

**Seasonal effect on semen quality of Gorno Altai and  
South African indigenous goats.**

**by**

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## DECLARATION

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Mbulaheni Hector Dombo

Date

Pretoria

## DEDICATION TO MY FAMILY

- To my mother, Vho-Puledi Dombo for the love and excellent guidance in my life. You have been a never-ending source of inspiration and a great mother to me. God bless you.
- To my brother M.J and sister M.E. Dombo, although it was tough, you have taken complete care of us since we were young. Your support and encouragement is what made me what I am today. God bless you.
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## LIST OF ABBREVIATIONS

G.....	Gorno Altai
R.....	Rural /Indigenous goat
N-living.....	Number of living spermatozoa
N-dead.....	Number of dead spermatozoa
TLS.....	Tailless spermatozoa
CT.....	Coiled tail spermatozoa
SC.....	Scrotal circumference
BN.....	Broken at neck spermatozoa
BM.....	Broken at mid-piece spermatozoa
DF.....	Degrees of freedom
SS.....	Sum of squares
MS.....	Mean square
F value	
PR> F.....	Probability of F
R-square	
CV.....	Coefficient of variation

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## CHAPTER 1

**PROJECT THEME:** Reproductive management of the domestic animals.

**TITLE:** Seasonal effect on semen production of Gorno Altai and the South African indigenous goats.

**AIM:** Quantification of the effect of season on the quality of semen in Gorno Altai and the South African indigenous bucks.

**MOTIVATION:** Indigenous goats in the rural areas of South Africa are only utilized for traditional and sacrificial purposes. The recent importance of the commercialisation of goats through value adding to meat, milk, skins and fibre make improved reproduction an integral part of the economic viability of commercialisation. It is assumed that the low fertility of goats observed during summer is linked primarily to the quality of the sperm produced during that period and not necessarily to the decrease in fertility of the doe. Periods of low sperm motility have also been noted to be correlated to periods of low fertility, suggesting that the fertilizing capacity of viable spermatozoa becomes drastically reduced for certain months of each year, especially during the non-breeding season. The resource-poor rural farmers do not necessarily have to replace their already existing animals, as they are perceived to be inferior. Gaining access to superior genetic material is thus of great importance, and access to such material is limited due to large financial constraint that stud animals impose on the resource poor farmer. As a first step, it is imperative to characterize the reproductive seasonality of the indigenous and Gorno Altai male goats. It is postulated that the success rate of artificial insemination can also be improved if the semen is processed and frozen at the most viable time, which is season related.

## INTRODUCTION

Semen of good quality is essential for the success of an artificial insemination programme. Sperm production rates and epididymal storage capacity have been documented for bulls, boars, rams and rabbits. Knowledge on sperm production rates would be useful in any animal breeding programme which aims at exploiting males with superior genetic potential. However reports on sperm production rates of the caprine specie are scanty.

Morphological evaluation of spermatozoa must be conducted in order to ensure semen of high quality (Roca *et al*, 1992). A high proportion of abnormal spermatozoa adversely affect the fertility of domestic animals. Semen quality of indigenous goats is reported to be acceptable all year round, as the proportion of abnormal spermatozoa is well within the accepted range for normal fertility. It has also been postulated that the quality of semen in goats varies according to the breed, geographical location and the season of the year. The type or occurrence of sperm abnormalities showed great variation during the experimental period (Eaton and Simon, 1953, Corteel, 1981).

A significant season X ejaculate interaction on sperm density and number was reported. Amir *et al* (1986) also recorded sperm density to be lower in June and August to September, compared to November to December. It was also postulated that the percentage of abnormal spermatozoa (mainly coiled tails, acrosomic defects and protoplasmic droplets), was significantly higher in March than in June and August to September. In addition it was found that 75 % of the abnormal spermatozoa were those with coiled tails in March, 60 % of the abnormal spermatozoa had acrosome defects in June, while the prevailing abnormality in August to September was the presence of protoplasmic droplets. In November to December, 80 % of the abnormal spermatozoa were evenly distributed between coiled tails and protoplasmic droplets. Motility has been said to be lower in March, than in the other months. Semen volume was found to decrease significantly corresponding to the decrease in sperm density and sperm number which was found to be significantly greater in March, June and November-December than in

August to September (Amir *et al*, 1986). Thus the literature on seasonality of semen quality is conflicting.

The effects of frequent ejaculation and of season on the semen characteristics of rams have been studied by several workers. In these studies it was reported that successive ejaculation affected the quantity, but not the quality of spermatozoa. It was also stated that the effect of season on sperm quality in the trials was characterized by a significant increase in the percentage of abnormal cells and a decreased motility in ejaculates during March and by an increased number of ejaculated spermatozoa in November-December (Salamon, 1962; 1964; Amir and Volcani, 1965; Amir, 1966; Lightfoot, 1968; Jennings and McWeeney, 1976; Tomkins and Bryant, 1976).

Colas (1983) and Amir and Volcani (1965) reported similar effects in Ile-de-France and Awassi rams. The morphological evaluation of spermatozoa is the most accurate test available to test the quality of the ejaculate. The incidence of the highest percentage of abnormal spermatozoa and the lowest conception rate in March may be indicative of the role that rams play in the lower fertility obtained in ewes during this month.

Fulkerson *et al* (1982) have indicated that a single artificial insemination requires twice as many spermatozoa for conception equivalent to natural mating. Assuming that semen of equal quality is used, the only difference between artificial and natural mating appears to be the absence of normal courtship behaviour of the ewe and the stress associated with handling and strange surroundings, when A.I is used. During natural service, rams often deposit fewer spermatozoa per service that are adequate for conception.

Conception becomes related to the number of times a ewe is served or the more rams with which the ewe mates, this can possibly be explained on the basis that inadequate numbers of spermatozoa are deposited in a single insemination (Mattner and Braden, 1967; Knight and Lindsay, 1973).

Scrotal circumference has a great role to play, as it influences the quantity of spermatozoa that can be produced by the intact ram. Scrotal circumference is positively correlated to the testicular weight. The colour of semen is indicative of the concentration of the ejaculate. Testicle measurements are used as indicators of both reproductive status and spermatogenic capacity of small ruminants, for example in rams and bucks (Bongso *et al*, 1982).

Among the important factors in the evaluation of male reproductive capacity, is sexual behaviour particularly in artificial insemination programmes where an artificial vagina is used to collect semen. In small ruminants living at high and mid-latitudes, reproductive factors appear to be influenced by environmental conditions throughout the year particularly day length and ambient temperature (Almeida & Pelletier, 1988; Langford *et al*, 1989). Simplicio *et al* (1982) postulated that in Brazilian Somali rams, significant seasonal differences were observed for ejaculate volume, % mass motility, progressive motility score and concentration of spermatozoa. All other characteristics of semen and testes varied between rams. It was also stated that the total number of spermatozoa per ejaculate did not vary according to season, and the magnitude of the seasonal effect was not sufficient to prevent the rams being used for breeding throughout the year. The seasonal variation in the colour and consistency of semen, proportion of dead spermatozoa and the scrotal volume were of smaller magnitude and the total number spermatozoa in the ejaculate did not differ between seasons. According to these findings, rams showed marked changes in the body weight and it was related to all testes measurements and the motility score of the spermatozoa.

There is an increase in the percentage of abnormal spermatozoa and a decrease in motility with increasing duration of heat. This also reflected an increase in both volume and density. The percentage abnormalities in semen starts with the coiling of the tail, followed by the appearance of tailless heads. Reaction time has been said to be the longest in animals that are exposed to high ambient temperatures for a long period of time. In a trial by Karagiannidis *et al* (1999), five Chios and five Friesian rams were used to study the effect of season on semen production. It was found that the best quality semen was produced mainly during autumn and the worst quality during spring. It was

also stated that the magnitude of these seasonal effects was not sufficient to prevent rams from being used for breeding throughout the year.

However, the existence of differences among rams within and between each breed in semen quality and quantity, makes it necessary to perform a semen evaluation in order to select the best males before being used for breeding. Significant differences were not recorded between the two breeds in any trait. Season affected both the quantity as well as the quality of semen. Semen volume was lower during spring, compared to summer, autumn and winter. During a transitional period, the percentage of motile spermatozoa was lower during summer, compared to autumn, winter and spring. A gradual increase motile sperm was observed with the highest value being recorded during autumn. Semen characteristics were generally higher during summer (onset of improvement) and autumn (peak of improvement), compared to during winter (onset of decline) and spring (lowest quality).

When considering that summer and autumn are seasons of decreasing day length (breeding season) and winter and spring are seasons with increasing day length (the non-breeding season), it is obvious that bucks are sensitive to photoperiodism. Ibrahim (1997) in a similar study with Chios and cross-bred rams raised in the United Arab Emirates, reported that semen characteristics were improved mainly during the winter. The variation of quantitative and qualitative sperm production, as well as the time of the year in which the unfavourable effect of photoperiodism occurs, varies between several latitudes and between breeds. It was also confirmed that breeds used for industrial crossing appear to be more sensitive to photoperiodism than the hardy breeds.

Contrary to the general consideration that semen quality is higher during the breeding season, it was also found that progressive motility of sperm was lower in summer, compared to the other three seasons of the year. According to Mandiki *et al* (1998), semen motility does not vary greatly over seasons, while a sporadic decrease is observed during spring. Colas (1983) suggested that photoperiod has an effect on the sperm motility. Karaginnidis *et al* (1999), concluded that semen characteristics of rams showed



a significant seasonal variation in semen quantity and quality and best semen is produced during late summer and autumn (breeding season).

In another study by Perez and Mateos (1996) with Verata and Malaguena bucks, it was postulated that there were differences between the breeds in semen characteristics, with higher semen production and better semen quality being observed in the Malaguena bucks. The influence of the photoperiod on both semen quantity and quality was observed in the Verata breeds, with a noticeable improvement in all semen characteristics during the periods of decreasing photoperiod. Differences were also observed in bucks of both breeds with regards to semen production, but only in the Malaguena bucks with regards to semen quality. Perez *et al* (1996) cited significant differences between breeds in almost all seminal characteristics. The Malaguena bucks gave higher semen volumes, higher total sperm per ejaculate, higher percentage of normal acrosomes and lower percentage of abnormal acrosomes, than Verata bucks. These bucks presented a higher concentration than the Malaguena breeds. Variations in semen production caused by photoperiod are more or less marked, depending on the latitude.

In studies performed in zones above 40°, these variations are very marked with significant increases in semen production during periods of decreasing photoperiod (Corteel, 1981). Greyling and Grobbelaar (1983) reported that, in latitudes between 30° and 40°, there are seasonal, but not very marked variations with a higher production during summer and autumn. In zones below 30°, bucks do not present seasonal variation in sperm production. It was also recorded that Verata breeds undergo a seasonal increase in testosterone secretion, which is greater than that of the Malaguena breed. This difference in sensitivity to photoperiod between breeds may be due to the different latitudes. As a consequence of their testicular size, Malaguena bucks have a higher seminal production due, possibly to their being less sensitive to photoperiod. This seminal production was more consistent throughout the year than that of Verata breed. Perez and Mateos (1996) concluded that photoperiod did not have the same effect on semen characteristics in the two breeds studied. Delgadillo and Chemineau (1992) confirmed that goat bucks show seasonal variation in semen quality, mainly influenced

by the photoperiodic changes. These results differ from results from other researchers, in that the minimum percentage of motile sperm cells and movement occurs during spring and summer. It is thus clear that studies in other species, as well as some studies with goats have alluded to the effect of photoperiod, season, breed, geographic location, frequent ejaculation, scrotal circumference (which is directly correlated to nutrition), sexual behaviour and temperature of the environment, as factors that can affect semen quality.

The objective of this study is thus to observe the effect of some of these factors on the semen quality of two breeds of goats (one breed exotic and one breed indigenous to South Africa) over a 1 year period in South Africa. This will provide information that may be used in subsequent semen freezing and artificial insemination programmes.

## CHAPTER 2

### LITERATURE REVIEW: FACTORS THAT INFLUENCE REPRODUCTION IN MALE ANIMALS

#### 2.1 Anatomy of the male reproductive system

The normal male sexual organs includes the testicles, which are considered essential as they produce the male sex cells or gametes, as well as the hormones responsible for male sexual behaviour and masculine character. The accessory male organs assist in conducting the male sex cells from the testicles to the female genital tract and these are comprised of the epididymis, vas deferens, seminal vesicle, prostate, bulbourethral gland and urethra, which are largely contained within the copulatory organ or penis (Setchel, 1977; Bearden and Fuquay, 1980; Hafez, 1980).

##### 2.1.1 The testicles/ testes

The testes are the primary male organs of reproduction. In all domestic male animals, the testicles are located at the inguinal region within the scrotum. Daily spermatozoa output has been found to be correlated to testes weight (Boyd and Van demark, 1957) and with the number of spermatozoa in the epididymis of bulls (Amann and Almquist, 1961).

During foetal development, the testicles move posteriorly from their original position adjacent to the kidneys and ultimately descend into the inguinal canal and come to rest within the scrotum. Gubernaculum plays a conspicuous role and the changes it undergoes are probably under hormonal control. At this time androgens are believed to promote testicular descent and oestrogen to inhibit it. Cryptorchidism is a condition in which one or both testicles fail to descend into the scrotum. It is probable that cryptorchidism is due to disordered endocrine secretion that is genetically determined. Abdominal retention of the testicles is associated with aspermia and, consequently, when both testicles fail to reach the scrotum the animal is sterile. Before the testicle passes from the abdomen to the scrotum, there occurs a special out pouching of the parietal peritoneum, which passes

from the internal inguinal ring. This flask shaped sheath or peritoneum is the tunica vaginalis reflexa or processus vaginalis. Muscle fibres, which have their own origin near the internal inguinal ring, are inserted into both tunicae and constitute the external and internal cremaster muscles. Contraction of these muscles occurs when the testicle is handled, and causes it to be pulled towards the inguinal canal. The spermatic cords consist of the internal cremaster muscle, the vesicles and nerves that run to the testicles in the double layer of mesorchium and the vas deferens. The testicle itself has no sensory nerve supply but the tunica vaginalis reflexa is richly supplied with sensory nerves from the third lumbar spinal nerve (Setchel, 1977; Hafez, 1980; Frazer, 1968).

### **2.1.2 Seminiferous tubules, epididymis and vas deferens**

The seminiferous tubules of the testicles are the source of spermatogenesis and they empty their contents by way of several straight tubes into the head of the epididymis. The epididymis is a cylindrical organ that is closely attached to the testicles and is essentially very long (30-155 m). It is an intricately coiled tube which is continuous at the tail of the epididymis with the vas deferens. The middle of the vas deferens is the body of the epididymis.

The epididymal tube is muscular and the epithelium lining its lumen, is ciliated. A peristaltic contraction of the epididymal tube propels the sperm through the organ in a matter of 12 days. During their passage through the epididymis, sperm lose their cytoplasmic or protoplasmic droplet and acquire a protective lipid covering.

Clinically the tail of the epididymis forms the most conspicuous part of that organ. In the bull it projects from the lower end of the testicle resembling half a walnut. The vas deferens passes up into the posterior part of the spermatic cord and enters the abdomen at the internal inguinal ring from which it runs posteriorly to empty into the urethra, where the urethra joins the neck of the bladder. The vas deferens has a strong muscular wall, but its epithelium is not ciliated (Frazer, 1968; Bearden and Fuquay, 1980).

### 2.1.3 The penis

The penis or male copulatory organ, is mainly cylindrical in shape and extends from the ischium to near the umbilicus. It is composed of three erectile structures, the two corpora carvenosa, connected to the ischium by the ischiocarvenosus muscles, and the corpus spongiosum, which contains the extra pelvic urethra. Erection of the penis is caused by the contraction of the ischio carvenosus muscle which compresses the penis against the ischium, thus obliterating its dorsal veins. The vascular spaces of the erectile tissue which may be linked to massive capillaries, becomes distended with blood. The retractor penis muscle passes from the ischium to the ventral surface of the penis and its contraction pulls the penis back into the sheath (Arthur *et al*, 1982; Bearden and Fuquay, 1980; Setchel, 1977).

The free end of the penis or glans is contained within the sheath and is partly covered by the prepuce or the foreskin, which is continuous with the inner lining of the sheath. The urethra of the horse terminates in a short free process which is contained in a small fossa, while the urethra of the ram and buck projects beyond the glans penis as a vermiform appendix. The end of the buck penis is coiled, especially during copulation. During copulation the penis undergoes a marked increase in size but the mechanism of this enlargement varies with the species.

In the bull, ram, buck and boar, vascular erection is not a very prominent feature of erection. The main factor, which increases the length of the penis during erection, is the obliteration of the sigmoid flexure of the organ.

The sigmoid flexure of the bull, goat and ram is post-scrotal, whereas in boars it is in front of the scrotum. During erection the penis of the bull, boar, buck and ram protrudes fully from the fore skin and the lining of the sheath and becomes completely erected (Setchel, 1977; Bearden and Fuquay, 1980).

#### **2.1.4 Accessory male organs**

The internal male reproductive organs comprise the seminal vesicles, the ampullae, the prostate gland, the bulbourethral gland and the pelvic urethra. Ampullae are the dilated terminations of the vas deferens and each enters the urethra through an opening in common with the seminal vesicle of the same side. The ampullary glands contribute to the ejaculate and are present in the terminal portions of the vas deferens of bulls, rams, dog and stallions. In addition the ampullae stores sperm suspension from the testes and the epididymis. In the stallion and boar the seminal vesicles are sac-like, but in the bull and ram the vesicles are lobulated and firmer. The secretion is watery and in some normal bulls it has a yellow tinge. The prostate gland is related to the intra-pelvic urethra, and has two parts. The prostate secretion is also watery. The bulbourethral gland or Cowper's gland of the stallion, bull and ram are small, round structures lying between the anus and the urethra. Their watery secretion is discharged prior to coitus and is thought to cleanse the urethra of urine. In many bulls and some stallions a small uterus masculinus – representing remnants of the mullerian ducts, can be found dorsal to the neck of the bladder (Bearden and Fuquay, 1980; Hafez, 1980).

#### **2.1.5 The scrotum**

Spermatogenesis takes place in the testes in the scrotum, normally at 4 to 7°C below normal body temperature. The scrotum is a pouch-like structure found hanging below the two legs of the buck. The main function of the scrotum is to protect the testes against any unfavourable environmental conditions, by regulating the temperature through contraction and expansion of the muscles during hot and cold days (Makerachian *et al*, 1984; Swanepoel and Heyns, 1985; Courot, 1984; Lino, 1972). In cold conditions the warming mechanisms are generally sufficient to maintain proper testicular temperature and the production of spermatozoa. However, in extremely hot conditions the cooling mechanism may not be able to cope with the production of spermatozoa and this leads to temporary sterility of the male due to heat stress. The scrotum can be divided into a neck,

a narrowing that attaches the scrotum to the body of an animal, and a septum that separates the two testicles.

Many sweat and sebaceous glands are contained in the skin of the scrotum and the outermost layer is covered with hair. The sweat glands in the skin allow for the evaporative cooling of the scrotum and also of the testes. The closeness of the testes to the abdomen is regulated by external cremaster muscles which connect the tunica vaginalis to the abdomen. Maxwell (1962) postulated the wall of the scrotum to consist of the skin and two membranes, the tunica dartos and the tunica vaginalis. The tunica dartos is the outer layer that provides most of the support to the testes. Each testis and its epididymis is enclosed by the tunica vaginalis.

In colder environments, the dartos contracts and pushes the testicles towards the inguinal ring with the consequent puckering of the fundus of the scrotum. In warm environments the dartos relaxes and allows the testicles full dependence in the scrotum. Interference with this thermoregulatory function such as clothing the scrotum in a heat-insulating bag or by causing a wide spread lesion of the scrotal skin, causes an interference of spermatogenesis. For this reason the abdominal testicle of the cryptochord is aspermatic (Bearden and Fuquay, 1980; Hafez, 1980).

## **2.2 PHYSIOLOGY OF MALE SEXUAL REPRODUCTION**

Normal reproductive activity in the male is comprised of the production of semen containing normal spermatozoa in adequate numbers, together with the desire and ability to mate. These sexual functions are under intrinsic control of hormones and the central nervous system, but environmental factors such as temperature, daylight length, nutrition, change in surroundings and systematic diseases may influence these reproductive functions.

Sex drive is also largely dependent on endogenous male hormones. The functions of the testicles are spermatogenic and endocrine. There is a close relationship between the two functions and generally males show reduced sexual desire and less active spermatogenesis in the non-breeding season (Arthur *et al*, 1984; Hafez, 1980).

### **2.2.1 Spermatogenesis**

Spermatozoa can first be seen in the seminiferous tubules of the testis of the bull at seven and a half months and in the ram, buck and boar at five months, although normal fertility is not achieved until a few months later. In the young male, attainment of the state of normal sexual desire with the ability to mate and to produce normal sperm is called puberty.

The complete cycle of spermatogenesis in the bull takes 50-60 days. As a result of the reduction division (meiosis) during spermatogenesis, two genetically different spermatozoa carrying either the X or the Y chromosome are produced and this is the basis of sex determination. In any one tubule the spermatogenic activity may vary at different points along its length and in different tubules some may be active and others resting. In the case of rams and bucks testes there are seasonal variations in spermatogenic activity with the greatest activity occurring in autumn (Asdell, 1968; Arthur *et al*, 1982; Hafez, 1980).

### **2.2.2 Sperm maturation in the epididymis.**

During spermatogenesis, a drop of protoplasm is extruded by each sperm and becomes attached at the junction of the sperm head and the body. Sperm reaching the head of the epididymis from the testicle show these proximal beads but, as they transverse across the epididymis, the droplet moves posteriorly. On arrival in the epididymal tail, most of the sperm have lost their protoplasmic droplets, although a minority may still show distal beads.



In normal semen beaded sperm is seldom seen. As the sperm pass through the epididymis they are nourished by the secretions of the epididymal tubule, and in addition acquire a protective lipoid covering. At this time their activity increases slightly and their fertility improves with the distance from the testis. The tail of the epididymis is the main storage house of sperm. The time taken by the sperm to traverse the epididymis is from 4 to 12 days and their average effective life span is about 40 days. The peristaltic contractions that occur in the tube of the epididymis are mainly responsible for moving the sperm through the epididymis.

Frequent sexual activity accelerates this movement, in which case immature or beaded sperm may appear in the ejaculate. Large quantities of fluids pass from the testes into the head of the epididymis but are resorbed before reaching the tail of the organ (Hafez, 1980; Arthur *et al*, 1982).

### **2.2.3 Function of the accessory organs**

The vas deferens is a muscular tube that conducts sperm from the epididymis to the urethra. In horses and ruminants the vas deferens dilates near the urethra to form ampullae that serve as an additional storage house of the sperm. The vas deferens is also secretory and contributes to the ejaculate. The bulbourethral gland or Cowper's gland in boars discharges a secretion before the orgasm and this fluid is thought to cleanse the urethra of urine in the boar. However, the secretion is of a highly viscous nature and, in the genital tract of the sow, it reacts with the watery secretion of the seminal vesicle to form a jelly-like mass. This gland is absent in dogs.

The secretion of the seminal vesicle and prostate gland provides a fluid bulk for sperm transport through the urethra; These secretions activate the relatively quiescent sperm from the epididymis, both by raising the temperature and supplying electrolytes. By contributing citric acid, they provide a buffering effect and furnish energy in the form of fructose for sperm metabolism.

In ruminants the volume of the accessory secretion in aggregates and the true (epididymal) sperm suspension are nearly equal, whereas in the stallion and boar the accessory secretion is greatly in excess (Bearden and Fuquay, 1980; Arthur *et al*, 1982).

## 2.2.4 Normal semen composition

Semen comprises aggregate fluid discharged by the male during ejaculation/ coitus. It is a milky suspension of sperm cells, the volume and concentration of which varies greatly between species.

**Table 2.2.4.1 Main characteristics of normal semen**

<i>Animal</i>	<i>Colour</i>	<i>Volume (ml)/ejaculate</i>	<i>Sperm concentrationx (10<sup>6</sup>/ml)l</i>	<i>Features</i>
Stallion	Greyish white	75 ml	1-2	Comprised of viscis fraction from the seminal vesicles
Bull	Milky-Creamy	3.5 ml	1	
Ram	Creamy	1.5 ml	2-3	
Boar	Greyish-white	200ml	100 000	Contains topica like lumps
Dog	Greyish-white	2-19ml	125 000	

*Adapted from Arthur, Noakes & Pearson (Veterinary Reproduction & Obstetrics) 1982*

## 2.2.5 REPRODUCTIVE ABNORMALITIES IN MALE ANIMALS

### 2.2.5.1 Lack of libido

Puberty occurs in bulls at 9 to 10 months, in stallions at 16 to 24 months, in boars, rams and bucks at 5 to 7 months of age. Full libido may be attained before normal spermatogenesis initiates. This is the reason why animals are not put to mate until a few

months after puberty. Sexual desire can be affected by age, heredity, environment and diseases.

\*Over-fatness is an occasional cause of lack of libido and the present practice of late suckling as well as the feeding of concentrates (e.g. show animals) should be condemned. Rams, bucks and bulls which are slow to serve where hand service is employed may be stimulated by teasing. A lack of sexual desire may also be shown by males of the other domestic species and require the same clinical approach as the one usually recommended for bulls (Arthur *et al*, 1982).

#### **2.2.5.2 Inability to copulate**

\*The inability to mate despite normal sexual desire is a fairly frequent cause of ram and buck infertility. It occurs most frequently in young males when first allowed to mate. In some cases it is due to skeletal or visceral pain, in other cases it could be due to lesions of the genital organs. In those that do not have lesions, the nervous control of copulation is believed to be defective (Arthur *et al*, 1982).

#### **2.2.5.3 Impotency due to penis defects**

This may be classified as an erectile failure, inability to protrude because of lesions of the penis or the prepuce and deviation of the protrusion. An inability to protrude may be classified into two forms, the penile frenulum and the rupture of the corpus carvenosum penis. These pose a potentially serious disorder in bulls. Injury to the preputial mucosa has become a normal feature in certain breeds of cattle. Long (1969) found that eversion occurred in 85% of polled bulls, but only in 1.4% of horned animals. Long and Hignett (1970) suggested that the condition is associated with aplasia or hypoplasia of the retractor muscle of the sheath, which normally stabilises the preputial epithelium during penile movement. Eversion is associated primarily with the non-erectile movement of the

penis within the sheath (Long *et al*, 1970) and is most likely to occur in breeds with long sheaths (Lagos and Fitzhugh, 1970).

#### **2.2.5.4 Penile tumors in males**

<sup>x</sup>Tumor-like masses observed on the ram and buck's penis are likely to be associated with infertility <sup>x</sup>(Arthur *et al*, 1982).

#### **2.2.5.5 Inability to fertilize**

The inability to fertilize, due to seminal defects, forms the largest category in bull infertility. This inability to fertilize is associated with disease changes that occur, contributing to the seminal ejaculate and is more directly related to the seminal micro-pathology. This phenomena has been found to be much more complex than a lack of libido or impotence, in that each of the problems is obvious male defect, while failure of fertilisation may involve the doe or the buck.

It is highly imperative to evaluate semen in respect to its fertilizing potential and representative samples must be collected and subjected to laboratory evaluations. Regular clinical investigations of the male must be carried out with respect to those organs that contribute to semen production (Arthur *et al*, 1982).

## 2.3 GENITAL DISEASES AFFECTING SEMEN FERTILITY

In ruminants, the testicular and accessory glands contribute approximately equally to the ejaculate, whereas in other domestic species, the accessory secretion is generally in excess. Diseases of either the testes or accessory glands may influence the quality of the semen, but lesions of the testes have a marked and important effect on semen. Genital diseases may be congenital or acquired.

Cryptorchidism and testicular hypoplasia are the most common as well as the most important congenital diseases, while testicular degeneration is the most troublesome acquired condition. Heredity, as well as other endocrine glands are often involved in diseases of the testes (Arthur *et al*, 1982).

### 2.3.1 Cryptorchidism

Cryptorchidism is frequently seen in foetal monsters, particularly those of the bovine species showing gross developmental abnormalities-for example, Schistosomes and two headed calves (William, 1939). The species most affected by cryptorchidism are horses and pigs. In pigs the incidence has been estimated to be between 1-2%. In dogs there is an estimated incidence of 0.05-0.1% and in some cattle e. g the Red Danish breed, 0.7%.

Cryptorchidism is uncommon in cats and sheep. However in sheep it has been noticed that, while the majority of the flocks are unaffected, in the occasionally affected flock, up to 5% of the rams may be cryptochids. In most of the ruminants it has been speculated that there is a possible association of the polled character with testicular retention. In many field and experimental observations, it has been pointed out that there is a hereditary basis for cryptorchidism in which recessive genes are involved (William, 1939; Arthur *et al*, 1982; Perez and Mateo, 1996).

### **2.3.2 Orchitis**

Inflammation of the testes is often followed by ischaemic necrosis and sometimes by abscess formation. Generally the spermatogenic function of the inflamed testicle is irrevocably destroyed and the preservation of the other testicle is achieved by the early removal of the infected gonad. Previously infectious agents that caused bovine orchitis were *Brucellus abortus*, *C. pyogenes* and *M.tuberculosis*. Males with acute orchitis are not likely to be used at stud, and also because of their reluctance to serve, it is impossible to collect semen from them. Seminal degeneration soon occurs (Arthur *et al*, 1982).

### **2.3.3 Testicular hypoplasia**

Testicular hypoplasia implies the incomplete development of the germinative layers of the seminiferous tubules. Different degrees of infection do occur, from showing only poor sperm production and defective sperm morphology, to cases where there is complete aspermia and markedly small testes. The less severe types may show only seminal abnormalities and are often unsuspected, until their failure to fertilize has become apparent.

There is little doubt that testicular hypoplasia is the most common cause of infertility in young bulls when first mated to females. With regard to sperm morphology, almost every type of abnormal sperm can be seen in hypoplastic bulls.

Tailless and looped tail defects, together with a variation in size and shape of the sperm head are common, but the most characteristic is the occurrence of immature sperm. The presence of more than 5% of sperm with proximal beads in the semen of a young bull usually indicates hypoplasia. The milder cases of hypoplasia may improve with age or under treatment with gonadotrophic hormones, but the more severe types are not healed by treatments (Arthur *et al*, 1982; Smith *et al*, 1989).

#### **2.3.4 Testicular degeneration**

Testicular degeneration involves retrogressive changes in the germinal epithelium of the seminiferous tubules. Great variation in severity can be seen in cases ranging from those showing mild defects of spermatogenesis to instances of gross testicular atrophy, where only the spermatogonia and Sertoli cells remain. Hypoplastic animals never attain normal testicular function, whereas those affected with testicular degeneration have a variable period of successful use (Arthur *et al*, 1982).

It is considered that testicular degeneration is a late manifestation of a defective endocrine system, whereas hypoplasia is an early indication of the similar but more severe disorder. There are numerous and diverse causes of testicular degeneration and anything that interferes with the thermoregulatory function of the scrotum will cause degeneration of the testes. It has been demonstrated that, by enclosing the bull's scrotum in a heat-insulating bag for 4 to 5 days, sperm motility and morphology are adversely affected. It then takes 4 to 6 weeks before the semen becomes normal. Sexual desires and the ability to copulate are not affected by the degeneration of the testes (Arthur *et al*, 1982).

### **2.4 REPRODUCTION IN FEMALES**

#### **2.4.1 Infertility with age**

Fertility in females is determined largely by the number and frequency of eggs shed, for there is little foetal atrophy. The number of eggs ovulated depends on the level of gonadotrophic hormones in the blood and this varies between breeds. Fertility may also decline with age and with increasing parity beyond a certain number of pregnancies. In the majority of instances, cattle, sheep, goats and pigs are not retained in the breeding herd long enough for this condition to be exhibited (Roca *et al*, 1997; Arthur *et al*, 1982).

However, reproductive decline with age has been clearly indicated in laboratory animals after repeated pregnancies and some of the underlying factors have been indicated in the literature (Jones and Krohn, 1960; Biggers *et al*, 1962; Adams, 1970). Although the shedding of potentially defective oocytes may contribute to decrease reproductive performance with advancing parity, Biggers *et al* (1962), Biggers (1969) and Adams (1970) considered defects in the uterus to be mainly responsible for reduced fertility with advancing age. Biggers *et al* (1969) also indicated that in rabbits, mice and hamsters, the increased embryonic death underlying small litter size in old females occurs after implantation, and would appear to implicate the uterus rather than the embryo.

With the use of the embryo transfer technique to insert embryos from young females into the uterus of the aged laboratory animals. Biggers (1969) and Adams (1970) found that a condition in the endometrium which forms cystic glandular hyperplasia was particularly prominent in aging rabbits, while others have cited an increased deposition of collagen in the aging tract as a cause underlying embryonic death.

Changes in the uterine vascular wall and impairment of capillary blood flow may also be important and must still be critically examined. However, in sheep, Laing (1970), suggested that the maximum birth rate is reached at about 5 to 6 years of age, after which there is a gradual decline. Goats show a higher fertility and have a shorter generation interval than cattle. Both of these traits contribute to a higher reproductive efficiency. Some breeds of goat reach puberty as early as 4 to 6 months, but full sexual maturity is generally reached at 6 to 8 months of age in females and 8 to 10 months in males (Devendra, 1978; Doney *et al.*, 1982).

The average age at first kidding can be affected by breed. Majid *et al* (1993) reported the average age at first kidding for five breeds of goats in the southern United States to be 12.4, 12.5, 13.1, 12, and 12 months, for Alpine, La Mancha, Nubian, Saanen and Toggenburgs respectively. Wilson (1976) also postulated that the average age at first kidding in tropical conditions to vary between 8 to 10 months in West African Dwarf (WAD) goats and 18 to 24 in east African goats. This is comparable with findings of



Wilson and Murayi (1988) who found crosses between local East African goats and Alpine goats to kid earlier (557 days) than crosses with the Anglo-Nubian (766 days).

Gross anatomical aberrations of the female genital tract are nearly always manifested at the time of birth. Conditions such as a short luteal phase or alternatively, persistent corpus luteum, development of cystic ovaries comprised of follicular or luteal cysts and an-ovulatory oestrus or silent heat tend to increase in occurrence with age.

#### **2.4.2 Oestrous and the oestrous cycle in goats**

The critical event leading to the onset of puberty in cattle and sheep is the pre-pubertal increase in LH pulse frequency, that results from the decrease in responsiveness of the hypothalamic-pituitary axis to oestrogen negative feedback in cattle and sheep (Kinder *et al*, 1987).

A normal oestrous cycle in goats lasts 18 to 22 days. Oestrus lasts approximately 24 to 36 hours with ovulation occurring towards the end of oestrus i.e. 30 to 36 hours after the onset of oestrous. It was also found that the behavioural and physical signals of oestrous receptivity were more pronounced in goats than in sheep, thereby allowing for easier detection of oestrous and the use of artificial insemination (Doney *et al*, 1982).

Under tropical conditions, the West African Dwarf goat was observed to have an average duration of oestrous of 2.3 days, with an average oestrous cycle length of 22.4 days (Kinder *et al*, 1987; Doney *et al*, 1982).

#### **2.4.3 Prolificacy and fecundity in the female**

Oppong and Yebuah (1981) recorded a significantly higher number of multiple births (59 %), compared to single births (41%) in two flocks of West African Dwarf goats. Prolificacy in some herds of goats have been reported to be as high as 2.5 and 3.0 kids, but averaged between 1.8 and 1.9 (Chemineau and Xande, 1982). Reproductive efficiency

increases with parity and age. Ngere and Mbap (1982) reported that multiple births increased from 13% at first kidding to 64% by the third pregnancy. Devendra (1978) claimed that the percentage of multiple births increased from 19 % at first kidding to as high as 79% for second and later kiddings.

#### **2.4.4 Infertility in goats**

Female infertility may be due to structural, functional or infectious factors. Although it is often assumed that goats are relatively fertile animals, farmers have become accustomed to animal loss from infertility of 5%. Because of the low numbers of individual goats showing infertility, and the fact that the infertile does are not recognized until the end of the kidding season, veterinary advice on the cause of the infertility is not sought. Goats are usually kept under more extensive natural conditions than cattle, and the fact that bucks are run with does during the breeding season, rather than the sexes being segregated as in cattle, favours fertility. Infertility can be discussed under three causes: infertility due to structural defects, infertility due to functional factors and infertility due to infections. Structural defects of the genital organs are generally uncommon in goats. Emady *et al* (1975) found 72 % of the does with microscopic defects of the gonads in abattoirs.

Owing to the rarity of anastomosis of the adjacent allantoic vessels in twins, the freemartin condition is unlikely in goats, but incidences of 0.23 to 1.1 % have been recorded. Occlusion of the uterine tubes and hydrosalpinx is an occasional cause of ovine and caprine infertility. Other developmental abnormalities of the genital organs of goats are rare (Dain 1971, Long, 1980).

#### **2.4.4.1 Infertility due to functional factors**

Except in the case of inefficient does, sub-oestrus and anoestrus are believed to be uncommon in goats. In fact, when the bucks are turned out with the rest of the flock it is the rule for most of the does to be mated within a month.

The first oestrous of the breeding season of some does is not accompanied by ovulation and, according to Dutt (1954), does more frequently fail to be fertilized at earlier than at later mating. Hammond (1921) cited embryonic death or resorption to be a conspicuous feature in sheep infertility. By comparing the number of corpora lutea with the number of foetuses, the incidence of the condition was estimated to be between 8 and 13 %. Sporadic cases of abortion and of foetal mummification are occasionally seen in sheep. A few cases of cystic ovaries have been recorded, but in sheep they probably form a very small part of the infertility complex.

A specific environmental cause of sheep and goat infertility due to the grazing of females on pastures of subterranean clover was described in Australia. These clovers contain large amounts of estrogenic substances (genistein) the ingestion of which leads to cystic degeneration of the endometrium and permanent sterility.

#### **2.4.4.2 Infertility due to infections**

Endometritis sometimes follows dystocia and retention of the after-birth. This usually resolves in the following 6 months, before the doe is bred again. Endometritis is again followed by salpingitis. From time to time enzootics of abortion occur on sheep farms, and much veterinary research has been undertaken to eliminate the aetiology of these outbreaks.

Three specific microbial abortion diseases in sheep are well recognized namely Vibriosis, Salmonella and Viruses or Rickettsial. It has been established that toxoplasmosis is an important cause of both abortion and the perinatal death in lambs. Tick-borne fever is a

\* rickettsial disease that affects both sheep and goats. Indigenous sheep and goats are relatively immune but, when susceptible animals are introduced into tick-infested areas, they develop a febrile illness for about 10 days. If animals are pregnant at that time, abortion may occur in up to 50 % of the animals and mortality may be as high as 20 %. Susceptible sheep and goats should not be brought into tick-infested areas but, where this is unavoidable, an appropriate dipping routine should be followed (Arthur *et al*, 1982).

## \* 2.5 SEASONALITY OF REPRODUCTION IN GOATS

The breeding season of most small species appears to be controlled not only by a single factor, such as periodicity, but by a combination of external stimuli, including behavioural factors, which vary in different species. Nevertheless, these factors act through sense organs on the internal rhythms of the individual. In vertebrates this mediation operates through the central nervous system (CNS), the hypothalamus and anterior-pituitary which secretes appropriate gonadotrophic hormones. It has been well recognised that a variety of domestic species limit their breeding activities to specific seasons.

Whether reproduction is taken to mean mating or parturition, it is clear that when seasonal reproduction occurs, the newborn are provided with environmental circumstances that favour survival until they reaches puberty. In seasonal species, the mating is intensive in the breeding, season and subdued or absent during the remainder of the year. These remarkable switches in the breeding drive are naturally observed in both sexes. In breeds of sheep which breed throughout most of the year, it is recorded that the intensity and duration of oestrus has significant seasonal variation (Joubert, 1962; Shelton and Morrow, 1965).

In the female, the duration and intensity of oestrus have been observed to change with seasons. In cattle, degrees of seasonal reproduction are recognised most markedly in most tropical breeds (Kohli and Malik, 1960). Erb and Waldo (1952), stipulated that, with

constant temperature changes associated with climate appear to be able to affect reproductive behaviour. This has also been observed with cattle in breeding programmes, that a sudden spell of cold weather is associated with a sudden drop in the number of cows in oestrus.

✕ Some goat species indigenous to areas with equal climates, but with marked rainfall, show degrees of seasonal breeding in relation to the rainy seasons. In indigenous cattle it has been observed that breeding becomes intensified with rainfall and the associated rapid growth of herbage. This is in line with some native breeds of cattle in West Africa reported to show increased reproductive activities in relation to the rainy seasons.

In some captive animals, far removed from their natural habitats, the rhythms of breeding are often maintained and correlate, not with local environmental factors, but with the rainy seasons of their origin (Bodenheimer, 1938). In contrast, Parer (1963) found that goats translocated from tropical Africa to northern USA show a breeding season typical of all other goats at that latitude. Among domestic species, sheep and goats are short-day breeders. Many breeds of sheep and goat at tropical and near tropical latitude breed in all seasons (Ferguson, 1964; Hafez *et al*, 1955; Hill, 1960; Symington, 1961).

✕ It appears to be a general rule that, among species which manifest seasonal reproduction, long-day breeders are those which have long gestation periods (9 to 11 months) and short-day breeders, have short gestation periods (5 to 7 months).

✕ The breeding season of goats is limited to the autumn months (Phillips *et al*, 1946; Doney *et al*, 1982). Goats are spontaneous ovulating, polyoestrous animals that display a pronounced seasonality in breeding with increased latitude.

✕ Under a tropical environment, conception in goats can occur all year round. However, seasonal distribution patterns do occur (Oppong and Yebuah, 1981; Chemineau and Xande, 1982; Chemineau, 1983). Oppong and Yebuah (1981) cited that year round conception is evident under tropical conditions. It is possible to obtain two kiddings per year or at least three kiddings in two years, instead of the usual one per year obtained under a temperate environment.

## 2.6 ENVIRONMENTAL FACTORS AFFECTING FERTILITY

### 2.6.1 Photoperiodism

Among the environmental factors, light plays a major role in the annual periodicity of sexual activities in sheep. Salisbury and Van Demark (1961) described the effects on conception rate of climatological factors such as temperature, humidity, solar radiation, atmospheric pressure, precipitation, and day length as the most outstanding environmental clues for reproductive responses of a seasonal nature. In animals, some developmental processes are controlled by the length of the day. The annual cycle of reproduction or the beginning of the breeding rest period can be controlled by day light length. Photoperiods are due to the length of the day or night more than light quality. In a study on hamsters, Brown et al (2001) stated that male hamsters (*Mesocricetus auratus*) are seasonal breeders and show marked testicular regression when exposed to short autumn photoperiods. They remain sexually quiescent for several months. Photoperiod can be simulated when the length of the natural day is extended by artificial lighting of very low intensity and a breeding response induced. It was further found that by mid-winter, however, the hamsters show a loss in responsiveness to the inhibitory influence of short photoperiods, and the testes begin to recover. In a study with other animals, the exposure to a short photoperiod of 12 weeks resulted in a marked decrease in testicular mass and serum testosterone level. After 22 weeks, these reproductive parameters were once again significantly elevated.

Bunning (1964) claimed that the duration of photoperiod is more important than the intensity of light. Other researchers stated that there is no cause in the environment for seasonal breeding rhythms. In another experiment with female hamsters, Wade *et al* (1986) found that exposure of the animals to shorter photoperiods increased body weight and interrupted the oestrous cycles. Thus, body weight appears to respond to the relative timing of the two light pulses rather than to the absolute amount of light and darkness just, as does reproduction. Breeding seasons are in different months in the northern and

southern hemispheres for several domestic species. Evidence of seasonal breeding is often taken as the time when most females come into oestrus (Bearden and Fuquay, 1980; Frazer, 1968; Lincoln and Short, 1980; Shelton, 1978; Mellado *et al*, 1991).

### § 2.6.2 Temperature effects

• Scott (1961) postulated that seasonal depression of fertility due to heat stress, is attributable primarily to females. Environmental conditions associated with subtropical areas contribute to lower reproduction and milk production (McDowell, 1972). Heat stressful environments are associated with alterations in the hormonal balance such as an elevation in plasma progesterone, prolactin and reduced adrenal responsiveness to ACTH. Temperature of the reproductive tracts are associated with transitory changes in the plasma estradiol, approaching oestrus and ovulation.

• Daily peaks of mating activities are apparently associated with fluctuations in the male sex drive. Basirov (1960) observed that bulls and buffaloes exposed to high temperatures during the summer season showed a marked reduction in libido, particularly when heat stress occurred. It was noted that libido was inhibited in all buffaloes, and greatly reduced in bulls, at ambient temperatures of 40°C to 50°C.

• Libido quickly returned to normal after affected animals were cooled by wetting. MacFarlane *et al* (1959) indicated that rats subjected to high ambient temperatures mated less frequently than did control rats at room temperature. Ulberg and Burfening (1967) reported that pregnancy rates declined from 61% to 45 % when the rectal temperature, (12hours post-insemination), increased by 1°C. Alliston *et al* (1965) also reported that poor reproductive performance was associated with thermal stress. This may be due to high ambient temperatures acting directly on the developing embryo and through the maternal endocrine imbalances. It was also suspected by some that climatic hyperthermia in the boar causes a reduction in the mating drive. Burger (1952) claimed that it was common in South Africa to find boars extremely inactive during the hot hours of the day. Sometimes totally ignoring the presence of the oestrous sow. It was also suggested that

liberal wetting with cold water is usually effective in improving the sexual activity of such boars. Hafez (1952) ascertained experimentally that such wetting is highly effective in reducing hyperthermia in pigs.

In Holstein heifers under cool climatic conditions (20°C), the average length of the oestrous cycle was 20 to 21 days, compared to 25 days under controlled hot climatic conditions. This indicates that hot climatic conditions significantly lengthen the length of the oestrous period. In Europe, the heat of summer days in central southern Europe can adversely affect the reproductive functions in dairy cattle. Dutt and Bush (1955) and Dutt and Simpson (1957) have shown that fertility in sheep was improved early in the season when rams were kept at cooler temperatures during the summer months. It can also be concluded that changes in temperature, e. g. high temperature, appear more likely to affect sexual activities during day time in the tropics and warm climates (Frazer, 1968; Setchel, 1971). The effect of high ambient temperature extremes and environmental control on the milk production of dairy cows has also been demonstrated (Johnston, 1966). Hahn (1969) indicated that climatological interrelationships have been found which allow for the prediction of milk production losses during summer within certain geographical areas of the United States. The conclusion can be drawn that excess body heat inhibits libido, but is only temporary in effect.

### **2.6.3 Daily ambient temperatures**

Colder temperature is considered to have a slightly beneficial effect on reproductive behaviour of certain ungulates and this is especially apparent in certain seasonal species (Dutt and Bush, 1955). They described the effect of low environmental temperature on the initiation of the breeding season in sheep. Semen quality and fertility in the male are depressed during summer in most locations and exposure of bulls to high temperature, impairs spermatogenesis and several weeks are required for semen quality to return to normal (Swiestra, 1970; Courot, 1984).



Cold ambient temperature appears to hasten the onset of reproductive activities, but effects seem slight. Darling (1937) found that, in the red deer, sudden cold weather close to the onset of the breeding season stimulates breeding activity dramatically. It was also found that in the insemination of cattle in Bavaria, weather conditions and the occurrence of oestrus were related. Increased blood progesterone levels were recorded in heat stressed females that indicates an altered hormonal status, which may contribute to reduced fertility.

In the females, heat stress delays puberty, causes anestrus, depresses estrual activity, lowers conception rate, induces abortions and increases peri-natal mortalities. High temperatures can also have a direct detrimental effect on developing spermatozoa and fertility. Good weather leads to more cows showing oestrous but poor weather can lead to a reduction in the incidences of oestrus (Swiestra, 1970; Courot, 1984).

#### **2.6.4 The effect of humidity on reproduction**

Gwazdauskas *et al* (1973) have evaluated the influence of humidity and environmental temperature on reproduction, plasma progesterone and corticoid concentrations on reproduction and determined the interrelationships of these factors on conception rates. Johnston and Branton (1953) illustrated that temperature and humidity are related to the fertility of bulls. In their study they indicated that as temperature and humidity increased, fertility decreased and *vice versa*. Light appears to be the most important controlling factor.

The fertilization rates in ewes exposed to elevated humidity on the 12<sup>th</sup> day of the cycle before breeding was significantly lower than that of the control ewes. The heat treatment also resulted in an increase in the percentage of abnormal ova in ewes. It was further indicated that only 3.7 % of ova from the control ewes examined 3 days after breeding were classified as morphologically abnormal, compared to 44.2 % from ewes under hot conditions. Fertilization and survival rate of the early embryo in the ewe appear to be more susceptible to heat damage.

High humidity, such as that found in the tropics and subtropics, has a definite depressing effect on the expression of oestrous in female animals. Anoestrus in the female has become a more important breeding problem under hot climatic conditions than under cool climatic conditions. However, light's effect is influenced by temperature, humidity, and, in many cases, by the nutritional status of the animal involved.

Temperature appears to be the second most important factor involved in seasonal fertility. The effect of humidity appears to be largely one of causing greater discomfort that accompanies high humidity at high temperature. Nutrition is an important seasonal factor, only if undernutrition results from the effects of the season.

#### **2.6.5 Effect of season of the year on reproduction**

Under semi extensive conditions in which the bulls remain with the cow herd all year round, mating takes place during the late spring and summer months in northern USA. In the study to test the effect of season on fertility in cows in Canada, Mercier and Salisbury (1947), found the conception rate to be significantly correlated with the mean monthly daylight length, with a lag of one to two months from the longest days to maximum fertility. The fertility of cows at various ages during each season of the year was also recorded. Findings indicate that heifers and cows up to about 7 years of age tend to be low in fertility during winter and summer and higher in spring and fall.

The effect of season in cows has been indicated, especially in areas farthest from the equator. The effects of long hours of daylight in the spring tended to lower the age of conception in heifers. It has also been deduced that, in areas closer to equator, the difference in long daylight length from one season to the next are not big and the effect of season on age of calving is less pronounced. The variation in this report concerning the effects of season on the fertility of bulls may also be due to variations in the age of the bull studied. Mercier and Salisbury (1947) found that on the average, the fertility of bulls was lowest in winter, gradually improving in spring, and reaching a peak in summer and

fall. Fertility was also found to be significantly correlated with daylight length, with one to two months lag as found for cows.

Fertility of bull under 4 years of age was at a peak in winter, declined with the coming of warmer weather, and improved in fall. It was concluded that young bulls were probably more subject to extraneous factors (especially temperature) than were older bulls.

## **2.7 PHYSIOLOGICAL FACTORS INFLUENCING REPRODUCTION**

The most widespread form of depressed fertility and sterility, with the exception of the pathological types, is that resulting from physiological causes. Some causes, such as the changes brought about by age and the variations in light and temperature, which occur in different seasons of the year, are largely uncontrollable. These directly or indirectly affect the general endocrine balance within an animal, causing changes in the levels of the controlling hormones (Cole and Garrett, 1980).

There are many instances where the reproductive potential of an animal is reduced by an endocrine imbalance. It has been found that the effects of age and season on fertility are complex, as there are so many interacting factors involved. Salisbury and Van deMark (1961) postulated that heifers and young bulls increase in fertility as they grow older. In heifers this increase continues up to 4 years of age, levels off at about 6 years of age, then gradually declines as the animal grows older.

Bulls have been seen to reach peak fertility at about 2 years of age, thereafter there is a slow decline with increasing age. However, many older cows and bulls remain highly fertile throughout a lifetime that is much longer than the average for cattle (Cole and Garrett, 1980; Van DeMark, 1961).

### **2.7.1 Endocrine imbalances in the male**

Disturbances in the endocrine system, which seem to influence reproduction, centre around the gonadotrophic hormones of the anterior pituitary (FSH, LH and prolactin), and the hormones of the testes. It is assumed that bulls producing low numbers of spermatozoa may be deficient in the pituitary hormone, FSH, which stimulates spermatogenesis (Rhind *et al*, 1986; Foster and Olster, 1985; Gomes and Joyce, 1975). Bulls that become lazy or lose their sex drive are assumed to have a deficiency of androgens which, in turn, may be caused by a lack of LH from the pituitary. When stimulating sexual activities in low breeding bulls by the use of gonadotropins, Asdell (1949), in his review, cited that a favourable response was obtained by injecting testosterone propionate.

### **2.7.2 Psychological disturbance in males**

Psychological aberrations resulting in lower fertility of farm animals have been received with great scepticism. Recently it was observed and evidence found it more and more apparent that gentle handling can enhance the chances of a successful reproduction performance. Mistreatment and abuse of both males and females may lower fertility or even cause sterility (Bauman and Currie, 1980; Salisbury *et al*, 1978; Bearden and Fuquay, 1980).

The pathways involved in lowered fertility from mistreatment involves mainly the hormone of the adrenal medulla, epinephrine, and the hormone from the pituitary, ACTH, which causes the adrenal cortex to release the adrenocortical hormones. The effect in cows of psychological disturbances has been recorded by the degree of nervousness displayed when inseminated artificially. Pouden and Firebaugh (1956) have rated cows during insemination as completely quiet, slightly nervous-1, moderately nervous-2 or jumpy-3.

It has long been known that mistreatment, abuse, or an unfavourable experience on the part of a bull at the time of mating or at semen collection with an artificial vagina or electro-ejaculator may cause it to develop psychological inhibitions that will prevent it from mounting and ejaculating. Some claims have been made that transportation stress can impair fertility. Willet and Larson (1953) claimed that transportation has a marked detrimental effect on the fertility of males. In this work, it was found that the average fertility of the bulls was slightly higher over a two-month period following translocation than before. Although transportation stress may occasionally affect fertility, it would, on the average, appear to be of no consequence. Evidence indicates that much can be gained by gentle treatment and much can be lost as a result of abuse (Willet and Larson, 1953).

### **2.7.3 Age of an animal and reproduction**

The effects of age on the fertility of males and females have been difficult to assess as such effects are complicated by many interactive factors. The age at puberty is breed dependent and varies between individuals. Environmental factors, such as the season of the year (climate), managerial factors, the presence of other males or females and sexual status frequently affect one age group more markedly than another group.

**Table 2.7.3.1 Average age at puberty in different animal species**

<b>Species</b>	<b>Puberty (m)</b>	<b>Variation (m)</b>
<b>Bull</b>	<b>10-12</b>	<b>4-18</b>
<b>Ram</b>	<b>6-7</b>	<b>4-12</b>
<b>Stallion</b>	<b>18-24</b>	<b>12-24</b>
<b>Boar</b>	<b>4-5</b>	<b>4-8</b>

*Adapted from Courot M (1984). The male in farm animal reproduction.*

Tanade and Salisbury (1946) reported that cows ranging from 1 to 2 years of age, fertility increased up to 4 years of age, levelled off to 6 years of age and then gradually declined.

A larger sample of cattle bred by artificial insemination was surveyed by Herman (1956) and his findings confirmed the report by Tanade and Salisbury (1946). However, several studies have shown that fertility improved slightly after the first one or two pregnancies and decreased after about the 10<sup>th</sup> pregnancy in cattle. Mercier and Salisbury (1947) reported that aged cows differed notably in their response to season from that of younger groups. It has also been reported that the fertility of bulls declines with advancing age.

Tanade and Salisbury (1946) found that the fertility of bulls used in an artificial insemination programme reached a peak at 2 years of age, and then declined gradually. Nevertheless, many older bulls, remaining after rigid selection in an artificial insemination system are as fertile as young bulls (Herman, 1956).

## **2.8 STRESS FACTORS AND FERTILITY**

Transportation of males from place to place has led to the assumption that the stress of such transportation was deleterious to semen production and fertility. The incidences of shipping or transport fever and exposure to infections resulting in transitory fever in such instances, probably contributes to the feeling that the transport of males is to be avoided. It is recommended that some type of suspensory support be provided for the testis to avoid testicular damage (Courot, 1984). This practice results in an increase in testicular temperature by removal of the natural heat-regulating mechanism for a period of more than 4 hours. This in itself proves damaging to spermatogenesis and ultimately in fertility.

## 2.9 EFFECT OF LIGHT INTENSITY ON FERTILITY

Light appears to be the primary factor involved in seasonal effects on fertility. In areas where the season results in equal periods of daylight and darkness, the highest fertility appears to occur in the spring, with the increasing hours of daylight (Salisbury and VanderMark, 1961). The role of external factors in seasonal reproduction, with photoperiodicity well known, as the principal agent is well recognised (Hammond, 1944; Yeates, 1954; Amoroso and Marshal, 1960).

The breeding seasons of sheep and goats are determined in most instances by the periods when the majority of females come into estrus. Watson and Radford (1960) have shown differences in breeding responses between breeds under similar environmental conditions. Clegg and Ganong (1959) also reported that gradual light reduction could induce oestrous in goats. It was also stated that extra-seasonal breeding could be induced in the goats treated with an artificially controlled light: dark ratio. Reduced lighting is also fairly effective in the induction of oestrous in goats but, conversely, increasing light results in the cessation of oestrus cycling in goats.

An important element of the seasonal phenomenon appears to be breed-specific sensitivity to some quantity of darkness per day. It is, therefore, assumed that males will have their reproductive efficiency seasonally influenced in a manner which will coincide with the females' breeding season. Pepelko and Clegg (1965) observed that, in rams presented to an oestrous ewe for one hour twice a month over a period of a year, the average number of ejaculates varied significantly according to the month and the season. Summer heat tend to counteract the beneficial effects of long daylight hours, and the lowest fertility often occurs during the summer. Humidity and nutritional status can completely mask or reverse the normal effects of light and temperature. A number of the physiological causes of lowest fertility also involve endocrine disturbances.

## 2.10 EFFECT OF CHANGE OF CLIMATE ON REPRODUCTION

Changes in weather such as cold, rain or heat can be responsible for temporary alterations in the breeding behaviour. Early reports by Mercier (1946) claimed that the seasonal effect on spermatogenesis was largely conditioned by the hours of the daylight and temperature variation. In a study Burgress (1953), no significant monthly or seasonal differences in conception rates was found. In Israel, it was found that the conception rate was lowest in September when the lowest sperm concentration and survival rate were recorded. It is also suggested that hours of daylight and temperature could be correlated with semen quality changes and also with fertility. However, when the seasonal differences in temperature and the length of day are small, marked changes in semen quality or fertility are not expected.

Marked seasonal differences in semen quality over a period of one year have been reported by Johnston and Branton (1953), but there were no corresponding significant differences in non-return rates.

Casady *et al* (1953) also obtained an adverse effect on semen quality by subjecting bulls to high environmental temperatures (30°C to 36°C). Patrick *et al* (1959) subjected bulls in the southern part of the USA to varying conditions of atmospheric temperature and humidity but found no change in semen production or fertility. In a study on the effect of season on fertility over a 5 year period, Erb and Waldo (1952) found the conception rates (non-return) to be highest in September to November and lowest in January to April.

The season of the year in which estrus is first exhibited (when puberty is reached), is the first point of importance. More permanent breeding habits among ungulates, however, occur with adaptation to more long-term weather changes. A slight tendency has been noticed for cycle length to vary with the season in the case of high-plane fed animals, longer cycles being recorded during summer than the winter months. The majority of the



high-plane fed animals reached puberty during winter while 85.7 % of the low-plane fed heifers demonstrated oestrous for the first time during the summer.

Low-plane frequencies during the late summer months are considered to be indicative of nutritional effects. It indicated that the low-plane fed animals reacted almost immediately on the stimulating effects of early summer grazing. An environment which could be unfavourable to heifers could be created when a season is associated with severe conditions which last for a considerable period of time. Where severe weather persists for a lengthy period of season, it has been found that indigenous species do not breed at that time which would result in births taking place during a season of inclement weather. Therefore breeding responses can be inhibited as an evolved adaptation.

Throughout Africa, the breeding season of the native ungulates usually varies according to the climate and the nutrition of the region. With the exception of the thermal factor, the tropics can be considered as regions of maximum climatic stability and do not restrict reproductive activities to the same extent as many other regions of the globe (Frazer, 1968). This indicates that sheep first restore the depleted body tissues, before the reproductive function recovers to normal (Joubert, 1962). The cycles tend to be shorter in midwinter and increase in length with the approach of summer, finally reaching a peak during late summer.

Generally as soon as the nutritional levels became critical, the low-plane fed heifers relapse into a state of sexual inactivity and usually remain in that state until late summer. High-plane fed heifers show little variation over the year-thus confirming the generally accepted principle that the domesticated cow under normal feeding and management conditions will breed at any time of the year (Joubert, 1962).

## **2.11 EFFECT OF NUTRITION ON REPRODUCTION ✓**

Throughout the world, the nutrition of sheep and goats has long been a factor of concern due to its effect on the reproductive performance of the animals. Where nutrition is adequate, reproductive performance might also be expected to be adequate, but this indicates little about the specific nutritional requirements at the different phases of reproduction. This information is vital for the optimisation of reproductive performance in a nutritionally fluctuating environment. Only when this information is available can grazing and feeding management be adjusted in the necessary tactical manner (Gunn, 1983).

Gunn (1983) indicated that energy nutrition is of major importance in reproductive performance and three major aspects need to be considered. There is the long term energy intake which includes the foetal stage throughout the growth and development of the animal until sexual and body maturity. The medium term energy intake entails the period in a given annual cycle in the mature animal and short-term energy intake included the immediate pre-mating and mating periods.

### **2.11.1 Nutrition in relation to the ovarian activity in females**

The effects of limited feed intake on the age of puberty, the age of first production of spermatozoa and ovum, and the rate at which mature semen and ovum production is reached are due primarily to diminished gonadotropin output by the pituitary gland. On the other hand, liberal feeding brings males and females into active sperm-cell as well as ovum production and causes active secretion of the accessory glands earlier than does limited feeding. Research that has been done with sows (Robertson *et al*, 1951; Christian and Nofziger, 1952; Haines *et al*, 1959; Zimmerman *et al*, 1960) and the ewe (El-Sheik 1955; Foote *at al*, 1959) has shown that energy levels have a marked effect on ovarian activity.

Prolonged restriction to dietary energy also results in the delayed onset of puberty, disruption of cyclicity in sexually mature animals, cessation of ovarian activity in cycling heifers and lengthening of the post-partum anestrus period in domestic animals (Joubert, 1954; Wiltbank *et al*, 1957; Reid, 1960; Keith, 1992; Louw *et al*, 1987).

Low levels of energy result in ovarian inactivity and postpartum anoestrous in suckling young cows, regardless of the level of protein. Wiltbank *et al* (1957) have also shown that energy has a marked effect on the ovarian activity of the sow, ewe and young beef females. Wiltbank *et al* (1962) have also demonstrated that changes in body weight and body condition are influenced significantly by the different feeding levels. It is further shown that prior to calving cows in the High-High and the High –Low groups gained weight and maintained their body condition, while cows in Low-high and Low-low groups lost weight and condition and were thin at calving.

Levels of energy following calving had a marked effect on changes in body weight and body condition scores. Treatment before calving had little or no influence on calving difficulties or the percentage of stillborn calves (Zimmerman *et al*, 1960). The average birth weight of calves born from cows in the Low-Low and Low-High groups was 5 kg less than those from cows in the High-High and High-Low groups.

### **2.11.2 Effect of feeding levels on the reproductive tract, ovarian activities and the intra-uterine environment of female animals**

Nutritional levels affect the size and weight of the reproductive tract of ewes. In dairy cows, as indicated by Butler and Smith (1989), fertility becomes confused with milk production during the postpartum period. Concerning the relationship between excess body condition or the feeding of excess energy and fertility, some experiments have reported a negative relationship as well as no relationship at all.

The study by Wallace (1954) indicated that feeding ewes on a high energy level caused a significant increase in the number of corpora lutea and in the size of the largest follicle. It

appears to be an accepted fact that cows losing condition at breeding have decreased fertility, whereas those gaining weight at breeding showed improved fertility (Butler *et al* 1981).

From the foetal stage, until maturity, nutrition in female animals may influence its subsequent reproductive performance by affecting the time or age of the onset of the first viable oestrus, by affecting the fertility and fecundity at this first oestrus or by residual effects on reproductive performance during the remainder of its reproductive life. It has been demonstrated that the uterine horns of the ewes fed on a higher energy level were larger than the horns of the ewes on only roughage. That certain reproductive phenomena in swine can be altered by varying the quantity of feed, has been indicated by Robertson *et al* (1951), Christian and Nofziger (1952), and Burger (1952).

Robertson *et al* (1951) and Christian and Nofziger (1952) also reported higher ovulation rates and lower embryo survival rates with a high energy level of feeding, compared to a moderate level-indicating that a level favourable to ovulation was not favourable to embryo survival. These results are also supported by El-Sheik *et al* (1955), who found that by increasing ovulation through increased feeding levels, embryo mortalities are also increased when measured at days 40 of gestation in cattle. It is well known that under-nourishment of ewes during late pregnancy can depress birth weight and lamb vigour, irrespective of the number of foetuses carried.

When comparing the pregnant uterine horns only, it was shown that ewes which had twin embryos (each in a separate horn) had significantly larger and heavier horns (cotyledons removed) than pregnant horns of ewes carrying a single embryo. Crown to rump length of the embryos, weight of the embryos, weight of the amnions, weight of the chorion, weight of the amnionic fluid and the size of the amnionic vesicle were greater for single than for twins under both nutritional levels of feeding (El-Sheik *et al*, 1955). These differences are known to remain pronounced in early life and are associated with lower milk intake of the smaller lambs and the inadequate ewe nutrition which is likely under such circumstances. Gunn (1977) and Alleden (1979) stated that when restriction of feed is severe and long term, compensatory growth does not overcome the initial restriction in

growth and mature size. This is either because the adult high plane of nutrition is inadequate or because the low plane of nutrition during rearing produces animals incapable of compensating. It was further indicated that the early limitations had long-term persistent effects on the adult reproductive rate in both high and low adult nutritional environments.

However, it has been found that body size and maturity is not the whole story. In a study of Alleden (1979), an unrestricted plane of nutrition for as short a period as the first 8 weeks of life prior to a lengthy period of restriction was clearly effective when compared with restricted nutrition from birth, in establishing a high reproductive potential. The results suggest that there may, therefore, be a critical development period which is highly sensitive to nutrition and which markedly affects the extent to which the genetic potential is achieved in later life. As cells, tissues, organs and systems are all initiated during gestation in a sequential development pattern, it has been suggested that the factors operating during these intrauterine formative stages may well modify subsequent productive possibilities (Everitt, 1969). Parr *et al* (1982) have shown concerns of a long-term effect of maternal undernutrition in early pregnancy on the adult productive life of the progeny.

To summarise, underfeeding (i. e energy restriction), appears to affect the hypothalamic and ovarian functions. A negative energy balance may be exacerbated by feeding excess protein because, as pointed out by Tyrrel *et al* (1970), it requires energy to metabolise excess protein. Research by Ferguson *et al* (1987) indicated that postpartum fertility was significantly enhanced by supplementation.

### **2.11.3 Nutritional effects on the occurrence of oestrus and uterine involution in female goats ✓**

There is a trend for the uterus of cows receiving a low energy diet either before or after calving, to involute more slowly. Wiltbank *et al* (1962) stated that treatment does not significantly affect the interval between calving and involution of the uterus.

The occurrence of oestrous after calving was significantly influenced by the level of nutrition. The level of energy provided before calving appears to be more important to effect the occurrence of oestrous. It was further found that a higher proportion of cows cycled by 90 days post calving, and oestrous was exhibited sooner after calving in cows receiving a high energy diet prior to calving.

The majority of cows fed a low energy diet failed to show oestrous, while most of the cows (85 %) on the low to high regime cycled by 90 days post partum. The interval from calving to first oestrus in the low-high energy group, was however significantly longer. Thus, it would appear as if a low level of energy prior to calving had an effect on the length of the interval from calving to first oestrous which was not readily overcome even though the cow received a high level of energy after calving.

### **2.11.4 Short term energy effect on fertility**

The period immediately prior to and at mating sensitive to short-term energy effect. Ovulation rate appears to respond to a short-term energy intake only within a specific intermediate body condition range. Above and below this range, it is the condition achieved that matters and there is no additional positive or negative effect of the energy intake (Brahma, 1996; Andrew and Orskov, 1970).

The practice of flushing females by improvement of their nutritional status for a short period of time immediately prior to breeding is often recommended as a method of increasing lamb crops (Foote *et al*, 1959). Here it was also found to increase ovulation

rate of mature ewes, due to flushing for a period as short as 3 weeks. Gosset and Sorensen (1959) reported that conception rate was somewhat lower in cows on a low level of energy after calving. By daily changing the diet from 3.63 to 7.26 kg of total digestible nutrients (TDN) per head, half of the cows that had not shown oestrous within 90 days post calving, initiated estrus and ovulation. Joubert (1954) showed that the main reason of supplemental feeding is in decreasing the length of the interval from calving to first oestrous. The energy levels after calving may also affect the subsequent conception rates. Lack of energy per se can also be a limiting factor for reproductive performance.

Body condition manipulation and experimental treatments involving several weeks of flushing, have recorded ovulation rate responses in groups of ewes. Work in swine has shown an increase in the ovulation rate when excess energy is fed for a short period of time (Zimmerman *et al*, 1960). During flushing, ewes need to have a greater appetite drive over mating and ewes which are not in such a physiological state, and which have a lower appetite drive, will not respond to flushing, no matter how long this is provided.

In another study, ewes in a poor body condition have been observed to eat 35 % more at mating than those which are in a good condition. Such a response to flushing may be interpreted as being associated with the increase in voluntary feed intake shown by the ewes in a poorer, but not poor body condition. Workers have shown that the ovaries of underfed rats and guinea pigs are responsive to gonadotropic hormone (Mulinos and Pomerantz, 1941; Stephens and Allen, 1941). It is clearly shown that ewes should not be losing weight at the time of mating. If well enough nourished to at least maintain body weight, ewes will produce more lambs than if they had been losing weight. Mann (1960) reported that the underfeeding of young bulls alters the semen composition and the effects can be prevented or reversed by an injection of gonadotrophic hormones.

### **2.11.5 Medium term-energy effects**

Considerable attention has been paid to the effect of level of the feeding and type of diet on sexual maturity, fertility and semen production. Jones and Krohn (1960) demonstrated a delay in sexual maturity in young animals (bulls) raised on alfalfa hay and reduced energy intake. In work done by James (1950), with twin bulls fed on two different nutritional levels from twenty weeks to two years of age, he found that, at 15 to 25 months of age, the bulls in the low plane group had a reduced level of total spermatozoal production, and smaller testes than in the higher plane group. He further reported reduced spermatozoal production and retarded onset of spermatogenesis in the calf on the low plane of nutrition.

A loss of energy stored in the body is due to the fluctuations in energy intake during the annual cycle. Energy is stored in the body in the form of fat. Since there are considerable differences in the storage of energy within the body according to the stage of the cycle, provision of energy in a grazing situation may fail to match the requirements. MacGregor and Swanepoel (1992) have concluded that under-nutrition (low body mass) was a greater cause of low fertility in young animals than over-nutrition (high body mass). Though there may well be interactions with long-term energy effects and genotypes, the most important period in relation to reproductive performance is that between the end of one pregnancy and lactation and the start of the next.

Lamond (1970) and Meaker *et al* (1980) inferred that adequate nutrition, both pre- and post-partum, is of vital importance in order to sustain good reproductive performance in beef females. This has been referred to as the recovery period, since it is the time that the body tissue reserves utilized during the previous pregnancy and lactation are replenished.



#### \* 2.11.6 Influence of nutrition on testicular growth

Production of spermatozoa and sex hormones are the main functions of the testes, and these are sensitive to changes in nutrition. During the developmental period in well-fed ram lambs, the reproductive organs increase rapidly in size parallel to the enhancement of the endocrine function (Skinner *et al*, 1968), leading to the attainment of puberty at about 4 to 7 months of age and about 28 to 36 kg live weight (Dun, 1955; Watson *et al*, 1956; Pretorius and Marincowitz, 1968). In a state of under-nutrition, testes size declines, whereas the testes of rams in a good nutritional regime are relatively large (Setchell *et al*, 1965).

In Merino rams, Oldman *et al* (1978), Knight and Lindsay (1973), Lindsay *et al*, (1979) have shown that spermatozoa are produced at a relatively constant rate of about  $20 \times 10^6$  spermatozoa/ gram testes per day, irrespective of the size of the testes.

Therefore, larger testes can be expected to produce more sperm per day. Wilson *et al*, (1977) stated that supplementation increases the daily output in pigs. Lindsay *et al* (1979) also indicated that rams are capable of doubling their size of their testes in 8 weeks after consuming feed supplemented with protein. Spermatozoa output is also correlated with both scrotal circumference and testes weight in bulls (Willet and Ohms, 1955; 1957).

The size of the testes in rams can be influenced by several factors, including season, the protein and energy content of the diet. Oldman *et al* (1978) concluded that testes appear to be particularly sensitive to changes in nutrition and that rams may gain or lose testicular volume at a greater rate than live weight. The practical value of their findings is that males should receive additional protein supplementation on natural dry pastures prior to and during the mating season particularly when the breeding season is relatively long.

### **2.11.7 Influence of protein on fertility**

Animals require protein as a source of essential amino acids and as a nitrogen source for rumen microflora. The effect of excess protein on fertility may be expressed through the reproductive hormones (Swanson, 1989). In an experiment with Holstein cattle, data indicated that the administration of GnRH in the preceding oestrous cycle increased serum progesterone during the luteal phase of the subsequent cycle in cows fed a 15 % crude protein (CP) diet, but not in cows fed a 22 % crude protein diet.

Jordan and Swanson (1979) reported plasma progesterone to be higher on day 15 of the oestrous cycle in cows fed a 12 % crude protein diet, compared to cows fed a 23% crude protein. It has also been observed that urea concentrations in uterine secretions are 2.7 times higher in cows fed excess protein. Visek (1982) has shown urea to be harmful to spermatozoa.

### **2.11.8 Influence of vitamins and minerals on fertility**

Several vitamins and minerals can influence fertility (Hurley, 1989). In lactating cattle, high-producing cows are probably subjected to minor deficiencies in minerals or vitamins, which together may be detrimental to fertility. Folman *et al* (1987) reported excess  $\beta$ -carotene to be harmful to fertility. Julien *et al* (1976) and Trinder *et al* (1973) indicated that deficiencies in selenium and vitamin E were associated with retained placentas. Tsou *et al* (1977) indicated copper to have a direct effect on the hypothalamus, stimulating the release of LHRH.

Ingraham *et al* (1987) indicated that a combination of Copper and Magnesium were effective in improving fertility in dairy cattle whereas those supplemented with Copper or Magnesium alone were not. A Vitamin A deficiency was found to lead to the birth of dead calves, with frequent retained placentas and these conditions somewhat resemble that resulting from Brucellosis infection (Madsen and Davis, 1949). The usual clinical signs of vitamin A deficiency includes night blindness, ophthalmia and diarrhoea, as well

as loss in body condition. Bull calves kept on a low vitamin A or carotene diet showed a degeneration of the germinal epithelium in the testes and the absence of spermatozoa. The influence of vitamin C (ascorbic acid) deficiency upon reproduction is not yet clear. Vitamin E (tocopherol) has been reported to be an important factor in bovine reproduction and, if fed in sufficient quantities, many cases of infertility can be cured (Madsen and Davis, 1949).

The primary requirements of minerals are for sodium chloride, phosphorus and calcium. A general mineral deficiency observed in the field is that of phosphorus. Phosphorus deficiency tends to occur when diets low in protein are fed, under conditions more or less resembling those found on the range when the grass is dry.

Phosphorus deficiency in the male has an effect on reproduction and is primarily noticed by a reduced appetite and consequent low intake of all nutrients. The phosphorus drain for semen production is far less than that resulting from heavy lactation. The amount of phosphorus required by the adult animal for efficient reproduction is approximately 10 to 12 mg per day. Calcium and phosphorus play a large role in the health and reproductive efficiency of the animal. The calcium–phosphorus ratio is of vital importance (Maynard and Loosli, 1962). The usual recommendation is to maintain this ratio between the limits P: Ca and 2:1 for maximum efficiency. Phosphorus deficiencies also occur in the soil and where animals are not fed adequate protein and mineral supplements. Reproduction begins to suffer when the animal start showing clinical signs of the deficiencies which include a rough coat, and an appetite for bones, soil and wood.

It has been reported that, on many farms where a phosphorus deficiency occurred, less than one calf was obtained every two years, even though there were no more than the usual number of abortions (Eckles *et al*, 1935). It was further reported that heifers often failed to come into oestrous until two years old. Palmer *et al* (1941) reported a delayed first oestrous in heifers which had been raised on phosphorus deficient diet. Conception occurred, but calving tended to be difficult and the calves born were either weak or dead. In more severe cases the growth and maturation of ovisac is hampered, possibly through a

reduction in the secretion of the follicle-stimulating hormone (Maynard and Loosli, 1962).

Low manganese intakes, on a diet of poor timoty hay, corn grain and corn-glutin, reduced the spermatozoa concentration and motility. The feeding of the toxic element, molybdenum, to bull calves results, in addition to the usual symptoms reminiscent of copper deficiency, in a complete lack of libido and inhibition of spermatogenesis and interstitial cell development (Salisbury and Van Demark, 1961).

## CHAPTER 3

### 3.1 MATERIAL AND METHODS

The experimental study was conducted at the Agricultural Research Council in Pretoria (25° 55' S; 28° 12' E), South Africa. The area is situated in a typical highveld climate at an altitude of 1525 m above sea level. The weather condition ranges from hot days and cool nights in summer to moderate winter days with cold nights. Two breeds of male goats were used in the experiment, namely Gorno Altai and indigenous goats which were randomly selected. The Gorno Altai bucks were 4 years of age and the indigenous bucks 5 years of age and weighed between 45 to 65 kg. The experimental animals were housed in enclosed pens with covered roofing and an open-air area. Every morning, the bucks were fed lucerne and hay, while water and mineral licks were provided *ad libitum*. The trial commenced in January 2000 and was completed at the end of December 2000.

Monthly air temperatures were obtained from the Weather Bureau Services of South Africa in Pretoria. To determine the effects of environmental temperatures on semen production, the average daily temperatures were calculated according to the following formula (Loubser *et al*, 1983):

Average daily temperatures (°C) =  $\frac{\text{Maximum temperatures} + \text{Minimum temperatures}}{2}$   
and converted to a monthly average (Loubser *et al*, 1983).

### 3.2 PREPARATION OF BUCKS FOR ELECTRO-EJACULATION

Semen was collected during the second week of each month. Before semen was collected, the prepuce of the penis was washed with a sterile gauze swab in order to remove dirt and excess urine from the sheath. Preputial hairs were clipped and the preputial orifice was cleansed thoroughly.

Semen collection is similar to harvesting any crop in a season. The effective harvesting of semen involves obtaining the maximum number of sperm of the highest possible quality from superior males. This involves proper semen collection procedures with males sexually stimulated and prepared. <sup>Δ</sup>

### ✕ 3.3 SEXUAL STIMULATION

Sexual stimulation prior to semen collection was induced by parading the teaser doe for several minutes amongst the males. Sexual stimulation was done in order to increase the number of spermatozoa per ejaculate, for the highest possible quality ejaculate. False mounts without ejaculation were allowed in order to enhance the degree of sexual excitement. Sexual stimulation was conducted according to the method of Hale and Almquist (1960) and Collins *et al* (1951) in order to increase semen production as sexual stimulation and preparation increases the semen volume and sperm concentration of the ejaculates in males. Ejaculates with a larger volume, high concentration, and higher motility are generally considered to have a higher fertility rate. <sup>Δ</sup>

### ✕ 3.4 PREPARATION OF THE ELECTRO-EJACULATOR

Semen was collected by means of an electro-ejaculator. The apparatus consisted of a bipolar electrode and a variable source of alternating electrical current. The voltage ranged from 0 to 30 volts with a low amperage (0.5 to 1.0). The electrode had negative and positive conductors running longitudinally along the electrode. Prior to the rectal insertion of the rectal probe, the electrode was lubricated with KY-jelly ease insertion.

Lubrication was also done in order to avoid injuries and causing stress to the male animal. The electrode was placed in the rectum (12 cm) immediately above the accessory sex glands in order to stimulate reproductive system. Three levels of voltage were applied with the buck responding at a peak of 8 volts (Maxwell and Evan, 1987; Bearden and Fuquay, 1980). The process of electro-ejaculation was accomplished with the buck on its side. <sup>Δ</sup>

### 3.5 SEMEN COLLECTION USING ELECTRO-EJACULATOR

Semen was collected in a graduated glass collection tube. Semen was collected from each buck once a month over a period of a year. Immediately following collection, the semen sample was placed in a water bath at 35°C. Each ejaculate was examined for volume, percentage progressive motile sperm and sperm concentration.

The semen volume was measured in a collection tube enclosed in a holding swab and the colour evaluated. Semen was also taken to the laboratory as soon as possible. A microscope was mounted with a hot stage in order to maintain the body temperature of the ejaculate.

The slide was allowed to warm up to 38°C. The intensity of the wave motion (WM) in an uncovered drop was estimated microscopically on a warm stage and rated between 0 to 5 (very turbulent). Motility was observed under a light microscope at a magnification of 100 X. Several fields were examined in order to estimate the percentage of sperms that were progressively motile. Measurements that were observed were mainly motility or wave motion. \*

### 3.6 ASSESSMENT OF SPERM CONCENTRATION WITH THE AID OF A HAEMOCYTOMETER

Semen concentration was determined with the use of a haemocytometer. A sample of 0.01 ml semen was diluted with 4 ml of physiological saline solution. A cover slip was placed on the counting grid of the haemocytometer. The edge of the counting chamber was touched with the tip of the pipette to allow a drop of diluted semen to run under the cover glass.

Spermatozoa were allowed to settle for 5 to 6 minutes before the haemocytometer was placed on the warm stage of the microscope. The haemocytometer was placed on the microscope under direct lighting with a magnification 400X to cover a large square on the grid. At least five large squares were counted and the total number of spermatozoa recorded.

### **3.7 STAINING OF THE GLASS SLIDES WITH SPERM**

A staining agent was obtained from the Onderstepoort Faculty of Veterinary Medicine, University of Pretoria. The agent was composed of 1 % eosin and (Nigrosin 5 %) compounds. Both eosin and the background stains were dissolved in 2.9% sodium citrate dihydrate buffer. The slides with sperm were dried quickly on a warmed plate (55°C-60°C) with a small electric fan directed across the plate. This was done to overcome early death of spermatozoa and the penetration of the staining agent before the slide was completely dry. Several fields at random were examined and sperm counted under a microscope.

### **3.8 ASSESSMENT OF PERCENTAGE DEAD AND ABNORMAL SPERMATOZOA.**

A clean slide was placed on the warm stage of the microscope. A drop of eosin-negrosin stain was placed on the slide and a drop of pure semen was mixed with the staining agent for ten seconds. The mixture was left to stand for approximately 50 seconds. The mixture was spread and drawn and a thin film was made on the microscope slide. The preparation was stored away from humidity for later microscopic analysis.

### **3.9 Semen evaluation**

#### **3.9.1 Visual appearance of semen**

When semen is visually examined in a collection tube, semen samples should be a homogeneously milky or creamy fluid and free of pus, urine, blood and dust. A watery semen sample contains few sperm and is almost certainly without a fertilizing capacity. Whey-like specimens have low sperm counts and are usually associated with infertility. Cryptochord males and those with bilateral epididymitis yield a sperm-free fluid, while cases of marked hypoplasia or degeneration of the testes show low sperm density. Purulent samples usually come from cases of seminal vesiculitis or epididymitis. In bulls,



semen with a yellow tinge is obtained, but the colour is due to vesicular secretions and it is a familial trait of no consequence (Arthur *et al*, 1982).

### **3.9.2 Evaluation of sperm motility**

Sperm motility is markedly influenced by changes in temperature. So for example, in winter semen placed on a slide immediately after collection cools down very rapidly and motility soon approaches zero, whereas in summer, air temperatures are sometimes sufficiently high for good motility to be maintained throughout the whole period of evaluation. Motility should be assessed as quickly as possible, either on the farm or immediately on return to the laboratory.

In order to make accurate comparisons, it is necessary to warm the specimens to body temperature immediately before the motility estimation. In the laboratory, the tube of semen and the glass slides are placed in the incubator for several minutes at 37°C. The microscope is then prepared with respect to focus and illumination. A drop of semen is quickly transferred from the tube to the slide, a warm cover slip applied and the specimen evaluated under the two-thirds objective.

A constant elevated temperature for more prolonged evaluations may be obtained by employing a thermostatic slide carrier or a hot stage. Walton (1939) postulated that, in respect to the warming of the slides, it is important to understand that temperatures above 60°C are lethal to sperm. At body temperature, under low magnification, normal semen exhibits mass sperm movement in the form of recurrent swirling waves. Under high magnification, sperm make an active, progressive motion that can only be followed momentarily. Poor semen samples indicate only rotatory or oscillatory activity of a minority of the sperm, while the occasional specimen is completely devoid of motility. Absence of motility is found in cases of orchitis and epididymitis, while various grades of poor motility are observed in instances of testicular hypoplasia and degeneration (Arthur *et al*, 1982).

### **3.9.3 Determination of sperm concentration**

The number of spermatozoa per millilitre of semen may be determined by means of the Thoma/ Neubauer haemocytometer and an appropriate diluting pipette-in the same method used for doing erythrocytes counts. The sperm head will be included only if spermatozoa are lying across the boundary lines of the large square.

Cleaning of the haemocytometer is followed by the second reloading and a second count made. The mean of the two sperm counts is taken. As a simple alternative to haemocytometer counts, sperm density may be estimated with sufficient accuracy by employing the comparison with a standard opacity tube. Low sperm density is a feature of testicular hypoplasia and degeneration (Arthur *et al*, 1982).

### **3.9.4 Evaluation of the percentage dead and live sperm**

Hancock (1952) reported that immediately after the motility estimation, a drop of semen can be mixed on a warm slide with two drops of warm negrosin-eosin stain and a thin smear of semen made with a cover slip, in a same manner used for making blood smears. Maxwell and Evans (1987) found that dead sperm take up the eosin stain whereas live sperm do not, and this difference may be assessed against the dark background imparted by the negrosin-eosin and a percentage count made of the dead sperm.

Good samples of semen have an average of 25 % dead sperm. A high percentage of dead sperm usually indicate severe infection of the testicles (Arthur *et al*, 1982).

### 3.10 MORPHOLOGICAL EVALUATION OF SPERMATOZOA

#### 3.10.1 Tailless sperms and sperms with looped tails

The most common sperm abnormalities are the detachment of the sperm head (tailless) and bending of the middle piece and a tail around and over the sperm head (looped tail). Looping of tails is sometimes caused by sudden temperature changes. Tailless and bending of the tails over the head also arises from rough manipulation of the semen, but these are indications of sperm weaknesses under standard conditions. Both types of sperm abnormalities are seen most frequently in the ejaculate of sterile males. In normal bulls it has been found that both tailless sperm and sperm with looped tails are present at an average percentage of 40%. In a specific hereditary sterile condition of Guernsey bulls, there are nearly 100% of tailless sperm. Hammond (1940) deduced that sperm might survive storage in the tail of the epididymis for approximately 60 days. It was further stated that it is reasonable to assume that changes occur in older sperms and that such changes will render them more fragile. This was based on the findings of a high percentage of tailless spermatozoa in the ejaculate of fertile bulls after long sexual rest, for example, 23 %, after a sexual rest of 72 days. That such tailless sperms are not necessarily dead is shown by the motility of the headless tails.

Tailless sperm may reach the oviduct but they will be incapable of penetrating the ovum. It has also been found that semen samples with numerous tailless sperms often showing a relatively high percentage of the looped or coiled tails is intrinsic sperm weakness including aging (Arthur *et al*, 1982).

### **3.10.2 Sperm with coiled tails**

The coiling of sperm tails come in two forms. The first involves the coiling of the tail at the extremity of the tail only, and the second involves the coiling of the whole of the tail and sometimes the middle piece as well.

In the coiling of the tail and the middle piece, it may appear as a tight coil adjacent or surrounding the sperm head. In the coiling of the extremities of the tail, motility can be seen, whereas motility cannot be seen in the coiling of the complete tail. Coiling of the middle piece with the head where there is no motility is regarded as a serious abnormality, and occurs mainly in the semen of infertile bulls (Zemjanis, 1962; Arthur *et al*, 1982).

### **3.10.3 Immature or unripe sperm**

Immature sperm are characterized by the presence of the droplet of protoplasm (cytoplasmic droplet) at the junction of the sperm head with the middle piece (the neck). The presence of such droplets is physiological and they are discarded along the way as they progress to the epididymis. Immature sperm with or attached to cytoplasmic droplets usually exhibit a low motility. A large number of immature sperm have been recorded in the semen of many infertile bulls, including young animals with hypoplasia and older bulls with a degeneration of the testes. It is generally considered that the appearance of these sperm in the ejaculate is an indication of the dysfunction of the testis and epididymis (Arthur *et al*, 1982).

### **3.10.4 Abnormal acrosomes**

The importance of the abnormality of the sperm head must be recognized, as this is where the chromosomes genetic material is contained. Sperm shapes and sizes vary depending on whether the sperm has been obtained from a fertile or an infertile bull. Some authors

have classified head defects, in contrast to those of the middle piece and the tail as the primary sperm abnormalities (Perez and Mateos, 1996; Arthur *et al*, 1982).

Very small and unduly large heads, short, narrow and pear-shaped heads, detachment, loosening or distortion of the galela capitis (acrosome cap), shrunken, misshapen or abnormally staining heads are all observed to a large extent in those sperm that have defects in middle piece (Hancock, 1949). It was further stated that sperm with double heads and others with an abaxial attachment of the middle piece, could also be seen. The integrity of the acrosome is an important prerequisite for the fertilizing ability, as it contains enzymes, which assist penetration of the ovum. One such enzyme which occurs only in an intact acrosome is acrosin.

Other varieties of defective sperm include an unduly thick middle piece. In other occasions its proximal portion appears as a filiform structure. The sperm tail sometimes shows an angular junction with the middle piece. Red blood cells are rarely seen but leucocytes are present in cases of seminal vesiculinitis, prostates, epididymitis and orchitis.

### **3.11 The following data was recorded on a monthly basis**

Buck body weight (W)

Semen volume (V) in ml

Wave motion of the spermatozoa (WM) (on a scale of 1-5)

Concentration of the spermatozoa (Cont)

Number of live spermatozoa (L)

Number of dead spermatozoa (D)

Number of normal spermatozoa (N)

Total number of spermatozoa (N)

Semen colour (Consistency)

Scrotal circumference in centimetres (SC)

### **3.12 Measurement of sperm morphological abnormalities**

- Number of tailless spermatozoa (TLS)
- Number of spermatozoa with enlarged heads (EH)
- Number of spermatozoa with small heads (SH)
- Number spermatozoa with coiled tails (CT)
- Number of spermatozoa with cytoplasmic droplets (CD)
- Number of spermatozoa with tapered heads (TH)
- Number of spermatozoa broken at neck (BN)
- Number of spermatozoa broken at mid-piece (BM)
- Number of spermatozoa with abnormal acrosomes (AA)

### **3.13 STATISTICAL ANALYSIS**

The results were analysed using the SAS package [SAS Institute Incorporation; User Guide Version 8.Cary.N.C:NC SAS Institute. IN. 1999]

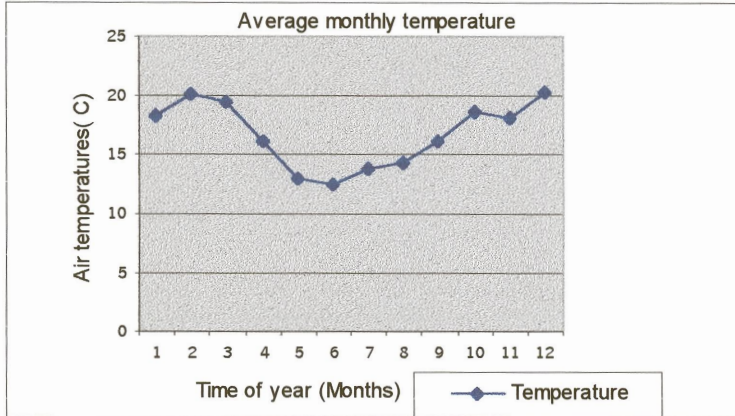
## CHAPTER 4

### RESULTS AND DISCUSSION

In mammals, nutrients are utilized by the tissues involved in the maintenance and growth during which body reserves, including energy stores (lipids), glucose reserves (glycogen), and amino acids reserves (labile protein), are established.

Seasonality of reproduction is a common feature in sheep and goat breeds in the temperate latitudes, and photoperiod appears to be the key factor controlling reproduction (Shelton, 1978, Ortavant *et al*, 1985, Mellado *et al*, 1991, Chemineau *et al*, 1992, Webb *et al*, 1998). Livestock in the tropics have characteristically low fertility due to tropical degradation particularly in winter season (Bonsma, 1980). Sexual behaviour and semen production of bucks are influenced by several factors such as individual characteristics (breeding and age), environment (photoperiod and temperature) and management (nutrition, social environment), which result in large variations in their reproductive capacity throughout their lifespan. Environment and, in particular, heat stress leads to a lower feed intake, due to the animal seeking shade so that less heat is produced (MacKinnon *et al* 1990). In tropical environmental conditions, emphasis should be placed on improved management, nutrition and possibly cross-breeding to improve reproduction efficiency.

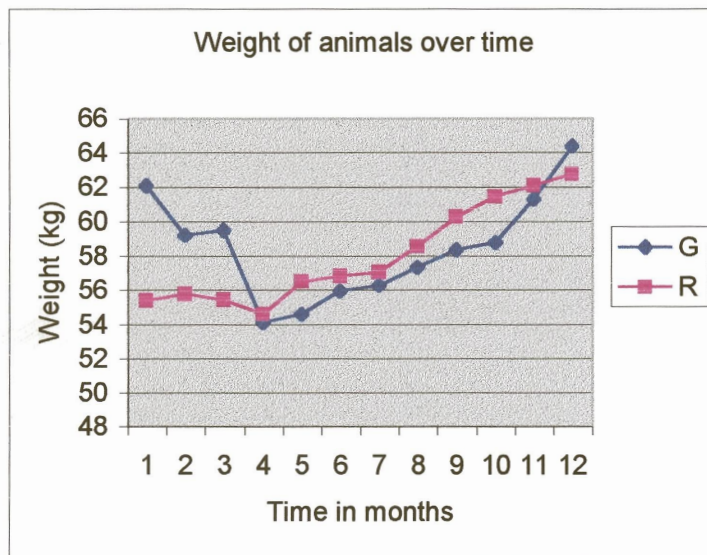
**Figure 4, Ambient temperature distribution over a period of one year (January - December 2000)**



Monthly distribution of air temperatures and its effects on reproduction over a period of twelve months.

The effects of month of the year on the body conditioning of the male Gorno Altai and the rural indigenous bucks is depicted in Figure 4.1.

**Figure 4.1: Effect of time on body weight for the Gorno Altai and rural indigenous buck breeds.**



Body weight is correlated to scrotal circumference and this is a function of birth mass, pre-weaning growth rates as well as age (Roca *et al*, 1992). This suggests that a larger



scrotal circumference (early puberty) and faster growth rate is compatible in young animals. From Table 4.1. it has been shown that time of the year (months) had significant ( $P < 0.05$ ) influence on the body weight of the bucks under the prevalent condition. Within the Gorno Altai, there has been a decrease in body weight during the beginning of Autumn until the end of winter season. Such decrease in body weight of the bucks is related to increase sexual activeness when buck are entering into their usual breeding season.

Body condition relates to weight and, for the two breeds, there was no significant variation in body weight that could have led to the increase or decrease in semen production. Climatic conditions that were thought to have an impact on the quality and quantity of forage available were the main reasons causing a greater variation in body weight.

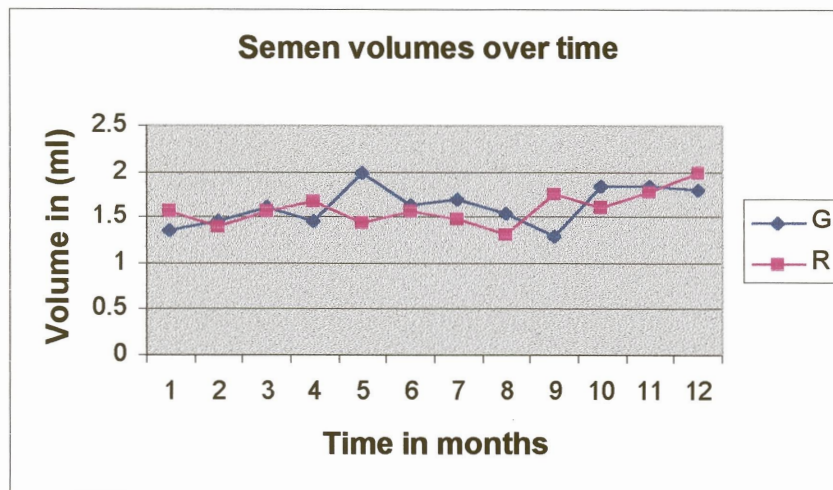
Body weight increases with age. According to Swanepoel and Heyns (1986) an increase in body weight leads to an increase in the body size of the animal. This increase in body size suggests that there is an increased deposition of body fat that would result in improved fertility and the production of good quality semen of the bucks.

The ability of the buck to maintain its body weight without jeopardizing its fertility status increases with age, and that is reflected by improved semen production during the time of forage unavailability. Reports in the literature are controversial concerning the relationship of excess body conditioning or feeding, excess energy and fertility especially in tropical environments (Roca *et al*, 1992).

There is a tendency for the bucks to loose weight during the breeding season due to spending a considerable amount of time courting and fighting. This has also been confirmed by Tomkins and Bryant (1976). It appears to be a standard rule that males during the breeding season lose weight and will eventually have decreased fertility. Those gaining weight duringt breeding are expected to show improved fertility. In Figure 1.1, it can be seen that it may seem profitable to condition score  $55.95 \pm 6.4$ ) in Gorno Altai bucks during the breeding season as there is a marked decrease in weight changes over a

period of one year with the resultant increase in weight after the breeding season. A constant maintenance in body weight of the animals can be attributed to the constant distribution of the light and temperature within the region which affect forage production.

*Figure 4.2 Effect of season (Time) on semen volume.*



Spermatogenesis is a continuous phenomenon which produces spermatozoa. For breeding soundness and sperm production, scrotal circumference is an important component for examining the reproductive potential of males (Maree, 1979). The size of the testes also has an impact on the quality and quantity of semen produced. In the post-pubertal period, testicular volume increases as body weight increases. A strong correlation exists between body weight and testicular volume. This direct relationship has been observed in bulls by Hahn (1969). It was further stated that the correlation is stronger in the young animals than in the adult, where semen production is subject to various external factors. In Figure 4.2, there is a significant variation in the volume of semen produced between the two breeds with the Gorno Altai ( $2 \pm 0.52$ ), ( $1.55 \pm 0.24$ ) producing more ( $P < 0.05$ ) semen than the indigenous breeds ( $1.43 \pm 0.4$ ), ( $1.32 \pm 0.15$ ) only during May and August respectively. The indigenous breed also showed an increased semen volume in these months and September which was ultimately maintained until the summer months. Such variation was also observed during the beginning of September with a significant ( $P < 0.05$ ) increase in the indigenous bucks ( $1.77 \pm 0.3$ ) than the Gorno Altai ( $1.28 \pm 0.25$ ) semen production.

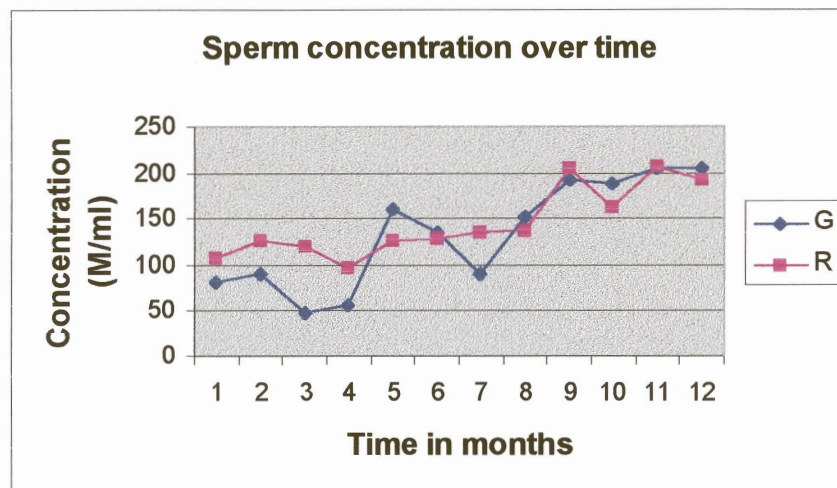
There has been a tendency for semen volumes to fluctuate among the indigenous bucks in response to increased pasture availability.

This semen response is also affected by an increased availability of nutrients in spring, compared to winter when the forage begins to degenerate. When forage production increases at the onset of spring, it is accompanied by an increase in the body weight of the animals. When electroejaculation is employed as a means of collecting semen, it should be borne in mind that the differences in the volumes of the semen in different months could also be attributed to the skill of the electroejaculator operator. As they are subjected to the tropical environmental conditions, the volume of semen produced will vary from season to season especially in seasonal breeders such as the Gorno altai.

Scrotal circumference as an indicator of testes size is highly correlated with sperm production in growing males (Rossouw, 1975, Curtis and Amman 1981, Coulter, 1982).

Many factors such as breed, age, season and body mass influences testes size and scrotal circumference so that they have a negative or a positive impact on the quality of semen produced (Makarechian *et al*, 1984).

**Figure 4.3 Effects of months on sperm concentration.**



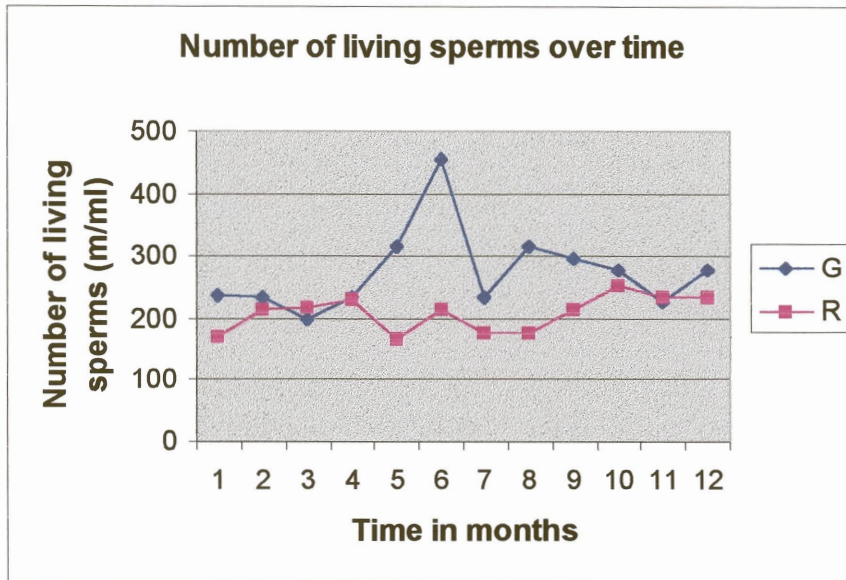
Bucks with good quality semen contain  $2.5-5.0 \times 10^9$  spermatozoa/ ml. In South African climatic conditions there are two breeding seasons per annum i.e the autumn and the spring breeding season. Fig 4.3 indicates a gradual increase in sperm concentration (numbers) with the two genotypes over a one year period. There is a highly significant differences ( $P < 0.05$ ) in sperm concentration between the indigenous bucks ( $121.5 \pm 64.02$ ) than in Gorno Altai bucks ( $48.17 \pm 21.87$ ) that occurs only during March as the beginning of autumn. Such a significant variation has only been observed in the indigenous breed that produces more sperm than the Gorno altai. The decrease in sperm output from February and March can be attributed to the increased mating pressure.

Figure 4.3 shows a sudden decrease in the sperm concentration of the ejaculate. This also explains the effects of increased mating leading to increased volumes of ejaculates but reduced cell numbers per milliliter. Figure 4.3 also indicates the tendency of sperm numbers to increase ( $464.33 \pm 201.2$ ) in winter especially for the Gorno Altai than the indigenous buck ( $221 \pm 104.9$ ) breeds. Between the two breeds of bucks, there is a gradual increase in the number of sperm from the winter until the December, when the ambient temperature reaches its peak.

Neely *et al* (1982) indicated scrotal measurements to have a high positive genetic correlation with testes size, weight and total sperm production. This finding is supported by Amann and Almquist (1961) and Almquist *et al* (1961). Daily spermatozoan output was found to be correlated to testis weight in the rabbit. The selection for greater scrotal circumference and diameter should thus result in an increased testes size, weight and total sperm output.

During the winter season, there was a sharp increase in the total number of sperm produced for the seasonal breeders. Figure 4.3 indicates that season has a positive influence on the numbers of sperm produced within the breed especially the Gorno Altai. Spermatozoa output is also correlated with both scrotal circumference and testis weight in bulls (Willet and Ohms 1955; 1957).

**Figure 4.4 Effect of season on spermatozoa produced**

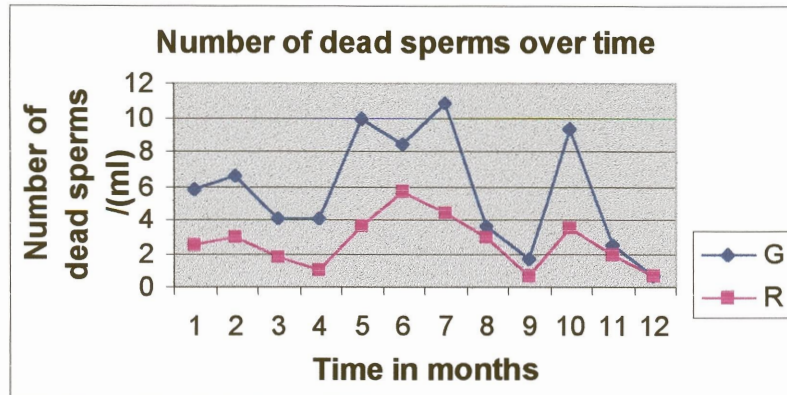


There should be an increase in the number of live spermatozoa and a decrease in the abnormal sperm from birth to the adult stage in bucks. The presence of cytoplasmic droplets is an indication of poor epididymal maturation occurring in the testes. Figure 4.4 indicates a significant increase ( $455.83 \pm 204.9$ ) and ( $215.67 \pm 100.6$ ) in the number of live spermatozoa produced in the months of May and June in both Gorno Altai and the indigenous bucks respectively. In both months the Gorno Altai have shown a capability of producing more live sperm than the indigenous breeds. Spermatogenesis is a process that is affected by fluctuations in air temperature, with decreasing testicular activity during the hot months. Figure 4.4 also indicates the significant differences in live sperm for the Gorno Altai and the indigenous breeds responding positively to reduced photoperiod. Such increases in the number of live spermatozoa can be attributed to the short day period. This demonstrates that photoperiodism has a positive influence on the number of live spermatozoa.

In comparison, Figure 4.4 reflects a much more stable production of the number of live sperm in the indigenous breeds and this shows that, under tropical conditions. Gorno Altai have the ability to produce good quality spermatozoa, maintaining fertility in a

foreign environment. An increase in the number of live spermatozoa is also dictated by an increase in the total number of spermatozoa in the ejaculate.

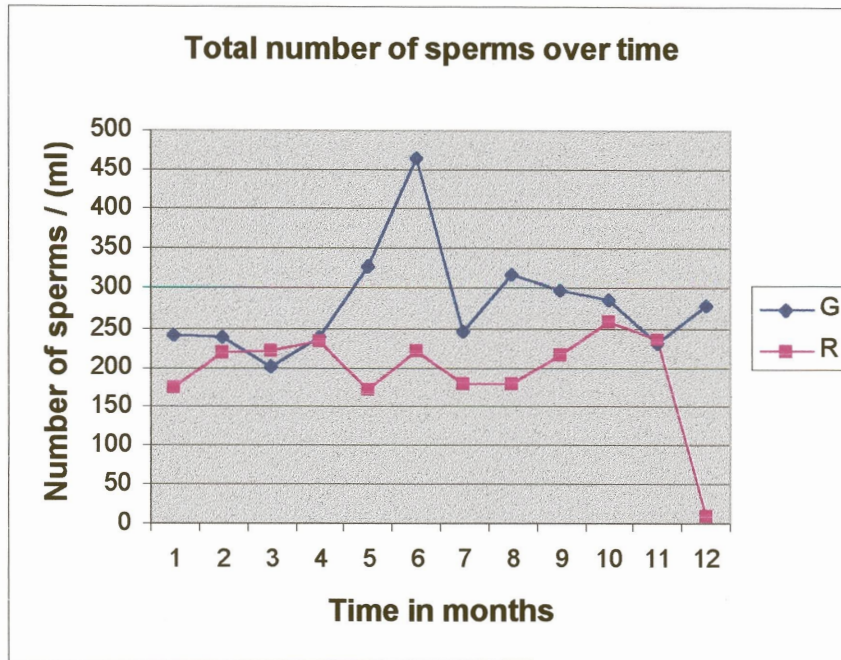
**Figure 4.5 Effect of season on the number of sperm mortalities**



Dead spermatozoa in an ejaculate lead to the complete failure of fertilization and reproduction. The death of sperm can be fuelled by a number of factors such as infection and high temperatures. In Figure 4.5, there is a constant production of the dead spermatozoa ( $5.8 \pm 3.5$ ) with no significant variation, especially during the non breeding season-with a marked increase during the breeding season from the sexual quiescent stage in Gorno Altai. An increase in volume of ejaculate during the breeding season also increases the number of spermatozoa that are dead.

As a result of the shorter photoperiod, (Figure 4.5), an increase in the number of dead spermatozoa were recorded from April until September in the Gorno Altai than the indigenous bucks. There was a sudden reduction in the number of dead cells during August month. This could suggest that, during winter, when spermatozoa are exposed to cold shock, the survival capacity is shortened by exposure to extremely low ambient temperatures. During winter months, it has also been observed that the number of tailless spermatozoa increased. This can be associated with the increased production of the immature spermatozoa due to sexual pressures in the Gorno Altai bucks.

**Figure 4.6 Effect of season on the total number of spermatozoa produced**



The number of spermatozoa produced by the adult bucks are correlated with the size of the testes ( $12 \times 10^6$  spermatozoa/ gram of testicular parenchima). The quality and quantity of semen, however, is modified during the year by environmental factors, mainly day light length. The increase of the number of the sperm within an ejaculate could lead to the improvement of conception rates in females.

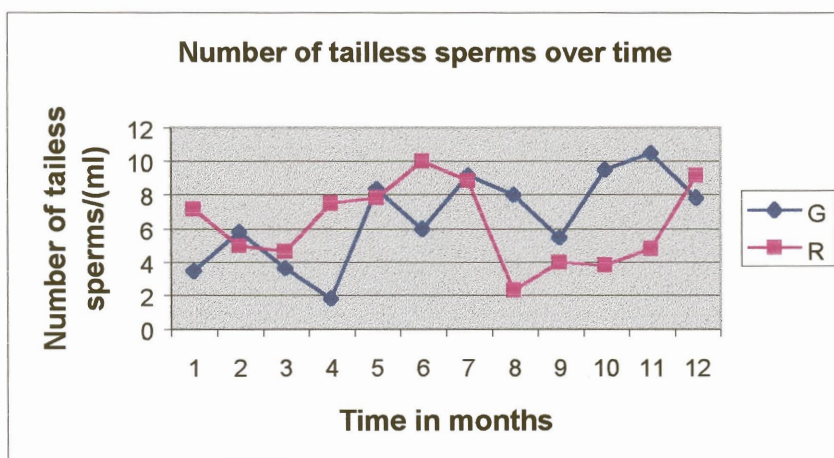
Large semen volumes with low numbers of sperm culminate in the failure of the fertilization process. Figure 4.6 shows a constant production of spermatozoa in the two breeds during the summer months. Total sperm numbers differed significantly ( $P < 0.05$ ) at the beginning of May, with the Gorno Altai showing an increase in the total number of sperms produced, compared with the indigenous counterparts. One contributing factor in this regard has been identified as the influence of low temperatures and shortened day light length in the tropical environment at that time on the Gorno Altai.

There was a tendency for the Gorno Altai to produce more spermatozoa during the winter, months as they are seasonal breeders compared to the indigenous bucks. This is another indicator of animals that are quality sperm producers during the winter periods because of their coating that regulate temperature changes. An increase in the number of

sperm produced as observed from winter until the end of spring-with a sudden reduction in sperm numbers when temperature began to rise. Indigenous bucks also demonstrated an increase in the number of sperm produced irrespective of the increase in the environmental temperatures. This corresponds to an increase in the production of the number of dead spermatozoa during this particular season.

After heat shocks, Gorno Altai bucks demonstrated an ability to recover gradually in terms of the number of spermatozoa produced, until full recovery was attained in February and March early during the breeding season.

**Figure 4.7 Effect of season on the number of tailless spermatozoa**



Failure of females to conceive is also a factor contributed to by the male. Large numbers of sperm without tails have a negative effect on the reproduction capacity of all animal species. An increase in temperature and reduced availability of feeds are also major contributors to the production of tailless, immature and deformed spermatozoa. It has long been known that some inherited sperm defects are associated with infertility (Taylor, 1995). Land (1978), confirmed that the most common cases of abnormalities are detached sperm heads in Guernsey cattle and abnormal acrosomes in Friesland bulls.

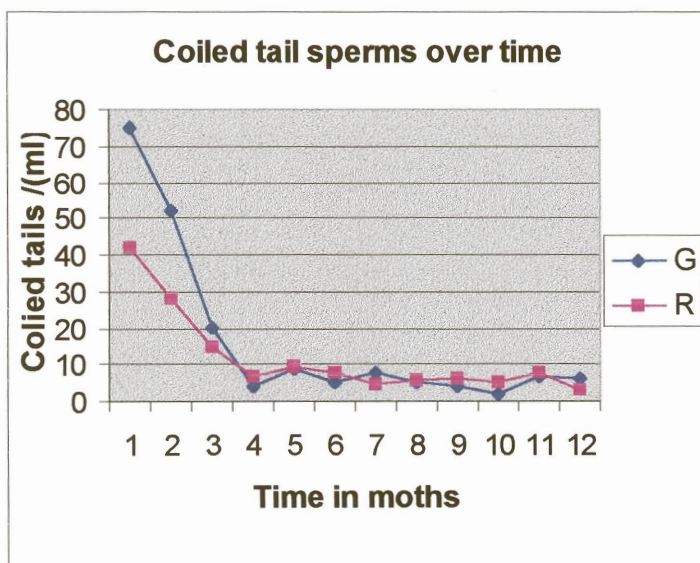
Within the two goat breeds under evaluation, there was a slight increase in the number of tailless spermatozoa in Gorno Altai at the onset of August (late winter), when



temperatures started to rise. It has been suggested that a cause is the high demand in the number of sperm needed for fertilization during the breeding season. Figure 4.7, also shows a significant ( $P < 0.05$ ), increase in the number of tailless spermatozoa from the beginning of September to December in Gorno Altai bucks ( $8 \pm 8.2$ ). Such an increase in the number of tailless spermatozoa has been attributed to the high temperature effects (Roca *et al*, 1992; Tegegne *et al*, 1994). It suggests that high temperatures have a negative effect on the quality of semen produced in a certain environment.

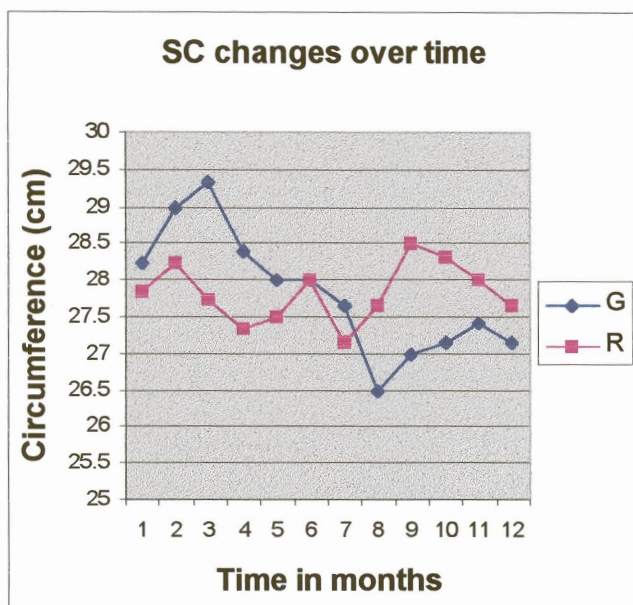
This also gives an indication that indigenous goats are more fertile due to quick recovery regardless, of temperature.

**Figure 4.8 Effect of season on the number of coil-tailed spermatozoa**



Normal spermatozoa have a tail or a flagella to swim or move towards the ovum. Females fail to conceive as a result of large numbers of spermatozoa deposited in the vagina lacking motility. Such tail defects e.g coiled tails are formed as a consequence of abrupt changes in temperature. Although there were no significant difference between the two breed regarding the tail defects during January and February, big differences in the number of coiled tail sperms between the Gorno altai and the indigenous breeds existed for December, January and February (Roca *et al*, 1992).

**Figure 4.9. Effect of season on scrotal circumference of Gorno Altai and S.A Indigenous bucks.**



Scrotal circumference is highly correlated with testes weight and sperm output in growing bulls and also to the percentage of normal sperms (Coulter, 1982). An increase in scrotal circumference also increases the possibility of the production of quality semen (Tegegne *et al*, 1994; Roca *et al*, 1992).

The pattern of testicular development, endocrine function and semen traits are influenced by genetic and environmental factors such as disease, nutrition, season and lactation (Rekwott *et al*, 1998, Hernandez *et al*, 1991, Godfrey *et al* 1990). In Figure 4.9, a constant increase and decrease in the scrotal circumference of the Gorno Altai breed was apparent with significant ( $P < 0.05$ ) scrotal changes ( $29.3 \pm 1.4$ ) taking place during March. In the two breeds, there was an apparent increase in scrotal size in March (onset of the breeding season). This increase in scrotal size is associated with the increased production of sperm and the spermatozoa storage in the epididymis, as these breeds entered into a season of increased sexual activity. Figure 4.9 also shows that, during winter (June and July), scrotal circumference is reduced until it recovers, with the approaching next breeding season.

A tendency within the indigenous bucks, in a reduction of scrotal circumference was recorded during the non-breeding season. This is also attributed to the lower production of spermatozoa. This fact is emphasized by the findings of Coulter (1982), who reported an increase in scrotal circumference to increase the probability of yearling bulls in having acceptable semen quality. Body weight and scrotal circumference increase with age in both the goat genotypes.

Season affected changes in scrotal circumference in both the goat genotypes during the month of March-with the introduction of shorter photoperiods. Table 4.9 depicts a significant ( $P < 0.05$ ) variation at the beginning of autumn. The difference could be initiated by the approaching breeding season for the Gorno Altai (seasonal breeders), when the day light length starts getting shorter, with the commencement of the breeding season. Table 4.9 shows a gradual increase in the scrotal circumference of the Gorno Altai compared to the indigenous bucks during March, followed by a gradual decrease during the subsequent months. Variation in the scrotal size of the indigenous bucks can also possibly be attributed to the skill of an operator.

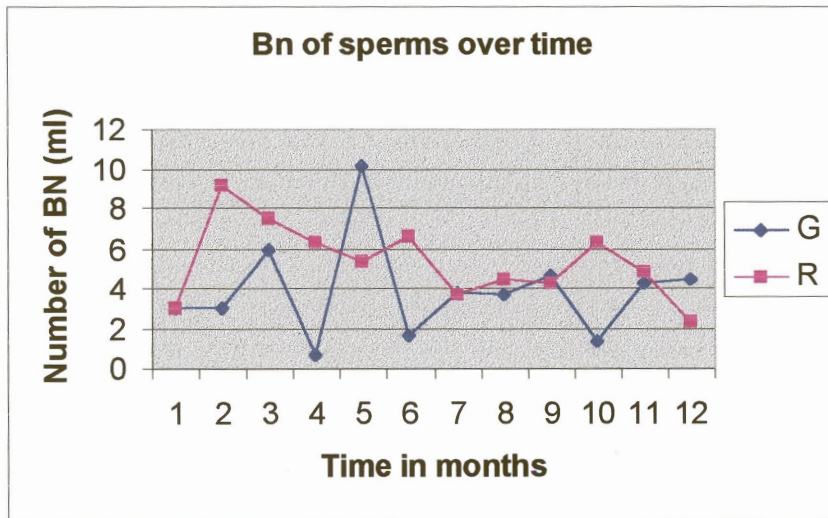
The rural indigenous buck maintains a constant scrotal circumference during all the seasons. Fields *et al* (1982) found little difference in post-pubertal testicular development between genotypes. Perry *et al* (1991) reported no differences in the patterns of testicular development between different genotypes. Table 4.9 indicates that the two goat genotypes react differently to season with respect to semen volume and sperm concentration. Goats with a small scrotal circumference of less than 25 cm do not produce semen of satisfactory quality (Coulter *et al*, 1987). Knight *et al* (1984) postulated that in general, semen have been found to have medium to low heritability.

Although data on the effect of season on semen trait is not consistent, Tegegne *et al* (1994) stated that, semen characteristics of Boran bulls were highest during the short rainy season and lowest during the dry season. The present findings of this trial are also supported by Tegegne *et al* (1994), who observed that Boran bulls had better semen traits

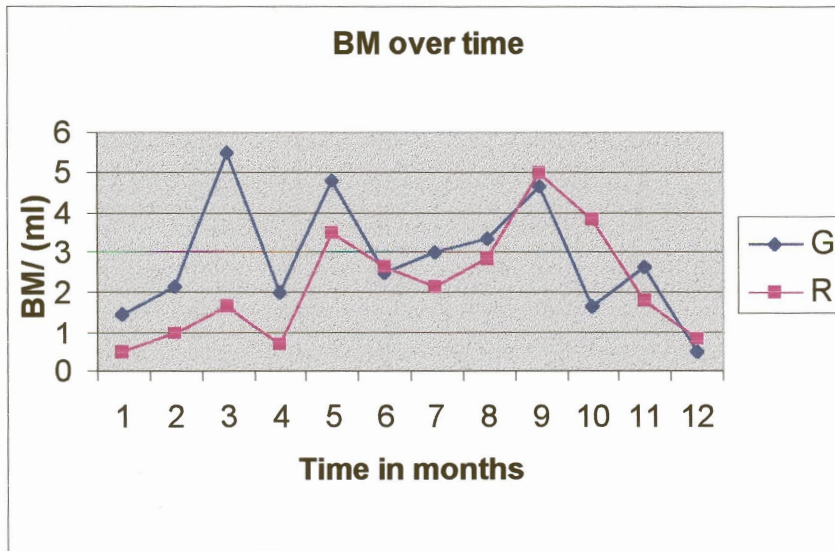
than Boran X Fresian bulls during the dry season and *vice versa*. Genotypes differences were minimal during the short rainy season.

Seasonal differences in semen traits and daily sperm production have been reported for different breeds of bulls (Field *et al* 1979, Wildeus *et al* 1984, Cardoso and Godinho 1985, Godfrey *et al* 1990, Goswan *et al* 1991). In contrast, Hernandez *et al* (1991) were not able to detect seasonal variation in semen traits for 4 Zebu breeds in Mexico. Table 4.9 indicate that testicular growth and semen quality were more affected during the hotter months in Gorno Altai breeds than in the indigenous goat breeds. Godfrey *et al* (1990) reported that season and location accounted for differences in semen quality and endocrine functions in Herefords and Brahman bulls. In tropical regions testicular function, semen output and quality, and mating behaviour of bucks can vary from season to season, depending mainly on the availability and quality of feed and climatic conditions.

**Figure 4.10 Effect of season on the number of spermatozoa broken at the neck**



**Figure 4.11** Effect of season on the number of spermatozoa broken at the mid-piece



The maturity stage of the spermatozoa plays a vital role in regulating the durability of the sperm. An increase in the number of sperm produced due to high sexual demands leads to an increase in the number of sperm abnormalities (Tegegne *et al*, 1994; Roca *et al*, 1992). (Figure 4.7, Figure 4.10, Figure 4.11). Figure 4.10 shows a significant variation in the number of sperms produced and broken at the neck. Such abnormalities are more pronounced in the Gorno Altai than the indigenous bucks, particularly in the months of June and October. All of these sperm abnormalities can be attributed to changes in ambient temperature. Figure 4.11 indicates no significant variation between the two breeds in terms of the number of spermatozoa produced when broken at mid-piece.

Temperature differences, whether cold or hot have a particular influence on the morphological state of the spermatozoa (Roca *et al*, 1992; Tegegne *et al*, 1994). An increased sexual desire activates spermatogenesis, resulting in the production of poor quality sperm. Significant ( $P < 0.05$ ) differences have been observed during June and October for the two goat breeds. There is a tendency in the Gorno Altai for an increase in the number of spermatozoa that are broken at the neck when the ambient temperature rises.

A tendency has also been observed in the Gorno Altai and the indigenous bucks regarding a higher incidence of spermatozoa that are broken at mid-piece during the breeding season. This phenomenon is also attributed to highly increased environmental temperatures (Tegegne *et al*, 1984).

For all the reproductive parameters studied, there was individual variation between bucks. Variation in semen quality throughout the year took place in a similar manner for each individual, irrespective of whether data was evaluated monthly or seasonally. It was also found that a similar seasonal pattern in a tropical zone showed the existence of a positive relationship existing between testis diameter and the photoperiod in male goats.

Although ambient temperature has been found to be a secondary factor that influences testes size in temperate climates, it indicates an obvious correlation with seasonal changes in scrotal diameter. It suggests that day length is not the only factor likely to affect testes size throughout the year. It is very difficult to promote day length as a primary inductor of seasonality in the geographic areas where seasonal variation in photoperiod is not so intense. In such circumstances testicular regression and recovery should be attributed to other climatic factors such as those which can change with the season such as ambient temperature and humidity.

Bongso *et al* (1982) postulated that in addition to day length, the influence of nutritional levels and their change with the season should not be ignored, as testes size has been reported to be slightly influenced by body weight and to be closely related to body growth. Similar results have been obtained in studies carried out on male goats in other subtropical and tropical climates.

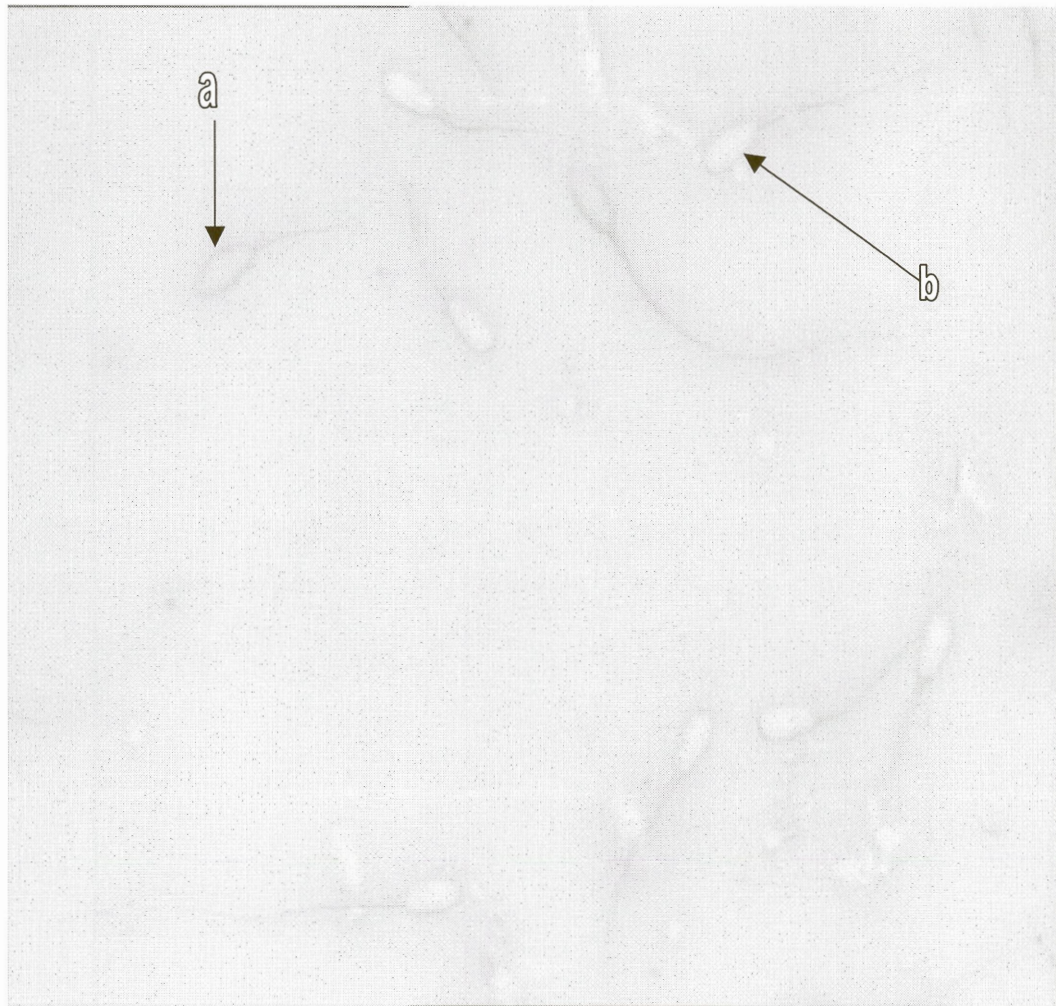
Table 4.1 Correlations on the influence of body weight on semen volume, sperm concentration, % live sperm, colour code and scrotal circumference.

		Weight	Volume	Concentration	% Live
Weight	Person Correlation	1			
	Sig. (2-tailed)	0.0			
	N	152			
Volume	Person Correlation	0.067	1		
	Sig. (2-tailed)	0.413	0.0		
	N	152	152		
Concentration	Person Correlation	0.089	0.274	1	
	Sig. (2-tailed)	0.278	0.001	0.0	
	N	152	152	152	
% Live	Person Correlation	-0.242	0.220	0.323	1
	Sig. (2-tailed)	0.003	0.006	0.000	0.0
	N	152	152	152	152
Colour code	Person Correlation	-0.327	0.139	0.323	0.411
	Sig. (2-tailed)	0.000	0.088	0.000	0.000
	N	152	152	152	152
SC	Person Correlation	0.098	-0.020	-0.107	-0.101
	Sig. (2-tailed)	0.230	0.810	0.188	0.216
	N	152	152	152	152

Other researchers (Neely *et al*, 1982; Willet and Ohms, 1955; 1957; Almquist, 1961; Almquist *et al*, 1961; Coulter, 1982; Tegegne *et al*, 1984; Roca *et al*, 1992) claimed that there is a strong correlation between body weight and scrotal circumference. In this study, Table 4.1 indicate that body weight did not influence the % live sperm, sperm concentration, wave motion or scrotal circumference in either breed. Although there is no significant correlation in the traits measured in this study, it is important to bear in mind that animals in different geographic locations respond to different environmental stimuli differently.

*(Plates) 4.12, Indication of different types of morphological abnormalities in goat semen as influenced by seasonal variation*

Plate 4.12.1 Dead (coloured red) and live buck sperm



A-Represents (coloured) dead sperm

B-Represents (uncoloured) live sperm



A- Plate 4.12.2 Abnormal acrosome



C-An abnormal accrosome on a dead sperm



Plate 4.12.3 Different sperm abnormalities

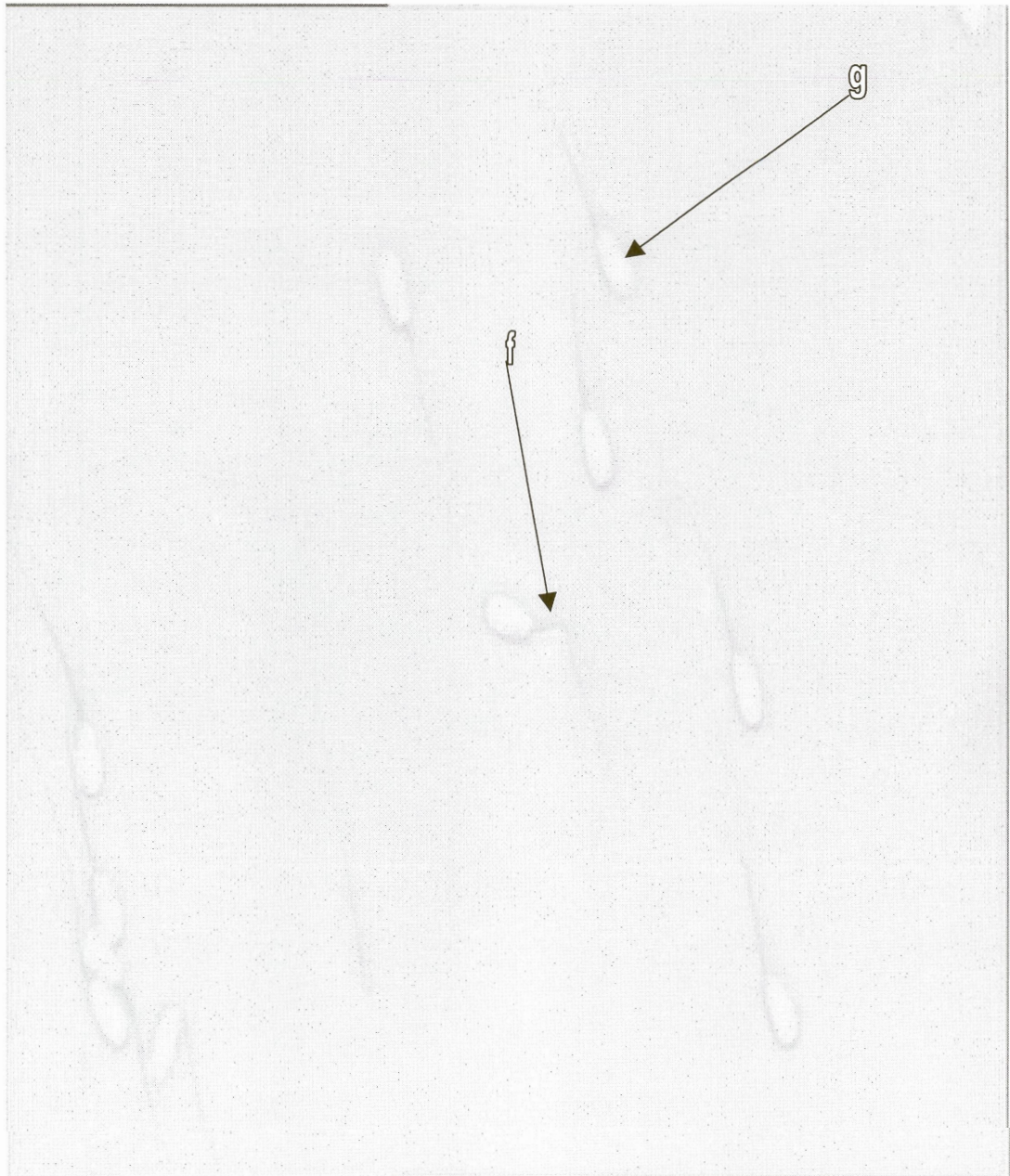


D-Sperm with a coiled tail

E-Dead sperm



Plate 4.12.4 Broken at mid piece-spermatozoa

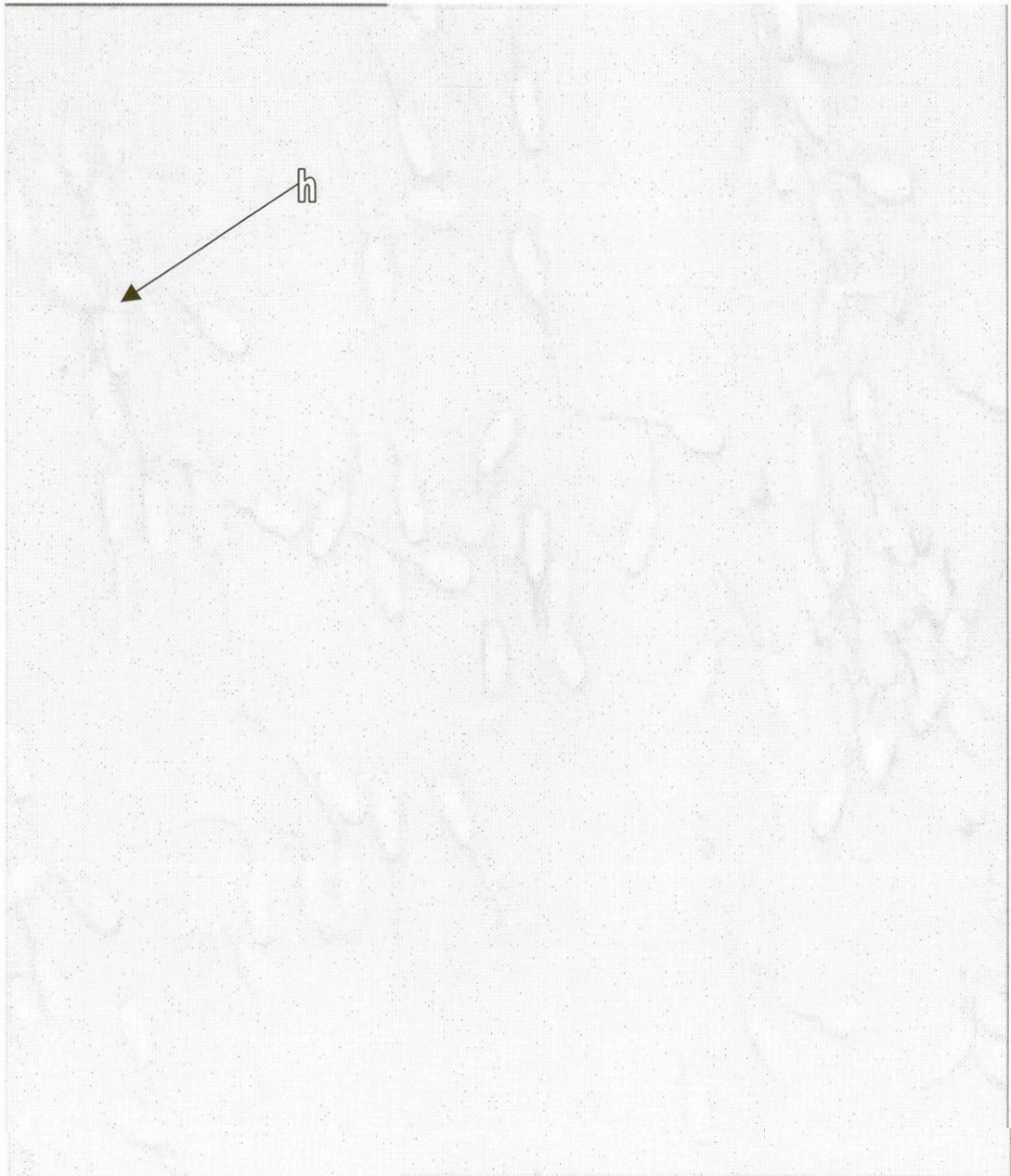


F-Sperm that is broken at the mid piece

G-Normal sperm



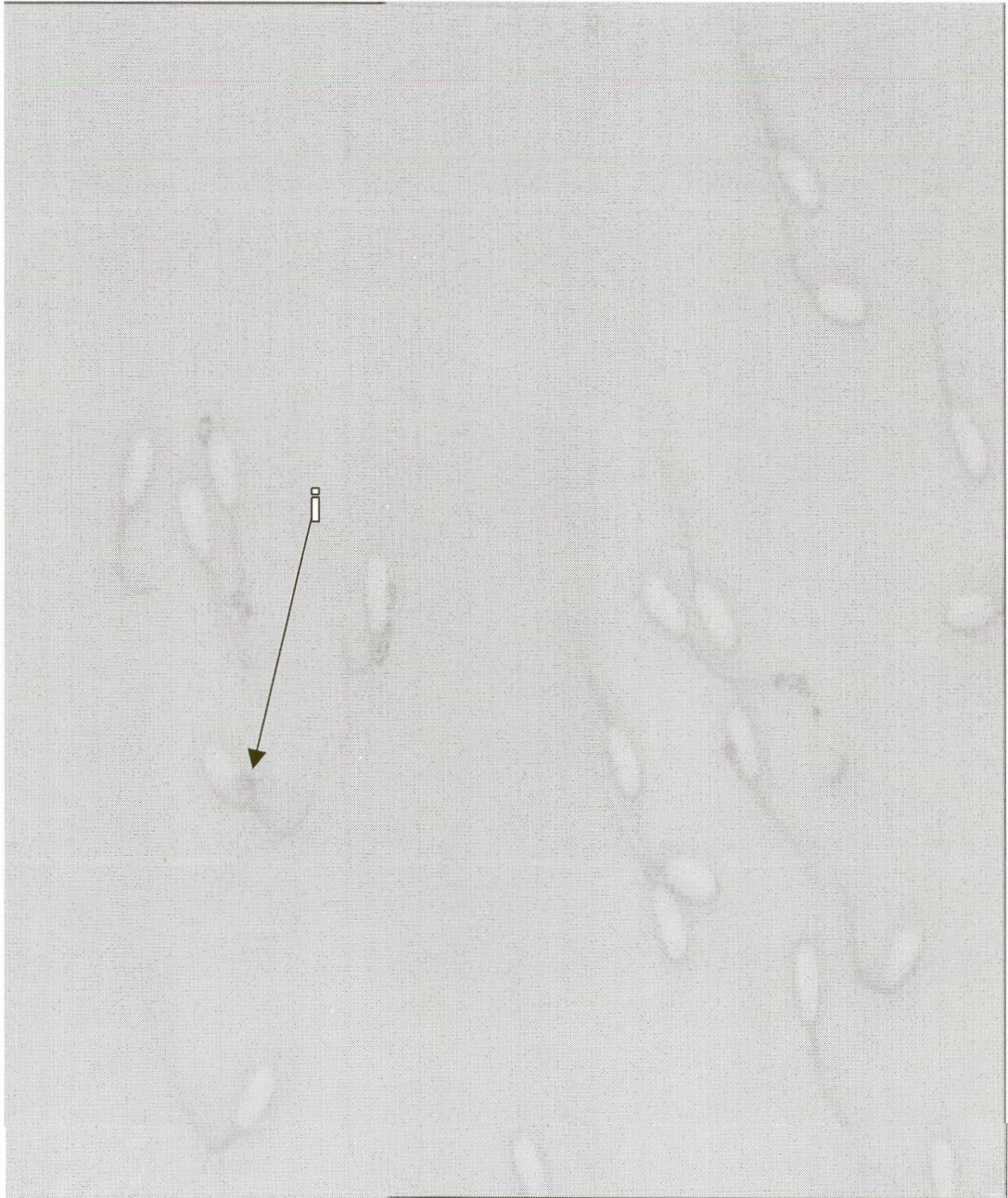
Plate 4.12.5 Coiled-tail spermatozoa



H-Sperm with coiled tail



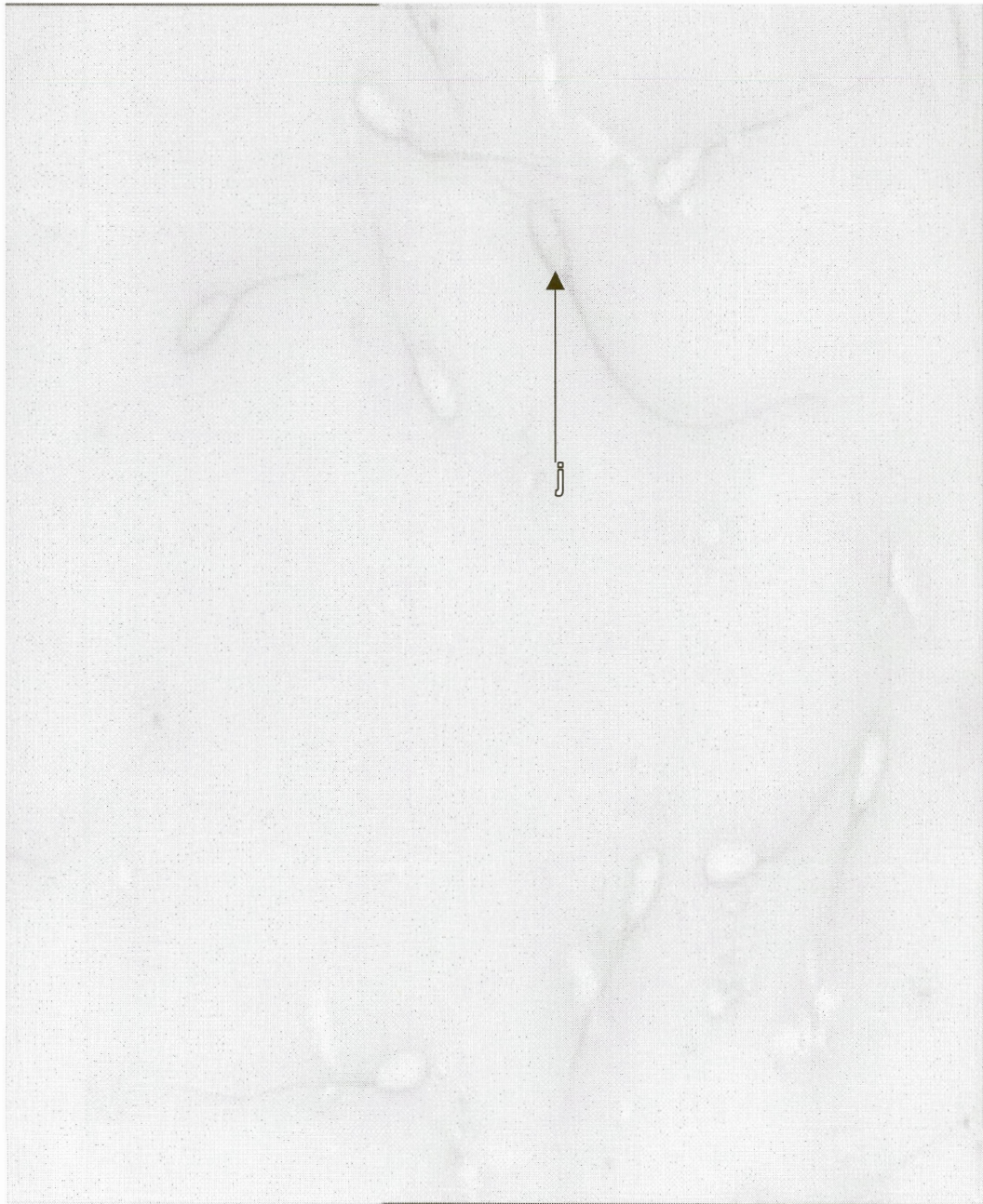
Plate 4.12.6 Cytoplasmic droplet



I-Sperm with a cytoplasmic droplet

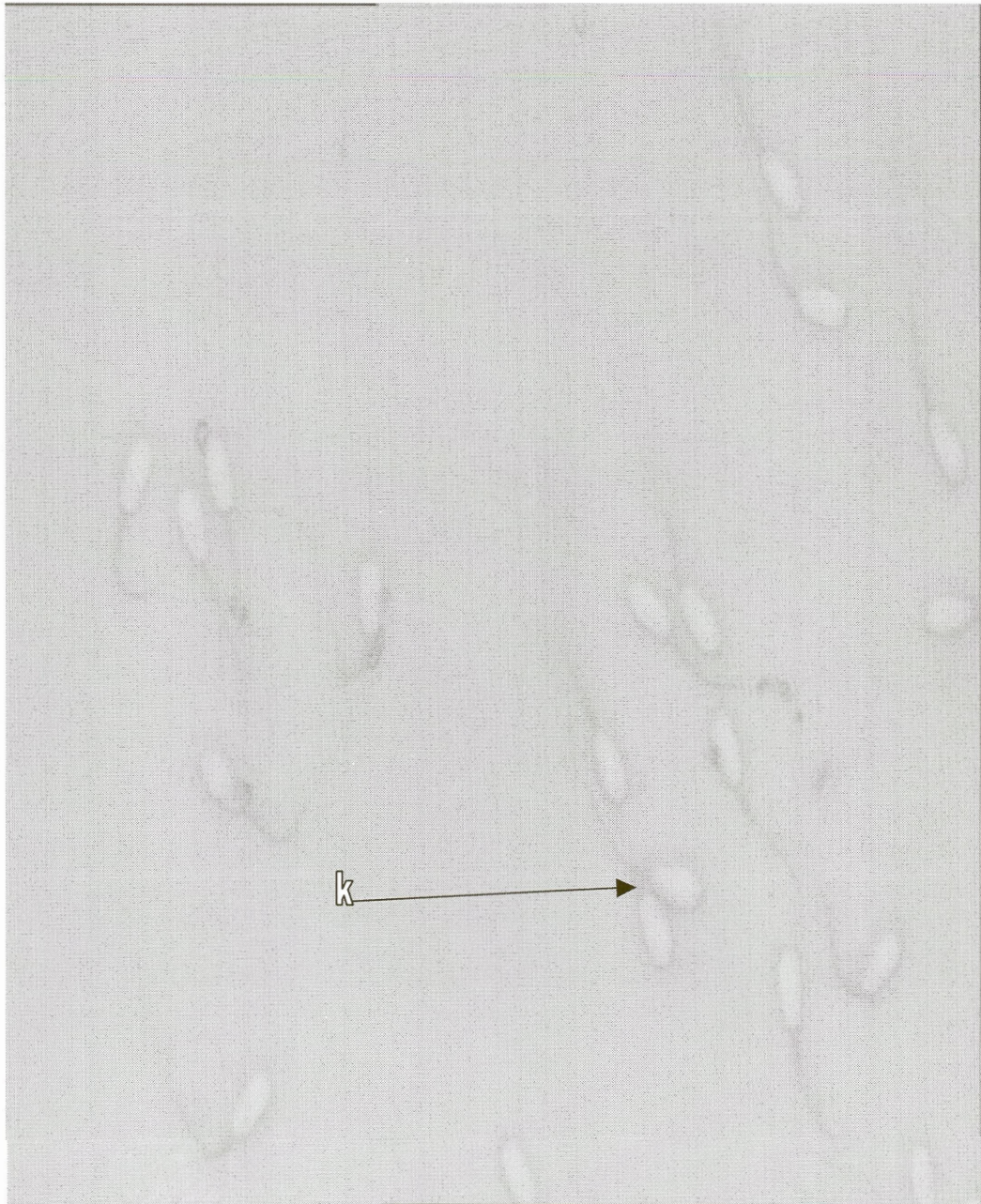


Plate 4.12.7 Dead spermatozoa



J-dead sperm

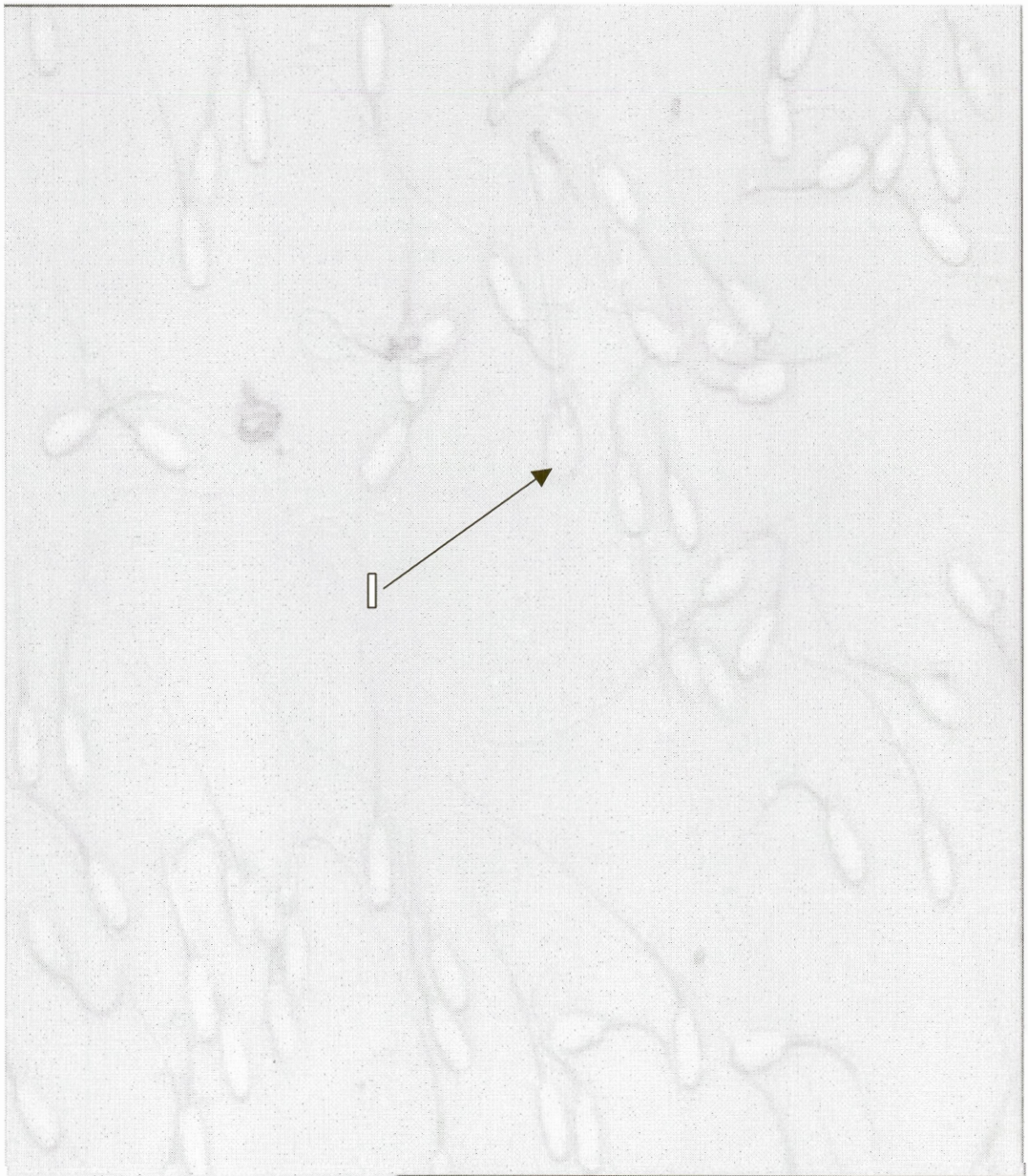
Plate 4.12.8 Double headed spermatozoa



K-Sperm with double heads



Plate 4.12.9 Enlarged head spermatozoa



L-Sperm with an enlarged head





Plate 4.12.10 Live spermatozoa



This plate represents live sperm



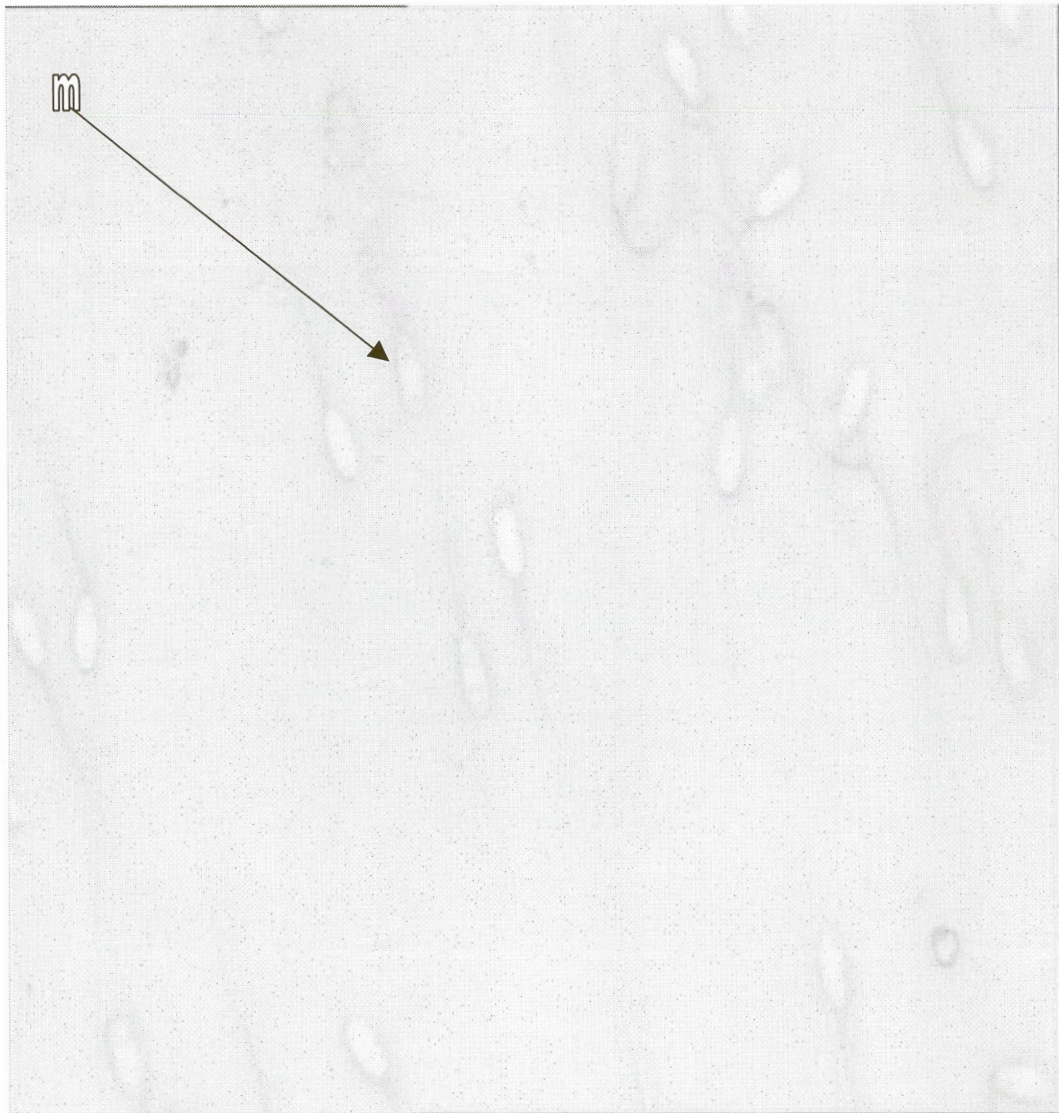
Plate 4.12.11 Normal sperm



This plate represents normal sperm



Plate 4.12.12 Tapped spermatozoa



M-Sperm with a tapped head

*The sketches above represent different structures of sperm morphological abnormalities influencing the reproductive as well as the fertilizing capacity of the bucks.*

## CHAPTER 5

### CONCLUSIONS AND RECOMMENDATIONS

Numerous studies have shown that variation exists in the fertility of males within the same breed. It is also known that, for the same animal, the fertilizing potential of semen does not remain constant throughout the year. The determination of the factors that affect the quality of semen and the extent of the effect is of vital importance. It is interesting to note that an increase in abnormal sperm cells, and a reduction in fertility in exotic breeds in the tropics as a result of high ambient temperatures is often reported.

In goats, as in many other species, the quality of semen is influenced by the age of the animal. The initial ejaculate contains a great number of abnormal cells but large differences exist between and within breeds. The abnormalities consist for the most part of head malformations, which indicate incomplete spermatogenic activity and incomplete epididymal maturation. Semen quality also depends on daylight length, the environment and the potential to fertilize is subjected to similar external factors. A young male is not necessarily a good sire and the use of such sires often results in a lower prolificacy rate.

It is also known that the sexual activity of male goats are subject to seasonal variations, while the extent of this effect varies from breed to breed. These variations can be due to a number of defects of the reproductive system, particularly the quality of semen and its fertilizing capacity. The most critical environmental factors in this regard are daylight length and temperature. Adverse environmental conditions directly affect the head piece of the spermatozoa, which often results in an increase in the proportion of pyriform heads, damaged acrosomes, and tailless spermatozoa. In bucks exposed to heat treatment, a fall in fertility appears as early as a few days post-treatment and the extent of the effect depends on the duration of the treatment. Large variation exists between breeds in both the sperm morphology and fertilizing ability of semen in spring and autumn.

From a practical point of view, this means that fertile bucks with high semen quality should be selected in spring and autumn in order to achieve high conception rates. A limitation in this regard would be the need to determine precisely which buck to select in the non-breeding season. It was previously reported that the morphological picture of semen from a buck is fairly repeatable from one year to the next. The highest prevalence of semen abnormalities tend to occur every year at about the same time. Exposure of the scrotum alone or of the whole animal to high temperature always results in changes in the morphological profile of the sperm, but considerable variations also occur between individuals in the extent of the effect.

Based on this trial, the following conclusion can be drawn with regards to the effects of season on semen production within the two goat breeds. Since semen production changes with temperature, humidity, daylight length, nutrition and the extent of these effects, the indigenous bucks were found to adapt better to all the adverse factors in the tropics. The results also indicate a constant production of semen throughout the year in the indigenous goats. This is confirmed by the fact that the sperm concentration remained constant in the indigenous goats. This phenomenon renders indigenous goats more usable than the Gorno Altai in the tropical environments-with the Gorno Altai producing a varying quantity of semen. As the number of live spermatozoa per ejaculate is important, it affects fertility. In winter and during the beginning of spring there is a large production of spermatozoa in Gorno Altai, compared to the indigenous goats. This gives a clear indication that the Gorno Altai respond well to seasonal cues. There was no increase in the number of dead spermatozoa in the two breeds, but there was a tendency between goats in the fluctuating numbers of dead sperms over seasons-with marked fluctuations in the Gorno Altai. The results also show a sharp decrease in the number of dead sperm for the Gorno Altai during the mid-summer season. Such a phenomenon is due to the time of sexual quiescence in the exotic breeds, during which their sexual vigour is reduced to zero. These fluctuations also indicate that although exotic breeds produce large amounts of spermatozoa in the winter season, their use is limited to that specific season. Hence, these bucks cannot be utilized for semen collection during the non-breeding seasons at an

artificial insemination station. It has also been found that, in the tropics, the exotic breeds produce a large proportion of dead sperm cells in the non-breeding season.

It is concluded that the use of exotic buck breed for semen collection in an artificial insemination programme is limited to a certain season of the year primarily when it is cooler in the tropics. The indigenous breeds are fertile throughout the year. Semen production and fertility can also be assessed with the use of a scrotal circumference size as an indicator in animals. In a selection process of the best animals, indigenous goats maintained a consistent scrotal circumference size during the year, while the exotic breeds showed a decrease or fluctuation in scrotal circumference over the entire period. This correlated with consistent sperm concentrations in the indigenous and fluctuating sperm concentration in the Gorno Altai. It is evident that the morphological evaluation of spermatozoa is, to date, the most accurate test available to evaluate the quality of semen. Based on this study, it is concluded that the semen from indigenous goat breeds could be used throughout the year for artificial insemination programmes whereas the use of the Gorno Altai should be limited to their natural breeding seasons. This will result in the collection and freezing of the highest quality semen in both breeds.

## CHAPTER 6

### IMPACT AND SOCIO-ECONOMICS OF THE STUDY.

To meet consumer demands and the increasingly severe competition between to maximize returns from resources invested, animals of the future must for efficiency. Desirable genetic traits must be increased and this can be done by selecting the most suitable individuals to become the parents of the next generations through breeding. In any efficiently operating artificial insemination unit, the determination of volume of an ejaculate, estimation of the number of spermatozoa/ ml contained and the proportion of cells exhibiting vigorous motility should be routine. The information obtained from such semen evaluation in the rural communities will not establish bases for day-to-day decisions making or provide knowledge necessary for extending the semen and processing it for distribution, but will furnish a continuous record of the semen-producing ability of each male which can then be used to select the most fertile males.

To counteract the influence of season on the reproductive output of animals, well-adapted breeds showing extended period of ovarian activity and semen of high quality must be selected or imported. These breeds may, at many latitudes, yield two crops of offspring per year (Land and McLelland, 1971).

The goat, because of its origin, distribution and unique anatomy and physiology, represents an under-utilized resource of animal protein, and simultaneously a great potential for increasing milk meat, skin and fibre production in the tropics. As the goat originated from tropical and sub-tropical areas, it not only has the ability to survive under harsh tropical conditions but also the ability to produce and reproduce.

Generally goats are more efficient than other domesticated ruminants in digesting low-quality roughage in both tropical and arid environments and are more efficient than sheep in the temperate zones. Sharma and Rajora (1977) reported that goats utilized high-grade

clover hay better than sheep, buffalo and cattle. Goat production is also a less risky enterprise and is more suited to farmers on small holding (1.6 ha), who often lack credit and other inputs needed e.g. cattle production.

The demand for animal protein and energy sources particularly milk and milk products is on the rise in the tropical developing world. Devendra and McLeroy (1982) suggested that the increased demand could be easily met by increasing the ruminant livestock population. Attempts to increase milk production through an increase in cattle numbers as occurred in developing tropical countries will have limited success owing to the high maintenance requirements and low productivity of cattle due to carrying capacity of the land.

Attempts to increase milk production by importing high milk producing temperate cattle have resulted in failure, due to the animals' inability to adapt to tropical environments, poor quality forage, diseases (trypanosomiasis), poor reproductive performance, low levels of management. An increase in the number of females bred increases the flock size, hence increases milk production within the herd. Goat milk production has a comparative advantage to small enterprises since it involves a relatively smaller initial investment and, therefore, carries less overall risk.

It is also suggested that the smaller investment in goats represents a corresponding smaller risk of financial loss through individual death, when compared to cattle or buffaloes. The small body size of goats is an immediate advantage with respect to housing (cheaper, less capital intensive) and, in addition, there is a faster turnover of capital owing to the earlier maturity specie, shorter generation interval and the more efficient use of labour to manage them. Goat meat is a potential by-product of the milk enterprise, which is acceptable to all religions and preferred by some. Goats are often used as a form of capital investment and insurance in many rural societies. The ease with which they are managed allows for the participation by all members of the community in the rearing of these animals and, as a result, is an important source of supplementary income.



In conclusion the, it is suggested that the benefits of goats over other livestock species is apparent. Their use could be encouraged by improved access to superior, adapted genetic materials. For this, artificial insemination programmes would be advantageous. Thus, this study has shown that semen to be used artificial insemination programmes can be collect throughout the year from indigenous goats, but should be limited to the breeding season only, in the exotic imports.

## CHAPTER 7

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## CHAPTER 7 B

### APPENDIX

*Table 7.1 Analysis of variance for the effect of season on the weight gains of the Gorno Altai and the indigenous bucks*

Weight					
Source	DF	SS	MS	F Value	PR>F
Error	120	5021	41.84		
Total	143	6199			
Breed	1	6.77	6.77	0.16	0.688
Month	11	895.26	81.39	1.95	0.04
Month X Breed	11	275.91	25.08	0.6	0.826
R-square		CV			
	0.19	11.098			

*Table 7.2 Analysis of variance for the effect of season on the semen volume of the two breeds*

Volume					
Source	DF	SS	MS	F Value	PR>F
Error	120	15.89	0.13		
Total	143	21.27			
Breed	1	0.029	0.029	0.22	0.64
Month	11	2.84	0.26	1.95	0.04
Month X Breed	11	2.51	0.23	1.72	0.076
R-square		CV			
	0.25	22.57			

**Table 7.3 Analysis of variance of the effect of season on the concentration of spermatozoa produced**

<b>Concentration</b>					
Source	DF	SS	MS	F Value	PR>F
Error	120	578669.67	4822.24		
Total	143	901118			
Breed	1	5208.02	5208.03	1.08	0.301
Month	11	282692	25699.29	5.33	<0.0001
Month X Breed	11	34548.97	3140.82	0.65	0.78
R-square		CV			
0.36		49.5			

**Table 7.4 Analysis of variance of the effect of season on the number of live spermatozoa produced**

<b>N-Living</b>					
Source	DF	SS	MS	F Value	PR>F
Error	120	2799042	23325		
Total	143	3319013			
Breed	1	158802.25	158802.25	6.81	0.01
Month	11	169409	15400.82	0.66	0.77
Month X Breed	11	191759.75	17432.71	0.75	0.69
R-square		CV			
0.157		62.92			

**Table 7.5 Analysis of variance of the effect of season on the number of dead spermatozoa produced**

<b>N-Dead</b>					
Source	DF	SS	MS	F Value	PR>F
Error	120	2485.67	20.71		
Total	143	3732			
Breed	1	324	324	15.64	0.0001
Month	11	754.33	68.58	3.31	0.0005
Month X Breed	11	168	15.27	0.74	0.701
R-square		CV			
0.334		109.23			

**Table 7.6 Analysis of variance of the effect of season on the number of total spermatozoa produced**

<b>Totals</b>					
Source	DF	SS	MS	F Value	PR>F
Error	120	2794800.83	23290.01		
Total	143	3335297.83			
Breed	1	173402.84	173402.84	7.45	0.0073
Month	11	174103.91	15827.63	0.68	0.756
Month X Breed	11	192990.24	17544.57	0.75	0.6854
R-square		CV			
0.16		61.81			

**Table 7.7 Analysis of variance of the effect of season on the number of tailless spermatozoa produced**

<b>TLS</b>					
Source	DF	SS	MS	F Value	PR>F
Error	120	5925.17	49.38		
Total	143	6807.66			
Breed	1	5.06	5.06	0.1	0.75
Month	11	390.58	35.51	0.72	0.72
Month X Breed	11	486.85	44.26	0.9	0.55
R-square		CV			
0.13		108.92			

**Table 7.8 Analysis of variance of the effect of season on the number of coiled-tail spermatozoa produced**

<b>CT</b>					
Source	DF	SS	MS	F Value	PR>F
Error	120	30191.67	251.59		
Total	143	74801.31			
Breed	1	793.36	793.36	3.15	0.078
Month	11	39391.14	3581.03	14.23	<0.0001
Month X Breed	11	4425.14	402.29	1.6	0.108
R-square		CV			
0.596		110.77			

**Table 7.9 Analysis of variance of the effect of season on the scrotal circumference of the two breeds**

SC					
Source	DF	SS	MS	F Value	PR>F
Error	120	314.88	2.62		
Total	143	369.54			
Breed	1	0.04	0.04	0.02	0.898
Month	11	23.98	2.18	0.83	0.609
Month X Breed	11	30.64	2.79	1.06	0.398
R-square		CV			
0.148		5.82			

**Table 7.10 Analysis of variance of the effect of season on the number of broken at neck spermatozoa produced**

BN					
Source	DF	SS	MS	F Value	PR>F
Error	120	3055.17	25.46	1.26	0.2088
Total	143	3793.99			
Breed	1	73.67	73.67	2.89	0.092
Month	11	284.24	25.84	1.01	0.4375
Month X Breed	11	380.91	34.63	1.36	0.201
R-square		CV			
0.195		109.26			



*Table 7.11 Analysis of variance of the effect of season on the number of broken at mid piece spermatozoa produced*

<b>BM</b>					
Source	DF	SS	MS	F Value	PR>F
Error	120	722.66	6.02		
Total	143	1015.83			
Breed	1	15.34	15.34	2.55	0.113
Month	11	212.07	19.28	3.2	0.0008
Month X Breed	11	66.24	6.02	1	0.45
R-square		CV			
0.29		96.78			