SEASONAL VARIATION IN SEMEN QUALITY OF DORPER RAMS USING DIFFERENT COLLECTION TECHNIQUES

by

Cosmas Masankosa Malejane

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Supervisor: **Prof. J.P.C. Greyling**

Dedication

- To my wife, Orebotse, and my sons Bakang and Keetla Malejane.
- To the Late, Dr L.M.J. Schwalbach, our mission is accomplished (May your soul rest in peace).

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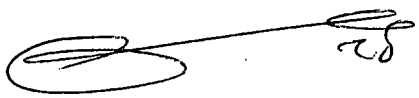
Declaration

I hereby declare that this dissertation submitted by me to the University of the Free State for the degree, **Magister Scientiae Agriculturae,** is my own independent work and has not previously been submitted by me at another University. I furthermore cede copyright of the dissertation in favour of the University of the Free State.



Cosmas Masankosa Malejane

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List of Abbreviations

%	Percentage
°C	Degree Celcius
µm	Micron
AI	Artificial insemination
ARC	Agricultural Research Council
ATP	Adenosine triphosphate
AV	Artificial vagina
BCA	Botswana College of Agriculture
BCS	Body condition score
BLUP	Best linear unbiased prediction
CASA	Computer assisted sperm analysis
cm	centimeter
e.g.	for example
EBV	Estimated breeding value
EE	Electro-ejaculation
et al.	And others
FSH	Follicle stimulating hormone
GLM	Generalized linear model
GnRH	Gonadotropin releasing hormone
h	Hour
ICSH	Interstitial cell stimulating hormone
im	intramuscular
SAFA	South African Feedlot Association
SAS	Statistical analysis system
SSH	Spermatogenesis stimulating hormone

kg	Kilogram
LH	Luteinizing hormone
mg	milligram
ml	milliliter
N	North
pH	Potential hydrogen
S	South
SAVSEG	South Africa veterinary semen and embryo group
SD	Standard deviation
STH	Somatotrophic hormone
UDRAW	Unit for the Development of Rhetorical and Academic Writing
UFS	University of the Free State
USA	United States of America

Chapter 1

Introduction

Sheep production plays an integral role in the South African agriculture. There are then different indigenous breeds of sheep, which include amongst others the South African Mutton Merino, Dohne Merino, Dorper, Dormer, Merinolandskaap, Walrich, Letelle, van Rooy, Afrikaner, Namakwa Afrikaner and Meat Master and other exotic breeds for example the Finish Landrace, Ile de France, Suffolk etc, to name but a few (Scholtz, 2010). The current study focused on the Dorper breed, because of its international renowned adaptability, hardiness, veld utilization, good mothering ability and high overall general demand in certain regions worldwide. The Dorper sheep as such has been described as the ideal breed which can survive, thrive, produce and reproduce under sub-optimal and optimal climatic conditions (Lategan, 2012). The Dorper sheep population then represents approximately 28% of the 22.2 million sheep in South Africa. The breed does not only rank second in terms of sheep numbers, but also realises superior prices at sales and auctions – the record price of R250 000 paid for a white Dorper ram in 2006, for example reflects the demand and popularity of the breed (Milne, 2008). Further the Dorper breed also plays a very important role in extensive sheep production and has established it as a source of income. It acts as a good source of animal protein, while providing diversity, as well as source of foreign exchange. Many South African rural communities also depend solely on sheep production (which includes the Dorper) for their livelihood (Schoeman *et al.*, 2010).

As mentioned earlier, the Dorper breed is in high demand – therefore it needs to be distributed more effectively in the market. The easiest and fastest way of disseminating genetic material of superior males may then be through artificial insemination (AI). For AI to take place, semen then needs to be collected from the male and later be deposited in the female in a fresh or frozen form (Bourdon, 2000). The first step in the creation of a cryopreservation semen bank is then the use of an effective method of semen collection. There are different methods of semen collection – some technicians prefer the artificial vagina (AV), while others prefer or

are forced to use electro-ejaculation (EE). The question which may follow is which is the most reliable method of semen collection for Dorper rams? This question was addressed in the current study by assessing semen quality e.g. colour, sperm concentration, sperm motility of Dorper rams, using both collection techniques. When addressing the above problem, the element of seasonality was also to be considered, as sheep are known to be seasonal breeders (Hafez and Hafez, 2000; Senger, 2003). Seasonality has generally been studied in sheep at high latitudes (greater than 35°) (Rosa and Bryant, 2003; Leahy *et al.*, 2010; Ridler *et al.*, 2012). On the other hand, Greyling and Grobbelaar (1983) also reported there to be little information regarding the fertility of rams throughout the year (seasonality) in South Africa.

In sheep flocks the lambing percentage or rate should be between 100 and 120%, but due to poor reproductive performance the annual lambing percentage in South Africa is reported to only be approximately 77% (Greyling and Schwalbach, 2002). To reduce this poor reproductive performance, the quality of ram semen needs to be assessed before the ram is introduced to the flock or be used for AI. The delay or absence can then jeopardize the economic sustainability of sheep production in general. Also owing to the high purchasing price of rams, transportation and rearing risks, the quality of semen needs to be checked or even be stored before the ram is moved from one farm to the next. Noakes *et al.* (2009) reported that breeding soundness examinations of the male must be part of the purchasing agreement.

The aim of the study was therefore to evaluate the seasonal variation of semen quality in Dorper rams, using different collection techniques and ultimately to identify a preferred time for controlled breeding and AI in the male.

The trial offered the opportunity of increasing knowledge regarding the concept of seasonality in male sheep – the reproductive physiology changes and terms used to explain seasonality. The candidate also learned the terms and the practical skills of semen collection and evaluation. Further the Dorper ram's reproductive performance and capabilities was also evaluated. In addition to the above intrinsic rewards, the empirical and rhetoric towards writing scientific papers were also learned, leading the researcher to have hopes for reasonable extrinsic rewards (Mouton, 2011).

Books, monographs, conference proceedings, reference material, journals, news papers and magazine articles, technical reports, theses, dissertations, visits to discipline specialists were used for the following purposes: to avoid repeating or duplicating previous research; to find more recent data regarding the research field; and to find the most widely accepted empirical finding in the field of study. Further also to learn regarding instrumentation currently being used and to learn how to use the correct terminology for the seasonal variation in semen quality of Dorper rams, using the different collection techniques (Mouton, 2011).

Chapter 2

Literature review

2.1 Background on the Dorper breed

The Dorper was originally developed for mutton production by cross-breeding the Dorset Horn and Blackhead Persian, with this development being performed in the arid and semi-arid regions of the North Western Cape (Campbell, 1989). This cross-breeding programme was initiated in the early 1930's, because of surplus sheep numbers (mutton), which could not be absorbed locally or exported because of a poor carcass quality. According to Milne (2000), the South African sheep breeds could generally not penetrate the English market because of the strange and fat tail type sheep which were not desirable to the consumers. The upgrade of the Dorper breed from the early 1930's to 1942, was however not a one man endeavor, as many researchers and producers were involved and Lategan (2012) reported research and trials in this regard to be performed at agricultural colleges, experimental farms and in co-operation with farmers. Emanating from these trials it was established that the Dorset Horn x Blackhead Persian crossbred produced the most acceptable and desired carcass quality. It was also reported that the name "DORPER" emanated after much deliberation and has a bilingual connotation of the first syllable of the two sheep breeds involved – Dorset horn ram + Persian ewe = Dorper. The main aim for developing the Dorper was thus to produce a hardy mutton sheep, capable of surviving, adapting and producing lambs off the veld in low rainfall areas (Lategan, 2012).

Through selection the Dorper breed exhibits two distinct coat colours. The traditionally black headed animals being called the Dorper (Plate 2.1), with the Dorper being a white sheep, with black head or head and neck – in stud breeding no further colouration being allowed than where it touches the shoulder or breast-bone of the animal. Complete pigmentation around the anus or reproductive organs and the hooves, is however compulsory.

Plate 2.1: Dorper ram (Milne, 2002)

Plate 2.2: White Dorper ram (Milne, 2010)

Emanating from breeding and selection a complete white sheep was then called the White Dorper (Plate 2.2). The White Dorper being fully pigmented around the eye-lids, under the tail and on the teats – this being the ideal. In the White Dorper breed standards allow a limited number of black spots, generally on the ears and the underline. According to Milne (2000), there is basically no difference in breed standards between the Dorper and the White Dorper, except regarding the colour and pigmentation. Despite this difference, the term "Dorper" is generally used to describe both breed types.

The Dorper breed has then since its inception spread throughout Southern Africa and has adapted well to various extreme arid environmental conditions, exhibiting a high fertility rate. According to De Waal and Combrinck (2000), at present the Dorper breed is numerically second to Merino in terms of numbers as the largest sheep breed in South Africa. Schoeman *et al.* (2010) recorded 643 active Dorper stud breeders, followed by 305 Merino stud breeders in South Africa. The total population of the Dorper breed in South Africa is estimated at over 7 million animals (Milne, 2000; Ramsay *et al.*, 2001).This breed is adaptable to a wide range of climatic conditions, from hot and dry to humid and cold environments – hence its popularity worldwide. The sheep generally puts on limited wool in the colder months and sheds it in the warmer weather – without the need of human assistance (shearing) (Simmons and Ekarius, 2001).

2.1.1 Advantages of the Dorper breed

2.1.1.1 Reproduction and meat/skin production

According to Schoeman (2000), this Dorper breed has an acceptable high fertility rate, which tends to be higher than those of the wooled sheep breeds in South Africa. Compared to the South African Mutton Merino (SAMM) and Dohne Merino (DM), it was recorded that the Dorper ewe lambs 3 months of age earlier – due to reaching sexual maturity earlier (Campbell, 1989). The ewes are thus highly fertile with a high percentage of multiple births. The ewes were also found to be able to be remated two months before weaning of their offspring. Under intensive production systems Dorper ewes were highly prolific – the ewes adapting well to lamb three times in two years. The lambs are generally sold directly from the dams at 3 to 4 months of age, under extensive conditions. The mating age of maiden ewes is generally at the two-tooth stage, (12 months of age) and the marketing period of slaughter lambs and culled young ewes is also generally before 12 months of age (Scholtz, 2010). Dorper rams have been reported to have a high libido – e.g. in a 24 hour period, 14 young Dorper rams were reported to mate 50 estrous ewes on average 19.7 times, with a range of 12 to 30 ewes (Cloete *et al.*, 2000).

Early maturity has also been reported in the breed regarding carcass quality and growth of the rams. So for example the Dorper is renowned for winning carcass competitions – due to the body conformation and even fat distribution over the entire carcass being a characteristic for this achievement. The Dorper breed then also produces world class skins for high quality leather clothing and gloves (Lategan, 2012). In Table 2.1 certain attributes of the breed and production traits are set out.

Table 2.1: Performance traits of the Dorper breed (Ramsay *et al.*, 2001)

	Male	Female
Mature body weight	100-120 kg	75-85 kg
Birth weight	5 kg	4 kg
100-day body weight	35 kg	31.5 kg
Average carcass weight (fat lamb production)	18 kg	

2.1.1.2 Adaptability and hardiness

From the Blackhead Persian, the Dorper inherited important traits such as hardiness, easy walking ability and pigmentation. As mentioned earlier the Dorper breed is fairly evenly distributed throughout South Africa and also further afield. Campbell (1989) however reported the Dorper to be better suited to desert, semi-desert and savanna veld environments. This breed is thus generally found in veld types that are arid to dry, with grass, Karoo bush species, shrubs and low bushes – with the Dorper being tolerant to extreme ambient temperatures – cold, wind, rain and high summer temperatures (Ramsay *et al.*, 2001). The breed can thus survive under severe drought conditions (Plate 2.1), and also under the snowy conditions in the United States of America and Switzerland. The Dorper is also generally known to survive health hazards and disease outbreaks –characterized by low mortality rates, high reproductive rates, high growth rates, while the ewe may maintain a reasonable body condition for raising her offspring – to be ready for the next mating cycle (Lategan, 2012).

Table 2.2: Geographic distribution of the Dorper breed in the four Provinces of South Africa (1963 to 1987) (Marais and Schoeman, 2011)

Province	1963/64	1976	1987
Cape	74.8%(1 988 692)	77.9%(4 042 725)	41.7% (2 766 871)
Free State	17.2% (457 293)	15.9% (825 152)	47.1% (3 125 171)
Gauteng	7.3% (194 084)	5.5% (285 430)	9.6% (636 977)
Kwa Zulu Natal	0.6% (15 952)	0.9 (46 707)	1.6% (106 163)
Total:	2 658679	5 189 634	6 635 182

In Table 2.2, the geographic distribution of Dorper in the four Provinces of South Africa from 1963 to 1987 is set out (Marais and Schoeman, 2011). Despite the fact that the Dorper breed was bred and developed for the South African environment and also declared indigenous to South Africa, the breed has found its way and is distributed throughout the world – for example in Australia, New Zealand, the United

Kingdom, Middle East, China, Canada, Germany, Switzerland, Brazil, Argentina, South America, Mexico, the United States of America, and various African countries (www.dorpersa.co.za., 2011).

2.1.1.3 Veld utilization

The grazing habits of these non-woolled sheep have made them adaptable to a wide range of environmental conditions (Plate 2.3). The Dorper generally utilizes small shrubs and perennial herbage, between 2.5 to 20 cm in height – better than cattle. Similarly it has been reported that Dorpers may walk approximately 7.52 km per day, compared to 5.31 km by the Merino or Karakul. Generally the Dorper sheep graze more selectively, as such the animals do not suffer deficiencies, compared to other farm animals. The Dorper has been reported to convert low quality roughage into high quality lamb (Oberholster, 2010).

Plate 2.3: Dorper ewes and lambs grazing (adaptability, hardiness, veld utilization and good mothering ability)

2.1.1.4 Good mothering ability

Dorper ewes have been reported to possess a good mothering ability (Plate 2.3). When left alone, they lamb easily, retain and look after their offspring. The survival rate for singles has been reported as being 92%, while for multiples the survival rate is 90%. The Dorper are reported to rarely reject their lambs, even if farmers interfere with the young soon after lambing. Thus the Dorper ewes will generally nurture their lambs under different conditions, resulting in low lamb mortalities (Cloete *et al.*, 2000).

2.1.2 Disadvantages of the Dorper breed

Care should however be taken when intensively feeding the Dorper (feedlot). Studies have shown that even although the Dorper is one the most popular meat producers, it is not economically suited for the feedlot environment – as it is more prone to depositing fat at an early stage (Scholtz, 2010; Van der Merwe, 2010). The wool industry, supported by The South African National Wool Growers' Association previously launched a campaign to ensure that the Dorper should not be crossed with woolled sheep. The reason for this being to protect the quality of wool (contamination with kemp). The Dorper was found to produce spotted and even black lambs, when crossbred with Merino sheep (Schoeman, 2000).

2.2 Potential utilization of the Dorper ram for semen freezing, artificial insemination and the exporting of genetic material

Artificial insemination (AI) is generally known as a reproductive technique to accelerate genetic progress. It involves the collection of semen from the males to then be deposited in the females – in either the fresh or frozen semen form (Bourdon, 2000). AI was then the first assisted reproductive technique to be used to maximize the utilization of superior males and to distribute their genes extensively amongst a female population. The first documented AI in sheep in South Africa was performed in 1932, while AI was first practiced in an organized manner in 1949. AI is still today regarded as the most dramatic technology used by farmers to increase the genetic potential and accelerate genetic progress in farm animals (Ferreira, 2009). AI has then also gained widespread acceptance in mainly the dairy cattle industry in most developing countries, but is still lagging behind with regard to sheep and goat breeding. In sheep production most farmers generally still prefer natural mating (Evans and Maxwell, 1987). The storage of semen, particularly in a frozen state, was reported to cause ultrastructural, biochemical and functional damage to sperm, resulting in a reduction of sperm motility, viability, impaired transport and lower fertility – research has then been devoted in addressing these problems (Leboeuf *et al.*, 2000). Owing to the world interest in the Dorper breed this is still ongoing, and hence the current trial being justified.

In South Africa laparoscopic AI was introduced at Ramsem, an AI station in the year 1985, and the technology has helped to improve the conception rate in small stock when using frozen semen. Laparoscopic AI is then used to deposit semen directly into each of the uterine horns, and is also used for insemination and flushing of superovulated ewes during embryo transfer programmes. Embryo transfer as such then also offers a viable alternative for Dorper sheep gene dissemination (Ramsay *et al.*, 2001). The technology of embryo transfer and its associated practices (splitting of embryos, sexing of embryos, semen sexing, laparoscopy, cloning – to name but a few), is being improved continually, and livestock farmers are implementing certain of these reproductive techniques (Mitchell and Doak, 2004).

2.3 Reproductive physiology of the ram

2.3.1 Anatomy of the male reproductive tract

Greyling (2009) defined reproduction as a series of complicated processes and to comprehend reproduction, there is also a need for an understanding of the anatomy, physiology and endocrinology of the male reproductive tract. The male reproductive tract then includes the testis, epididymis, deferent duct (vas deferens), the urethra which is continued into the penis and the accessory genital glands – vesicular seminales (vesicular gland), bulbo-urethral (Cowper's gland) and prostate gland (Gerneke, 1986). This reproductive tract is then illustrated in Figure 2.1.

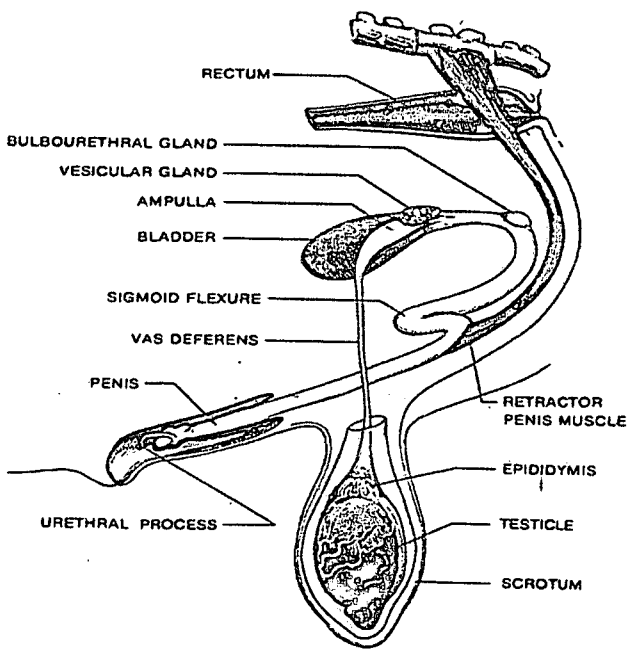

Figure 2.1: The reproductive tract of the ram (Mitchell and Doak, 2004)

2.3.1.1 The testis

The testis or testicle (Figure 2.2) is the primary male reproductive gland or gonad. The testis contains the seminiferous tubules, tubuli recti, rete testis and epididymis – being a very sensitive and delicate organ which should be handled with care during soundness tests. Testis size as such is a highly heritable trait, but is also influenced by external factors such as age, breed and level of nutrition of the animal. The testis size is generally then also seen as a good indicator of sperm production – with the number of sperm produced being correlated with the size of the testis (Milne, 2010; Van Wyk, 2010). Thus testes size is noteworthy – as sperm output is proportional to testis size (Gordon, 1997). Rams possessing large symmetrical testes free of defects are then likely to produce semen of good quality. Rams with large testes then generally also sire daughters which start cycling earlier in the breeding season and produce more twins, compared to daughters of rams with small testes (Milne, 2010). The testis as a gland performs two major functions, namely:

- The production of the male gametes (exocrine)
- The production of the male sex hormone (endocrine).

The testes of the ram are generally very large and could weigh between 200 and 300 g each, in a healthy adult ram (Evans and Maxwell, 1987). A ram testes weight of 500 g has also been reported (Senger, 2003). In this trial, testes size was also included as a parameter by measuring the testes volume and scrotal circumference. It is generally difficult to record the separate (left and right) testis parameters, as these reproductive organs are contained together within a pouch called the scrotum (Guyton and Hall, 2011). The testes size of the ram may then vary according to season, reaching a maximum in the middle of the natural breeding season.

Each testis is then covered with a tough, fibrous- elastic membrane called the tunica albuginia, which supplies blood by way of the testis arteries and veins. The testes also contain the Sertoli cells and the interstitial cells of Leydig – each with a specific function (Gerneke, 1986; Evans and Maxwell, 1987).

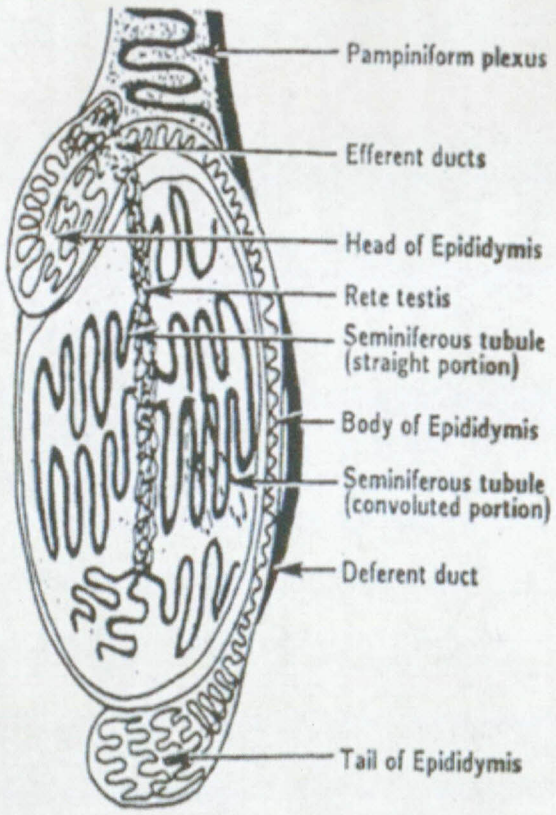

Figure 2.2: Diagrammatic presentation of the testis (Penner, 1993)

2.3.1.2 The scrotum

The scrotum as such not only supports and protects the testes, but also has an important role to play in temperature regulation. The scrotum size is generally utilized by the ram breeder, to superficially test for breeding soundness. According to Scholtz (2010), scrotum size serves as an excellent indicator in the evaluation of the animal's reproduction potential. When evaluating the contents of the scrotum, the temperature, size, texture, evenness of the testes and epididymis should be examined (Noakes *et al.*, 2009). The scrotum must then also preferably hang symmetrical and the testes should move freely within the scrotum. The septum which separates the left and right testis should also be felt. Too much wool on the scrotum should be recorded, as this could make the ram more susceptible to heat stress, and this in turn will affect the efficiency of spermatogenesis. Excess wool should thus be removed at shearing to keep the testes cool (Gouletsou and Fthenakis, 2010). On the other hand care should also be taken in the evaluation, as long hair and a small scrotum could indicate lower quantities of the reproductive (gonadotrophic) hormones being produced (Scholtz, 2010). Arthur *et al.* (1996) reported the measurement of the scrotal circumference to be a useful tool in estimating male fertility. Scrotal circumference in mature rams generally vary between 28 and 40 cm.

Scrotal circumference in relation to the age of the Dorper ram, has also been reported, with the minimum scrotal circumference quoted being:

- 10 months of age – 30cm
- 2 tooth – 32cm
- 4 tooth – 33cm
- 6 tooth and older – 34 cm or greater (Lategan, 2012)

A two tooth ram under feedlot conditions should exhibit on average, a testes circumference of 35-39 cm. As previously mentioned, circumference of the testes has then been shown to be well correlated with sperm production (Campbell, 1989). In cattle, scrotal size has been correlated to the quality and quantity of sperm, pregnancy and yearling weight (Scholtz, 2010)

The cremaster muscle may extend or contract to keep testes at a temperature of 4 to 7 °C below the abdominal temperature, while the pampiniform plexus also helps to cool the blood supply to the testis (Evans and Maxwell, 1987). The lower scrotal temperature is generally a result of the surface evaporation of moisture, the convection, circulation of lower air temperature, and heat loss by radiation. Under natural conditions a ram with a scrotal circumference of 30 cm or more can be successfully mated to 80 - 100 ewes, provided that the ram is in good health and has a high serving capacity (Gouletsou and Fthenakis, 2010).

Scrotal circumference has been positively associated with increased semen production and a decrease in age at puberty of heifer progeny. This increase in scrotal size has then also been associated with an increase in sperm motility, the percentage of normal sperm, total sperm concentration and a decrease in the percentage of abnormal sperm (Van Wyk, 2010). It is accepted that scrotal circumference should always be measured at the widest part of the scrotum (Kafi *et al.*, 2004; Fourie *et al.*, 2005).

2.3.1.3 Epididymis

The epididymis is responsible for the transport, storage and final maturation of the sperm cells. Sperm in the epididymis receive nutrients from epithelial secretions. While in the epididymis the sperm attain a slight degree of motility and over 90% of

the fluid leaving the testis is absorbed. This may result in a negative pressure in the testis, which then helps in the sperm transport when the fluids are absorbed. The sperm matures in the epididymis during a period of two to three weeks (Gerneke, 1986).

The caudal portion or tail of the epididymis is the major site of sperm storage. In dead wildlife or slaughtered animals live sperm may be collected from the caudal portion of the epididymis. These caudal epididymis sperm must however be collected within 72 hours following slaughter, provided the gonads are stored at 4°C (Lone *et al.*, 2011). The sperm contained in the epididymis may be termed the extragonadal reserves, even although only those in the distal part of the tail are ejaculated. The sperm cell matures in the epididymis with the protoplasmic droplet on the sperm cell generally acting as an indicator of the degree of sperm maturity. Sperm exhibiting a protoplasmic droplet are judged as not yet mature. The tail of the epididymis generally has the capacity to store approximately 70 billion sperm cells. From the epididymis, the sperm are then transported to the vas deferens during ejaculation (Hafez and Hafez, 2000).

2.3.1.4 Vas deferens

The vas deferens is a tube with a wall consisting of a mucosa, a muscularis and an adventitia or serosa layer. The mucosa is generally rich in elastic fibres, while the muscularis has a layer of smooth muscle fibres. The serosa contains the blood vessels, nerves and longitudinal muscle fibres (Gerneke, 1986). This structure transports the sperm from the epididymis to the urethra in the penis. Blockage or severing of the vas deferens will ultimately prevent the semen from being secreted during ejaculation. The surgical removal of the vas deferens dorsal to the scrotum in the male to make teaser rams or bulls, is termed a vasectomy (Evans and Maxwell, 1987). By involuntary muscular contractions, the vas deferens is also then involved in semen ejaculation (Salisbury *et al.*, 1978)

2.3.2 The accessory sex glands

The accessory sex glands of the male include the ampulla, the seminal vesicles, the bulbo-urethral or Cowper's gland and the prostate. The development and the

activities of these accessory sex glands are stimulated by the androgens (testosterone), and retarded by estrogen (Gerneke, 1986).

2.3.2.1 Ampulla

The last 3 to 4 cm of the vas deferens is enlarged to form the ampulla. This thickening of the vas deferens being caused by branched tubular glands, situated in the mucosa of the prepuce or from the penis. The ampulla serves as a temporary storage organ for the sperm and contributes only slightly to the volume of the seminal plasma (Arthur *et al.*, 1996; Noakes *et al.*, 2009).

2.3.2.2 Seminal vesicles

The seminal vesicles are situated adjacent to the neck of the bladder and lateral to the ampulla. These glands secrete a colourless fluid that contributes substantially to the volume of the ejaculate. This secretion is then gelatinous in structure and rich in globulins, fructose and flavins (Gerneke, 1986). The secretions of the vesicles as such then make the semen more alkaline and activate the sperm and provide nutrients to the sperm. The secretion of the fluid occurs because of contractions of muscles in the connective tissue of the seminal vesicles (Salisbury *et al.*, 1978).

2.3.2.3 Bulbo-urethral or Cowper's glands

The bulbo-urethral are paired glands covered by the muscularisbulbo-glandularis and surrounded by a fibro-muscular capsule. These two glands lie on each side of the pelvic urethra. The bulbo-urethral gland produces a viscid (thick and sticky), mucus-like lubricating substance (Salisbury *et al.*, 1978). This lubricating fluid being secreted first – just prior to coitus and is considered to cleanse the urethra of urine and adjust the pH for the semen that will follow ejaculation (Arthur *et al.*, 1996). The secretions of the Cowper's glands are the first fluid to be secreted during ejaculation. The secretions tend to be more alkaline than that of the other accessory glands (Mitchell and Doak, 2004).

2.3.2.4 The prostate gland

The prostate gland is composed of two parts, namely the body of the prostate and the disseminate prostate. The prostate as such is located near the neck of the

bladder, while the disseminated prostate surrounds the urethra (Mitchell and Doak, 2004). The prostate gland then produces the prostatic fluid that drains into the urethra via several small excretory tubules. This gland is the source of the male antagglutin, and it is believed to secrete a fluid high in minerals (Salisbury *et al.*, 1978). The prostate gland is high in sodium and citrate and is a major source of zinc. Approximately 25% to 40% of the semen volume reported is reported to be prostatic fluid (Mitchell and Doak, 2004).

2.3.3 Penis

The penis consists of a root, a body and the glans. The penis is generally seen as the copulation organ for deposition of the semen into the vagina and also the excretion of urine. It composed of varying quantities of erectile tissue – when the male is sexually stimulated, this erectile tissue is filled with blood. This engorgement then causes the penis to enlarge and become rigid, thus enabling it to penetrate the vagina (Salisbury *et al.*, 1978). The penis of the ram can be extended up to 30 cm during copulation in the ram. The penis is normally held in the 'S' position by the retractor muscles – during copulation, the retractor muscle extends and the sigmoid flexure subsequently straightens. According to Milne (2010) before breeding (part of soundness evaluation), the penis of the ram needs to be inspected and the following aspects warrant attention:

- The urethral process has not been removed (shorn off) or injured. The urethral process rotates rapidly during ejaculation and sprays the semen around the anterior vagina, near the opening of the cervix.
- There are no ulcers or swellings on the lining of the penis.
- The penis as such, is not broken.

The penis of the ram can easily be protruded from the prepuce with the ram in a sitting position on its haunches for inspection, or even during the electro-ejaculation technique (Noakes *et al.*, 2009).

2.3.4 Morphology of the sperm cell

Sperm (Figure 2.3) are highly specialized, free-swimming cells, specifically adapted to finding and fertilizing the ovum in the Fallopian tube (at ampullary-isthmic junction). In simple terms the sperm cell, is the male sex cell or gamete. It consists of a head, a short flexible neck and a movable tail (Gerneke, 1985). In the ram the head is flat and ovoid and is mostly made up entirely of a nucleus – the nucleus then contains the chromosomes which are responsible for passing on paternal genetic information. The head is protected by the acrosome which contains the hydrolytic enzymes, for example acrosin, hyaluronidase, zona lysine, estrases and acid hydrolases. These hydrolytic enzymes are then set free during fertilization, thereby making it possible for the sperm to penetrate the corona radiata and zona pellucida of the ovum. The tail is the locomotory organelle of the sperm and as such is used for propulsion of the sperm in the seminal fluid (Senger, 2003). The ram or buck sperm cell is reported to be approximately 60 micron (60 µm) in length. The head alone being 8 to 10 µm long, 4 µm wide, and 1µm thick (Evans and Maxwell, 1987).

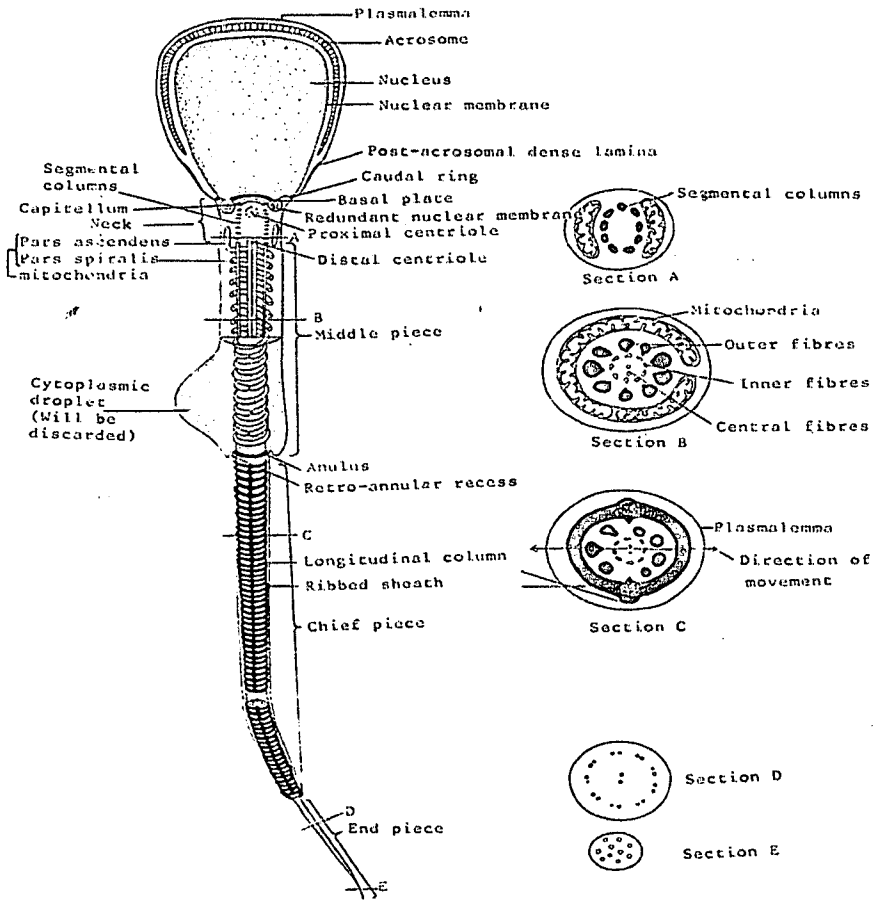

Figure 2.3: Diagrammatic presentation of sperm cell (Gerneke, 1985)

2.3.5 Spermatogenesis

Spermatogenesis (Figure 2.4) or the production of sperm cells occurs in the seminiferous tubules within the testes – the process initiated at puberty and which usually continues until the death of the male. Even although the release of motile sperm occurs at puberty, the process of spermatogenesis starts during the foetal stage. During early foetal development, the stem-cell spermatogonia are formed from the primodial germ cells and become established in the walls of the seminiferous tubules. The spermatogonia then remain inactive until sexual maturity or puberty is reached (Gerneke, 1985; Bester, 2006) The seminiferous tubules are lined with these spermatogenic cells, between which are located the nutritive and supporting Sertoli cells (Hafez and Hafez, 2000). The efficiency of spermatogenesis is generally influenced by the amount of germ cell degeneration, pubertal development, season of the year or aging of the male (Johnson *et al.*, 2000)

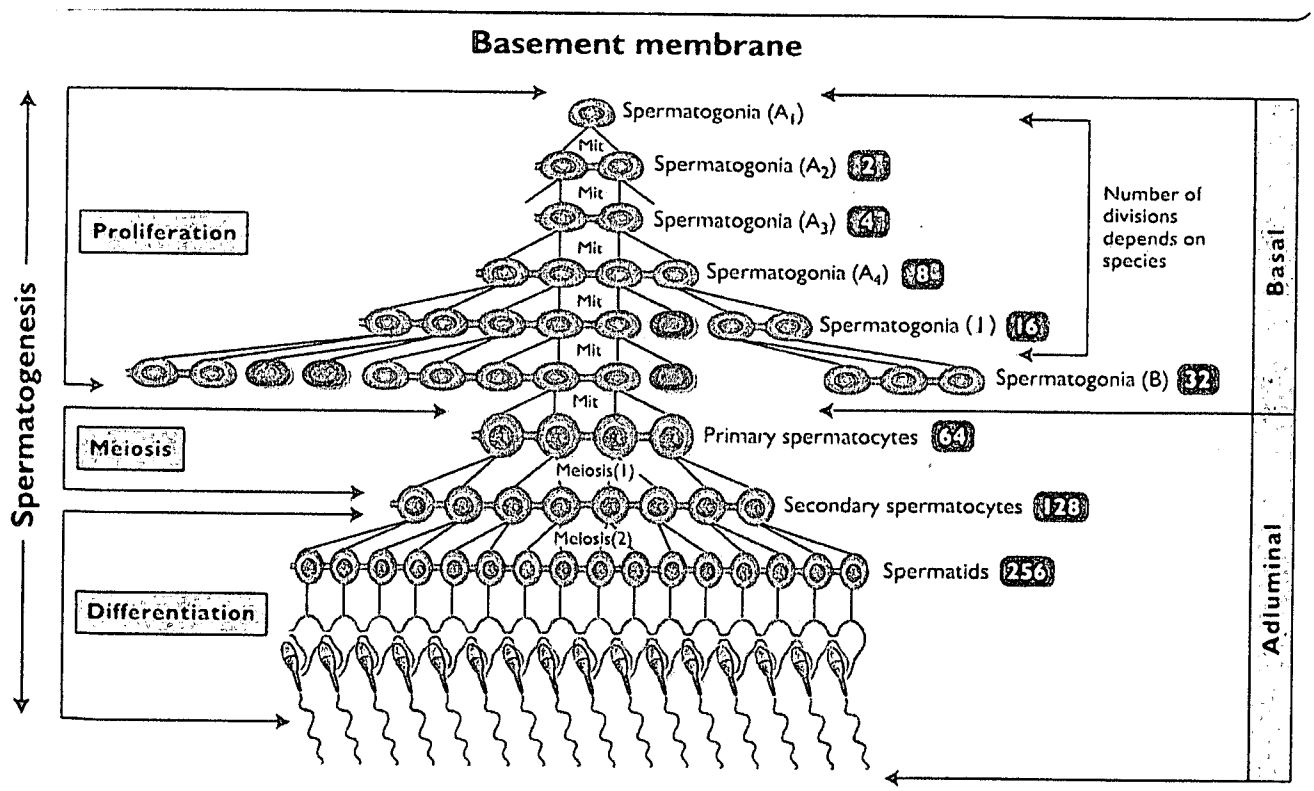

Figure 2.4: Schematic presentation of spermatogenesis (Senger, 2003)

The Sertoli cells are somatic cells situated in the seminiferous epithelium and are generally believed to govern the process of spermatogenesis. These cells are sometimes also called the nursing cells. The Sertoli cells provide nutrition and support to the spermatogenic cells, while the key function of the Sertoli cells lies in

the formation of the blood- testis-testis barrier, which amongst others prevents the body from setting up an immune reaction against newly formed sperm cells (Johnson *et al.*, 1997; 2008). Other functions also include providing structural support to the developing sperm cells and phagocytosis of degenerating germ cells. The Sertoli cells also secrete oestrogen, inhibin, a GnRH-like peptide, protein, lactate, pyruvate and tubular fluid – being quoted as being equivalent to the follicular granulosa cells in the female (Noakes *et al.*, 2009).

The main goal of spermatogenesis is ultimately to provide the male with a continual supply of male gametes through stem cell renewal, provide genetic diversity, provide billions of sperm each day to maximize reproduction by both natural service and artificial insemination and provide an immunologically safe site where germ cells are not destroyed by the male's immune system. On daily bases the ram can produce 10 x 10^9 sperm, compared to 6 x 10^9 sperm per day in a beef bull (Senger, 2003).

The process of spermatogenesis as such can be subdivided into two stages, namely spermatocytogenesis and spermiogenesis. Spermatocytogenesis as such is the development of spermatogonia to spermatids and can be subdivided into the following phases: the proliferation, growth and maturation phases (Gerneke, 1985). In a report by Senger (2003), the process of spermatogenesis was reported to be subdivided into three phases, namely the proliferation phase, the meiotic phase and the differentiation phase.

2.3.5.1 Proliferation phase (spermatocytogenesis)

The proliferation phase involves the spermatogonia or the germ cells. These spermatogonia are specialized diploid cells, located in the basal compartment of the seminiferous epithelium (Johnson *et al.*, 1997). At puberty the spermatogonia begin to divide mitotically and undergo several divisions to produce more diploid spermatogonia. An important part of this proliferation phase is stem cell renewal (Senger, 2003). As shown in Figure 2.4, the spermatogonia pass through 6 divisions to form primary spermatocytes – these divisions being the spermatogonia A_1 to A_4, spermatogonial and spermatogonia B. This proliferation phase thus involves the mitotic cell division to increase the yield of spermatogenesis and primary

spermatocytes by proliferation of type A-spermatogonia, through a number of successive mitotic divisions. Some of the type A-spermatogonia continually form type B-spermatogonia, with more vesicular nuclei – while the rest remain as proliferating type A-spermatogonia. Briefly the nucleus is the control center of the cell, which contains large quantities of deoxyribonucleic acid (DNA), which make up the genes. The type B-spermatogonia divide by mitotic divisions to form preleptotene spermatocytes (Johnson *et al.* 1997). An important event of the preleptotene phase is complete DNA replication forming tetrads without separation (Senger, 2003).

2.3.5.2 The meiotic or growth phase

Meiosis is known as the process by which genetic material is exchanged between homologous chromosomes to produce haploid spermatids. The primary spermatocytes (diploid) undergo two quick successive meitotic divisions, the first division halving the chromosome numbers (reduction division) i.e. separating the homologous pairs to form spermatocytes with a haploid number of chromosomes. The sheep has 54 chromosomes, thus the haploid number of chromosomes is 27 (Bester, 2006). The second division then comprises the splitting of the centromeres and separation of the daughter chromosomes to form the haploid spermatids (Johnson *et al.*, 1997).

2.3.5.3 Differentiation phase (spermiogenesis)

The differentiation phase of spermatogenesis is commonly been called spermiogenesis in reproductive physiology terms. This differentiation refers to a series of changes which the haploid spermatids, as a syncytial groups of cells, undergo to form sperm. These changes take place while the spermatids are imbedded in the distal invaginations of the Sertoli cells. Spermatids then differentiate from spherical cells with spherical nuclei, to cells that have a streamlined sperm head, containing a penetrative enzyme and also condensed nucleus carrying the male genome and a tail that is necessary for motility (Johnson *et al.*, 1997; 2000). Immediately after completion of meiosis, the spermatids undergo a period of ribonucleic acid (RNA) synthesis. Differentiation then produces a highly sophisticated, self-propelled package of enzymes and DNA. Spermiogenesis as

such consists of four developmental phases: the Golgi, cap, acrosomal and maturation phases (Gerneke, 1985; Bester, 2006; Noakes *et al.*, 2009). The spermiogenesis phases are illustrated in Figure 2.5.

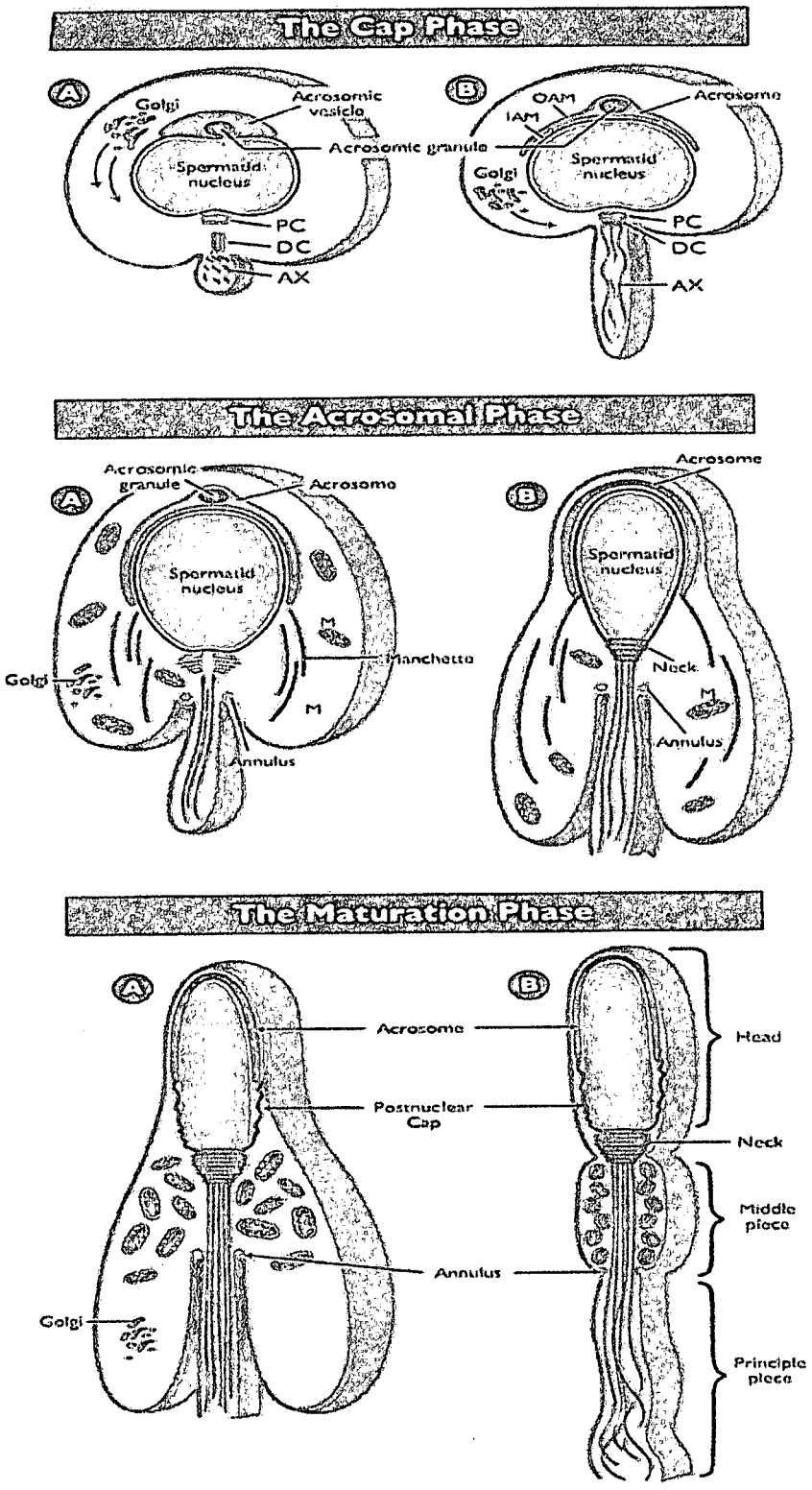

Figure 2.5: Schematic spermiogenesis phases (Senger, 2003)

2.3.5.3.1 The Golgi phase

The Golgi phase is the first step in the development of the acrosome (sperm cap). The acrosome being a vesicle containing a hydrolytic enzyme covering the nucleus of the spermatogonia. The Golgi phase spermatid contains a prominent Golgi apparatus that produces membrane-bound enzymes at its mature phase. The small vesicles then fuse to form the acromic vesicle, adjacent to the nucleus (Johnson *et al.*, 1997).

2.3.5.3.2 Cap phase

The acrosomic vesicle spreads progressively caudally to cover the spermatid nucleus. This spreading continues until nearly two-thirds (⅔) of the front portion of the nucleus is covered by a thin, double-layered membranous sac, so that it closely adheres to the nuclear envelope (Hafez and Hafez, 2000).

2.3.5.3.3 The acrosomal phase

During the acrosomal phase there are major changes in the nucleus, the acrosome and the tail of the developing spermatids. The spermatic nucleus begins to elongate and the acrosome eventually covers the majority of the anterior nucleus. The acrosome, also condenses and elongates to correspond to the shape of the nucleus. The remaining Golgi apparatus and cytoplasm containing the centrioles then move caudally. The shape of the spermatid has now changed from a round to an elongated structure (Gerneke, 1985; Hafez and Hafez, 2000).

2.3.5.3.4 Maturation phase

During the maturation phase or the final phase of spermatid development, the manchette migrates caudally where it may provide a shaft that supports the flagella canal. The mitochondria then migrate towards and cluster around the flagellum in the region posterior to the nucleus (Johnson *et al.*, 1997). Mitochondria are the "powerhouse" of the cell, as such without mitochondria the cells would not be able to get enough energy from the nutrients, and essentially all cellular activities would cease. In the mitochondria the liberated energy is used to synthesize a high energy substance called adenosine triphosphate (ATP). At this stage there is also the reshaping of the nucleus and acrosome of each spermatid, initiated in the acrosomal phase, producing the sperm characteristics for each species. The proximal centriole

as such remains behind the nucleus and is believed to participate in the first cleavage division after fertilization. At the end of the maturation phase there is the formation of the residual body, and the elongated spermatid is ready to be released as a sperm cell (Senger, 2003).

2.3.5.4 Duration of spermatogenesis

The time that it takes from the activation of the stem cell, to the release of free sperm into the lumen of the seminiferous tubules, is approximately 46 to 49 days in the ram (Schutte *et al.*, 1986). This time includes all the phases of spermatogenesis. From the seminiferous tubules the sperm then move into the epididymis and the sperm take between 8 to 14 days to migrate through the epididymis (Gerneke, 1986; Noakes *et al.*, 2009). In other words mature sperm that are produced are utilized or ejaculated in approximately two months.

Spermatogenesis follows a wave-like motion through the seminiferous tubules. To understand the cycle and development of these germ cells throughout spermatogenesis, it may be useful to compare spermatogenesis to that of a college student – from year 1 to year 4 (Figure 2.6). Throughout the processes of spermatogenesis certain stem cells fail to reach maturity and subsequently degenerate (Hafez and Hafez, 2000). Spermatogenesis can generally only terminate in the male due to senility, general weakness or disease. Some other factors which may affect spermatogenesis include atrophy of the seminiferous tubules, Vitamin A and E deficiencies. The release of the germ cell from the Sertoli cells into the lumen of the seminiferous tubules, is referred to as spermiation and is analogous with ovulation in the female. Spermiation occurs as a result of growth pressure, fluid secretion in the seminiferous tubules and contractions of the myoid fibroblasts which surround the seminiferous tubules (Gerneke, 1985). From the seminiferous tubules the sperm then move into the epididymis for maturation (as previously described in 2.3.1.3).

Spermatogenesis and sexual activity in the ram essentially never stop, as opposed to ovulation and oestrus in the ewe, which is time limited. According to Guyton and Hall (2011) sperm cells that are not ejaculated are presumed to be re-absorbed by the epithelial cells in the epididymis, or are passed out in the urine. Other research

by Salisbury *et al.* (1978) has reported unejaculated sperm to be lost through spontaneous seminal emissions.

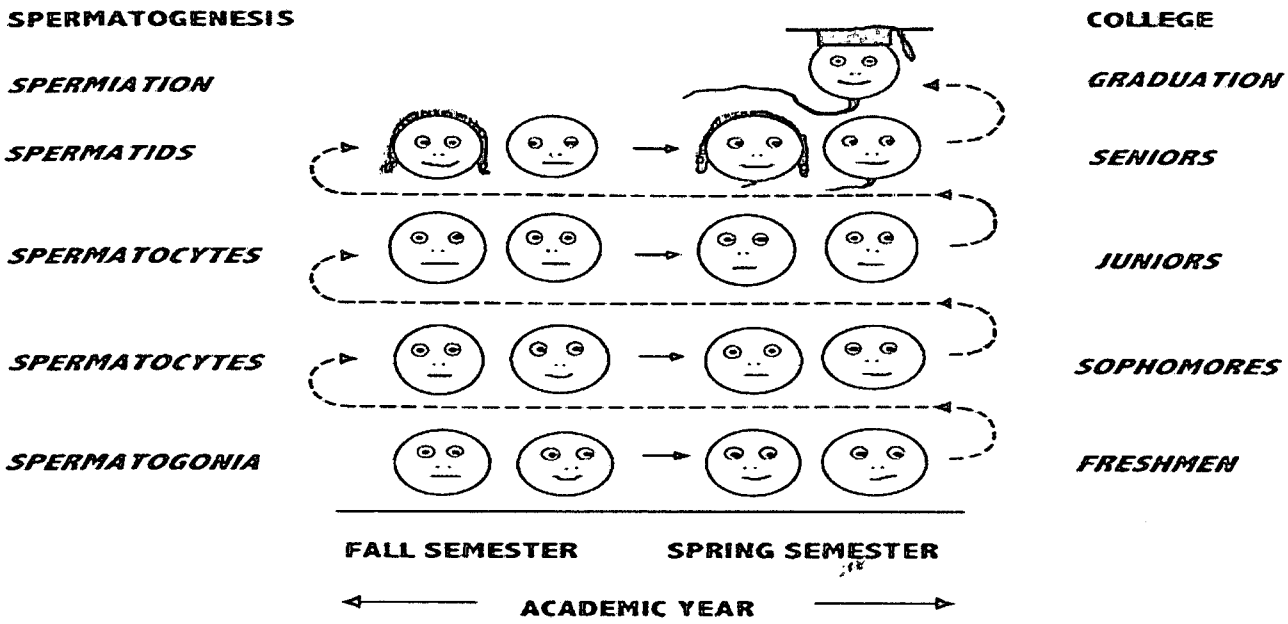

Figure 2.6: Spermatogenesis analogous with a college student (Hafez and Hafez, 2000)

2.3.5.5 The hormonal control of spermatogenesis

The functions of the reproductive organs are controlled by the nervous and endocrine system. Under the influence of the hypothalamus, the anterior pituitary gland synthesizes and discharges several endocrine hormones. These endocrine hormones can then be seen as chemical agents synthesized and secreted by the specialized glands and carried by the blood to other parts of the body – where they act on specific tissues or organs (Guyton and Hall, 2011). The two hormones that are mainly responsible for regulating testes function are follicular stimulating hormone (FSH) and luteinizing hormone (LH). These hormones are called gonadotrophic hormones, as they act on the gonads (Senger, 2003; Mitchell and Doak, 2004). These hormone secretions are again regulated by a feedback system – negative or positive, as illustrated in Figure 2.7.

2.3.5.5.1 Follicle stimulating hormone (FSH)

Follicle-stimulating hormone (FSH), also known as spermatogenic stimulating hormone (SSH) is secreted by the anterior pituitary gland. This hormone then stimulates the Sertoli cells to help convert spermatids to sperm (Bester, 2006).

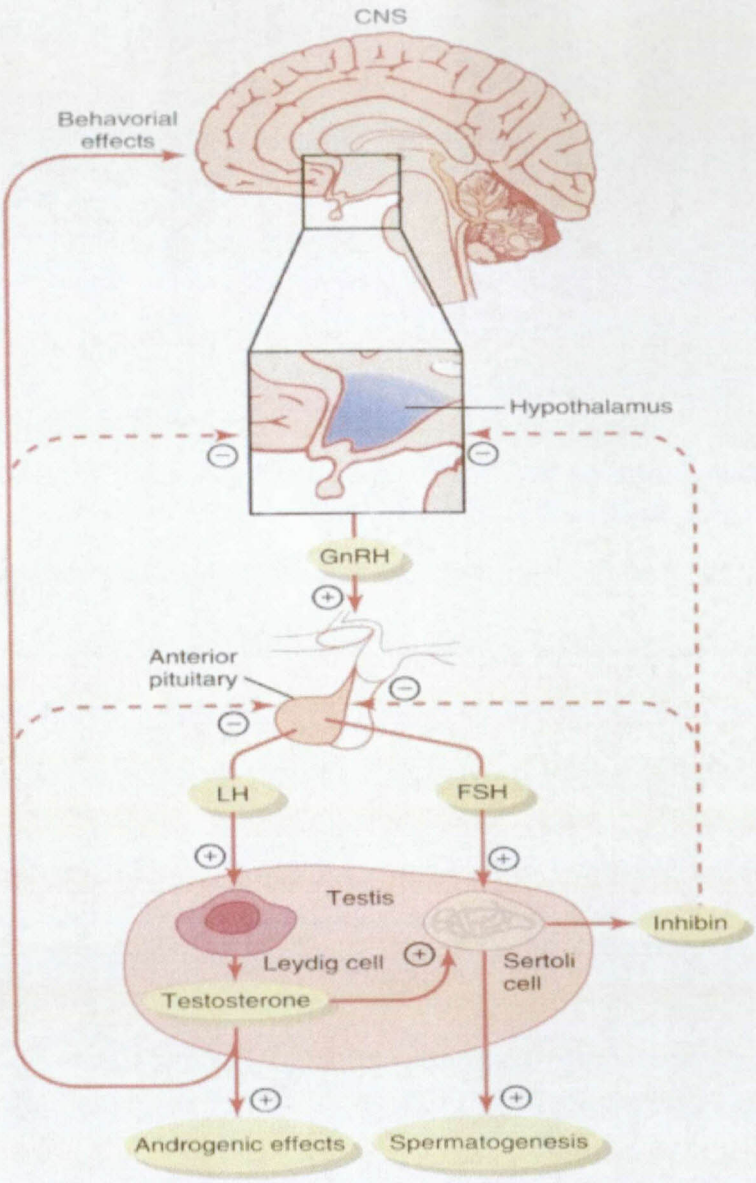

Figure 2.7: Hormonal control in male reproduction (Guyton and Hall, 2011)

2.3.5.5.2 Luteinizing hormone (LH)

Luteinizing hormone (LH) also known in the male as interstitial cell stimulating hormone (ICSH) is also produced by the anterior pituitary gland and stimulates the interstitial cells of Leydig in the testes to secrete testosterone (Bester, 2006).

2.3.5.5.3 Testosterone

The androgen testosterone is secreted by the interstitial cells of Leydig in the testicles - the interstitial cells of Leydig produce progesterone, most of which is then converted to testosterone. Testosterone as such controls the development of the secondary male sex glands, is responsible for the maintenance of the male genital duct, the sex characteristics, libido and spermatogenesis. Blood testosterone levels

may also serve as an indicator of seasonal seminal differences and may also be expressed in the efficiency of the semen collection technique (Senger, 2003).

2.3.5.5.4 Estrogen level

The liver in the male has been reported to produce 80% of the total estrogen in the body. The rest is believed to be formed in the Sertoli cells by converting testosterone to estrodiol. Estrogen as such has been found to be essential during the differentiation phase (spermiogenesis) (Guyton and Hall, 2011).

2.3.5.5.5 Growth hormone

Growth hormone also called somatotrophic hormone (STH) is also produced in the anterior pituitary. This hormone is mainly responsible for the metabolic functions, body growth and protein synthesis. Growth hormone specifically promotes early division of the spermatogonia. In its absence, as in pituitary dwarfs, spermatogenesis is severely deficient or absent, leading to infertility (Guyton and Hall, 2011).

2.3.5.5.6 Inhibin

Inhibin is secreted by the Sertoli cells and the target tissues are the gonadotrophs of the anterior lobe of the pituitary (Senger, 2003). Inhibin has a direct effect on the anterior pituitary gland, mainly to inhibit the secretion of FSH and it possibly has a side-effect on the hypothalamus in inhibiting the secretion of GnRH. Apart from the regulating pituitary FSH, inhibin related proteins also regulate the Leydig cell function (Hafez and Hafez, 2000).

2.4 Preparation of rams for semen collection

Rams should be taken care of throughout the year to maximize their productive longevity (Ridler *et al.*, 2012). Several weeks (6-8) before the onset of semen collection, attention should especially be paid to the body condition of the rams. The rams should also be treated for both internal and external parasites, vaccinated, sheared and crutched. Rams should also be properly identified by e.g. eartags for easy identification and record keeping. The age of the rams should always be borne in mind. Water and feed should then always be readily available throughout the breeding season or period of semen collection (Evans and Maxwell, 1987).

As mentioned earlier, prior to semen collection rams should be examined for soundness – to check that e.g. the testes are well developed for the breed and age. All males used for semen collection should thus be selected and be certified by a veterinarian for breeding soundness (Scholtz, 2010). Stud animal breeders are able to interpret the estimated breeding values (EBV's), which therefore enables them to use the Best Linear Unbiased Prediction (BLUP) to choose the most suitable males for breeding (Van Wyk, 2010). Stud breeders should convince themselves that the males selected for semen collection are genetically superior to their counterparts. At the end of the day, these males should then also be free of disease or any conformation abnormalities (Bourdon, 2000).

2.5 Semen collection

It has been researched that acceptable fertility results attained with AI, starts with a good semen collection technique. It is important to note that semen quality cannot be improved in the laboratory (Penner, 1993). Thus the initial step in creating a sperm cryobank, is the use of an effective method of semen collection. This semen collection can then be performed by the use of the artificial vagina and/or electro ejaculator. It is thus important to describe these methods of semen collection (Maule, 1962; Matthew *et al.*, 2003).

2.5.1 The artificial vagina (AV)

The artificial vagina can be seen as an imitation or simulation of the vagina of the ewe – which ultimately provides the correct temperature and pressure to stimulate the penis of the male and induce ejaculation. The AV method of semen collection is then generally considered to be the fastest and most hygienic of the various semen collection methods available – e.g. aspiration from the vagina of a recently bred ewe, electro-ejaculation and collection from the caudal portion of the epididymis (Maule, 1962; Lone *et al.*, 2011). However this technique of using the AV, requires the training of the rams. The rams to be collected by the AV method are thus trained, familiar to the collector and the collection environment – the collector must preferably even feed and water the animals. It has also been found by Salisbury *et al.* (1978) that semen collected using the AV method, is generally fairly uncontaminated, and similar to the natural ejaculate. According to Leboeuf *et al.* (2000) the presence of a

female in estrus can be used to facilitate the training of the ram and the collection of semen. To avoid pregnancy, the teaser ewes used can even be ovariectomized (Kafi *et al.*, 2004). The training of rams for AV semen collection may be successful within a week, depending on the libido of the ram (Hafez and Hafez, 2000). According to Maule (1962) it may take 2 days to train Dorset Horn, Romney Marsh, and Australian Merino rams which have never been handled in intensive husbandry. The artificial vagina then generally yields ejaculates with a smaller semen volume and denser concentration, when compared to that of electro-ejaculation.

2.5.1.1 The basic configuration of the artificial vagina

The artificial vagina (Figure 2.8) consists of an outer casing of rubber or plastic (15 to 20 cm x 5 to 6 cm) and an inner liner made of rubber or latex. A watertight jacket is formed inside the cylinder by turning back both ends of the latex over the outer casing cylinder on either side. The latex has thick ends which secures it tight on the casing – to avoid water spillage. The casing on its cylinder surface has a water inlet, which is used to pour in warm water to warm the inside of the AV. The AV can also be inflated through the water inlet to exert more pressure. The water jacket of the AV is normally filled with warm water between 50°C to 60° C, and a valve used to close the water inlet. The temperature on the inside of the liner should be 42°C to 45°C. At one end of the AV a thin coating of sterile, water-soluble lubricating jelly is applied to facilitate the penetration of the penis, while at the other end a collecting tube is inserted (Mitchell and Doak, 2004; Bester, 2006). Prior to the use of the AV for semen collection, the temperature needs to be monitored with a thermometer.

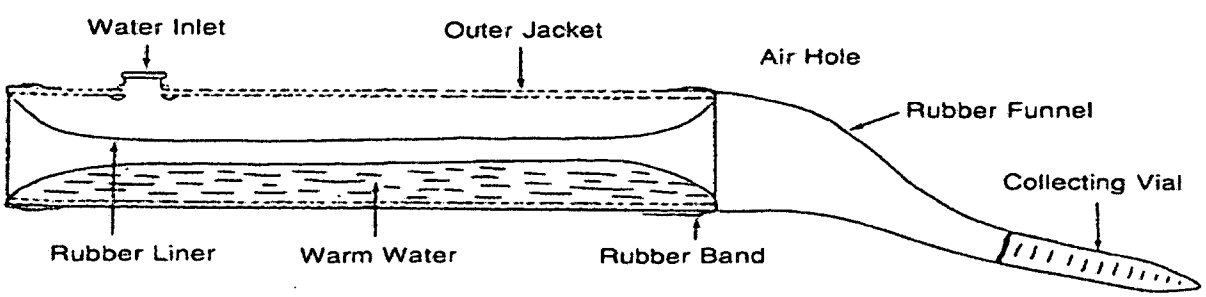

Figure 2.8: Cross-sectional view of artificial vagina (Mitchell and Doak, 2004)

Evans and Maxwell (1987) recommended four steps in the training of rams and bucks for semen collection using the artificial vagina. These steps can be summarized as follows:

- Bring the males into the holding pen (s) in the shed for a period of 5-10 days to allow them to get accustomed to the surroundings.
- Introduce one or two estrous females into the pen (s) and allow the males to mount.
- Accustomize the male to mounting an estrous female fixed in the collection pen. This should be done in the presence of the operator. When the male mounts, the operator should accustomise the animal to handling, by touching the sheath of the penis. If the male shows no interest in the female when left alone with her, the ram may be stimulated by either changing the teaser, or by allowing an active male into the pen. It is best to continually remove a difficult or reluctant male and re-introduce the ram for a short period, rather than persist for a prolonged period of time. Each time the ram is re-introduced it provides a new stimulus.
- Rams and bucks which regularly mount the teaser female in the presence of the operator can be trained to serve into an artificial vagina. Rams are considered trained if they mount and ejaculate into the artificial vagina.

2.5.2 Electro-ejaculation (EE)

Electro-ejaculation (EE) stimulation was pioneered in 1936 to collect semen in rams which were not able to naturally mate. The electro-ejaculation technique has since been used in other instances and is generally implicated for animals not being able to ejaculate (O'Kelly, 2011). Whitlock *et al.* (2012) reported electro-ejaculation to facilitate men with spinal cord injuries and reduced reproductive capabilities. It is generally accepted that the use of electro-ejaculation is an alternative when males are not trained to utilize the AV, or for game/wild species – electro-ejaculation may thus be a viable method of repeatedly collecting ejaculates, without being lethal (Jiménez-Rabadán *et al.*, 2012).

The major disadvantages when using electro-ejaculation, apart from injury, a lower sperm concentration, and possible urine contamination, are also the difficulty of further semen collection within a short interval, should one collection be

unsatisfactory. Electro-ejaculation has however attracted criticism as being inhumane and causing pain to the male (Palmer, 2005). Ortiz-de-Montellano *et al.* (2007) rejected EE on the basis of animal welfare. Changes in the behavioral patterns, vocalizing, struggling and the displaying of muscular contractions have also been reported. Matthews *et al.* (2003) was reported to prefer electro-ejaculation, because of it being more practical – as it does not require previous training of the ram. Salisbury *et al.* (1978) also found electro-ejaculation not to cause harmful side-effects, no loss in body condition, no change in temperament, and no special disinclination (unwillingness) to further applications of the electro-ejaculator.

According to Maule (1962) electro-ejaculation however should be used with care, as there exists a possible injury risk to the male, due to the physical reactions. Electro-ejaculation should only be used in extreme cases such as lameness or old males that have temporary lost their desire or ability to serve the AV. To take care of pain and stress, Jiménez-Rabadán *et al.* (2012) suggested males being sedated with xylacine (0.2 mg/kg Rompun®2% im). Palmer (2005) reported in the United Kingdom that electro-ejaculation without anesthesia is being discouraged and banned in many European countries.

Males raised under extensive conditions in most cases initially reject AV training for semen collection, due to lack of contact with humans – leaving the alternative of electro-ejaculation for semen collection in these animals (Ortiz-de-Montellano *et al.*, 2007).

2.5.2.1 The basic configuration of the electro-ejaculator

The electro-ejaculator should be of a solid state (cylindrical electrode having on its surface four longitudinal metal strips) and of low-amperage type, with complete grounding of the electronics (Salisbury *et al.*, 1978). The rectal probe is placed into the rectum and is used then to stimulate the sacral plexus, hypograstric nerve and parasympathetic outflow via the pundendal nerve (Noakes *et al.*, 2009). Electro-ejaculation is a two phase process. The first emission phase involves the stimulation of the lumbar sympathetic nerves, which form the hypogastric nerve and which subsequently supply the vas deferens and ampulla. The second ejaculatory phase then involves the contraction of the urethral muscles, which are supplied by the

sacral parasympathetic nerves (forming the pelvic and internal pudendal nerves). The clean lubricated rectal probe is inserted to a depth of 15 to 20 cm in the rectum of the ram, with care to avoid injury to the ram. By passing a few 5 to 10 seconds rhythmic electric stimuli through the electrodes, an ejaculation reaction is induced and the ram ejaculates in the semen collection tube (Bester, 2006). The electro-ejaculator stimulus must be maintained, with particular attention paid to possible short circuits and a current that should not exceed 1 ampere. The recommended voltage to be used should not exceed 15 volt. If the EE is used responsibly, it is generally humane and may cause the minimal discomfort to the ram (Mitchell and Doak, 2004; Noakes *et al.*, 2009).

2.6 Semen handling post collection

Senger (2003) and Noakes *et al.* (2009) stated that the collected semen should be handled at 30 to 37°C, following collection. The semen must not be brought into contact with metals and only glass or plastic collection vials should preferably be used. Equipment used for semen collection must then also be free of chemicals, bacteria, disinfectants, the presence of cells in the semen, excess air and the collecting equipment must be dry (Greyling, 2009). Collection equipment must be sterilized for approximately 15 minutes and then be stored in a dust free environment. Detergents are not usually necessary, but if used, then extra rinsing is important. Fresh undiluted semen should be processed within 10 to 15 minutes and exposure to direct sunlight should be avoided at all times (Zamiri *et al.*, 2010). For the handling and evaluation of semen a clean, dust free shed could easily be turned into a field laboratory (Evans and Maxwell, 1987).

2.7 Semen evaluation

After collection of the semen, the quality and quantity of each ejaculate should be carefully evaluated before use. The purpose of semen evaluation being to ascertain whether the number of functional normal sperm present in an ejaculate are sufficient to induce pregnancy, and whether the ram has the capacity to produce enough sperm to achieve the required pregnancies. The general macroscopic and microscopic parameters to be observed are the volume of semen, the colour of the ejaculate, sperm wave motion, odour of the semen, motility of the sperm, concentration of the sperm, and morphology of the sperm cells (Leboeuf *et al.*, 2000;

Purdy, 2006). According to Hafez and Hafez (2000) no single parameter can accurately predict the fertility of a semen sample.

2.7.1 Volume of the ejaculate

Ejaculation is the actual process of expulsion of semen through the urethra into the vagina of the female. Senger (2003) subsequently reported ejaculation to be a neural reflex that expels sperm from male reproductive tract. This neural reflex comes as a result of intromission, stimulation of the glans penis and forceful muscle contraction. During ejaculation, the sperm are transported from the tail of the epididymis via the vas deferens to mix with the seminal plasma in the pelvic urethra. Ejaculation in the ram generally occurs very fast (1-2 seconds) and factors affecting ejaculation, generally include breed, age of the male, nutritional status, reproductive management, method of semen collection, the frequency of semen collection, skill of semen collector, season of the year and the responsiveness of the ram (Theron, 2001: Greyling, 2009). A normal mature ram may mount and ejaculate 10 to 15 times per day, but the quality and quantity of the semen generally decreases as the frequency of semen collection increases. Gil *et al.* (2003) recorded a general decrease in semen volume per day if the ram was collected 3 times per day. However it has been recommended that 3 to 5 semen collections per day for a 4 to 5 day period, as being acceptable, if providing a 2 to 3 day rest period (Evans and Maxwell, 1987). The volume of a ram ejaculate generally ranges between 0.5 to 2 ml in mature rams, while younger rams produce 0.5 to 0.7 ml (Plate 2.4). A higher ejaculate has been recorded when electro-ejaculation was used, compared to the artificial vagina. This greater volume with electro-ejaculation could be ascribed to the greater contribution of the accessory sex glands, following the electrical stimuli (Jiménez-Rabadán *et al.*, 2012). Matthews *et al.* (2003) using 18 young post pubertal Dorper rams recorded a volume of 1.1±0.4 ml when using the artificial vagina and a volume of 1.3±0.4 ml when using the electro-ejaculator. Contradictory results have however also been reported by Marco-Jiménez *et al.* (2005), with the artificial vagina yielding more semen compared to electro-ejaculation (1.2±0.1 ml and 1.0±0.1 respectively) – however statistically no significant difference was recorded in semen volume between the artificial vagina and the electro-ejaculation technique of collection.

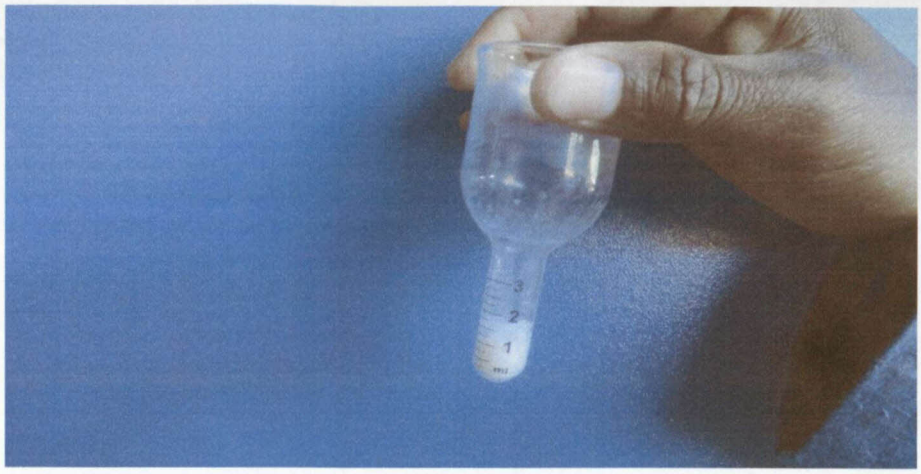

Plate 2.4: Semen collected in a calibrated collection tube

Semen volume as such is a very important parameter, as the semen volume can be used to calculate the number of ewes to be inseminated, or the number of straws/pellets to be cryopreserved. To determine the overall number of sperm per ejaculate, the formula of semen volume x density (concentration) is generally used (Talebi *et al.*, 2009). A lower semen volume has then also been recorded in the winter months (Talebi *et al.*, 2009; Zamiri *et al.*, 2010). According to Noakes *et al.* (2009), rams should not be classified as unsound on the basis of a low ejaculate volumes following electro-ejaculation, until it is certain that the sample is indeed representative of the ram's potential.

2.7.2 Colour and smell of semen

The colour of ram semen varies from milky-white to pale cream. The colour of the semen sample generally serves as an indicator of its density (concentration), as well as possible contamination. The general concentration of ram semen is set out in Table 2.3. Colours due to contamination which may be observed are e.g. a red colour, being indicative of the presence of blood, a urine smell and a yellow colour indicative of the presence of urine and a grey colour indicating pus (inflammation) being present (Greyling and Grobbelaar, 1988). The presence of blood or pus may be due to injury or disease of the penis or reproductive tract. The urine smell generally originates from the urine of the male, produced during the semen collection process. All these foreign substances may then be detrimental to the sperm and the contaminated semen sample should be discarded (Greyling, 2009). To evaluate the colour and smell, the senses of sight and smell are used respectively, by the person

evaluating the semen. In general a hygienic and clean ejaculate has a slightly sweet and aromatic smell (Penner, 1993)

Table 2.3: Concentration of ram semen as assessed by colour of the ejaculate (Evans and Maxwell, 1987).

Score	Consistency	Mean	Range
5	Thick creamy	5.0	4.5- 6.0
4	Creamy	4.0	3.5-4.5
3	Thin creamy	3.0	2.5-3.5
2	Milky	2.0	1.0-2.5
1	Cloudy	0.7	0.3-1.0
0	Clear (watery)		insignificant

Number of sperm ($x10^9$/ml)

2.7.3 Semen pH

The pH of semen is indicative of the acidity or alkalinity of the semen sample. Ram and buck semen pH vary between 6.4 and 6.9. Hafez and Hafez (2000) reported ram semen pH to range between 5.9 and 7.3. An alkaline semen pH may be assumed to be an indication of inflammation of the accessory glands (Greyling and Grobbelaar, 1988). The semen pH should generally be checked immediately after collection, as a delay may induce the semen pH to become acidic – a result of the sperm in the semen degrading the fructose under the anaerobic conditions that normally exists in the narrow collection tubes. In general, large changes in semen pH can result in sperm damage, infertility, or sperm mortalities (Hafez and Hafez, 2000).

2.7.4 Semen wave motion

The sperm cells are generally negatively charged and as such they repel each other to form circular motions, as if chasing one another. This wave-like motion can be observed with the aid of a microscope. The negatively charged characteristic is due to the carbohydrate moieties attached to the outer surface of the cells (Guyton and Hall, 2011).This wave motion is generally scored on a scale of 0 to 5 as follows: 0: being no movement and 5: being vibrant semen wave movement (Jiménez-Rabadán *et al.*, 2012). For wave assessment, a fresh, undiluted semen droplet is generally placed on a pre-warmed slide (32°C) and observed microscopically under low-power

(x10 magnification). O'Hara *et al.* (2010) reported that semen ejaculates scoring >3 regarding wave motion on a scale of 0 to 5 are generally acceptable. Hafez and Hafez (2000) set out the 0 to 5 scale for semen wave motion as follows:

- 0 Totally immotile
- 1 Individual sperm movement
- 2 Very slow wave movement
- 3 General wave movement, slow amplitude of the wave
- 4 Rapid wave motion, no eddies
- 5 Rapid wave motion, eddies present

2.7.5 Sperm motility

Sperm motility is generally defined as the ability of the sperm cell to swim progressively forward, with the rate of sperm motility being defined as the speed at which the sperm cell travels (Senger, 2003). Kozdrowski *et al.* (2007) regarded sperm motility as one of the most important parameters in the assessment of semen quality. Sperm motility as such, is also used when calculating the number of potential doses per ejaculate. Thus sperm motility is either made by evaluation of the wave motion characteristics of the semen sample, or the proportion of progressively motile sperm in the sample. Evans and Maxwell (1987) reported sperm motility to be markedly influenced by temperature – thus temperature control during semen handling is of the utmost importance (semen should be handled at a temperature of between 30 and 37°C).

Progressive sperm motility may then be assessed by using an arbitrary scale of 0 to 5 or be expressed as a percentage e.g. – 1 (25%), 2 (25-50%), 3 (50-70%), 4 (70-90%) or 5 (90-100%) motile sperm (Zamiri *et al.*, 2010). According to Talebi *et al.* (2009) a score of 0 to 5 in gross sperm motility may be described as follows:

- 0: no discernable motion.
- 1: weak undulated or oscillatory motion.
- 2: slow progression, including a stop and start of sperm motion.
- 3: steady progressive sperm motion.
- 4: rapid progressive sperm motion.
- 5: very rapid and vigorous sperm motion.

To assess the individual progressive motility of sperm, the semen sample generally needs to be extended. Many extenders – e.g. skim milk-based extenders, AndroMed, TRIS-based extenders have subsequently been reported (Paulenz *et al.*, 2003; O'Hara *et al.*, 2010; Jiménez-Rabadán *et al.*, 2012). In most reports skimmed milk-based extenders have been used. The most important characteristics of semen extenders being that they must serve as a buffer, serve as source of nutrients to the sperm, serve as a protectant for the sperm, must have the right pH, must be isotonic to seminal plasma, serve as a cryoprotectant during the freezing of semen and contain antibiotics to stop bacterial growth. When assessing semen quality a progressive sperm motility of ≥ 75% is acceptable for a semen sample to be processed further (Paulenz *et a*l., 2003; 2005).

2.7.6 Sperm cell concentration (semen density)

The sperm cell concentration is indicative of the number of sperm cells per unit volume (ml) of semen. Knowing the total number of sperm in the ejaculate will then also be indicative of how many inseminations may be performed with one ejaculate. Evans and Maxwell (1987) reported different methods of assessing the sperm cell concentration. These methods were based on the consistency or appearance of the semen sample, using a haemocytometer, the colorimeter or an electronic particle counter. The concentration of sperm may then also vary significantly from species to species, and from male to male. Researchers generally use the haemocytometer for accurate semen density determinations (Salsbury *et al.*, 1978; Evans and Maxwell 1987; Mitchell and Doak, 2004). The haemocytometer has been reported to be accurate and easy to implement and is then also very useful in the training technicians to make visual estimates. The haemocytometer has however also been reported to be slow (time consuming), although more accurate and useful under field conditions, to estimate the sperm concentration per ejaculate (Noakes *et al.*, 2009). Matthews *et al.* (2003) found a significant difference in sperm cell concentration using the artificial vagina, compared to electro-ejaculation for semen collection. Most studies have recorded a sperm cell concentration of 2000 to 3000 x 10^6/ml. Good quality ram semen has been reported to contain 3000 to 6000 x 10^6 sperm/ml (Evans and Maxwell, 1987). Paulenz *et al.* (2005) and O'Hara *et al.* (2010) stated ≥2.5 x 10^9

sperm/ml to be considered as the normal range, and semen with this sperm concentration could be processed and used for AI.

2.7.7 Sperm viability and morphology

For the evaluation of the morphology of the sperm cell, an eosin/nigrosin stain is generally used immediately after semen collection. Morphological abnormal sperm may be defined as any sperm shape deviating from the norm. Under the microscope (x1000 magnification; oil-immersion) a person may be able to identify dead sperm and also the abnormal sperm (Kafi *et al.*, 2004). With this stain the live sperm will colour white, while dead sperm will colour red. Sperm cell abnormalities like loose heads, double heads, broken or bent necks and mid-pieces, double tails, protoplasmic droplets can also be identified. The morphology of the sperm cell is very important to evaluate, as generally abnormal sperm will not be able to reach the ovum and hence no fertilization will result (Noakes *et al.*, 2009).

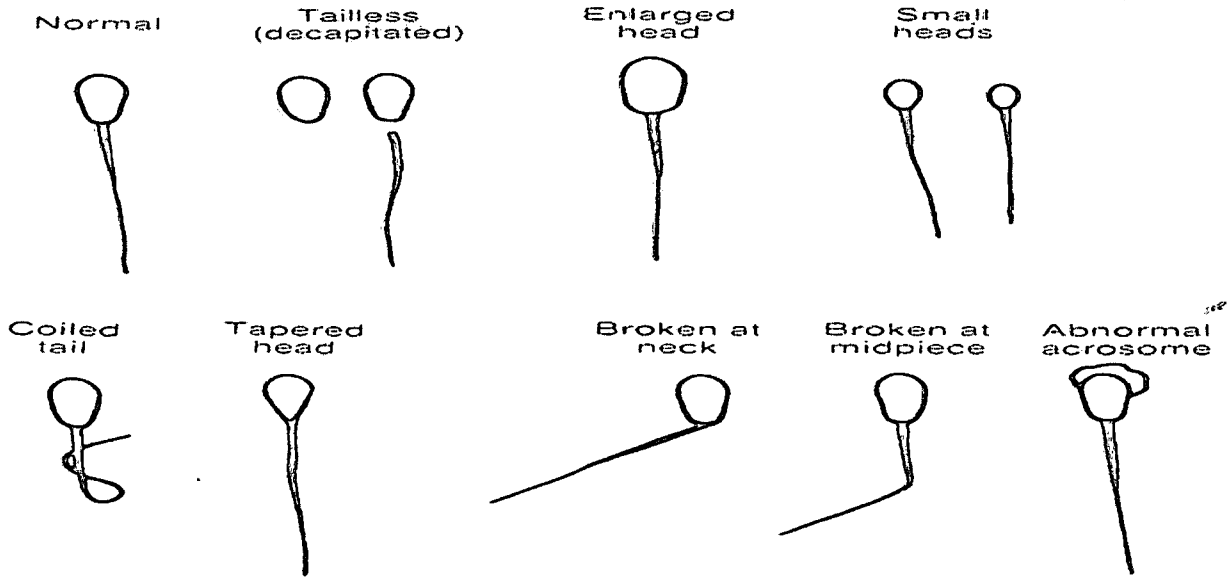

Figure 2.9: Diagrammatic presentation of a normal sperm and certain morphological defects in sperm (Evans and Maxwell, 1987)

It is important to note that every semen ejaculate generally contains a small portion of abnormal sperm (Hafez and Hafez, 2000). The sperm morphology (Figure 2.9) thus serves as an indicator of the health status of the seminiferous tubules and to a certain extent of the epididymis. Handling of rams may influence the morphology of the sperm cells. So for example rams exposed to heat stress, high relative humidity

and transportation may exhibit a higher occurrence of sperm abnormalities. A normal ejaculate should record >85% normal, with <20% abnormalities generally being seen as acceptable for a ram ejaculate (Arthur *et al.*, 1996). Matthews *et al.* (2003) recorded 94.3±3.4% and 95.6±2.3% normal sperm in semen collected with the aid of the AV and EE, respectively (no significant difference). According to Greyling and Grobbelaar (1983) no significant difference was also recorded in the sperm morphology between artificial vagina and electro-ejaculation collected ram semen samples.

2.8 Libido

Libido or sex drive has been defined as the behavioral drive associated with the desire to copulate in the male, and can be seen as a process under the control of the hormone, testosterone. Libido is generally not dependant and related to the scrotal circumference and should be evaluated separately (Scholtz, 2010). To detect estrous ewes, rams use olfactory cues (pheromones) and according to Perkins and Roselli (2007) rams exhibit several courtship behaviors e.g. by sniffing the genital area of the ewe, pawing, nuzzling, licking and nibbling the the ewe's flank and the ano-genital region. These actions are usually followed by the Flehmen stance (after sniffing, ram arches its head up and retracts the upper lip until it curls completely away). Regarding the above courtship behaviors, Simitzis *et al.* (2006) reported the ram to follow the ewe at a distance of less than 0.5 meter. The male is then progressively aroused and then frequent erections of the penis occur, followed by an unsuccessful attempt to mount the female (Arthur *et al.*, 1996). As a response, the estrous female displays a characteristic mating position, known as lordosis. The sexual behavior of the female include increased walking activity, phonation, nervousness and attempts to mount other animals (Senger, 2003). A good libido should terminate in copulation, and it is important to note that ram's libido is highly related to the overall flock fertility. Greyling (2009) reported libido to practically be measured using the following parameters:

- Measuring the reaction time (sexual response recorded in seconds)
- Time interval between consecutive matings – in other words, the duration of the recovery phase between matings.
- Number of matings per unit time– i.e. the mating frequency of a ram.

- In practice libido can also be measured by recording the number of ewes mated by a specific ram, in a given period of time.

In the current research trial, libido was evaluated based on the reaction time of the ram. The scoring system of libido was, as adapted from Theron (2001), being as follows:

0-Mounting and ejaculating in more than 150 seconds
1-Mounting and ejaculating in more than 120 seconds, but less than 150 seconds
2-Mounting and ejaculating in more than 90 seconds, but less than 120 seconds
3-Mounting and ejaculating in more than 60 seconds, but less than 90 seconds
4-Mounting and ejaculating in more than 30 seconds, but less than 60 seconds
5-Mounting and ejaculating in less than 30 seconds.

Ungerfeld and Lacuesta (2010) stated reproductive male behavior to be generally influenced by the social environment in which the ram lambs are reared. Early exposure to females, the presence of ewes in estrus, age, social hierarchies, breeding season, semen collection technique are some of the factors known that can affect the libido of the ram. Other factors which may affect libido include e.g. previous breeding experience of the ram, method of restraint of the ewes in estrus (semen collection), shyness of the rams to the test procedures and sexual attractiveness of the ewe (Gouletsou and Fthenakis, 2010). Young rams that shows sexual inactivity should be exposed to ewes for up to three evaluations within a 10-day period. The testing pen should also contain no trough with feed or water. Each ram may be exposed to two or three ovariectomized ewes for a 20 minute period, and then the number of mounts and successful matings recorded (Kafi *et al.*, 2004). Hafez and Hafez (2000) reported no relationship between libido and semen quality.

2.9 Factors affecting semen quality

2.9.1 Puberty

Puberty is the first phase in the sexual activity of the male and entails the acquisition of gonadotrophin secretion, spermatogenesis, gonadal steroid secretion, reproductive behavior and the development of the secondary sex characteristics (Senger, 2003). In small stock, puberty is generally related to age and body mass. Most rams attain puberty at an age of 6-7 months and 40-60% of mature body

weight. At puberty the ram lambs become sexual active for the first time, but there are however many factors which may affect the onset of puberty (Ridler *et al.*, 2012). These include genetic factors, nutrition, disease, seasonality, wool production, imbalances in hormone secretion and variations in ambient temperature (Hafez and Hafez, 2000). The onset of puberty then depends on the ability of specific hypothalamic neurons to produce GnRH in sufficient quantities to promote and support spermatogenesis. Ram lambs that are born early in the breeding season and exhibit a satisfactory growth rate may reach puberty in early autumn, while those born later and do not grow so fast and will not reach puberty until the following year. Even although puberty is reached in ram lambs at the age of 6-7 months, semen collection should also not commence before 12 months of age (Noakes *et al.*, 2009).

2.9.2 Seasonality of semen production

Gordon (1997) reported the reproduction activity in sheep to be seasonal. Sheep and goats are short daylight breeders, with the change in photoperiod operating via the retina of the eye, through the optic nerve, to the pineal gland and then the secretion of melatonin. Apart from the pineal gland, melatonin is also produced in the retina, intestines and salivary glands (Rosa and Bryant, 2003). Melatonin stimulates the hypothalamus and pituitary to secrete higher levels of the gonadotropic hormones (FSH and LH), and hence induce a higher sexual activity (Senger, 2003). Melatonin is generally synthesized and released at night. Therefore the inhibition on the pinelocytes is decreased due to the longer dark period. Melatonin ultimately stimulates the release of GnRH and thus initiates cyclicity (Greyling, 2009). It is important to stress that under certain rearing conditions (e.g. extensive management), nutritional stress may have a greater effect than photoperiod on semen production (Kafi *et al.*, 2004).

According to Rosa and Bryant (2003), sheep become sexually active in response to the decreasing day light length in late summer to early autumn. On the other hand Rosa *et al.* (2012) found no increase in semen volume or sperm concentration after treating rams with melatonin. The study suggested that rams implanted with melatonin during late spring, without a priming period of long days preceding the treatment, was not sufficient to increase testes growth and subsequent sperm production.

Greyling and Grobbelaar (1983) divided the South African year into four seasons, namely:

- Summer (December-February)
- Autumn (March-May)
- Winter (June-August)
- Spring (September-November)

According to Hafez and Hafez (2000) rams generally produce sperm throughout the year and this enables them to mate all year round. Dorpers ewes demonstrate a long or extended breeding season, with only 1 month of real anestrus (October) (Cloete *et al.*, 2000). Generally sheep breeds coming from countries between latitude the 35° N and 35° S are likely to breed all year round (Ridler *et al.*, 2012). The same sentiments have been shared by Rosa and Bryant (2003) who further explained that at latitude of greater than 35°, ewes are shown to be seasonally polyoestrus. Sheep breeds from the temperate climates in the mid or high latitudes are reported to be seasonal breeders, although a clear seasonal trend was not recorded when monitoring e.g. Boer goat bucks (Theron, 2001). It is generally accepted that rams show seasonal fluctuations in semen quality.

A decrease in day length is generally accompanied by an increase in the gonadotrophic hormone production and testosterone levels. It has been established that long days inhibit and short days stimulate the reproductive activity in sheep and goats. Sperm quality (sperm wave motion, progressive sperm motility, percentage live sperm) has been reported to be high in autumn, while dead sperm values are high in winter (Talebi *et al.*, 2009; Arrebola *et al.*, 2010). The same sentiments have been shared by Zamiri *et al.* (2010) who stated ejaculate volume, sperm density, progressive motility and the percentage normal sperm to be high in autumn and low during winter – however, the decline in semen quality in winter could be a limiting breeding factor. The magnitude of these seasonal effects is generally not so marked, as to prevent rams from being used for breeding purposes throughout the year (Bester, 2006).

2.9.3 Ambient temperature

It has been suggested that the negative effect of ambient temperature on scrotal circumference is a direct result of reduced spermatogenesis. So for example an elevated temperature (above 45°C) recorded a negative effect on spermatogenesis, while a sudden drop in temperature (below 10°C), also causing irreversible loss in sperm viability. When the ambient temperature is so high that the gradient required for normal spermatogenesis cannot be maintained, degeneration of the spermatogenic tissue subsequently results (Evans and Maxwell, 1987). A high ambient temperature (averaging 25°C), particularly in combination with increasing daylight length during the summer months have been demonstrated to result in a reduction of the semen quality – thus temperature is a seasonal component affecting the fertility in rams (Talebi *et al.*, 2009)

It is important to stress that seasonal reproduction occurs mainly in wild species, as a result of natural selection. Animal domestication and artificial selection however has contributed to minimizing the effects of season on reproductive activity. Similarly it has been found that the ram's sensitivity to photo period to be different from that of the ewe. It has been shown that reproduction in sheep may be influenced by ambient temperature, level of nutrition and social relationships (Rosa and Bryant, 2003). According to Karagiannidis *et al.* (2000) in Greece, late summer and autumn are considered to be the natural breeding season in sheep – winter and spring again as the non-breeding season for sheep. Hafez and Hafez (2000) reported that ewes which were allowed to run with rams throughout the year produced lambs every 6 ½ months.

2.9.4 Nutritional status

For the animal to perform its physiological functions, nutrients are essential. According to Aganga and Nsinamwa (1997) feed is any material which is ingested by animals, capable of being digested, absorbed and utilized. These components capable of being utilized by the animals are thus described as nutrients. Animals then utilize the nutrients for maintenance, growth, production, reproduction and to carry out the many metabolic reactions involved in performing body functions. The 6 basic essential nutrients required include water, proteins, carbohydrates, lipids,

minerals and vitamins. The rams used in the current trial were fed a maintenance diet, so as to avoid the animals becoming too obese or on the other hand losing body weight during the trial. The scrotal circumference, scrotal fat, testes weight and volume may thus be influenced by the nutritional status of the ram (Fourie *et al.*, 2004).

According to Senger (2003), nutrition plays a very important role in reproduction. Animals which are properly fed tend to produce adipocytes, which stimulate the production of a hormone called leptin that enters the blood. Leptin may then stimulate the neuropeptide Y neurons or directly stimulate the gonadotrophin releasing hormone (GnRH) neuron. Blood leptin can thus reflect the nutritional status of the animal. Nutrition generally affects the endocrine, rather than the spermatogenic function of the testis (Hafez and Hafez, 2000). The greater the amount of adipose tissue, the greater the amount of leptin.

Overfeeding, under-feeding or malnutrition also plays a role in reproduction. Overfeeding may lead to the accumulation of fat in the reproductive tract, which then interferes with its development and functionality. So for example too much fat in the scrotum could lead to the under development of the testes. Fat accumulation in the scrotum may also lead to an increased testicular temperature, which again leads to lower sperm production (Greyling, 2009) Under-nutrition generally leads to a reduction in the secretion of GnRH, which in turn will affect the production of follicular-stimulating hormone (FSH) and luteinising hormone (LH) (Guyton and Hall, 2011). The function of FSH in the male is spermatogenesis, while LH stimulates in the release of testosterone. Thus testosterone is essential for the maintenance of the male genital duct, the male sexual characteristics and libido, as well as spermatogenesis. Under-nutrition may thus ultimately affect libido. Under-conditioned rams will generally exhibit a low fertility, while over-fat rams will normally be lazy and unable to mount the females.

2.9.5 Body temperature

The body temperature of all homeotherms is controlled by homeostasis. The term homeostasis is then generally used by physiologists to define the maintenance of a constant internal environment in the body (Guyton and Hall, 2011). According to

Radostits *et al.* (1994) the normal body temperature of sheep is 39 °C. Lowe *et al.* (2001) reported that this temperature could vary by 0.5 to 1.2°C on either side of 39 °C. An increase or decrease in body temperature then generally indicates health problems. Diseases which may cause fever in rams include amongst others, blue tongue and heartwater – which could then induce temporary sterility in the male. It is very important to monitor the body temperature, as the testes must be maintained at 4 to 7°C lower than the body temperature

2.9.6 Body weight and body condition score (BCS)

Body weight needs to be monitored because, apart from acting as an indicator of the nutritional status and maturity of the animal, it can also serve as an indicator of the health status. Post natal penis development, development of the genital glands, descent and development of the testes, the onset of semen production and spermatogenesis are all influenced by age, but are closely linked to body weight (Van der Merwe, 2010).

The body weight of animals may be measured using both objective and subjective methods. An objective method can be defined where a recognized measurement is used, while a subjective method is where e.g. the body condition of the animal is scored (i.e. too thin or too obese) on a scale of 0 to 5 (half point increments may also be used). Using a scale to record the actual body weight is a more reliable indicator of body condition. However due to the wide variation in mature body size between individuals, it becomes difficult to use weight to determine a reliable body condition (Vatankhah *et al.*, 2012). Similarly, compared to eye appraisal, body condition score (BCS) is said to be a more accurate indication of the body condition of the sheep. By knowing the BCS, managers can make adjustments to the nutritional programme – not only to monitor body weight, but also to feed the animals more efficiently. Briefly Upton and Soden (1991) and Lategan (2012) have summarized aspects to be considered and facilitate body condition scoring sheep on a scale 0 to 5, according to their body condition:

- Score 0 – Sheep are extremely emaciated. No fat or muscle detectable and the sheep are at the point of dying.

- Score 1 – The backbone feels very sharp in both the vertical and transverse processes. Eye muscle is shallow, with no fat cover. The spine is sharp and prominent.
- Score 2 – The muscle tissue is of sufficient depth to give the spinous processes a smooth and rounded feeling, but there is little fat cover. It is possible to pass the fingers under the ends of the transverse processes with a little pressure. Eye muscle have a little fat cover, but is full.
- Score 3 – Pressure is needed to feel the individual spinous processes. The spine can be felt as smooth and rounded. The loin muscle is full, with a moderate fat cover. Eye muscle is full, with some fat cover.
- Score 4 – The ends of the transverse processes cannot be felt. Spine can be detected as a line. The eye muscle is full with a thick fat cover.
- Score 5 – The spine and transverse processes cannot be detected, there is a fat dimple over the spine. The sheep are very fat. The eye muscle is very full, with a very thick fat cover.

Body condition scoring as such is thus based on the feeling of the level of muscling and fat deposition over and around the vertebrae in the loin region, behind the last rib and in front of the hip bone. Regarding BCS, a score in rams of 3.5 to 4 is ideal. It has to be borne in mind that during the breeding season or semen collection, rams are likely to lose at least 0.5 units (Gouletsou and Fthenakis, 2010). Vatankhah *et al.* (2012) reported that the BCS at mating should range between 3 to 3.5, for optimal, sustainable profitability. The sperm production and libido of the rams generally decreased with a BCS of more than 4 (on the scale of 0-5). BCS being based on feeling the level of muscling and fat deposition over and around the vertebrae, as described earlier. Figure 2.10 demonstrates the most important body areas to be identified when evaluating the BCS of an animal.

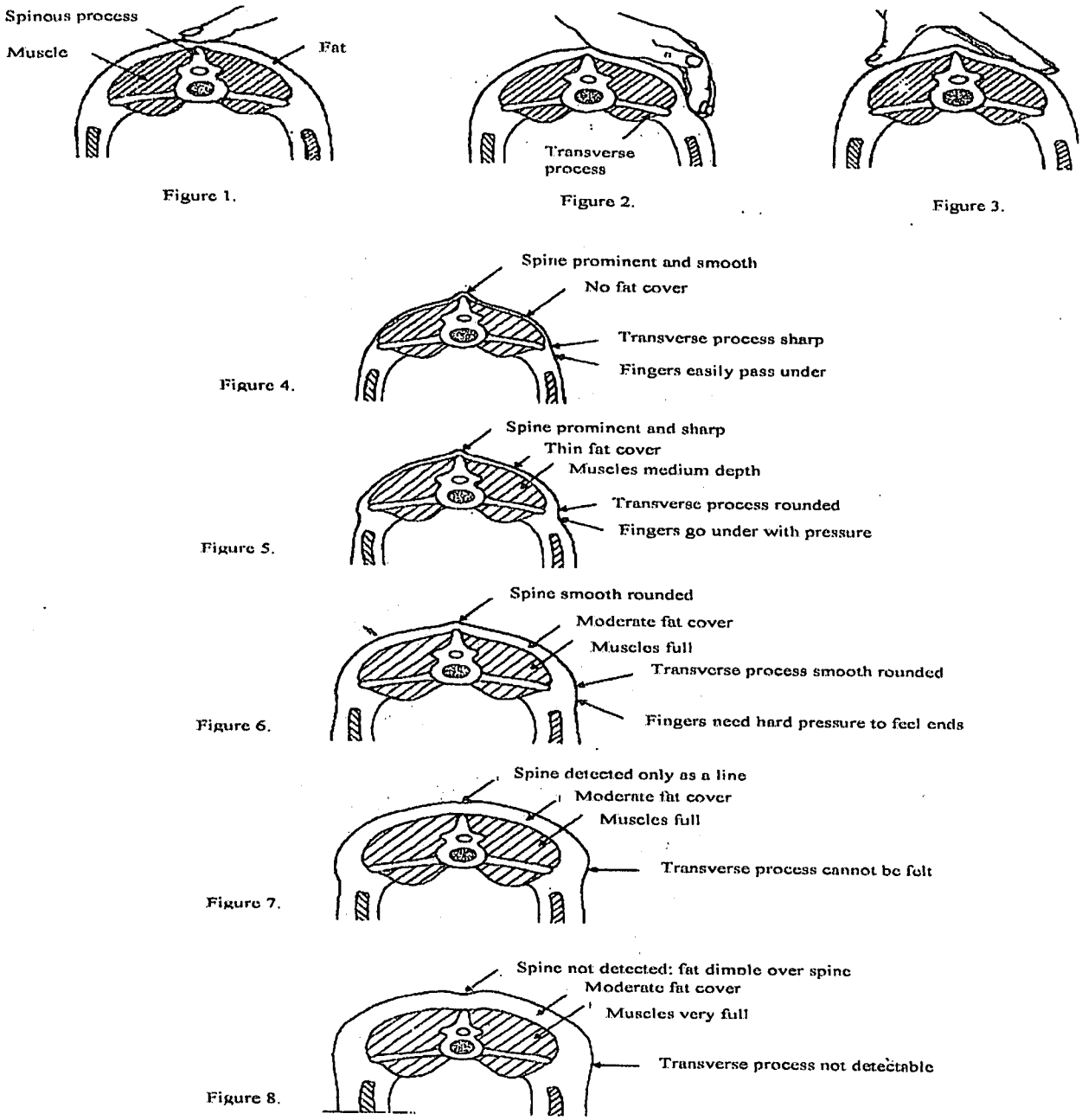

Figure 2.10: Body areas to evaluate for body condition scoring (ARC, 2009)

2.9.7 Stress

According to Ortiz-de-Montellano *et al.* (2007), electro-ejaculation without anesthesia in humans was found to be painful. Animals unlike humans, cannot convey how painful electro-ejaculation is. However it is based on the assumption that, what is painful in humans may also cause pain in animals. It is thus assumed that electro-ejaculation is painful to animals. This pain subsequently causes stress to the animal.

It is not the purpose of this research to evaluate the pain and stress level as such, however to be in pain is defined as suffering from a destructive body state, and it may therefore be necessary to link the pain and stress caused by electro-ejaculation to the reproductive physiology. Radostits *et al.* (1994) defined stress as any stimulus, internal or external, chemical or physical or emotional, that excites the neurons of the hypothalamus to release corticotrophin-releasing hormones at a rate greater than it would naturally occur.

There are other conditions which may also put the ram under stress. These include for example high ambient temperature or humidity, changes in the environment or diet, parasites or disease. Even routine management activities may cause stress to the ram (to a greater or lesser degree) – these may include hoof trimming, deworming (drenching), shearing, crutching, dipping and transportation. Rough handling of rams e.g. using a prodder, beating, chasing, shouting are some of stressful factors which may affect the behavior of the ram (Evans and Maxwell, 1987)

Reproductive performance is often affected by stress. The energy that is normally available for processes like growth, the immune response or reproduction may be channeled into reducing stress. Stress has been reported to adversely affect fertility. Examples have been quoted of serious consequences on the reproduction performance of women who are e.g. involved in long distance running (Goos and Consten, 2002). Stress may also decrease the plasma LH and hypothalamic GnRH in the male. As mentioned previously LH stimulates the cells of Leydig in the testes to secrete testosterone – which has many reproductive implications (Guyton and Hall, 2011).

2.9.8 Age

The Dorper (or any breed for that matter) ram lambs' testes weight and the diameter of the seminiferous tubules increase with age. Semen quality (dead or abnormal sperm) of ram lambs have also demonstrated an increasing tendency with age. The sperm concentration was also recorded to increase markedly after the age of 140 days (most growth in the testicular artery occurs between 6 and 12 months of age) (Bester, 2006). The mean sperm concentration of ram lambs at 6 to 8 months of age

was reported between 1.2 to 2.0 x 10^9 per ml, with the semen volume ejaculate varying between 0.3 to 1.0 ml. The above figures illustrate that ram lambs attain fertility at a relatively early age. It is thus recommended that ram lambs be weaned at 100 days of age to prevent them from mating the dams (Hafez and Hafez, 2000).

The length of the productive life of sheep is generally reported to be 8 to 10 years of age and this is essentially determined by the condition of their permanent incisors. Post pubertal rams should be used for breeding or semen collection. Young immature rams because of their inexperience and a limitation in sexual behavior, may generally sire a lower number of offspring. However in older rams, semen quality, libido and the ability to serve are likely to decrease with age – from the age of 7-8 years (Noakes *et al.*, 2009).

2.9.9 Hormonal abnormalities

Fetal and postnatal hormone deficiencies can cause serious clinical abnormalities e.g. cretinism and dwarfism. Cretinism results from the congenital lack of the thyroid gland to produce thyroid hormone, and is characterized by the failure in body growth (dwarfism) (Gerneke, 1985). Animals with pan-hypopituitary dwarfism do not reach puberty and hence never produce enough quantities of gonadotrophic hormones to develop sexual activity. If the fetus does not secrete sufficient quantities of the thyroid hormone, growth and maturation of the brain both before birth and afterward are greatly retarded, and the brain remains smaller than normal. The importance of the brain in relation to reproductive physiology has already been explained in this review (Figure 2.9), illustrating the feedback regulation of the hypothalamic-pituitary-testis axis in males. A deficiency of hypothalamic-pituitary hormones in males then generally leads to a failure or a low reproduction rate (Guyton and Hall, 2011).

2.9.10 Breed

As mentioned earlier the Dorper breed exhibits an extended breeding season (longer than most British sheep breeds). The superior characteristics of the Dorper breed has already been emphasized regarding mutton production, adaptability and hardiness, veld utilization and good mothering quality (2.1.1.4). In general each sheep breed is said to have its own unique qualities.

2.9.11 Disease

Disease can be defined as the inability of the animal to perform certain physiological functions at normal levels, even though the nutritional and other environmental factors are at adequate levels. Thus disease implies changes in the normal physiological functions or a deviation from the norm. When many cells in an organ are affected, organ failure generally occurs and the subsequent involvement of other organs may affect the entire body system – resulting in the disruption of the body functions, manifested as a subclinical and later clinical status (Radostits *et al.*, 1994).

Rams with defects such as cryptorchidism, asymmetrical testes size, testes hypoplasia, spermiostasis, or varicoele should not be used for semen collection. Other defects which should be discriminated against may include, amongst others, umbilical, inguinal or scrotal hernia and brachynathismor prognathism (Gouletsou and Fthenakis, 2010). Many diseases and/or defects may be spread via the semen. The World Organisation for Animal Health requirements for disease testing in semen of sires used in AI programmes have provided a list of ram diseases to be evaluated/monitored by a veterinarian, before semen of a particular ram can be used. These diseases in sheep and goats include amongst others caprine and ovine brucellosis, ovine epididymitis, contagious agalactia, peste des petits in ruminants, leptospirosis, paratuberculosis, scrapie, maedi-visna, caprine arthritis/encephalitis, blue tongue, tuberculosis, border disease and contagious caprine pleuropneumonia (Noakes *et al.*, 2009). To ensure acceptable semen quality, it is desirable that each country establishes a mechanism to ensure that institutions involved in the international movement of genetic material be reliable. In South Africa quality control is performed by the South Africa Stud Book and Livestock Improvement Association, and if there is any doubt pertaining to a particular animal or persons, the South Africa Veterinary Semen and Embryo Group (SAVSEG) could be consulted – everyone has to conform to a certain code of ethics (Ramsay *et al.*, 2001).

Chapter 3

Material and methods

3.1 Study area and period

The trial was conducted in the metabolic building at the University of the Free State (UFS) campus in Bloemfontein, South Africa. The study site is located 28.57° south longitude, 25.89° east latitude and at an altitude of 1304 m above sea level. The trial was carried out from January 2012 to January 2013 (summer to summer)

The Bloemfontein area falls within the Grassland Biome, with a rainfall gradient that generally corresponds to the relative environmental conditions made up by a sweet and sour grass cover. The area is dominated by *Themeda triandra, Eragrostis* species, *Aristida* species, *Digetaria* species, *Chloromelas* species and *Enneapogon* species. Among the most common trees and shrubs are the *Acacia* species (*A. karroo*, *A. erioloba*, *A. nilotica)*, *Boscia foetida*, *Cussonia paniculata*, *Buddleja saligna*, *Olea europaea*, *Searsia lancea*, *Ziziphus mucronata* and *Diospyros lyciodes*, to name but a few (Van Wyk *et al.*, 2008). The mean annual rainfall ranges between 500 and 550 mm, with precipitation occurring predominantly during the summer months (December to April). The mean daylight length varies from 13.2 hours in mid-summer, to 9.8 hours in mid-winter (Smit, 2009). Climatological information for this location during the experimental year is summarized in Table 4.1.

3.2 Study animals and management

This trial had been previously approved by the Animal Ethical Committee of the University of the Free State (project no: 02/12). The five rights of animals in a feedlot, as coded by South African Feedlot Association (SAFA), were adhered to. The basic and fundamental requirements being that animals be treated humanely during the intensive housing. The five animal rights are then set out as: the right to freedom of movement; the right to free access to fresh feed and water at all times; the right to appropriate health care; the right to freedom from injury and suffering; and the right to freedom from harassment (www.safeed.co.za., 2010).

Eleven mature Dorper rams, recording a mean body weight of 69.6 ± 9.2 kg (range between 57.5 kg and 83 kg) and mean age of 18 ± 4.7 months (range between 14 and 24 months) were sourced locally, where they had been maintained extensively on veld. Determining the age in the rams was performed by evaluation of their teething status (Oberholster, 2010).

Upon arrival, the rams were allowed 5 days to recover and adapt before any activity was performed. Fresh water and feed were provided in separate pens to help relief the rams from transportation stress. The rams were then vaccinated for pulpy kidney and this was followed by a pasteurella vaccine, weighing of the animals and treatment for internal and external parasites. Thereafter a general management schedule for disease prevention and hoof trimming was followed.

The rams were clinically examined for soundness by a veterinarian (Gouletsou and Fthenakis, 2010; Scholtz, 2010). After passing the ram soundness test, the rams were ear-tagged, divided into 2 groups and randomly placed in separate pens. The rams were maintained in individual mobile pens (measuring 3 m x 1.4 m x 1.3 m) and allowed to exercise twice weekly. A group of 6 rams were then trained (during the adaptation period) for semen collection with the aid of the AV, while the remaining 5 ram's semen was collected with the aid of the EE. The pens were also provided with rubber mats to provide the necessary bedding and insulation. 2 Dorper ewes were then sourced from the University of the Free State west campus (Bloemfontein) to be used as teaser ewes for the training in the AV semen collection and the libido testing of the rams. These ewes were also kept in a pen (3 m x 3 m x 1.3 m), in close proximity of the rams. The rams were managed uniformly, maintained at the natural ambient temperature and under natural photoperiod, for the entire trial period.

The rams used in this trial were fed a maintenance diet (8.5 MJ ME/kg) to avoid the animals becoming too obese or losing body weight during the observation period. Water was provided *ad libitum* throughout the trial. The composition of the diet used in the research trial is set out in Table 3.1.

Table 3.1 Feed composition of the maintenance diet fed during the trial period (NUTRI feeds, 2012)

Ingredient	**g/kg**
Protein (min)	120
Ammonium chloride (max)	10
Total protein derived from Ammonium chloride (max)	13.67%
Moisture (max)	120
Fibre (max)	250
Fibre (min)	120
Calcium (max)	15
Fat (min)	15
Fat (max)	70
Phosphorus (min)	3

During the first 2 weeks of the adaptation period, the rams were fed the maintenance pellet diet, mixed with ground lucerne. This inclusion of lucerne was performed to reduce the stress and facilitate the adaptation process, as well as keeping the rumen functional (Van der Merwe, 2010). Thereafter the rams were offered a restricted amount of 2.5 kg feed (half given at 7:00 to 8:00 and the other half at17:00 to 18:00) pellets per day, throughout the trial – the nutrition thus remained uniform and constant throughout.

All rams were checked regarding their welfare twice daily (minimum), between 7:00 to 8:00 and then again 17:00 to 18:00. Apart from feeding, observations were done to check for clinical signs of discomfort and also to accustomise the rams to the researcher.

The water and feed troughs were placed 3 m apart and 30 cm from the ground to allow the rams to drink and eat freely – also to prevent contamination of the feed with manure. No stale or mouldy pellets were allowed throughout the trial – good feed bunk management being adhered to (Van der Merwe, 2010). The cleaning of the pens was performed twice weekly to prevent the occurrence of flies, coccidiosis and also to prevent any unpleasant odours. The rams had never before been used in

any breeding programme, and during the course of the study as such were considered to be inexperienced.

3.2.1 Brief description of the facilities

The metabolic building where the animals were housed, are under the management and supervision of the Department of Animal, Wildlife and Grassland Sciences. This building as such had been designed for the handling of research animals (small stock, pigs, poultry). Generally the roofed structure was specious, well ventilated, dust free, with a concrete floor that facilitated cleaning. This structure also generally protected the research animals from inclement weather and any uninvited visitors (providing safety and eliminating stress) and was provided with a water and electricity. The facility included mobile pens, weighing scales (for animals and animal feeds), tables, and many other facilities for different types of experiments. The area had previously been approved by the Animal Ethical Committee of the University of the Free State, as animal housing.

3.3 Different techniques of semen collection

3.3.1 Artificial vagina (AV)

Most materials used for semen collection and evaluation were purchased from Ramsem. Ramsem (a small stock AI and Embryo Transfer Station) is located 10 km from Bloemfontein and renders a full-time artificial insemination and embryo-transfer service to sheep and goat breeders – under supervision of a team of veterinarians (Ramsay *et al.*, 2001).

From the first day of arrival the researcher started to interact with the rams, by feeding, cleaning the pens and touching the animals. For the first few days the rams were wary, but by day 10 after arrival all the rams (including the EE group) responded well to touch – more specifically touching the scrotum. Prior to training and semen collection, the long wool or hair was removed from around the sheath and prepuce to avoid semen contamination. Training of the rams for semen collection using the AV started on day 10 after arrival. Two days before training was started, the teaser ewes were injected with 1ml Estrumate (prostaglandin) i.m. to induce estrus. The training procedure used was as described in detail in 2.5.1.1

(Evans and Maxwell, 1987) and Plate 3.1 illustrates ram training for semen collection, using the AV.

The AV was prepared as described in 2.5.1.1 (the basic configuration of the artificial vagina) for semen collection. The procedure entailed the water in the AV being heated to a temperature of between 50°C and 60°C. The pressure inside the AV being adjusted and the AV then dried of any excess water spilled during filling. The one end of the AV was lubricated with a thin coat of a non-toxic sterile K.Y. jelly. Excessive lubrication of the AV was avoided as this could cause contamination of the semen (Salisbury *et al.*, 1978).The temperature inside the AV was checked using a thermometer and this temperature generally ranged between 42°C and 45°C. Thereafter a pre-warmed (32°C) semen collection tube was placed at the other end of the AV. When the ram mounted, the penis was directed into the AV – care being taken not to touch the penis itself. When the ram's penis contacted the warm, lubricated surface of the AV, the ram generally gave a forward thrust. A vigorous upward and forward thrust signified that ejaculation had occurred. Only semen collected in the collection tube was accessed, as semen in the surface of AV lining (water jacket portion) generally resulted in thermal damage to the sperm cells (Mitchell and Doak, 2004). This ejaculation generally occurred within 1 to 2 seconds. Semen was deposited into the collection tube and the tube then removed from the AV, placed in a water bath (32°C), for semen evaluation to be performed within 10 to 15 minutes (Zamiri *et al.*, 2010).

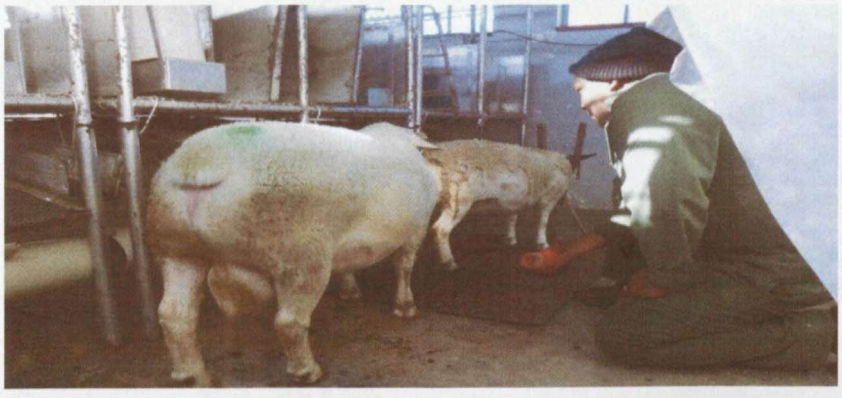

Plate 3.1: Ram training for semen collection using the AV

3.3.2 Electro-ejaculation (EE)

The basic principles of EE are set out in 2.5.2.1. (Mitchell and Doak, 2004; Noakes *et al.*, 2009). In brief, the ram was placed on its haunches (buttocks) and the penis prolapsed through the prepuce and held, using a clean white gauze behind the glans penis. The ram was then restrained in a lateral position on the floor and glans penis and urethral process inserted into a collection tube – Plate 3.2. The K.Y. jelly was used to lubricate the clean rectal probe which was then inserted to a depth of 15 to 20 cm into the rectum of the ram and directed towards the floor of the pelvis – caution being taken not to injure the ram. After insertion of the rectal probe into the rectum, the ram was given a few seconds to relax. An electrical stimulation was applied at intervals of 3 to 5 seconds – alternated with periods of rest, of the same duration. The electrical current was gradually increased, until semen was produced. Care was taken, as over-stimulation tends to induce urination – urine is found to be the major contaminant when using this method of semen collection. Generally after several stimuli, semen was produced – from the prolapsed penis, semen was collected into a pre-warmed (32°C) collection tube which was then removed and placed in a water bath (32°C) (Evans and Maxwell, 1987). The entire process of semen collection was performed in a period of approximately 1 minute, and a minimum of 4 people were involved to restrain and collect semen. After collection the ram was given enough time to voluntarily stand, as EE has been shown to cause a temporary slight motor inability of the hind quarters and limbs (Salisbury *et al.*, 1978).

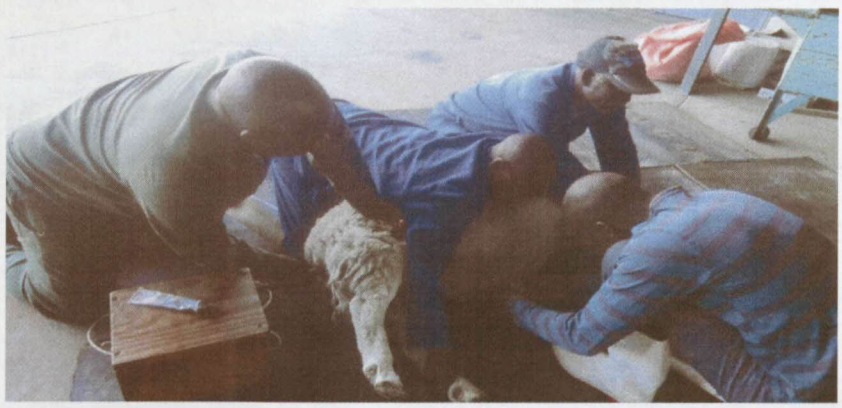

Plate 3.2: Collection of ram semen by electro-ejaculation

3.4 General routine

A laboratory (Plate 3.3) to perform the semen evaluation was set up adjacent to the semen collection area in the metabolic building. This was done to facilitate and provide a clean dust free environment for the semen processing and evaluation. Care was taken to prevent cold shock and damage to the sperm cells during and after collection (Salisbury *et al.*, 1978.) Semen evaluation was performed every Thursday ranging between 8:00 and 11:00. Care being taken that all parameters were recorded by the same technical person. The manufactures' instruction on how to utilize equipment was followed, as laid down in manual, and the expiry date of the consumables was also constantly checked.

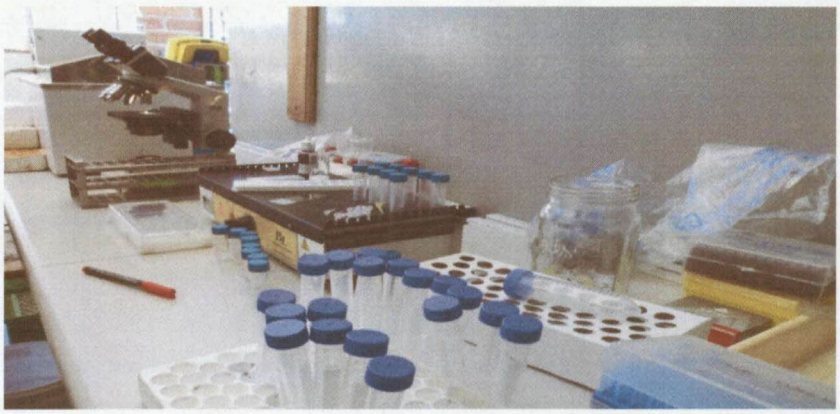

Plate 3.3: Laboratory for semen evaluation

3.5 Semen evaluation

3.5.1 Semen volume

The volume of the ejaculate was recorded directly from the calibrated tube (Plate 2.2), immediately after collection – before placing the collection tube in a warm bath at 32°C. All readings were taken at the bottom of the meniscus. Care was also taken to make sure that the tube was kept in an upright position.

3.5.2 Colour and smell of ejaculate

The first macroscopic parameter to be recorded of the semen sample was colour. The concentration of an ejaculate was firstly assessed according to the colour of the

ejaculate and also for the possibility of contamination (Table 2.3), according to Evans and Maxwell (1987). Thus in addition to the ejaculate colours specified, any peculiar or foreign substance e.g. wool in the semen was recorded. For the determination of any peculiar smell, olfactory evaluation was used e.g. smelling of the urine (Penner, 1993).

3.5.3 Semen pH

The semen pH was determined with the aid of a digital pH meter (Hand-held pH/mV/ Temperature/RS 232 Meter). The probe was always immersed in an electrode storage solution when not in use. The probe was rinsed with distilled water before use to remove any impurities. Care was taken that the probe was acceptably immersed in the semen sample when taking the pH readings. After taking the pH reading, the probe was rinsed with distilled water and again placed in the electrode storage solution (Anon, 2003).

3.5.4 Semen wave motion

To assess the wave motion, 10µl semen was drawn with a pipette and placed on a pre-warmed microscope slide (32°C), and observed under a low-powered (Olympus) microscope (x10 magnification). Wave motion was microscopically assessed on a scale of 0 to 5, using the guidelines as laid down by Hafez and Hafez (2000) – for details see 2.7.4 (semen wave motion). The wave motion was recorded immediately after collection, as a delay generally leads to a decrease in semen wave motion.

3.5.5 Sperm motility

For the determination of sperm motility, a volume of 10µl of semen was diluted in 990µl (1:100) pre-warmed (32°C) skimmed milk. Thereafter 10µl of the mixture of semen and skimmed milk was placed onto a pre-warmed microscope slide (32°C) and covered with a pre-warmed (32°C) cover slip – to assess sperm motility. A low magnification of a phase contact microscope was used (x40 magnification) for this microscopic evaluation. The arbitrary scale of 0-5 as set out by Zamiri *et al.* (2010) – see 2.7.5 (progressive sperm motility), was used. A total of 100 sperm cells were recorded for each semen sample, as either motile or non-motile sperm and then expressed as a percentage.

3.5.6 Sperm concentration (semen density)

Sperm concentration was determined with the aid of an improved Neubauer haemocytometer (depth 0.100 mm and 0.0025 mm^2). A 10µl semen was diluted in 990µl (1:100) distilled water, to dilute and also kill all the sperm. For sperm counting, the microscope (x100 magnification) was used. The 10µl of the mixture of semen and distilled water was drawn up into a pipette and placed on the improved Neubauer haemocytometer and covered with a cover slip. The sample was allowed a period of 5 to 6 minutes for the sperm (in mixture) to settle. All visible sperm cells in the 5 designated (5/25) diagonal squares were recorded. Sperm counting was performed immediately, or some time (24 hours) later. If performed later the mixture were stored in a refrigerator at a temperature of 5°C. On the haemocytometer the 25 large squares contained 16 smaller squares (Figure 3.3), which mainly facilitated in the counting of the sperm cells. The total number of sperm cells per ml was then calculated as the number of sperm cells counted in the 5 diagonal squares x 5 x 10^6 sperm/ml. After every sperm count the haemocytometer and cover slips were cleaned with 70% alcohol and dried with paper towels (Evans and Maxwell, 1987; Mitchell and Doak, 2004)

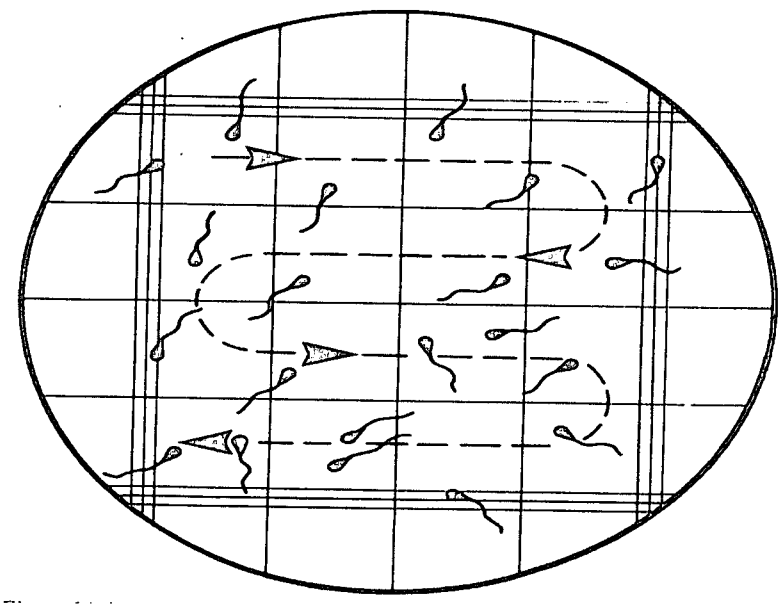

Figure 3.1: Enlarged counting grid of a haemocytometer chamber (Evans and Maxwell, 1987)

3.5.7 Sperm viability and morphology

The sperm viability (live vs dead sperm) and morphology were determined by making a semen and eosin-nigrosin stain. Sperm that were presumed to be dead

stained red, while sperm that were alive did not stain and remained white. Thus all sperm cells that stained light red were classified as dead. The procedure entailed taking 10µl of semen and diluting it with 100 µl (10:100) of a pre-warmed (32°C) eosin-nigrosin stain and mixing it well. A droplet of 10µl semen and stain mixture was then placed on a clean pre-warmed (32°C) microscope slide with another slide used as a spreader – so that a thin film of semen smear was formed on the microscope slide. It is important to push rather than pull the edge of the spreader at a 30° to 45° angle with the first slide – excessive pressure must however be avoided (Salisbury *et al.*, 1978). The microscope slide with the smear is then immediately dried on a warm plate (32°C) for less than a minute. The smeared slides were then stored in a clean tray for counting.

For viability and morphology the slides were examined at x1000 magnification (oil-immersion). A representative sample of 100 sperm on each slide, from randomly selected areas was then counted to determined the percentage live (white vs red sperm cells) – as well as for the determination of the percentage abnormal sperm and expressed as a percentage. Sperm cell abnormalities like e.g. loose heads, double head, broken or bent neck and mid-pieces, double tail and protoplasmic droplets were expressed as a percentage of the normal sperm cells (Evans and Maxwell, 1987; Theron, 2001; Mitchell and Doak, 2004).

3.6 Body parameters measured

3.6.1 Scrotal circumference

The scrotal circumference of each ram was recorded every week (Tuesday) between 8:00 and 10:00. Three people were needed – 1 person to hold the ram by the neck in a standing position, the second person to hold the scrotum between the hind legs with the measurements being taken by the third person. The testes were secured at the base of the scrotum by grasping the scrotal neck. A flexible measuring tape was placed over the widest point of the scrotum. Caution was paid when handling the scrotum, to ensure that both testes were firmly located in the scrotum (Fourie *et al.*, 2005; Noakes *et al.*, 2009).

3.6.2 Scrotal volume

The scrotal volume was measured from the rear, between the ram's legs in a standing position, weekly (Tuesday) between 8:00 and 10:00. The scrotal volume being taken as an indicator of the testes size. Testes volume was measured using a volumetric measuring cylinder. The water for measuring scrotal volume was maintained at a temperature of 25°C. The ram's scrotum was first soaked in water (25°C) to wet it, then the testes were held at the base by grasping the scrotal neck and submerging the scrotum into the container of water. The water which was displaced when the testes were submerged was measured to estimate the scrotal volume (Fourie *et al.*, 2004).

3.6.3 Body temperature

The body temperature of the rams was taken weekly on a specific day (Tuesday) between 8:00 and 10:00. The temperature was taken by inserting a clinical thermometer into the rectum and it being left in place for 2 minutes. Prior to insertion the mercury in the thermometer was shaken and the bulb moistened to facilitate penetration into the anus. Care was also taken to ensure that the thermometer bulb was held against the mucosa – if the bulb of the thermometer is surrounded by dung the temperature measured may be inaccurate. Care was taken to avoid exciting the rams prior to the insertion of the thermometer (Radostis *et al.*, 1994).

3.6.4 Body weight recordings

The body weight was used as an indicator of nutritional status and health of the rams. The body weight of the rams was taken weekly (Tuesday) between 8:00 and 10:00 with the aid of an electronic scale. No overnight fasting was implemented to avoid digestive disorders, which could have affected the performance of the rams (Bester, 2006).

3.6.5 Body condition score (BCS)

Although body weight is a reliable indicator of the condition of the animal, there exists a wide variation in mature size between individual animals and breeds, which makes it difficult to use body weight to determine an accurate estimation of body condition (Vatankhah *et al.*, 2012). The BCS was recorded every 2 weeks (Tuesday) between 8:00 and 10:00. The body condition score in this trial was based on

palpation of the tips of both the spinous and transverse processes of the vertebrae, and the fullness of the muscle and fat cover over and around the vertebrae in the loin region. The BCS system used was adapted from Upton and Soden, (1991) and Lategan, (2012) – as previously described in 2.9.6, the same technician was used to evaluate the BCS throughout the trial.

3.7 Environmental measurements

3.7.1 Ambient temperature, daylight length and relative humidity

The ambient temperature, daylight length and relative humidity data for the entire trial period was obtained from the Department of Agrometeorology (UFS weather station). The weather station being situated approximately 100 m east of where the rams were kept. Environmental measurements (climatology) are set out in Table 4.1.

3.8 Age of the rams

The age of rams were unknown and the teething method was thus used to estimate the age of the rams. Teething was performed at the beginning of the trial using the teething system as set out by Oberholster (2010), which entailed the following:

- Full set of milk teeth – 12 months old
- 2 teeth – 15 to 18 months old
- 4 teeth – 24 months old
- 6 teeth – 30 months old
- 8 teeth – 36 months or older
- Eroded teeth – ≥ 6 years

3.9 Libido testing

Every 2 weeks (Tuesday) between 8:00 and 10:00 the ram's libido was evaluated by measuring the ram's reaction time (30 to 150 seconds) when exposed to the 2 ewes in estrus (Theron, 2001). The ram was removed immediately when trying to mount and the reaction time recorded. No successful mating was allowed, to avoid pregnancy. A libido testing pen was erected in the vicinity of the rams to stimulate them, all being able to see the ewes. No feed or water was made available in this 3 m x 3 m x 1.3 m pen. The observer was positioned at the gate to remove the ram as

soon as the ram started to mount. The ewes were not restrained and rams were put into the ewe's pen one by one (Gouletsou and Fthenakis, 2010).

3.10 Statistical analyses

The results were statistically analyzed with PROC GLM procedures of SAS (1995). The one-way analysis of variance (ANOVA) was used to compare treatments. Parameters were considered significant at the confidence level $P<0.05$. Where treatment means differed significantly for a specific trial, the Tukey's method for multiple comparisons was used to determine which mean ultimately differed significantly (Fair, 2009; 2011).

Chapter 4

Results

This study was conducted mainly to determine the seasonal variation in semen quality of Dorper rams, using different collection techniques. It was however considered necessary to perform certain additional evaluations and recordings in order to clarify the effect and relationship of certain body parameters of the rams with method of semen collection and ultimately semen quality.

4.1. Climatological information

The climatological information for the region is set out in Table 4.1,Figure 4.1 and Figure 4.2. The summer months showed high ambient temperatures with a mean of 24.5°C. Maximum ambient temperatures of 36.4°C were recorded in December (summer), while the lowest ambient temperatures were recorded in July (winter) at −3.6°C. Autumn and spring recorded a mean ambient temperature of 17.2°C and 18.6°C, respectively. The summer months also recorded the longest day light length. The longest day light length recorded was in December – being 14.0 h. There was then a decrease in day light length from December to June (summer to onset of winter), followed by an increase from July to November. June (onset of winter) recorded the shortest day light length of 10.1 h.

Table 4.1 Seasonal climatological information for the region during the trial year as obtained from the Department of Agrometeorology (UFS weather station)

	Ambient temperature (°C)			**Relative humidity (%)**			**Day light length (hours)**		
Season	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Summer	8.4	36.4	24.5	5.9	93.2	54.0	12.4	14.0	13.4
Autumn	-1.8	32.9	17.2	8.8	92.6	47.6	10.2	12.4	11.2
Winter	-3.6	29.2	10.9	7.1	94.0	47.1	10.1	11.3	10.5
Spring	1.7	34.1	18.6	5.3	90.5	36.5	11.3	13.5	12.6

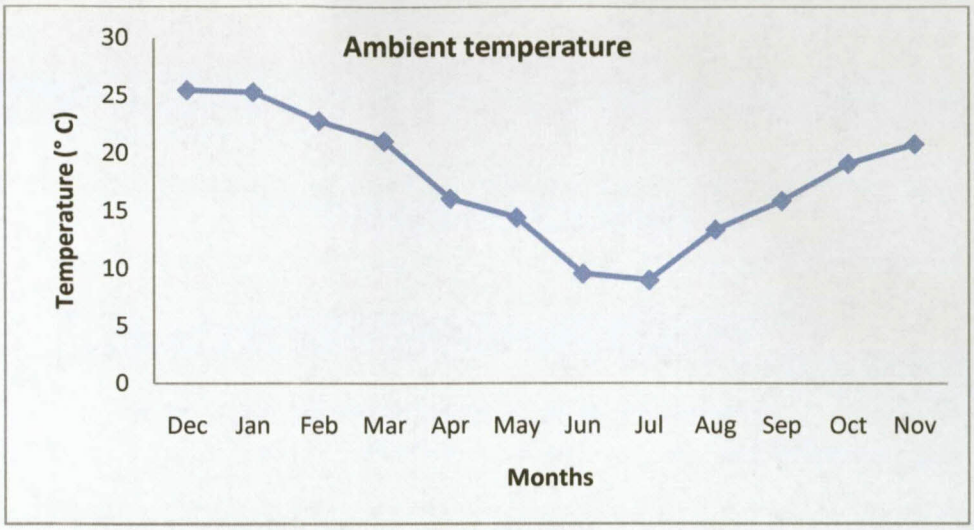

Figure 4.1 Average monthly ambient temperatures recorded (Department of Agrometeorology, UFS)

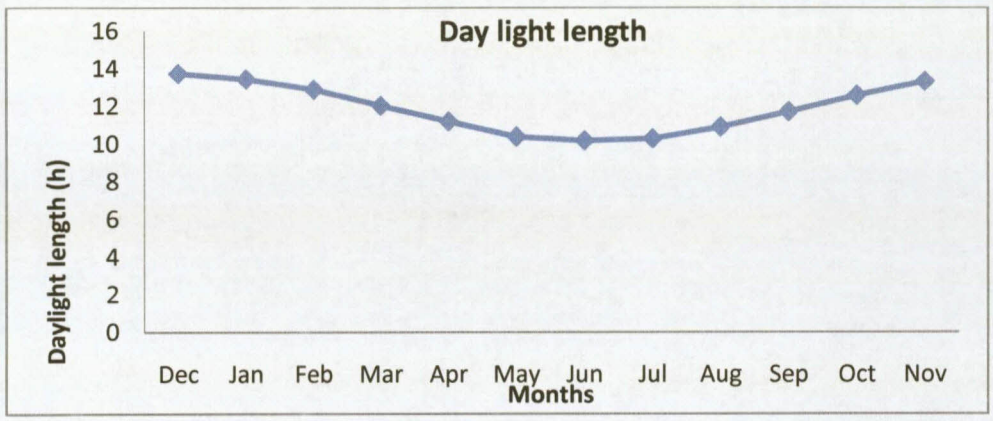

Figure 4.2 Average monthly day light length recorded (Department of Agrometeorology, UFS)

4.2 Macroscopic semen parameters

4.2.1 Semen volume

The semen volume collected throughout the year with the aid of artificial vagina and electro-ejaculator is set out in Table 4.2 and Figure 4.3. Table 4.2 presents the seasonal overall means, while Figure 4.3 illustrates the monthly means. Except for winter, there was a significant ($P<0.05$) difference in the semen volume recorded following semen collection using the AV, and that recorded using the EE technique. With regard to season, no significant ($P>0.05$) difference was recorded for the overall values for semen volumes collected using the AV, however with EE collection a significant ($P<0.05$) difference was recorded between seasons. The average for

seasons were however the same (1.1 ml) for both collection techniques. When using the AV, the semen volume for each month stayed relatively constant throughout the year ranging from 1.06 ml to 1.17 ml. However the EE values fluctuated, with values that ranged from 0.48 ml to 1.42 ml being recorded. Semen collection started in February (summer) and ended in January 2013 (summer), thus covering all four seasons of the year.

Table 4.2 Mean (±S.D.) macroscopic seasonal variation in certain semen parameters, following AV and EE collection in rams for the observation period

Semen parameters	Season	AV	EE
Semen volume (ml)	Summer	$^{x}1.1\pm0.2^{a}$	$^{y}1.0\pm0.4^{b}$
	Autumn	$^{x}1.1\pm0.3^{a}$	$^{z}0.7\pm0.4^{b}$
	Winter	$^{x}1.1\pm0.3^{a}$	$^{x}1.2\pm0.6^{a}$
	Spring	$^{x}1.1\pm0.3^{a}$	$^{x}1.3\pm0.4^{b}$
Colour (0-5)	Summer	$^{y}3.6\pm0.3^{a}$	$^{x}2.8\pm0.7^{b}$
	Autumn	$^{x}3.9\pm0.4^{a}$	$^{x}2.5\pm0.83^{b}$
	Winter	$^{z}2.8.0\pm0.4^{a}$	$^{y}1.9\pm0.6^{b}$
	Spring	$^{y}3.6\pm0.3^{a}$	$^{x}2.6\pm1.0^{b}$
Semen pH	Summer	$^{x}6.8\pm0.1^{a}$	$^{x}6.8\pm0.1^{a}$
	Autumn	$^{x}6.8\pm0.1^{a}$	$^{x}6.9\pm0.2^{a}$
	Winter	$^{y}6.7\pm0.3^{a}$	$^{y}6.8\pm0.3^{a}$
	Spring	$^{y}6.7\pm0.2^{a}$	$^{y}6.7\pm0.2^{a}$

^{a,b} Means in the same row with different superscripts, differed significantly ($P < 0.05$)

^{x,y,z} Means in a column with different superscripts, differed significantly ($P < 0.05$)

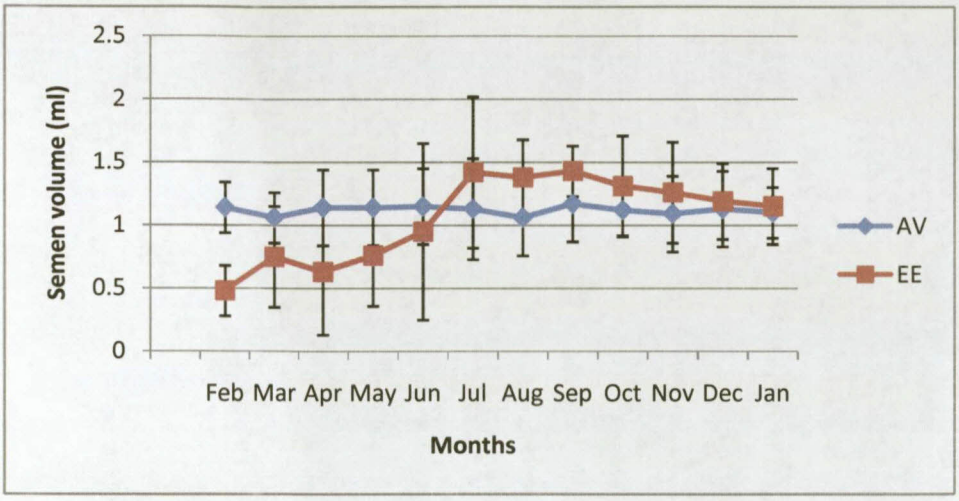

Figure 4.3 The mean (±S.D.) monthly semen volume (ml) of Dorper rams using the different collection techniques (AV and EE) for an entire year

4.2.2 Semen colour

The effect of season on semen colour (indicative of semen density) using the AV and EE is set out in Table 4.2 and Figure 4.4. Table 4.2 shows the seasonal variation, while Figure 4.4 represents the monthly tendency. There was no indication of contamination in all the semen samples collected – the main challenge in the exercise was to draw a distinct line between the milky-white colour and a pale cream colour of the semen sample, with the naked eye. Although the different techniques showed little variation in colour as such, there was a definite trend ($P<0.05$) for the AV group to maintain a higher colour code and therefore a more dense semen sample. There was thus a significant ($P<0.05$) difference between the AV and EE semen collection techniques, as well as overall mean for the different seasons. The lowest seasonal semen colour measurements were recorded in winter – on the scale of 0 to 5. The AV semen collection recorded a mean of 2.8 ±0.4, while the EE recorded 1.9±0.6. No unfamiliar smell was detected in any of the semen samples (no contamination).

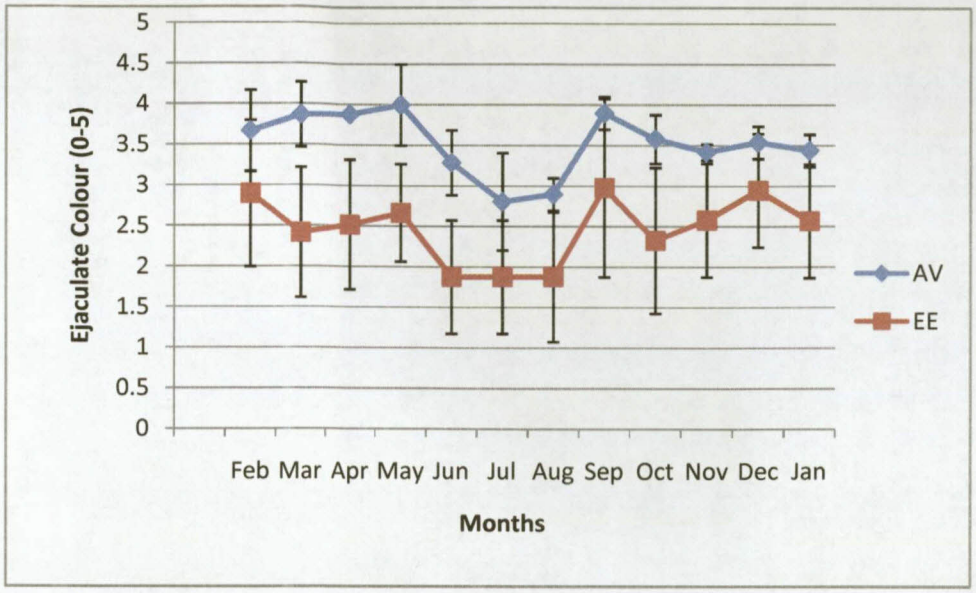

Figure 4.4 The mean (±S.D.) monthly semen colour recorded in Dorper rams using different collection techniques (AV and EE), for an entire year

4.2.3 Semen pH

The effect of season on semen pH using the AV and EE is set out in Table 4.2. There was no significant ($P>0.05$) difference in the semen pH recorded following collection using the AV, and the values for those collected using the EE technique. When comparing the semen pH by season, spring maintained the lowest pH, recording 6.7±0.2 for both collection techniques. The overall seasonal mean pH of the ejaculates recorded in this trial ranged from 6.7±0.2 to 6.9±0.2.

4.3 Microscopic sperm parameters

4.3.1 Sperm wave motion

The effect of season on sperm wave motion using the AV and EE is set out in Table 4.3 and Figure 4.5. Table 4.3 represented the seasonal variation, while in Figure 4.5 the average monthly sperm wave motion is reflected. The values showed an almost similar trend to that of the semen colour. The overall sperm wave motion recorded a significant ($P<0.05$) difference between the two collection techniques, and also between seasons. Winter recorded the lowest sperm wave motion value in both collection techniques.

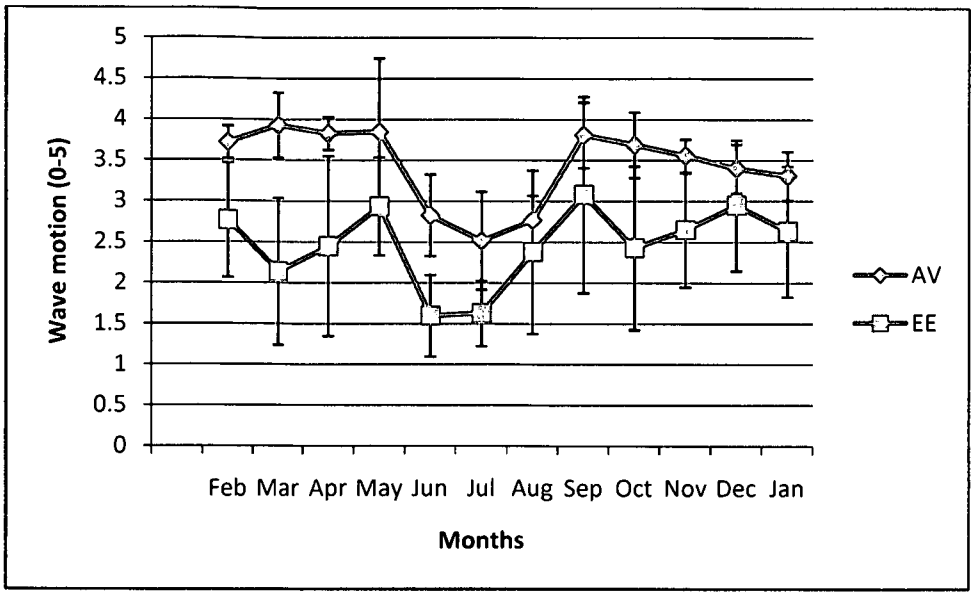

Figure 4.5 The mean (±S.D.) monthly sperm wave motion recorded for both semen collection techniques (AV and EE)

Table 4.3 Mean (±S.D.) microscopic seasonal semen characteristics for the AV and EE semen collection techniques

Semen parameters	Season	AV	EE
Sperm motility (%)	Summer	$^{x}76.7\pm5.3^{a}$	$^{x}68.8\pm8.0^{b}$
	Autumn	$^{x}76.7\pm8.14^{a}$	$^{x}65.8\pm10.2^{b}$
	Winter	$^{y}52.2\pm15.3^{a}$	$^{y}32.6\pm15.8^{b}$
	Spring	$^{x}77.8\pm5.2^{a}$	$^{x}63.3\pm18.5^{b}$
Sperm wave motion (0-5)	Summer	$^{y}3.5\pm0.3^{a}$	$^{x}2.8\pm0.7^{b}$
	Autumn	$^{x}3.9\pm0.3^{a}$	$^{x}2.5\pm1.0^{b}$
	Winter	$^{z}2.7\pm0.7^{a}$	$^{y}1.9\pm0.6^{b}$
	Spring	$^{x}3.7\pm0.4^{a}$	$^{x}2.7\pm1.7^{b}$
Sperm abnormalities (%)	Summer	$^{y}7.1\pm2.4^{a}$	$^{y}6.2\pm2.6^{a}$
	Autumn	$^{z}5.3\pm4.0^{a}$	$^{z}4.2\pm3.0^{a}$
	Winter	$^{x}9.0\pm2.4^{a}$	$^{x}8.5\pm2.9^{a}$
	Spring	$^{z}4.4\pm1.9^{a}$	$^{z}4.6\pm2.8^{a}$
Sperm viability (%)	Summer	$^{y}78.5\pm4.5^{a}$	$^{y}74.3\pm6.9^{b}$
	Autumn	$^{x}84.2\pm9.1^{a}$	$^{x}82.2\pm10.6^{a}$
	Winter	$^{z}48.0\pm17.3^{a}$	$^{z}39.0\pm14.6^{b}$
	Spring	$^{x}81.3\pm5.3^{a}$	$^{y}72.8\pm16.3^{b}$

^{a,b} Means in the same row with different superscripts, differed significantly ($P < 0.05$)

^{x,y,z} Means in a column with different superscripts, differed significantly ($P < 0.05$)

4.3.2 Sperm motility

The sperm motility results for the AV and EE semen collection techniques recorded throughout the year are set out in Table 4.3 and Figure 4.6. A significance ($P<0.05$) difference in sperm motility between the two collection techniques was recorded throughout the year – with the AV generally producing sperm with a higher motility. When evaluating seasonal variation regarding sperm motility for the AV collection technique, no significant ($P>0.05$) difference was recorded for autumn and spring. For both collection techniques, winter (June, July, August - winter) recorded the lowest sperm motility (Table 4.6).

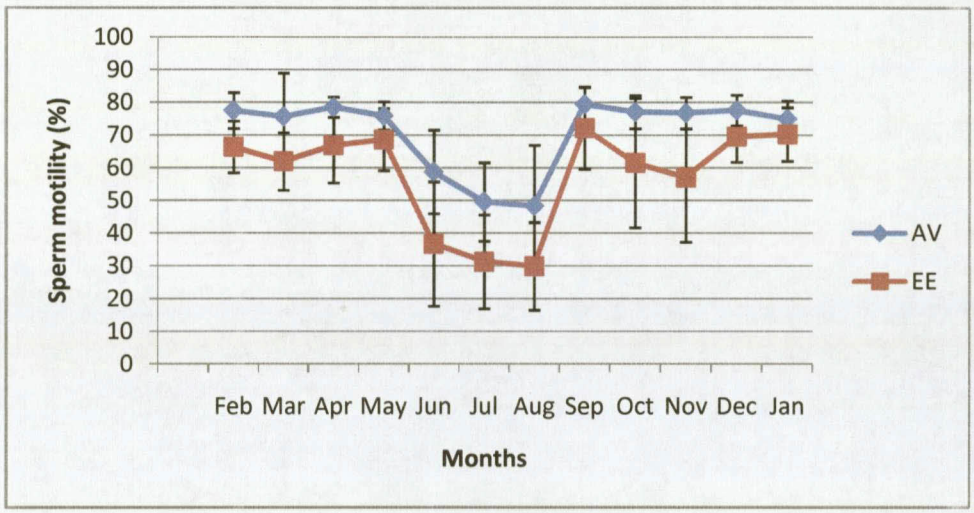

Figure 4.6 The mean (±S.D.) monthly sperm motility (%) of Dorper rams using different collection techniques for all the months of the year

Table 4.4 The mean (±S.D.) effect of season on sperm density ($x10^9$ sperm /ml) for the different semen collection techniques in Dorper rams

	Artificial vagina			Electro-ejaculator		
Season	Mean	Min	Max	Mean	Min	Max
Summer	$^{y}2.65\pm 0.33^{a}$	1.95	3.60	$^{x}1.97\pm0.67^{b}$	0.59	2.99
Autumn	$^{x}3.28\pm0.55^{a}$	1.90	4.36	$^{y}1.64\pm0.68^{b}$	0.15	2.89
Winter	$^{y}2.67\pm0.71^{a}$	1.23	3.73	$^{y}1.57\pm0.70^{b}$	0.05	2.97
Spring	$^{x}3.45\pm0.52^{a}$	2.33	4.68	$^{y}2.19\pm0.10^{b}$	0.24	4.30

^{a,b} Means in the same row with different superscripts, differed significantly ($P < 0.05$)

^{x,y,z} Means in a column with different superscripts, differed significantly ($P < 0.05$)

4.3.3 Sperm cell concentration

The sperm cell concentration (sperm density) obtained following the different semen collection technique is set out in Table 4.4 and Figure 4.7. From Table 4.4 semen collected by the AV method recorded a significantly ($P<0.05$) higher sperm cell concentration, compared to EE method of collection for the entire observation period. When comparing the overall seasonal means of the AV with regard to the sperm concentration, at the end of the day there was no significant ($P>0.05$) difference in sperm density between that collected in summer and that in winter, similarly also for autumn and spring, when using the AV method of collection. The EE collected semen recorded no significant ($P>0.05$) difference for summer and spring and also for autumn and winter. Figure 4.7 illustrates an increase in semen concentration from February to May (autumn or natural breeding season) and also another increase from July to October (winter/ spring) for the AV semen collection technique.

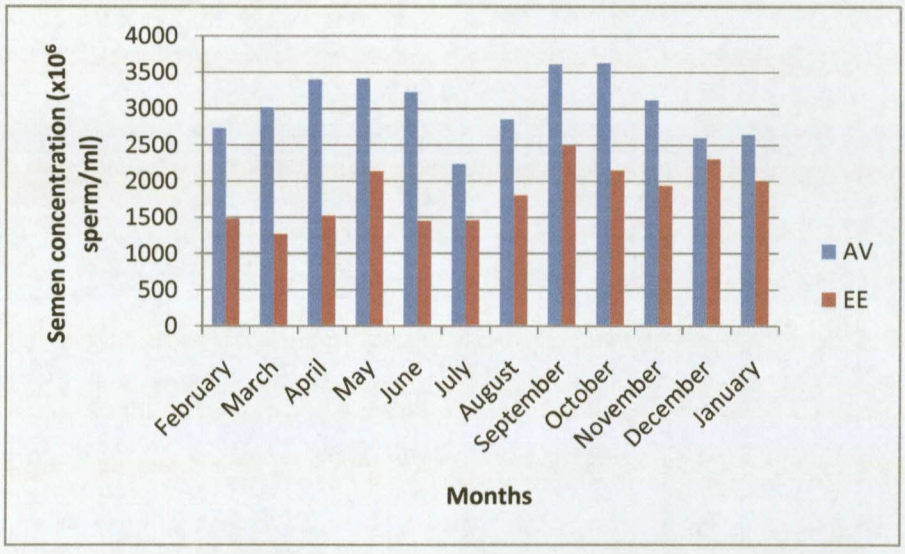

Figure 4.7 The mean monthly semen concentration ($x10^6$ sperm /ml) of Dorper rams, using different collection techniques for an entire year

4.3.4 Sperm viability

The sperm viability recorded by the different semen collection techniques is set out in Table 4.3 and Figure 4.8. In both collection techniques there was no significant ($P>0.05$) difference for semen collected in autumn, while other seasons recorded a significant ($P<0.05$) difference. Thus on a seasonal basis, there was no significant ($P>0.05$) difference regarding sperm viability for autumn and spring (AV collection technique), while the EE group recorded no significant ($P>0.05$) difference for the

summer and spring seasons. Thus the winter season generally yielded semen of low quality, for both the semen collection techniques.

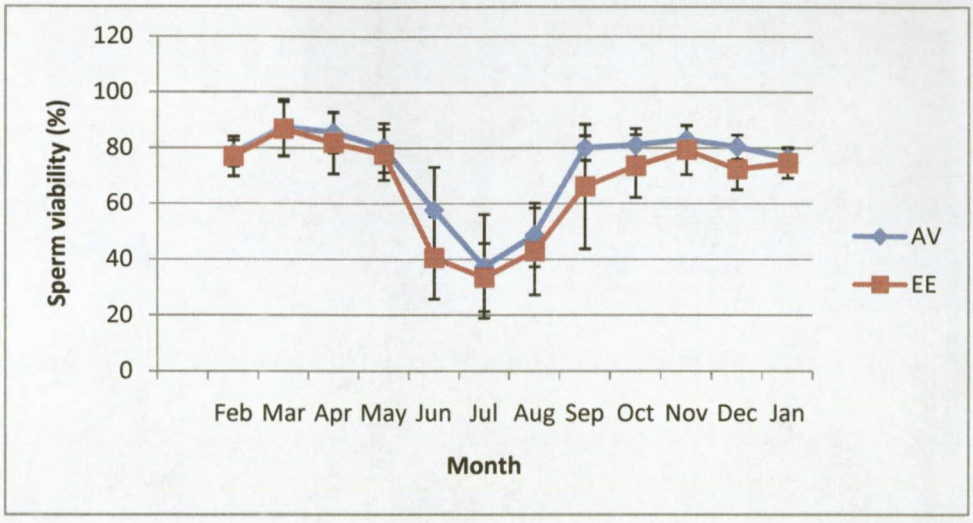

Figure 4.8 The mean monthly sperm viability (%) of Dorper rams using different collection techniques for the entire year

4.3.5 Sperm morphology

The sperm morphology (abnormalities) data for the AV and EE collection techniques recorded throughout the four seasons are set out in Table 4.3 and Figure 4.9. No significant ($P>0.05$) differences were recorded for the two collection techniques for the entire observation period. Seasonally there was however distinct ($P<0.05$) differences within each technique. The highest mean monthly sperm abnormality rates of 10.1±2.1 % and 10.3±2.5 % were recorded in the month of July (winter) for the AV and EE semen collection techniques, respectively. The highest mean seasonal sperm abnormalities were also recorded for winter (9.0±2.4 % and 8.5±2.9 % for AV and EE semen collection techniques, respectively). Generally the percentage sperm abnormalities were low, indicating acceptable techniques used to stain and also to collect the semen.

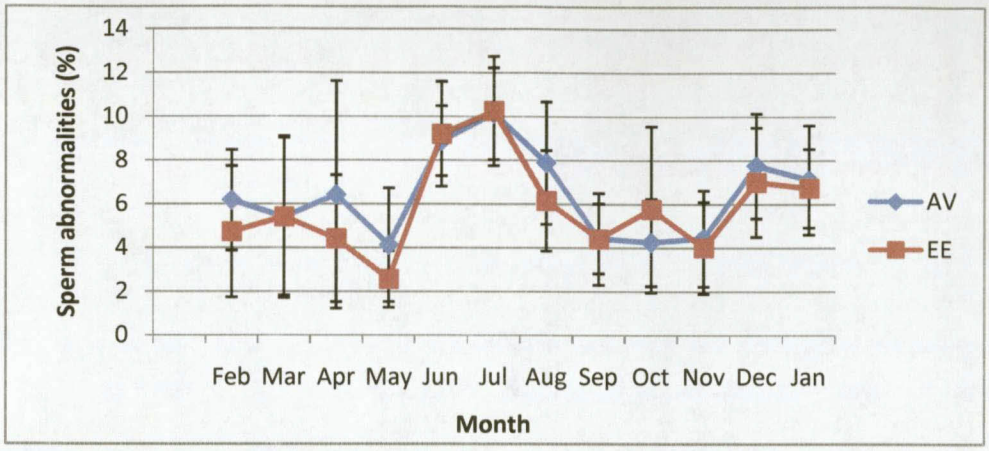

Figure 4.9 The mean (±S.D.) monthly sperm abnormalities (%) of Dorper rams using different collection techniques for an entire year

4.4 Libido

The libido of rams during the observation period is set out in Figure 4.10. The four seasons of the year and the methods of semen collection had no significant (P>0.05) effect on the libido of the rams in both groups. The libido generally remained high throughout the year – on the scale of 0 to 5, mean libido was recorded to be between 4.5±0.7 and 4.9±0.4 for the AV and EE groups, respectively. The superficial monitoring of libido in this study is to be kept in mind.

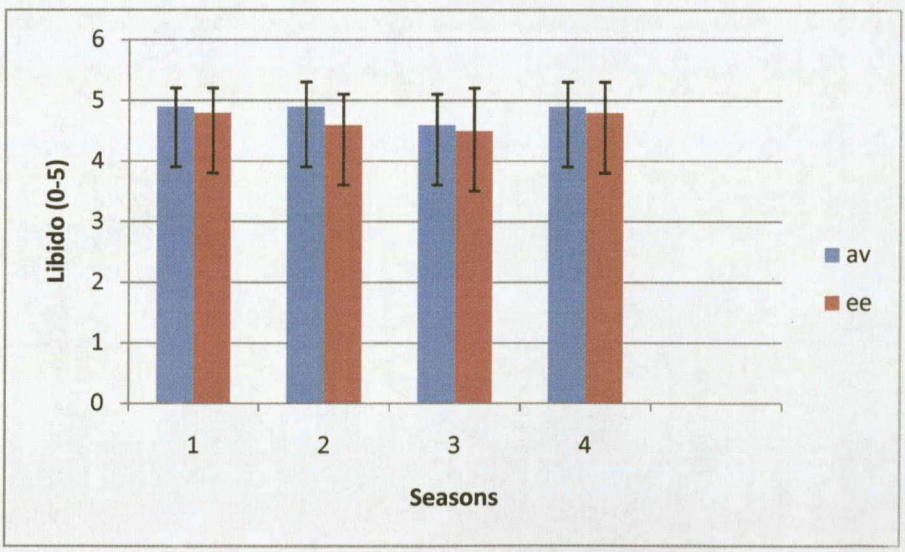

Figure 4.10 Mean (±S.D.) libido (0-5) of Dorper rams using different collection techniques for an entire year (1= summer, 2 = autumn, 3 = winter, 4 = spring)

4.5 Body parameters

4.5.1 Body weight

The mean body weight of the rams used in the trial is set out in Table 4.5 and Figure 4.11. The mean body weight of the rams for the two groups did not show any significant (P>0.05) changes throughout the year, as would be expected of adult (grown) animals. At the onset (February) of the trial the monthly mean body weight was 76.0±11.1 kg for the AV and 72.8±1.8 kg for the EE group, respectively. The final (January) monthly body weight data were 97.5±5.8 for the AV and 95.9±4.0 for the EE groups. On a seasonal average the rams body weight increased by 5.7 kg (5.8 AV and 5.5 EE respectively). The yearly body weight averages recorded were 89.9 kg for the AV group, while the EE group recorded 87.1 kg – leading to a body weight difference of only 2.8 kg between the two collection groups (not significant).

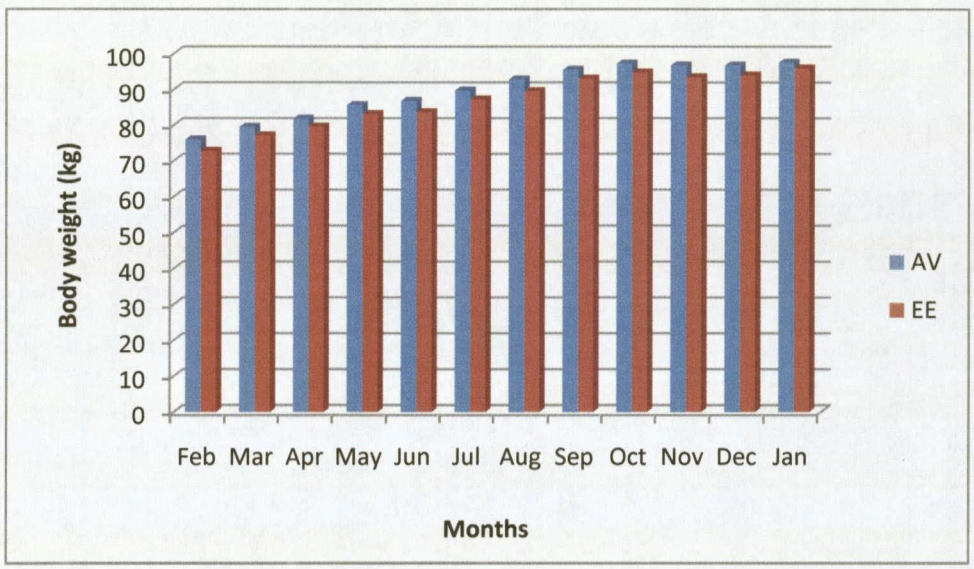

Figure 4.11 Mean monthly body weight of rams used for AV and EE semen collection during the observation period

4.5.2 Body condition score

The mean body condition score of the rams during the observation period is set out in Figure 4.12. The body condition score was used to indicate the degree of fatness, while the body weight indicated more the skeletal size. No significant (P>0.05) differences were recorded between the two groups of rams (AV and EE groups). The four seasons recorded no significant (P>0.05) difference for both collection techniques. The measurements for autumn tended to be lower, compare to the other

seasons (may be due to human error as the researcher was still learning how to score the rams). Generally nutrition played no role as the rams were feed a uniform diet throughout the observation period.

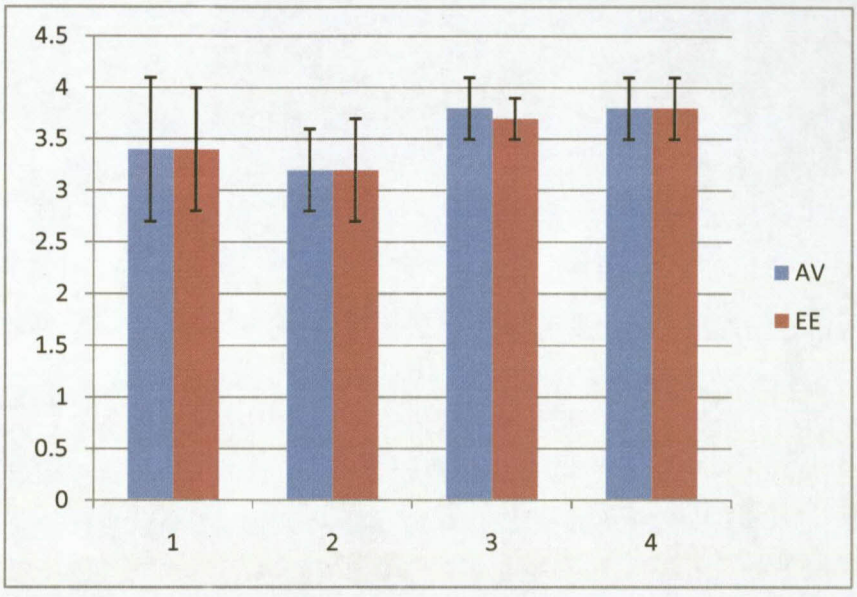

Figure 4.12 Mean (±S.D.) body condition score (0-5) of Dorper rams using different collection techniques for an entire year (1= summer, 2 = autumn, 3 = winter, 4 = spring)

4.5.3 Body temperature

The mean body temperatures of rams in the AV and EE collection groups is set out in Table 4.5.The seasonal body temperatures of the rams ranged between 38.9±0.2 °C and 39.0±0.3 °C for the AV and EE groups, respectively. Thus no significant ($P>0.05$) difference was recorded between the two groups of rams for semen collection and also no significant ($P>0.05$) difference in body temperature for the four seasons of the year. Also because of the similar measurement recorded the values are only presented in Table 4.5 and not in a figure form. Evident was the ability of the Dorper breed to maintain a constant body temperature throughout the year.

4.5.4 Scrotal circumference

The mean seasonal scrotal circumference for AV and EE collection methods is set out in Table 4.5. No significant ($P>0.05$) difference was recorded between the collection techniques for the entire observation period. On a year average (overall) the scrotal circumference for both collection techniques were almost identical. The AV technique group's average was 35.4 cm and the EE technique measured 35.5

cm, with a difference of only 0.1 cm. As can be expected, scrotal circumference is a function and related to body size – thus the similar measurements. The scrotum of all rams used in this trial hung symmetrically, the testes freely moving within the scrotum and their scrotums not covered by excess wool.

4.5.5 Scrotal volume

The mean seasonal scrotal volume for the AV and EE collection group are set out in Table 4.5. Like in the case of scrotal circumference, there was no significant ($P>0.05$) difference between the rams in the two methods of semen collection throughout the year. The average mean for the four seasons was 748.9 ml and 744.7 ml for the AV and EE methods respectively. The overall difference between the two collection groups only being 4.2 ml.

Table 4.5 Mean (±S.D.) body parameters recorded for Dorper rams in the AV and EE collection groups, throughout the four seasons of the year

Body parameter	Season	AV	EE
Body weight (kg)	Summer	$^{y}90.7\pm12.4^{a}$	$^{y}88.2\pm11.4^{a}$
	Autumn	$^{z}82.3\pm8.8^{a}$	$^{z}79.9\pm5.7^{a}$
	Winter	$^{y}89.8\pm6.4^{a}$	$^{y}86.7\pm4.3^{a}$
	Spring	$^{x}96.5\pm5.8^{a}$	$^{x}93.7\pm3.7^{a}$
Body temperature(°C)	Summer	$^{x}39.0\pm0.3^{a}$	$^{x}39.0\pm0.3^{a}$
	Autumn	$^{x}39.0\pm0.2^{a}$	$^{x}39.0\pm0.3^{a}$
	Winter	$^{x}38.9\pm0.2^{a}$	$^{x}39.0\pm0.3^{a}$
	Spring	$^{x}38.9\pm0.2^{a}$	$^{x}38.9\pm0.3^{a}$
Scrotal circumference (cm)	Summer	$^{x}36.0\pm2.4^{a}$	$^{x}36.4\pm2.2^{a}$
	Autumn	$^{y}34.4\pm1.8^{a}$	$^{y}34.4\pm0.7^{a}$
	Winter	$^{y}35.1\pm1.8^{a}$	$^{y}34.9\pm1.0^{a}$
	Spring	$^{x}36.1\pm2.1^{a}$	$^{x}36.1\pm1.9^{a}$
Scrotal volume (ml)	Summer	$^{x}807.6\pm191.2^{a}$	$^{x}816.4\pm155.7^{a}$
	Autumn	$^{y}658.5\pm138.8^{a}$	$^{y}650.4\pm78.9^{a}$
	Winter	$^{y}711.1\pm160.8^{a}$	$^{y}706.4\pm92.1^{a}$
	Spring	$^{x}818.2\pm158.8^{a}$	$^{x}805.5\pm128.6^{a}$

^{a,b} Means in the same row, with different superscripts, differed significantly ($P < 0.05$)

^{x,y,z} Means in a column with different superscripts, differed significantly ($P < 0.05$)

4.6 Correlation coefficient

The correlation coefficient (r) between the various semen and body parameters are set out in Table 4.6. The negative values recorded for example semen volume and sperm wave motion or sperm abnormalities in the AV group indicate that the higher the semen volume the lower the sperm wave motion or abnormalities (not significant). The EE group also showed a tendency for higher semen volume to record lower sperm motility (Table 4.6). The only significant (P<0.0001) correlations were as expected between scrotal volume and body weight and also scrotal volume and scrotal circumference – in both the AV and EE groups

Table 4.6 Correlation between various semen and body parameters of the rams

Parameters	Correlation coefficient (AV)	Correlation coefficient (EE)
Semen volume x semen colour	0.07743	0.03954
Semen volume x sperm motility	0.03470	-0.06672
Semen volume x body weight	0.18019	*0.41947
Semen volume x sperm density	0.21569	0.29547
Semen volume x sperm wave motion	-0.01657	0.13152
Semen x volume x sperm abnormalities	-0.12434	0.06724
Scrotal volume x body weight	*0.47510	*0.54576
Scrotal volume x scrotal circumference	*0.80748	*078280

*P<0.0001

Chapter 5

Discussions

5.1 Adaptation and training of ram

The rams used in this trial were sourced locally, where they had been maintained extensively on the veld. To facilitate their adaptation and good health, the rams were vaccinated for pulpy kidney, pasturella and treated for internal and external parasites. The management of the rams was explained in detail in section 2.5 and 3.2. The adaptation and humane handling (welfare) was implemented to help make the trial achieve the aim of evaluating the natural variation in semen quality of Dorper rams, using the different collection techniques.

After the rams had been adapted to their new environment, the animals were randomly divided into two groups (the AV and EE semen collection groups) – the AV group being trained for semen collection. Training of the rams for AV semen collection was successfully performed within a week (an indication of the libido). So for example 4/6 (67%) of the rams responded to the AV collection within 2 days of training. This training time frame was in agreement with that quoted by Hafez and Hafez (2000) and Maule (1962), respectively. The rams were always eager to be collected using the AV (indicative of libido) and the AV also simulated the natural mating (ejaculation) process (Salisbury *et al.*, 1978). On the other hand the rams in the EE collection technique group were not trained for semen collection with this technique being less reliable and repeatable.

5.2 Semen evaluation

5.2.1 Semen volume

Semen volume is a very important parameter and it is generally used to calculate or estimate the number of ewes that can be inseminated, or the number of straws/ pellets to be cryopreserved. There are many factors which may affect the semen volume (ejaculate). Generally these factors include breed, age of the male, nutritional status, reproductive management, method of semen collection, the frequency of semen collection, skill of semen collector, season of the year and

responsiveness of the ram (Evans and Maxwell, 1987; Theron, 2001; Greyling, 2009).

Figure 4.3 demonstrated that during the first 5 months (February to June) of EE semen collection, ejaculation was characterized by a relative low semen volume. Lack of training for the EE collection technique and inadequacies in the skill of stimulation may have contributed to this low semen volume recorded from February to June. The low semen volume by EE in these months could thus be attributed to the researcher not yet fully skilled on how to collect semen using the electro-ejaculator (Evans and Maxwell, 1987; Theron, 2001; Greyling, 2009). Also in Figure 4.3, when using the AV, the volume for each month stayed relatively constant throughout the year – as the collection of semen with an artificial vagina resembles or simulates natural ejaculation. Collecting semen using the AV generally required the active participation of the ram, leading to the involvement of the nervous and endocrine systems. Under the influence of these systems the gonadotrophic hormones (FSH and LH) are reported to be produced in sufficient quantities to regulate the reproductive system of ram (Guyton and Hall, 2011).

Also during these months (February to June), in the EE group, there was ram vocalization – this vocalization being associated with the pain said to be caused by electro-ejaculator (Palmer, 2005). On average 20% of the 5 rams stimulated by EE vocalized, this being lower than the 55.6% reported by Whitlock *et al.* (2012), who researched the stress effect of electro-ejaculation on bulls. Even although vocalization stopped during the fifth month, it did not imply that electro-ejaculation was no longer painful, but possibly the rams became accustomed to the pain inflicted by the EE. It could also imply that the rams were getting used to being handled (Ortiz-de- Montellano *et al.*, 2007).

When applied gently, electro-ejaculation was reported not to cause harmful effects – no loss in body condition, no change in temperament, and no special disinclination (unwillingness) to further application (Salisbury *et al.*, 1987). Contrary to Salisbury *et al.* (1978), there has been disinclination to further applications of the electro-ejaculator. The rams did demonstrate a degree of resistance (unwillingness) when herded to the place of semen collection. The EE collection process was

characterized as reported by struggling and muscular contractions during collection, which was followed by a slight temporary limp after collection. Generally the perennial area of the rams remained inflamed after collection – inflammation being a sign of pain and as such this trial cannot deny that the EE technique is a painful treatment (Palmer, 2005). The general tendency in the current trial was that the EE was not the preferred method of semen collection.

Apart from the problem of the EE, this trial also showed that semen of Dorper rams could be collected using the AV and EE method throughout the year (inside and outside the natural breeding season). Generally spermatogenesis can only terminate in the male due to senility, general weakness or disease (Geneke, 1985). From Figure 4.2 it was also evident that on a yearly basis, both collection techniques yielded on average the same volume of 1.1 ml of semen. Studies previously performed to compare the two collection techniques and the current trial was in agreement with that of Matthews *et al.* (2003); Marco-Jiménez *et al.* (2005), who recorded no significant difference in semen volume between the two collection techniques. The findings regarding ejaculate volume of the current trial also fell in the range of that for mature rams of 0.5 to 2 ml (Hafez and Hafez, 2000). All rams used in the present study adapted well to the new environment and facilities – luckily no mortalities were experience for the entire experimental period. Thus the data of all 11 rams were used throughout.

5.2.2 Semen colour

The colour of a semen sample generally serves as an indicator of the sperm density (concentration), as well as possible contamination (blood, dirt, pus, urine). No physical contamination was however recorded for both collection techniques. The absence of blood or pus in this trial may be postulated to serve as indicator of no injury or disease of the penis or reproductive tract in the rams. This result was also attained by acceptable hygiene and proper ram care – the collection place always being kept clean and all rams kept free of faeces prior to collection (Evans and Maxwell, 1987). Table 4.2 recorded that the semen obtained from the EE group generally yielded a thin cream to milk colour (1.9±0.6 to 2.8±0.7 – on a scale of 0 to 5) semen sample, which was less concentrated, compared to the AV group (Evans and Maxwell, 1987).The winter semen records of the AV collection group and all

samples from the EE group recorded a milky to cloudy colour. Autumn recorded a slight increase in semen colour for the AV collection technique, which generally indicated a higher sperm count (concentration) (Greyling and Grobbelaar, 1988). No slightly sweet or aromatic smell of the semen sample was detected as reported by Penner (1993), therefore this parameter was not further considered.

5.2.3 Semen pH

Semen pH (indicator of acidity and alkalinity) has been studied in different breeds of rams (Greyling and Grobbelaar, 1983; Hafez and Hafez, 2000) – these studies have recorded a ram semen pH of 5.9 to 7.3, and 6.4 to 7.1, respectively. The current trial (mean semen pH of 6.7±0.3 to 6.9±0.2 for the AV and EE groups, respectively) was within these ranges. From this trial the electro-ejaculator may be assumed not to have caused inflammation of the accessory glands – as inflamed accessory glands would generally result in a more alkaline semen pH (Greyling and Grobbelaar, 1988). The semen pH in this current trial may also indicate that the 10-15 minutes for the fresh undiluted semen sample following the collection period (to prevent further sperm cell metabolism), is a factor to be seriously considered. The delay in the processing of fresh undiluted semen may induce the semen pH to become more acidic, due to degradation of fructose by sperm (Hafez and Hafez, 2000; Zamiri *et al.*, 2010).

5.2.4 Sperm wave motion

Sperm wave motion was assessed using a sample of fresh undiluted semen and being scored on a scale of 0 to 5, with 0: being no movement and 5: representing vibrant sperm motion. Among the other factors to be considered when assessing sperm wave motion that have been reported are e.g. temperature (30-37 °C) and the time of storage of the undiluted semen (10-15 minutes). Sperm wave motion as such then estimates the motility of semen and the viability (Evans and Maxwell, 1987; O'Hara *et al.*, 2010). Table 4.3 recorded a significant difference between the two collection techniques with the AV producing a higher sperm wave motion. Sperm wave motion in both groups were significantly ($P<0.05$) lower during winter. The AV method of semen collection yielded sperm with a wave motion of >3 in summer, autumn and spring, while the EE technique yielded a score of <3 during all seasons of the year. Semen scoring of >3 regarding the sperm wave motion on the scale of 0

to 5 is generally accepted as suitable for AI or cryopreservation (O'Hara *et al.*, 2010). Regarding sperm wave motion in this trial, all the AV samples obtained, except for those collected in winter could or should be used for AI or cryopreservation – while all samples from the EE group would generally be seen as inferior and be discarded. This parameter ruled out the EE technique as a method for semen collection and also demonstrated that ram semen should not be collected in winter (Arrebola *et al.*, 2010; Zamiri *et al.*, 2010).

5.2.5 Sperm motility

High sperm motility is generally regarded by semen evaluators to be a good indication of good semen quality, with fertilizing ability and high ram fertility. Sperm motility as such could also be used when estimating the number of doses per ejaculate. The sperm motility in both groups was significantly ($P<0.05$) lower during the winter period in this trial (Table 4.3). These findings are in agreement with the findings of Talebi *et al.* (2009) and contrary to that of Karaniannidis *et al.* (2000). The AV method yielded a sperm motility of more than 75 % in summer, autumn and spring, while the EE technique yielded less that 70% in all the seasons. Paulenz *et al.* (2003; 2005) reported that when assessing semen quality a progressive motility of ≥75% is acceptable for semen to be processed for AI or cryopreservation. The winter records for sperm motility (Table 4.3) for AV collection technique and for the EE method were contrary to Karagiannidis *et al.* (2000). Karagiannidis *et al.* (2000) reported the percentage of motile sperm to generally range from 70 to 90%. Ambient temperature as such has been reported to affect sperm motility – the winter temperatures in Bloemfontein were recorded to be very low (-3.6°C) (Table 4.1), which could have played a role in affecting the sperm motility. Evans and Maxwell (1987) reported an ambient temperature of below 10°C to affect sperm quality negatively. High summer temperatures were generally found not to affect sperm motility too much (Zamiri *et al.*, 2010).

5.2.6 Sperm cell concentration

The sperm cell concentration or sperm density is an indication of the number of sperm cells per unit volume (ml) of seminal plasma. Thus for the overall number of sperm per ejaculate, the formula of semen volume x concentration is generally used (Talebi *et al.*, 2009). From Table 4.4 it is evident that the semen collected by the AV

method recorded a significantly ($P<0.05$) higher sperm concentration, compared to the EE method of collection, throughout the year (all samples). These findings are in agreement with previous findings (Mathews *et al.*, 2003). The sperm concentration measurement for the AV technique throughout the year, fell within the acceptable sperm cell concentration of $\geq 2.5 \times 10^9$ sperm/ml as reported by Paulenz *et al.* (2005) and O'Hara *et al.* (2010). Thus on a practical seasonal basis, all semen samples collected by the AV could be processed and further utilized – while the samples collected by EE would have to be discarded or used to a limited degree. From Table 4.4 and Figure 4.7 it is evident that even although there was a decline in sperm concentration during June (winter), the AV values for this month remained high and recorded an acceptable sperm cell concentration of $\geq 2.5 \times 10^9$ sperm/ml (Paulenz *et al.*, 2005; O'Hara *et al.*, 2010) – leading to the winter still recording an acceptable concentration, compared to summer. Kafi *et al.* (2004) and Talebi *et al.* (2009) on the other hand recorded a higher sperm concentration in winter, compared to the summer. However these findings were contrary to expectations, especially based on effect of low ambient temperatures in winter on sperm viability (Evans and Maxwell, 1987). The maximum sperm concentration recorded in Table 4.4 does however not rule out the electro-ejaculator for use in semen collection in Dorper rams – especially where necessitated. From Table 4.4 it may be postulated that the rams in this trial should or could not be classified as unsound or low fertile on the basis of low sperm cell concentration recorded, even after using the EE (Noakes *et al.*, 2009).

5.2.7 Sperm viability

The evaluation of sperm viability (percentage dead or live sperm) was performed with the aid of an eosin/nigrosin stain. With this stain live sperm coloured white, while dead sperm were stained red. Zamiri *et al.* (2010) reported a wide range of the percentage live ram sperm – varying between 60 and 90%. Fourie *et al.* (2004) reported mean values of 74.6±26% and 68.5±3.7% for extensively and intensively managed groups of Dorper rams, respectively. Table 4.3 in the current trial recorded 84.2±9.1% and 82.2±10.6% for the percentage live sperm in the AV and EE collection groups in autumn (natural breeding season), respectively. The autumn values recorded no significant ($P>0.05$) difference for both collection techniques. The

winter percentage for the sperm viability (% live sperm) in both collection techniques was however lower than that reported by other researchers. The current sperm viability results (Table 4.3 and Figure 4.8) may then lead to the speculation that the winter in Bloemfontein is not a good time for semen collection in the Dorper breed. In addition to the decline in sperm viability during winter, there were also decreases in the semen characteristics such as colour, sperm motility, wave motion, while the sperm abnormalities increased. It may be necessary to also link the effect of winter to photoperiod, ambient temperature and ultimately spermatogenesis. It is also worth noting that generally spermatogenesis is active throughout the year in the ram to a greater or lesser degree, but at the same time there are certain seasonal variations in semen quality which may also vary from region to region (Zamiri *et al.*, 2010).

5.2.8 Sperm morphology

According to the sperm morphology or abnormalities recorded (Table 4.3 and Figure 4.9), overall the semen quality in this trial may generally be seen as satisfactory. This trial recorded no significant difference in sperm morphology for both the AV and EE method – this was in agreement with the study of Greyling and Grobbelaar (1983) and Mathews *et al.* (2003). It is important to note that every semen ejaculate generally contains a small portion of abnormal sperm. The seasonal trend recorded (Table 4.3) as being ≤ 10% abnormalities, is similar to that recorded by Karagiannidis *et al.* (2000). Sperm abnormalities of up to 20% have been reported as being acceptable. Based on the morphology of the semen samples collected by both techniques in this trial throughout the year, it could be recommended that all of the semen evaluated could be used for AI or be cryopreserved. The most common abnormalities recorded throughout the trial were mainly tail-less and coiled tailed sperm – coiled tails being the most common in winter. Sperm morphology may then be affected by heat stress, transportation, temperature, collection frequency, age, season of the year, disease and nutrition. From the results of the sperm morphology recorded it may also be speculated that rams used in this trial were healthy and fertile, as sperm morphology serves as an indicator of healthy seminiferous tubules and to a certain extent of the epididymis (Hafez and Hafez, 2000). The relatively low percentage sperm abnormalities then also reflect on the staining technique and also the semen collection method being satisfactory.

5.3 Photoperiod and ambient temperature

The photoperiod and ambient temperature in the current trial is set out in Table 4.1 and Figure 4.1 and Figure 4.2. The process of spermatogenesis was explained earlier in section 2.4.6. In sheep a decrease in day light length increases the rate of spermatogenesis – due to the production of melatonin which ultimately stimulates the release of GnRH (Greyling, 2009). Melatonin which is reported to be released from the pineal gland, the retina, intestine and salivary glands stimulates the hypothalamus and pituitary to secrete higher levels of the gonadotrophic hormones (FSH and LH), and hence induce a higher sexual activity (Rosa and Bryant, 2003; Senger, 2003; Guyton and Hall, 2011). From Table 4.1 and Figure 4.1 all rams were expected to produce good quality semen from autumn to spring because of the decrease in day light length. However this trend was interrupted during winter, possibly because of the low ambient winter temperatures (<10°C) recorded – which have been reported to be detrimental to the process of spermatogenesis (Evans and Maxwell, 1987). In spring, because of an increase in the ambient temperature, this trial found a corresponding increase in semen quality (Figure 4.2, Figure 4.3 and Table 4.4). It may thus be postulated that the Dorper rams used in this trial produced good quality semen in summer, autumn and spring, mainly because of the decreasing day light length (Rosa and Bryant, 2003; Talebi *et al.*, 2009).

5.4 Libido

Figure 4.10 sets out the libido of rams recorded throughout the observation period. Libido is generally associated with the desire to copulate in the male – libido or sex drive is then mainly under the control of the male sex hormone, testosterone. Research has been carried out on many of the factors affecting libido in rams – these include early exposure to females, the presence of ewes in estrus, age of the male, social hierarchies, breeding season, previous breeding experience of the ram, shyness of the rams to the libido test procedures and sexual attractiveness of the ewe . All these libido tests are however generally superficial and could be influenced by many exogenous or endogenous factors. The importance of this trait is however not to be underestimated and is highly hereditary (Gouletsou and Fthenakis, 2010; Ungerfeld and Lacuesta, 2010).

On the scale of 0 to 5, this trial recorded the mean libido to range between 4.5±0.7 and 4.9±0.4 irrespective of the treatment group (this score is highly satisfactory). Even although not significantly different (P>0.05), the libido of the rams in the current trial was affected to a certain extent by the low winter temperatures of Bloemfontein. To detect estrous ewes, rams use the sight and olfactory cues (pheromones).The most common overt signs of libido seen throughout the trial were the sniffing of the ram and the ram following the ewe at a distance of less than 0.5 metres for a period of time (Simitzis *et al.*, 2005; Perkins and Roselli, 2007). These findings of a high libido throughout the year, even although a variation in semen quality was recorded, suggest that there is no relationship between libido and semen quality. The rams remained sexually active throughout the year, suggesting that when the ram is left with the ewe throughout the year, lambs may be produced – as a good libido generally ends in copulation (Hafez and Hafez, 2000). In addition to libido there are other factors which affect copulation and these could include for example the ability to mount (mating ability), ability to achieve an erection, the ability to achieve intromission and then the ability to ejaculate. It is worth noting that the nutrition of the rams was monitored and maintained constant throughout the current trial, and this diet may have contributed to the high libido as reported by Rosa and Bryant (2003) and Senger (2003). Cloete *et al.* (2000) reported Dorper rams to generally exhibit a high libido – so for example in a 24 h period, 14 young Dorper rams have been reported to mate 50 estrous ewes.

5.5 Body parameters

5.5.1 Body weight and body condition score (BCS)

The rams used in the current trial were physically mature (approximately 2 years of age and not in the active growing stage) and as such their body weight did not fluctuate much during the trial (Table 4.5 and Figure 4.11). The effect of nutrition on the body weight of the experimental rams was recorded and monitored by use of a scale and body condition scoring. The rams were then maintained at a body weight of approximately 90 kg (Table 4.5). This was lower than the performance information on the Dorper breed (100 to 120 kg for mature rams) reported by Ramsay *et al.* (2001). On the scale of 0 to 5, the body condition score of the rams used in the trial was generally relatively moderate (3.2±0.4) in autumn (a month after the trial started), but generally increased and reached its maximum value of 3.8±0.3 in

spring. Among the factors that could have contributed to this variation in BCS may have been human error – as body condition score is a subjective method of scoring and prone to human error. However these findings of BCS in Dorper rams in the current trial fell within the range of that for mature rams of a BCS of 3 to 4. Using both the scale and BCS, a better monitoring system was attained. The body weight then generally has two components – the basic skeletal size which is reflected by the scale, and the degree of fatness (body condition) which is determined by palpation of the tips of both the spinous and transverse processes of the vertebrae (Gouletsou and Fthenakis, 2010; Vatankhah et al., 2012). Apart from acting as an indicator of the nutritional status and maturity of the animal, the body weight then also served as an indicator of the health status of the animal. The onset of puberty and spermatogenesis are generally closely linked to body weight (Van de Merwe, 2010). Nutrition generally affects the endocrine system – animals which are properly fed tend to produce adipocytes which stimulate the production of a hormone called leptin – leptin again stimulates the GnRH neurons to increase the LH pulses (Senger, 2003).

It is worth noting that body weight of the rams is affected by many factors including genetics, age of the animal, nutrition, environmental and temperature fluctuations, hormones, season of the year, disease, and daily activities (Senger,2003; Greyling, 2009; Guyton and Hall, 2011). This trial was carried out with the hypothesis that the scrotal circumference, scrotal fat, testes weight and volume amongst others can be influenced by nutrition. Too obese or too thin rams may then influence the process of spermatogenesis. Obesity as such has been reported to interfere with the thermoregulatory mechanisms of the testes, while accumulation of fat in the scrotum could then decrease the cooling efficiency of the scrotum and pampiniform plexus. This may in turn affect the process of spermatogenesis (Johnson *et al.*, 2000; Fourie *et al.*, 2004). Thus in this trial changes in semen quality could mainly be attributed to the season of the year and/ or the semen collection technique and not to a change in body condition or body weight.

5.5.2 Body temperature

The body temperature of the rams in the two groups was not influenced to any degree by either seasonal changes and/or the method of semen collection. As

reported by Radostis *et al.* (1994), the body temperature of the rams used in this trial was close to the norm (39 °C). The recorded range (Table 4.5) fell between 38.9±0.3°C and 39.0±0.3 °C, well within the variation reported by Lowe *et al.* (2001) for normal, healthy animals – an indication of the adaptability of the breed to ambient temperature. Ultimately body temperature has been recorded to monitor the health status of the rams – any increase above the normal body temperature could be as a result of fever, which could negatively affect the process of spermatogenesis. High body temperatures generally affect the sperm by increasing the metabolic rate, which exhausts the energy reserves of the cell and ultimately decrease the life-span (Evans and Maxwell, 1987; Johnson *et al.*, 2000). Spermatogenesis in rams has been quoted to take approximately two months to complete (stem-cells spermatogonia to spermatids takes approximately 46 to 49 days in the seminiferous tubules and the sperm take between 8 to 14 days to migrate through the epididymis) (Gerneke, 1986; Schutte *et al.*,1986; Noakes *et al.*, 2009). According to the body temperatures recorded all the rams used in this trial may be assumed to have been healthy and therefore speculated to be able to produce good quality semen.

5.5.3 Scrotal circumference and volume

The scrotal circumference and volume were recorded as indicative of testes size (Table 4.5). The testes size is highly heritable trait, but is also influenced by external factors such as age, breed and level of nutrition of the animal. These measurements can thus serve as an excellent indicator in the evaluation of the males' reproductive potential (Scholz, 2010). Research carried out on these parameters have shown the scrotal circumference of mature rams generally to vary between 34 and 39 cm. This then depends on the breed type and management of the rams (Campbell, 1989). The current trial (Table 4.5) recorded the scrotal circumference to be on average 35.4 cm (AV) and 35.5 cm (EE), which is in agreement with a normal, sound adult Dorper ram (Lategan, 2012). Research performed by Arthur *et al.* (1996), Noakes *et al.* (2009) and Gouletsou and Fthenakis (2010) reported large testes to be indicated by the actual scrotal circumference and to serve as an indicator of sound rams – which are then postulated to be fertile. The rams used in this trial did not have long hair on the scrotum and/or have a small scrotum – which could be indicative of small quantities of the reproductive hormones being produced (Scholz, 2010). Large testes have been positively associated with increased semen production and a

decrease in the age at puberty. The current mature scrotal circumference and scrotal volume data may then lead to the speculation that rams in this trial had the potential to produce semen with an increased sperm motility, increased percentage of normal sperm, increased sperm cell concentration and a decrease in the percentage abnormal sperm count (Van Wyk, 2010) The rams in this trial recorded almost similar (no significant difference) testes sizes – as such any difference in semen quality would then be attributed to season or the method of semen collection and not to the testes size. These results could also serve as an indication that the management of the rams throughout the year was not biased, as rams were managed uniformly.

It is worth noting that stress factors which were considered possible were controlled and minimized throughout the year. These factors included ambient temperature, changes in the environment and diet, parasites and disease (Evans and Maxwell, 1987). As previously mentioned the rams were managed under the five rights of animals in a feedlot. These humane conditions were provided to make the trial reach its aim in evaluating the natural seasonal variation of semen quality in Dorper rams, under stress-free conditions, using the different semen collection techniques.

Chapter 6

Conclusions and Recommendations

From the study it may be concluded that the current demand for Dorper sheep may encourage animal scientists (reproduction technicians) to embark upon disseminating the genetic material or semen of Dorper rams in South Africa and far afield. It has been researched that acceptable fertility resulting from AI start with an acceptable semen collection technique. Thus the initial step in creating a sperm cryobank is the use of an effective method and time of semen collection. The results from the trial recorded that both the artificial vagina and electro-ejaculation collection methods were able to yield semen from rams throughout the year. However the artificial vagina collection method generally yielded significantly better semen quality than electro-ejaculation collection method. Out of eight semen parameters tested (semen volume, semen colour, semen pH, sperm cell concentration, sperm motility, sperm wave motion, sperm abnormalities and sperm viability) six recorded a significant ($P<0.05$) difference, while two parameters remained similar (semen pH and sperm abnormalities) throughout the year. This trial concluded that the AV be used as a method of preference, because it was shown to be the most humane and the rams were always eager to be collected using the AV method. The rams rejected the EE method and always showed a certain degree of resistance and discomfort when herded to the semen collection area. However technicians are also encouraged to learn how to use the EE, as there could arise circumstances warranting or necessitating its use. The absence of a reliable, repeatable technique when using the EE is lacking.

The reproductive activity in sheep has been reported to be seasonal – the one part of this trial was then to evaluate the seasonality of the Dorper breed, as literature reported little information regarding the fertility of these rams throughout the year in South Africa. From the records of this trial, generally semen of significantly ($P<0.05$) higher quality was recorded in summer, autumn and spring. This report may then recommend that semen should only be collected during these seasons. The cold winter of Bloemfontein was recorded to be detrimental to semen quality.

Other parameters, including libido, body weight, body condition score, body temperature, scrotal circumference and scrotal volume generally did not show any significant variation throughout the year – leading the researcher to conclude that when undertaking a project of this nature, the age of the rams should be considered. In addition to these parameters this trial showed that nutrition of the rams should be considered as important, as most parameters are affected (directly or indirectly) by nutrition. The literature linked nutrition to growth, age at puberty, spermatogenesis and health status of the male.

The objectives of this research in increasing the knowledge and insight regarding the concept of seasonality in male sheep, the reproductive physiology of the ram and the practical aspects of semen collection and evaluation were ultimately met.

From this trial the following aspects may be recommended:

1. Further studies must be conducted using more rams as large numbers of experimental animals may allow a more meaningful statistical comparison.
2. Objective test of semen evaluation for example using the Computer Assisted Sperm Analyser (CASA), may be used to reduce human error as the techniques used in this trial were not always repeatable.
3. To reduce the pain caused when using the EE collection method, animals should be sedated. Even although this trial found EE collection method as inferior, it still remains an alternative method for animals with spinal or hind legs problems. Apart from being used on the untrained male, the electro-ejaculation technique also remains useful in wild animals and endangered species.
4. Blood samples should also be included to test for certain hormones, for example testosterone – to correlate to e.g. libido.

ABSTRACT

SEASONAL VARIATION IN THE SEMEN QUALITY OF DORPER RAMS, USING DIFFERENT COLLECTION TECHNIQUES

by

Cosmas Masankosa Malejane

Supervisor: Prof.J.C. Greyling

Department: Animal, Wildlife and Grassland Sciences

University of the Free State

Degree: M. Sc (Agric)

The aim of the study was to evaluate the seasonal variation in semen quality of Dorper rams using different semen collection techniques. The Dorper breed was used because of its renowned adaptability, hardiness, veld utilization, good mothering ability and high general global demand. The study was carried out on the University of the Free State campus, in Bloemfontein, South Africa from January 2012 to January 2013 (summer to summer). Eleven mature Dorper rams, recording a mean body weight of 69.6±9.2 kg and mean age of 18±4.7 months were used in this trial. The rams were clinically examined for breeding soundness prior to the study with the aid of a veterinarian, and then randomly divided into two groups. A group of 6 rams were trained for semen collection with the aid of the AV, while in the remaining 5 rams semen were collected using the EE. Two Dorper ewes were used as teasers during the training and for the entire duration of the trial in the AV group. A general management schedule for disease prevention was followed with water provided *ad libitum* throughout the trial and all rams fed a 2.5 kg maintenance diet (8.5MJ ME/kg) per day – the nutritional regime thus remained uniform and constant throughout the trial. A laboratory to perform the semen evaluation was set up adjacent to the semen collection area. Semen was collected weekly (Thursday) with the aid of the artificial vagina (AV) or electro-ejaculator (EE). Semen ejaculates were evaluated for semen volume, semen colour, semen pH, sperm wave motion, sperm motility, sperm cell

concentration, sperm viability and morphology. Other parameters evaluated included libido (sex drive), body weight, body condition score, body temperature, scrotal circumference and scrotal volume. Climatological information for the study area during the experimental period, was obtained from the Department of Agrometeorology (UFS weather station).The effect of method of semen collection technique (AV vs EE) and the seasonal changes on the above mentioned parameters were compared over seasons. The seasons were laid out as summer (December, January and February), autumn (March, April and May), winter (June, July and August), and spring (September, October and November). The semen and body parameter results were statistically analyzed with the aid of the PROC GLM procedures of SAS (1995). Parameters were considered significant at $P<0.05$.The results of this trial general showed that semen of Dorper rams may be collected using the AV and EE methods throughout the year. However a significant ($P<0.05$) difference in semen quality collected by the AV vs EE collection method was recorded. The AV collection method generally recording significantly better semen quality than the EE collection method. The rams were always eager to be collected using the AV method, while disinclination to further application of EE method was recorded in the Dorper rams throughout the year. Generally semen of significantly ($P<0.05$) better quality was also recorded in summer, autumn and spring (both semen collection techniques).The trial found that semen for AI or cryopreservation should not be collected in winter. The body parameters recorded in this trial generally were not significantly ($P<0.05$) different between the two treatment groups due to the maturity of the rams used and general uniform management of the rams. Therefore changes in semen quality in this trial could mainly be attributed to the season of the year and/or the semen collection technique and not to a change in body parameters. The general tendency in the current trial was that the EE technique was the inferior method of semen collection – thus the AV is recommended as the most acceptable method of semen collection in Dorper rams. If the circumstances are such for the use of electro-ejaculation is necessitated, operators must strive to collect semen by applying electrical stimulation in the most humane possible way.

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