in Denmark and Japan, with about 60% in the USA, although the percentage is much lower in less developed countries.

The contribution of artificial insemination to the genetic composition of highly productive bovine breeds, has led to the practice being considered a desirable tool in the breeding of other species (Corteel, 1981). As a result, many aspects of the semen preservation technology developed for bovine semen have been adapted for the preservation of semen of other species.

Bull semen was first used to artificially inseminate cows in Denmark in 1936, in the USA in 1937 and in Britain in 1942 (Betteridge, 1986). A brief history of the development of dilution and preservation techniques applied to bovine semen used in artificial insemination was given by Amann and Schanbacher (1983). By the mid-1950's cows were routinely inseminated with diluted semen kept in a liquid state at 5°C for up to two days. The bovine semen was diluted with an extender of either egg-yolk and citrate, or heated cow's milk, in which antibiotics (penicillin, streptomycin) were included .

In the late 1950's, two methods of bovine semen dilution were developed that extended the storage period to between three and five days, whilst maintaining fertility. In the first of these, an egg-yolk based diluent was used in combination with a complex buffer that was gassed with carbon dioxide, or with a buffer generating carbon dioxide from bicarbonate and citric acid. The second method involved the addition of glycerol to semen previously diluted with heated milk and cooled to 5°C.

Similar methods for the short-term preservation and storage of liquid bovine semen have remained useful in some situations. In New Zealand for example, egg-yolk diluents incorporating citric acid based buffers and caproic acid have been widely used.

Of greater importance to the role of artificial insemination in the breeding of cattle has been the development of semen freezing methods. Bull semen was first successfully frozen in 1949, with spermatozoa being protected from the lethal effects of the freezing process (Polge et al., 1949). Initially, frozen bull semen was packaged in sealed glass ampoules and stored in alcohol cooled with solid carbon dioxide (dry ice). This system of storing diluted semen was used commercially in the USA as well as other countries. However, the wide-spread use of frozen semen on an international level did not occur until after 1959. This was a result of the development of super-

insulated, cryogenic tanks suitable for field use, and the adoption of liquid nitrogen as a preferred storage medium.

Since that time, considerable research effort has been directed at the development of improved diluents, packaging and methodologies for the freezing and thawing of bull semen. In particular, the introduction in 1964 of plastic straws for the packaging of frozen semen, has improved the efficiency of storage and reduced losses of semen during the insemination procedure. Even with the best procedures for the freezing of bull semen, 20% or more of the spermatozoa do not recover motility after freeze-thawing. However, Amann and Schanbacher (1983) state that conception rates for cows inseminated with frozen-thawed semen are potentially equivalent to those obtainable with unfrozen semen or natural service.

The artificial insemination of other domestic animals with frozen semen has not been as well developed, partly because there have been fewer commercial incentives (Betteridge, 1986). A principle difference between cattle and other species has been in the number of females that may be inseminated with one ejaculate. Up to 1,000 cows may be inseminated with one ejaculate, whilst the ejaculates of boars, rams and stallions may only be used over five to fifty females (Amann and Schanbacher, 1983). In addition, the conception rates achieved in the past with the frozen semen of other species have been lower than those reported for cattle. It has been apparent that there are distinct species differences in the diluents required for optimum sperm survival in the freeze-thaw process. Interest in the application of artificial insemination and semen freezing has however been developing in other species such as pigs and sheep (Betteridge, 1986).

The insemination techniques used for goats are essentially derived from those developed for sheep (Doney et al., 1982) whilst the media used for the preservation of both sheep and goat semen are adapted principally from those developed for bull semen (Ritar, 1983).

2.3 Semen.

Semen is the generative fluid of the male. It is ejaculated within the genital tract of the female during copulation, facilitating the gamete transfer required for fertilisation to occur within the female. Semen is comprised of the male gametes, or spermatozoa, suspended within the seminal plasma. Thus semen has a cellular component and a

secretory fluid component. However, the final combination of the spermatozoa and seminal plasma only occurs during the process of ejaculation (Harper, 1986).

Mammalian spermatozoa are produced in the testes by the processes of spermatogenesis (Setchell, 1977; Amann and Schanbacher 1983; Setchell, 1984; Hochereau-de Reviers, 1987). The cycle of the seminiferous epithelium has been characterised in goats by Bilaspuri and Guraya (1984). In post-pubertal males, spermatozoa are released from the Sertoli cell lining in the walls of the seminiferous tubules, which ramify throughout the testicular tissue. Harper (1986) outlines the transport of spermatozoa in the male tract. Spermatozoa released from the seminiferous epithelium are passively transported to the rete testis suspended in the fluid secretions of the Sertoli cells. The spermatozoa pass from the rete testes to the efferent ducts and thence to the epididymides. Fluid flow and transport of the suspended spermatozoa is achieved by the action of cilia, water reabsorption, cellular secretory activity and muscular contraction in duct walls.

The fluid environment in which the spermatozoa are suspended changes with transport along the genital tract. Initially the spermatozoa are suspended in the fluid of the rete testes. The composition of the fluid in the seminiferous tubules and rete testes is distinct from that of the blood plasma and testicular lymph (Setchell, 1984). This difference is maintained by the specialised junctions between the Sertoli cells that create the blood-testes-barrier, in combination with the specific secretory activity of the Sertoli cells (Waites, 1980). Testes fluid transports the spermatozoa from the germinal epithelium to the epididymides in a medium containing substrates required for sperm survival. In addition, this fluid contains compounds such as acrosomal proteinase (acrosin) inhibitors, androgen-binding protein, inhibin and steroid hormones. In domestic species such as cattle and sheep the relationships between specific Sertoli cell secretions and sperm quality have not been comprehensively investigated (Hochereau-de Reviers, 1987).

The spermatozoa that leave the testes are both immotile and infertile (Setchell, 1984). The capacity for both functions is acquired on passage through the three regions of the epididymis, the caput, the corpus and the cauda epididymis. The composition of the fluid in which the spermatozoa are suspended, changes considerably on passage through these regions of the epididymis.

Thus the epididymis provides an environment for both the functional maturation of the spermatozoa and their maintenance under optimal conditions prior to ejaculation

(Amann, 1987). Sperm cells from the proximal and distal cauda epididymis are equivalent to ejaculated spermatozoa in terms of fertilising capacity. However, although spermatozoa from the cauda epididymis are considered functionally mature, these cells are further altered on contact with seminal plasma.

The cells of the epididymal epithelium, like Sertoli cells, are polyfunctional and are involved with secretory, accumulative and absorptive processes (Waites, 1980). Along the length of the epididymis the lumenal fluid changes in ionic and organic composition (Setchell, 1984). This is achieved within the epididymis by the transport of small molecules, the transport and synthesis of large molecules and the metabolism or modification of existing molecules, as described by Amann (1987). Like testicular fluid, the lumenal fluid of the epididymis has a unique composition (Waites, 1980).

The involvement of the epididymis with the maturation of spermatozoa is evident from the acquisition of fertilising capacity, the acquisition of forward motility, changes in the morphology of spermatozoa, and changes in sperm membrane structure and metabolism in the epididymal environment (Amann and Schanbacher, 1983). However, the biology of maturation has been poorly understood, as are the mechanisms of sperm maintenance in the cauda epididymis prior to ejaculation or evacuation (Amann, 1987).

The protein content of epididymal fluid is important to sperm maturation, as it appears likely that some changes in the protein composition of the spermatozoa result from interaction with the surrounding fluid (Setchell, 1984). Epididymal proteins have been observed to accumulate at the sperm membrane surface (Waites, 1980). Acidic glycoproteins secreted in the caput and corpus epididymis become coated onto sperm surfaces in the cauda region of the epididymis, and these have been implicated in the processes of sperm maturation. Additionally, a protein associated with the acquisition of progressive motility, described as forward motility protein, is present in the epididymal fluid at much higher concentrations than it is in seminal plasma.

Concentration of the suspension of lumenal fluid and spermatozoa may also be important to the processes of maturation. It has been estimated that 98 % of fluid is absorbed out of the lumen on passage from the rete testis to the cauda epididymis in the goat, with 93 % of fluid being absorbed between the rete testis and the caput epididymis (Jindal, 1984). The latter author suggested that this absorption may increase the probability of spermatozoa contacting maturation factors, or may condense such factors to critical concentrations.

Spermatozoa that pass through the epididymis are either ejaculated or voided during urination (Amann and Schanbacher, 1983). Resorption is not a major form of sperm removal from the genital tract. During seminal emission and ejaculation, muscle contractions in the duct walls rapidly transport the suspended spermatozoa from the two cauda epididymides to the vasa deferentia and then into common urethra (Harper, 1982). In the urethra, the stored secretions of the accessory glands (the vesicular glands, prostate gland and bulbo-urethral glands) are released and mixed with the spermatozoal suspension from the epididymis. This combined fluid is forcibly ejaculated from the penile urethra by the actions of the ischiocavernosus and bulbospongiosus muscles which surround the urethra in both the pelvis and penis. The involvement of muscular activity and blood flow during erection and ejaculation in the goat buck was discussed by Beckett et al. (1978).

The fluid in which the ejaculated spermatozoa are suspended is thus comprised of the various secretions from the testes, the epididymides and the accessory glands, and is collectively described as the seminal plasma. In most mammals up to 95-98% of the seminal plasma volume may be derived from the accessory glands (Setchell, 1977; Hogarth, 1978). The seminal plasma provides a medium for the transfer for spermatozoa, further activates sperm motility and provides a buffered, nutrient-rich medium to aid sperm survival after ejaculation (Mann and Lutwak-Mann, 1981). This medium may contain considerable quantities of potassium, sodium and chloride ions, with the major organic components being fructose, sorbitol, inositol, citric acid, glycerylphosphorylcholine, phospholipids, prostaglandins, riboflavins and proteins (Evans and Maxwell, 1987).

An interesting constituent of goat seminal plasma is the enzyme phospholipase-A which originates in the Cowpers (or bulbo-urethral) glands. This enzyme hydrolyses the lecithin of egg yolk to fatty acids and lysolecithin, which are toxic to spermatozoa (Roy, 1957; Aamdal et al., 1965; Ritar and Salamon, 1982). This reaction has important repercussions for the use of egg yolk in the storage of goat semen, as will be discussed in a later section. Phospholipases and lysophospholipases have been implicated in the support of the spermatozoa throughout the maturation phase in the epididymis (Atreja and Anand, 1985). Glycolysable substrates are not available to the spermatozoa in the epididymis and energy must be derived from the oxidation of other substrates such as phospholipids. Phospholipases and lysophospholipases are produced by goat epididymal spermatozoa, although their activity decreases on transit from the caput to cauda epididymis.

Mendoza et al. (1981, 1989) characterised the concentration of fructose, glucose, lactic acid, citric acid, glycerylphosphorylcholine (GPC), glycerophosphate (GP), glycerol, and riboflavin present in the seminal plasma of Angora bucks. The concentrations of fructose and lactic acid were high enough for them to be considered important substrates for sperm metabolism. Lactic acid is rapidly formed from the metabolism of fructose by sperm in ejaculated semen and can be oxidised to provide an energy source. Interestingly, it was suggested that the lactic acid was derived from the accessory glands, since sperm metabolism was arrested on dry ice at the point of collection in this study.

Although citric acid was present in significant concentrations, it is not a source of metabolic energy that can be utilised by mammalian semen. Instead citric acid may be an important component of the buffering system of the seminal plasma. In contrast, glucose can be utilised as an energy substrate but was present in only low concentrations. This difference in the concentration and potential metabolic importance of glucose and fructose is consistent with other reports (Anand, 1973).

The concentration of GPC in the seminal plasma was correlated with the concentration of spermatozoa in the ejaculates, suggesting an epididymal origin (Mendoza et al. 1989). This GPC was not regarded an energy substrate for spermatozoa, although breakdown products may possibly be utilised to some extent in the uterus.

This study also indicated that the high concentrations of riboflavin in the seminal plasma were responsible for the yellow colouration that was obvious in over 60% of the ejaculates collected. Riboflavin content was strongly correlated with the concentrations of fructose and citric acid indicating a common origin, probably the seminal vesicles.

The seminal plasma also contains reproductive hormones such as inhibin and prostaglandin. Inhibin, a hormone associated with the feedback regulation of pituitary hormone secretion has been described in goat seminal plasma (Miyamoto et al., 1987). The concentration of inhibin in the seminal plasma is distinctly seasonal and probably involved with the seasonal variations in spermatogenesis. Prostaglandins are also present in higher concentrations in goat seminal plasma than in many other species (Mann and Lutwak-Mann, 1981). These have been suggested as an aid to sperm

transport within the female reproductive tract, as prostaglandins stimulate uterine contractions.

2.4 Semen Collection.

Semen may be collected by ejaculation into an artificial vagina or by electrical stimulation of the genital tract (Corteel, 1981). In general, the approaches to semen collection have not changed greatly since the 1950's (Amman and Schanbacher, 1983). One exception is the development of improved forms of electrical stimulators.

Routine collection methods for most domestic livestock depend upon the induction of ejaculation into an artificial vagina (Betteridge, 1986). This can be achieved after introduction of the male to a teaser female. Evans and Maxwell (1987) and Holt (1987) describe the collection of semen from sheep and goats. The same procedures may be used for both species. The artificial vagina used for sheep and goats is similar to that used for bulls, although shorter to accommodate the shorter penis of the ram or buck. It consists of a rigid outer jacket and an inner liner of rubber. A tap in the outer casing allows the envelope formed between the inner and outer jackets to be filled with warm water and pressurised. At one end the liner may be lubricated, whilst at the opposing end a glass collection vessel is held in place.

Prior to collection, the males are trained in the presence of the operator to mount a teaser female held in a restraint. Most males can be trained to mount live teasers whether in oestrus or not (Betteridge, 1986). Successful collection can depend upon attention to the individual mounting habits of the male. This can be with respect to the preparation of the teasers, the preparation of the artificial vagina and the actions of the operator.

As Evans and Maxwell (1987) note, the artificial vagina stimulates the male to ejaculate using both thermal and mechanical stimuli. The penis is directed into the artificial vagina as the buck rises to mount the teaser. At this point the temperature of the artificial vagina should be between 42 and 45 °C and the water-jacket should be under pressure. Ejaculation rapidly follows extension of the penis along the length of the artificial vagina and is often accompanied by a pronounced pelvic thrust. Inversion of the artificial vagina allows the semen to drain into the collection vessel ready for processing.

Semen collection using electro-ejaculation is also described by Evans and Maxwell (1987) and Holt (1987). The electo-ejaculator is a rectal probe with attached bipolar electrode. This probe is inserted into the rectum of the male when the latter is restrained in a lateral position on the floor. The penis is manually exposed from the prepuce by straightening the sigmoid flexure and manipulating the glans penis. Once exteriorised, the glans penis is gripped with a cotton swab and the urethral process is directed into a collection tube. Voltage is then applied in several brief periods of stimulation until ejaculation occurs. The electro-ejaculator directly stimulates the ampullae and seminal vesicles (Betteridge, 1986).

Semen Quality.

In many respects the artificial vagina method of semen collection is regarded as preferable to electro-ejaculation (Evans and Maxwell, 1987; Gomes, 1977; Holt, 1987; Ritar, 1987). The former is simple, quick and does not stress the buck. Electro-ejaculation can result in signs of stress in the animals from which semen is collected. Memon et al. (1986) observed increased vocalisation, excessive muscular contractions of the rear legs and increased rate and depth of respiration in electro-ejaculated bucks.

Semen collected by electro-ejaculation is usually of poorer quality than that collected by the artificial vagina method because of the comparatively lower concentration of spermatozoa. Memon et al. (1986) compared goat semen collected by artificial vagina and two types of electro-ejaculator, reporting that both forms of electro-ejaculator gave ejaculates of greater volume than those collected by artificial vagina. However, the electro-ejaculates also had a lower sperm concentration, containing a greater amount of seminal plasma than artificial vagina ejaculates. Austin et al. (1968) reported similar differences between ejaculates collected by these two methods. Memon et al. (1986) also found that whilst the mass activity (or wave motion) and pH was greater in the ejaculates collected by artificial vagina, no differences in sperm motility were recorded. However, the percentage of normal acrosomes was greater in ejaculates collected by artificial vagina than those produced by one type of electro-ejaculator.

These results obtained for bucks are similar to those reported for rams (Mattner and Voglmayr, 1962). These authors attributed the lower wave motion of electro-ejaculated semen to the lower sperm concentration, but found no effect of collection

method on the proportion of live or actively progressing spermatozoa. Acrosome morphology was also unaffected by the collection method.

In addition to modifying the proportion of sperm cells to the volume of seminal plasma, electrical stimulation may also alter the composition of the seminal plasma . Iritani and Nishikawa (1964b) reported lower concentrations of fructose, citric acid and protein in the seminal plasma of electro-ejaculated buck semen. This contrasts with the finding of Mattner and Voglmayr (1962) that electro-ejaculated ram semen contained a higher concentration of fructose than semen collected by artificial vagina. Quinn and White (1966b) also found that electro-ejaculated ram semen contained higher concentrations of sodium, potassium and calcium. The electro-ejaculated buck semen assessed by Iritani and Nishikawa (1964b) also displayed six times more egg-yolk coagulating enzyme activity. These results suggest a proportionally different contribution of seminal plasma constituents from the secretions of the accessory glands during electro-ejaculation compared to artificial vagina collection. Differences in ejaculate pH may be related to sperm concentration, with pH tending to be lower in higher sperm concentration ejaculates (Mattner and Voglmayr, 1962).

Electro-ejaculated semen may be less suitable for subsequent processing than semen collected by artificial vagina. Ram semen collected by artificial vagina is more resistant to cold shock than that collected by electro-ejaculation, with regard to effects on sperm motility and structural integrity (Quinn et al., 1968). These authors established that dilution of an ejaculate with seminal plasma increased cold shock succeptibility, but that the difference in cold shock tolerance between the two methods of semen collection could not be explained purely by differences in sperm concentration. When sperm collected by artificial vagina and electro-ejaculation were suspended in seminal plasma from either collection method, both were more resistant to cold shock in the seminal plasma from artificial vagina collection. This suggests that seminal plasma composition contributes to differences in cold shock susceptibility, possibly being related to pH and buffering capacity (Quinn et al., 1968). Cold shock susceptibility declines with decreasing pH, and since electro-ejaculated semen contains a greater proportion of seminal plasma, its buffering capacity may be greater, retarding the decline in pH. Additionally, the lower concentration of spermatozoa in electro-ejaculates is likely to reduce the metabolic production of lactic acid, further reducing acid decline.

In general, semen collected by the artificial vagina method is regarded as more closely resembling the ejaculate deposited in the female during natural mating (Lightfoot,

1968) and gives more consistent results than electro-ejaculation in terms of the number of sperm collected (Mattner and Voglmayr, 1962). Electrical stimulation of the urogenital tract may also introduce urine contamination into the ejaculate (Evans and Maxwell, 1987).

Collection Efficiency.

Electro-ejaculation has the advantage of being readily applied without significant animal preparation. However, sperm concentrations in the ejaculate are lower, and the total number of spermatozoa collected per ejaculate are similar to that collected by artificial vagina, only if sufficient semen volume is obtained during electrical stimulation (Memon et al., 1986, Mattner and Voglmayr, 1962). Indirect evidence has also suggested a significant loss of spermatozoa during electro-ejaculation of bulls, by retrograde flow into the bladder and subsequent loss in the urine (Dooley et al., 1986). In this study the mean loss of spermatozoa was 21 percent but ranged from 1 to 50 percent. Whilst this is a significant loss, no evaluation was made of the losses that may have occurred during natural mating or with artificial vagina collection, consequently the comparative importance of this finding cannot be determined with regard to collection efficiency.

Whilst artificial vagina collection may be the preferred method of semen collection, there are also potential problems associated with this procedure. As Corteel (1981) notes, this form of collection depends upon the libido of the buck and some may never display any breeding behaviour at all. Corteel reported one study of 46 Alpine and Saanen goats at 5 to 6 months old, of which 8.7 percent showed a lack of libido. Low libido is regarded as a problem in Australian Angora bucks but not in Cashmere bucks (Scheurmann, 1985). Whilst low libido may cause problems in semen collection, high libido may not always be associated with high semen output (Fielden and Barker, 1964).

Libido may also be affected by the seasonality of reproductive behaviour, affecting the capacity to collect semen. Goats are generally regarded as short-day breeders with the maximum incidence of breeding activity occurring in autumn and early winter (Moore, 1985). In equatorial regions, where daylength is relatively constant, breeding activity is not seasonally limited by photoperiod and goats may display year-round sexual activity (Doney et al., 1982). Chemineau (1986) reported that tropical Creole goats in Guadeloupe are not seasonal breeders. In behavioural tests, the number of

matings per test and the time from the start of the test to the first mating did not vary with month, although the number of mountings per test did. Conception, combining fertility and sexual activity similarly did not vary between three times of year. Corteel (1981) states that in non-seasonal breeds seasonality cannot limit libido and therefore semen collection.

In temperate regions (higher latitudes) goats show a considerable seasonal difference in reproductive activity (Shelton, 1978). Although goats may be capable of mating throughout the year, there are distinct seasonal variations in the expression of sexual activity related to photoperiod (Thimonier, 1981). Despite this seasonality of mating behaviour, semen can be collected throughout the year in some seasonal breeds (Corteel, 1981). This is the case where the animals have been well trained such that mounting and ejaculation become reflex activities.

Other environmental factors also reportedly influence sexual desire to produce a seasonal effect. In the male Damascus goat, Elwishy et al. (1971) assessed sexual desire in terms of the reaction time (time taken from introduction to mating), the number of mounts per ejaculation and the number of refusals when introduced to an anoestrous teaser doe. Whilst maximum sexual activity occurred in autumn, the significant effect of month on sexual activity was regarded as being influenced more by relative humidity than by photoperiod or temperature.

Sexual performance during collection can be influenced by methods of sexual stimulation or teasing. Price et al. (1984) examined the effects of three stimuli compared with an unstimulated control. The stimuli consisted of having another male watching from an adjacent area, observing another male court and copulate with the teaser, or a combination of both.

After introduction to the teaser female, the time to mounting and ejaculation was significantly shortened by teasing. Although no effect of stimulation on semen concentration or volume was observed, these results have implications to the efficacy of the collection process. Fielden and Barker (1964) recorded a variable effect of teasing on semen output from individual bucks with no increase across the whole experimental group. However, teasing increased the number of ejaculates that were lost through rapid ejaculatory responses, questioning the value of the procedure. However, the skill of the technician can limit such losses.

2.5 Ejaculate Quality.

Ejaculate quality assessment includes measures of volume and sperm concentration, and assessment of the mass activity and motility of the population of spermatozoa within the ejaculate (Corteel, 1981; Evans and Maxwell, 1987).

Seasonality.

Ejaculate characteristics vary with season in breeds that display seasonality of reproductive behaviour. Eaton and Simmons (1952) found that ejaculate volume was greatest in Summer and Autumn and lowest in Spring, whilst concentration of spermatozoa was conversely higher in Spring and lowest in Autumn. Ejaculate volume ranged from 0.74ml in autumn to 0.49ml in Spring. The concentration of spermatozoa ranged from 2.26 to 3.28 billion per ml in Autumn and Spring respectively. Doney et al. (1982) state that ejaculate volumes in sexually active bucks may vary widely, but commonly fall within the range of 0.6 to 0.9ml, with an average sperm concentration of about 4 billion sperm per ml.

The total number of spermatozoa per ejaculate may also vary with season, in addition to the negative correlation of ejaculate volume and sperm concentration (Corteel, 1981). Eaton and Simmons (1952) reported that total sperm number per ejaculate was highest in Summer and lowest in Spring. Miyamoto et al. (1987) similarly found that total sperm number per ejaculate increased from Spring to Summer and decreased in winter. These changes reflect seasonal change in the endocrinological status of the male. In the Damascus goat, Elwishy et al. (1971) reported no seasonal change in spermatogenic activity as indicated by the total number of spermatozoa per ejaculate. Seasonal variation in other reproductive traits were reported for this breed, but were not regarded as being controlled by the light environment. In the aseasonal Creole goat, Chemineau (1986) also observed no variation in spermatogenic activity with time of year.

The pituitary hormones LH (luteinising hormone) and FSH (follicular stimulating hormone) are involved in the control of spermatogenesis and steroidogenesis in the male. LH primarily stimulates the synthesis and secretion of testosterone from the interstitial cells of the testes, whilst FSH promotes the growth of the seminiferous tubules and is important in the first stages of spermatogenesis (Baird, 1975). LH-induced testosterone secretion is required for the development and maintenance of the

male reproductive system and the processes of spermatogenesis (Edey, 1983; Gower, 1979). Secondary sexual characteristics and libido are also promoted by testosterone. FSH primarily acts upon the Sertoli cells that are intimately associated with the developing spermatocytes, eliciting a range of metabolic changes in the Sertoli cells (Means et al., 1980). There is a functional dependence of the germ cells on the Sertoli cells and the latter exhibit changes in function during the cycle of the seminiferous epithelium (de Kretser, 1990). However, as the latter author notes, the intercellular communication is two-way, with the presence of germ cells also eliciting changes within the Sertoli cells.

FSH and LH have integrated functions, with FSH stimulating androgen-binding protein (ABP) production in the Sertoli cells, which is subsequently secreted into the lumen of the seminiferous tubules. This may help maintain a constant testosterone concentration near the androgen-dependent germinal epithelium, against the background of fluctuating Leydig cell secretion of testosterone in response to the episodic release of LH (Hogarth, 1978; Jegou et al., 1979; Waites, 1980).

In the ram, Courot and Ortavant (1981) confirmed the importance of LH, FSH and testosterone in the control of spermatogenesis and identified some areas of sensitivity in the developing germ cells. These authors concluded that undifferentiated spermatogonial stem cells require no hormonal support *per se*, although their division to renewing spermatogonia was depressed by hypophysectomy. In contrast, the more differentiated intermediate and type B spermatogonia were highly sensitive to pituitary hormone deprivation, being subject to degeneration or dormancy. Primary spermatocyte production thus eventually ceases, although the division to young spermatids from the existing primary spermatocytes was quantitatively insensitive to pituitary hormone deprivation. The most highly differentiated germ cells, the spermatids, were subject to degeneration after hypophysectomy, although their active life-span after hypophysectomy was greater than that of the spermatogonia, and degeneration was delayed until the more sensitive stages of chromatin condensation and nuclear elongation.

In bucks displaying reproductive seasonality, there are distinct changes in endocrinological status according to the time of year. Racey et al. (1975) reported that LH secretion increased during summer and autumn, and suggested that the elevated testosterone levels also observed in autumn, resulted from the combined action of higher levels of circulating LH and LH-induced trophic changes in testicular responsiveness. Muduuli et al. (1979) similarly found that under natural photoperiod

mean LH and testosterone concentrations in serum increased with decreasing daylength. LH concentrations increased as a result of higher baseline levels between the pulsatile release of the hormone and increased release frequency. Testosterone was also released episodically and rises in serum concentrations followed those of LH. In contrast, serum FSH concentrations showed no episodic pattern of release, but did rise at a similar time to LH. However, the peak in FSH concentration occurred during a time of increasing daylength.

Miyamoto et al. (1987) did not fully characterise hormone levels during the period of increasing photoperiod, but did define simultaneous peaks in both LH and FSH one month after the summer solstice. These authors also examined the activity of inhibin in the seminal plasma. Inhibin is secreted by the Sertoli cells (Steinberger and Steinberger, 1976) and is a negative feed-back control of FSH production at the pituitary-hypophyseal axis. Taking into consideration the time lag between the initiation of spermatozoal development and storage in the tail of the epididymis, there was a positive correlation between inhibin activity in the seminal plasma and sperm number in the ejaculate two months later (Miyamoto et al., 1987).

Rams also display seasonality of reproductive characteristics, behaviour and endocrinology (Sanford et al., 1977; Tulley and Burfening, 1983). The cytological effects of this photoperiodicity were examined by Hochereau-de Reviers et al. (1985). No seasonal change in the total numbers of Leydig cells, Sertoli cells or stem cells was recorded, although it was found that Sertoli and Leydig cell size, spermatogenic efficiency and daily sperm production were all reduced under long-day conditions. Cytological changes were positively correlated with FSH and LH concentration changes. These observations link the changing endocrinology of the seasonal male with some of the observed differences in ejaculate characteristics.

In addition to sperm concentration and ejaculate volume, reproductive seasonality may also affect the motility of the collected semen. Eaton and Simmons (1952) described reduced motility scores (mass activity or wave motion) in non-breeding season ejaculates, with motility scores highest in Summer and Autumn and lowest in Winter. Elwishy et al. (1971) also found a seasonal variation in both the mass activity and the initial motility (% motile) of ejaculates. These characteristics were greatest in summer, during what was regarded as the breeding season for the Damascus goat. Corteel (1981) states that in the non-breeding season, sperm motility is severely depressed in diluted and cooled semen and also in diluted frozen-thawed ejaculates. This author suggested that the seasonal coincidence of low sperm motility and low

levels of peripheral blood testosterone indicated that some androgen-dependent mechanism may be affecting sperm motility. GnRH (gonadotrophin releasing hormone) therapy can significantly effect the reproductive characteristics of out-of-season rams, increasing the percent motile sperm and testosterone levels (Schanbacher and Lunstra, 1977).

Seasonal changes in sperm motility may result from changes in the seminal plasma composition. Baas et al. (1983) note that it is well documented that seminal plasma sustains the motility of spermatozoa, but that it may also be detrimental to sperm survival. In bulls, motility is affected by seminal plasma constituents that have been implicated in inducing both a reactivation of motility in sperm deactivated by Ficoll washing, and also in reducing the subsequent period of sustained motility. In goats, there is a seasonal variation in the seminal plasma constituents (Iritani and Nishikawa, 1964a) and also in the effect of seminal plasma on sperm the survival (Nunes et al., 1982). The latter authors found that seminal plasma from the non-breeding season had a negative affect on sperm survival in diluted semen incubated at 20°C and 4°C. Bulbo-urethral gland secretions were suggested as the source of the inhibitory effects on sperm survival, with these negative effects being counteracted by vesicular gland secretions during the breeding season.

Another aspect of quality, the proportion of morphologically abnormal sperm, may also be effected by the season of collection. In the breeding season, the average ejaculate contains 85 to 95 % morphologically normal sperm (Corteel, 1981). The percentage of abnormal sperm has been reported as being lowest in the breeding season and highest in Summer and Spring (Eaton and Simmons, 1952). Yao and Eaton (1954) and Elwishy et al. (1971) similarly characterised seasonal changes in the percentage of abnormal spermatozoa. In contrast, Skalet et al. (1988) found no such seasonal variation in Nubian bucks of mixed ages and regarded this as supportive of the literature in other domestic species.

Negative correlations between motility and the percentage of abnormal spermatozoa have been reported (Eaton and Simmons, 1952; Elwishy et al., 1971). However, in seasonally quiescent rams, Schanbacher and Lunstra (1977) reported that whilst GnRH therapy increased sperm motility (% motile) there was only a slight reduction in the percentage of abnormal sperm.

Status of the individual.

Seasonality is not however the only factor affecting ejaculate quality. Ejaculate volume, sperm concentration and total number of sperm per ejaculate are variable with breed and age of the buck (Corteel, 1981). This author regarded that in the first breeding season the number of sperm per ejaculate may be only 60 % of that in the mature animal. Eaton and Simmons (1952) found no effect of age on ejaculate characteristics other than motility and the occurrence of sperm abnormalities. In animals older than one year, motility decreased with age whilst the number of abnormal spermatozoa increased. These authors did however note a significant effect of breed (comparing Toggenburgs with a local American breed) on semen volume, sperm concentration, total number of sperm and the percentage of sperm abnormalities. The ejaculate volume appeared to be related to the weight of the buck. In Nubian bucks, Skalet et al. (1988) characterised a high incidence of sperm abnormalities (64.6 %) at the onset of puberty at 141 days. By eight months the percentage of sperm abnormalities had declined considerably (12.5 %), although subsequently there was no significant age effect on the type or percentage of abnormalities in bucks up to four years old.

The health of the animal is also a determinate of semen quality. Environmental stressors such as heat, humidity and energy intake, all affect ejaculate volume and concentration (Corteel, 1981).

Collection.

As already discussed, collection method affects a range of ejaculate quality factors. The frequency of collection also influences some ejaculate characteristics. In the early 1950's the exploitation of males (the bull in particular) as a sperm factory was limited by the notion that collection frequencies greater than once per week would damage testicular function and be deleterious to the health of the male (Amann and Schanbacher, 1983). This assumption was dispelled in various collection studies, and the latter authors state that collections may be made daily or even several times daily without deleterious effects on fertility in a range of domestic species.

In goats, collection two to three times daily on alternate days using an artificial vagina is regarded as a normal regime of collection (Evans and Maxwell, 1987). Even so,

ejaculate characteristics do vary between collections. Fielden and Barker (1964) found that ejaculate volume and sperm concentration declined with successive collections in a trial studying the effects of frequent collection. On average 62% of the total yield of spermatozoa on any one day was collected in the first two ejaculates, whilst some of the animals were collected up to 12 times on the collection day. Corteel (1981) regarded that it is not worth collecting smen from bucks more than twice a day.

At the point of collection and immediately afterwards, before there is any influence of method of preservation, semen quality may be affected by a number of environmental factors (Evans, 1987; and Evans and Maxwell, 1987). Collected semen is sensitive to a range of factors that effect the environment of the sperm cells and cause cellular damage or impaired function. Semen is adversely effected by temperatures in excess of the 37.5°C at which it is ejaculated. Such temperatures may quickly exhaust the energy reserves of the spermatozoa and cause cellular death. The spermatozoa are also sensitive to rapid reductions in temperature that cause irreversible loss of viability (cold-shock). Direct exposure to sunlight, ultra-violet light or even strong fluorescent light can also reduce sperm viability.

In terms of the liquid environment, spermatozoa are affected by changes in the pH of the seminal plasma with pH values above or below 7 reducing the viability of spermatozoa. Such changes may result from the oxidative metabolism of the spermatozoa, with the consequent production of lactic acid and carbon dioxide. During the collection process, contact with contaminants such as urine, dust or bacteria, and contact with metal or disinfectants used to sterilise equipment are all deleterious to the survival and viability of the spermatozoa, and should be avoided.

Some types of artificial vagina liner may also have toxic reactions with collected semen. Bovine spermatozoa are adversely affected by rubber as opposed to polyethylene liners (Flick and Merilan, 1988; Salhab and Merilan, 1989). Although the toxicity effects were not initially observable, the rubber liner reduced the survival of spermatozoa stored at 4 °C. Toxic effects were apparent within 3 hours of collection and peaked after 60 hours. Bovine semen was frozen under the same conditions after collection with both types of liner, and at least 50% of the motile spermatozoa collected using polyethylene survived after freeze-thawing, whereas not more than 30% collected with rubber liners survived.

2.6 Semen Handling after Collection.

A normal ejaculate contains many more spermatozoa than is needed to achieve acceptable fertility when deposited in the female genital tract (Evans, 1987). The ejaculate may thus be divided into a number of insemination doses, providing the potential to distribute the available semen over a much greater number of females than occurs by natural mating. Dilution of the semen is necessary to give an inseminate volume that may be conveniently handled during AI. This inseminate volume must contain a sufficient number of spermatozoa to meet fertility requirements whilst not wasting the available sperm resource (Memon and Ott, 1981). Undiluted semen can be used where the males and females are being reared together, although it is necessary for the inseminator to work quickly to avoid exhaustion of sperm energy reserves and desiccation of the ejaculate (Corteel, 1981).

However, diluting semen may also confer benefits beyond the modification of sperm concentration. A diluent or extender may be used to modify the environment of the sperm cells in the period between collection and insemination in order to improve sperm survival and inseminate fertility (Foote, 1988). The nature of the diluent depends upon whether the semen is processed for use as short-term liquid-stored semen or as frozen-thawed semen.

In general terms, the extender must perform a number of functions as outlined by Evans (1987) and Memon and Ott (1981). The semen extender must fully or partially replace the seminal plasma to provide a vehicle for the transfer of the spermatozoa and improve or maintain their viability during processing. Thus the extender should be a medium that provides a source of metabolisable energy and also contains a buffer against pH shifts resulting from metabolic production of lactic acid. The medium may also contain protectants against cold-shock and freezing damage. Even where no cooling is intended, protective substances may be included to reduce the effects of any accidental cold-shock. The extender may contain antibiotics to inhibit bacterial growth in the inseminate and must maintain the osmotic pressure and electrolyte balance of the sperm cells after dilution. Evans (1987) states that the diluent should be isotonic for fresh semen. However, Salamon and Ritar (1982) found that a hypertonic extender increased the percentage of motile spermatozoa after freeze-thawing.

Regardless of the method of semen preservation, there are some general processing considerations, as described by Evans and Maxwell (1987). Immediately after collection the semen should be protected from cold-shock and held at 30°C in a water-bath whilst preliminary assessment of concentration, motility and volume is made. Only satisfactory ejaculates should be processed further. During this time the ejaculate should also be protected from direct sunlight and contact with water or airborne contaminants. After assessment, dilution should be undertaken with extender at the same temperature as the semen. Dilution should be gradual, particularly where hypotonic solutions are used, so that osmotic shock to the spermatozoa is avoided. Following dilution, the semen may be subject to further processing, such as washing, cooling and freezing.

2.7 Liquid Storage of Semen.

For semen that is extended and stored at temperatures above 0°C, spermatozoa survive for only a few days (Corteel, 1981). In addition, the fertilising capacity of sperm is maintained for a shorter period than motility (Corteel, 1981; Evans and Maxwell, 1987).

The use of liquid-stored semen is thus restricted with regard to the time period between collection and insemination. As a result, the flexibility of the insemination programme may be reduced by geographical and seasonal considerations and also by the capacity of the sire to produce semen on demand. Anderson (1969) notes that insemination with commercially traded liquid goat semen is limited by distance and to areas where goat populations are relatively dense. It is of interest to note though, the wide-spread application of ambient temperature storage of bovine semen in New Zealand (Gomes, 1977).

Fresh Semen.

Fresh semen for use immediately after collection can be diluted and held at 30°C in a number of media, although preferred diluents are heat-treated skim milk or a Tris-based buffer that contains no cryoprotectants (Ritar, 1987). Cows milk provides a simple diluent that can be prepared from whole, skim or powdered milk, or directly used in the ultra-heat-treated (UHT) form (Evans and Maxwell, 1987). Synthetic diluents may include an energy substrate such as glucose or fructose and antibiotics,

particularly if the semen is to be used for intrauterine insemination. Synthetic diluents for fresh semen may also contain constituents that protect sperm against accidental cold-shock. A simple synthetic is phosphate-buffered-saline (PBS), a completely inorganic medium that avoids potential immunological reaction from the injection of biological material into the uterine wall during laparoscopic insemination. However, PBS alone offers no protection against accidental cold-shock, and strict control of the inseminate temperature is critical.

Chilled Semen.

The main purpose of diluting fresh semen is the dissemination of spermatozoa and not preservation. However, semen can be stored to some extent in a liquid form after cooling. Chilled semen can be satisfactorily used within six hours of collection, providing greater flexibility than fresh semen, without the technical requirements and expense of semen freezing (Ritar, 1983; Ritar, 1987). However, chilled semen is similarly restricted to use in situations where the semen is for use with readily accessible recipient does.

The objective of semen storage is to prolong the fertilising capacity of the spermatozoa, an aim that may be achieved by reducing or arresting metabolic function (Evans and Maxwell, 1987). As these authors note, liquid storage of semen depends upon the reversible reduction of the motility and metabolic activity of spermatozoa at 5 to 15°C.

In cattle an early finding was that the addition of egg yolk to the diluted semen prior to cooling increased fertility and sperm survival during storage (Foote, 1988). The first types of extenders were yolk-phosphate based media, which were superseded by modifications of yolk-citrate media (Gomes, 1977) which form the basis for the widely used diluents for sperm preservation. Ritar (1987) commented that the chilled storage of goat semen has received little attention, probably as a result of the limitation of sperm survival by the interaction of egg yolk with the seminal enzyme phospholipase-A.

Ritar (1983) did however review a number of reports on sperm survival during chilled storage of goat semen. Roy (1957) found that buck spermatozoa remained motile for a period of 3 days when diluted with a yolk-citrate media and cooled to 3°C. After this time there was complete immobilisation of the sperm cells and coagulation of the

inseminate. Blockhuis (1962) found that sperm survival at 4°C was better in 3% sodium citrate than skim milk. Schindler et al. (1972) investigated a range of media and found that the media most conducive to sperm survival was one containing sodium citrate, glycine, fructose and egg yolk. Boar spermatozoa are sensitive to differences in osmotic pressure, with motility being preserved longer in slightly hypotonic diluents (Stevermer et al., 1964).

Despite the storage restrictions associated with the use of chilled semen, the technique may still be of benefit in situations where the equipment for semen freezing is either not available or not warranted (Corteel, 1981). This may be the motivation for more recent studies concerning liquid storage of semen. Sing et al. (1982) reported that the duration of storage had a highly significant effect on sperm motility and survival over a period of 96 hours at 5°C, and that there was also a significant effect of diluent. After 48 hours of storage, the semen diluted in egg yolk-citrate medium had more live spermatozoa than tris-yolk-glycerol and citric acid-whey diluents. At the same rate of dilution (1 in 10) and under the same storage conditions, Borgohain et al. (1985) found though that milk based diluents gave consistently greater sperm motility percentages than egg yolk based media.

Extended periods of chilled sperm preservation have been claimed by some authors (Matthew et al., 1984). These workers diluted semen in tris-yolk extender, varying egg yolk concentration (5,10,15,20,25 and 30 %), pH (6.5, 6.75 and 7.0) and storing at 3-5°C or 6-8°C. Sperm motility was maintained for up to 10 days at the lower temperatures and up to 13 days at the higher temperatures. The best egg yolk concentrations were 25% and 5% respectively at 3-5°C and 6-8°C. The performance of the high egg yolk diluent at the lower temperatures is interesting, suggesting limited phospholipase activity. Only at the higher storage temperatures was there an effect of pH, with semen diluted at pH 7 having greater motility than that diluted with media of lower pH. The more basic media probably reduce the metabolic decrease in pH at temperatures above 5°C. However, the fertilising capacity of the sperm after such storage was not determined.

Another possible means of liquid storing semen involves the reversible inactivation by chemical treatment. Using ram semen inactivated by washing with a Ficoll density gradient, Tervit et al. (1982) reported that 80% of spermatozoa were motile following reactivation in a milk-based diluent after 3 days of ambient temperature storage. Washing in Ficoll causes a loss of spermatozoan motility whilst maintaining the integrity of the plasma membrane (Jansen et al., 1982). The inactivated spermatozoa

are also resistant to the effects of cold shock, a result that may be related to Ficoll induced changes in the lipid composition of the plasma membrane. These authors recorded 90% forward motility after reactivation in the presence of egg yolk and incubation for 25 minutes. Motility was also restored after incubation in the presence of individual phospholipids such as phosphatidyl-choline.

Short chain fatty acids can also induce a reversible inactivation of goat sperm at specific concentrations, and have a positive effect on motility after sperm reactivation with an egg yolk-citrate media (Nimbulkar et al., 1982) . The motility increased as the number of carbon atoms increased from 2 to 6 in chains with an even number of carbons, and decreased with chains with uneven numbers of carbon atoms. Acetic acid and caproic acid in an egg yolk-citrate diluent had the greatest effects, preserving the semen for up to 14 days with a 60 % motility after 4 days storage at ambient temperatures.

Although fertility results relating to the various methodologies of semen storage will be discussed in the next chapter, it is important to note that the relationship between sperm cell motility and fertilising capacity is not a simple one. Whilst cell viability is required for fertilisation it does not follow that all viable cells are capable of fertilisation. Corteel (1981) states that since sperm cells survive longer than sperm cell fertilising capacity, the former cannot be used to predict fertility. Ritar (1983) also noted that studies indicate that the motility of spermatozoa is not a reliable indicator of the fertility of chilled-stored spermatozoa. Aspects of semen and inseminate quality as they affect fertility and fertility prediction will be discussed more fully in the next chapter.

2.8 Cryopreservation of spermatozoa.

2.8.1 Methods of Semen Freezing, Packaging and Storage.

Semen from farm animals has been commercially frozen, packaged and marketed as: (1) glass ampoules (0.5 to 1.2 ml); (2) polyvinyl chloride straws (0.25 to 0.5 ml); (3) or as pellets (0.1 ml). Semen was initially frozen in a mixture of dry ice and alcohol and packaged in ampoules, but Gomes (1977) notes that it later became standard practice for ampoules to be frozen in liquid nitrogen, with straws being frozen in liquid nitrogen vapour and pellets on blocks of dry ice. The frozen semen was then

stored in either mechanical freezers or in dry ice and alcohol. However, it was the adoption of liquid nitrogen as a storage medium and the development of suitable insulated storage containers that led to the widespread international use of frozen semen (Amman and Schanbacher, 1983).

The semen of sheep and goats is commercially frozen in straws, but is more commonly frozen and stored in the form of pellets, which offer both a relatively simple method of semen processing and also generally better post-thaw results than straws (Evans, 1987). Packaging semen in polyvinyl chloride straws does however offer other advantages, mainly with respect to the individual identification of the straws and the complete enclosure of the semen as a safeguard against contamination (Evans, 1987; Evans and Maxwell, 1987). Freezing semen in straws or pellets, as opposed to ampoules, allows much faster rates of freezing as a result of the larger ratio of surface area to volume (Memon and Ott, 1981). This also provides a greater flexibility of control over the thermodynamics of the freezing process. Foote (1988) stated that semen packaging should be designed to allow good thermal transfer, as epitomised by the 0.25 ml straw (Watson and Martin, 1975).

Pellet-Frozen Semen.

The standard procedure for the production of pellet-frozen semen is for chilled semen to be pipetted onto dry ice (Memon and Ott, 1981). This method was developed by Japanese workers, and although its application to bovine artificial breeding was limited, it has been widely used for other domestic species (Gomes, 1977). The procedure for pellet freezing semen is described in detail by Evans and Maxwell (1987). A solid block of dry ice with a smooth surface must be obtained, and onto it depressions engraved with a metal stencil. Semen that has been chilled to 2 to 5°C is then rapidly pipetted (3 to 8 drops; 0.1 to 0.3 ml) into each of the depressions. Once the pellets are frozen, as indicated by a distinctive change in the pellet appearance, they are plunged into liquid nitrogen. Prior to freezing, rapid changes in the temperature of the semen must be avoided. The temperature of the chilled semen must be maintained at 5°C (or the temperature to which it is cooled) either by processing in a cool room or by using cooled-water jackets in which the vessel containing the liquid semen is immersed. The glass pipettes should also be cooled with each batch of semen to be frozen.

Pelleted semen is thus frozen in two stages. Initially the semen is frozen to -79°C, the temperature of dry ice, and then to -196°C upon plunging into liquid nitrogen. Some control may be exerted over the rate of freezing, particularly in the first stage, by variation of the pellet size or volume. However, no effect of pellet volume was observed on the post-thaw survival of sperm cells in studies by Salamon (1970; 1973) in either sheep or pig semen (over the range of 0.03 to 0.3 ml) or by Lightfoot and Salamon (1969a) and Visser (1974) with ram semen over even wider ranges in pellet size (0.03 to 0.86 and 0.6 ml respectively). Visser (1974) noted that even though the rate of semen freezing varied significantly with pellet size, with larger pellet volumes reducing the freezing velocity, the survival and acrosome morphology of the frozen spermatozoa remained similar. This is in direct contrast with straws, where faster rates of freezing are superior to slower rates (Graham, 1976; as cited by Memon and Ott, 1981).

Straw-Frozen Semen.

The use of plastic straws for semen freezing was introduced in 1964 (Amann and Schanbacher, 1983) and applied widely to bovine semen freezing. Jondet (1964; cited by Memon and Ott, 1981) described a method for freezing bull semen, where straws were suspended on a rack in liquid nitrogen vapour 4 cm above a liquid nitrogen surface. This method was applied directly to other species such as sheep (Andersen and Aamdal, 1972) and goats (Anderson, 1969), and is the basis for the straw freezing technology that exists today.

When filling straws in preparation for freezing, an air gap should be left at the end of the straw to prevent splitting during the freezing process (Evans and Maxwell, 1987). The straws may be sealed with polyvinyl chloride plugs, nylon plugs, steel balls or by electrostatic compression (Gomes, 1977). Evans and Maxwell (1987) describe a simple procedure where straws are placed horizontally onto a cold rack (at 5°C) and lowered into liquid nitrogen vapour 3 to 4 cm above the liquid nitrogen surface in a suitable container, such as a styrofoam container. After 7 to 8 minutes the frozen straws are then immersed in liquid nitrogen. Care should be taken to avoid temperature fluctuations in the straws at cooling and after freezing. Goat semen has been frozen in both 0.25 ml and 0.5 ml straws (Hahn, 1972; Memon et al. 1985; Deka and Rao, 1986; Andersen, 1969 and Waide et al. 1977).

An alternative to simpler techniques of straw freezing in static vapour is available in the form of programmable forced vapour freezers. Parkinson and Whitfield (1987) demonstrated the use of such freezers in the optimisation of freezing conditions for the semen of individual bulls, particularly those that show poor sperm survival after conventional freezing methods, but noted that the use of such freezers dates back to the 1960's and 1970's. Controlled freezing chambers have been used in the experimental freezing of ram semen (Watson and Martin, 1975; Fiser et al. 1986).

The rate of freezing in straws can be more easily controlled than in pellets. This is achieved by use of a programmable freezer or by altering the height of freezing above the liquid nitrogen surface in static vapour chambers. Using a programmable freezer, Watson and Martin (1975) found no change in the survival of ram sperm when the rate of cooling was varied from 6 to 24°C/min over the temperature range of 5 to -55°C, prior to plunging in liquid nitrogen.

With bull semen there are critical regions of the freezing curve that have different cooling rate requirements for optimal sperm survival (Parkinson and Whitfield, 1987). These authors found that a slow rate of cooling was beneficial between the initial holding temperature of 5°C and equilibration at an intermediate temperature of -5°C, with both cooling rate and intermediate temperature significantly affecting sperm survival. The initial cooling rate had a quadratic relationship with sperm survival, the optimum being -12.2°C/min.

Polge (1952) suggested that rapid freezing is required from -15 to -25°C and Parkinson and Whitfield (1987) found a trend of increased sperm survival with very rapid cooling from the intermediate temperature to a second intermediate temperature of -30°C. Losses of sperm below -30°C were found to be small and although only a narrow range of slow cooling rates were examined, these were not regarded as critical when straws were further cooled to -80°C. However, a small but significant improvement in sperm survival was indicated when the second intermediate temperature was extended to -45°C prior to slower freezing to -80°C.

Parkinson and Whitfield (1987) concluded that the region of the cooling curve of greatest importance to sperm cell survival is between -2 and -20°C. It is in this initial part of the freezing curve that the diluted semen changes phase. Watson and Martin (1975) found that a pause in cooling at the temperature of crystallisation had no affect on sperm survival and concluded that the latent heat of crystallisation in straws, and the consequent temperature differential between the semen and the freezing cabinet,

had no detrimental effect on the survival of spermatozoa. In contrast, Parkinson and Whitfield (1987) found a negative correlation of sperm survival, with the integrated area of the freezing curve that represents the magnitude and duration of the temperature differential caused by latent heat evolution. These authors stated that three areas of the freezing curve must be considered important; the rate of cooling from the equilibration temperature, the degree of supercooling that occurs and the efficiency of latent heat dissipation. Limiting supercooling and allowing temperature equilibration at the point of crystallisation benefit sperm survival.

Programmable freezers allow control of the freezing requirements across the whole freezing curve, and make adjustments to limit temperature fluctuations. Supercooling can also be controlled by inducing crystallisation (seeding) in response to brief periods of very rapid cooling (-500°C/min). In conventional static vapour freezing chambers only the initial chamber conditions are set. Some of the problems associated with the use of the latter in the study of freezing conditions were discussed by Robbins et al. (1976). These include the constantly changing rate of cooling with change in temperature, and different degrees of supercooling and latent heat dissipation in response to changes in the initial static state. A practical consideration is also the influence of the heat load placed on the freezing chamber, which may retard latent heat dissipation.

It is important to note however that freezing rate and the degree of cryoprotection are interactive. The effect of the freezing rate is modified by the level of glycerol inclusion in the freezing medium and the period of glycerol equilibration (Fiser and Fairfull, 1986; Drobnis et al., 1982). However, generally fast freezing rates such as -5 to -100°C/min give better survival of sheep and goat than slower rates of 1 or 2°C/min (Fiser and Fairfull, 1986; Drobnis et al., 1982). Colas (1975) used a static freezing chamber, but also found that a broad range of higher freezing rates (as indicated by lower initial chamber temperatures) gave the best sperm survival for frozen ram semen.

Cell death occurs as a result of different mechanisms when frozen at fast or slow rates (Parkinson and Whitfield, 1987) . During rapid freezing, cell death may result from the formation of intracellular ice and possibly extracellular ice. With slow cooling, cell death occurs as intracellular water is diffused into the surrounding diluent which becomes increasingly hypertonic as the diluent water freezes. This causes intracellular osmotic damage to the cell as it dehydrates. There is therefore an optimum freezing rate at which cell damage is minimised from either process, and this

is a function of the cell's surface area to volume ratio, the fragility of the cell membranes and the nature and concentration of the cryoprotectant used.

Generally the rate of sperm survival is higher for sheep and goat semen frozen as pellets than as straws (Evans and Maxwell, 1987; Shirbhate and Honmode, 1982; Hunton 1987), despite the slower rates of freezing with the latter.

2.8.2 Protection of Spermatozoa During cooling and Freezing.

Freezing and thawing inevitably reduce the motility and viability of sperm populations (Evans, 1987). The damage from both processes combine, but may be examined separately since their effects on the spermatozoa occur at separate stages of semen processing.

Freezing, can be considered to be comprised of a series of steps, with losses in viability due to cell damage at each of these stages. The semen is cooled from the temperature of collection to a point where crystallisation occurs, and is then further cooled to eventually reach the temperature of the liquid nitrogen storage medium. In practice this is seldom a continuous progression along a cooling curve. Instead, it may frequently comprise cooling to 5°C, before freezing to a set temperature and then transfer to liquid nitrogen.

Cooling.

Cooling, and especially rapid cooling, of sperm to temperatures near 0°C can result in a loss of sperm motility, loss of membrane lipids, increased membrane permeability, calcium influx, damage to the acrosome and other structures and a reduction in fertility, in addition to the desired reduction in metabolic activity (Foote, 1988). The cholesterol and polyunsaturated fatty acid content of sperm membranes is related to cold-shock sensitivity, and sperm that have low ratios of cholesterol to phospholipid and a relatively high polyunsaturated fatty acyl component in there membranes (such as bull and ram sperm) tend to be more sensitive to cold shock. The deleterious effects of cooling prior to crystallisation may be minimised by the addition of protective materials such as egg yolk and phospholipid to the semen diluent, and also by control of the cooling rate.

Memon and Ott (1981) note that sheep and goat semen is cooled slowly to avoid cold shock, with diluted semen generally being cooled to 5°C at a rate of 0.5°C/minute. Salamon and Ritar (1982) cooled extended goat semen from 30°C to 5°C in a period of 1.5 hours. Similar approaches are reported elsewhere, with buck semen being cooled from 35°C to 5°C over 1.5 to 2 hours (Deka and Rao 1986, Memon et al., 1985). Comparing cooling from 35 to 5°C in 0.5, 1.5 and 2.5 hours, Deka and Rao (1987a) found little difference in motilities before and after freezing, but found that the incidence of acrosomal changes, particularly acrosomal swelling, was lower at the slower rate of cooling.

Although slow cooling of the semen is generally recommended, studies with ram semen have shown that rapid cooling from 30 to 16°C may not adversely effect spermatozoa (Fiser et al., 1987). Semen treated in this manner was then cooled to 5°C at a rate of 0.2°C/minute before freezing, and compared with semen cooled only at 0.2°C/minute. The advantages of rapid early cooling obviously lie in reduced semen processing time. However, rapid cooling to temperatures below 15°C is associated with a progressive decrease in the percentage of spermatozoa that are motile and have intact acrosomes prior to freezing.

Lipoproteins and lecithins (phospholipids) present in egg yolk are commonly used to give cold shock protection (Memon and Ott, 1981). Watson and Martin (1975) concluded that egg yolk improved the post-thaw motility and reduced acrosome damage in frozen ram semen, and that the beneficial effects of egg yolk were conferred during both cooling and freezing. It was suggested that the greatest effect occurred during cooling, although this was based on egg yolk removal prior to freezing, after addition at dilution, a process that may not have been complete. Although acrosome damage apparently increased slightly when egg yolk was present during cooling and freezing, as opposed to during either cooling or freezing, damage was still significantly reduced compared to the control containing no egg yolk. Very low concentrations of egg yolk are required, and a level of only 1.5% provided satisfactory protection.

Whilst not examining the separate effects of egg yolk on cooling or freezing, Ritar and Salamon (1982) reported a beneficial effect of egg yolk concentrations of 1.5 to 12% on the post-thaw survival of washed goat spermatozoa. However, the use of egg yolk in the preservation of goat semen is subject to the complicating influence of the egg yolk interaction with phospholipase-A. This interaction of egg yolk and a coagulating factor in goat semen was characterised by Roy (1957). As noted by Ritar

and Salamon (1982), the enzyme phospholipase-A originates in the Cowpers or bulbo-urethral glands and causes agglutination of the semen storage medium and produces toxins to sperm. Enzymatic hydrolysis of egg yolk lecithins produces fatty acids and lysolecithins that are toxic to spermatozoa, causing sperm immobilisation (Corteel et al., 1984; Aamdal et al., 1965). Even so, Ritar and Salamon (1983) observed that egg yolk had a protective effect on frozen unwashed spermatozoa, as indicated by initial post-thaw motilities. Toxic effects became apparent with incubation of the semen and were greater with increasing egg yolk concentration. At 1.5% egg yolk however, a positive effect on sperm survival was carried through the first 2 hours of incubation.

In milk based diluents such as those used by Corteel (1974), cold shock protection is also attributed to lipoprotein and lecithin fractions (Memon and Ott, 1981). O'Shea and Wales (1966) found that casein and lecithin had a separate and additive effect on the viability of ram semen cooled to 5°C. The mode of cold shock protection appears to be related to associations with the sperm cell membranes. Quinn et al. (1980) characterised a reversible interaction of phospholipid with the plasma membranes of ram spermatozoa. Protection was conferred against cold shock, as indicated by an increased percentage of motile sperm. The interaction between the phospholipid and sperm that was immediate and reversible with centrifugal washing. The speed of the acquisition of the protective factor is strongly indicative of a plasma membrane association. Earlier work by Watson (1975) using a fluorescent probe, similarly indicated that an egg yolk fraction was reversibly bound to the plasma membrane surface.

In contrast, Cookson et al. (1984) used antisera to characterise a firm binding of a lipoprotein fraction to bull sperm, suggesting an irreversible interaction. It was suggested that a lipoprotein fraction is the major egg yolk component that binds to bovine sperm and confers cryoprotective properties, however no examination of cold shock succeptibility was made. Watson (1981) compared the interaction of ram sperm with lecithin liposomes and a lipoprotein fraction of egg yolk and established that whilst both gave equal protection from the brief and severe stress of cold shock, lipoprotein provided superior protection to lecithin during cooling and cold storage. This difference may be related to the strength of the binding interactions. Indeed the latter author discussed that the observed differences in the degree of protection offered by phospholipid and lipoprotein may be interpreted as evidence of two distinct protective factors. These have been regarded as a resistance factor present in both lipoprotein and phospholipid preparations (possibly lecithin), and a storage factor more related to the protein fraction of the lipoprotein complex. However, an

alternative explanation was offered that related the difference in protection during cooling to the lower solubility of lecithin liposomes. Whilst lipoprotein preparations form a true solution, lecithin liposomes are dispersions, so that at low temperatures they may reagregate to effectively reduce the surface area available to interact with the sperm cell.

The protection that results during cooling from the binding of phospholipid and lipoprotein fractions of egg yolk at the sperm cell surface, is believed to involve the stabilisation of the sperm membranes (Cookson et al., 1984). However, the exact mechanism of this association remains unclear, particularly with regard to observed differences in the strength of the phospholipid and lipoprotein association with the sperm membranes (Foote, 1988). It appears that the initial effects of cold shock damage are associated with a primary lesion in the plasma membrane, with other events such as acrosomal swelling being regarded as subsidiary to this (Quinn et al., 1980). Quinn and White (1966b) reported that cold shocking ram and bull semen caused an increase in sodium content of the spermatozoa and a decrease in potassium and magnesium contents, indicating a partial loss of membrane function. These effects were greatest after dilution, after which the calcium content of the spermatozoa also rose. The addition of lecithin to the diluent depressed the rise in calcium and sodium after cold shocking. Robertson and Watson (1987) also reported an unstable flux of intracellular calcium in response to slow cooling of ram spermatozoa and that egg yolk initially enabled spermatozoa to retain the capability to extrude calcium ions after slow cooling or cold shocking. This benefit was not evident after 24 hours of cold storage. Incubation of sperm at 37°C for 3 hours also reduces the protective effects of phospholipid included in the media, suggesting some storage-related change in the succeptibility of the spermatozoa to cold shock.

Freezing.

In addition to any cellular damage that is incurred during cooling, spermatozoa may also suffer damage as a consequence of the freezing process. Amann and Schanbacher (1983) note that freezing damage is probably the result of the interactive effects of ice crystal formation that physically damage the spermatozoa, and increases in solute concentration as pure water is withdrawn by crystallisation of the extracellular solvent. These effects, and thus the degree of cellular damage, are related to the rate of cooling and freezing (Parkinson and Whitfield, 1987). Foote (1988) noted that a factor in the successful cooling of sperm was the temperature at

which phase change occurred, and that the control of the cooling rate may be important in the provision of sufficient time for orderly phase changes. A balance must be achieved between the effects of intracellular and extracellular ice formation, and intracellular osmotic damage as the cell dehydrates.

Glycerol is commonly added to extenders to protect mammalian sperm against the lethal effects of freezing (Corteel, 1981; Gomes, 1977; Memon and Ott, 1981; Ritar, 1983). Other agents such as ethylene, propylene glycol, dimethyl sulphoxide, and high concentrations of non-permeating sugars have been investigated, but are less protective than glycerol (Gomes, 1977; Waide et al., 1977. The cryoprotective benefits of glycerol were first observed in 1949 and Smith and Polge (1950) reported that goat semen diluted to contain 10 and 15% glycerol and then frozen to -79°C, displayed good motility after thawing.

Glycerol acts by binding to water and reducing ice formation during freezing, and by permeating the sperm so that the cells are not exposed to high osmotic stress until a lower temperature is reached (Foote, 1988). At this lower temperature it was suggested that the effects of osmotic stress are less critical.

Various concentrations of glycerol have been reported as the optimum, and it appears that the level of cryoprotectant required may be interactive with the method of freezing. Waide et al. (1977) found that 14 to 16% glycerol was required for goat semen frozen in dry ice and alcohol in either ampoules or straws. Also freezing semen in ampoules, Fraser (1962) indicated that 7 to 8% glycerol was needed. When semen was frozen in pellets, Salamon and Ritar (1982) found that glycerol interacted with the method of dilution (1-step or 2-step) and the time allowed for equilibration. However, the best mean post-thaw recoveries and the best individual treatment involved dilution with 4% glycerol as opposed to 2.5 or 5.5%. Deka and Rao (1986) reported that 6.4% glycerol gave significantly higher percentage of sperm that were progressively motile, compared with either 4 or 9% glycerol. In contrast, Drobnis et al. (1980) found that 4% glycerol best conserved sperm motility in straws, with motility reduced at higher concentrations.

Studies of ram semen have indicated that there is an interaction between the level of cryoprotectant required and the rate of freezing (Watson and Martin, 1975; Fiser and Fairfull, 1986). The glycerol requirement is reduced as the freezing rate is increased, or as Fiser and Fairfull (1986) stated, increasing the glycerol content results in a downshift in the range of optimal cooling velocities. Since more rapid cooling limits

the exposure of the sperm cell to concentrated solute as water freezes out of solution (Foote, 1988), this may reduce the potential benefit of the added glycerol. The protective action of glycerol is largely attributed to a salt buffering capacity which minimises electrolyte damage of the sperm cell (Memon and Ott, 1981).

In addition to freezing rate, Watson and Martin (1975) noted that increased levels of egg yolk in the freezing diluent also induced reductions in the glycerol requirement. These authors observed though that the degree of acrosomal damage was directly related to the glycerol concentration. Although acrosome damage was reduced by the inclusion of glycerol, increasing the glycerol level from 2.5 to 7.5% increased the proportion of acrosomes damaged. Deka and Rao (1986) reported a similar response with goat semen, but also observed that maximal post-thaw motility and acrosome integrity were not coincident. Whilst motility was greatest at 6.4% glycerol, least acrosome damage was observed at 4% glycerol. Spermatozoa cannot tolerate unlimited exposure to protectants such as glycerol (Foote, 1988) and Fiser and Fairfull (1986) found that glycerol was toxic to ram spermatozoa at concentrations above 8%.

Glycerolisation of the spermatozoa may generally be achieved by either 1-step or 2-step dilution methods (Evans and Maxwell, 1987; Ritar, 1983). In the first situation the freezing medium containing glycerol is added at dilution immediately after collection. Alternatively the semen may be initially diluted with a non-glycerol diluent after collection, cooled to 2 to 5°C, and the glycerol added in a final dilution step prior to freezing. The latter constitutes the general approach to 2-step dilution, although Salamon and Ritar (1982) also examined the effect of 2 dilution steps at 30°C. Memon and Ott (1981) commented that whilst glycerol has been added at either 30 or 4°C, protection is conferred at lower temperatures, implying that inclusion at higher temperatures in the cooling curve is an unnecessary complication. However, glycerolisation after collection actually simplifies media preparation and reduces semen handling (Lightfoot and Salamon, 1969a).

Goat semen has been diluted by both the 1-step (Hahn, 1972; Memon et al., 1985) and 2-step methods (Deka and Rao, 1986; Drobnis et al., 1980; Lyngset et al. 1965), but 1-step dilution at 30°C gave higher post-thaw motilities (Salamon and Ritar, 1982). Similar results have also been reported for ram semen (Lightfoot and Salamon, 1969b).
Equilibration time at 5°C prior to freezing was also implicated as a factor affecting the post-thaw recovery of frozen semen. Salamon and Ritar (1982) found there was an interaction between equilibration time and method of dilution used and between

equilibration time and glycerol concentration in the freezing medium. For 1-step dilution, equilibration for 1.5 hours was beneficial at 2.5 and 4% glycerol, but had no effect at 5.5% glycerol. However, longer equilibration at all 3 levels of glycerol reduced the percentage of motile spermatozoa after thawing. When glycerol was added at 5°C, equilibration for at least 3 hours improved post-thaw recoveries regardless of the concentration of glycerol used. Deka and Rao (1986) reported that equilibration for 5 hours increased the percentage of motile spermatozoa after glycerolisation at 5°C. These results tend to indicate that the time of sperm cell contact with the glycerated medium is important. However, the effects due to glycerol and temperature equilibration need to be more clearly elucidated. Foote (1988) stated that the affect of equilibration time was a response to temperature and not glycerol, since glycerol appears to penetrate the sperm cell quickly in contradiction to the studies illustrated above.

9. Thawing.

Since frozen semen must be thawed before assessment can be made, the damaging effects of both freezing and thawing are integrated and cannot easily be partitioned. Faster rates of thawing are more beneficial than slow rates, possibly due to reduced opportunity for ice crystal formation during rapid thawing (Foote, 1988).

In the cryopreservation of bull semen, Robbins et al. (1976) found that the rate of thawing had a more significant effect on the post-thaw recovery of sperm cells than did a wide range of freezing rates. Indeed these authors suggested that freezing rate was relatively unimportant within the parameters investigated. Faster thawing gave a higher percentage of motile sperm and percentage of sperm with intact acrosomes, although this depended on the level of glycerol used in the freezing medium. Foote (1988) also stated that optimal rate of thawing may vary with the medium used.

Ritar (1983) commented that the general recommendation for goat semen is to thaw as rapidly as possible, although thawing between 30 and 40°C is most practical. Anderson (1969) reported that thawing goat semen at 75°C for 10 seconds was superior to thawing at 35°C for 30 seconds. Shirbhate and Honmode (1982) similarly found that sperm thawed at 60 degrees centigrade for 15 seconds had greater vitality than did that thawed at 40 degrees centigrade for 25 seconds. These authors also found that the difference between thawing rates was greater for pellets than for straws. Although sperm motility may be improved by rapid thawing, the rate of acrosome

damage may not be significantly effected (Deka and Rao, 1987b). Clearly however, the benefits of faster thawing are moderated by the freezing process and damage that has already occured (Fiser et al., 1986).

10. Sperm Washing.

Washing of the spermatozoa may be undertaken to remove most of the seminal plasma from the sperm cell environment by dilution and centrifugation prior to cooling and freezing (Corteel, 1981). This procedure removes factors in the seminal plasma that are detrimental to the spermatozoa. Seminal plasma effects the metabolism of sperm and does not provide an optimal environment for sperm storage in domestic species such as cattle and sheep (O'Shea and Wales, 1966). The toxic interaction of enzymes in goat seminal plasma with egg yolk lecithins, also suggests that egg yolk-containing media cannot be used to freeze goat semen (Corteel et al., 1984) . Whilst this is open to dispute, washing of the spermatozoa directly after collection does enhance the ability of the spermatozoa to withstand freezing in egg yolk based media.

Ritar and Salamon (1982) found that buck seminal plasma provided a poorer environment for the maintenance of spermatozoa than ram seminal plasma, and that this environment further deteriorated after dilution with an egg yolk-containing medium. Washing of the semen prior to freezing improved the survival of frozen-thawed spermatozoa. Intensively washed spermatozoa tolerated a wide range of egg yolk concentrations (1.5 to 12%).

Washing immediately after collection improves survival of frozen-thawed spermatozoa (Corteel et al., 1984). Memon et al. (1985) concluded that seminal plasma removal was beneficial in preserving the structural integrity and post-thaw motility of frozen goat semen regardless of the diluent used (egg-yolk or milk based). Corteel et al. (1984) also stated that washing reduced the deterioration of sperm revival with time of frozen storage of semen frozen in milk-based diluent. Ritar and Salamon (1991) recently reported that storage time reduced the viability of frozen sperm, regardless of whether semen was washed or not, or included egg yolk or not. This study examined the effects of storage over a period of 6 months, whereas Corteel et al. (1984) observed no deterioration in sperm revival for up to 5 years, after washing and freezing in a milk-based diluent.

The effect of seminal plasma on the viability of frozen goat semen is variable with season of collection. The role of semen washing in the preservation of frozen semen may thus vary in importance and application according to time of year. Ritar and Salamon (1991) found that the post-thaw survival of unwashed Angora semen was effected by both egg yolk concentration and the month of semen collection. The concentration of egg yolk in diluted semen that gave the best sperm survival declined from 12% in March (Southern Hemisphere) to none at all in August. This may result from seasonal variation in seminal enzyme activity, and the negative effects on sperm survival may be overcome by washing the sperm. It was also suggested that the post thaw recovery of sperm varied with season, with best results obtained between April and July.

Nunes et al. (1982) reported that seminal plasma from the non-breeding season had a negative effect on chilled or frozen-thawed sperm survival, when compared to breeding season seminal plasma. However, washing of the sperm was beneficial to cell survival regardless of season of collection.

Despite the benefits of sperm washing, its adoption in semen processing may be subject to other constraints. Corteel (1981) notes that washing complicates the process of semen collection, and that the negative effects of seminal plasma would be more satisfactorily avoided biochemically, given better understanding of the mechanisms involved. Washing is also wasteful of spermatozoa since a proportion of cells is removed with the decanted supernatent after centrifugation, and it may be preferable to use a simpler method that does not involve washing but achieves acceptable levels of fertility (Ritar, 1988).

Ideally the preferred method of freezing goat semen would be in polyvinyl straws, using a simple, quick, one step dilution and freezing technique.

CHAPTER 2.

INSEMINATE ASSESSMENT

AND ARTIFICIAL

INSEMINATION

2. INSEMINATE ASSESSMENT AND ARTIFICIAL INSEMINATION.

2.1 Methods of Sperm Quality and Fertility Assessment.

Assessment of the ejaculate and the processed semen is an important component of both routine semen collection for commercial purposes, and the development and comparison of semen processing methods. At collection, semen is assessed for the motility of the population of sperm cells (as indicated by the degree of wave motion observed in a sample of the ejaculate examined using microscopy), the concentration of sperm cells within the ejaculate, the volume and colour of the ejaculate. This information is used to establish whether the ejaculate is suitable for processing, and is a crude form of quality assessment, used to screen for abnormal ejaculates before further time and effort has been invested in them. However, closer examination of a semen sample is required after processing and prior to insemination, to establish whether acceptable numbers of viable sperm are present in the inseminate. There are a range of techniques available for such assessment, including insemination itself.

2.1.1 Visual Assessment.

With visual assessment, a sample of the semen is examined using microscopy at a higher level of magnification than that used in the assessment of the neat ejaculate. The proportion of the sperm population displaying progressive forward motion can be determined, indicating the percentage of live motile sperm. This assessment is subjective, and consistency may be achieved by individual operators, although between operator accuracy may be a another matter. This problem of accuracy and standardisation can be addressed by use of image analysis equipment, which can provide information on the percentage motile sperm and their forward velocity (Salamon, 1987; Anzar et al., 1991). Such systems offer the advantage of objective quality control to commercial enterprises. Alternatively, spectrophotometry has been proposed as a simpler means of objectively establishing the forward motility of populations of sperm cells (Majumder and Chackrabarti, 1984).

Staining methods that discriminate between live and dead cells also reduce the subjectivity associated with the visual assessment of the proportion of live sperm cells. However, no information is provided about progressive motility, and the variability of this method may also be unacceptably high (Salamon, 1987).

Morphological studies may provide additional information about the percentage of abnormal sperm cells in either the ejaculate or the processed semen. This may relate to both the motility and the functional capacity of the spermatozoa. However, the time consuming nature of these studies generally means that a detailed evaluation of the prevalent sperm abnormalities is only undertaken as a precursor to the inclusion of a sire in an artificial breeding programme (Evans and Maxwell, 1987).

Incubation Studies.

Incubation studies are a commonly used in the evaluation of semen processing techniques. The proportion of motile spermatozoa is visually assessed repeatedly over a period of 4 to 6 hours during incubation at 37°C. These incubation studies take some account of the effect latent injury may have on sperm function, as expressed by continued progressive motility (Salamon, 1987). The period over which motility is maintained may be more related to fertilising capacity than the initial percentage of motile cells.

Motility and Fertilising Capacity.

Since the quality assessment of semen thus largely involves a routine visual determination of the motility of a sperm population, the importance of sperm motility *per se* warrants discussion. In the female, sperm transport is achieved by smooth muscle contractions of the genital tract, cilliar beats, fluid currents and flagellar motility of the sperm (Hawk, 1983; Hogarth, 1978). However, the rapid transport of sperm to the oviducts after insemination indicates that in relative terms, sperm motility is an inconsequential means of transport.

However, sperm motility appears necessary for the sperm to enter the cervical crypts and folds (Hawk, 1983). It has been postulated that sperm entering cervical crypts can reach the uterus by avoiding exposure to the body of the cervical canal, and to the mucus flowing back to the vagina (Mullins and Saake, 1982). Indeed in the ewe, dead sperm enter the cervix in only small numbers, do not enter the cervical folds and are quickly lost from the genital tract, probably as a result of drainage to the exterior (Lightfoot and Restall, 1971). However, despite this, Hunter (1986) stated that there is no evidence to indicate that the spermatozoa that enter the cervical crypts are directly involved in fertilisation. In the oviduct, motility may also be important after

activation of the sperm, as hyperactive motility may assist in the location of an ovum (Cummins, 1982).

Whilst sperm motility is used to compare the relative vigour of sperm populations, it is not a requirement of fertilisation. It is possible that an immotile sperm cell that is damaged only in the tail section may be capable of fertilisation if brought into contact with a zona-free ovum (Salamon, 1987). Thus whilst motility may provide a common basis for comparing sperm cell survival after various semen processing methods, this information may not necessarily allow comprehensive prediction of fertility after insemination. Corteel (1981) stated that the percentage of motile sperm and their progressive motility is generally only poorly related to fertility. This is illustrated by some literature with respect to the post-thaw motility of frozen semen (Davis and Foote, 1987), and perhaps most dramatically with regard to ram sperm stored after chemical inactivation (Tervit et al., 1982). In the latter study, a high proportion of motile sperm at insemination achieved only a very poor conception rate. However, the effect of sperm motility on conception may be confounded by the insemination conditions.

2.1.2 Tests of Sperm Function.

The observation that motility is not the only aspect of sperm function important to fertilising capacity, is obvious from an understanding of the role of the sperm in fertilisation.

Once deposited in the female genital tract, the spermatozoa must contact and penetrate the oocyte to initiate the nuclear fusion that occurs during fertilisation. However, it is interesting to note that the population of sperm cells delivered to the female is not initially capable of fertilisation. Indeed, during natural mating the sperm cells reach full functional maturity only after entering the female genital tract.

Sperm exposed to the female tract undergo capacitation, where they attain the functional maturity required for fertilisation (Bedford, 1982). This is associated with the activation of the sperm (observed as a change in the beat of the flagellum), the removal of a decapacitation factor from the sperm surface and the activation of acrosomal enzymes (Anderson, 1977; Courtens et al., 1984). Capacitated sperm have the ability to undergo the acrosome reaction, a prerequisite for fertilisation, in which the reacted acrosome breaks down releasing the enzymatic contents, (notably

hyaluronidase and acrosin) that facilitate penetration of the ovum. Thus fertilisation involves a complex of characteristics and some of these can be assessed using a variety of different tests that look beyond motility.

Acrosomal Integrity.

Evaluation of the population of sperm cells with potential viable acrosome reactions has been investigated as a means of predicting sperm fertility (Berger, 1990). The acrosomes of caprine sperm were stained with fluorescein isothiocyanate-labelled pisum sativum agglutinin, providing comparatively accurate assessment of acrosome integrity in frozen sperm. Whilst results from this technique were significantly correlated with transmission electron microscope observations, the percentage of lost acrosomes was underestimated by staining.

The next step in such assessment, determining the capacity to undergo the acrosome reaction, has also been investigated as a means of fertility assessment, using a variety of exogenous molecular agents that stimulate the acrosome reaction (Meizel, 1985). Davis and Foote (1987) reported that the percentage of acrosome reactions induced after incubation of frozen-thawed sperm with such chemical agents may be used to predict fertility in bovines. The percentage of already acrosome reacted sperm was negatively correlated with the non-return rate, whilst the rate of increase in acrosome reactions with incubation (indicating the capacity to react) was positively correlated with the non-return rate, giving an R2 of 0.95 in the best circumstance.

Davis and Foote (1987) found that the best prediction of the non-return rate (R2=0.97) was obtained using rate of change in the percentage of both motile and acrosome reacted sperm. This accounted for the greatest amount of variation in sire fertility and was interpreted as indicating that both factors are independently related to fertility.

Membrane Integrity.

Membrane integrity is a requirement of normal cell function. Assessment of membrane damage offers a means of determining the extent of functional disruption induced in sperm cells by processing techniques, and this can then be related to potential fertility. The interaction of fluorescent probes such as carboxyfluorescein

diacetate and propidium iodide with immobilised sperm cells can be readily used to distinguish cells with disrupted plasma and organelle membranes, using fluorescent microscopy (Harrison and Vickers, 1990). These authors indicated that this methodology allows a more accurate estimate of the proportion of viable sperm cells than either the assessment of motility or acrosomal integrity.

Oocyte Penetration.

Oocyte penetration tests have also been used to evaluate sperm cells functionally and predict fertility in farm animals. These tests are based on the observation that zona-free hamster eggs will interact *in vitro* with sperm cells from other species. Bousquet and Brackett (1982) adapted this technique to assess frozen-thawed bovine sperm, by determining the average number of sperm attached to and inside the vitteli, and observing the formation of the male pronuclei and the percentage of vitteli interacting with sperm from initial binding onwards. The results from this assessment were positively correlated with the 60 day non-return rates from 2 bulls, with the exception of pronuclear development which was negatively correlated with fertility. It was postulated that this latter result was due to subtle damage to the sperm cells leading to more rapid acrosome reactions and pronuclear development which may result in less effective interaction of the sperm cells with the vitteli.

These authors concluded that quantitative and qualitative differences in the interaction of zona-free hamster ova with frozen-thawed bovine sperm provide a basis for assessment of fertilising capacity. Significant correlations were observed between motility at insemination and the percentage of vitteli interacting with sperm and also between the average number of sperm attached to the vittelus and the number and percentage of sperm with intact acrosomes.

Eaglesome and Miller (1989) similarly reported a positive correlation of both the percentage of sperm interacting with ova and the mean number of sperm bound to the vitteli, with the fertility rates of 2 bulls of differing fertility. However, this applied only to frozen-thawed sperm and not fresh semen. Despite these correlations, the authors were unable to define test results indicating high or low rates of fertility, due to the variation between and within the ejaculates assessed. This limits the current effectiveness of the technique with regard to any quantitative prediction of fertility, although comparisons between sires can be made.

Previous studies (Pavlock and Flechon, 1985) have also suggested that in addition to variability in the interaction of sperm cells with ova, the test is not logically related to *in vivo* fertility. This is due to the small percentage of the tested sperm population that interact with the ova (<0.2%). Thus the test provides little information about the remaining sperm, unless they are irreversibly immotile or morphologically defective and therefore unable to fuse with ova. It was also noted that sperm penetration tests are not sensitive tests of defective motility.

Ova penetration tests have been adapted for use with frozen-thawed caprine sperm, with a positive but not significant correlation of penetration with normal acrosome ultrastructure (Berger, 1989). This lack of significance was postulated to be due to small sample size, or because different aspects of sperm fertility are assessed by the two techniques.

Other tests of post-thaw sperm quality include assessment of sperm penetration of cervical mucus. However, whilst this test provides a good assessment of motility it does not provide an adequate indication of fertility (Galli et al., 1991).

One of the greatest limits to the adoption of artificial insemination technology is the lack of simple tests that allow prediction of fertility prior to insemination (Corteel, 1981). Whilst there has been much research into the development of tests that assess the functional quality of sperm populations, these quality assays are still not accurate fertility predictors (Salamon, 1987). Fertility trials in the truest sense, where conception rates are assessed after insemination, are the most direct tests of fertilising capacity of sperm populations. Although costly, time consuming and subject to many other influences, these field trials are the best basis for comparisons between different methods of processing semen.

2.2. Artificial Insemination.

2.2.1 Artificial Insemination Technique.

As with sheep, goats can be inseminated using either the cervical or laparoscopic methods, both of which are described in detail by Evans and Maxwell (1987).

Cervical insemination is a relatively simple procedure commonly used with fresh diluted semen. Females to be inseminated are restrained with their hind legs over a rail and the body inclined head down, a position allowing the best presentation of the cervical canal. A speculum is inserted through the vulva and into the vagina, and a lightsource is used to visualise the cervix and passage of the inseminating pipettes. Mucus may obscure the cervix interfering with insemination, but can be removed by draining the genital tract as the forequarters are elevated.

Semen is loaded into a plastic inseminating pipette using a syringe connected by tubing to the pipette. The semen is held in the pipette behind an air buffer included to allow complete expulsion of the semen during insemination. The inseminating pipette has curved tip that can be introduced to the cervix and gently manipulated to pass as far as possible through the cervical canal. Once further passage through the cervix cannot be achieved, the semen is deposited by depression of the syringe. Gradual withdrawal of the speculum allows closure of the tract, preventing backflow of the semen.

In sheep the cervical canal has a highly tortuous path and is not easily penetrated. The situation is different with goat does and deeper penetration of the cervix and even penetration to the uterus is possible. A simpler modification of this method is vaginal insemination, in which semen is deposited before the cervix. However, this so-called "shot in the dark" method is not recommended as it limits potential fertility.

Laparoscopic insemination involves minor surgery and semen can be deposited intra-uterine without full laparotomy (Killen and Caffery, 1982). In this more complicated and more expensive procedure, the female recipients can be restrained in specially designed inseminating cradles. In these, the sedated or locally anaesthetised animals are presented belly-up and inclined head down at a 45° angle. The belly is shorn and prepared for laparotomy, during which the peritoneal cavity is penetrated at two sites on either side of the midline, using a scalpel inscision, trochar puncture and cannulation. At one puncture, the cannula contains a valve attached to a gas-line, and carbon dioxide is used to inflate the abdomen and allow insertion of an endoscope. In the opposite puncture the cannula is open and allows access for a probe or inseminating pipette. With the peritoneal cavity inflated, the endoscope and probe can be manipulated to locate the uterus.

The inseminating pipette consists of a length of narrow bore glass tubing with a sharp elongate tip, drawn under a hot flame. A syringe is attached by tubing to the pipette

and 2 doses of semen, one for each horn, are loaded into the pipette separated by an air buffer on either side.

On location of the uterine horns, the pipette tip is stabbed through the uterine wall and into the uterine lumen, half way between the bifurcation of the uterus and the utero-tubule junction. Once the tip of the pipette penetrates to the lumen, the dose of semen can be expelled by depressing the syringe to a pre-set level. The passage of the semen can be observed through the endoscope. This procedure is repeated for the other horn and the pipette and cannulae removed.

Between inseminations the surgical equipment should be immersed in an antiseptic solution. However, contact of this solution or water with the inseminate must be avoided. The pipettes should be rinsed with semen diluent and wiped clean of any fluids before re-use.

2.2.2 Preparation of Females for Insemination.

In addition to the normal consideration that should be applied to the condition of the females prior to mating, insemination demands further preparation. As Evans and Maxwell (1987) note, insemination is only successful when performed at specific times relative to ovulation. As a result it is necessary that oestrous be either detected or induced. Females may be drafted out of a herd for insemination after detection of a natural oestrous, or oestrous may pharmacologically induced at a known time.

The oestrous cycle of goats has a duration of 19-21 days, with oestrous being a 32-48 hour period in this cycle (Shelton, 1978; Doney et al., 1982). Thus during the breeding season when the oestrous cycle is fully active, a ratio of 2/21 of the herd will be in oestrous on any one day. Oestrus does may be detected using vasectomised bucks or testosterone treated wethers wearing harnesses and crayons. Does marked with crayon can then be drafted out of the herd for insemination. However, this method gives no control over the number or identification of the animals to inseminated on any day, limiting the organisation of the insemination programme.

Natural Synchronisation.

Some degree of synchronisation may be achieved naturally using the male or buck effect, similar to that observed in sheep, although methods of oestrus detection must

still be employed. However, this technique of synchronisation is generally regarded as useful only at the start of the breeding season before spontaneous ovulation occurs, and requires management of the females such that there is no contact with males prior to the desired synchronising introduction of teaser males (Evans and Maxwell, 1987).

The stimulation of ovulation in response to rutting males involves a range of stimuli including sight, sound and odour (Shelton, 1980; Chemineau, 1987). Each of these stimuli exert an effect, although this effect is maximal when all are operative and the does are in the presence of the rutting male. This contact can have an immediate effect on the hormonal activity of the does (Chemineau, 1987). It is also interesting to note that it has been suggested that such contact may influence already cycling does, possibly by causing luteolysis and thus early ovulation (Chemineau, 1983).

The male effect has no effect in the anoestrous period, but can stimulate early reproductive activity in the transition period between the anoestrous and the breeding season. Ott et al. (1980) described this phenomena in dairy goats late in the anoestrous period, observing that the majority of does displayed oestrus an average of 5.5 days after introduction to males, whilst unstimulated controls remained inactive. Normal oestrous was observed in the majority of does, although a proportion displayed short cycles, or standing oestrus with no accompanying rise in progesterone levels. The controls were later shown to be similarly responsive to the male presence. Shelton (1980) reported a similar response for Angoras in this transitional period, with 80% of does ovulating within 10 days of exposure to rutting males. This was significantly different to the surveyed incidence of spontaneous ovulation over the same time-period (14%) in a group of control animals. However, the variability of this response was indicated by a lack of observed stimulation in a subsequent year of study.

Pharmacological Synchronisation.

Greater control of oestrus may be obtained by pharmacological means, although this is more expensive and commonly involves synchronisation using progesterone or prostaglandin treatments.

The prostaglandin method operates by destroying active corpora lutea present on the ovary. This removes the inhibitory effect of progesterone, produced by the corpus luteum, on the production of pituitary gonadotrophins. Increasing gonadotrophin

concentrations subsequently stimulate follicular growth and ovulation. Synchronisation of a herd requires 2 injections of prostaglandin administered 10-14 days apart (Evans and Maxwell, 1987). Two administrations are required because the corpus luteum is only sensitive to prostaglandins between days 6-17 of the oestrous cycle. Thimonier (1981) stated that control of oestrus in a population may be achieved by injecting 8 to 11 days apart, and Ishwar and Pandey (1990) reported good synchronisation with injections 11 days apart. This method appears to only provide a means of synchronising oestrus and successfully breeding already cyclic animals (Alacam et al., 1985). A high proportion of does ovulate 2-3 days after the second prostaglandin injection. It should be noted that prostaglandins are not effective as a means of synchronising oestrus, during periods of anoestrous resulting from either season, parturition or sexual immaturity (Thimonier, 1981).

Progesterone or progestagen administration allows oestrus synchronisation by simulating the effects of the corpus luteum (Evans and Maxwell, 1987). The progesterone supplement inhibits the pituitary hormones during the period of exposure, whilst after withdrawal, the reactivation of the pituitary stimulates follicular growth and oestrus. Exogenously applied progesterone has no effect on existing corpora lutea and so the treatment must be applied for at least the life-span of a normal corpus luteum (18 days).

Progesterone or progestagens can be applied as intravaginal pessaries, such as sponges or CIDR's (controlled internal drug release devices), or as subcutaneous implants in order to synchronise oestrous (Ritar et al., 1984; Ritar et al., 1989, Bretzlaff and Madrid, 1985).

In addition to oestrous synchronisation, ovarian stimulation may be desired to either to ensure that ovulation occurs during induced oestrus, or to stimulate ovulation in the anoestrous or early breeding season.

Ovulation can be stimulated by administration of PMSG (pregnant mare serum gonadotrophin), which provides a supplement of pituitary hormones. Ritar et al. (1984) reported that PMSG was necessary to stimulate satisfactory ovulation in Angoras synchronised with sponges during both the breeding and non-breeding seasons. Few does ovulated in response to synchronisation treatments in the absence of PMSG supplementation. At the start of the breeding season, Alacam et al. (1985) similarly reported that intravaginal sponges only synchronised oestrus in already cycling does. Cashmere types also display a poor ovulatory response to CIDR

treatment without PMSG in the early breeding season (Ritar et al., 1989). PMSG is used to reduce the variability in the ovulatory response and ensure that most females ovulate (Ritar, 1984). This may be necessary to help maximise the potential benefit of insemination and justify the cost involved.

2.2.3 Factors Affecting Fertility.

When fertility trials are conducted, it is important to be aware of the variety of factors that may affect conception rates, so that these may be accounted for in any experimental work comparing methods of semen processing or artificial insemination. Fertility may be effected by: the hormonal treatments used to synchronise and stimulate oestrus; the timing of insemination relative to ovulation; the method of insemination and site of inseminate deposition; the condition, age and genotype of the stock; and the quality and quantity of sperm in the inseminate.

Pharmacology.

Some of the analogues of progesterone used in synchronisation of oestrous may adversely affect the transport of sperm within the female genital tract (Hawk, 1983). Thus different types of synchronising agents may have an effect on fertility, although this may depend on the insemination technique. Ritar et al. (1989) found no difference in kidding percentage or litter size after intrauterine insemination of does synchronised with either sponges containing fluorogesterone acetate or CIDR's containing progesterone. However, Moore and Eppleston (1979) suggested that does cervically inseminated after sychronisation with cronolone (progestagen) sponges or prostaglandin were less fertile than does inseminated after detection of natural oestrus. All three groups of does were only inseminated after oestrous detection.

Prostaglandin induced oestrus has also been associated with variable fertility results. Thimonier (1981) reported poor rates of fertility after insemination and hand-mating, although it was noted that the use of fixed time inseminations may be a confounding problem in this comparison. In contrast, Ishwar and Pandey (1990) reported no deleterious effects after natural mating of prostaglandin synchronised does, although only a small number of animals was used. Problems associated with the use of prostaglandins are the incidence of short oestrous cycles and reduced sperm transport from the cervix, both of which may result in reduced fertility (Thimonier, 1981).

Administration of ovulatory stimulants such as PMSG before insemination may also effect fertility and fecundity. Ritar et al. (1989) observed a significant dose related effect of PMSG on ovulation rate. In fact PMSG may directly stimulate ovulation in the absence of any other treatment and induce superovulation in goats (Cameron and Battye, 1988; Cameron et al. 1988).

Timing.

The timing of insemination with respect to the initiation of oestrus and ovulation is another factor affecting the survival and interaction of sperm cells and oocytes, and hence fertility (Corteel, 1981). Jabbour and Evans (1991) found that in sheep there is an effect of time of insemination relative to time of ovulation and the fertilising life of the inseminated sperm in the female tract. Sperm fertility declined after 24 hours, although this effect was interactive with the method of semen processing. Frozen-thawed sperm had a shorter fertile life than fresh sperm.

In the goat, Moore and Eppleston (1979) reported an interaction between the time of oestrus detection after pessary removal and time of insemination. These authors reported that in total there was no significant effect on fertility of inseminating either 0-2 or 12-14 hours after oestrus detection following synchronisation. However, there was a significant interaction with the time of oestrus detection after pessary removal. Later insemination (12-14 hours) was favoured when oestrus was detected up to 24 hours after pessary removal, whilst early insemination (0-2 hours) gave greater fertility when oestrus was detected greater than 60 hours after pessary removal. Ritar et al. (1989), and Ritar and Salamon (1983) were unable to detect any significant effect of time of insemination relative to sponge removal between 40, 48 and 50 hours after sponge removal. It should be noted that the timing of ovulation with respect to the initiation of oestrus varies with the means of synchronisation, and is advanced with the use of PMSG (Ritar et al., 1989, 1984).

Site of insemination.

The method of insemination, or more accurately the site of inseminate deposition, may also have a significant effect on fertility. This applies in the broadest sense to the differences between cervical and laparoscopic intra-uterine insemination. The

combination of method of insemination and the type of semen processing effects fertility. In particular, the fertility of frozen-thawed semen tends to be lower following cervical insemination (Maxwell, 1985). This may be due to an insufficient population of functional sperm in the cervix, and impaired transport along the female genital tract (Lightfoot and Salamon, 1970).

An important factor concerning the cervical method is the ability to penetrate to the uterus or deep within the cervix (Shelton, 1978). Whilst in sheep it is very difficult to penetrate the cervix, it is possible to penetrate deep into the cervix or into the body of the uterus in most goat does (Moore, 1985). Ritar and Salamon (1983) reported that fertility was improved with increasing depth of cervical insemination, and that this was more apparent with frozen-thawed semen than fresh-diluted semen. In dairy does, Ritar et al. (1989) found that uterine penetration could be achieved in some 40% of does inseminated, and that these had higher fertility compared to does inseminated in the cervix.

To circumvent the postulated sperm transport problems associated with cervical insemination, the technique of laparoscopically locating the uterus and directly injecting semen into the uterine horns was developed in sheep (Killeen and Caffery, 1982). Laparoscopic insemination has been successfully applied to goats (Ritar et al., 1989). This method of insemination has led to the wider use of frozen-thawed in sheep and goats. Fertility rates after using laparoscopicaly deposited frozen-thawed semen, have been as effective as cervical insemination with fresh semen (Maxwell, 1985). However, Jabbour and Evans (1991) observed that the site of laparoscopic insemination may also be important. Under conditions of late insemination after ovulation, these authors reported that fertility of frozen-thawed semen was greater when inseminated at the oviduct as opposed to the uterine horn. This observation was suggested to be a result of impaired sperm transport.

Breeding season.

Periods of post-partum and seasonal anoestrus may also effect fertility after insemination after induced oestrus. The return to oestrus after kidding is related to lactation but appears to be dependent on seasonal effects as well (Lawson et al., 1984). These authors found that restricted suckling advanced the return to oestrus in the transition periods between the non-breeding and breeding seasons. This post-partum recovery of ovarian activity appears to be related to suckling stimulated

release of opioid-peptides that inhibit GnRH (Gonadotrophin Releasing Hormone) from the hypothalamus, maintaining a low LH (luteinising Hormone) release frequency such that maturation of ovarian follicles does not occur (Nett, 1987). Ritar et al. (1989) reported a practical observation that may be related to this effect, with fertility results apparently lower with reduced time between weaning and insemination in the early breeding season.

Some breeds of goat originating in the temperate higher latitudes regions of the globe display a pronounced seasonality of reproductive activity, and are regarded as short-day breeders (Doney et al., 1982; Shelton, 1978; Evans and Maxwell, 1987). The breeding season, characterised by spontaneous ovulation, is limited to the Autumn months This seasonality is believed to mediated by the duration of dark phase (night) secretion of melatonin. This acts to reduce the intensity of oestradiol inhibition of LH release stimulated by GnRH under short-day conditions. This results in increased LH release frequency in the breeding season, stimulating ovarian activity such that complete oestrous cycles are observed (Chemineau et al., 1988a). Conversely, under long-days there is enhanced negative feed-back of oestradiol on LH secretion (Mori et al., 1987). Other factors such as the reproductive status of herdmates (males) and nutrition (as may be effected by environment) may also modulate seasonality (Restall, 1987).

Hormone treatments can however artificially stimulate reproductive activity in the anoestrous or early breeding season. This can be achieved by treatment with GnRH (Knight et al., 1988), melatonin (Earle et al., 1987; Chemineau et al., 1988b; McGregor et al., 1989), and PMSG in conjunction with synchronising agents (Ritar et al., 1984; Corteel et al., 1988). However, fertility rates may be reduced compared to those achieved in the breeding season (Corteel et al., 1988).

Livestock.

The animals themselves also obviously play a significant role in the fertility results. Fertility may be affected by plane of nutrition, genotype and whether the does are pluriparous or primiparous. With natural mating, the fertility and prolificacy of goats is generally high relative to other domestic ruminants, although this is variable with genotype (Shelton, 1978; Devendra and Burns, 1970; Terrill and Foote, 1987). Dairy breeds such as the Anglo Nubian are regarded as having the highest fertility (Devendra and Burns, 1970; Doney et al., 1982). Australian feral goats, from which

the Australian Cashmere type is derived, display high fertility and ovulation rates (Holst, 1981). However, Angoras may exhibit lower reproductive performance, and display a range of congenital problems, with high rates of abortion being reported in some populations (Scheurmann, 1985; Gifford, 1982).

The age of the female should also be considered with respect to puberty. Alacam et al. (1985) reported that greatly reduced fertility in maidens compared to pluriparous does, following insemination at the start of the breeding season in which the maidens were expected to become reproductively active. This puberty effect may interact with the type of hormonal treatment used prior to insemination. In general, fecundity increases with age (Devendra and Burns, 1970).

2.2.4 Fertility Results.

A critical appraisal of semen freezing technology from reported fertility trials is difficult for a number of reasons. As introduced in the previous section, fertility percentages may be influenced by a large number of factors besides the fertilising capacity and number of apparently functional sperm (generally assessed as the number of motile sperm inseminated) in the inseminate. The intrusion of these other factors, means that direct comparison of the effects of the inseminate alone is not valid between trials. This situation is compounded by the lack of within trial comparisons made between the wide range of semen processing methods available. The appraisal of fertility must therefore encompass the entire insemination procedure being reported.

A further problem associated with many trials using goats is the small number of animals on which fertility results are reported. Conception or pregnancy is scored as either a positive or negative result, and the binomial variance alone results in a 10% standard deviation in a sample of 25 animals, indicating that a large number of animals must be inseminated if fertility is to adequately measured (Foote, 1988). This author also regards that if qualitative differences are to be measured between the sperm being inseminated, then the inseminate dose must approach the critical numbers of sperm required to achieve normal fertility.

The following section indicates some of the fertility results that have been reported for the broad categories of semen processing. These are fresh semen, liquid-stored semen and frozen-thawed semen.

Fresh and Liquid Stored Semen.

Fresh semen refers to undiluted semen with a short fertile life, whilst liquid stored semen is diluted with a media to extend its life and increase the efficiency of insemination by distibuting the collected sperm more effectively. Neat semen is of limited use in insemination programmes, as it must be used quickly before it desiccates or agglutinates (Evans, 1987). Diluted semen stored as liquid is more practical, but also exhibits only a short period (a few days) of sperm cell survival and fertilising capability, similarly limiting flexibility of insemination (Corteel, 1981, Ritar, 1987).

Lyngset et al. (1965) cervically inseminated goat does with semen diluted with egg yolk containing extender, 2 to 6 hours after semen collection. A conception rate of only 60% was reported, although it was considered that the synchronising agent, methyl-acetoxy-progesterone (MAP) may have reduced fertility. Schindler et al. (1972) reported that 50% conception was obtained after multiple insemination during natural oestrus, with semen diluted using an egg yolk-citrate extender and used soon after collection. In another trial, these authors observed 67% fertility with semen cooled to 10°C and stored for up to 5 hours, under similar insemination conditions. Further commercial field trials indicated that short term storage of diluted semen achieved approximately 50% conception.

Early field observations of 40% conception after insemination with liquid stored semen, were reported by Kupferschmied (1972). Problem areas were identified as sperm damage during transport, poor timing of insemination, poor oestrus detection, and lack of inseminator skill. In contrast, under experimental conditions, Moore and Eppleston (1979) achieved up to 67% conception after insemination with electroejaculated semen during natural oestrus. However, this semen was only diluted if insufficient semen was available for insemination with a fixed volume of inseminate. Synchronisation depressed conception, which may also have been influenced by the use of electroejaculated semen as opposed to that collected by artificial vagina (AV). Ritar and Salamon (1983) used AV collected semen, diluted and used fresh, and recorded up to 75% fertility after cervical insemination in the second oestrus (a natural oestrus) after synchronisation. Conception rates varied between trials, and were not improved by double insemination with twice the number of sperm in one experiment, but were in another, although there was no significant

difference with single insemination of either 60x106 or 120x106 motile sperm in a further experiment.

Frozen-Thawed Semen.

Freezing and thawing semen reduces the motility and viability of spermatozoa, and this effects fertility (Maxwell, 1985), which is reduced after cervical insemination relative to fresh or chilled semen (Maxwell, et al., 1980; Ritar and Salamon, 1983). Early reports indicated very poor fertility (22%) after insemination with frozen-thawed semen (Lyngset et al., 1965) although rapid improvements have been claimed (Anderson, 1969; Hahn, 1972). Field trials have indicated that fertility percentages of 50-70% can be achieved (Bowen, 1988; Corteel et al., 1987).

Ritar and Salamon (1983) found that fertility of frozen semen was more sensitive to the depth of insemination in the cervix than fresh semen. However, when semen was deposited into the uterus, both types of semen achieved similar fertility. This implies a sperm transport problem associated with the use of frozen semen and cervical insemination. Despite this, up to 62% fertility was achieved with cervical insemination and PMSG administration. Lawrenz (1986) found that 60-70% fertility could be achieved using non-surgical intrauterine deposition of frozen semen in does selected for the capacity to penetrate the uterus. This was similar to the results of Ritar and Salamon (1983), when considering the proportion of animals where semen was deposited in the uterus. Thus, cervical insemination with frozen-thawed semen can give acceptable results under some conditions (Lawrenz, 1986; Corteel et al., 1988), but has also yielded very poor results (Moore et al., 1989).

To avoid the problems associated with cervical insemination of frozen semen, the laparoscopic method of insemination was developed to by-pass the cervix and deposit sperm directly in the uterus (Maxwell, 1985). The latter author notes that fertility using this method is comparable to cervical insemination with fresh semen, or natural mating over one synchronised oestrus. Restall et al. (1988) recorded only a poor fertility (43%) after intrauterine insemination , compared with similar insemination with fresh semen (82%), although the semen was of different origin. Moore et al. (1989) found that whilst cervical insemination with frozen semen even during a natural oestrus gave only poor (34%) fertility, compared with fertility after laparoscopic insemination (65%). The former was regarded too unreliable, while laparoscopic insemination gave acceptable results with frozen semen. After

synchronisation of does, Ritar et al. (1989) also reported acceptable fertility (65%) using laparoscopically inseminated frozen semen. However, as noted by Corteel et al. (1987) there is room to improve beyond acceptable fertility rates.

Simple techniques for freezing ovine semen in straws have developed that produce fertility comparable to that achieved with pellet frozen semen (Hunton and Flecker, 1987). Straws offer a number of advantages over pellet frozen semen, including individual identification, standard volume, ease of handling and storage.

CHAPTER 3.

INSEMINATE ASSESSMENT AND

ARTIFICIAL INSEMINATION.

3. GROWTH AND SEXUAL DEVELOPMENT.

3.1 Introduction.

The characterisation of sexual development in domestic species is of a practical interest, particularly as it relates to puberty and the expression of reproductive behaviour in young stock. The stage at which reproductive activity is successfully expressed can help determine the age structure of the herd; sets a limit on the rapidity with which genetic gains may be made (particularly in intensive breeding systems) by determining the generation interval; may effect the period over which surplus stock are retained before initial selection on reproductive parameters; and sets a physiological timescale for the separation of the sexes.

Compared with many domestic ruminants, goats rank highly with respect to their reproductive performance (Terrill and Foote, 1987). In Australia, both the feral population and commercial Cashmere types exhibit a high reproductive rate. However, in the Angora population, a range of congenital problems may modify or prevent reproductive function (Scheurmann, 1985). In breeds such as the Angora where congenital conditions are known to be a problem, specific knowledge of the processes of normal sexual development may provide a means for a more effective selection of the best potential sires on reproductive characteristics (Ozsar et al., 1990).

Many studies of reproductive development and function have concentrated on the role of the female. However, selection for factors that indicate good sexual development in the male not only ensures that functional sires are retained, but may also determine the reproductive potential of the future herd. The high heritability of testis size (0.67) means that the progeny of sires with large testes will also tend to have large testes, a trait which is highly correlated with sperm production (Foote, 1990). In addition to indicating reproductive fitness, this characteristic may be desirable if sires are to be selected for artificial breeding purposes. Furthermore, testis size of the sire may be related to the reproductive characteristics of the female progeny. In cattle, sire testes size has been correlated with higher fertility and younger age at puberty of female progeny (Coulter and Foote, 1979; Toelle and Robison, 1985; Smith et al., 1989).

However, the relationship of testes size with reproductive performance is confounded with similar associations with body size (Coulter and Foote, 1979). Haley et al. (1990)

reported a positive correlation between sire testes size and the day of the first oestrus in the second breeding season of ewe progeny, and also with the litter size per ewe mated (attributed to a change in fertility). It was suggested that there is general evidence for a positive association between testes size and the reproductive performance of female progeny. However, as these authors state, any implicit effect of testes size needs to be examined in the absence of the confounding influences of body-size and live-weight. Even so, the improvement in fertility with selection for testes size indicated an associated selection for improved rates of conception. Consequently knowledge of the normal development of the testes may be of significant interest to commercial breeders, in selecting improved sires.

3.2 Puberty.

Puberty is a term that has been used to describe the point at which various stages of sexual development have been achieved. The definition of puberty appears to vary between investigations. However, in a strict sense puberty describes the earliest stage at which sexual maturity is achieved, this being the point at which the individual is capable of successfully mating. Thus the most direct assessment of puberty must relate the individual to the its ability to reproduce progeny in the absence of any limitation other than its own sexual competence. In practice however, it may be valid to characterise only certain aspects of the process of sexual development and use these as an indication that puberty has been reached, although the difference between this and a physical determination of puberty should be made clear.

The development of sexual maturity involves many interactive factors that must operate in concert for sexual competency to be expressed. Functional competence of all components of the reproductive system is not achieved simultaneously and puberty can be described as the stage at which all components are at a sufficiently advanced stage to facilitate reproductive activity (Ashdown and Hancock, 1974). This situation is well illustrated in the female. In prepubertal female lambs as young as 12 weeks old, follicular development has been observed with the presence of antral follicles and large steroidogenic follicles (Sonjaya and Driancourt, 1989). These authors also observed that at 14 to 16 weeks LH-sensitive follicles were present and that ovulation could be stimulated by supplementation with exogenous hormone.

Reproductive development in the male is characterised by the growth and development of the genital tract and accessory glands, the development and descent of the testes, the

initiation of the spermatogenic cycle and release of mature spermatozoa and the development of sexual desire and the erectile capability of the penis (Ashdown and Hancock, 1974). These changes are regulated by reproductive hormones. This endocrine system is comprised of the hypothalamus that produces gonadotrophin releasing hormone (GnRH); the anterior pituitary which produces luteinising hormone (LH) and follicle stimulating hormone (FSH); and the gonads that produce the gametes in addition to the steroid sex hormones, testosterone, oestrogen, progestin and inhibin (Adams and Steiner, 1988).

The onset of puberty in the male is not an abrupt development, but rather a gradual process that can be characterised by observations such as the onset of spermatogenesis, the appearance of sperm in the ejaculate, and the capacity for intromission (Skalet et al., 1988). Adams and Steiner (1988) note that puberty has been variously described according to: (1) phenotypic changes involved in the sexual differentiation of male and female, including the development of secondary sexual characteristics and the rapid increase in size of the sexual organs; (2) neuroendocrine development such that the hypothalamus-hypophysial axis competently coordinates the reproductive hormone responses; and (3) the integration of physiological and behavioural responses whereby functional gametes are produced and sexual activity is displayed.

In sheep, a working definition of male puberty can be taken as the point at which reproduction first becomes possible, and this is regarded by many authors as the developmental period over which the testes become responsive to the pituitary hormones and release mature spermatozoa (Adams and Steiner, 1988). Ortavant et al. (1977) suggested six phases of pubertal development placed in the context of the progressive establishment of the spermatogenic cycle: (1) the impubertal phase, during which gonocytes migrate to the genital ridges; (2) the prepubertal phase, where cellular differentiation from the gonocytes first occurs; (3) puberty, where mature spermatozoa are released from the seminal epithelium; (4) the first post-pubertal phase, in which spermatogenic production increases; (5) the second post-pubertal phase, during which adult spermatogenic activity is observed in the still expanding testes; and (6) adulthood, at which mature testis size (subject to seasonal variation) is achieved.

As suggested by the above developmental protocol, the point at which puberty is reached is not the conclusion of reproductive development. Maximal reproductive capacity is only achieved after subsequent post-pubertal development of the reproductive system (Ashdown and Hancock, 1974). Corteel (1981) stated that in early-maturing breeds of goat, the numbers of sperm in the ejaculate in the first

breeding season are only 60% of those characteristic of adult production in the second breeding season.

3.3 Endocrine development.

Whilst the reproductive organs undergo some maturation during foetal life, final maturation and functional development occurs post-natally (Adams and Steiner, 1988). These authors reviewed the reproductive endocrinology of lambs from birth to puberty.

Initially, the mean concentrations of circulating LH are low in the new-born ram lamb. These concentrations increase between the second and fourth months of post-natal life, before declining in the fifth month. These fluctuations in mean LH concentrations are reflective of the frequency of pulsatile LH release. Pulsatile release may be observed as early as the first week of post-natal life, with the onset of pulsatile release being related to the growth rate of the animal. Foster et al. (1978) described that the LH pulse frequency increased sharply up to week eight and then declined slightly over the subsequent eight weeks in the young ram. However, Adams and Steiner (1988) note that the LH content of the pituitary increases from birth until week 12 and remains high. At all ages examined, the pulsatile release of LH is followed by an increase in the circulating concentrations of testosterone.

The ontogeny of the increase in pituitary FSH is similar to that of LH, although serum FSH concentrations apparently remain constant during the prepubertal phase (Adams and Steiner, 1988). Prolactin is another hypophysial hormone that remains relatively constant throughout puberty, although peak concentrations between weeks 10 and 12 are coincident with the onset of rapid testicular growth and the initiation of spermatogenesis.

Testosterone is the predominant androgen observed in the circulating plasma and within the testes of the prepubertal ram. As already noted, testosterone production is responsive to the pulsatile release of LH. However, the fluctuations in testosterone production that result from the pulsatile nature of LH release may be reduced by the activity of androgen binding protein (ABP) within the testis. The ABP may help maintain a constant testosterone concentration within the androgen-dependent germinal epithelium of the seminiferous tubules, despite the background of fluctuating testosterone secretion by the Leydig cells (Waites, 1980). The concentration of testosterone in the testes increases two weeks prior to the onset of spermatogenesis,

whilst a significant increase in circulating testosterone concentrations occurs at the onset of spermatogenesis.

In goats, Chakraborty et al. (1989) described the changes in serum concentrations of testosterone, LH and FSH in Anglo-Nubian bucks from birth to 44 weeks of age. These observations were related to the onset of puberty, defined in this study as the stage at which motile spermatozoa were first collected by electro-ejaculation. These authors found that serum concentrations of LH were initially elevated between birth and week 20, and then declined till week 44. This decline in LH concentration was coincident with an increase in serum testosterone concentrations, which were initially low until week 20 and then increased to a peak at the time that puberty was determined. This observation is probably a result of a system of feedback control of LH by testosterone. After reaching puberty, the serum concentrations of testosterone declined to levels characteristic of the adult. These authors also found that serum FSH levels followed a pattern similar to LH, although the decline after puberty was followed by an increase to prepubertal levels. This contrasts with the constant levels of FSH ascribed to the prepubertal ram by Adams and Steiner (1988). Chakraborty et al. (1989) stated that testosterone is an important regulator of the processes of sperm maturation and puberty onset.

In the Angora breed, Ozsar et al. (1990) compared a range of reproductive characteristics across a group of randomly selected males which was divided into two subgroups, those that achieved puberty in the first breeding season and those that did not or displayed delayed pubertal development. The two groups were distinguished on the basis of differences in the observed serum concentrations of testosterone and LH. During the non-breeding period both groups had similar serum concentrations of both hormones. In Autumn, at an age of approximately six months, the animals that were judged to achieve puberty normally experienced a significant increase in LH and testosterone levels. At the end of Autumn testosterone returned to the base levels. Serum LH concentrations followed a similar pattern to the increases in testosterone, although there was significant individual variation.

The animals that experienced delayed pubertal development exhibited significantly lower fluctuating levels of testosterone and LH during the first breeding season. However, both groups showed parallel increases in live-weight and testis size (scrotal circumference) in the breeding season, although there were significant differences in magnitude between the two groups. Whilst no supporting data was presented, Ozsar et al. (1990) stated that the expression of sexual behaviour was related to the observed

serum concentrations of testosterone, and differences were apparent between the two groups of animals studied. It was concluded that the onset of puberty in the Angora is characterised by increases in circulating LH and testosterone concentrations. This data also supports the proposition that pubertal development in goats is limited by live-weight and testis size thresholds, as has been previously suggested in the ram and buck (Foster et al., 1985; Chemineau and Thimonier, 1986). These thresholds apparently over-ride the developmental stimuli associated with the transition to the breeding season.

3.4 Development of the Reproductive Tract.

Yao and Eaton (1954) studied aspects of the post-natal development of the reproductive tract in goats. Of particular interest in this regard is the separation of the penis during puberty to allow protrusion and erection of the penis. Initially the urethral process and glans penis are completely adhered to the mucosa of the prepuce. These adhesions are regarded as an indication of sexual immaturity, and the separation of the penis related to the activity of the testicular hormones (Elwishy and Elsawaf, 1971).

Separation of the penis from the prepuce begins at the filiform appendage or urethral process, which is initially situated in a groove across the head of the glans penis (Elwishy and Elsawaf, 1971). Separation continues in stages with the adhesions of the glans penis breaking down after the separation of the urethral process (Skalet et al., 1988). The state of adhesion or separation of the penis can be scored according to three established stages (Madani et al., 1989). These are: (1) the infantile stage in which the penis is small and completely adhered to the preputial mucosa; (2) the separation stage during which adhesions break down and the glans penis becomes partially visible through the preputial orifice; (3) and the final stage at which the penis is fully detached from the prepuce and can be exposed.

In the late maturing Damascus goat, the separation of the penile appendages (age 243 days) occurs significantly in advance of the age of puberty (age 509 days) as estimated from the observation of sperm within the ejaculate (Elwishy and Elsawaf, 1971). The volume of the testes at separation and puberty was also substantially different (60 ml and 139 ml respectively).

In direct contrast, Skalet et al. (1988) reported that the time of complete penile separation in Nubian bucks was coincident with their estimate of puberty, defined as the

time at which sperm are first produced. Examining two genotypes of Libyan goat, Madani and Rahal (1988) reported a time lag of 30 to 50 days between penile separation and the observation of sperm in electro-ejaculates. This characteristic alone does not seem to consistently provide an adequate indication of pubertal development across different breeds.

3.5 Development of the Testes.

As already noted, reproductive development through the pubertal period is a gradual and continuous process (Skalet et al., 1988). Adams and Steiner (1988) regard puberty as a continuum of development between the activation of the endocrine function of the testes and the release of mature spermatozoa in the final stages of puberty. The testes present an obvious external feature that perhaps best reflects this progressive growth and development, and thus may provide the most valuable indicator of pubertal development.

The testes may be parametrically assessed using a variety of techniques. Chemineau and Thimonier (1986) suggested that testicular volume may be determined by palpation with an orchidometer, measurement of the testis diameter with a calliper, or by water displacement. Of these methods, Oldham et al. (1978) found that comparative palpation was best correlated against testis volume at slaughter in rams, with a correlation coefficient of 0.96, whilst the water displacement and external dimension measures had correlation coefficients of 0.83 and 0.86 respectively. Madani and Rahal (1988) reported a much stronger correlation (R2=0.95) of scrotal diameter with testis weight in Libyan goats. Scrotal circumference has also been used as a measure of testicular size during the development of young goats (Bongso et al., 1982, Oszar et al., 1990). This measure has similarly been strongly correlated (R2=0.96) against testis weight and volume in goats (Wolde-Michael et al., 1989), and is a commonly used and accurate predictor of testicular weight in bulls (Coulter and Foote, 1979).

Testis assessment is an accepted component of breeding soundness examinations in the adult male (Ott and Memon, 1980; Foote, 1990). Testis consistency is examined for evidence of abnormalities or clinical problems that may affect reproductive function. However, a factor also considered is testis size, as this is strongly correlated with sperm production. This correlation is frequently between 0.85 and 0.95 in most species studied (Foote, 1990), although the relationship between testis weight and daily sperm

output in rams has been reported as having a correlation coefficient of 0.83 (Lino, 1972).

Testis measurements may thus provide some indication of comparative reproductive potential, particularly where sires are selected for AI purposes and sperm production rates assume greater importance. Scrotal circumference measures provide substantial information about the reproductive capacity of bulls (Coulter and Foote, 1979) and are used in the determination of breeding soundness examination scores for bulls under natural mating conditions (Larsen et al., 1990). The latter authors stated that scrotal circumference is an important parameter in bovine fertility prediction. However, the relationship is apparently characterised by a threshold effect, such that above a threshold circumference other factors become more important to the prediction of breeding success. When threshold levels are surpassed, the relationship between scrotal circumference and fertility indices may be non-significant or even negative. Regardless of this, Larsen et al. (1990) maintain that testis measures should not be ignored as indicators of normal development and adequate sperm production.

Dun (1955) also regarded that an examination of testis size and consistency in combination with penile mobility can be used to determine sexual maturity in the young ram. However, it should be noted that testis size appears to be of doubtful value in predicting libido, probably because threshold androgen levels may be maintained even by testes of subnormal size (Foote, 1990).

Testicular growth or expansion continues from birth through the period of pubertal development, but is characterised by periods of more rapid expansion (Bongso et al., 1982; Baishya et al., 1987; Madani and Rahal, 1988). Testis size also fluctuates with season in the mature animal in many breeds of sheep and goat that exhibit seasonal reproductive cycles (Evans and Maxwell, 1987).

The growth and development of the testis is characterised by the expansion and development of the seminiferous tubules and the associated cells that produce androgens (Leydig cells) and participate in spermatogenesis (Sertoli cells) within the testicular tissue. Baishya et al., (1987) found that seminiferous tubule diameter is significantly and positively correlated with testis weight. Rapid increases in tubule diameter and corresponding increases in testicular size, as measured by scrotal circumference, are indicative of the onset of spermatogenesis (Bongso et al., 1982). The onset of spermatogenesis is also synchronous with the initiation of seminiferous

tubule vacuolation, the degree of which increases with increasing testis size in the young buck (Madani and Rahal, 1988).

During testis expansion in the ram, the population of Sertoli cells initially increases and is followed by increases in cellular and nuclear size of the Sertoli cells (Monet-Kuntz et al., 1984). In prepubertal lambs however, little increase in the cytoplasmic and nuclear size of the Leydig cells was observed. The increase in the interstitial stroma of the testes occurred as the population size of Leydig cells increased.

As the testes grow and develop so the spermatogenic cycle commences, concludes and continues in the active seminiferous tubules. As reported by Watson et al. (1956) from studies of the developing ram, the formation of a distinct seminiferous tubule lumen is rapidly followed by, or coincident with, the observation of primary spermatocytes. Following this, tubules containing secondary spermatocytes and then spermatids are observed to predominate within the testis. The initiation of spermatogenesis in the testis is then completed with the release of spermatozoa into the tubule lumen, free from the Sertoli cell cytoplasm, and then passage of the spermatozoa to the rete testis and the epididymis.

Relationships with age and weight.

In many domestic species, there is evidence of a general relationship between growth and reproductive performance, as indicated in studies of female reproduction (Brien, 1986). In males, reproductive development similarly exhibits a strong relationship with body size. As the site of both testosterone and gamete production, the growth and development of the testes is an obvious parameter with which to assess male reproductive development. In rams, testicular development has been more closely related to body-weight than age, and as a result puberty has been associated with live-weight thresholds (Dun, 1955; Watson et al., 1956; Madani et al., 1989). Female development to puberty has been similarly related to the achievement of critical live-weights (Foster et al., 1985).

In male goats, testicular circumference has also been more strongly correlated with body-weight than with age (Bongso et al., 1982). Wolde-Michael et al. (1989) also reported a significant correlation of scrotal circumference and testis weight at slaughter with body-weight, but found no significant relationship with age. The relationships between testis size and body-weight were stronger if the comparison was made using

buck weight as a proportion of dam weight, to adjust the relationship for the proportion of estimated potential mature weight achieved by the individuals.

Testicular growth curves against age and body-weight are typically described as curvilinear, sigmoidal or triphasic for young rams and goat bucks (Watson et al., 1956; Bongso et al., 1982; Madani and Rahal, 1988; Madani et al., 1989). All are terms used to describe the changing rate of testicular growth as the animal matures. Initially and terminally there are periods of relatively slow growth, whilst there is an intermediate period characterised by rapid testicular growth. It should be noted that testicular development does not cease at puberty (Watson et al., 1956).

Madani and Rahal (1988) reported a comparatively poor relationship between testis development and body-weight. Instead it was suggested that the sigmoidal development of testis diameter with age and body-weight was indicative that other factors were also determinants of testicular development. These authors contended that photoresponsiveness to decreasing daylength was the stimulus for the observed periods of increased testis development. In females of breeds that exhibit seasonality of reproductive behaviour, photoperiodism is important to sexual maturation with decreasing daylength stimulating early onset of puberty (Amoah and Bryant, 1984; Foster et al., 1985). However, since it has been observed that not all animals achieve puberty under the same photoperiodic conditions (Ozsar et al, 1990) such a developmental response appears to be conditional on live-weight, indicating a role for both factors.

Ultimately, the development of the spermatogenic cycle is best related directly to testis development. However, indirect measures of development such as live-weight are required to practically measure spermatogenic development. The value of these predictive measures lies in their relationship with testis weight and ease of application. Both testis weight and live-weight have been used to define the different stages of development of spermatogenesis (Skinner et al., 1968; Madani and Rahal, 1988).

3.6 Development of Sexual Competence.

The observation of reproductive activity in the young male is the most direct indication that puberty has been reached and sexual competence achieved. However, it is possible that the conditions of such an assessment may supress the expression of this behaviour. This may occur if the animal is removed from herdmates, introduced to a foreign environment, or artificial vagina collection attempted at the same time. Care must be

taken to exclude such inhibitory influences if sexual competence *per se* is to be accurately determined as a part of puberty assessment.

The expression of sexual behaviour can be scored according to the type of reaction observed. In young males, mounting attempts are common even prior to the onset of puberty (Ozsar et al., 1990). After the infantile behavioural stages, the sexual reactions of the males may be described as : (1) smelling the external genitalia of the female; (2) "lazy" mounting without protrusion; (3) mounting with erection and short protrusion, but with no penile movement and no intromission or ejaculation; and (4) where mounting was accompanied by erection, protrusion, intromission and ejaculation (Elwishy and Elsawaf, 1971). Ejaculation is indicated by an obvious pelvic thrust followed by a backward retraction of the head (Madani and Rahal, 1988).

In the initial stages of sexual development, Elwishy and Elsawaf (1971) observed that the young males that showed interest in the female but did not mount, might smell and lick the prepuce of other males and mount them, occasionally with erection. Such expressions of homosexual behaviour have been implicated as a contributing to the failure of males reared in unisexual groups to breed as adults, and has been associated with sexual inhibition and extended reaction times during semen collection (Orgeur et al., 1984; Price and Smith, 1984/85).

Orgeur et al. (1984) examined the effect of social group during rearing on the subsequent expression of sexual activity. These authors reared French dairy goat males in either individual pens after weaning (according to the practice of the artificial insemination centre) or in a mixed group of males and females of the same age. The two groups did not differ in their sexual reactions at puberty, although the mixed group gave ejaculates of higher volume and lower sperm concentration. The latter observation was attributed to a stimulation of the accessory glands in response to the presence of the females. The contact with female age-mates did not offer any apparent advantage to the reproductive development of the young males. These authors concluded that this indicated that group rearing of males appears to be acceptable. However, the experiment did not address the potential problems associated with rearing the males in unisexual groups, as the control animals were individually housed. Thus no assessment could be made regarding the value of heterosexual rearing as opposed to post-weaning rearing in groups of males only, in which homosexual bonds might be established.

Sexual behaviour in terms of mate choice and sexual performance (achievement of ejaculation) has been related to the occurrence of male-male mounting in goats reared in unisexual groups (Price and Smith, 1984/85). These authors studied 24 yearling dairy type males that were heterosexually inexperienced and reared together. The behaviour of these subjects when exposed to stimulus mates (male and female) allowed their classification as : (1) sexually inactive (17%), showing no sexual interest in the stimulus animals; (2) bisexual (25%), willing to mount and copulate with either sex; and (3) female orientated (58%), only mounting females when offered a choice of mates.

Of the males that were observed to be sexually active when introduced to a stimulus animal, 70% were female orientated despite many of these actively displaying homosexual behaviour in the rearing group. Price and Smith (1984/85) regarded that this indicates that most male goats inately prefer, or rapidly learn to prefer female sexual stimuli. Bisexual behaviour was related to the frequency of mounts received in the rearing group. Males classified as bisexual received a greater number of mounts from a wider range of conspecifics, but interestingly were not distinguished by the frequency with which male-male mounts were initiated by the individual in question. Those males that were judged sexually inactive in the mate selection tests, directed large numbers of mounts toward a specific herdmate in the rearing group. The authors suggested that the development of sexual attachments to specific individuals in all-male rearing groups may be a significant reason for reproductive failure, rather than the failure to develop normal mating skills. Thus the experiences that result from unisexual rearing may adversely modify reproductive behaviour.

The expression of sexual competence as the successful act of copulation and conception is the strongest evidence of puberty and sexual maturity. Other measures of sexual maturity such as the presence of free spermatozoa in the testes, the degeneration of penile adhesions and sperm present in ejaculates collected by electroejaculation or artificial vagina, are easier to collect but vary in chronology. These differences in method of puberty determination can produce great variety in estimates of weight and age at puberty (Madani and Rahal, 1989).

In the Damascus goat, Elwishy and Elsawaf (1971) found that ejaculates could be obtained by artificial vagina collection from bucks 388-549 days old. This was 1-3 weeks in advance of the appearance of live spermatozoa in the ejaculate, in the majority of bucks assessed. Ejaculate quality varied with the individual, with initial ejaculates containing no spermatozoa in some animals, and only non-motile sperm in others. The

timescale of the production of sperm bearing ejaculates and the progressive development of sperm motility varied between individuals. These events occurred significantly later than the degeneration of penile adhesions in this late maturing breed.

In contrast, Skalet et al. (1988) observed that full penile separation was approximately coincident with the appearance of sperm in the ejaculate which occurred at a much younger age in the Nubian buck (141 days). In Libyan Fat-Tailed lambs, Madani et al. (1989) found that penile separation occurred after free sperm were observed in testicular sections, whilst sperm in ejaculates were observed some time after this. However, Skalet et al. (1988) also found sperm in electro-ejaculates prior to the first successful artificial vagina collections. Madani and Rahal (1988) similarly found that the appearance of sperm within electro-ejaculates occurred prior to the first successful mating. Clearly direct comparisons of age and weight at puberty must be interpreted in the context of the method used to determine puberty or sexual competence.

CHAPTER 4.

SEMEN FREEZING EXPERIMENTS: DEVELOPMENT OF A STRAW FREEZING METHOD FOR CAPRINE SEMEN

4. SEMEN FREEZING EXPERIMENTS: DEVELOPMENT OF A STRAW FREEZING METHOD FOR CAPRINE SEMEN.

Introduction.

Sheep and goat semen frozen in straws generally exhibits poorer post-thaw recovery of spermatozoa than pellet frozen semen (Evans and Maxwell, 1987; Hunton et al., 1987; Maxwell et al., 1980; Ritar, 1987; Shirbhate and Honmode, 1982). However, these differences may not be significant, and fertility results in sheep can be similar after intrauterine insemination with semen frozen as straws or pellets (Hamilton and Killeen, 1987; Hunton et al. 1987; Hunton, 1988).

The practical advantages of cryogenic preservation of sperm outlined in Chapter 1, are perhaps maximised with the use of polyvinyl straws. Compared to pellets, polyvinyl straws are more easily packed for transport and storage, allow clear identification of the inseminate, provide greater protection from environmental contamination, and confer good control over the thermodynamics of freezing (Evans and Maxwell, 1987; Ritar, 1987; Watson and Martin, 1975; Wilson, 1988). Additionally, it is often an export requirement that semen be frozen in straws. The advantages of accurate identification of polyvinyl straws benefit both the vendor and the purchaser, and in combination with automated semen analysis, may offer some form of quality assurance.

The advantages of freezing semen in polyvinyl straws have been well recognised in bovine artificial breeding, and straws have been adopted as the preferred means of packaging and marketing semen (Amann and Schanbacher, 1983). In bovine artificial breeding, straws have been fully integrated with the method of insemination, with the straw becoming a component of the insemination equipment.

Simplicity is an advantage to the wider application of artificial breeding technology. The technique progressively developed within these experiments, is designed to be simple and not require access to expensive equipment. One-step dilution and freezing has been applied to ram semen, saving time and reducing the complexity of semen processing (Wilson, 1988). However, goat seminal plasma is less conducive to sperm survival than that of the ram, and seminal plasma removal improves post-thawing percent motile (Corteel et al., 1984; Memon et al., 1985; Ritar and Salamon. 1982).

As a result, complicated methods of processing goat semen have been devised, though at a cost in time and lost sperm (Ritar, 1988).

Corteel et al. (1984) stated that egg yolk cannot be used in goat semen diluent, due to the reaction with the seminal plasma enzyme phospholipase-A, and the subsequent release of fatty acids and lysolecithins that are toxic to sperm (Roy, 1957). However, low concentrations of egg yolk have been successfully used to freeze goat semen (Ritar and Salamon, 1982; 1983). These experiments further establish the credibility of the technique employed.

A series of 4 experiments was undertaken to develop a simple one-step method of diluting and freezing caprine semen in polyvinyl straws, using a freezing medium containing egg yolk.

The experiments investigated the height of straw freezing in vapour above a liquid nitrogen surface, the freezing media and rate of dilution, the temperature of straw filling, the type of freezing surface, and finally a comparison of straw and pellet frozen semen.

Materials and Methods.

Each of these experiments were conducted during the breeding season (southern hemisphere) using semen from a pool of mature cashmere type bucks, on an artificial breeding station (Hadspen Park, Hadspen, Tasmania).

Collection Procedure.

For ease of collection, teaser does were induced to display behavioural oestrus on the day of each collection. Does were treated with intravaginal progesterone pessaries, type G CIDR's (330mg progesterone, AHI plastic moulding company, New Zealand) for 7 to 21 days. These were removed 36 hours prior to collection and the does injected with a 0.5mg dose of oestradiol benzoate.

At collection the teaser does were restrained in a metal crush in a quiet area of the collection shed. All males used in these experiments were trained for semen collection well in advance of the experimental work. Bucks familiar with the routine

of semen collection were introduced singly to the collection area and the restrained doe, from adjacent pens. The bucks were allowed to mount the teaser doe and the penis guided (touching only the sheath) into a warm water filled artificial vagina (AV) of the same dimensions as that used for the collection of ram semen.

Following ejaculation, the semen was drained into the warm tulip glass within the AV. The tulip glass was then removed from the AV and care taken to protect the semen from light and cold shock whilst being transported to a 30°C water-bath. Immediately following collection, the semen was visually assessed for motility, colour, density and volume, as described by Evans and Maxwell (1987). This was undertaken to determine if the ejaculate was of sufficient quality to warrant inclusion in the experiment. As soon as practicable after collection, each ejaculate was diluted in accordance with the experimental procedure.

The semen was diluted at 30°C, using diluent pre-warmed in the same water-bath that the semen was transferred to after collection. The diluent was prepared from a double strength stock solution (itself prepared minus egg yolk a day prior, and refrigerated) to give the concentrations of media constituents in the diluted semen shown in table 4.1 (unless otherwise stated). The egg-yolk was added on the day of collection, and the stock diluted with distilled water. In experiments using more than one media concentration, all were diluted from the same stock solution. Table 4.2 illustrates the constituent concentrations in the dilution media used. The semen was either diluted in the collection glass, or pipetted into test-tubes and diluted where more than one dilution regime was used. The diluent was added slowly to the semen in warmed pipettes and the dilution mixed.

In each experiment, ejaculates from four bucks were used as replicates. As such, semen from these ejaculates were divided between the experimental treatments either at processing or freezing, depending on the experiment. The time between collection and dilution of the semen, and hence subsequent cooling was kept to a minimum within the technical constraints of the treatments employed.

The methodology of processing and freezing is described separately for each experiment.

Table 4.1 Recommended concentrations of media constituents in diluted semen.

Constituent	Concentration
Tris(hydroxymethyl) methylamine	250mM
Citric Acid	82.7mM
Glucose	27.7mM
Glycerol	4.0% (v/v)
Egg Yolk	1.5% (v/v)

Table 4.2 Estimated tonicities and concentrations of media constituents in diluent modified for dilution rate.

Modified diluent	Estimated tonicity KPa	Tris (mM)	Citric acid (mM)	Glucose (mM)	Glycerol (% V/V)	Egg yolk (% V/V)
M 1:2	948	375.0	124.0	41.6	6.0	2.25
M 1:5	758	300.0	99.2	33.3	4.8	1.8
M 1:11	689	272.7	90.2	30.3	4.4	1.64
M 1:23	660	260.9	86.3	28.9	4.2	1.57

Experiment 1.

In this first experiment, the basic method of freezing caprine semen in polyvinyl straws was investigated. This was done in a 4x4x4 factorial experiment examining the effect of the height and time of exposure of 0.25ml straws to the vapour above a liquid nitrogen surface, in a simple static vapour chamber, prior to plunging into the liquid nitrogen.

The semen collected from four bucks was diluted at a rate of 1:5 (1 volume of semen to 5 volumes of diluent) using diluent M1:5, and held at 30°C. At this temperature the straws (previously labelled with the treatment identification) were filled by aspiration and heat sealed. These straws were cooled in a horizontal position in a water jacket, over a period of 1.5 to 2 hours. This was achieved by placing the straws in water from the 30°C water bath and cooling in a forced fan cooler equilibrated at 5°C. The rate of cooling was manipulated by altering the volume of the water jacket.

Once the semen in the straws had reached 5°C, as assessed by measuring the temperature of the water jacket, the straws were prepared for freezing by removing from the water and rapidly drying in an open work area at the top of the cooler, using cooled tissues.

Prior to this, the polystyrene freezing chamber was prepared for freezing the straws at the four heights. The height of the freezing platform (a wire grid with a 1cm2 mesh) was manipulated by placing it on top of a wire basket immersed in the liquid nitrogen, and adjusting the level of the liquid nitrogen relative to the upper surface of the freezing platform. The straws were frozen at heights of 0.25, 1, 4 or 16 cm for 10, 30, 90 and 270 seconds. The straws were frozen in the enclosed freezing chamber and rapidly plunged into the liquid nitrogen after appropriate exposure to the vapour.

The same freezing chamber was used for each of the treatment combinations, with the height of the platform being adjusted down from the 16cm level (initially), by the addition of liquid nitrogen to the freezing chamber. With each height adjustment the freezing chamber was allowed to equilibrate before introduction of the straws.

Following freezing, the straws were transferred to a liquid nitrogen container and stored for one to two weeks prior to assessment. Once frozen, care was taken to avoid temperature fluctuations in the straws during subsequent handling.

Experiment 2.

This was a 3x2x3x4 factorial experiment examining the effects of filling straws at three temperatures, (either 30°C, 22°C or 5°C) with two types of straw (0.25 or 0.5ml) and three types of freezing platform.

The semen collected from four bucks was diluted at a rate of 1:5 with media M1:5 at 30°C. However, in this experiment both 0.25 and 0.5ml straws were filled at either at 30°C in the water-bath immediately after collection, after cooling to 22°C in a cooler, or after continued cooling to 5°C. After filling at 30°C or 22°C the straws were sealed with a water reactive polyvinyl chloride gel and cooled lying horizontally in water jackets (at the same initial temperature as the straws). These straws and an aliquot of the semen in a test tube, were cooled to 5°C over a period of 1.5 hours in the cooler.

All semen was thoroughly mixed to fully suspend the spermatozoa prior to filling and sealing. Temperature shock was avoided by ensuring that the mixing pipettes and straws were at the same temperature as the semen. Straws filled with 5°C semen were filled in an open topped work area of the cooler, and also sealed with water reactive polyvinyl gel. Immediately before freezing, all straws were dried with cool tissues inside the cooler.

The straws were frozen on one of three platform types at a standard height of 4cm for 1.5 minutes in the freezing chamber described in experiment 1. The freezing platforms used were the 1cm2 wire mesh of experiment 1, a wire frame offering only two points of contact at either extreme of the straws and a perforated metal plate. The platforms were regarded as varying according to the degree of contact with the straws at freezing, this being six to eight points with the mesh, two points with the frame and near continuous contact with the perforated metal plate. Adequate time was allowed for the platforms to equilibrate in the freezing chamber before use.

After freezing in the liquid nitrogen vapour of the sealed freezing chamber, the straws were plunged into the liquid nitrogen reservoir and transferred to an storage container. The straws were stored under liquid nitrogen for one to two weeks before assessment.

Experiment 3.

The effects of dilution rate and dilution media were examined in this 4x4x4 factorial experiment. Semen from four bucks was diluted at rates of 1:2, 1:5, 1:11 and 1:23, with each of the media M1:2, M1:5, M1:11 and M1:23 (table 4.2). Only when the dilution rate and the dilution media were appropriately matched, were the recommendations for the concentration of diluent constituents in diluted semen (table 4.1) achieved. With all other combinations of dilution rate and dilution media these recommendations were not met, and table 4.5 illustrates the effects of the dilution regimes on the concentrations of tris(hydroxymethyl)methylamine.

After collection and initial assessment, the semen from each of the four bucks was accurately divided into 16 aliquots in clean dry test tubes at 30°C. As rapidly as possible, this semen was diluted with each of the four diluents at each of the four dilution rates. Care was taken to ensure that temperature fluctuations were avoided during this handling of the semen.

Once appropriately diluted, the semen was loaded by aspiration into 0.25ml straws at 30°C and the straws heat sealed. The straws were then cooled to 5°C in a water jacket over a period of 1.5 to 2 hours.

At 5°C the straws were dried within the cooler in preparation for freezing, and then rapidly transferred in groups of 16 (one replicate block) to the freezing rack. The straws were frozen at a height of 4cm in the vapour above the liquid nitrogen surface for two minutes on the perforated freezing platform described in experiment 2. Immediately following this period of vapour freezing, the straws were plunged into the liquid nitrogen reservoir and subsequently stored in a liquid nitrogen container for one to two weeks prior to thawing and assessment.

Experiment 4.

Five types of freezing and packaging semen were compared over four rates of dilution. Semen was frozen in 0.25 and 0.5ml straws and also as 0.1, 0.2 and 0.4ml pellets. Semen diluted at rates of 1:2, 1:5, 1:11 and 1:23 with media M1:2, M1:5, M1:11 and M1:23 respectively, was used with each method of packaging.

After collection, semen from each of the four bucks was divided into four aliquots of measured volume and diluted in test tubes at each of the four rates. At 30°C the 0.25 and 0.5ml straws were filled with the diluted semen and heat sealed. The straws were then placed in a 30°C water jacket and cooled to 5°C in a horizontal position over 1.5 to 2 hours. The semen to be frozen as pellets was retained in the test tubes after dilution and similarly cooled to 5°C.

Straws were frozen as they were in experiment 3, at 4cm for two minutes on the perforated metal freezing platform and plunged into liquid nitrogen. Semen was also frozen as pellets according to the method of Salamon and Ritar (1982). The different volumes of semen were dropped rapidly from pasteur pipettes (cooled with the semen) into depressions already formed in a block of dry ice. After freezing, observed as the semen changed colour, the pellets were plunged into liquid nitrogen and the subsequently stored under the same conditions as the frozen straws. As in previous experiments, the frozen semen was stored for a period of one to two weeks before thawing and assessment.

Freezing rates.

The rates of semen freezing were characterised in 0.25ml straws frozen at the different heights used in experiment 1, and also in the 0.5ml straws and 0.1. 0.2 and 0.4 ml pellets compared in experiment 4. This was achieved by placing a thermocouple probe (Digisense, Cole-Palmer, Chicago, with type K probe) into the straws at 5°C before placement on the freezing platform, or into the centre of the pellets held on dry ice. The time taken for the completion of crystallisation of the semen in straws frozen on the 3 freezing platform types used in experiment 2, was also recorded.

Assessment.

These semen freezing experiments were assessed in the laboratory by comparing visual estimates of the percentage of live motile sperm over an incubation period of 6 hours. In the assessment of each experiment, the pellets and straws of frozen semen were thawed in a random order in a water-bath at 37°C.

Pellet frozen semen was thawed by rapid agitation in a dry test tube immersed within the water-bath for approximately one minute. Straw frozen semen was thawed by

direct immersion of straws within the 37°C water-bath for 20-30 seconds. Immediately following thawing, the straws were rapidly wiped dry with tissues, and both ends cut to release the straw contents into clean dry test tubes. Thawed semen was transferred to test tube racks in 37°C water baths for incubation.

All semen to be assessed within each experiment was thawed at the same time, over as short a time-span as possible (approximately 20 minutes depending on the size of the experiments). Assessment was begun once all semen had been thawed, at time 0 (the first assessment) and at intervals of two hours thereafter (at 2, 4, and 6 hours).

The samples of semen for assessment were prepared independently of the assessor, initially in a random order that was subsequently repeated at each assessment time. The test tubes containing the aliquots of frozen-thawed semen were shaken to fully suspend the spermatozoa, and a droplet removed with a warmed pipette and deposited on a clean microscope slide and rapidly covered with coverslip. Both slide and coverslip were pre-warmed to avoid temperature shock. The percentage of live motile spermatozoa was then visually estimated to the nearest 5% under a 40x objective on a warm stage at 37°C.

This procedure of slide preparation was repeated at each time of assessment. Between assessments the test tubes were covered with foil to limit evaporative desiccation of the small volumes of frozen-thawed semen. The semen was incubated under normal atmospheric conditions in a clean laboratory environment and under fluorescent lighting. All glassware was washed in pyroneg and thoroughly rinsed with distiled water and dried before use.

Analysis.

All of the semen freezing experiments were analysed using Genstat IV. The data was subjected to an arcsine transformation and analysis of variance conducted on the factorial treatment design. The repeat measure component involved in the assessment of the same experimental units at each incubation time was accounted for, with the latter being a sub-plot in a split plot design. Where significant interactions were determined between buck identity (ie. the replicate) and an experimental factor, the interaction mean square was used to test for factor significance.

Results.

Experiment 1.

The mean % of live spermatozoa for each combination of height of vapour freezing and time of exposure to the vapour, is illustrated in figure 1, averaged over time of incubation and over the four replicates (bucks).

The % of post-thaw motile spermatozoa was affected by both the height of straw freezing in the liquid nitrogen vapour and time of exposure to the vapour freezing, and there was a significant interaction ($P<0.001$) between these two factors. Time of incubation and the identity of the buck also significantly ($P<0.001$) effected post-thaw % live sperm.

Means over all treatment combinations and the full period of incubation, indicated that a freezing rack height of 4cm and a vapour freezing time of 270 seconds gave the best post-thaw % of live spermatozoa. Indeed this combination of treatments also gave the best individual result.

At the 0.25, 1 and 4cm freezing heights, the greatest increases in % live sperm were recorded between vapour freezing times of 10 and 30 seconds, with differences between times greater than 30 seconds being comparatively small. In contrast, at the freezing height of 16cm freezing times of 10 to 90 seconds gave uniformly poor post-thaw recoveries of live spermatozoa. The mean % of motile sperm increase dramatically between 90 and 270 seconds, after which time the results of freezing at this height were comparable to the other freezing heights.

Post-thaw % live sperm was poor at 10 seconds regardless of freezing height, but for 30 seconds and greater the results after freezing at 4cm were better than at all three other heights.

Experiment 2.

The type of straw (0.25 or 0.5ml), the temperature of straw filling and the type of freezing platform all affected post-thaw % live sperm.

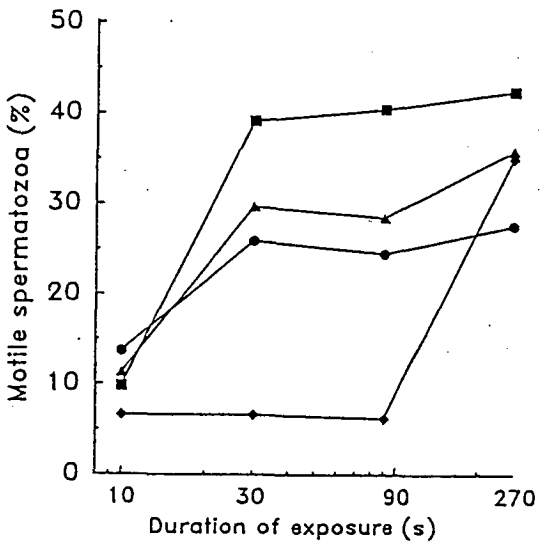

Fig. 1. Experiment 1: Effect of straw height in vapour above liquid nitrogen level (● 0·25 cm; ▲ 1 cm; ■ 4 cm; ◆ 16 cm) and duration of exposure to liquid nitrogen vapour on the mean percentage of motile spermatozoa during 6 h post-thawing incubation.

Table 4.3 shows the mean % of post-thaw live sperm for 0.25 and 0.5ml straws filled at semen temperatures of 5, 22 and 30°C averaged across time of assessment. There was an interaction ($P<0.05$) between each of these factors. Whilst the mean % live sperm was similar for both the straw types over all treatment combinations, the responses to filling temperature differed. The best results for both straw volumes were recorded with straw filling at 30°C, but the poorest results were with filling at 5°C for 0.25ml straws and at 22°C for 0.5ml straws.

In addition to producing the highest initial (Time=0) % of motile sperm, straws filled at 30°C also displayed a slower rate of decline in the % live sperm across the incubation period. Filling at 5°C and 22°C gave the greatest declines in live sperm with incubation for 0.25 and 0.5ml straws respectively.

There was also a significant interaction ($P<0.001$) between the type of freezing platform and the time of incubation (table 4.4). The multi-point contact of the straws with the wire mesh and the perforated metal plate gave better mean post-thaw % live sperm than the two point contact offered by the wire frame. This was a result of poorer initial recoveries after freezing on the two point contact frame, and also of a greater rate of decline in the % of live sperm with incubation. The semen frozen on the wire mesh and perforated plate responded similarly to incubation time.

Again there was a significant ($P<0.05$) effect of the individual bucks on the post-thaw recovery and survival of the frozen spermatozoa.

Experiment 3.

In this experiment the % post-thaw live motile sperm was effected by a significant interaction ($P<0.01$) between the four media described in table 4.2 and the rate of dilution of the semen. The means across the full six hours of incubation (four times) are give in table 4.5.

Over all the dilution media used, dilution at a rate of 1:23 gave the greatest mean % of motile sperm. The 1:2 dilution media gave the best results over all rates of dilution.

Table 4.3 Experiment 2: The effects of temperature of straw filling and straw volume on the post thaw survival of incubated spermatozoa.

Straw volume and Straw filling Temperature (°C)	Motile spermatozoa (%) after incubation for:				
	0hr	2hr	4hr	6hr	Mean
0.25ml straws					
5	28.2	25.4	24.2	23.9	25.4
22	30.8	31.4	28.7	28.5	29.8
30	33.1	34.2	32.5	31.7	32.9
Mean	30.7	30.3	28.5	28.0	29.4
0.5ml straws					
5	32.8	34.1	32.6	27.9	31.9
22	30.3	28.9	25.8	23.9	27.2
30	33.4	34.8	33.4	32.1	33.4
Mean	32.2	32.6	30.6	28.0	30.8
Overall mean	31.4	31.5	29.5	28.0	30.1

Table 4.4 Experiment 2: The effects type of freezing rack on the post thaw survival of incubated spermatozoa.

Type of freezing rack	Motile spermatozoa (%) after incubation for:				
	0hr	2hr	4hr	6hr	Mean
Two point	29.7	28.6	27.4	25.0	27.7
Wire grid	32.2	31.6	30.6	29.7	31.0
Perforated plate	32.5	34.2	30.7	29.4	31.7
Mean	31.4	31.5	29.5	28.0	30.1

Table 4.5 Experiment 3: The relationship between dilution rate and diluent type on the mean percentage of motile spermatozoa after post-thawing incubation for 6 hours.

Type of diluent	Mean percentage of motile spermatozoa by dilution rate (semen:diluent)				
	1:2	1:5	1:11	1:23	Mean
M1:2	33.4 (250)	37.7 (313)	37.3 (344)	36.3 (359)	36.2
M1:5	24.9 (200)	35.3 (250)	39.4 (275)	42.3 (288)	35.5
M1:11	13.7 (182)	32.5 (227)	38.4 (250)	38.8 (261)	30.9
M1:23	9.7 (174)	31.4 (217)	34.5 (239)	38.0 (250)	28.4
Mean	20.4	34.2	37.4	38.9	32.7

Table 4.6 Experiment 4: The effects of freezing process and dilution rate on the survival of incubated spermatozoa.

Type freezing or dilution rate (semen:diluent)	Mean percentage of motile spermatozoa by dilution rate				
	0hr	2hr	4hr	6hr	Mean
0.25 ml straw	35.8	33.7	33.3	30.3	33.3
0.50 ml straw	37.6	35.6	34.0	29.8	34.3
0.10 ml pellet	39.6	38.8	38.1	36.0	38.1
0.20 ml pellet	40.1	40.0	38.8	37.4	39.1
0.40 ml pellet	41.8	39.9	39.9	38.3	40.0
1:2	38.3	37.7	37.7	35.9	37.4
1:5	39.0	38.5	37.3	35.0	37.5
1:11	40.1	38.3	37.4	34.0	37.4
1:23	38.5	36.0	34.9	32.6	35.5

At dilution rates of 1:2 and 1:5, the maximum % of post-thaw motile sperm was recorded after dilution with media M1:2, whilst media M1:5 gave the best results at dilution rates of 1:11 and 1:23.

The greatest % of post-thaw motile sperm was achieved at a dilution rate of 1:23 with media M1:5, whilst the poorest result was recorded after dilution at 1:2 with media M1:23. Indeed dilution at 1:2 with any media other than M1:2 produced especially poor results, and the range in results across the various media used was greater for dilution at 1:2 than at any other dilution rate.

As with the other experiments, the effect of buck on the % of post-thaw live sperm was significant ($P<0.01$).

Experiment 4.

The results of the comparison of semen frozen in the two straw types and three pellet sizes after dilution at rates of 1:2, 1:5, 1:11 and 1:23 with media M1:2, M1:5, M1:11 and M1:23 respectively, are shown in table 4.6.

Across the pellets and straws used to package frozen semen in this study, pellet frozen semen gave better recovery and survival of sperm than semen frozen in straws ($P<0.001$). Post % of motile sperm increased slightly with increasing pellet size ($P<0.05$), but there was no difference with straw volume. There was also an interaction ($P<0.01$) between freezing method and incubation time. The decline in cell survival with incubation was greater for the straws than pellets, and was greater for the 0.5ml than 0.25ml straws, whilst there was little difference between the three pellet sizes during incubation.

Dilution rate apparently had little effect, with mean % live sperm only being significantly reduced after dilution at 1:23 ($P<0.05$). However, the rate of decline in the % live sperm with incubation was greater for semen diluted at the higher rates of 1:11 and 1:23 (interaction $P<0.05$).

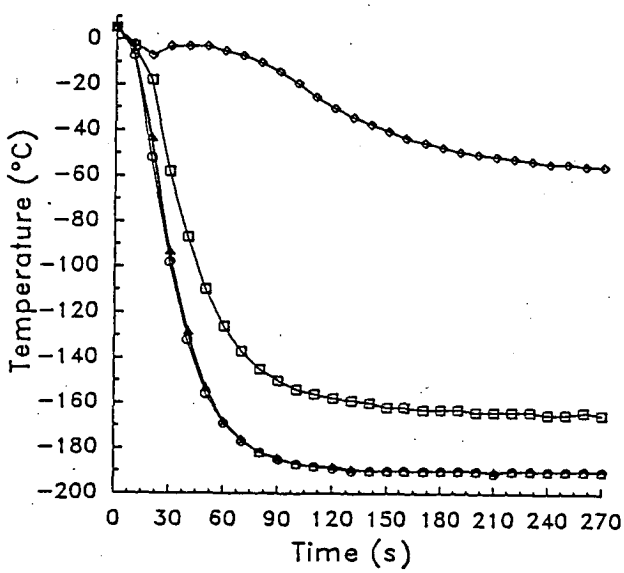

Fig. 2. Experiment 1: Temperature of semen in the centre of 0·25 mL straws placed on a pre-cooled wire grid in vapour at different heights above liquid nitrogen level. (○ 0·25 cm; △ 1 cm; □ 4 cm; ◇ 16 cm.)

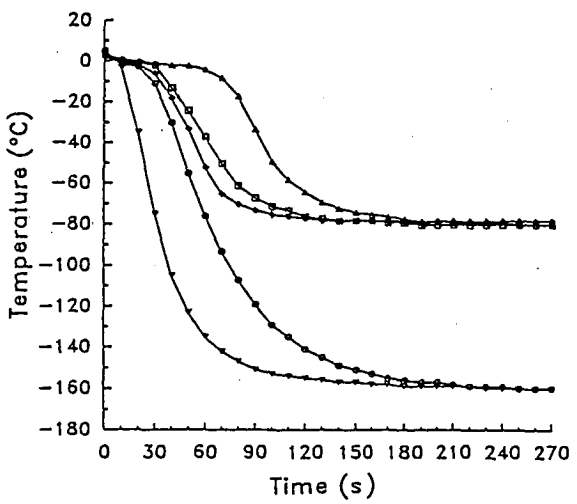

Fig. 3. Experiment 4: Temperature of semen in the centre of straws frozen in vapour 4 cm above liquid nitrogen level and in pellets frozen on dry ice. (▽ 0·25 mL straw; ○ 0·50 mL straw; ◇ 0·10 mL pellet; □ 0·20 mL pellet; △ 0·40 mL pellet.)

Freezing Rates.

The freezing curves for 0.25ml straws frozen at heights of 0.25, 1, 4 and 16cm are shown in figure 2. These varied in both the rate of decline in temperature and also the final plateau temperature. Straws frozen at 0.25 and 1cm had very similar rates of freezing and declined to a temperature of -190°C in 240 seconds. The temperature of straws frozen at 4cm reduced to -165°C in 210 seconds whilst straws frozen at 16cm cooled at a much slower rate and reached a final temperature of only -55°C after 270 seconds. A noticeable feature of the latter cooling curve, not observed at lower freezing heights, was an increase in temperature after the initial sub-zero decrease in the temperature of the straws.

Figure 3 shows the freezing curves recorded for the straws and pellets compared in experiment 4. Whilst the 0.25 and 0.5 ml straws frozen under the same conditions reached the same plateau temperature, the initial rate of cooling was slower for the larger straws. The pellets froze at much slower rate compared to the straws, with increasing volume similarly reducing the rate of cooling. After two minutes of vapour freezing (the point at which the straws were plunged into liquid nitrogen), the 0.25ml and 0.5ml straws reached temperatures of -155°C and -141°C In contrast, the 0.1ml, 0.2ml and 0.4ml pellets reached temperatures of -78°C -76°C and -64°C respectively after two minutes of freezing on dry ice. These pellets equilibrated to the temperature of dry ice after approximately 2, 2.5 and 3.5 minutes after dropping on to the dry ice block.

The visual observations on the time taken to effect crystallisation in the straws frozen on the three freezing racks used in experiment 2 are presented in table 4.7. Both the 0.25ml and 0.5ml straws displayed a significant ($P<0.001$) decrease in the time taken for crystallisation to occur with greater contact of the straws with the freezing surface.

Discussion.

This series of experiments examined the effects of a variety of factors on the post-thaw revival and subsequent survival during incubation, of goat spermatozoa frozen in straws. The % of live spermatozoa was sensitive to the method of processing and handling the semen prior to and during freezing. The results illustrate the importance of paying attention to a number of different aspects of the method of semen processing, when attempting to achieve higher percentages of live sperm.

The basic technique employed was a simple one that is comparatively rapid and may be readily applied in the absence of sophisticated equipment. In brief, neat semen was diluted in a single step with an egg-yolk based diluent after collection, and then frozen in static liquid nitrogen vapour, prior to complete immersion in liquid nitrogen.

Cooling Rate and Temperature of Vapour Freezing.

The survival of the spermatozoa during freezing in 0.25ml straws was dependent on both the rate of freezing in the liquid nitrogen vapour and also the temperature of the straws at immersion in the liquid nitrogen. This is indicated by the results of experiment 1, where there was an interactive effect of the height of straw freezing in the vapour above the liquid nitrogen reservoir and the time of exposure to that vapour.

Examination of the freezing curves of the straws, (illustrated in figure 2) shows that the rates of freezing were similar for straws held at 0.25 and 1cm in the vapour, whilst differences were obvious when the straws were held at either 4 or 16cm. The final temperature of the straws at immersion within the liquid nitrogen was determined by both the height of the straws in the vapour and the time of exposure (up to a plateau or equilibration point). For straws held at 4cm or lower, the decline in temperature past 90 seconds of exposure to the vapour was small, whilst straws at 16cm continued to exhibit a decline in temperature well past this point.

This latter point probably explains the comparatively small increases in the % post-thaw live sperm after 90 seconds of exposure for straws held at 0.25, 1 and 4cm. In contrast straws at 16cm displayed a substantial increase in the % of live sperm between 90 and 270 seconds of exposure, reflecting the continued decline in temperature of the straws.

Examining the survival of sperm from straws frozen after different times within each height of freezing, critical temperatures prior to plunging may be deduced. From the temperatures after 30 seconds of exposure at heights 0.25, 1 and 4cm, and after 270 seconds at 16cm, it appears that straw temperatures of between at least -95°C and -55°C are required for any significant rates of sperm survival. Higher temperatures prior to plunging, as illustrated by the uniformly poor results obtained at 10 seconds exposure and at up to 90 seconds at a height of 16cm, were detrimental to sperm survival. This may be related to the temperature change that occurs in the straws as

Table 4.7 Experiment 4: Freezing times (seconds) observed for 0.25ml and 0.5ml straws on three types of freezing rack.

Freezing rack	0.25 ml straw	0.5ml straw
Two-point	8.1 ± 0.38	12.2 ± 0.61
Wire grid	6.8 ± 0.19	11.4 ± 0.47
Perforated metal plate	6.3 ± 0.20	9.6 ± 0.50

they are plunged into the liquid nitrogen. Some improvement in the % post-thaw motile sperm was observed with reductions in temperature prior to plunging after longer than 30 second exposure to the vapour freezing.

From the results shown in figure 1, it is apparent that sperm survival was greatest when the straws were frozen at a height of 4cm. At this height straws were subject to a rapid rate of cooling, but one slower than at heights of 0.25 and 1cm. Rapid cooling of straws during the freezing process has been associated improved post-thaw recovery in ram semen, although reportedly over a wide range from 10-100 c/minute (Fiser et al., 1886; Fiser and Fairfull, 1986). Indeed, at high rates of freezing bull semen, Robbins et al. (1976) regarded freezing rate as insignificant.

The straws frozen at 16cm cooled at a much slower rate, and only achieved a comparable survival rate at the plateau temperature for that height of freezing. Thus whilst on average this slower rate of freezing appeared poor in comparison to the faster rates of freezing, this may have been due to the overriding effects of the critical temperature at immersion. In view of this incomplete examination of the effect of the slowest cooling rate, it may be worth investigating cooling to temperatures below the final temperature reached at 16cm, at a similar rate of cooling. However, this would require the use of another type of freezing unit than the static vapour chamber used here, such as a programmable freezer.

Freezing Rack.

The freezing process was also influenced by the type of freezing rack. Ice crystallisation was initiated within the straws at the point or points of contact between the semen filled straws and the pre-cooled freezing platforms. As a result, the pattern of crystallisation along the full length of the straws varied with the number of contact sites. On the 2-point freezing frame, crystallisation was initiated at the extremes of the straws and spread along the length of the straw to meet in the centre. This pattern was repeated at each of the multiple sites of contact along the straws frozen on the wire mesh and metal plate. Consequently the time taken for complete crystallisation of the semen within the straws was least for the metal plate, followed by the wire mesh and then the 2-point frame.

The average % of motile sperm was less for straws frozen on the wire frame than either of the other freezing platforms. This result was manifest as a lower initial

recovery and a higher subsequent rate of decline in sperm survival with incubation. This may be explained in terms of less cellular disruption and damage with the more rapid crystallisation along the length of the straws, where there were more points of contact with the freezing rack. Fiser and Fairfull (1986) note that during rapid freezing, smaller less harmful ice crystals may form. Alternatively, the more rapid crystallisation may increase the rates of dissipation of the latent heat associated with the phase change, reducing the effect of any temperature fluctuation in the semen.

Temperature at Straw Filling.

The effect of the temperature of straw filling varied with straw capacity. The lowest average % of motile sperm was recorded after filling 0.25ml straws at 5°C and 0.5ml straws at 22°C. The best results were obtained after filling at 30°C for both straw capacities. These results may reflect a reduced opportunity for temperature fluctuations in the semen prior to freezing, if straws are filled at 30°C rather than after the commencement of cooling. Such an effect would not apply where semen is cooled and packaged in controlled temperature cool-rooms. However, this observation may be important to a simple method that does not require expensive equipment.

Dilution Rate and Media Concentration.

The factorial combinations of dilution rate and dilution media of experiment 3 indicated that a simple adjustment of the concentrations of the diluent constituents in the media on the basis of dilution rate to give a recommended final concentration in the diluted semen (Salamon and Ritar, 1982), did not necessarily give the best results. The most appropriate dilution media varied with dilution rate. The highest average % of live sperm diluted at a rate of 1:2 and 1:5 was achieved with media M1:2, whilst media M1:11 was best for dilution at rates of 1:11 and 1:23. In general hypertonic media (M1:2 and M1:5) were better tolerated than hypotonic media (M1:11 and M1:23).

Although the ranges of concentrations of media constituents in the diluted semen were not repeated over the four rates of dilution, it appeared that the sperm cells were sensitive to concentrations lower than those illustrated in table 4.1. In contrast, increases in the concentration of media constituents in the diluted semen were either beneficial or at least not detrimental. It appears possible that the recommended

concentration for this tris-based media (in the diluted form) may be increased with some benefit.

The differences in the average % of motile sperm between dilution rates, probably reflect the differences in the tolerance to higher or lower media concentrations. The semen diluted at rates of 1:23 and 1:11 performed better since they were not subject to lower media concentrations. Even so, there did appear to be a positive effect of increasing rate of dilution on sperm survival when equivalent media concentration comparisons were made. It would be of interest to further examine this comparison across a range of concentrations.

These differences in the effect of dilution rate, after semen was diluted with media to achieve the final concentrations of media constituents shown in table 4.1, were not repeated in the treatment averages of experiment 4 (table 4.6). Here, the averages across all pellet and straw types indicated no differences between dilution rates of 1:2, 1:5 or 1:11, although dilution at the highest rate of 1:23 gave significantly ($P<0.05$) lower % motile sperm.

Straws and Pellets.

In the comparison of semen frozen as pellets on dry ice or as straws frozen in liquid nitrogen vapour at a height of 4cm, the pellet frozen semen gave a greater % of surviving sperm cells after thawing and incubation. Such differences between pellets and straws have been previously reported for ram semen (Hunton et al., 1987; Hunton, 1988; Maxwell et al., 1980). Within each type of packaging of the frozen semen, the volume of the straws or pellets had little effect on the post-thaw recovery and survival of spermatozoa. This is in agreement with reports of varying pellet volume (Lightfoot and Salamon, 1969; Salamon, 1970, Salamon, 1973). This result is interesting since pellet or straw volume had quite a large effect on the measured cooling curves (figure.2). Volume varied the rate of cooling during the freezing process and also the temperature at which the semen as plunged into liquid nitrogen.

This indicates that differences in rate of cooling and temperature at plunging within the range imposed by these differences in volume, were limited in their effects on the recovery and survival of the sperm cells after freeze-thawing. There was a trend of better results at the slower rates of cooling within straws or pellets, and the significantly better results from pellets compared with straws were of course

associated with a lower rate of cooling. However, the differences in sperm survival between the straws and pellets were comparatively small, when considered in the context of the differences in rate of cooling. This tends to the support the observation of experiment 1, in which the temperature at which the semen was plunged into the liquid nitrogen appeared more important than the rate of cooling to that temperature.

However, even small differences in the % of motile live sperm may be significant in insemination programmes, if it means the difference between the genetic gains warranting the expense of insemination. Certainly these experiments indicate that once semen has been cooled below some threshold temperature before plunging, the slower rates of freezing may be beneficial.

In spite of the differences between pellets and straws illustrated in experiment 4, the simple technique for freezing caprine semen in polyvinyl straws used here gave comparatively good results in terms of the % of post-thaw motile sperm. Certainly the results from the straw frozen semen indicated that inclusion in insemination trials was warranted, in view of the potential benefits offered by packaging frozen semen in straws.

This series of experiments indicates that acceptable post-thaw recoveries of motile sperm may be achieved after freezing caprine semen in straws, using a simple one-step method of dilution and freezing, after dilution with a medium containing egg yolk. The importance of adjusting media concentration to the rate of dilution is illustrated, and some effects of freezing rate and temperature at immersion in liquid nitrogen observed.

CHAPTER 5.

ARTIFICIAL INSEMINATION EXPERIMENTS WITH CASHMERE GOATS.

5. ARTIFICIAL INSEMINATION EXPERIMENTS WITH CASHMERE GOATS.

Introduction.

The application of artificial insemination, particularly in conjunction with the use of frozen semen, can offer a range of benefits to breeding programmes (Evans and Maxwell, 1987). Because of the relative simplicity of the techniques used, artificial insemination (AI) technology probably allows for the widest artificial dissemination of desired genotypes. This is evident the widespread and routine adoption of AI, and appreciation of the increased genetic impact of improved sires, in the commercial breeding of other species such as bovines (Amann and Schanbacher, 1983; Betteridge, 1986; Corteel, 1981). AI increases the efficient utilisation of male genotypes, whilst embryo transfer increases the reproductive impact of the female, although the process is more complex (Maxwell, 1985). The use of frozen semen at insemination offers a wider choice of male genotypes as semen donors, and making available otherwise inaccessible sires, further adding to the potential for genetic advance.

The acceptance and adoption of artificial insemination techniques depends in part on the fertility rates that can be achieved. This results from the combination of the quality of the inseminate and the efficacy of the method of insemination.

The following four experiments were conducted to investigate the effects on fertility and fecundity of the method of processing and freezing sperm, the method of oestrus synchronisation, the number of motile spermatozoa in the inseminate, the time of insemination relative to ovulation and two methods of insemination. Account was also taken of the age of the females inseminated.

Materials and Methods.

Experimental Design.

Experiment 1.

The preparation of females for insemination, the time of insemination and sperm dose were examined for effects on the fertility and fecundity of adult does after cervical

insemination with pellet frozen semen, in this 2x2x3 factorial experiment. Oestrus was synchronised by intravaginal treatment with either CIDR's or sponges and insemination conducted either before or after the estimated time of ovulation (respectively 45 and 55 hours after CIDR removal, and 55 and 65 hours after sponge removal). Does were inseminated with 80, 120 or 160 million motile sperm, from semen diluted at a rate of 1:2 (semen:diluent) and frozen on dry ice as pellets.

Experiment 2.

In this 2x2x2x3 factorial experiment, both adult does and maiden hogget does were inseminated laparoscopically. The same intravaginal treatments and times of insemination described in experiment 1 were used, and the semen was also diluted and frozen in the same manner. However, the inseminate doses were adjusted to 15, 30 and 60 million motile sperm.

Experiment 3.

This 3x2x3x2x3 factorial experiment examined the effect of insemination regimen, as well as method of semen processing and freezing, on the fertility and fecundity of kid does, maiden hogget does and adult does after laparoscopic insemination. Insemination of these three age groups was conducted either 39 or 45 hours after CIDR removal. The semen used at insemination was diluted at rates of 1:2, 1:5 and 1:11 and frozen in either pellets or straws, and the inseminate dose consisted of either 5, 10 or 20 million motile sperm.

Experiment 4.

Here a simple 2x3 factorial experiment further investigated the effects of semen freezing method and sperm number in the inseminate, on the fertility of maiden hogget does that were laparoscopically inseminated Semen was diluted at a rate of 1:23 and frozen either as pellets or straws. The does were inseminated with 1, 5 or 25 million motile sperm.

Semen Collection and Freezing.

Semen was collected on an artificial breeding station at Hadspen, Tasmania, during the period of January to April in each year of insemination. The donor bucks were

five adult (18 months or older) Cashmere bucks in experiments 1 and 2, and 2 adult and 4 young (6 to 8 months) bucks in experiments 3 and 4. In each of the experiments, the semen was collected using an artificial vagina (AV), after the bucks were presented with a doe induced to display oestrus and restrained in a head bale.

During the collection period, the individual bucks were collected up to three times per day on three days in each week (Monday, Wednesday and Friday). Teaser does were prepared by removal of a CIDR and administration of 0.5mg of oestradiol benzoate 36 hours prior to the start of collection.

The semen used for insemination was collected and processed according to the methods described in chapter 4. After collection, the semen deposited in the graduated tulip glass of the AV was protected from light and cold shock whilst being transferred as rapidly as possible to a 30°C water-bath for assessment and processing.

At 30°C the semen was assessed for volume (ml), colour (white to bright yellow) and concentration of the ejaculate (score 1 to 5). Motility, or the degree of wave motion in the ejaculate, was also assessed (score 1 to 5) by observing a droplet of semen on a microscope warm stage (37°C) using a low magnification objective. Abnormal ejaculates, such as those either excessively dilute or displaying poor initial motility, were rejected prior to further processing. However, despite the intensive periods of collection before each insemination trial, very few ejaculates were rejected on these grounds.

After collection, the semen used in experiments 1 and 2 was diluted at a rate of 1:2 with the appropriate diluent illustrated in table 4.2. This gave the concentrations of media constituents in the diluted semen illustrated in table 4.1. The diluted semen was cooled to 5°C in test tubes over a period of 1.5 to 2 hours, and frozen as pellets on dry ice before plunging into liquid nitrogen.

For experiment 3, the collected semen was diluted at rates of 1:2, 1:5 and 1:23 using the appropriate media from table 4.2. Thus there were equivalent concentrations of media constituents in the diluted semen at each dilution rate. Individual ejaculates were diluted at one rate only, and split between straws and pellets. The low ejaculate volumes and six combinations of processing treatment meant that it was impracticable to further divide the ejaculates between the treatments during the course of the routine collection for insemination. Instead, alternate collection days were assigned to each dilution rate.

After dilution of the semen, 0.25ml poly vinyl straws (I.M.V. L'Aigle, France) were filled by aspiration at 30°C and heat sealed. These were then immersed in a water jacket at the same temperature, and cooled to 5°C over a period of 1.5 to 2 hours. At 5°C the straws were dried and placed onto a perforated metal plate 4cm above a liquid nitrogen reservoir in the freezing chamber described in chapter 4 (appendix 1). The straws were allowed to freeze in the sealed chamber for 1.5 to 2 minutes before plunging into liquid nitrogen. The pellet frozen semen was similarly cooled to 5°C in test tubes and frozen on dry ice, prior to plunging into liquid nitrogen.

The semen frozen in 0.25 ml straws for experiment 4 was processed as in experiment 3, with the exception that the semen was diluted only at a rate of 1:23 with the appropriate diluent from table 4.2.

All frozen semen was transferred to a liquid nitrogen container for storage of between 1 and 12 weeks prior to insemination. In this time a pellet or straw from each collection batch was assessed for initial % post-thaw live motile sperm. Pellets were thawed in a 37°C water bath by rapid agitation in a dry test tube until melted. Straws were directly immersed in the water bath and agitated for approximately 15 seconds before drying and release of contents into a warmed dry test tube. Only collection batches exhibiting at least 35% post-thaw live sperm in the sampled pellet or straw, were retained for insemination. After this assessment, the pellets and straws were stored in pooled batches by buck and processing treatment, in the liquid nitrogen container.

Preparation of Females for Insemination.

Each insemination experiment was conducted on the "Woolnorth" property near Smithton, Tasmania (40° 45'S, 144° 45'E). Females used for insemination were Australian Cashmere types either imported from mainland Australia (New South Wales) or bred on the property from imported stock. Insemination was conducted only in the breeding season in each year of the trial (experiments 1 and 2, April 1987; experiment 3, March 1988; experiment 4, April 1989). The does were weighed either 2 weeks after insemination (experiments 1 and 2) or at the time of intravaginal device insertion (experiments 3 and 4) and the live-weights for each age group (maiden kids, maiden hoggets and primiparous and multiparous adults) are presented in table 5.1.

Oestrus was synchronised by intravaginal treatment of the does with either Chronogest sponges (45 mg fluorogestone acetate, Intervet Pty Ltd, Australia; experiments 1 and 2) or with type G CIDR devices (330 mg progesterone, AHI plastic Moulding Co., New Zealand; experiments 1, 2, 3 and 4) for between 15 and 20 days (depending on the day of insemination). At the time of intravaginal device removal, the does were injected intramuscularly with 200 iu PMSG (Folligon, Intervet Pty Ltd, Australia, Experiments 1 and 2; Pregnecol, Heriot Agvet Pty Ltd, Melbourne, Experiments 3 and 4).

At the time of inserting intravaginal devices, testosterone treated wethers were introduced to the does at a ratio of 1:20 (wethers:does). Wethers were treated at this time with 150 mg testosterone enanthate (2 ml Tesgro, Merck, Sharp and Dome Pty Ltd, Australia) injected intramuscularly. This treatment was repeated at the time of intravaginal device removal, and was designed to avoid problems associated with the induction of short oestrous cycles in does following the initial introduction of males to the herd (Chemineau, 1987). With the introduction of the back-up males 15 days after insemination, the wethers were removed.

Insemination Procedure.

Prior to insemination, the does were housed off-pasture over-night. For both cervical and laparoscopic insemination, the does were intravenously injected with 3 mg of xylazine hydrochloride (Rompun; Bayer Leverkussen, Germany) 10 to 20 minutes before insemination.

With cervical insemination, the hind legs of the does were held over a padded rail and the body held at a 45° angle. Semen was deposited as deeply as possible within the cervical canal or the opening of the uterus, using a flexible plastic inseminating pipette with an angled tip. During insemination the cervical canal was exposed using a duck-bill speculum with affixed light source. The depth of cervical insemination was recorded.

Laparoscopically inseminated does were subject to simple laparotomy after being restrained on inseminating cradles and their belly hair removed. The semen was deposited into the uterine lumen, half-way between the body of the uterus and the utero-tubule junction, using a sharp tipped glass inseminating pipette. Half of the

total number of motile sperm in the inseminate dose was deposited in each uterine horn.

With both cervical and laparoscopic insemination, the dose of inseminate was regulated using calibrated syringes. Inseminate doses were separated in the inseminating pipettes by air buffers on either side, to facilitate full expulsion of the inseminate. Between each insemination, the pipettes were rinsed with freezing diluent minus the glycerol and egg yolk.

Insemination was conducted near the time of ovulation as estimated from previous determinations of ovulation in does after synchronisation with both sponge and CIDR treatment (Ritar et al., 1984, 1989). The females were randomly allocated within each age group, to the various experimental treatments. Semen from the different bucks used in each experiment was evenly represented across treatments within each experiment.

Semen was thawed in a water-bath at 37°C by agitation of 4 pellets in each test tube until melted, or by direct immersion of straws for 15 to 30 seconds. A sample of each thawed batch of semen was assessed microscopically at insemination, to allow detection of any mishap during semen handling (eg. failure of water-bath thermostat). The semen was held at 30°C for up to 15 minutes prior to use. The sperm dose was manipulated by adjusting the volume of the inseminate according to the rate of dilution. It was assumed that 40% of the sperm recovered after thawing, from an average concentration of sperm in the original ejaculate.

Data Collection.

In experiments 1, 2 and 3, fertility (assessed as pregnant or not to the AI) and fecundity (the number of foetuses) was determined from ultrasonic examination of the does 75 to 80 days after insemination. Testosterone treated wethers and entire bucks fitted with sire harnesses and crayons were also used to monitor the returns to service up to day 57 in experiments 1 and 2. In experiment 4, the fertility data was determined from udder examination 160 days after insemination, whilst fecundity could not be accurately measured.

Statistical Analysis of Data.

Fertility results were tested for statistical significance by X2 analysis, whilst fecundity data was subjected to analysis of variance. Where results of post-thaw recovery of sperm are presented (% live), differences were examined using the students t-test.

RESULTS.

Experiment 1.

Results for the cervical insemination of adult does with pellet frozen semen diluted at a rate of 1:2 are presented in table 5.2. The results are pooled across the five bucks used in the experiment, there being no significant differences between sires in the non-return rates, fertility and fecundity results.

No significant differences were recorded in fertility between the intravaginal treatments with sponges or CIDR's. Time of insemination relative to the cessation of intravaginal treatment did however have a effect on fertility rates. Does inseminated prior to the estimated time of ovulation (45 and 55 hours for CIDR's and Sponges respectively) had significantly ($P<0.05$) higher fertility rates than those inseminated afterwards. This difference was greater for does treated with CIDR's (49.0% v 32.0%, $P<0.05$) than sponges (38.5% v 36.3%, not significant), although there was no significant interaction recorded between intravaginal treatment and time of insemination. No significant differences were observed between the numbers of motile sperm in the inseminate dose. In contrast to the fertility data recorded from ultrasound observations, the non-return rates did not differ significantly with any of the main effects.

Both the non-return and fertility rates varied significantly ($P<0.05$ and $P<0.01$, respectively) with the depth of deposition of the inseminate in the reproductive tract. Fertility rates increased as the inseminate was deposited from 1 cm within the cervix, to between 1.5 and 3 cm within the cervix, or within the uterus. Fecundity was also significantly lower ($P<0.05$) when the inseminate was released at 1 cm within the cervix compared to deeper insemination. However, depth of cervical insemination was an observation at the time of insemination and not an experimental treatment. As a consequence, the numbers of animals within each depth range differed widely, with

Table 5.1 Mean live-weight (kg) and range of maiden kids, maiden hoggets and adult does used in each insemination experiment (n).

Experiment	Maiden Kid does (6-8 months old)	Maiden Hogget does (18 months old)	Adult does (2.5-7.5 years old)
Expt 1 (cervical)			31.8±0.23 (398) 22-45
Expt 2 (laparoscopic)		27.9±0.29 (203) 18-39	31.4±0.25 (357) 22-47
Expt 3 (laparoscopic)	17.8±0.11 (318) 14-26	25.6±0.45 (65) 19-38	31.1±0.21 (479) 21-47
Expt 4 (laparoscopic)		28.4±0.20 (254)	

Table 5.2 Experiment 1: Reproductive performance of does after cervical insemination with pellet frozen semen diluted at a ratio of 1:2 (semen:diluent).

Main Treatment	No. does inseminated	% does non-return	% does pregnant	Fecundity (fetus/pregnant doe)
Intravaginal treatment				
CIDR	204	46.6	40.7	1.75±0.078
Sponge	198	47.5	37.4	1.92±0.08
Time of insemination				
before ovulation	200	48.5	44.0	1.89±0.07
after ovulation	202	45.5	34.2	1.75±0.09
No. motile speratozoa ($x10^6$ sperm)				
80 (dose=0.2ml)	131	52.7	42.0	1.84±0.10
120 (dose=0.3ml)	134	41.8	34.3	1.83±0.10
160 (dose=0.4ml)	137	46.7	40.9	1.82±0.09
Depth of insemination				
To 1.0cm in cervix	69	31.9	23.2	1.56±0.16
1.5-3.0cm in cervix	79	46.8	34.2	1.82±0.10
Into uterus	254	51.2	44.9	1.87±0.07
Totals and means	402	47.1	39.1	1.82±0.06

17.2% inseminated up to 1 cm within the cervix, 19.7% between 1.5 and 3 cm in the cervix and 63.2% of the animals being inseminated within the uterus.

The incidence of short cycles following insemination, as detected by the harnessed testosterone treated wethers up to day 15 after insemination was 6.7% (27/402). Over all does inseminated, the fertility rate to the AI assessed from the ultrasonic scanning was 8 % less than the rate of non-return to service.

Experiment 2.

As in experiment 1, the non-return rates, fertility rates and fecundities of laparoscopically inseminated maiden hoggets and adult does did not significantly vary between the individual bucks used. The results, pooled across all bucks used, are presented in table 5.3.

No significant differences were observed in fertility rates between the maiden hoggets and the adult does. Similarly there were no significant differences in fertility between does intravaginally treated with CIDR's or sponges. However, time of insemination relative to the estimated time of ovulation did significantly ($P<0.01$) effect fertility after insemination, with higher fertility recorded with insemination prior to this time rather than afterwards. No interaction of time of insemination with the type of intravaginal treatment was observed. No difference in fertility was detected with the number of motile sperm in the inseminate, across the range examined here. There were no significant differences in fecundity between treatments.

The rates of non-return to service and the fertility rates recorded by ultrasonic scanning were similar, although the latter were 3.5% lower than the non-return rates. The total incidence of short cycles recorded in the 15 days after insemination was 4.0%, this being similar for both the maiden hoggets and the adult does (9/211 and 14/358 for hoggets and adults respectively).

Experiment 3.

No significant differences in fertility were detected between bucks. The fertility rates assessed by ultrasonic scanning are pooled across sire for the laparoscopically inseminated kids, hoggets and adults in table 5.4.

Table 5.3 Experiment 2: Reproductive performance of does after laparoscopic insemination with pellet frozen semen diluted at a ratio of 1:2 (semen:diluent).

Main Treatment	No. does inseminated	% does non-return	% does pregnant	Fecundity (fetus/pregnant doe)
Age of does				
Maiden (18 months)	221	69.2	63.0	1.73±0.06
Adult (2.5-7.5 years)	358	66.1	64.0	1.82±0.05
Intravaginal treatment				
CIDR	290	68.6	64.5	1.84±0.05
Sponge	279	65.6	62.7	1.73±0.05
Time of insemination				
before ovulation	283	72.4	69.6	1.83±0.05
after ovulation	286	61.9	56.7	1.74±0.05
No. motile speratozoa ($x10^6$sperm)				
15 (dose=0.0375ml)	189	65.6	66.7	1.74±0.07
30 (dose=0.075ml)	189	63.5	58.7	1.80±0.07
60 (dose=0.15ml)	191	72.3	65.4	1.81±0.06
Totals and means	569	67.1	63.6	1.79±0.04

Table 5.4 Experiment 3: Reproductive performance of does after laparoscopic insemination.

Main Treatment	No. does inseminated	% does pregnant	Fecundity (fetus/pregnant doe)
Age of does			
Kid (6-8 months)	318	34.0	1.17±0.04
Maiden (18 months)	65	55.4	1.22±0.07
Adult (2.5-7.5 years)	479	63.9	1.27±0.03
Semen freezing process			
Pellet	439	51.7	1.24±0.03
Straw	423	52.5	1.24±0.03
Dilution rate (semen:diluent)			
1:2	294	52.7	1.29±0.04
1:5	311	50.8	1.19±0.03
1:23	257	52.9	1.24±0.04
Time of insemination (after CIDR removal)			
39hr	479	51.6	1.27±0.03
45hr	383	52.7	1.20±0.03
No. motile speratozoa ($x10^6$sperm)			
5	301	53.8	1.22±0.03
10	285	51.9	1.24±0.04
20	276	50.4	1.26±0.04
Totals and means	862	52.1	1.24±0.02

The age of the does inseminated had a significant effect on fertility. The kid maidens had a significantly lower ($P<0.001$) fertility rate than the combined class of hoggets and adults, with pregnancy rates being 34.0% (108/318) and 62.7% (341/544) respectively. The difference between the fertility of the hoggets and the other age groups was not significant, although this comparison was limited by the small number of hoggets inseminated (n=65). The fecundity of the pregnant does increased significantly ($P<0.05$) with increasing age between the three age groups.

No significant differences in fertility rates were detected between the semen freezing process (pellet or straw), dilution rate of semen, the number of motile sperm in the inseminate and time of insemination relative to CIDR removal. There was however a significant difference ($P<0.05$) in fecundity with time of insemination. Does inseminated 39 hours after CIDR removal had a mean fecundity greater than does inseminated at 45 hours. No significant interactions between treatments were observed.

Samples of semen taken from each freezing batch were thawed and assessed prior to pooling, and the resultant recovery of motile sperm is presented as a mean in table 5.5. Recoveries were similar across dilution rates for straws and pellets.

Experiment 4.

Fertility rates expressed as the percentage of maiden hogget does kidded 160 days after laparoscopic insemination, are presented in table 5.6. No differences in fertility were observed between bucks, and the results presented are means across the four sires used in the insemination programme.

For semen diluted at a rate of 1:23, insemination with approximately 1, 5 and 25 million motile sperm did not give any statistically significant difference in fertility ($P>0.05$), despite a trend of declining fertility with the reduction in the dose of motile sperm. This reduced fertility was most apparent for does inseminated with pellet frozen semen, although there were no significant interactions. There was no significant difference in fertility after insemination with semen frozen by the pellet or straw methods ($P>0.05$).

Post-thaw assessment of semen used for insemination indicated that significantly more sperm recovered motility after pellet freezing than after straw freezing (43.1 ±4.9% and 31.3 ±2.1% respectively; $P<0.05$). Since the dose rates of the motile

Table 5.5 Experiment 3: The recovery of motile sperm (%) after diluting semen at three rates and freezing as straws or pellets

Freezing method	Dilution Rate (semen:diluent)		
	1:2	1:5	1:23
Pellet	41.8±1.21	45.0±1.09	42.5±1.44
Straw	38.8±1.13	40.3±1.12	42.9±2.14

Table 5.6 Experiment 4: Kidding percentages after laparoscopic insemination with semen frozen as pellets and straws.

Freezing method	Number of motile sperm inseminated (x 10^6)			
	1	5	25	Means
Pellet	52.2 (46)	58.7 (46)	60.0 (40)	56.8 (132)
Straw	47.7 (44)	52.5 (40)	52.6 (38)	50.8 (122)
Means	50.0 (90)	55.8 (86)	56.4 (78)	53.9 (124)

sperm used in the experiment were based on an assumed recovery of 40% in addition to an assumed concentration, the poorer viability of the straw frozen semen would have substantially reduced the number of motile sperm in the inseminate dose.

DISCUSSION.

This series of experiments examined the effects on fertility of a variety of insemination conditions, in addition to the effects of the semen freezing processes used. Whilst the majority of the data was collected from laparoscopically inseminated does, an attempt was made to relate these findings to cervical insemination with frozen semen, a practice generally not recommended.

Cervical and Laparoscopic insemination.

Experiments 1 and 2 were conducted concurrently using comparably run females randomly selected from the same herd, although only adult does were used in experiment 1, whilst both adults and hogget maidens were used in experiment 2. However, no significant differences or interactions were recorded with doe age in experiment 2. This allows comparison between the two methods of insemination under similar conditions.

Whilst the cervically inseminated does received on average 3.5 times more motile sperm than laparoscopically inseminated does, the fertility rate was substantially less after cervical than laparoscopic insemination (39.1% vs 63.6% respectively). Over the widest range of inseminate doses used in experiments 1 and 2, the cervically inseminated does received 10.5 times more motile sperm than laparoscopically inseminated does, whilst fertility rates were 40.9 % and 66.7 % respectively. Although comparisons between the cervical insemination of experiment 1 and any other experiments are not strictly valid, (due to differences in time and the animals used) the lowest dose rate used in laparoscopic inseminations still gave better fertility (50 % from 1x106 motile sperm in experiment 4) than the best cervical result achieved.

Such differences in fertility between cervical and laparoscopic insemination, have been postulated as resulting from problems associated with the transport of frozen-thawed sperm within the female reproductive tract (in particular across the cervix)

after cervical insemination (Evans and Maxwell, 1987). Certainly the results from experiment 1 indicated a positive relationship between fertility and increasing depth of cervical insemination. However, although 63% of the does were inseminated into the uterus via the cervix, fertility was still poor with only 45% of these pregnant to the AI.

It seems rational to suggest that frozen-thawed sperm cells may less competently travel within the reproductive tract, as has been suggested in the ewe (Lightfoot and Salamon, 1970; Allison and Robinson 1971). Even subtle differences in the placement of the inseminate within the female can effect fertility. Killeen et al. (1982) indicated that fertility was greater when semen was deposited as a split dose in both horns rather than as one in a single horn. In superovulated ewes, Jabbour and Evans (1991) also found that the % of fertilised ova recovered was greater after insemination with frozen-thawed semen in the oviduct than the uterus. No such difference was observed with fresh semen.

However, poorer sperm motility, in conjunction with deposition of the inseminate farther from the site of fertilisation, cannot be blithely attributed responsibility for the lower fertility recorded for cervical than laparoscopic insemination. This assumes that the sperm cell itself is a determining factor in transportation to the site of fertilisation, whilst studies on sperm transport within the female reproductive tract have not been conclusive (Hawk, 1982).

Other possible explanations of the poorer fertility after cervical insemination, may relate the method of insemination to the functional state of the sperm cell at the time of interaction with an ovum. In experiments 1 and 2, both types of insemination were undertaken at the same times relative to the estimated time of ovulation. However, it seems likely that the interactions between the sperm cells and ova occur within different time-scales, according to the method of insemination.

Although some studies have indicated that sperm cells are rapidly transported within the female reproductive tract, there are conflicting results depending on the method of study (Hawk, 1982; Hunter, 1986). Thus the AI method may effect the chronology of the interaction of sperm cell interactions in the female, and the subsequent capacity of the sperm cells to initiate and sustain the processes of fertilisation. This may result from differential time of exposure of sperm cells to the female environment, exposure to different environments within the female reproductive tract, or inherent time related loss of function.

The reasons for the reduced fertilisation of cervically inseminated does needs to be more thoroughly elucidated in terms of the interactive gametes, before rational steps can be taken to improve the situation.

Time of Insemination.

The time of ovulation of goat does treated with CIDR's and sponges has been previously determined (Ritar et al., 1989; 1984). The timing of insemination relative to the estimated time of ovulation indicated that insemination prior to ovulation gives better fertility results than insemination afterwards. Fertility was improved after insemination 45 and 55 hours after CIDR and sponge removal, compared with insemination 55 and 65 hours after their respective removal. This relationship was evident for both methods of insemination used. Further advancing insemination to 39 hours after the removal of CIDR's gave no improvement in fertility of laparoscopically inseminated does, although fecundity was increased. These observations may indicate a relationship in which the gamete interaction is optimised by the timeliness of that interaction, relative to the exposure of the gametes to the uterine environment.

No differences were observed in fertility between the two types of intravaginal treatment used, provided that account was taken of the different timing of ovulation relative to the cessation of such treatment. This is consistent with previous reports (Corteel et al, 1988; Ritar et al., 1989).

Inseminate Dose.

Within each of the four experiments undertaken (cervical and laparoscopic), the dose of motile sperm in the inseminate did not significantly effect fertility or fecundity. Thus over the range of approximately 160 to 80 million motile sperm there was no reduction in fertility of cervically inseminated does. Whilst the full range of laparoscopic dose rates (from 60 to 1 million motile sperm) was not examined in any one experiment, and comparisons between different experiments are not valid, the lack of significant differences within experiment indicate that no threshold level was identified. Further reductions in the dose rate of motile sperm in the inseminate seem possible, suggesting that each collected ejaculate may be extended even further,

improving the efficacy of sperm collection and dissemination. However, it would be interesting to assess whether there are any significant falls in fertility rates over the entire range in the one experiment.

The results of these studies suggest that substantial reductions in the number of frozen-thawed motile sperm in the inseminate may be achieved without significantly impacting on fertility. Other researchers have reported similar findings for both sheep and goats, though not down to the low doses examined here (Corteel et al., 1988; Findlater et al., 1991; Ritar and Salamon, 1983). Such a technical observation may be of significant commercial value. In experiment 4, very low sperm numbers in the inseminate were successfully achieved using a very high rate of dilution of the semen (1:23). The impact of further distributing the revived sperm on fertility, and on the commercial success of the operation must be carefully considered.

In all experiments the dose rate of motile sperm in the inseminate was achieved by manipulating the inseminate volume. As a consequence any effects of inseminate volume on fertility are confounded with the results discussed for dose rate. However, in experiment 3 there was no effect of dilution rate, and thus of inseminate volume, on fertility after laparoscopic insemination. Since the recovery of motile sperm was similar across dilution rate for the semen used in this experiment, inseminate volume did not apparently have an effect on the laparoscopic insemination.

Straws and Pellets.

Goat semen processed by simple one-step freezing and dilution has previously achieved acceptable fertility (Ritar and Salamon, 1983; Ritar, et al., 1989). However, one step freezing of semen in straws has not been compared with pellets in fertility trials, although similar fertility rates have been reported with ram semen (Hunton, 1987). In experiments 3 and 4, no significant differences in fertility were recorded between semen frozen in pellets or 0.25ml straws, although the recovery rate of straw frozen sperm was significantly less in experiment 4. This suggests that there were no inherent differences in the viability and fertilising capacity of the recovered sperm frozen by the two methods. Indeed the lower recovery rate of the sperm frozen in straws in experiment 4 meant that the does were subsequently inseminated with a smaller number of motile sperm compared to those inseminated with pellet frozen semen, and yet this did not significantly effect fertility. However these results may only indicate that the inseminate dose was sufficiently in excess of the lower threshold

of motile sperm required for adequate fertility, to mask any qualitative effect of the spermatozoa (Foote, 1988).

The straw freezing method used in these studies illustrates that goat semen can be quite simply frozen in polyvinyl straws, and that this method provides a viable alternative to the packaging of semen in pellets. These fertility trials also further demonstrate that goat semen can be successfully frozen according to a simple one-step method of dilution and freezing, in a diluent milieu that contains egg yolk (although only at low concentrations). Both the methods of freezing semen in straws and pellets may be readily applied with a minimum of specialist equipment.

Whilst this technique of processing and freezing semen may produce adequate results, a comprehensive fertility trial comparison with semen subjected to other forms of processing (multi-step dilution, centrifugal washing, freezing in milk-based diluents) would be of value in determining whether there is a commercial advantage of the simpler technique. Comparisons between incubated samples of frozen-thawed sperm are not sufficient to make such determinations.

Of the different age groups of does used, only the kid maidens were less fertile and fecund after laparoscopic insemination. These does were approximately 54% of mature live-weight and 6 to 8 months old, whilst 18 month old hogget maidens that were a mean of 89% (experiment 2) and 77% (experiment 3) of mature live-weight, appeared to exhibit fertility and fecundity similar to adult does. Only adult does were cervically inseminated, so no effect of age was determined, although reduced ease of insemination and fertility would be expected with younger nulliparous does.

Where data was collected, the returns to service after insemination over-estimated the % of pregnant does, as determined by ultra-sound pregnancy diagnosis. Clearly it is important that fertility be adequately defined in such fertility trials.

CHAPTER 6.

FERTILITY OF GOATS AFTER CERVICAL INSEMINATION WITH HIGHLY CONCENTRATED FROZEN-THAWED SEMEN

6. THE FERTILITY OF GOATS AFTER CERVICAL INSEMINATION WITH HIGHLY CONCENTRATED FROZEN-THAWED SEMEN.

Introduction.

Cervical insemination is a simpler, quicker and cheaper method of insemination than laparoscopy, and its practice is less restricted to specialist technicians (Evans and Maxwell, 1987; Ritar, 1987). However, these advantages of the cervical method are countered by generally poorer fertility rates, particularly when frozen-thawed semen is used (Maxwell et al., 1980; Ritar, 1987).

In sheep, a highly concentrated inseminate dose containing a large number of motile spermatozoa can improve fertility after cervical insemination (Allison and Robinson, 1971). However, freezing at low dilution rates to give high sperm concentrations, reduces the proportion of sperm cells surviving the freezing process (Visser and Salamon, 1974). Reconcentration of spermatozoa for insemination after freeze-thawing has been examined as a means of circumventing this problem (Salamon, 1977). There are differences in the cervical anatomy between the goat doe and the ewe, that make the former more penetrable during insemination (Evans and Maxwell, 1987; Moore, 1985). These differences may influence fertility, and acceptable rates of fertility may be obtained after insemination with frozen-thawed concentrated semen in the goat doe.

Two experiments were conducted to investigate the effects of freezing highly concentrated semen on the % of motile sperm after incubation, and fertility after cervical insemination. It was hypothesised that a small volume of highly concentrated sperm deposited within the cervix might improve fertility, compared to larger inseminate volumes containing similar numbers of motile sperm.

Materials and Methods.

Experimental Procedure.

Experiment 1. Semen Freezing.

In this 3x3x4 factorial experiment, buck semen was diluted in three media (Blow, Bmid, Bhigh; table 6.1), at three rates of dilution (semen:diluent; 1:0.5, 1:1, 1:2), for semen collected from four bucks. All semen was frozen as pellets on dry ice.

Experiment 2. Cervical Insemination.

In the breeding season (April, 1989), this 2x3x2 factorial experiment examined the fertility of Cashmere does after cervical insemination with frozen-thawed semen at two dilution rates (semen:diluent; 1:0.5, 1:2), at three times of insemination (single insemination at 36 hours, single insemination at 45 hours and double insemination at 36 and 45 hours) after CIDR removal (Type G CIDR, 330mg progesterone, Carter Holt Harvey plastic Products, New Zealand), and with two inseminate doses (60x106 and 120x106 motile sperm).

Semen Collection and Processing.

Semen for experiment 1 was collected by artificial vagina (AV) from four bucks during the early breeding season (January). Immediately after collection, the semen was transferred to a 30°C water bath and assessed for volume (ml), colour, initial motility (wave motion, score 1-5), and optical density. Optical density was used to determine sperm concentration by relation to a standard curve of optical density and sperm concentration determined by haemocytometer counts.

Semen from each buck was held at 30°C and divided into nine measured aliquots for dilution at the three rates (1:0.5, 1:1 and 1:2) with each of three media to achieve the concentrations of diluent constituents in the diluted semen as detailed in table 6.1. Thus nine media preparations were required, a Blow, Bmid and Bhigh adjusted for each of the three dilution rates, as shown in table 6.2. All diluents were prepared from

Table 6.1 Concentrations of diluent constituents in buck semen after dilution with media B_{low}, B_{mid} and B_{high}.

Media	Tris* (mM)	Citric Acid (mM)	Glucose (mM)	Glycerol (% v/v)	Eggyolk (% v/v)
B_{low}	200	66.1	22.2	3.2	1.2
B_{mid}	250	82.7	27.8	4.0	1.5
B_{high}	300	99.2	33.3	4.8	1.8

* Tris(hydroxymethyl)methylamine

Table 6.2 Concentrations of diluent constituents used to dilute buck semen with media B_{low}, B_{mid} and B_{high} at dilution rates 1:0.5, 1:1 and 1:2 (semen:diluent).

Media		Tris* (mM)	Citric Acid (mM)	Glucose (mM)	Glycerol (% v/v)	Eggyolk (% v/v)
1:0.5						
	B_{low}	600	198.4	66.6	9.6	3.6
	B_{mid}	750	248.1	83.4	12.0	4.5
	B_{high}	900	297.6	99.9	14.4	5.4
1:1						
	B_{low}	400	132.2	44.4	6.4	2.4
	B_{mid}	500	165.4	55.6	8.0	3.0
	B_{high}	600	198.4	66.6	9.6	3.6
1:2						
	B_{low}	300	99.2	33.3	4.8	1.8
	B_{mid}	375	124.1	41.7	6.0	2.3
	B_{high}	450	148.8	50.0	7.2	2.7

* Tris(hydroxymethyl)methylamine

one concentrated stock solution, by dilution with distiled water. Dilution of the semen was conducted at 30°C with media and glassware equilibrated at this temperature. Rapid semen processing was required to avoid desiccation of the aliquots of semen during the dilution procedure.

Once diluted, semen from each of the bucks was cooled to 5°C over a period of 1.5 to 2 hours, by placing the semen in test-tubes surrounded by a water jacket initially at 30°C, into a 5°C forced-fan cooler. At 5°C the semen was frozen as pellets on dry ice. The pellets were then immersed in liquid nitrogen and stored in this manner for approximately seven days prior to assessment.

Semen for use in experiment 2 was collected in the breeding season (March to April) according to the protocols described above, with the exception that semen was only diluted at rates of 1:0.5 and 1:2 with diluent Bmid (Table 6.2). Semen was collected from a pool of six Cashmere bucks, on three days in each week of collection (Monday, Wednesday, Friday). In the routine collection of semen for insemination, individual ejaculates were diluted at one rate only, and alternate collection days assigned to each dilution rate. Fresh diluent was prepared on each day of collection. After cooling to 5°C, up to three ejaculates from each buck were combined and thoroughly mixed prior to freezing as pellets (O.9 to 0.12ml) on dry ice. Ejaculates were combined to reduce the proportion of highly concentrated semen lost during handling (adherence of the highly viscous inseminate to glassware) and that used in assessment. One pellet from each freezing batch was assessed prior to pooling of pellets by buck and dilution rate, and storage under liquid nitrogen. Only ejaculates with an initial concentration of at least $2.5x10^6$ sperm (as determined by optical density) were retained for insemination.

Thawing and Assessment.

In experiment 1, pellet frozen semen was thawed in clean dry test-tubes (2 pellets per tube) by agitation in a 37°C water bath. The pellets were thawed in a random order in pre-labelled test-tubes, and transferred to another 37°C water bath for incubation over 6 hours. After all pellets had been thawed, assessment of the % of motile sperm was conducted by visual estimate (to the nearest 5%) initially at time 0 hours and thereafter at 2, 4 and 6 hours.

Samples for assessment were prepared independently of the assessor, such that their identity was unknown. The test-tube containing the semen was agitated and a drop of semen removed with a pipette, placed on a glass slide and rapidly covered with a coverslip. All glassware contacting the semen was maintained at 37°C. Assessment was conducted under a 40x phase contrast objective, with the sample slide on a 37°C warmstage. Samples awaiting assessment were held on a similar warmstage. After thawing in a random order, the samples of semen were repeatedly assessed in the same order, and the semen protected from desiccation during incubation by covering of the test-tubes with aluminium foil.

Prior to insemination in experiment 2, pellet frozen semen was thawed as described above, although three to four pellets were thawed in each test-tube. Immediately following thawing, the semen was transferred to a 30°C water bath, motility was rapidly assessed under a microscope, and the semen used within 10 to 15 minutes of thawing.

Preparation of Females and Insemination.

Intravaginal CIDR devices were inserted into adult Cashmere does (n=222) 18 to 19 days prior to removal, on the Woolnorth property near Smithton, Tasmania (40°45'S, 144°45'E). At CIDR insertion the does were weighed (32.0 ± 0.28Kg) and testosterone treated wethers (150mg testosterone enanthate; Tesgro, Merck, Sharp and Dohme, Australia, Pty Ltd) were introduced to the herd at a ratio of 1:20 (wethers:does). These wethers were treated with testosterone again at insemination, and remained with the does until replaced by entire bucks 14 days after insemination.

At CIDR removal the does were injected intramuscularly with 200 iu PMSG (Pregnecol, Heriot Agvet Pty Ltd). Insemination was conducted either 36 or 45 hours after CIDR removal, or at both times where does were inseminated twice.

The does were randomly allocated to insemination treatments, divided between six bucks. Within each time of insemination, the treatments of sperm dose and dilution rate, were rotated between bucks. The dose of motile sperm inseminated was manipulated by adjustment of the inseminate volume, calculated on the basis of the average ejaculate concentration, and the assessment of the % motile sperm at each dilution rate, determined from the samples assessed in the laboratory after freeze-thawing of each semen batch.

At insemination, the does were presented with the hind-quarters resting on a padded rail and the body inclined at a 45° angle. A duck-billed speculum with attached light-source was then inserted into the vagina, and semen deposited as deeply as possible into the cervical canal, using a plastic inseminating pipette with a blunt angled tip.

Fertility Data.

At 160 days after insemination, pregnancy to the artificial insemination was determined by examination of the does for evidence of parturition. This consisted of an udder examination and observation of the vulva and tail region for evidence of post-natal bleeding.

Statistical Analysis.

In experiment 1, the post-thaw % of motile sperm data was subjected to angular transformation, and then analysed by analysis of variance. This was done for a split-plot design, with incubation time being the sub-plot. Where significant interactions between bucks (the replicates) and treatment factors were revealed, the interaction mean square was used to test for significance of the treatment factor.

In experiment 2, the fertility data, scored as pregnant or not to the artificial insemination, was examined for significant treatment effects using X2 analysis.

Results.

Experiment 1.

There was an interaction ($P<0.01$) of rate of semen dilution with incubation time on the post-thaw % of motile sperm. The post-thaw % of motile sperm was higher after dilution at 1:2 than either 1:1 or 1:0.5, with post-thaw survival declining with lower rates of dilution. The progressive reduction of motile sperm with increasing incubation time, was greater for sperm frozen at a rate of 1:0.5.

Table 6.3 shows the % of post-thaw sperm survival for the three dilution rates with each dilution media, averaged over the six hours of incubation. There was no interaction of treatments with incubation time. Media Bmid gave better ($P<0.01$) post-thaw survival than media Blow and Bhigh at each rate of dilution, and there was no significant interaction.

No significant differences in sperm survival after freeze-thawing were observed between bucks.

Experiment 2.

The fertility results after insemination are presented in table 6.4. Fertility did not differ significantly with either the dilution rate of frozen semen, the time of insemination or the number of motile sperm in the inseminate. Fertility was slightly higher after single insemination than double insemination (37.2% and 25.7% respectively, $P<0.10$), whilst the best results were achieved after insemination at 36 hours after CIDR removal, with semen frozen at a rate of 1:2 (fertility = 19/41, 46.3%).

The age of the bucks used in the insemination programme had no significant effect on fertility. After freezing semen for insemination, the % of motile sperm was assessed immediately after thawing sample pellets. From this compiled data, semen diluted and frozen at rates of 1:0.5 and 1:2, had post-thaw recoveries of 38.4 ± 1.56% and 43.1 ± 4.9% motile sperm, respectively.

Discussion.

Freezing buck semen at low rates of dilution (1:0.5 and 1:1, semen:diluent) significantly reduced the post-thaw % of motile sperm after incubation, compared to semen diluted and frozen at a rate of 1:2. The survival of motile sperm diluted at 1:0.5 was both initially lower than that diluted at 1:2, and also more sensitive to the time of post-thaw incubation. The latter observation may indicate that sperm frozen at lower rates of dilution were either less viable or were more subject to the accumulation of debilitating factors (such as respiratory products) during incubation.

Table 6.3 Mean percentage of motile spermatozoa assessed over six hours of post-thaw incubation following freezing of buck semen at three dilution rates with dilution media B_{low}, B_{mid} and B_{high}.

Media	Mean % of motile sperm Dilution rate (semen:dilution media)			
	1:0.5	1:1	1:2	Mean
B_{low}	31.0	33.6	33.5	32.7
B_{mid}	33.0	35.8	37.2	35.3
B_{high}	28.0	30.7	35.3	31.3
Mean	30.7	33.4	35.3	33.1

Table 6.4 Fertility of Cashmere does following cervical insemination.

Factor	Number of does kidded/ number of does inseminated	% of does kidded
Dilution rate (semen:diluent)		
1:0.5	32/109	29.4
1:2	42/113	37.2
Time(s) of Insemination after CIDR removal		
36 hours (single)	30/80	37.5
45 hours (single)	25/68	36.8
36 and 45 hours (double)	19/74	25.7
Number of motile spermatozoa in the inseminate		
60×10^6	33/114	28.9
120×10^6	41/108	38.0
Age of bucks		
18 months (n=3)	42/118	35.6
2.5 years (n=3)	32/104	30.8
Total	74/222	33.3

However, whilst this reduction in motile sperm for semen diluted at 1:0.5 compared to 1:2, was assessed as 6% in experiment 1 and 10% in experiment 2, the concentration of motile sperm was approximately 50 to 70 % higher.

The rate of dilution of the semen did not have any apparent effect on the interaction of sperm with the constituents of the three media concentrations used. At each dilution rate the media concentrations previously recommended (Evans and Maxwell, 1987; Salamon and Ritar, 1982) and used in other experiments reported in this manuscript, gave the best % post-thaw motile sperm.

The reconcentration of diluted semen after freeze-thawing has previously been recommended as a means of depositing large numbers of sperm cells in the cervical canal and achieving adequate fertility in sheep (Salamon, 1977). It has been suggested that a limit to cervical insemination is the establishment of an adequate population of sperm cells within the cervical canal (Lightfoot and Salamon, 1970), however excessively increasing the volume of the inseminate may not provide a satisfactory means of achieving this, as much of the inseminate may effectively be lost to back-flow (Evans and Maxwell, 1987).

In this study the effect of sperm concentration in the inseminate was examined independently of the number of motile sperm used. This was achieved by adjusting the inseminate volume to take account of the dilution rate and the survival of motile sperm. Despite the substantially higher concentrations of motile sperm in the inseminate diluted at 1:0.5, fertility was reduced compared to semen diluted at 1:2, though not significantly. This was not effected by the dose of motile sperm inseminated. This poorer fertility result may be due to interactions of high cell concentrations with either dead sperm, seminal plasma or diluent constituents. Alternatively, the lower fertility may reflect poorer sperm fitness that resulted from freezing at such low dilution rates, but was not apparent in the assessment of post-thaw motility alone.

An interesting observation was that double insemination with a split dose of semen reduced fertility. This may be due to the additional stress of double handling of the animals at insemination. Whilst the reduction in fertility associated with reducing the number of motile sperm in the inseminate from 120x106 to 60x106 was not significant, it was substantial enough to indicate a trend of reduced fertility between the two dose rates.

Reduced fertility over this range was reported by Ritar and Salamon (1983), although there was no difference in fertility after insemination with 80x106 or 160x106 motile sperm in experiment 1 of chapter 5. Reducing the number of sperm inseminated is of interest as it can increase the efficacy of insemination if fertility is not similarly reduced.

These results emphasise the low fertility that may result from cervical insemination with frozen semen, as has been previously reported in sheep and goats (Lyngset et al., 1965; Maxwell et al., 1980; Ritar and Salamon, 1983). Significantly increasing the concentration of sperm during freezing, and subsequent insemination with high concentrations of sperm recovered from such treatment provided no benefit in fertility. Other avenues of improved gamete fitness or interaction in the female genital tract must be explored, if the advantages of this simple method of insemination are to be more fully realised.

CHAPTER 7.

GROWTH AND REPRODUCTIVE DEVELOPMENT IN YOUNG MALE CASHMERE AND ANGORA KIDS

7. GROWTH AND REPRODUCTIVE DEVELOPMENT IN YOUNG MALE CASHMERE AND ANGORA KIDS.

Introduction.

Characterising reproductive development and puberty in males is of practical interest for a number of reasons. Such information can be used to avoid unplanned matings, and may aid the in recognition of sub-normal reproductive development or provide some early indication of reproductive potential. Early culling on reproductive indices may be objectively considered, as may the potential advancement of breeding programmes and reduction of the generation interval by the earlier use of replacement sires.

In this study, growth and reproductive development of Cashmere and Angora males reared in three husbandry regimes, was measured for animals assigned to 5 age at slaughter groups. The relationships between age, live-weight, carcase weight, testicular circumference and testes weight were examined, and the effects of husbandry and breed considered. Development of the seminiferous tubules was observed, and pubertal development was assessed on the appearance of spermatozoa in the seminiferous tubules.

Materials and Methods.

Husbandry.

In June and July of 1989 approximately 105 Angora does and 165 Cashmere does were naturally mated at the Cressy Research Station, Cressy, Tasmania. These animals were allocated to three groups after stratification on the basis of their live-weight prior to joining. These groups were then randomly allocated to one of three rearing strategies. The does were mated in the two breed groups and subsequently combined to run together as one mob until immediately before the start of kidding. At this time the does were divided into the three rearing groups and kidded in adjacent paddocks. Kids were born in October and November, and kidding extended over a period of six to eight weeks.

During the kidding period the animals were checked daily and new-born kids tagged. Data collected at birth for all kids included the date of birth, the birth-weight, sex, sibling status and the identification of the dam. Over the period from kidding to weaning, the does and their progeny were run in the separate rearing groups. After weaning onto pasture the kids were run as a single group.

The rearing strategies applied to the progeny from the three groups were: (1) normal weaning; (2) early weaning; and (3) intensive rearing on a foster feeder.

(1) Normal weaning.

The kids were weaned according normal farm practice at approximately 10 to 12 weeks of age. These animals were weaned on the 31/1/90, except for six animals weaned after weighing greater than 14 Kg on 3/1/90. The majority of the normal weaned kids were weaned directly to improved pasture. However, six animals were regarded as too light (<10kg) to wean to field conditions and were weaned to the supplementary feed area provided for early weaned and foster-fed kids. These animals were removed from the supplementary feed area with the early weaned group.

The normal weaned kids in the second age at slaughter group (14 weeks old) were allowed to remain on their mothers until slaughter. This slaughter was planned to coincide with the weaning on the 31/1/90, but was unavoidably delayed for a period of two weeks.

(2) Early weaning.

The early weaned kids were run with their mothers for six to eight weeks. To reduce the effects of age differences within the group on weaning age, these kids were weaned in four sub-groups. The criterion for weaning was primarily age, although it was decided that a weight requirement of 10 Kg should also be met. Kids born between 13/10/89 and 22/10/89, 23/10/89 and 29/10/89, and 30/10/89 and 28/11/89 were assigned to weaning on the 6/12/89, 14/12/89 and 22/12/89 respectively. Kids born outside these times (two born in December) and those not meeting the weight requirement at assigned weaning times, were finally weaned on 3/1/90.

Early weaned kids were given ad-lib access to hay and pelletised feed for an average of 7 weeks after weaning. All kids that weighed 12 Kg or greater on the 3/1/90 were removed from the supplementary feed area on 22/1/90. The remaining kids were allowed access to the feed area until the 12/2/90, when they were removed to improved pasture and run as one group with weaners from all rearing groups.

(3) Intensive rearing on a foster feeder.

Intensively reared kids were removed from their mothers 24 hours after birth and given access to milk substitute (Superkid, Barastoc) delivered from a foster-feeder machine (Alfa Laval). These animals were reared in a shed with a raised slatted floor. The kids were divided between three groups in the foster-feeder area. These constituted age groups of animals at different stages of competence on the foster feeder.

Initially the kids were placed in a nursery pen with access to foster-feeder teat banks and over-head heat lamps. Kids in this area were introduced to the foster-feeder teats and bottle-fed if necessary. The newly introduced kids were thus separated from the competition of older and more robust kids. Once kids were observed to suckle vigorously from the foster-feeder teats and judged to be adequately competitive, they were successively removed to two other groups . The first of these was a group of peers adapted to the foster feeder, and from there the kids were introduced to a more advanced group. This strategy was implemented to ensure that access to the teat banks (see appendix 1) was not restricted by competition factors resulting from the age structure of the group. Outside the nursery pen, the kids were provided with pelletised feed, and those in the advanced group were also given access to improved pasture.

The intensively reared kids were weaned from the foster-feeder according to the same weight and age criteria described for the early weaned kids. At weaning, the foster-fed kids were also removed to the same supplementary feed area. An exception to the weaning strategy described was that all intensively reared kids were removed from the foster-feeder by the 22/12/89. This meant that some of the late-born kids were weaned at a younger age than their peers. This situation was imposed by management constraints. Intensively reared kids were removed from the supplementary feeding area by the 12/2/90, with the early weaned kids (see appendix 1). However, as with the latter group, kids weighing 12 Kg or greater were removed on the 22/1/90.

Once all kids from the three rearing regimes had finally been weaned to pasture (after the 12/2/90), they were run as a single mixed group of males and females. The males were subsequently separated from the females on the 8/3/90, although kids from all three rearing strategies continued to run together. Once weaned onto pasture, the kids were subject to the normal husbandry practice of the farm.

Experimental Procedure.

The males from the three rearing strategies described were left entire and aspects of their growth and development assessed. This assessment consisted of repeated measures of live-weight, scrotal circumference and horn length, and a slaughter study from which aspects of reproductive and carcase development were also characterised.

Once kidding had concluded, all surviving male Cashmere kids were allocated to one of five slaughter groups. Allocation was conducted such that within each of these slaughter groups the kids were of similar age, and that the between group distribution of singles, twins and triplets was similar. These factors were considered in order to limit the effect of the extended period of kidding on the planned age comparisons, and to reduce any effect of litter size on group performance. To achieve these objectives the male kids in each rearing strategy were stratified into five groups on the basis of birth date and sibling status. These groups were combined across rearing strategies according to this ranking.

However, because the kids were born into their rearing groups, the size and age structure of each rearing group was predetermined. This was a compromise resulting from the integration of this trial within an existing breeding trial. Orphaned kids reared on the foster-feeder were not allocated for slaughter.

After allocation of Cashmere kids to the five groups, these groups were in turn randomly allocated to five slaughter ages. Initially the kids were to be slaughtered at approximate ages of 6, 12, 18, 24 and 30 weeks. In the course of the experiment however, it was decided to defer the final two slaughter times until ages of 30 and 36 weeks. This decision was made in order to allow comparison of differences in both age and live-weight between these and other slaughter times. Approximate slaughter dates were calculated from the mean ages of the slaughter groups. The timing of the second slaughter was advanced due a delay at the slaughter house.

In total, 83 Cashmere kids were allocated to the five slaughter groups, and 79 of these survived to slaughter. The small number of male Angora kids (11) that survived could not be adequately distributed across the five slaughter times, preventing sampling across different age and weight groups. These animals were all assigned to slaughter with the final Cashmere age group.

Live-Measures.

Although the extended period of kidding restricted the start of the weighing programme, frequent weighing was conducted throughout November, December and January. Initially this was every two weeks, and then weekly during the weaning period, with live-weights being taken on the 1/11/89, 16/11/89, 28/11/89, 6/12/89, 13/12/89, 20/12/89, 3/1/90 and 31/1/90. Measures of testes circumference and horn length were also taken on the 6/12/89 and 31/1/90, immediately prior to the planned slaughter of the first two Cashmere age groups. At these times, data was collected from all available kids, in addition to those in the slaughter group. However, because the second slaughter was delayed the measures from this group were retaken immediately prior to slaughter.

All further weight, testis and horn data was collected one day prior to slaughter of an age group. This data was thus collected from all available animals on the 8/3/90, 9/4/90, 21/5/90 and 18/6/90. The slaughter planned for the 10/4/90 (24 week group) was deferred as explained previously. Thus data collected on the 9/4/90 and weights from the 9/5/90 (to monitor the progress of the deferred group), did not immediately precede a slaughter.

Live-weight data was initially measured using a set of tared hanging-bucket scales, in the same manner as the birth-weight data. When the kids became too large to be weighed in this manner, weight data was collected using a set of walk-through clock-face scales. Measures of the largest testes circumference and horn length from the skull to horn tip were made using a flexible nylon measuring tape. Horn length was measured along the outer curvature of the growing horn.

Live-measure data is only presented for animals included in the slaughter program. This excludes data collected from animals that were not allocated to slaughter or died prior to slaughter. The data is thus a set of repeat measures from a reducing sample size (resulting from the slaughter programme).

Slaughter Data.

One day prior to each slaughter date, measures of live-weight, horn length and testes circumference were taken, providing a set of live-measures that could be directly related to the carcase and reproductive tract data collected post-mortem. The animals were then housed for approximately 24 hours before slaughter, and denied access to feed and water. On the day of slaughter the animals were weighed and this weight described as an empty weight. At the slaughterhouse, only the measure of hot carcase weight was initially taken.

After slaughter as much of the reproductive tract as possible was collected prior to skinning the animal (this included the scrotum and testes and the portion of the genital tract leading to and including the penis) and placed in a plastic bag identified to the kid. These samples were placed on ice in a styrofoam esky and transported back to the laboratory.

In the laboratory, both testes from each animal were removed from the scrotum and the *tunica albuginea*. The epididymis was then dissected away from the body of the testis and the spermatic cord removed at the hilus. The testes were individually weighed on a Mettler platform balance and their dimensions measured with vernier callipers. Measures were taken of the widest and narrowest widths of the testes in addition to their longest length.

The left testis from each animal was sectioned laterally, midway between the poles of the testis, for histological examination. This section was placed in a 70 ml sample jar and fixed in Bouins media for a period of 48 hours prior to storage in 70 percent alcohol (prepared from ethanol and distilled water). Where a complete section through the testis was too large for convenient storage, the section was halved or quartered.

On the day following slaughter, the cold carcases were weighed and a range of carcase measures taken. These included the GR (fat depth at the GR site) taken 120mm from the backbone at the right 12th rib; the length and width of the eye muscle between the left 12th and 13th ribs as measured with vernier callipers; and a carcase length measure taken from the gambol at the left leg to the sternum using a flexible measuring tape.

Histology.

Seminiferous tubule development was characterised across a sample of six Cashmere kids in each of the five slaughter times (a total of 30 animals). A further 10 sections from Cashmere animals were later examined to provide more data points on regression curves of interest. These additional sections were selected regardless of slaughter group to provide data points across the full range of testis weights when combined with the data already collected.

The testes sections fixed in Bouins media following slaughter were stored in 70 percent alcohol until the completion of the slaughter programme. Once all sections had been fixed, they were processed in the following manner for histological examination.

The testes sections were sub-sampled in both the horizontal and vertical planes and the resulting smaller sections placed in plastic tissue processing cassettes. Where the testis section was too large for a full plane to be processed, a sample was taken from the interior of the testis. The tissue cassettes were then placed in an automated tissue processor (Tissue Tek III, Vacuum Infiltration Processor) and subjected to a series of dehydrations in alcohol solutions. All of the testes sections were processed at the same time, such that they were subject to the same conditions of dehydration and tissue shrinkage.

In the final stage of tissue processing, the sections were held in a bath of molten wax until removal from the tissue processor. As soon as possible after the completion of tissue processing, the sections were embedded in wax tablets to allow sectioning for microscopy. The tissue sections were removed from the plastic cassettes and placed in an aluminium mould containing some cooling wax. The tissue was tamped down into this wax and then fully covered with additional molten wax. Part of the cassette was affixed to the wax tablet to form a base useful in the manipulation of the tablet during sectioning, and in identifying the section.

Once the tissue had been embedded, the surface of the tablets was wax sealed prior to storage. The tablets were prepared for sectioning by placement on a cold tray to harden. The tablets were then affixed to a microtone (Microm HM 340, Heidelberg), trimmed and 4 micron thick sections taken. The wax and tissue sections were

removed from the blade of the microtone and placed in a water-bath at 49°C and then carefully manipulated onto a glass microscope slide. The slide and affixed tissue was then dried and stained with haemotoxylin and eosin in an automated staining rack (Tissue Tek II, Histo Tek Slide Stainer). Finally, a coverslip was fixed over the stained tissue with resin and the slide allowed to dry once again, before the tissue sections were ready for examination.

Sections of processed testes tissue were viewed on a colour monitor using a camera (Minitron, CCD Camera MTV-1801CB) attached to a microscope (Nikon Optiphot) with a four times objective lens in place. The seminiferous tubules observed in cross-section were measured using an image analyser package (ImageMeasure, Version 4.02, Microscience Inc. W.A.). All measures were made with the microscope set as described, and the image analysis equipment calibrated in microns.

Care was taken to avoid measuring tubule sections that may not have been transverse sections. In this regard, measures were taken from circular or oval tubule sections but not those that were obviously elongate. Efforts were made to minimise any unconscious operator selection of the tubules measured (within the above constraints) by measuring all suitable tubule sections in any field of view. One slide was examined for each animal and 50 tubules were measured per slide. Measures were made of tubule diameter, total tubule area, tubule wall thickness, lumen diameter and lumen area. The linear measures taken were those estimated to be the minimum for each tubule, where symmetry was not observed.

The same sections used in the characterisation of seminiferous tubule development were also used in the assessment of the proportion of tubules containing late spermatids and/or released spermatozoa. The slides were examined using a phase contrast microscope at a magnification of 40 times. Again, 50 tubules were examined per animal and scored for the presence or absence of late spermatids and released spermatozoa.

RESULTS.

Live-Measure Data.

The progressive means for live-weight, age, scrotal circumference and horn length for each of the 5 slaughter groups at each time of data collection are presented in tables 7.1, 7.2, 7.3 and 7.4 respectively.

The mean live-weights of the 5 slaughter groups were similar at the initial weighings. The slightly lower live-weights of Cashmere groups 2 and 3 were associated with younger mean age, as illustrated in table 7.2. Also Cashmere slaughter group 2 comprised later-born kids and mean live-weights were similar over the period 1/11/89 to 16/11/89, during which kids were still being born into the group. Live-weight increased with age, although both Cashmere groups 4 and 5 suffered a check in growth in March-April. Whilst both groups increased in live-weight after this time, that increase was not substantial, and was followed by a reduction in live-weight in group 5 animals.

The Angoras slaughtered at a similar mean age to Cashmere group 5 also had similar live-weight to the group 5 Cashmeres up to age 76 days. The Angoras subsequently recorded slower increases in live-weight than the Cashmere group although following the same trend of live-weight change.

Initially the increases in scrotal circumference were similar between slaughter groups, relative to mean age. In Cashmere groups 4 and 5 the observed check in live-weight gain was also reflected in a check in the increase in scrotal circumference. However, the decrease in scrotal circumference in Cashmere group 5 was proportionally less than the decrease in live-weight.

Initially the mean scrotal circumference of the Angoras was similar to that of the group 5 Cashmeres of similar age. However, by age 100 days the Angoras had both a lower mean live-weight and scrotal circumference.

Horn growth did not exhibit the disassociation of growth with age recorded for live-weight and scrotal circumference. The rate of horn length appeared more linear with age than the other tissues. Compared with the Cashmeres of similar age the Angoras had longer horns.

Table 7.1 Progressive mean live-weight (kg) of each slaughter group of Cashmere and Angora kids at each time of data collection.

Data Collection	Date	Cashmere Slaughter Group 1	2	3	4	5	Angora Group
1	Birth	2.9±0.12	3.0±0.11	2.±0.10	3.0±0.12	2.8±0.08	2.9±0.10
2	01/11/89	4.3±0.21	4.9±0.28	3.4±0.18	4.7±0.19	5.2±0.22	5.00±0.27
3	16/11/89	6.4±0.34	4.1±0.51	4.9±0.28	6.9±0.31	7.1±0.38	7.1±0.38
4	28/11/89	8.7±0.44	5.6±0.55	7.3±0.45	9.4±0.51	9.1±0.53	9.4±0.51
5	06/12/89	10.1±0.57	6.9±0.60	8.4±0.51	10.4±0.51	10.2±0.65	10.4±0.55
6	13/12/89		7.9±0.62	9.9±0.59	11.3±0.47	10.9±0.98	10.9±0.53
7	20/12/89		9.3±0.57	11.0±0.64	12.1±0.41	11.7±0.72	11.9±0.67
8	03/1/90		10.4±0.70	11.7±0.46	13.2±0.42	12.7±0.80	12.5±0.71
9	31/1/90		13.1±1.05	14.4±0.52	16.2±0.59	15.5±0.91	14.3±1.03
10	08/3/90			16.5±0.58	19.3±0.73	18.0±0.93	15.5±0.94
11	09/4/90				19.2±0.79	17.8±0.90	14.7±0.83
12	09/5/90				20.2±0.71	19.1±0.81	16.3±0.92
13	21/5/90				20.9±0.76	20.3±0.80	17.4±0.96
14	18/6/90					18.0±0.76	15.2±0.85

Table 7.2 Mean age (days) at each time of data collection for each of the five Cashmere slaughter groups and the Angora slaughter group.

Data Collection	Date	Cashmere Slaughter Group 1	2	3r	4	5	Angora Group
1	Birth						
2	01/11/89	8±0.7	-	4±1.0	11±0.6	15±0.7	13±1.5
3	16/11/89	22±0.9	8±1.7	17±1.1	26±0.6	30±0.7	28±1.5
4	28/11/89	34±0.9	18±2.2	29±1.1	38±0.6	42±0.7	40±1.5
5	06/12/89	42±0.9	26±2.2	37±1.1	46±0.6	50±0.7	47±1.5
6	13/12/89		33±2.2	44±1.1	53±0.6	57±0.7	55±1.5
7	20/12/89		40±2.2	51±1.1	60±0.6	64±0.7	62±1.5
8	03/1/90		54±2.2	65±1.1	74±0.6	78±0.7	76±1.5
9	31/1/90		82±2.2	93±1.1	102±0.6	106±0.7	104±1.5
10	08/3/90			129±1.1	138±0.6	142±0.7	140±1.5
11	09/4/90				170±0.6	174±0.7	172±1.5
12	09/5/90				200±0.6	204±0.7	202±1.5
13	21/5/90				212±0.6	216±0.7	214±1.5
14	18/6/90					244±0.7	242±1.5

Table 7.3. Mean scrotal circumference (cm) for the five Cashmere slaughter groups and the Angora slaughter group at each time of data collection.

Data Collection	Date	Cashmere Slaughter Group 1	2	3	4	5	Angora Group
5	06/12/89	11.6±0.31	9.9±0.56	10.2±0.36	11.7±0.37	11.3±0.56	11.4±0.30
9	31/1/90		12.5±0.7	13.1±0.54	14.9±0.64	16.0±0.87	12.9±0.48
10	08/3/90			17.3±0.22	19.5±0.84	20.1±0.86	13.6±0.70
11	09/4/90				20.1±0.92	20.1±0.78	13.7±0.71
13	21/5/90				21.1±0.71	21.5±0.56	15.9±0.97
14	18/6/90					20.9±0.56	15.9±0.72

Table 7.4 Mean horn length (cm) for the five Cashmere slaughter groups and the Angora slaughter group at each time of data collection.

Data Collection	Date	Cashmere Slaughter Group 1	2	3	4	5	Angora Group
5	06/12/89	3.4±0.16	2.0±0.31	2.4±0.13	3.5±0.19	3.8±0.21	4.3±0.21
9	31/1/90		6.2±0.50	6.8±0.20	8.0±0.29	8.1±0.46	8.9±0.34
10	08/3/90			9.3±0.27	10.8±0.36	10.8±0.59	10.8±0.43
11	09/4/90				12.3±0.44	12.6±0.64	12.0±0.57
13	21/5/90				14.5±0.45	15.2±0.76	14.0±0.70
14	18/6/90					15.8±0.76	14.8±0.62

Regressions of this growth data are presented in table 7.5. From the live-measure data accumulated from all of the Angora and Cashmere kids studied, scrotal circumference was better correlated with live-weight than with age. In contrast, horn growth was better correlated with age. The slopes of the correlation curves indicated that the increase in live-weight and scrotal circumference was slower for the Angoras than Cashmeres. Regressions of the Angora data were generally poorer than those for the Cashmeres, although this is confounded with smaller sample size. The regressions of scrotal circumference exhibited the greatest breed difference.

Multifactor regression of this live-weight, scrotal circumference and horn data indicated that rearing strategy was not a significant factor.

Data collected at slaughter.

Table 7.6 presents the data collected at slaughter from the 79 Cashmere males allocated to the 5 slaughter groups, and the 11 Angora males slaughtered with the oldest group of Cashmeres. There were no significant differences or interactions with rearing group, and the data presented is pooled across the three rearing groups at each slaughter.

This data includes mean age, live-weight, empty weight, horn length, the weight and dimensions of the testes, hot and cold carcase weight, GR, eye muscle length and width, and carcase length. Age group comparisons are only possible between the Cashmere males, whilst a breed comparison of Cashmeres and Angoras can only be made at the fifth age of slaughter.

Age at slaughter was effectively an experimental treatment, and the five groups of Cashmere animals were slaughtered at mean ages of 43, 96, 133, 214 and 246 days. The Angoras slaughtered with the fifth group had a mean age of 243 days.

The mean live-weight and scrotal circumference of the Cashmeres increased significantly ($P<0.05$) with each successive slaughter up to age 214 days (group 4). Mean live-weight at 246 days (group 5) was significantly ($P<0.05$) less than that of the 214 day age group, although the decrease in scrotal circumference was not significant. The Angoras slaughtered at 243 days had a mean live-weight and testes circumference significantly ($P<0.05$) less than the Cashmeres aged 246 days.

Table 7.5 Regression analyses of livemeasure data.

Response Variable	Regression Type	Factor	Model	Correlation Coefficient	R^2
Cashmere data					
Live-weight	Simple	Age	Mult.	0.91	82.0
Scrotal circumference	Simple	Live-weight	Lin.	0.88	77.9
Scrotal circumference	Simple	Age	Lin.	0.81	66.3
Horn length	Simple	Live-weight	Lin.	0.84	70.3
Horn length	Simple	Age	Lin.	0.93	86.5
Angora data					
Live-weight	Simple	Age	Mult.	0.87	75.6
Scrotal circumference	Simple	Live-weight	Lin.	0.70	49.4
Scrotal circumference	Simple	Age	Lin.	0.59	34.4
Horn length	Simple	Live-weight	Lin.	0.71	50.7
Horn length	Simple	Age	Lin.	0.90	81.6

Mult. = Multiplicative, $Y= ax^b$

Lin. = linear, $y= a=bx$

Table 7.6 Mean age, live-weight, scrotal circumference, horn length, testis and carcase measures at slaughter of Cashmere and Angora males.

	Cashmere Group	Slaughter Group			Angora Group
	1	2	3	4	5
n	17	15	15	16	16	11
Age (days)	43.2±0.91	95.5±2.21	133.0±1.06	214.4±0.51	246.3±0.72	242.5±1.05
Live-weight (kg)	10.1±0.57	14.1±1.09	16.5±0.58	20.9±0.76	18.0±0.76	15.2±0.85
Testis circumference (cm)	11.6±0.31	13.7±0.79	16.9±0.87	21.4±0.65	20.9±0.56	15.9±0.71
Horn length (cm)	3.4±0.16	6.9±0.52	9.4±0.27	14.6±0.44	15.8±0.76	14.80.62
Testis measures						
Left weight (g)	3.7±0.29	14.5±2.87	40.1±5.22	65.5±4.40	60.5±2.76	23.6±3.67
Right weight (g)		14.4±3.08	38.1±4.06	58.6±6.45	58.7±4.39	24.0±4.00
Left length (mm)	24.9±0.69	36.8±2.44	50.3±2.97	63.0±1.76	61.7±1.26	45.7±2.23
Right length (mm)		36.9±2.70	52.4±2.53	60.6±3.33	60.5±2.14	46.7±2.59
Left width 1* (mm)	15.7±0.57	21.8±1.81	31.7±2.18	40.8±1.07	41.2±0.87	26.5±1.82
Right width 1* (mm)		21.4±1.81	32.2±1.70	38.2±2.11	40.2±1.41	26.5±1.91
Left width 2! (mm)	17.50.51	24.1±1.73	34.1±2.12	44.1±1.09	43.7±0.85	29.2±1.69
Right width 2! (mm)		23.9±1.74	34.7±1.85	43.0±1.44	42.4±1.40	29.5±1.84
Carcase measures						
Empty weight (kg)	9.4±0.50	12.6±1.04	15.1±0.59	18.7±0.74	17.0±0.69	14.6±0.81
Cold weight (kg)	4.7±0.26	5.8±0.54	6.1±0.29	8.0±0.33	7.4±0.30	5.5±5.5
GR (mm)	5.8±0.38	6.4±0.60	6.2±0.12	4.1±0.37	4.7±0.28	5.8±5.8
Length (cm)	60.6±0.87	58.9±1.44	66.1±0.84	71.2±0.88	69.6±1.14	66.0±1.02
Eye Muscle						
Width (cm)	18.8±0.50	14.6±1.04	17.4±0.65	16.7±1.02	13.5±0.64	10.7±0.67
Length (cm)	35.5±0.81	37.4±1.62	42.2±0.83	42.5±1.05	40.3±0.85	30.3±0.93

* = the narrow width measurement taken from the assymetrically oval testis.
! = the wide width measurement taken from the assymetrically oval testis.

Horn length increased significantly ($P<0.05$) between each of the slaughter groups of Cashmere kids. In the fifth slaughter group the mean horn length of the Cashmeres was less than that of the Angoras, although not significantly.

Mean testis weight of the Cashmeres increased significantly ($P< 0.05$) between 43 and 214 days. As with scrotal circumference, the mean testes weight at 246 days was less than that at 214 days, but not significantly. The Angoras at 243 days had a significantly ($P<0.01$) lower mean testis weight than the Cashmeres at age 246 days.

Carcase weight increased up to slaughter group 4. These increases were significant ($P<0.05$) except between groups 2 and 3, whilst carcase weight decreased between groups 4 and 5, although not significantly. Increases in mean GR were not significant, although there was a significant reduction in GR after group 3. Eye muscle measures showed no significant trends, other than a significant reduction in width between groups 4 and 5. Carcase length increased gradually with slaughter age. However, significant differences ($P<0.05$) were only recorded between groups 2 and 3, and between groups 3 and 4.

At age 243 days Angoras had significantly lower measures of carcase weight ($P<0.01$), eye muscle dimensions ($P<0.01$) and carcase length ($P<0.05$) than Cashmeres at age 246 days. In contrast, the GR of the Angoras was significantly ($P<0.05$) greater than that of the Cashmeres.

Regression analyses of the Cashmere data collected at slaughter are described in table 7.7.

Total testis weight (measured from slaughter 2) was slightly better correlated against live-weight than against age, and there were strong correlations of testis weight with scrotal circumference. Measures of testis parameters were strongly linear. Carcase weight was equally related to live-weight and empty (fasted) weight.

Table 7.7. Regression analyses of Cashmere data collected at slaughter.

Response Variable	Regression Type	Factor	Model	Correlation Coefficient	R^2
Live-weight	Simple	Age	Mult.	0.77	59.2
Scrotal circumference	Simple	Live-weight	Lin.	0.87	75.9
Scrotal circumference	Simple	Age	Lin.	0.84	70.3
Left testis weight	Simple	Scrotal circumference	Lin.	0.94	87.6
Total testes weight	Simple	Scrotal circumference	Lin.	0.95	90.9
Total testes weight	Simple	Live-weight	Lin.	0.78	61.9
Total testes weight	Simple	Age	Lin.	0.75	55.9
Left testis length	Simple	Left testis widest width	Lin.	0.93	86.4
Left testis length	Simple	Left testis narrow width	Lin.	0.92	84.0
Carcase weight	Simple	Live-weight	Lin	0.95	90.3
Carcase weight	Simple	Empty weight	Lin.	0.96	92.5

Mult. = Multiplicative, $Y = ax^b$
Lin. = linear, $y = a = bx$

Testis Histology.

Seminiferous tubule development in Cashmere kids.

Means for live-weight, age, left testis weight and seminiferous tubule measurements at slaughter of 30 Cashmere animals from the five age at slaughter groups, are presented in table 7.8. No significant effect of rearing strategy was detected for any of the factors examined.

The mean diameter and area measures of the seminiferous were tubules calculated from 50 measurements per individual, and averaged over the six animals in each slaughter group. Tubule wall thickness, lumen diameter and lumen area are presented as means for the number of vacuolated tubules in each group. Vacuolation is expressed as the proportion of assessed tubules from each group that possessed a lumen. The presence of late spermatids and/or released spermatozoa is expressed as the mean percentage of the tubules examined, in which either were observed.

Least significant differences indicated that mean live-weight differed significantly ($P<0.05$) between most successive slaughter groups. These differences were mostly increases associated with the significant increases in age. However, exceptions were that live-weight was not significantly different between groups 2 and 3, and that live-weight decreased significantly between groups 4 and 5.

Left testis weight increased significantly between all slaughter groups except between groups 1 and 2 where the increase was not significant, and between groups 4 and 5, where there was no difference. Measures of scrotal circumference were similar, except that increase between groups 1 and 2 was significant.

Analysis of variance indicated that the area and diameter of the seminiferous tubules, the proportion of vacuolated tubules, the wall thickness of vacuolated tubules, and tubule lumen diameter and area, were all significantly affected by slaughter group ($P<0.01$).

Mean seminiferous tubule area and diameter increased with each successive slaughter group. These increases were significant ($P<0.05$) between all groups except 4 and 5.

Vacuolated tubules were observed in one animal in slaughter group 1, in four animals in group 2 and in all six animals in all subsequent slaughter groups. The proportion of

Table 7.8 Mean age, live-weight, left testis weight and seminiferous tubule description at slaughter for 30 Cashmere kids.

Cashmere slaughter group	1	2	3	4	5
n	6	6	6	6	6
Age (days)	44.7±0.88	96.8±1.47	134.2±1.94	214.5±0.76	247.3±1.09
Live-weight (Kg)	11.2±0.98	14.8±0.95	16.8±1.01	21.2±0.87	18.6±0.44
Scrotal circumference (cm)	11.9±0.48	13.8±0.54	18.3±0.80	21.9±0.70	21.7±0.41
Left testis (g)	4.1±0.30	13.3±1.44	46.1±4.75	65.8±7.18	65.4±3.48
Seminiferous tubules					
Diameter (μ)	58.6±2.2	98.3±4.8	159.6±7.2	178.6±4.4	184.3±4.5
Area (μ^2)	3192±241	10309±955	26581±2291	31074±1662	33257±1601
Vacuolation					
n animals	1	4	6	6	6
tubules (%)	5.3	38±16.2	97.7±1.7	99.0±.68	100
Tubule wall					
thickness (μ)	25.3	37.6±1.8	52.1±2.2	55.7±1.6	56.5±1.8
Tubule lumen					
diameter (μ)	10.5	32.5±4.8	54.3±4.7	67.3±2.6	73.9±2.7
area (μ^2)	96	1117±366	3992±507	5500±397	6458±478
Tubules with sperm and late spermatids					
% observed	0	0	48	71	65

vacuolated tubules observed in the testes sections from these animals increased significantly ($P<0.05$) to group 3. At this point the proportion of vacuolated tubules had reached almost 100%.

Significant ($P<0.05$) increases in the wall thickness of vacuolated tubules were only detected between slaughter groups 1 and 3. The means increased between all other groups but not significantly.

Mean lumen diameter and area of the vacuolated seminiferous tubules also increased with each successive slaughter age. These differences were significant ($P<0.05$) between all slaughter groups except groups 4 and 5 for lumen diameter. However, mean lumen area was only significantly different ($P<0.05$) between groups 2 and 3, and between groups 3 and 4.

Late spermatids and released spermatozoa were first observed in animals from slaughter group 3, whilst the largest percentage of tubules containing either late spermatids or released spermatozoa was recorded in group 4. This latter mean was significantly greater than that for group 3, although not significantly greater than for group 5.

Tables 7.9 and 7.10 show the progressive mean live-weight and scrotal circumference of each Cashmere age group at each time of slaughter. The data shows that there was a decline in live-weight and slight regression of scrotal circumference experienced by the animals used to represent the final slaughter group in the histology study. This follows the trends in live-weight and scrotal circumference established for the larger slaughter groups.

The testis histology data collected from this sample of animals was used in a series of regression analyses detailed in table 7.11.

Simple regressions of seminiferous tubule area against live-weight and age indicated that there was stronger relationship with age. Seminiferous tubule area was also strongly correlated with scrotal circumference, although the best relationship was with testis weight. Regressions of seminiferous tubule diameter against these factors were similar to those for tubule area.

Table 7.9 Mean Live-weight (kg) for each slaughter group of six Cashmeres used in the assessment of testis histology at each time of slaughter.

	Slaughter group				
Slaughter time	1	2	3	4	5
1	11.2	6.8	8.7	10.9	10.2
2		13.3	15.0	16.3	15.7
3			16.8	19.2	18.0
4				21.2	21.2
5					18.6

Table 7.10 Mean scrotal circumference (cm) of each slaughter group of six Cashmeres used for assessment of testis histology at each time of slaughter.

	Slaughter group				
Slaughter time	1	2	3	4	5
1	11.9	9.1	10.5	12.1	11.8
2		12.4	13.7	15.8	16.7
3			18.3	20.7	21.0
4				21.9	22.2
5					21.7

Table 7.11 Regression analyses of seminiferous tubule parameters against live-weight, age and testis weight at slaughter of 30 Cashmeres from five age at slaughter groups.

Response Variable	Regression Type	Factor	Model	Correlation Coefficient	R^2
Seminiferous Tubule					
Area	Simple	Age	Mult.	0.96	91
Area	Simple	Live-weight	Mult.	0.84	71
Area	simple	Testis weight	Mult.	0.90	96
Area	Simple	Scrotal circ.	Linear	0.93	87
Diameter	Simple	Age	Mult.	0.96	92
Diameter	Simple	Live-weight	Mult.	0.85	71
Diameter	Simple	Testis weight	Mult.	0.98	96
Diameter	Simple	Scrotal circ.	Linear	0.94	88
Tubule Lumen					
Area	Simple	Age	Linear	0.87	76
Area	Simple	Live-weight	Linear	0.68	46
Area	Simple	Testis weight	Mult.	0.94	88
Area	Simple	Scrotal circ.	Linear	0.90	80
Diameter	Simple	Age	Mult.	0.90	81
Diameter	Simple	Live-weight	Linear	0.67	45
Diameter	Simple	Testis weight	Mult.	0.92	85
Diameter	Simple	Scrotal circ.	Linear	0.90	81
Tubule Wall					
Thickness	Simple	Age	Mult.	0.86	75
Thickness	Simple	Live-weight	Mult.	0.69	48
Thickness	Simple	Testis weight	Mult.	0.90	80
Thickness	Simple	Scrotal circ.	Mult.	0.84	71
Tubules					
% LS/RS*	Simple	Age	Linear	0.87	75
% LS/RS*	Simple	Live-weight	Linear	0.80	64
% LS/RS*	Simple	Testis weight	Linear	0.90	80
% LS/RS*	Simple	Scrotal circ.	Linear	0.91	83
% LS/RS*	Simple	Tubule area	Linear	0.93	87

* = The percentage of seminiferous tubules in which late spermatids and/or released spermatozoa were observed.
Mult. = Multiplicative, $Y= ax^b$
Linear, y= a=bx

Regression of the proportion of vacuolated tubules was not considered appropriate. The majority of values were either 0 or 50, with only nine of 30 values determined being in the intervening range. Vacuolation was essentially complete by age 134 days.

Simple regressions of lumen area, diameter and tubule wall thickness were strongest against testis weight, followed by scrotal circumference, age and live-weight respectively. Regressions against age and scrotal circumference were similar and regressions against live-weight were poor.

The percentage of tubules containing late spermatids and/or released spermatozoa was similarly related to scrotal circumference and testis weight. Both of these regressions were stronger than those against either age or against live-weight. However the strongest regression was against seminiferous tubule area.

DISCUSSION.

Live-Measure Data.

This data illustrates that the Cashmere groups allocated to each slaughter time had initially similar live-weight, scrotal circumference and horn length. Thus comparisons between the slaughter groups appear valid.

The model of increasing live-weight and age was complicated by a period of slowed growth and weight loss in the older animals. Whilst pasture quality, dry matter availability and parasitic worm burden were not monitored in this trial, each probably contributed to the observed curtailment of growth in groups 4 and 5. During the latter period of the trial, a decline in feed quality and availability was subjectively observed.

A further influence on weight gain may have been the expression of male behaviour within the bachelor herd. In the breeding season (Autumn) male goats may lose appetite causing a cessation of growth or loss of weight (McDowell and Bove, 1977). Even when feed supply is adequate, Autumn growth may be slow as sexual maturity is approached (Louca et al, 1977). The amplitude of this seasonal effect on appetite is reduced in the male by castration.

The growth rates observed in this trial were poor in comparison to those characteristic of the Swiss dairy, South African Boer, Damascus and Spanish breeds. These breeds are capable of pre-weaning growth rates in excess of 200g/day (Warmington and Kirton, 1990). However, growth rates are related to mature size and this can vary up to ten-fold between different breeds of goat. Goats generally have slower growth rates than sheep, even when account is taken of relative mature size (Thoney et al, 1987).

Holst (1981) recorded the live-weight of captured Australian feral animals, the foundation stock of the Australian Cashmere type. Six tooth bucks ranged in weight from 52.1 Kg to 34.1 Kg. No weights were recorded for fully mature animals. Harrington (1982) presented data attributing live-weights of up to 66Kg in broken mouthed ferals. In an unselected herd of Cashmere goats, Restall and Pattie (1989) reported relatively slow growth until at least age four years. One year old males averaged only 16.7 Kg. The kids in this study performed better, reaching 21 Kg in 212 days. However the intact male "bush" goats of Allan and Holst (1989) were outstanding in comparison, reaching 33.7 kg live-weight in only 180 days.

Pre-weaning growth rates of 140-170 g/day have been reported for Australian Cashmere goats (Allan and Holst, 1989), although McGregor et al. (1988) recorded a slower rate of 98 g/day for intact males to weaning at four months. The latter is similar to the 102 g/day achieved by the male kids at age 133 days in this trial.

Strong curvilinear relationships of scrotal circumference and diameter with live-weight and age, have been widely reported for various breeds of sheep and goats (Bongso et al., 1982; Braun et al., 1980; Chemineau and Thimonier, 1986; Madani and Rahal, 1988; Madani et al. 1989).

In this study, scrotal circumference was more closely related to live-weight than age as has been indicated for other breeds of goat (Bongso et al., 1982; Madani et al., 1989). In bulls, Coulter and Foote (1979) suggest that age accounts for less of the observed variation in scrotal circumference, when the latter is positively correlated with both age and live-weight. The stronger correlation of scrotal circumference with live-weight observed in this study was emphasised by a departure from the positive association of live-weight with age. In Australian goats, Wolde-Michael et al. (1989) also reported a strong relation of scrotal circumference with live-weight, although the R^2 of 50% for scrota circumference with age with was poor compared with the values reported here.

Measures of scrotal circumference are of value in the prediction of testes weights, the importance of which is discussed with the slaughter data.

Horn length was better correlated with age than live-weight, in contrast to scrotal circumference, and the relationship was linear. This is similar to the pattern of horn growth reported in Libyan goats by Madani and Rahal (1988). Horn growth has been linked to sexual development in bucks.

The live-weight, scrotal circumference and horn length data indicated that the animals across the three rearing groups performed similarly.

Comparing breeds, the birth weight, live-weight up to age 76 days and scrotal circumference to age 47 days of the Angoras was similar to that of the Cashmeres. However, subsequently the Angoras had consistently lower live-weight and scrotal circumference. This may indicate that compared with the Cashmeres this group of Angoras had a different growth pattern or lower growth potential, were less competitive, or were less tolerant of the environmental stressors.

Differences in mature body size may account for the observed differences in growth rates, as the progeny of larger breeds tend to grow faster (McGregor, 1985). Mature size may vary naturally between breeds or may result from inbreeding effects. The Australian Angora herd has historically had a narrow genetic base.

Slaughter Data.

As indicated in the live measure data discussed above, rearing group had no significant effect on the data collected at slaughter. Consequently the animals were considered in terms of age at slaughter groups only.

The lack of any significant rearing group effect, and in particular any negative effects of foster rearing or early weaning, probably reflects the experimental procedure employed. Compensatory husbandry practices were associated with both the foster rearing and early weaning treatments. Specifically this involved the provision of a post weaning supplement of proprietary pelletised feed and hay to the foster reared and early weaned kids. Furthermore the foster reared kids were provided with milk replacer *ad libitum*. This management strategy was employed as a consequence of the integration of these observations within an existing trial.

In this trial the dams and female progeny were run as a fibre producing herd. The male progeny examined here were subject to the normal management of the research farm, with the exception that they were not castrated. As a consequence, the animals were not managed to attain maximal growth and carcase development. As a result the growth of the animals studied was substantially lower than that reported in some of the literature.

Data collected at slaughter indicated that live-weight, scrotal circumference, testis weight, testis dimensions, the fasted or empty weight and carcase weight of the Cashmeres increased up to age 214 days. At age 246 days the slaughtered Cashmeres had lower live-weight, carcase and testis measures than the preceding age group. The live-measure data for this group confirm that these parameters declined over this period and did not result from a consistently poorer growth.

Across all groups, testis weight was more related to live-weight than age. These differences were most apparent as a result of a departure from the positive association of live-weight with age in the last two slaughter groups. However, reduced testes weight was proportionally less than the loss of live-weight. This indicates either that the reproductive material was buffered from the regression of the other body tissues, or that there was a time lag in expression at the testis level. In contrast, the increase in horn length was not effected and was strongly correlated with age across all groups.

The sigmoidal increase in testes weight typified by a period of rapid growth has also been widely reported in sheep and goats (Madani and Rahal, 1988; Monet-Kuntz et al., 1984; Skinner, 1975; Watson et al., 1956). Although following this model, the data presented here was not collected frequently enough to fully characterise this growth phase in the Cashmere animals studied.

The highly significant relationship between scrotal circumference and testis weight provides further evidence of the value of this parameter as an index of testicular development or growth. Wolde-Michael et al. (1989) reported an R^2 of 96% for this relationship, slightly stronger than the 91% observed in this trial. Indeed scrotal circumference measures are accepted as accurate predictors of testicular weight and are highly repeatable between technicians (Coulter and Foote, 1979).

There were equally strong relationships of left testis weight and total testis weight with scrotal circumference. This validates the use of correlations of left testis parameters against scrotal circumference in the histology study.

The testis dimensions measured indicated that there was no change in testis shape with increasing size. This information gives confidence in the association between measures of scrotal circumference and testis weight across a range of weights.

Changes in carcase weight with slaughter group reflected those in live-weight. Carcase development for this group of Cashmere kids was generally poor. The GR site measures were variable and probably inappropriate for the younger slaughter groups. In the latter, location of a standard measurement site on the very small carcase was questionable. Changes in the measures of the eye muscle dimensions were also variable, although they may have indicated declining body condition in groups 4 and 5.

Angoras slaughtered at a similar age to comparably run Cashmeres had significantly lower live-weight, testis and carcase measures. This data probably indicates that the Angoras had lower growth potential and vigour than the Cashmeres.

The exceptions in this breed comparison were that there was no significant difference in horn growth, and that GR of the Angoras was significantly greater. The Angoras also had a more obvious subcutaneous fat cover. These observations were made despite the lower live-weight of the Angoras, indicating differential growth and development. However, the relationship of testis weight with live-weight was similar between the two breeds.

Histology: Seminiferous tubule development in Cashmere kids.

Seminiferous Tubule size.

Whilst seminiferous tubule growth and development has commonly been characterised using light microscopy (Skinner et al., 1968; Watson et al., 1956), there are also precedents for the use of image analysis equipment in the examination of testicular tissue (Harayama et al., 1991) as used in this study.

The increases in mean area and diameter of the seminiferous were significant between all slaughter groups, except groups 4 and 5. Thus, the cross-sectional size of the seminiferous tubules increased significantly up to a mean age of 215 days. However, the trends suggest that tubule expansion continued across all five age groups.

Rapid tubule expansion was observed in animals between slaughter ages 45 and 134 days, although the greatest increase occurred between ages 45 and 97 days. By age 134 days, live-weight 16.8 Kg and a left testis weight of 46 g, the greatest proportion of tubule expansion had occurred. At this time the mean area of the seminiferous tubules was 80% of the maximum observed mean. Thus, most age or weight related effects on tubule size had been expressed by this stage. Live-weight however, increased significantly up to group 4, at age 215 days and mean live-weight 21.2 Kg. Indeed the largest weight and age differentials between slaughter groups were recorded over this period.

Similar rapid seminiferous tubule expansion has been reported in the literature. Watson et al. (1956) observed that seminiferous tubule expansion was sigmoidally related to live-weight and curvilinearly related to testes weight. The data collected in this trial followed a similar pattern although over a smaller range of values. In the former study growth continued up to a testes weight of 300 to 400 g. Bongso et al. (1982) regarded the sudden increase in the size of the seminiferous tubules as the point of initiation of spermatogenesis.

The attenuation of seminiferous tubule expansion at these relatively low testes weights may have been caused by the check in growth experienced by the kids slaughtered in groups 4 and 5. This may have delayed tubule development and altered the association with increasing live-weight.

However, the repeat measures of live-weight (table 7.9) indicate that further substantial live-weight increases occurred prior to the check in growth. Despite these increases in live-weight, the expansion of the seminiferous tubules attenuated. This probably reflects a slowing of tubule expansion with physiological maturity, rather than purely a reduction mediated by slowing growth rate.

The confounding of slowed tubule expansion with reduced growth and increasing age was greatest in kids slaughtered in group 5. Seminiferous tubule size increased slightly, despite the reduction in live-weight with increasing age. However, neither

the increase in seminiferous tubule size or the decrease in live-weight were significant between groups 4 and 5.

The kids slaughtered in group 5 lost an average of 2.6 Kg in the four weeks prior to slaughter. The mean tubule size observed in group 5 kids was associated with the same maximum mean live-weight as for kids slaughtered in group 4 and was resistant to the decline in live-weight.

This translates as a stronger association of seminiferous tubule expansion with age than live-weight. However, the strongest correlation was with testis weight. Watson et al. (1956) found that the general histology of the testes was closely related to testis weight and live-weight, although tubule size was more related to testes weight and live-weight than to age.

In the present study, the stronger correlation of tubule size with age than live-weight was due to the decline in live-weight whilst tubule area increased slightly or remained unchanged. Bongso et al (1982) reported a similarly strong association with age.

The stronger relationship of seminiferous tubule area with testis weight indicates that live-weight and age effects on seminiferous tubule development were probably mediated through effects on testis development.

Vacuolation.

The proportion of vacuolated tubules increased significantly over the period of greatest tubule expansion and testis weight increase. Vacuolation of the seminiferous tubules was completed between the mean ages of 97 and 134 days, and mean live-weights of 14.4 and 16.8 Kg. Further increases in mean seminiferous tubule size past age 134 days occurred as a result of the expansion of already vacuolated tubules.

Lumen size.

The differences in mean lumen area of the seminiferous tubules between successive slaughter groups were less distinct than the differences in tubule size. Although mean lumen area increased with each successive group, significant differences were only observed between groups 2 and 3, and between groups 3 and 4. In contrast, measures

of lumen diameter were less variable and the means were significantly different between groups, except 4 and 5. However, these measures of lumen size should be considered in conjunction with the occurrence of vacuolation.

In the early stages of vacuolation the lumen boundary was highly irregular, with the lumen extending out from the tubule centre in a star shape. More developed tubules had regular oval lumen boundaries.

Lumen size increased between groups 4 and 5 (though not significantly), despite a trend of declining live-weight and no change in testis weight. This suggests that tubule development continued over this period. The differences in lumen size between the age groups are indicative of continued tubule development.

As with tubule size, the lumen area and diameter were best correlated against testis weight and age. The correlation against live-weight was poor.

Wall Thickness.

The increases in seminiferous tubule size and lumen area after complete vacuolation were not associated with a significantly thicker seminiferous epithelium. Continued functional development of the tubules is indicated though from increasing lumen size.

As with other measures of testis histology, tubule wall thickness was best correlated against testis weight and age.

Presence of Late Spermatids and Released Spermatozoa.

Observations of spermatozoa or spermatids present within the seminiferous tubules have been used in puberty studies in sheep and goats (Wolde-Michael et al., 1989; Madani and Rahal, 1988; Bongso et al., 1982; Skinner et al, 1968). In this study, the percentage of tubules in which late spermatids and released spermatozoa were observed was used to indicate the presence of an active spermatogenic cycle. Tubules were scored for the presence of either spermatozoa within the tubule lumen, or differentiated spermatids arranged around the seminiferous epithelium. The presence and % of sperm and spermatids has previously been used to describe the stage of pubertal development (Wolde-Michael, 1987; Watson et al, 1956).

In the final stages of spermatogenesis, the initially round spermatids undergo spermiogenesis to assume the morphology of mature spermatozoa, and are arranged as a single layer around the inner surface of the seminiferous tubules (Bilaspuri and Guraya, 1984; Amann and Schanbacher, 1983; Ortavant et al., 1977; appendix 4). The release of mature spermatozoa from the seminiferous epithelium constitutes an end point of the spermatogenic cycle, although tubules containing spermatids with condensed and elongate nuclei are also regarded as exhibiting complete spermatogenesis (Lunstra and Echternkamp, 1988; Adams and Steiner, 1988).

An active spermatogenic cycle was first observed in the animals with a mean age of 134 days and a mean live-weight of 16.8 Kg, and subsequently in all older animals. The percentage of tubules in which the spermatids or spermatozoa were observed, increased significantly between animals aged 134 days, and both 215 and 247 day age groups. However, the animals aged 215 days had a higher percentage of active tubules than kids aged 247 days, although this difference was not significant.

The slightly lower (not significant) percentage of active tubules in the animals at mean age 247 days was thus associated with a significantly lower live-weight, but only slightly lower (not significant) mean testis weight, compared to those kids at age 215 days. Whilst all other measures of the seminiferous tubules tended to increase over this period, (though not significantly), it would appear that spermatogenic activity was negatively effected by the decline in live-weight.

Regression analyses indicated that the percentage of tubules in which late spermatids and released spermatozoa were observed was best correlated against testis weight, scrotal circumference and age than live-weight. In this trial, the percentage of active tubules were better correlated against age and live-weight than the R^2 of 55% reported by Wolde-Michael et al. (1989).

Other regressions indicated a very strong correlation (0.93) of active tubules against seminiferous tubule area, but a lower correlation (0.79) against tubule lumen area.

The expression of an active spermatogenic cycle was reached by a lower testis weight of approximately 46 g, an age of approximately 134 days, and a live-weight of 16 Kg. Puberty defined by 70% active seminiferous tubules, was reached at a testis weight of 66 g, age 214 days and live-weight 21 Kg. Madani and Rahal (1988) observed elongated spermatids at a lower testis weight of 29 g, whilst Wolde-Michael et al.

(1989) characterised puberty at approximately 15.5 Kg, or 40% of the dams mature weight. Allan and Holst (1989) commented that puberty in goats can be reached as early as 112 days old, although Bongso et al. (1982) observed complete spermatogenesis at 210 days. Skinner (1975) observed spermatozoa in the epididymis of Boer goats by age 120 days. Puberty in the present study was observed at a stage comparable to some of the literature.

Growth and reproductive development and activity are intimately associated and can be retarded by low nutrition after weaning (Brien, 1986; Foster et al., 1985). The latter may be related to low LH pulse frequency and amplitude (Suttie et al., 1991). Pretorious et al. (1968) observed that penis development, testes descent and puberty (assessed as the observation of sperm in the ejaculate) were retarded for 4 weeks by poor nutrition. With regard to the generally poor growth observed in the present trial, it is possible that puberty in these kids could have been further advanced.

Conclusion.

Scrotal circumference proved an accurate predictor of testis weight. Testis weight and scrotal circumference were both strongly correlated with live-weight and age. The large data set of live-measures indicated that scrotal circumference was more related to live-weight than to age. Total testes weight collected at slaughter was similar. However, left testis weight was better correlated with age in the histology study, and consequently so was the seminiferous tubule data. Differences between the relationships with age and weight were generally small.

Rearing group had no significant effect on growth and development, although it seems likely that there were other limits.

Angoras performed poorly in most regards compared with Cashmere kids studied.

Seminiferous tubule development continued to some degree across all five age at slaughter groups. Increases in the size of the seminiferous tubules and testis weights were greatest over ages 45 to 134 days. This indicates that tubule expansion was greatest up to a mean age of 19 weeks and slowed up to a mean age of 35 weeks. After 19 weeks there was no increase in the thickness of the seminiferous epithelium.

In contrast to the physical measures of tubule development, the observation of a functional spermatogenic cycle appeared to be more affected by live-weight than age. This was evidenced by the drop in the percentage of tubules with late spermatids and released sperm between ages 215 and 247 days. This coincided with a fall in live-weight that slightly affected testis weight, but appeared to have no deleterious effect on the other tubule measures. Testis weight appeared to be buffered in some part from the decline in live-weight.

Spematogenic activity was first apparent at age approximately 134 days, live-weight 16 Kg, and a testis weight of 46 g, corresponding to a scrotal circumference of 18 cm.

8. References.

Aamdal, J., Lyngset, O. and Fossum, K. (1965). Toxic effect of lysolecithin on sperm. Nordisk Vet.-Med., 17: 633-634.

Adams, L.A. and Steiner, R.A. (1988). Puberty. In "Oxford Review of Reproductive Biology, Vol. 10". (Ed. J.R.Clarke) pp. 1-52.

Alacam, E., Oszar, S., Kilicoglu, C., Guven, B., Izgur, H., Tekeli, T. and Glatzel, P. (1985). Induction of oestrus in Saanen goats at early breeding season by intravaginal progesterone sponges (MAP) or by prostaglandin F2 injections. Effect on different age groups. Theriogenology, 24: 283-291.

Allison, A.J. and Robinson, T.J. (1971). Fertility of progestagen-treated ewes in relation to the numbers and concentration of spermatozoa in the inseminate. Australian Journal of Biological Science, 24: 1001-1008.

Allan, C. J. and Holst, P.J. (1989). Comparison of growth and dressing percent between intact male, castrated male, and female kids of Australian Bush goats. Small Ruminant Research, 2: 63-68.

Amann, R.P. (1987). Function of the epididymis in bulls and rams. Journal of Reproduction and Fertility, Supp. 34: 115-131.

Amann, R.P. and Schanbacher, B.D. (1983). Physiology of male reproduction. Journal of Animal Science, 57 (Supp. 2): 380-403.

Amoah, E.A. and Bryant, M.J. (1984). Effect of pattern of lighting and time of birth on the occurrence of puberty in female goat kids. Animal Production, 38: 83-89.

Anand, S.R. (1973). The carbohydrates of buffalo and goat semen. Journal of Reproduction and Fertility, 32: 97-100.

Andersen, K. (1969). Insemination with frozen semen in goats. Nordisk Vet.-Med., 21: 625-628

Andersen, K. and Aamdal, J. (1972). Artificial insemination with frozen semen in sheep in Norway. World Review of Animal Production, 8: 77-79

Anderson, G.B. (1977). Fertilisation, early development, and embryo transfer. In "Reproduction in domestic animals". (Eds. C.R. Austin and R.V. Short) Third ed. pp. 286-314. Academic Press.

Anzar, M., Hassan, M.M., Graham, E.F., Deyo, R.C.M. and Sing, G. (1991). Efficacy of the Hamilton Thorn motility analyser (HTM-2030) for the evaluation of bovine semen. Theriogenology, 36: 307-317.

Ashdown, R.R. and Hancock, J.L. (1974). Functional anatomy of male reproduction. In "Reproduction in Farm Animals". (Ed. E.S.E. Hafez) pp. 3-23. Lea and Febiger, Philidelphia.

Atreja, S.K. and Anand, S.R. (1985). Phospholipase and lysophospholipase activities of goat spermatozoa in transit from the caput to the cauda epididymis. Journal of Reproduction and Fertility, 74: 687-691.

Austin, J.W., Leidy, R.B., Krise, G.M. and Hupp, E.W. (1968). Normal values for semen collected from Spanish goats by two methods. Journal of Applied Physiology, 24: 369-372.

Baas, J.W., Molan, P.C. and Shannon, P. Factors in seminal plasma of bulls that affect the viability and motility of spermatozoa. (1993). Journal of Reproduction and Fertility, 68: 275-280.

Baird, D.T. (1975). Reproductive hormones. In "Reproduction in Mammals, 3, Hormones in Reproduction". (Eds. C.R. Austin and R.V. Short) pp. 1-29. Cambridge University Press.

Baishya, G., Bhattacharya, M. and Ray, T.C. (1987). A correlative note on testis of local kids of Assam. Indian Veterinary Journal, 64: 989-990.

Beckett, S.D., Reynolds, T.M. and Bartels, J.E. (1978). Angiography of the crus penis in the ram and buck during erection. American Journal of Veterinary Research, 39: 1950-1954.

Bedford, J.M. (1982). Fertilisation. In "Reproduction in mammals: 1 Germ cells and fertilisation. (Eds. C.R. Austin and R.V. Short) Second edition, pp. 128-163. Cambridge University Press.

Berger, T. (1989). Development of a zona-free hamster ova bioassay for goat sperm. Theriogenology, 32: 69-77.

Berger, T. (1990). Pisum sativum agglutinin used as an acrosomal stain of porcine and caprine sperm. Theriogenology, 33: 689-695.

Betteridge, K.J. (1986). Increasing productivity in farm animals. In "Reproduction in Mammals, Volume 5 Manipulating Reproduction". (Eds. C.R. Austin and R.V. Short) Second Edition, pp. 1-47. Cambridge University Press.

Bilaspuri, G.S and Guraya, S.S. (1984). The seminiferous epithelial cycle and spermatogenesis in goats (*Capra hircus*). Journal of Agricultural Science, Cambridge, 103: 359-368.

Blokhuis, J. (1962). Artificial insemination in the goat. In "The Semen of Animals and Artificial Insemination". (Ed. G.P. Maule). Commonwealth Agricultural Bureau.

Bongso, T.A., Jainudeen, M.R. and Siti Zahrah, A. (1982). Relationship of scrotal circumference to age, body weight and onset of spermatogenesis in goats. Theriogenology, 18: 513-524.

Borgohain, A.C., Deka, B.C. and Rajkonwar, C.K. (1985). Preservation of buck semen. Indian Veterinary Journal, 62: 81-82.

Bousquet, D. and Brackett, G. (1982). Penetration of zona-free hamster ova as a test to assess fertilising ability of bovine sperm after frozen storage. Theriogenology, 17: 199-213.

Bowen, G.M. (1988). Experiences with artificial insemination in goats. Proceedings of the New Zealand Society of Animal Production, 48: 65-67.

Braun, W.F., Thompson, J.M. and Ross, C.V. (1980). Ram scrotal circumference measurements. Theriogenology, 13: 221-229.

Bretzlaff, K.N. and Madrid, N. (1985). Synchronisation of estrus and fertility in goats with norgestomet ear implants. Theriogenology, 24: 351-357.

Brien, F.D. (1986). A review of the genetic and physiological relationships between growth and reproduction in mammals. Animal Breeding Abstracts, 54: 975-997.

Cameron, A.W.N. and Battye, K.M. (1988). PMSG may directly stimulate ovulation in goats. Proceedings of the 20th Annual Conference of the Australian Society for Reproductive Biology, 20: 81.

Cameron, A.W.N., Battye, K.M. and Trounson, A.O. (1988). Time of ovulation in goats (Capra Hircus) induced to superovulate with PMSG. Journal of Reproduction and Fertility, 83: 747-752.

Chakraborty, P.K., Stuart, L.D. and Brown, J.L. (1989). Puberty in the male Nubian goat: serum concentrations of LH, FSH and testosterone from birth through puberty and semen characteristics at puberty. Animal Reproduction Science, 20: 91-101.

Chemineau, P. (1983). Effect on oestrous and ovulation of exposing Creole goats to the male at three times of the year. Journal of Reproduction and Fertility, 67: 65-72.

Chemineau, P. (1986). Sexual behaviour and gonadal activity during the year in the tropical Creole meat goat, II male mating behaviour, testis diameter, ejaculate characteristics and fertility. Reproduction, Nutrition and Development, 26: 453-460.

Chemineau, P. (1987). Possibilities of using bucks to stimulate ovarian and oestrous cycles in anovulatory goats, a review. Livestock Production Science, 17: 135-147.

Chemineau, P. and Thimonier, J. (1986). Methods for evaluation of reproductive and growth-rate performance in sheep and goats. World Review of Animal Production, 22(4): 27-33.

Chemineau, P.; Martin, G.B.; Saumande, J. and Normant, E. (1988a). Seasonal and hormonal control of pulsatile LH secretion in the dairy goat (Capra hircus). Journal of Reproduction and Fertility, 83: 91-98.

Chemineau, P., Pelletier, J., Guerin, Y., Colas, G., Ravault, J.P., Toure, G., Almeida, G., Thimonier, J. and Ortavant, R. (1988b). Photoperiodic and melatonin treatments for the control of seasonal reproduction in sheep and goats. Reproduction, Nutrition and Development, 28: 409-422.

Colas, G. (1975). Effect of initial freezing temperature, addition of glycerol and dilution on the survival and fertilising ability of deep-frozen ram semen. Journal of Reproduction and Fertility, 277-285.

Cookson, A.D., Thomas, A.N. and Foulkes, J.A. (1984). Immunochemical investigation of the interaction of egg-yolk lipoproteins with bovine spermatozoa. Journal of Reproduction and Fertility, 70: 599-604.

Corteel, J.M. (1974). Viabilite des spermatezoides de bouc conserves et congeles avec ou sans leur plasma seminal: effet du glucose. Ann. Biol. Anim. Bioch. Biophys., 14: 741-745.

Corteel, J.M. (1981). Collection, processing and artificial insemination of goat semen. In "Goat Production". (Ed. C. Gall) pp. 171-191, Academic Press.

Corteel, J.M., Barel, G. and Leboeuf, B. (1987). Development and application of artificial insemination with deep frozen semen and out-of-season breeding of goats in France. Proceedings of the Fourth International Conference on Goats, March 8-13, 1987, Brasilia, Brasil, Vol. 1: 523-547.

Corteel, J.M., Baril, G., Leboeuf, B. and Nunes, J.F. (1984). Goat semen technology. In "The Male in Farm Animal Reproduction", (Ed. M. Courot). pp. 237-256. Martinus Nijhoff, The Netherlands.

Corteel, J.M., Leboeuf, B. and Baril, G. (1988). Artificial breeding of adult goats and kids induced to ovulate with hormones outside the breeding season. Small Ruminant Research, 1: 19-35.

Coulter, G.H. and Foote, R.H. (1979). Bovine testicular measurements as indicators of reproductive performance and their relationshop to productive traits in cattle: a review. Theriogenology, 11: 297-311.

Courot, M. and Ortavant, R. (1981). Endocrine control of spermatogenesisin the ram. Journal of Reproduction and Fertility, Supp. 30: 47-60.

Courtens, J.L., Nunes, J.F. and Corteel, J.M. (1984). Induction of the acrosome reaction in the spermatozoa of the goat by secretions of the male accessory glands and milk. Gamete Research, 18: 191-199.

Cummins, J.M. (1982). Hyperactivated motility patterns of ram spermatozoa recovered from the oviducts of mated ewes. Gamete Research, 6: 53.

Davis, A.P. and Foote, R.H. (1987). Relationship of sire fertility to acrosome-reacted motile sperm after treatment with liposomes. Journal of Dairy Science, 70: 850-857.

de Kretser, D.M. (1990). Germ cell-Sertoli cell interactions. Reproduction Fertility and Development, 2: 225-235.

Deka, B.C. and Rao, A.R. (1986). Effect of glycerol level in tris-based extender and equilibration period on quality of frozen goat semen. Theriogenology, 26: 231-238.

Deka, B.C. and Rao, A.R. (1987a). Effect of cooling time on quality of frozen goat semen. Indian Journal of Animal Reproduction, 8: 25-27. Animal Breeding Abstracts, 56: 4495.
Deka, B.C. and Rao, A.R. (1987b). Effect of extenders and thawing methods on post thawing preservation of goat semen. Indian Veterinary Journal, 64: 591-594. Animal Breeding Abstracts, 57: 120.

Devendra, C. and Burns, M. (1970). In "Goat Production in the Tropics, Technical Communication No. 19". Commonwealth Agricultural Bureau.

Doney, J.M., Gunn, R.G. and Horak (1982). Reproduction. In " World Animal Science, Production System Approach, Sheep and Goat Production". (Ed. I.E. Coop) pp. 57-80. Elesvier Scientific Publishing Co.

Dooley, M.P., Pineda, M.H., Naurer, R.R. and Lunstra, D.D. (1986). Evidence for retrograde flow of spermatozoainto the urinary bladder of bulls during electroejaculation. Theriogenology, 26: 101-109.

Drobnis, E.Z., Nelson, E.A. and Burrill, M.J. (1980). Effect of several processing factors on motility and glutamic oxalacetic transaminase levels for frozen goat semen, III glycerol level. Journal of Animal Science, supp. 51: 439.

Drobnis, E.Z., Nelson, E.A. and Lin, Y.T. (1982). The effect of freezing rate on motility and GOT release of frozen goat spermatozoa. Proceedings of the Third International Conferance on Goat Production and Disease, University of Arizona, Tuscon, Arizona, January 10 to 15, pp. 282.

Dun, R.B. (1955). Puberty in Merino rams. The Australian Veterinary Journal, 31: 104-106.

Eaglesome, M.D. and Miller, S.A. (1989). Prediction of fertility of bovine semen: Preliminary studies with the hamster egg penetration test. Theriogenology, 31: 643-652.

Earle, C., McPhee, S., Williams, A., Dunstan, E., Tilbrook, A., Ayton, B. and Staples, L. (1987). Effect of melatonin treatment on the early reproductive performance of Angora bucks and does. Australian Society for Reproductive Biology, 19: 25.

Eaton, O.N. and Simmons, V.L. (1952). A semen study of goats. American Journal of Veterinary Research, 13: 537-544.

Edey, T.N. (1983). "A Course Manual in Tropical Sheeep and Goat Production. Australian Vice Chancellors Committee. AUIDP Canberra.

Elwishy, A.B. and Elsawaf, S.A. (1971). Development of sexual activity in male Damascus goats. Indian Journal of Animal Science, 41: 350-56.

Elwishy, A.B., Elsawaf, S.A., Elmikkawi, F. and Omar, A.A. (1971). Monthly and seasonal variation in sexual activity of male Damascus goats. Indian Journal of Animal Science, 41: 562-569.

Evans, G. (1987). Semen processing. The University of Sydney Post-Graduate Committee in Veterinary Science Refresher Course for Veterinarians Proceeedings No. 96, Artificial Breeding in Sheep and Goats. pp. 1-6.

Evans, G. and Maxwell, W.M.C. (1987). "Salamon's Artificial Insemination of Sheep and Goats". Butterworths, Sydney.

Fielden, E.D. and Barker, C.A.V. (1964). Semen collection studies and sperm reserves in the goat. Proceedings of the 5th International Congress of Animal Reproduction and Artificial Insemination, 3: 488-497.

Findlater, R.C., Haresign, W., Curnock, R.M. and Beck, N.F.G. (1991). Evaluation of intrauterine insemination of sheep with frozen semen: effects of time of insemination and semen dose on conception rates. Animal Production, 53: 89-96.

Fiser, P.S., Ainsworth, L. and Fairfull, R.W. (1987). Evaluation of a new diluent and different processing procedures for cryopreservation of ram semen. Theriogenology, 28: 599-567.

Fiser, P.S. and Fairfull, R.W. (1986). Combined effects of glycerol concentration, cooling velocity, and osmolarity of skim milk diluents on cryopreservation of ram spermatozoa. Theriogenology, 25: 473-484.

Fiser, P.S., Fairfull, R.W. and Marcus, G.J. (1986). The effect of thawing velocity on survival and acrosome integrity of ram spermatozoa frozen at optimal and suboptimal rates in straws. Cryobiology, 23: 141-149.

Flick, D.L. and Merilan, C.P. (1988). Toxicity of artificial vagina liners for bovine spermatozoa. Theriogenology, 29: 1207-1213.

Foote, R.H. (1988). Preservation and fertility prediction of spermatozoa. The 11th International Congress on Animal Reproduction and Artificial Insemination, 5: 126-134.

Foote, R.H. (1990). Use of reproductive biotechnologies to solve problems of reproduction in farm animals. Fifth AAAP 1990, 2: 282-304.

Foster, D.L., Mickelson, I.H., Ryan, D.D., Coon, G.A., Drongowski, R.A. and Holt, J.A. (1978). Ontogeny of pulsatile luteinizing hormone and testoterone secretion in male lambs. Endocrinology, 102: 1137-1146.

Foster, D.L., Yellon, S.M. and Olster, D.H. (1985). Internal and external determinants of the timing of puberty in the female. Journal of Reproduction and Fertility, 75: 327-344.

Fraser, A.F. (1962). A technique for freezing goat semen and results of a small breeding trial. Canadian Veteriary Journal, 3: 133-144.

Galli, A., Basetti, M., Balduzzi, D., Martignoni, M., Bornaghi, V. and Maffii, M. (1991). Frozen bovine semen quality and bovine cervical mucus penetration test. Theriogenology, 35: 837.

Gifford, D.R. (1982). The Angora goat. In "Goats for Meat and Fibre in Australia-SCA Technical Report Series No. 11". pp. 74-103. CSIRO.

Gomes, W.R. (1977). Artificial insemination. In "Reproduction in Domestic Animals". (Eds. H.H. Cole and P.T. Cupps) Third ed. pp. 257-285. Academic Press.

Gower, D.B. (1979). "Steroid Hormones". Croom-Helm, London.

Hahn, R. (1972). A contribution to the deep-freezing-preservation of goatbuck and ram semen. World Review of Animal Production, 8: 80.

Haley, C.S., Lee, G.J., Ritchie, M. and Land, R.B. (1990). Direct responses in males and correlated responses for reproduction in females to selection for testicular size adjusted for body weight in young male lambs. Journal of Reproduction and Fertilty, 89: 383-396.

Hamilton, P.A. and Killeen, I.D. (1987). A comparison of ram semen frozen in straws to that frozen in pellets and the effect of PMSG on fertility. Department of Agriculture South Australia; Artificial Breeding of Sheep with Frozen Semen Workshop, Charles Hawker Conference Centre, Waite Institute, Glen Osmond, South Australia, (Ed. W.M.C. Maxwell) pp. 12-15.

Harayama, H. Nanjo, I., Kanda, S. and Kato, S. (1991). Testicular development in Chinese Meishan boars. Theriogenology, 36: 637-643.

Harper, M.J.K. (1986). Sperm and egg transport. In "Reproduction in Mammals, Volume I Germ Cells and Fertilisation". (Eds. C.R. Austin and R.V. Short) Second Edition, pp. 102-127. Cambridge University Press.

Harrison, R.A.P. and Vickers, S.E. (1990). Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. Journal of Reproduction and Fertility, 88: 343-352.

Hawk, H.W. (1983). Sperm survival and transport in the female reproductive tract. Journal of Dairy Science, 66: 2645-2660.

Hochereau-de Reviers, M.T., Monet-Kuntz, C. and Courot, M. (1987). Spermatogenesis and Sertoli cell numbers and function in rams and bulls. Journal of Reproduction and Fertility, Supp. 34: 101-114.

Hochereau-de Reviers, M.T., Perreau, C. and Lincoln (1985). Photoperiodic variations of somatic and germ cell populations in the Soay ram testis. Journal of Reproduction and Fertility, 74:329-334.

Hogarth, P.J. (1978). Male Reproduction. In "Biology of Reproduction". pp. 8-35. Blackie, Glasgow-London.

Holst, P.J. (1981). Age, hair, colour, liveweight and fertility of two samples of Australian feral goat (Capra hircus). Australian Wildlfe Research, 8: 549-553.

Holt, N. (1987). Semen collection. The University of Sydney Post-Graduate Committee in Veterinary Science Refresher Course for Veterinarians Proceeedings No. 96, Artificial Breeding in Sheep and Goats. pp. 25-30.

Hunter, R.H.F. (1986). Transport, sequestration and activation of spermatozoa in relation to the time of ovulation in farm animals. World Review of Animal Production, 22: 85-89.

Hunton, J. (1987). Pregnancy rates following intra-uterine insemination with pellet or straw frozen ram semen. Department of Agriculture, South Austalia, Artificial Breeding of Sheep with Frozen Semen Workshop, Charles Hawker Conference Centre Waite Institute , Glen Osmond, South Australia 29-30 January 1987. (Ed. W.M.C. Maxwell) pp. 16-24.

Hunton, J.R. (1988). Frozen storage of ram spermatozoa in straws. Australian Association of Sheep and Goat Artificial Breeders; Proceedings of the Inaugural Meeting, Newcastle NSW, August 28 1988, pp. 7-9.

Hunton, J.R., Flecker, S.E. and Maxwell, W.M.C. (1987). Pregnancy rates following intra-uterine insemination with pellet or straw frozen ram semen. Journal of Agricultural Science Cambridge, 109: 189-191.

Iritani, A. and Nishikawa, Y. (1964a). Studies on the egg-yolk coagulating enzyme in goat semen, VI on the chemical properties of the ejaculated semen and the secretion of accessory sexual organs in the goat. Japanes Journal of Animal Reproduction, 10: 44-51.

Iritani, A. and Nishikawa, Y. (1964b). Studies on the egg yolk coagulating enzyme in goat semen, VIII the enzyme activity of the semen collected by electical stimulation and the semen depleted by the artificial vagina. Japanese Journal of Animal Reproduction, 10: 57-62.

Ishwar, A.K. and Pandey, J.N. (1990). Estrus Synchronisationand fertility behaviour in Black Bengal goats following either progesterone or prostaglandin treatment. Theriogenology, 34: 1015-1024.

Jabbour, H.N. and Evans, G. (1991). Fertility of superovulated ewes following intrauterine or oviductal insemination with fresh or frozen-thawed semen. Reproduction Fertility and Development, 3: 1-7.

Jansen, G.J., van Eerten, M.T.W. and Forrester, I.T. (1982). Biochemical events associated with Ficoll washing of ram spermatozoa. Proceedings of the New Zealand Society of Animal Production, 42: 95-97

Jegou, B., Dacheux, J.L., Garnier, D.H., Terqui, M., Colas, G. and Courot, M. (1979). Biochemical and physiological studies of androgen-binding protein in the reproductive tract of the ram. Journal of Reproduction and Fertility, 57: 311-318.

Jindal, S.K. (1984). Sperm concentration in different segments of the goat epididymis. Theriogenology, 22: 545-549.

Killeen, I.D. and Caffery, G.J. (1982). Uterine insemination of ewes with the aid of a laparoscope. Australian Veterinary Journal, 59: 95.

Killeen, I.D., Caffrey, G.J. and Holt, N. (1982). Fertility of ewes following intra-uterine insemination with the aid of a laparoscope. Proceedings of the Australian Society of Reproductive Biology, 14: 104.

Knight, C.H., Wilde, C.J., Mcleod, B.J. and Haresign, W. (1988). Exogenous GnRH induces ovulation in seasonally anoestrous lactating goats (Capra hircus). Journal of Reproduction and Fertility, 83: 679-686.

Kupferschmied, H. (1972). Experiments with AI in goats in Switzerland. World Review of Animal Production, 8: 81.

Larsen, R.E., Littell, R., Rooks, E., Adams, E.L., Falcon, C. and Warnick, A.C. (1990). Bull influences on conception percentage and calving date in Angus, Hereford, Brahman and Senepol single-sire herds. Theriogenology, 34: 549-556.

Lawrenz, R. (1986). Artificial insemination of Angora and Boergoats with deep-frozen semen. Journal of South African Veterinary Association, June 1986: 109-111.

Lawson, J.L., Forrest, D.W. and Shelton, M. (1984). Reproduction response to suckling manipulation in Spanish goats. Theriogenology, 21: 747-755.

Lightfoot, R.J. (1968). Studies on the number of ewes joined per ram for flock matings under paddock conditions: II The effect of mating on semen characteristics. Australian Journal of Agricultural Research, 19: 1043-1057.

Lightfoot, R.J. and Restall, B.J. (1971). Effects of site of insemination, sperm motility, and genital tract contractions on transport of spermatozoa in the ewe. Journal of Reproduction and Fertility, 26: 1.

Lightfoot, R.J. and Salamon, S. (1969a). Freezing ram spermatozoa by the pellet method: II The effects of method of dilution, dilution rate, glycerol concentration, and duration of storage at 5° C prior to freezing on survival of spermatozoa. Australian Journal of Biolical Sciences, 22: 1547-1560.

Lightfoot, R.J. and Salamon, S. (1969b). Freezing ram spermatozoa by the pellet method: III The effects of pellet volume, composition of the thawing solution, and reconcentration of the thawed semen on survival of spermatozoa. Australian Journal of Biological Science, 22: 1561-1572.

Lightfoot, R.J. and Salamon, S. (1970). Fertility of ram spermatozoa frozen by the pellet method: 1 Transport and viability of spermatozoa within the genital tract of the ewe. Journal of Reproduction and Fertility, 22: 385-398.

Lino, B.F. (1972). The output of spermatozoa in rams. Australian Journal of Biological Science, 25: 359-366.

Louca, A., Economides, S. and Hancock, J. (1977). Effects of castration on growth rate, feed conversion efficiency and carcass quality in Damascus goats. Animal Production, 24: 387-391.

Lunstra, D.D. and Echternkamp, S.E. (1988). Repetitive testicular biopsy in the ram during pubertal development. Theriogenology, 29: 803-810.

Lyngset, O., Aamdal, J. and Velle, W. (1965). Artificial insemination in the goat with deep frozen and liquid semen after hormonal synchronisation of oestrus. Nord. Vet-Med, 17: 178-81.

Madani, M.O.K. and Rahal, M.S. (1988). Puberty in Libyan male goats. Animal Reproduction Science, 17: 207-216.

Madani, M.O.K., Rahal, M.S., Zawia, M.T. and Eluwhaishi, B.A. (1989). Puberty and early sexual development in Libyan fat-tailed ram lambs. British Veterinary Journal, 145: 276-288.

Majumder, G.C. and Chakrabarti, C.K. (1984). A simple spectrophotometric method of assay of forward motility of goat spermatozoa. Journal of Reproduction and Fertility, 70: 235-241.

Mann, T. and Lutwak-Mann, C. (1981). "Male Reproduction and Function". Springer-Verlag.

Matthew, J., Raja, C.K.S.V. and Nair, K.P. (1984). Preservation of buck semen in Tris yolk diluent. Indian Veterinary Journal, 61: 964-968. Animal Breeding Abstracts, 53: 564.

Mattner, P.E. and Voglmayr, J.K. (1962). A comparison of ram semen collected by the artificial vagina and by electro-ejaculation. Australian Journal of Experimental Agriculture and Animal Husbandry, 2: 78-88.

Maxwell, W.M.C. (1984). Current problems and future potential of artificial insemination programmes. In "Reproduction in Sheep". (Eds D.R. Lindsay and D.T Pearce) pp. 291-298. Australian Academy of Science and Australian Wool Corporation, Canberra.

Maxwell, W.M.C. (1985). Recent developments in artificial breeding of animals. Proceedings of the Australian Association of Artificial Breeding and Genetics, 5: 71-76.

Maxwell, W.M.C., Curnock, R.M., Logue, D.N. and Reed, H.C.B. (1980). Fertility of ewes artificially inseminated with semen frozen in pellets or straws, a preliminary report. Theriogenology, 14: 83.

McDowell, R.E. and Bove, L. (1977). Cited by McGregor, B.A. (1985). McGregor, B.A. (1985). Growth, development and carcass composition of goats: A review. Goat In "Production and Research in the tropics. Proceedings of a workshop at University of Queensland, Brisbane, 6-8 February 1984. Ed J.W. Copeland. pp 82-90.

McGregor, B.A. (1985). Growth, development and carcass composition of goats: A review. Goat In "Production and Research in the tropics. Proceedings of a workshop at University of Queensland, Brisbane, 6-8 February 1984. Ed J.W. Copeland. pp 82-90.

McGregor, B.A., Mcphee, S.R., Williams, A.W., Earle, C.R. and Staples, L.D. (1989). Melatonin treatment improves kidding percentage in Angora does. Australian Society for Reproductive Biology, 21: 98.

McGregor, B.A., Wolde-Michael, J., and Holmes, J.G.H. (1988). The influence of energy supplementation, and zerenol implants on growth and carcass characteristics

of Australian feral goat kids. Proceedings of the Austrlian Society of Animal Production, 17: 234-237.

Means, A.R., Dedman, J.R., Tash, J.S., Tindal, D.J., Van Sickle, M. and Welsh, M.J. (1980). Regulation of testis Sertoli cell by FSH. Annual Review of Physiology, 42: 59-70.

Meizel, S. (1985). Molecules that initiate or help the acrosome reaction by their interaction with the mammalian sperm surface. The American Journal of Anatomy, 174: 285-302.

Memon, M.A., Bretzlaff, K.N. and Ott, R.S. (1985). Effect of washing on motility and acrosome morphology of frozen-thawed goat spermatozoa. American Journal of Veterinary Research, 46: 473-475.

Memon, M.A., Bretzlaff, K.N. and Ott, R.S. (1986). Comparison of semen collection techniques in goats. Theriogenology, 26: 823-827.

Memon, M.A. and Ott, R.S. (1981). Methods of semen preservation and artificial insemination in sheep and goats. World Review of Animal Production, 17: 19-25.

Mendoza, G., White, I.G. and Chow, P. (1989). Studies of chemical components of Angora goat seminal plasma. Theriogenology, 32: 455-466.

Mendoza-Esquivel, G., Chow, P.Y.W., White, I.G. and Salamon, S. (1981). Some chemical parameters of goat semen. Proceeding of the Austalian Society of Reproductive Biology, 13: 61.

Miyamoto, A., Umeza, M., Hamano, K. and Masaki, J. (1987). Seasonal changes in inhibin activity in seminal plasma and serum concentrations of FSH, LH and testosterone in the male goat (Capra hircus). Theriogenology, 28: 67-76.

Monet-Kuntz, C., Hochereau-de Reviers, M. and Terquil, M. (1984). Variations in testicular androgen receptors and histology of the lamb testis from birth to puberty. Journal of Reproduction and Fertility, 70: 203-210.

Moore, N.W. (1985). Manipulation of reproduction in the goat. In "Goat Production and Research in the Tropics, A.C.I.A.R. Proceedings No. 7. (Ed. J.W. Copeland) pp. 66-69.

Moore, N.W. and Eppleston, J. (1979). The control of oestrous, ovulation and fertility in relation to artificial insemination in the Angora goat. Australian Journal of Agricultural Research, 30: 965-972.

Moore, R.W., Dow, B.W. and Staples, L.D. (1989). Artificial insemination of farmed feral goats with frozen-thawed semen. Proceedings of the New Zealand Society of Animal Production, 49:

Mori, Y., Tanaka, M., Maeda, K., Hoshino, K. and Kano, Y. (1987). Photoperiodic modification of negative and positive feedback effects of oestradiol on LH secretion in ovariectomised goats. Journal of Reproduction and Fertility, 80: 523-529.

Muduuli, D.S., Sanford, L.M., Palmer, W.M. and Howland, B.E. (1979). Secretory patterns and circadian and seasonal changes in luteinizing hormone, follicle stimulating hormone, prolactin and testosterone in the male Pygmy goat. Journal of Animal Science, 49: 543-553.

Mullins, P.E. and Saake, R.G. (1982). A study of the functional anatomy of the bovine cervix. Journal of Dairy Science, 65 Supp. 1: 184.

Nett, T.M. (1987). Function of the hypothalamic-hypophysial axis during the post-partum period in ewes and cows. Journal of Reproduction and Fertility, 34: 201-213.

Nimbulkar, M.V., Chavan, V.P. and Honmode, J. (1982). Preservation of goat semen at ambient temperatures. Proceedings of the Third International Conferance on Goat Production and Disease, University of Arizona, Tuscon, Arizona, January 10 to 15, pp. 285.

Nunes, J.F., Corteel, J.M., Combarnous, Y. and Baril, G. (1982). Role of seminal plasma in the in vitro survival of goat sperm. Reproduction, Nutrition and Development, 22: 611-620.

O'Shea, T. and Wales, R.G. (1966). Effect of casein, lecithin, glycerol, and storage at 5 degrees centigrade on diluted ram and bull semen. Australian Journal of Biological Sciences, 19: 871-882.

Oldham, C.M., Adams, N.R., Gherardi, P.B., Lindsay, D.R. and Mackintosh, J.B. (1978). The influence of level of feed intake on sperm-producing capacity of testicular tissue in the ram. Australian Journal of Agricultural Research, 29: 173-179.

Orgeur, P., Signoret, J.P. and Leboeuf, B. (1984). Effects of rearing conditions upon the development of sexual activity and semen production in young male goats. In "The male in farm animal reproduction ". (Ed. M. Courot) pp. 135-140. Martinus Nijhoff, Netherlands.

Ortavant, R., Courot, M. and Hochereau-de Reviers, M.T. (1977). In "Reproduction in Domestic Animals". (Eds. H.H. Cole and P.T. Cupps) pp. 203-228. Academic Press.

Ott, R.S. and Memon, M.A. (1980). Breeding soundness examinations of rams and bucks, a review. Theriogenology, 13: 155-164.

Ott, R.S., Nelson, D.R. and Hixon, J.E. (1980). Effect of the presence of the male on initiation of oestrous cycle activity of goats. Theriogenology, 13: 183-190.

Ozsar, S., Guven, B., Celebi, M., Kalkandelen, G. and Van de Wiel, D.F.M. (1990). Testosterone and LH concentrations in the male Angora goat during puberty. Animal Reproduction Science, 23: 319-326.

Parkinson, T.J. and Whitfield, C.H. (1987). Optimisation of freezing conditions for bovine spermatozoa. Theriogenology, 27: 781-797.

Pavlok, A. and Flechon, J.E. (1985). Some factors influencing the interaction of ram spermatozoa with zona-free hamster eggs. Journal of Reproduction and Fertility, 74: 597-604.

Polge, C., Smith, A.U. and Parkes, A.S. (1949). Nature, 164: 666.

Polge, C. (1952). The storage of bull semen at low temperatures. Veterinary Record, 65: 557-559.

Price, E.O. and Smith, V.M. (1984/85). The relationship of male-male mounting to mate choice and sexual performance in male dairy goats. Applied Animal Behaviour Science, 13: 71-82.

Pretorius, P.S. and Marincowitz, G. (1968). Post-natal penis development, testes descent and puberty in Merino ram lambs on different planes of nutrition. South African Journal of Agricultural Science, 11: 319-334.

Price, E.O., Smith, V.M. and Katz, L.S. (1984/85). Sexual stimulation of male dairy goats. Applied Animal Behaviour Science, 13: 83-92.

Quinn, P.J, Salamon, S. and White, I.G. (1968). The effect of cold shock and deep freezing on ram spermatozoa collected by electrical ejaculation and by an artificial vagina. Australian Journal of Agricultural Science, 19: 119-128.

Quinn, P.J. and White, I.G. (1966a). Variation in semen cations in relation to semen quality and methods of collection. Fertility and Sterility, 17: 815-825. Animal Breeding Abstracts, 36: 21.

Quinn, P.J. and White, I.G. (1966b). The effect of cold shock and deep-freezing on the concentration of major cations in spermatozoa. Journal of Reproduction and Fertility, 12: 263-270.

Quinn, P.J., Chow, P.Y.W. and White, I.G. (1980). Evidence that phospholipid protects ram spermatozoa from cold shock at a plasma membrane site. Journal of Reproduction and Fertility, 60: 403-407.

Racey, P.A., Rowe, P.H. and Chesworth, J.M. (1975). Changes in luteinising hormone-testosterone system of the male goat during the breeding season. Journal of Endocrinology, 65: 8-9.

Restall, B.J. (1987). Reproduction in Australian goats. In "Working papers for SCA Workshop on the Role of Non-Milch goats in Agricultural Production in Australia, Surfair, Sunshine Coast, Queensland 23-27 August 1987". pp. 4.1-4.28.

Restall, B.J. and Pattie, W.A. (1989). he inheritance of Cashmere in Australian goats. 1. Characteristics of the base population and the effects of environmental factors. Livestock Producton Science, 21: 157-172.

Restall, B.J., Ritar, A.J., Milton, J.B.T. and Sripongpun, S. (1988). Fertility and kidding rate in Thai native goats inseminated with frozen semen. Proceedings of the Australian Society of Animal Production, 17: 306-309.

Ritar, A.J. (1983). PhD Thesis. Aspects of Reproduction, Storage of Semen and Controlled Breeding of the Goat. Department of Animal Husbandry, University of Sydney.

Ritar, A.J. (1987). Arificial breeding in goats. In "Working papers for SCA Workshop on the Role of Non-Milch goats in Agricultural Production in Australia, Surfair, Sunshine Coast, Queensland 23-27 August 1987".

Ritar, A.J. (1988). Procedures for freezing buck semen in straws. Australian Association of Sheep and Goat Artificial Breeders, Proceedings of the inaugural meeting, Newcastle, N.S.W., 28th August, 1988, pp. 10-11.

Ritar, A.J., Maxwell, W.M.C. and Salamon, S. (1984). Ovulation and LH secretion in the goat after intravaginal progestagen sponge-PMSG treatment. Journal of Reproduction and Fertility, 72: 559-563.

Ritar, A.J. and Salamon, S. (1982). Effects of seminal plasma and of its removal and of egg yolk in the diluent on the survival of fresh and frozen-thawed spermatozoa of the Angora goat. Australian Journal of Biological Sciences, 35: 305-312.

Ritar, A.J. and Salamon, S. (1983). Fertility of fresh and frozen-thawed semen of the Angora goat. Australian Journal of Biological Science, 36: 49-59.

Ritar, A.J. and Salamon, S. (1991). Effects of month of collection, method of processing, concentration of egg yolk and duration of frozen storage on viability of Angora goat spermatozoa. Small Ruminant Research, 4: 29-37.

Ritar, A.J., Salamon, S., Ball, P.D., O'May, P.J. (1989). Ovulation and fertility in goats after intravaginal device-PMSG treatment. Small Ruminant Research, 2: 323-331.

Robbins, R.K., Saacke, R.G. and Chandler, P.T. (1976). Influence of freeze rate, thaw rate and glycerol level on acrosomal retention and survival of bovine spermatozoa frozen in french straws. Journal of Animal Science, 42: 145-154.

Robertson, L. and Watson, P.F. (1987). The effect of egg yolk on the control of intracellular calcium in ram spermatozoa cooled and stored at 5 degrees C. Animal Reproduction Science, 15: 177-187.

Roy, A. (1957). Egg-yolk coagulating enzyme in the semen and Cowpers gland of the goat. Nature, 179: 318-319.

Salamon, S. (1973). Deep freezing of boar semen, III effects of centrifugation, diluent and dilution rate, pelletvolume, and method of thawing on survival of spermatozoa. Australian Journal of Biological Science, 26: 239-247.

Salamon, S. (1977). Fertility following deposition of equal numbers of frozen-thawed ram spermatozoa by single and double insemination. Australian Journal of Agricultural Research, 28: 477-479.

Salamon, S. (1970). The survival of ram spermatozoa following pellet freezing below -79°C. Australian Journal of Biological Science, 23: 459-468.

Salamon, S. (1987). Assessment of frozen-thawed semen. In "Artificial Breeding of Sheep with Frozen Semen Workshop, Charles Hawker Conference Centre Waite Institute, Glen Osmond South Australia. (Ed. W.M.C. Maxwell) pp. 5-11.

Salamon, S. and Ritar, A.J. (1982). Deep freezing of Angora goat semen: effects of diluent composition and method and rate of dilution on survival of spermatozoa. Australian Journal of Biological Sciences, 35: 295-303.

Salhab, S.A. and Merilan, C.P. (1989). Effects of liner composition, cooling profile and time of glycerolisation on survival of bovine spermatozoa. Theriogenology, 31: 623-629.

Sanford, L.M., Palmer, W.M. and Howland, B.E. (1977). Changes in the profile of serum LH, FSH and testosterone and in mating performance and ejaculate volume in the ram during the ovine breeding season. Journal of Animal Science, 49: 1382-1391.

Schanbacher, B.D. and Lunstra, D.D. (1977). Acute and chronic effects of gonadotrophin releasing hormone on reproductive characteristics of rams during the non-breeding season. Journal of Animal Science, 44: 650-655.

Scheurmann, E. (1985). Congenital abnormalities in Angora goats. Australian Association of Artificial Breeding, Proceedings of the fifth conference, pp. 363-368.

Schindler, H., Amir, D., Laor, M. and Barnea, R. (1972). Insemination trials with goats under field conditions. World Review of Animal Production, 8: 76.

Setchell, B.P. (1977). Male reproductive organs and semen. In "Reproduction in Domestic Animals". (Eds. H.H. Cole and P.T. Cupps) Academic Press.

Setchell, B.P. (1984). The functions of the testis and epididymis in rams. In "Reproduction in Sheep". (Eds. D.R. Lindsay and D.T. Pearce). pp. 62-72. Australian Academy of Science and Australian Wool Corporation.

Shelton, M. (1978). Reproduction and breeding of goats. Journal of Dairy Science, 61: 994-1010.

Shelton, M. (1980). Goats: Influence of various exteroceptive factors on initiation of estrus and ovulation. International Goat and Sheep Research, 1: 156-162.

Shirbhate, R.N. and Honmode, J. (1982). Observations on certain factors affecting semen quality during deep freezing of buck semen. Proceedings of the Third International Conferance on Goat Production and Disease, University of Arizona, Tuscon, Arizona, January 10 to 15, pp. 284.

Singh, M.P., Sinha, S.N and Singh, B (1982). Studies on preservation of buck semen. Indian Veterinary medical Journal, 6: 125-130.

Skalet, L.H., Rodriques, H.D., Goyal, H.O., Maloney, M.A., Vig M.M. and Noble, R.C. (1988). Effects of age and season on the type and occurance of sperm abnormalities in Nubian bucks. American Journal of Veterinary Research, 49: 1284-1289.

Skinner, J.D. (1975). Reproductive physiology of indigenous and exotic male animals in South Africa: puberty in South African goat breeds, including the Angora. Abstract, Animal Breeding Abstracts, 43: 4628.

Skinner, J.D., Booth, W.D., Rowson, L.E.A. and Karg, H. (1968). The post-natal development of the reproductive tract of the Suffolk ram, and changes in the gonadotrophin content of the pituitary. Journal of Reproduction and Fertility, 16: 463-477.

Smith, A. and Polge, C. (1950). Storage of bull spermatozoa at low temperatures. The Veterinary Record, 62: 115-116.

Smith, B.A., Brinks, J.S. and Richarson, G.V. (1989). Relationships of sire scrotal circumference to offspring reproduction and growth. Journal of Animal Science, 67: 2881-2885.

Sonjaya, H. and Driancourt, M.A. (1989). Ovarian Morphology and function in 12-week-old lambs from breeds with different ovulation rates as adults. Reproduction Fertility and Development, 1: 265-72.

Steinberger, A. and Steinberger, E. (1976). Secretion of an FSH-inhibiting factor by cultured Sertoli cells. Endocrinology, 99: 918-921.

Stevermer, E.J., First, N.L. and Hoekstra, W.G. (1964). Effect of osmotic pressure on boar spermatozoa. Journal of Animal Science, 23: 67-70.

Suttie, J.M., Foster, D.L., Veenvliet, B.A., Manley, T.R., and Corson, I.D. (1991). Influence of food intake but independence of body weight on puberty in female sheep. Journal of Reproduction and Fertility, 92: 33-39.

Terrill, C.E. and Foote, W.C. (1987). Estimating reproductive performance in goats. Proceedings of the 4th International Conference on Goats, Brazil, vol. 1: 577-583.

Tervit, H.R., James, R.W., Shannon, P. and Tillot, M. (1982). Fertility of inactivated ram sperm. Proceedings of the New Zealand Society of Animal Production, 42: 93-94.

Thimonier, J. (1981). Control of seasonal reproduction in sheep and goats by light and hormones. Journal of Reproduction and Fertility, Supp. 30: 33-45.

Thimonier, J. (1981). Practical uses of prostaglandins in sheep and goats. Acta Vetrinaria Scandinavia, Supp. 77: 193-208.

Thonney, M.L., Taylor, St C.S., McClelland, T.H (1987). Breed and sex differences in equally mature sheep and goats. 1. Growth and food intake. Animal Production, 45: 239-260.

Toelle, V.D. and Robison, O.W. (1985). Estimates of genetic correlations between testicular measurements and female reproductive traits in cattle. Journal of Animal Science, 60: 89-100.

Tulley, D. and Burfening, P.J. (1983). Libido and scrotal circumference of rams as affected by season of the year and altered photoperiod. Theriogenology, 20: 435-448.

Visser, D. (1974). The effect of pellet volume, dilution rates prefreezing and at thawing, and of thawing temperature on the survival and acrosome-morphology of frozen ram spermatozoa. South African Journal of Animal Science, 4: 147-155.

Visser, D. and Salamon, S. (1974). The effect of freezing method on the survival of ram spermatozoa. South African Journal of Animal Science, 4: 157-163.

Waide, Y., Niwa, T. and Asanuma, R. (1977). Studies on preservation of liquid and frozen semen of domestic animals, viability and fertility of frozen goat spermatozoa. Japanese Journal of Animal Reproduction, 23: 129-137.

Waites, G.M.H. (1980). Functional relationships of the mammalian testis and epididymis. Australian Journal of Biological Science, 33: 355-370.

Warmington, B.G. and Kirton, A.H. (1990). Genetic and non-genetic influences on growth and carcass traits of goats. Small Ruminant Research, 3:147-165.

Watson, P.F. (1975). The interaction of egg yolk and ram spermatozoa studiued with a fluorescent probe. Journal of Reproduction and Fertility, 42: 105-111.

Watson, P.F. (1981). The roles of lipid and protein in the protection of ram spermatozoa at 5° C by egg-yolk lipoprotein. Journal of Reproduction and Fertility, 62: 483-492.

Watson, P.F. and Martin, C.A. (1975). Effects of eggyolk, glycerol and the freezing rate on the viability and acrosomal structures of frozen ram spermatozoa. Australian Journal of Biological Science, 28: 153-159.

Watson, R.H., Sapsford, C.S. and McCance, I. (1956). The development of the testis, epididymis, and the penis in the young Merino ram. Australian Journal of Agricultural Research, 7: 574-590.

Wilson, H.R. (1988). Procedures for freezing ram semen in straws. Australian Association of Sheep and Goat Artificial Breeders; Proceedings of the Inaugural Meeting, Newcastle NSW, August 28 1988, pp. 4-6.

Wolde-Michael, T, Miller, H.M., Holmes, J.H.G., McGregor, B.A. and Galloway, D.B. (1989). Effect of supplementary feeding and Zeranol on puberty in feral Cashmere goats. Australian Veterinary Journal, 66: 124-126.

Yao, T.S and Eaton, O.N. (1954). Postnatal growth and histological development of reproductive organs in male goats. American Journal of Anatomy, 95: 401-431.

APPENDIX 1

Photo 1: Foster rearing facility at Cressy Research Station.

Photo 2: Foster reared and early weaned kids receiving hay & pellet supplement.

APPENDIX 2

Photo 3: Foundation stock at the Woolnorth property.

Photo 4: Cervical insemination at "Woolnorth".

APPENDIX 3

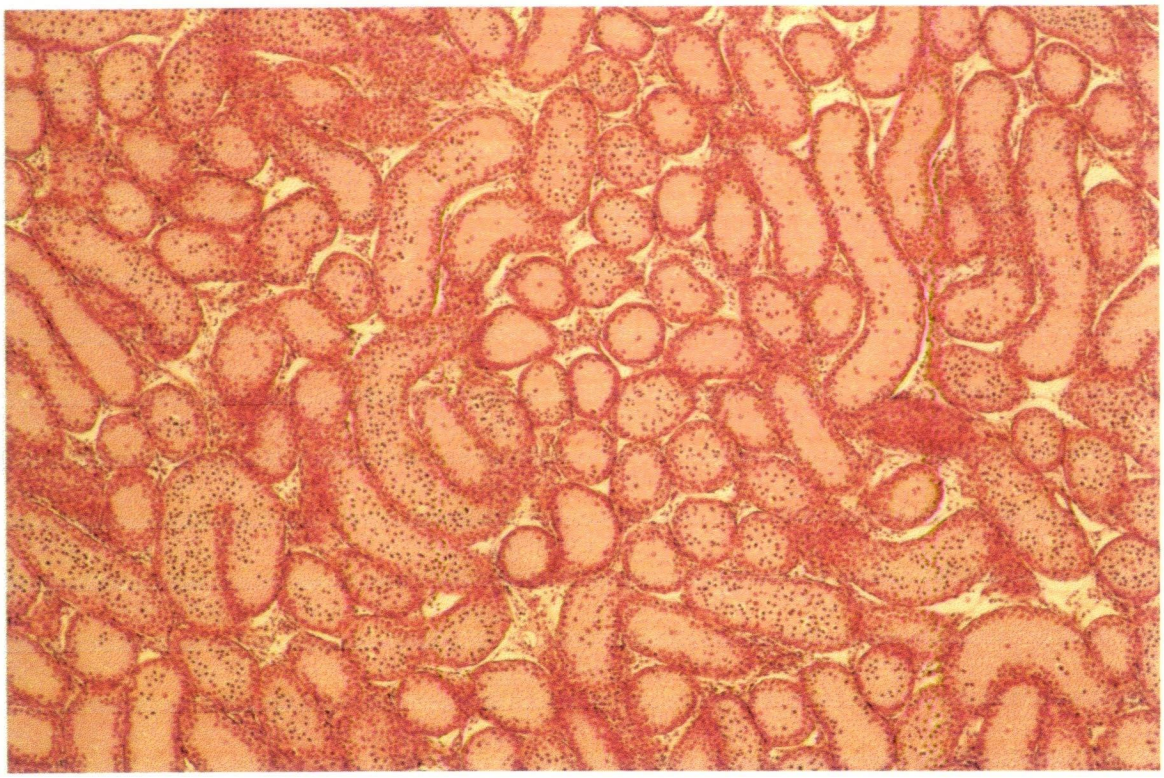

Photo 5: Testes section from Slaughter Group 1 (40x).

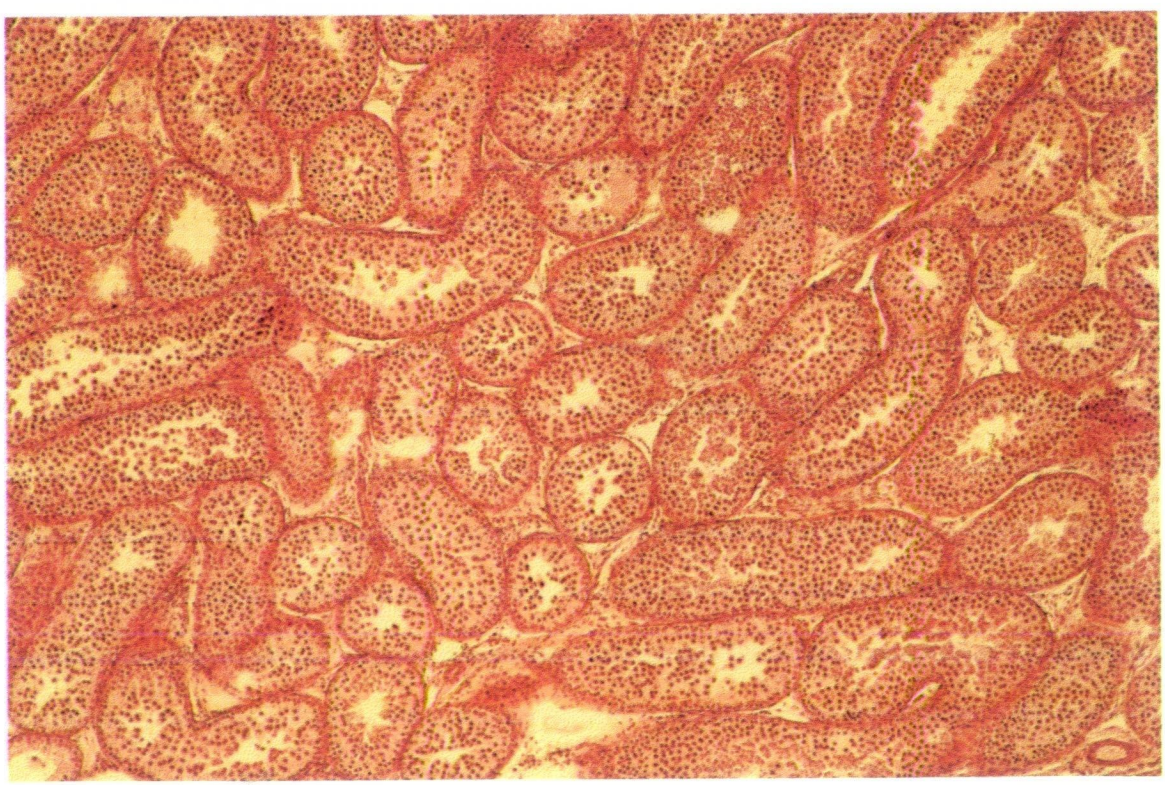

Photo 6: Testes section from Slaughter Group 3 (30x).

APPENDIX 3

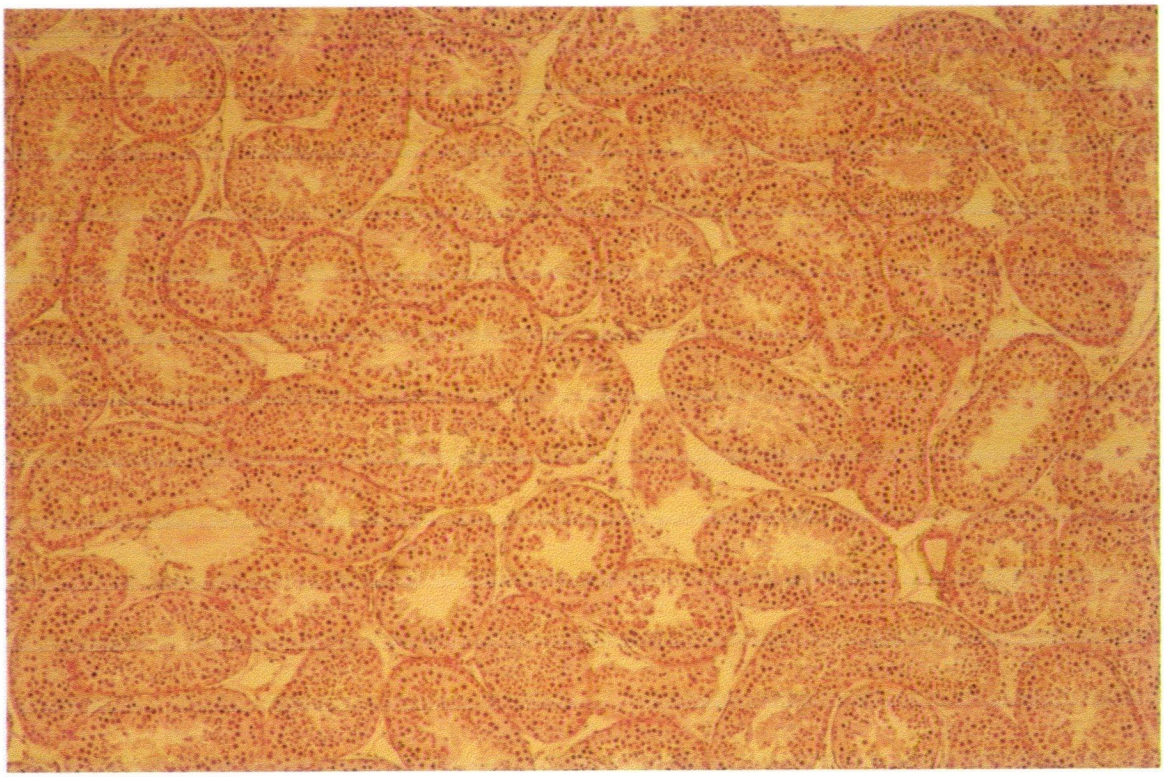

Photo 7: Testes section from Slaughter Group 5 (40x).

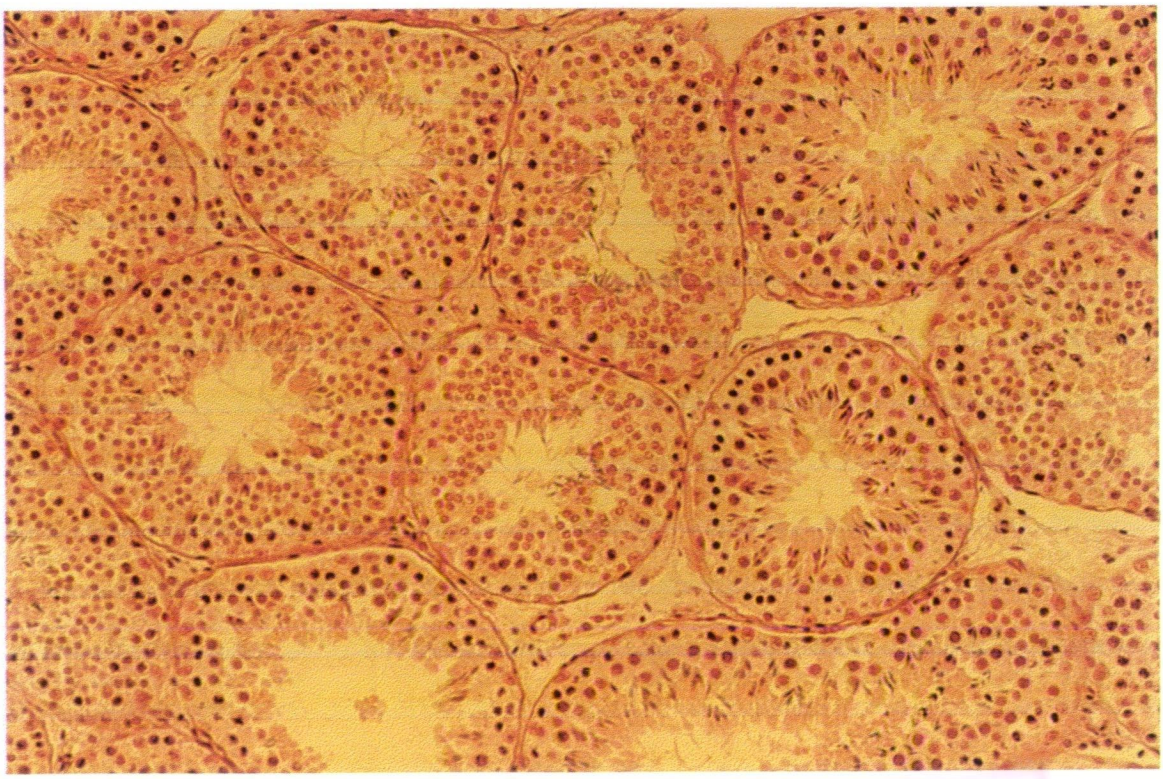

Photo 8: Testes section from Slaughter Group 5 (100x).