Effect of different dietary energy levels on productive and Reproductive traits in Dorper rams

by

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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 for a degree to any other university. I furthermore cede copyright of this thesis in favour of the University of the Free State.

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List of Abbreviations

ADG	Average daily gain
AI	Artificial insemination
AV	Artificial vagina
Co	Cobalt
CP	Crude protein
Cu	Coper
DM	Dry matter
DMI	Dry matter intake
DMSO	Dimethylsulfoxide
EE	Electro-ejaculation
FCR	Feed conversion ratio
FSH	Follicle stimulating hormone
GE	Gross energy
GnRH	Gonadotropin releasing hormone
He	High energy
ICSH	Interstitial cell stimulating hormone
Le	Low energy
LH	Luteinizing hormone
LN_2	Liquid nitrogen
Mn	Manganese
Me	Medium energy
ME	Metabolisable energy
NDF	Neutral detergent fibre

SC	Scrotal circumference
Se	Selenium
SSH	Spermatogenic stimulating hormone
UHT	Ultra-heat-treated
Zn	Zinc

Chapter 1

General Introduction

In South Africa, the poor reproductive performance of livestock is a major limitation for animal production. The annual lambing percentage is approximately 77% (Greyling & Schwalbach, 2002). Ideally, the annual lambing percentage in sheep flocks should be between 95 and 100%. The reproductive performance may be limited by several factors, of which the environment and particularly the nutritional status of the animal is often the most important. The effect of nutrition, particularly underfeeding and flush feeding on female fertility has been extensively studied (Helali *et al.*, 1990; Kleeman *et al.,* 1991; Lozano *et al.,* 2003). However, nutritional effects on male fertility have not yet received the same attention by researchers worldwide.

It is a common practice in South Africa to feed high energy diets to young rams during performance testing trials or during their conditioning for shows or auctions. However, very little is known about the effects of such diets on subsequent ram fertility. Some farmers and animal scientists are against the fattening of breeding rams, as it is believed that this reduces their fertility.

There are very few studies done on the effect of high energy diets on ram fertility. According to Brown (1994) overfeeding of sires can have serious detrimental effects on their future reproductive capacity. In a study in which 2 levels of dietary energy and/or protein were fed to mature Merino rams, Braden *et al.* (1974) found dietary energy level to significantly affect the daily sperm production and testes weight. However, protein

level was shown to have no significant effect on these parameters. In a recent study Labuschagné *et al.* (2002), demonstrated the detrimental effects of high energy diets on the fertility of young bulls. Both semen quantity and quality of high energy fed bulls were inferior, when compared to medium energy-fed bulls. This was probably a result of impaired testicular thermoregulation, due to fat accumulation in the scrotum, most of it over the vascular cone in the neck of the scrotum. Similar findings were reported by Fourie *et al.* (2003) in young Dorper rams.

In general, ram semen cryopreserves (freezes) unsatisfactorily when compared to bull or even human semen, and about 40 to 50% of the sperm die during the freeze-thawing processes (Watson, 2000). There is thus still much research needed to improve the efficiency of cryoperservation in ram semen and the factors affecting sperm survival. There are no reports in the literature on the effects of nutrition on the cryopreservation tolerance of ram semen. However, there is anecdotal evidence that suggests that high dietary energy levels may have detrimental effects on the cryotolerance of ram semen.

The aims of this study were:

(i) To evaluate the effect of high energy diets on the growth performance, carcass, scrotal and testiculr characteristics of young Dorper rams.

(ii) To determine the effects of dietary energy levels of young Dorper rams on their semen quantity, quality and cryotolerance (resistance to freezing).

(iii) To evaluate the reversibility of the hypothesised detrimental effects of high energy diets on testicular, scrotal and semen characteristics of Dorper rams during subsequent periods of nutritional restriction. In other words, the one aim was to evaluate if the detrimental effects induced during the conditioning (fattening) of rams (i.e. before a sale) are reversible once they are placed on veld.

This dissertation is presented in the form of three separate articles, augmented by a general introduction, a literature review and general materials and methods that eventually create a single unit. Although care had been taken to avoid unnecessary repetition, some has been inevitable.

Chapter 2

Literature Review

Factors affecting the quality and freezability of ram semen

The most important factors affecting testicular characteristics and semen quality and freezability in rams are reviewed in this chapter. Of particular importance are the effects of high energy diets on ram semen quantity and quality.

2.1 Factors affecting puberty and the onset of spermatogenesis

According to Laing (1955) puberty in the male is not reached suddenly, but gradually phases in after transition from the infantile state. While the testis may not be fully descended from the abdomen and sperm production does not take place, to the same level of the adult stage, both spermatogenic tubules and interstitial cells are actively producing sperm cells and the testicles are in the scrotum.

Salisbury *et al.* (1978) showed that at puberty the testes are subjected to increased levels of gonadotropins namely follicle stimulating hormone (FSH) also known as spermatogenic stimulating hormone (SSH) and luteinizing hormone (LH) also known in the male as interstitial cell stimulating hormone (ICSH), produced by the anterior pituitary gland. The sequence of hormonal events controlling spermatogenesis appears to be as follows:

1. At puberty, increased levels of the gonadotropin LH, stimulate the Leydig cells (interstitial cells) of the testes to produce androgens (testosterone).
2. The androgens, acting locally in the testes, initiate the early stages of spermatogenesis, perhaps acting in conjunction with FSH.
3. The gonadotropin FSH stimulates spermiogenesis and in the presence of androgens, completing the production of spermatozoa.

4. Continued spermatogenesis is maintained by a reciprocal balance of FSH, LH, œstrogen and testosterone.
5. In addition to its effect on the entire male sexual reproductive tract, testosterone also aids in maintaining optimum conditions for spermiogenesis, and semen deposition in the reproductive tract of the female for fertilization. There is no doubt that other hormones (for example growth hormone) are also involved as interacting agents in the processes of onset of puberty and spermatogenesis.

Lunstra *et al.* (1978) and Evans *et al.* (1996) consider that there is a clear chronological relationship between the development of the endocrine and gonadal systems in sexually maturing bulls. The increase in LH secretion, with elevated FSH secretion, appears to initiate testicular maturation and spermatogenesis. Increased testosterone secretion occurs as the most mature cells, resulting from spermatogenesis, are produced. Breed, age, feed intake and probably other factors influence the onset of puberty in rams.

The testes in rams usually start to increase in size at an age of 8 to 10 weeks and a body weight of between 16 to 20 kg. This coincides with the enlargement of the seminiferous tubules and the appearance of primary spermatocytes. At an age of between 4 to 6 months, with a live weight of 40 to 60 % of mature weight, copulation with ejaculation of viable spermatozoa may occur. There are however, some breed differences. The sexual development process may be accelerated in rams born in autumn – first exposed to long and then to short periods of daylight (Hafez & Hafez, 2000).

The effect of age and body weight on the ability of animals to produce semen has long been recognised and the effects of age and body weight is difficult to separate when semen-producing capacity is evaluated (Almquist & Cunningham, 1968; Hahn *et al.,* 1969). Brown (1994) found high energy intake to have beneficial effects such as an advancement of onset of puberty, as a result of enhanced reproductive development and increased testicular size and sperm production in both young and adult animals – whilst excessive intake can have detrimental effects on reproduction.

According to James (1968) semen production appears to be more related to liveweight than to age and Salisbury *et al.* (1978), reported growth rate of the ram to be the main factor influencing the early production of semen. Any event that limits the growth rate of rams during pre-pubertal development – such as nutrient deficiency, disease or injury – delays, but does not prevent the eventual manifestation of puberty. Puberty is reached at later ages and lighter body weights in rams retarded in growth. The highly significant correlation between testicular weight and age (approximately 80%) indicates that approximately 80% of the variation in testicular weight is associated with body weight in rams (Cloete *et al.*, 2000). However, Salisbury *et al.* (1978) reported that the relationship between testis size and body weight decreases or is diminished after puberty. Proper ram-lamb management is thus very important. After puberty, the effect of body weight and growth on semen production is still important for some time, but as maturity is reached, other factors begin to play a more important role and can change the relationship of body weight size and semen production.

In mature animals the relationship between body weight and testis size, and between testis size and sperm cell production, is lower. Coulter *et al.* (1997) reported testicular tone to decrease over time in bulls, while Pruitt and Corah (1986) found higher dietary energy levels not to hasten sexual development as measured by serum testosterone concentration, age at first mating or age at puberty in young bulls. Bulls that recorded higher serum testosterone concentrations at 1 year of age were younger at puberty. When corrected for age, there was no relationship between testosterone level and scrotal circumference.

2.2 Establishment of spermatogenesis in the young ram

According to Cole and Cupps (1969) there is a slow growth rate in ram lambs during the first 2 or 3 months after birth. Thereafter growth is more rapid, when spermatogenesis starts. At the end of the 5th month, testicular growth slows down as the initial spermatogenic process is completed. There are two types of cells present in the seminiferous tubules during the first period (2 to 3 months after birth): the supporting cells (with a small, highly stainable nucleus) which are located along the basement membrane and the gonocytes (with large and lightly stained nuclei) which are found in the central part of the seminiferous tubules. The supporting cells are transformed into Sertoli cells and the gonocytes into spermatogonia. The supporting cells start to multiply between 4 and 6 months after birth and then take the form of Sertoli cells, while the number of gonocytes continue to increase until adulthood. The number of gonocytes thus increase progressively, until maturity. In the lamb many of these gonocytes give rise to A-type spermatogonia, followed, at approximately the 105th day, by primary spermatocytes, and, approximately the

120-125th day, by spermatids. The last stages of the cycle of the seminiferous epithelium occur only towards the 140-150th day of spermatogenesis.

2.3 Hormones involved in the control of spermatogenesis

The functions of the testes, namely the production of spermatozoa and androgens, are regulated by specific hormones. These hormones, called gonadotropins, are released into the bloodstream by the pituitary gland, which is located in the base of the brain. The production of spermatozoa and androgens by the testes ceases without gonadotropin support. Production and release of gonadotropins by the pituitary are in turn controlled by other centres in the brain (hypothalamus), which also respond to environmental stimuli. The main gonadotropins maintaining and regulating spermatogenesis are FSH and LH (Evans & Maxwell, 1987).

2.3.1 Follicle stimulating hormone.

This hormone (FSH) is also known as spermatogenesis stimulation hormone (SSH) (Hafez & Hafez, 2000). Schoeman and Combrink (1987) showed that at puberty, the blood FSH concentration normally reaches a peak and causes hypertrophy of the Sertoli cells and an increase in diameter of the seminiferous tubules. FSH stimulates spermatogenesis in the seminiferous tubules (Laing, 1955). There however seems to be some controversy regarding the importance and role of FSH in maintaining spermatogenesis. According to Cole and Cupps (1969), FSH stimulates the seminiferous tubules to increase growth but does not hasten the appearance of mature spermatozoa. Evans and Maxwell (1987) stated FSH to be necessary to initiate the production of spermatozoa at puberty or at the begin-

ning of the breeding season, but does not appear to be required for the maintenance of spermatogenesis.

2.3.2 Luteinizing hormone

In the male this hormonae (LH) is known as interstitial cell stimulating hormone (ICSH) (Hafez & Hafez, 2000). LH acts on the Leydig cells (interstitial cells) of the testes and stimulates androgen (testosterone) production (Laing, 1955). The testosterone in turn acts on the seminiferous tubules to promote spermatogenesis (Evans & Maxwell, 1987). Foster *et al.* (1978) stated an increase in both volume and activity of the Leydig cells to be caused by the secretory pattern of LH. It may be possible that it is through testosterone secretion that LH has an effect upon the seminiferous tubules (Cole & Cupps, 1969).

2.3.3 The male sex hormone testosterone

The testes, and particularly the Leydig cells, produce the male sex hormone (androgen) testosterone. Although there is some local action of testosterone within the testes, (stimulation of spermatogenesis), most of this hormone drains via the blood vessels to the general circulation (Evans & Maxwell, 1987). Here it acts as a growth promoter, stimulating the male secondary sex characteristics, producing the characteristic male body conformation, and acting on the behavioural centres of the brain to promote male sexual behaviour (libido). This is in agreement with Laing (1955) who maintains that testosterone is also important for mating behaviour, the post-pubertal development and the later maintenance of functional integrity of the accessory reproductive organs. Testosterone levels begin to increase from well before puberty.

2.3.4 Stimulus for gonadotropin secretion in the ram

The main external stimulus that affects gonadotropin secretion in the ram, is daylight length (Evans & Maxwell, 1987). As daylight shortens, there is an increase in the secretion of gonadotropins by the pituitary gland, resulting in stimulation of testicular function in rams. Factors such as temperature, nutrition, disease and stress may also modify pituitary secretion.

Social factors are also involved in the regulation of spermatogenesis. The sudden introduction of an oestrous ewe is known to stimulate LH secretion in rams, presumably via olfactory and/or visual stimuli. Although changes in daylight length may reduce gonadotropin secretion during the non-breeding season, there is always sufficient gonadotropin released to maintain a relatively low level of production of spermatozoa and androgens. Cole & Cupps (1969) indicated that rams do not to become sterile during the non-breeding season, but rather show reduced testicular size, decreased production of spermatozoa and reduced fertility. There exists a variation in the degree of seasonality recorded within a breed. Since sex drive (libido) is also dependent on androgen production, this behavioural characteristic also varies with season.

2.4 The process of spermatogenesis

2.4.1 Spermatogenesis

During early foetal development, stem-cell spermatogonia are formed from primordial germ cells and become established in the walls of the seminiferous tubules. The spermatogonia remain inactive until puberty occurs. At puberty the stem-cells begin cellular divisions to produce spermatozoa and the population of stem-cell spermatogonia (numbering several million), remain constant throughout life. Stem-cell spermatogonia contain the full diploid number of chromosomes (2n=54) in sheep. At puberty, spermatogonia begin to divide mitotically, and undergo several divisions to produce more diploid spermatogonia. These new spermatogonia are distinct from the stem-cells, and after their final division transform into primary spermatocytes. The primary spermatocytes (2n) undergo two meiotic divisions, first becoming secondary spermatocytes, and finally haploid spermatids (n=27 chromosomes in sheep) are produced (Figure 1).

Each primary spermatocyte yields 4 spermatids, but the earlier division of the spermatogonia ensure that the number of spermatids resulting from each division of the stem-cells is much higher. However, the process of cell division is error-prone and sensitive to physiological changes brought about by disease or changes in nutrition, temperature, etc, and many potential spermatids are lost along the way (Evans & Maxwell, 1987).

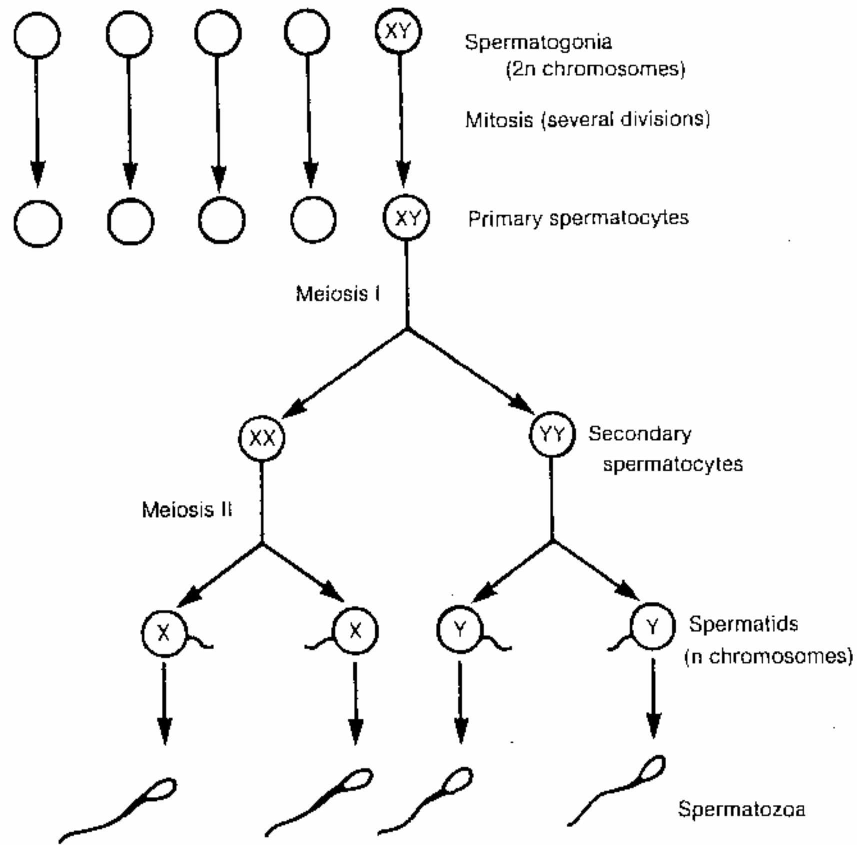

Fig 2.1 Schematic representation of spermatogenesis
(Evans & Maxwell, 1987)

Salisbury *et al.* (1978) state spermatogenesis to consist of two main phases. During the first phase, growth of spermatogenic tissue occur by simple cell division, followed by a reduction division (meiosis) that halves the cell's chromosome number (diploid to haploid). This phase of spermatogenesis is known as spermatocytogenesis. The formation of spermatids marks the end of phase 1. The allelic genes responsible for the inheritance of characteristics and carried by the chromosomes are distributed randomly among the spermatozoa in the reduction division. The second phase of spermatogenesis is called spermiogenesis. Completely formed spermatozoa are the result of metamorphosis of the

spermatids in this phase. The changes include the formation of distinct parts like the acrosome, the head, the midpiece, and the tail of the spermatozoa (Salisbury *et al.,* 1978).

Hafez and Hafez (2000) indicated spermiogenesis to be the process of transformation of round spermatids to elongated spermatozoa by a series of progressive morphologic changes. These changes include condensation of the nuclear chromatin, the formation of the sperm tail or flagellar apparatus, and development of the acrosomal cap. The different developmental stages of spermatid transformation are divided into 4 phases: the Golgi, cap, acrosomal and maturation phases. Spermatozoa are moved by internal pressure from the seminiferous tubules through the *rete testis* and *vasa efferentia* to the single epididymis duct, where the cells undergo a process of maturation. Spermatozoa are transported by contractions through the epididymis. Only when the spermatozoa reach the tail of the epididymis do they have the capacity of independent fertility and motility. Here spermatozoa remain fertile for a relatively long period of time compared to the length of fertile life in the female tract. Smooth muscular contraction beginning in the *vasa efferentia* and involving all the excurrent ducts and accessory glands are responsible for ejaculation of spermatozoa during copulation (Hafez & Hafez, 2000).

2.5 Duration of spermatogenesis

Various well-defined cellular associations that undergo cyclic changes can be observed when any cross-section of the seminiferous epithelium is studied under the microscope.

As many as 14 distinct cellular associations, or stages are identifiable in some species. A complete, time-dependent cycle of the stages, known as the cycle of the seminferous epithelium, has been defined as “a series of changes in a given area of seminiferous epithelium between two appearances of the cellular association or developmental stages.” Various stages of the cycle are used to classify the steps in spermiogenesis. The time necessary to complete a cycle of the seminiferous epithelium varies between domestic species. The duration of one cycle is about 10 days in the ram. Four to almost 5 epithelial cycles, depending on the species, are required before the type A spermatogonia from the first cycle have completed the metamorphosis of spermiogenesis (Hafez & Hafez, 2000).

The time that it takes from the activation of the stem-cell to the release of free spermatozoa in the tubules, is approximately 40 days in the ram. Passage through the epididymis takes a further 10 – 14 days, giving a total of 50 – 54 days to form a sperm cell (Evans & Maxwell, 1987). Salisbury *et al.* (1978) and Cameron *et al.* (1988) reported spermatogenesis takes 40 to 49 days to be completed, not considering the time necessary to pass through the epididymis. It will take a further 11 days to travel through the epididymis if the rams ejaculate regularly. If the cycle is interrupted by disease or any other stress factor, normal fertility will not be restored until a full spermatogenic cycle has occurred. This means that temporary sterility may persist for several weeks. Similarly, following initiation of a new cycle at puberty or at the onset of a breeding season, it takes some time before sperm production reaches a peak. The division of stem-cell spermatogonia do not occur at the same time, but each one commences a new wave of divisions at regular in-

tervals (10.5 days in the ram). As different stem-cells start a new development wave at different times, the spermatogenic process is continuous (Evans & Maxwell, 1987).

2.5.1 Factors affecting spermatogenesis, semen quantity and quality

An evaluation of semen quality and quantity as a means to evaluate the process of spermatogenesis and the potential fertility of the male, requires the collection of a semen sample. The semen collection method or technique used, may have a major effect on the quality of the sample. For this reason, a short description of the most important semen collection methods currently used in rams is given (Salisbury *et al,* 1978).

2.5.2 Semen collection techniques

Semen collection is like harvesting any other farm crop. It involves obtaining semen with the maximum number of sperm and of the highest possible quality in each ejaculate. The ultimate objective is to make maximum use of superior sires. To achieve these objectives, proper semen collection procedures, as well as sexually stimulated and prepared males are necessary. The male determines the initial quality of semen and this cannot be improved even with superior handling and processing methods. However, the quantity and quality of the semen can be significantly reduced by improper techniques (Bearden & Fuquay, 1980). Salisbury *et al.* (1978) reported the methods used for semen collection in animals to have undergone several modifications over the years, and many of these changes have been important steps in the improvement of artificial insemination.

2.5.1.1.1 The natural vagina

The vagina is the female organ in which semen is deposited during copulation in the ewe. It can also be regarded as a common passage for both the urinary and reproductive systems. The anterior part of the vagina, which contains the entrance to the cervix, forms a recess, called the vaginal fornix. During natural mating in sheep semen is deposited in this part of the vagina. Posterior to the vaginal fornix is the vestibule, a short, narrower portion that closes the vagina off from the exterior. The urethral orifice is located in the lower posterior part of the vestibule. The vestibule also contains secretory glands, which produce mucus to lubricate the vagina when the female is in oestrus (Evans & Maxwell, 1987).

2.5.1.1.2 The artificial vagina semen collection method

2.5.1.1.2.1 A basic model

Salisbury *et al.* (1978) found the artificial vagina (AV) to eliminate several of the disadvantages above the collection of semen from the natural vagina. The artificial vagina is easy to use, the semen collected is fairly clean and the ejaculate is similar to the natural sample. The AV consists of a rigid cylinder of rubber, PVC, or other material and a thin-walled rubber tube. A watertight jacket is formed inside the cylinder by turning back both ends of the thin-walled rubber tube over the outer cylinder on both sides. The jacket is filled with water hot enough (45-55 ºC) to bring the inside temperature of the artificial vagina to a few degrees above normal body temperature, through a screw-plug hole. According to Donovan *et al.* (2001) the warm water simulates the thermal and mechanical stimulation of the vagina over the *glans penis.* Into one end of the artificial vagina a graduated, glass semen collection tube of a slightly smaller diameter than the cylinder, is fit-

ted. A female of the same species (preferable in oestrus) is placed in a neck clamp and the male is allowed to mount. When the male mounts, the penis is manually deviated from the natural vagina into the AV, where the male ejaculates naturally. The major disadvantage of this method is that the rams have to be trained in advance for this method of semen collection (Matthews *et al.,* 2003).

2.5.1.1.2.2 Preparation of the artificial vagina

Salisbury *et al.* (1978) noted that it is important that all parts of the artificial vagina (AV) are clean, sterile and dry before being assembled. The reason for this is to avoid contamination of the semen sample and the transmission of disease from one ram to another. All rubber parts should be thoroughly scrubbed with soap and hot water, rinsed with hot water, then with alcohol, and finally with distilled water and dried. Some consider alcohol sterilisation insufficient and sterilise the rubber parts in boiling water. The AV should be dried and stored in a dust-free cabinet or case. The jacket is filled with water at the proper temperature and pressure. These aspects could vary with the air temperature, with the delay between filling and collection, and with individual rams. At the time of collection, a temperature of between 42 ºC and 44 ºC inside the artificial vagina is usually effective. Burkhart (1973) indicated that the water temperature could be between 58-60 ºC when the AV is filled. Salisbury *et al.* (1978) also found that water at 55 ºC or higher may be required at the time of filling in cold weather or when there is considerable delay between filling and semen collection. Only by making careful temperature checks during filling and just before collection, the correct temperature can be achieved. The semen collector should always make sure that the temperature of the AV is correct. This can

easily be determined with a thermometer just prior to collection. It is extremely important to prevent the AV being too hot or too cold, as it will determine the ram's willingness to mount and ejaculate. The semen collection tube should also be warm in order to prevent cold shock to the spermatozoa. The amount of water used to fill the AV must allow the artificial vagina to expand when the ram thrusts at the time of ejaculation. After the jacket is filled with water to the proper temperature and pressure, it is important to lubricate the lining. The first few centimetres of the interior of the liner should be thinly coated with a sterile lubricating jelly that is non-toxic to spermatozoa. One should avoid excessive lubrication as the jelly is carried through the AV by the ram's penis and can contaminate the semen sample.

2.5.1.2.3 Procedures before and during (artificial vagina) collection with an artificial vagina

There are important measures to be taken before and during semen collection to insure that the best ejaculate possible is obtained. Such measures include hygiene, adequate pre-collection sexual stimulation (to bring about maximal ejaculation), and maintenance of sex drive (by changing teasers and environment) and by avoiding distractions and stress. To avoid contamination of the semen sample, the ram from which semen is to be collected should be free of chaff and dirt. By clipping the long hair off the sheath, the underline is kept clean. However, too close clipping of the sheath is not desirable because of the possibility of skin irritation (from the short hairs) and as extremely short hairs do not protect the opening of the sheath. If washing of the underline is necessary, it should be done long enough prior to collection so that the animal is dry at the time of collection as a wet

underline is more conducive to the transmission of bacteria than a dry one (Salisbury *et al.*, 1978).

2.5.1.2.3.1 Pre-collection sexual stimulation

A question frequently asked is whether the ram should be allowed to work at will or be restrained at the time of collection. Lezama *et al.,* (2003) found that the volume of semen and the number of spermatozoa collected increased considerably if rams are restrained for a few minutes before serving in the AV.

Bearden and Fuquay (1980) claimed that sexual stimulation, by exposing males to the normal courtship situation prior to semen collection, increases the number of sperm cells per ejaculate in all species. The effect of sexual stimulation has been better defined in the bull than in the ram. There are two reasons for providing adequate sexual stimulation:

1. To insure that the ram will mount and ejaculate within a reasonable period of time.
2. To insure the collection of the maximum number of sperm with the highest possible quality per ejaculate.

In order to reduce the semen processing time, the cost per mating and to be able to prepare more breeding units (insemination doses) per ejaculate, it makes sense for ejaculates with a larger volume – which also have a higher sperm fertility, higher concentration and motility. Sexual stimulation can be accomplished by exposing the male to the teaser a-

nimal for several minutes. False mounts i.e. allowing the ram to mount the teaser animal without allowing penetration and ejaculation, enhance the degree of sexual excitement. The combination of a few false mounts prior to semen collection seems to provide adequate stimulation for most males (Bearden & Fuquary, 1980).

According to Salisbury *et al.* (1978), if sex drive is to be maintained at a level where rams will continue to work on a routine semen collection schedule of one or more ejaculations per week, teaser animals must be supplied and alternated when needed, and stress distractions be avoided.

2.5.1.2.3.2 Handling of the rams during semen collection

It is important to change teasers and environment. Occasionally rams go "stale" or become disinterested in mounting and serving the AV if the same routine is followed at each collection. Two techniques can be used to stimulate the sex drive. The first is changing the teaser female animals – the efficacy of this method has been recognised for many years. By changing the teaser animal, sexual interest is usually restimulated, and more semen collections are possible. The other technique used is to change the surroundings where semen collections are made. The collection area can be changed from indoors to outdoors to get a better response in slow-reacting rams. Other changes that can be made are changes in the staff, in their appearances and routines. The semen collector must be constantly on the lookout for signs of sluggishness or slowness on the part of the ram and make changes when necessary to keep sexual interest high (Salisbury *et al.,* 1978). One

extremely important aspect in the handling of the ram during collection, is to avoid distractions. An excited, mishandled, or distracted ram will frequently refuse to work. Gentle treatment of the ram is important at all times of collection. Avoid sudden moves at the time of semen collection. Movements are less distracting when dark or dull-coloured clothing is worn. Some movement by the teaser ewe will encourage some rams, while excessive shifting around may cause other rams to refuse to mount and can make handling of the AV difficult (Evans & Maxwell, 1987).

The semen collector should hold the AV at an angle to the ground so that its long axis is parallel to the line of the penis during collection. Excessive bending of the penis before or during the thrust can distract or injure the ram. One should never grasp the unsheathed penis, rather lightly grasp the sheathed portion in order to guide the penis into the AV. To attain maximum effectiveness at the time of semen collection, each ram's individual character must be learned. If the collector does not learn the individual whims of each ram, much less effective collections will result, and refusal to ejaculate in the AV may even occur (Salisbury *et al.,* 1987).

2.5.1.1.3 The electro-ejaculation method of semen collection

Gunn (1936), in Australia was the first to use the electo-ejaculation (EE) semen collection method. The method consisted of stimulating the spinal cord between the 4th lumbar and the first sacral vertebrae by placing one electrode in the rectum and the other in the back muscle. By passing a few 5-to10-second rhythmic electric stimuli through the elec-

trodes, an ejaculation was produced and the semen collected in a glass tube. The animals experienced no harmful effects, no loss of condition, no change in disposition, and no special disinclination to further application of the treatment. However, during the application of this method, the electric current produced general tetanic contractions of all body muscles, and a slight and temporary motor inability of the hindquarters and hind limbs, at the end of the treatment. A bipolar rectal electrode in contact with the floor of the rectum, was later introduced and its currently still used (Salisbury *et al.* 1978). Carter *et al.* (1990) described the EE as a two phase process. The first emission phase involves stimulation of the lumbar sympathetic nerves which form the hypogastric nerve and which supply the *ampullae* and *vasa deferentia*. The second ejaculatory phase involves contraction of the urethral muscles, which are supplied by the sacral parasympathetic nerves, forming the pelvic and internal pudendal nerves. Electro-ejaculators are electrical generators, which deliver an oscillating current of either sine-wave or pulse-wave to the nerve controlling the emission and ejaculation of semen. A sine-wave of 18 to 20 Hz has produced good results in bulls and rams. A pulse-wave enables the collection of semen from rams but is of limited value when used on bulls. Researchers claimed that EE usually produced ejaculates with larger volumes, but a lower sperm concentration, than that obtained using the AV. The total number of spermatozoa was comparable, and fertility levels also seemed to be comparable to that of ejaculates collected from the same rams with the AV (Bearden & Fuquay 1980). According to Matthews *et al.* (2003), semen collected with the aid of an AV produced a higher sperm concentration, but similar volume and morphological results, when compared to that collected by an EE. Carter *et al.* (1990) also compared the EE with the AV method, for semen collection in rams. The

repeatability of the volume of the ejaculate obtained, sperm concentration, total sperm number, percentage of normal sperm, and wave motion were slightly higher when using the artificial vagina technique. The major advantage of using EE is that no training is required by the ram using this method of semen collection.

2.7 Semen evaluation techniques

2.7.1 Semen evaluation

Sperm are unique among cells in their uniformity and function. Mature sperm are terminal cells, the end products of complex developmental processes that cannot undergo further division or differentiation. Examination of semen is the standard method of evaluating the potential fertility of breeding males, other than directly evaluating their ability to produce a pregnancy (Hafez & Hafez, 2000). The quantity of spermatozoa per ejaculate is dependant on the volume and the concentration of the semen sample (Evans & Maxwell, 1987). The qualitative characteristics of the semen include the motility and the morphology of the spermatozoa. These characteristics, as well as the colour and smell of the semen sample, should be evaluated as soon as possible after collection. No single characteristic can accurately predict the fertility of a semen sample, however examining various physical characteristics of sperm can determine the potential fertility (Hafez & Hafez, 2000).

2.7.1.1 Semen appearance and volume

Ram semen varies from milky-white to pale creamy in colour (Bag *et al.,* 2002). According to Hafez and Hafez (2000) there is a correlation between the colour and the concentration of the semen ejaculate (Table 2.1). The presence of blood in the semen is indicated by a pink colour of the semen (contamination) and can be due to injury or disease of the penis or reproductive tract. A grey or brown semen colour indicates contamination or infection of the ram's reproductive tract. Urine can be present when an electro-ejaculator is used and this is indicated by a yellowish discoloration of the semen, often diluted and with a strong odour. Contaminated semen samples should be discarded. Semen volume varies according to the method of collection. Larger volumes ussaully result from EE, compared to the AV method of the semen collection. However, Matthews *et al.* (2003), reported no significant differences between semen collected from Dorper rams by AV or EE in terms of volume. A significantly higher concentration and better percentage of live sperm cells were however recorded using the AV. This is in agreement with the findings reported by Matter and Voglmayr (1962). Hafez and Hafez (2000) indicated that ram age and condition, season, skill of the collector and frequency of collection to affect ejaculate volume. False mounts may increase ejaculate volume when an AV is used. The ejaculate volume ranges between 0.5 and 2 ml in mature rams, and 0.5 and 0.7 ml in young rams. The ejaculate volume will decrease if a ram is collected three or more times per day or for lengthy periods of time. Gil *et al.* (2003) using the AV to collect semen from rams, regarded a volume of between 0.75 and 2ml to be normal. Moderately frequent ejaculations (16 times daily) seem to assure a higher sperm output, while more frequent (20 times or more) ejaculations per day can induce possible tiredness or "block" of the ejaculatory process (Salamon, 1964). The volume of semen obtained can be assessed either in a cali-

brated collecting glass or, more accurately, using calibrated pipettes (Evans & Maxwell, 1987).

2.7.1.2 Sperm concentration

Table 2.1 indicates the colour of the semen ejaculate as an indication of sperm concentration in rams

Table 2.1 Subjective assessment of sperm concentration according to the colour of the semen sample

Score	Colour	Number of sperm (10^9)/ml	
		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	Insignificant

Source: Hafez and Hafez, (2000); Evans and Maxwell, (1987)

Hafez and Hafez (2000) reported that the accurate determination of the concentration and volume of an ejaculate determines the number of insemination doses and consequently the number of females that can be inseminated with an ejaculate. Sperm concentration in the ejaculate is measured with the aid of a haemacytometer, a colorimeter, or spectrophotometer. The haemacytometer is a microscope slide with precisely scored chambers (volume). A semen sample of the ejaculate is diluted in a fixed ratio with water to kill the sperm cells and thus render them immobile. The number of sperm cells lying in a chamber is counted under the microscope and multiplied by the dilution factor used (Loskutoff

& Crichton, 2001). This is a very accurate technique, but time consuming. The spectrophotometric or colorimetric methods can be used instead. The advantage of these methods is that they are accurate and fast to implement. A spectrophotometer, calibrated at 550nm, is preferred for determining sperm concentrations. A standard curve indicating concentration versus 0.5% increments of light transmittance gives the range needed to determine the concentration. Photometers are not accurate with contaminated semen samples, and the addition of cloudy extenders prior to estimation of concentration can also influence the results (Hafez & Hafez, 2000).

2.7.1.3 Sperm motility

The semen sample can also be evaluated according to the wave motion, and is thus referred to as the mass motility. The normal semen of rams exhibits a wave-like motion when examined for motility under a microscope. In fact, experienced inseminators are able to observe wave motion in the collection glass with a naked eye, but accurate assessment requires the use of a microscope. An estimate of the motility of sperm is made, based on the vigour of the wave motion or on the overall sperm activity if wave motion is not present. This is usually assessed on a 0 – 5 scoring system, where 5 is very good wave motion and 0 is when the sperm is motionless. Although this form of assessment is subjective, with practice an accurate estimate of the motility of semen can be achieved (Evans & Maxwell, 1987).

Hafez and Hafez (2000) reported sperm motility assessment to involve the subjective estimation of the viability of the sperm and the quality of motility. The light microscope

at low magnification (x200 to 400 magnification) is most commonly used to analyse sperm motility. Fresh, raw and extended semen can be used to evaluate sperm motility. High sperm concentrations in a fresh semen sample can hamper the assessment of motility, making it difficult to discern individual motility patterns. This limitation can be overcome by using an aliquot of diluted semen (concentration 25 $x10^6$ sperm/ml) in a good quality semen extender. Sperm performance in its own accessory gland fluid (seminal plasma) can be evaluated if a raw sample semen is used. Sperm motility is extremely susceptible to environmental changes (such as excessive hot ambient temperatures) – thus it is necessary to protect the semen from harmful agents or conditions prior to evaluation. An experienced person and a properly equipped microscope is necessary for a reliable estimation of semen motility. A drop of extended semen is placed on a glass slide and a glass cover slide is placed over the drop and then observed using a microscope with a built-in warmer stage and phase-contrast optics.

Parameters of sperm motility include the following (Hafez & Hafez, 2002):

Percentage of sperm motile (normal is 70 to 90% motile sperm)

Percentage of sperm, progressively motile (normal is 70 to 90% motile sperm)

Sperm velocity (based on a subjective scale of 0 to 4)

Longevity of sperm motility in fresh semen sample (at room temperature of 20 to 25 ºC), and in extended semen (at room temperature, or refrigerated temperature – 4 to 6 ºC).

Various patterns of sperm motility are visualised. Sperm motility in diluted semen samples is a long semi-arc pattern. The degree of motion is used to score a sample. Sperm motility can be influenced by many factors. In ram semen an initial motility of 90 % is acceptable for further processing and freezing (Bag *et al.*, 2002). Loskutoff and Crichton (2001) recommended the evaluation of motility in 200 individual sperm and the use of a calculated mean, using the following criteria:

0 = no movement

1 = head movement only (no forward sperm progression)

2 = slow forward sperm progression (usually with labored head movement)

3 = fast forward sperm progression

4 = faster forward sperm progression

5 = fastest, linear forward progression

The semen extender used may slightly alter the motility, usually by increasing velocity measures. After initial extension, a high percentage of sperm may exhibit a circular motility pattern, which usually resolves after 5 to 10 minutes in the extender. It would appear as if the sperm cells are reflecting light if excessive fluid is present between the slide and cover slide, while the sperm move forward. In the case of less fluid the cells may appear to move in a two-dimensional pattern. Sperm swimming in a tight circular motion could indicate cold shock. Oscillatory motion may indicate aged or dying cells. Infertility or sub-fertility in males may be correlated to the semen motility patterns. Several procedures have been developed for the objective evaluation of sperm motility, time-lapse pho-

tomicrography, frame-by-frame playback videomicrography, spectrophotome-try, and computerised analysis (Hafez & Hafez, 2000).

2.7.1.4 Sperm morphology

Salisbury *et al.* (1978) found aging of the sperm cells to result in morphological changes, even in semen kept under controlled temperatures. Sperm preparations should be made as soon as possible after the ejaculates have been obtained from a male. Care must be taken to prevent cold shock during and after collection, until the cells are fixed on the microscope slide. Cold shock will coil the sperm tails and make it impossible to distinguish artefacts due to handling from abnormal cells produced by the testes. Freshly ejaculated sperm cells are readily broken at the neck, and can produce a high proportion of tailless sperm heads. Hafez and Hafez (2000) stated abnormal sperm cells to be present in every semen sample. Morphologic abnormalities of sperm have the greatest relationship to fertility in livestock, with heat stress causing high percentages of damage to sperm. Periods of high ambient temperature, together with high humidity may render a male infertile for up to 6 weeks and many abnormal sperm cells may appear in ejaculates collected during the recovery period. Providing adequate shade and clean cool water can minimize the effect of heat stress. The ram's fertility is questionable when 20 % or more cells are abnormal in a semen sample. Semen with more than 15% abnormal sperm should not be used for artificial insemination (AI). Gil *et al.* (2003) regarded semen with less than 10% abnormalities, as normal in sheep. Seasonal variations influence the percentage of abnormal sperm, with the number being the highest in spring, declining as the breeding season advances. An eosin-nigrosin stain can be used to evaluate sperm morphology. Stai-

ned thin semen smear slides are examined with the aid of a high microscopic magnification (x1000). At least 150 spermatozoa must be examined. Abnormal sperm are classified into the following 5 categories (Hafez & Hafez, 2000):

Loose sperm heads

Abnormal sperm heads/abnormal sperm tail formations

Abnormal sperm formations

Abnormal sperm formation with a proximal cytoplasmic droplet

Abnormal sperm tail formation with a distal cytoplasmic droplet

According to Salisbury *et al.* (1978) abnormalities can occur in the head, neck, mid-piece, tail, or any combination of these parts of the sperm cell. Abnormalities of the head include twin, tapering or pyriform, round, shrunken, large, narrow, elongated, and diminutive heads. Abnormalities of the neck include broken necks and loose heads. The most common abnormalities of the mid-piece are bent, broken, short, enlarged or thickened, double, filiform, vestigial mid-piece, and abaxial attachment of the mid-piece to the head of the sperm. The principal abnormalities of the tail are coiled, twin, broken, crooked, kinky, and truncated tails. The researchers consider not much more accuracy to be gained by examining more than 100 sperm cells in a single semen smear.

Loskutoff and Crichton (2001) classify sperm abnormalities as follows:

Primary abnormalities (those occurring during spermatogenesis in the testis). These abnormalities included the following:

Sperm head:

-Microcephalic (small heads), Macrocephalic (large/swollen heads), Double heads

-Abnormal acrosome

Mid-piece of the sperm cell:

-Swollen, Elongated, Abaxial

Tail of the sperm:

-Double, Short

Secondary abnormalities (those occurring during maturation in the epydidymis). These abnormalities included the following:

Sperm head:

-Detached, Loose/damaged acrosomes

Mid-piece of the sperm cell:

-Bent, Protoplasmic droplets

Tail of the sperm cell:

-Bent, Shoe-hook, Protoplasmic droplets

Tertiary abnormalities (those resulting from poor handling of the semen – post-ejaculation). These abnormalities include the following:

-Reacted (dead) acrosomes,

-Coiled sperm tails

2.7.2 Semen quality

High quality ram semen is classified as semen with a motility of higher than 85 % and with less than 10% abnormal sperm (Evans & Maxwell, 1987; Hafez & Hafez, 2000; Gil *et al*. 2003). Gil *et al*. (2003) also classified sperm motility higher than 70 % to be normal. Fertilising ability however does not only rely on these two parameters alone. The total number of live sperm per insemination is more important than the percentage abnormal sperm. The limiting factor in semen fertility is the inability of a single sperm to penetrate the *zona pellucida* of the ova (Hafez & Hafez 2000). The normal concentration of an ejaculate varies from 3.5 to 6 X 10^9 sperm/ml in the ram (Evans & Maxwell, 1987; Hafez & Hafez, 2000). Gil *et al*. (2003) however considered a concentration of $\geq 2.5 \times 10^9$ sperm/ml to be normal and acceptable.

2.8 Factors affecting the fertility of rams

2.8.1 Age and frequency of collection

Cook *et al.* (1994) demonstrated that as the of bulla age increase, there is a significant increase in scrotal circumference, epididymal sperm reserves, vascular cone to skin distance, length and lumen diameter of the testicular artery. Most growth in the testicular artery occurs between 6 months and 1 year of age. Thickness of the testicular artery wall and arterial-venous distance decreases significantly with age and with proximity to the testicle. Hahn *et al.* (1969) also indicated that the ejaculate volume in bulls increase with age, while Almquist and Cunningham (1968) found an increase in sperm concentration with an increase in age of the bulls. These researchers suggest that the prediction of sperm output from the measurement of scrotal circumference could be valuable in young bulls, but it holds little value in older bulls. The residual standard deviation in volume,

mass sperm motility and semen concentration tended to increase with age in rams, but to a lesser extent than the means. Semen quality traits (dead and abnormal sperm) showed an increasing tendency with age (Rege *et al.,* 2000). Skinner *et al.* (1971) demonstrated that in Dorper ram lambs, sperm concentration increased markedly after the age of 140 days and indicated that the testicular weight and the diameter of the seminiferous tubules of Dorper ram lambs increased with age. Cloete *et al.* (2000) concluded that it is clear that Dorper ram lambs can fertilize ewes at an early age.

The volume of each ejaculate decreases with frequency of collections within a day or over several days (Evans & Maxwell, 1987). Severe underfeeding and overfeeding has been shown to reduce the sexual activity of bulls, with nutrition having the greatest effect in older bulls (Wodzicka-Tomaszewska *et al.*, 1981). Age has a pronounced effect in bulls, particularly regarding testis size. It was however not possible from the data previously available to determine to what extent body weight, as opposed to age, was associated with testicular development (Coulter & Foote, 1977). Branton *et al.* (1947) found short-term weight loss not to affect semen quality in mature Holstein bulls.

2.8.2 Scrotal characteristics and thermoregulation of the testes

The function of the scrotum is to help in maintaining the lower than abdominal temperature required for efficient spermatogenesis. Temperature differences of 7 ºC have been recorded in rams between the peritoneal/abdominal and the testes temperature (Salisbury *et al.,* 1978). James (1968) recorded similar temperature differences between the body

and the testes in rams. At a room temperature of about 18 ºC the temperature of the terminal part of the scrotum is 4 to 6 ºC lower than that of the testes. The lower scrotal temperatures result from surface evaporation of moisture, convection, circulation of lower-temperature air, and heat loss by radiation. A factor contributing to the cooling and warming of the arterial blood irrigating the testes is the heat-exchange mechanism as effected by the position of the testicular artery and the surrounding *pampiniform venous plexus*, which is separated only slightly by connective tissue within the spermatic cord. In either case, the result is a cooling or warming of the testis blood supply to midway between the abdomino-testicular temperature differences – which in turn depends on the environmental temperature. When ambient temperature is so high that the gradient required for normal spermatogenesis cannot be maintained, degeneration of the spermatogenic tissue result. The extent of degeneration being dependent on the level and duration of the increased temperature (Folch, 1984). Cook *et al.* (1994) reported arterial wall thickness and arterial-venous to distance decrease with both age and proximity to the testicle in beef bulls. Increased arterial wall thickness may impede heat transfer from the arteries to veins and to the scrotal surface, resulting in increased scrotal temperature. At the top of the vascular cone, the arterial blood is very warm and heat is readily transferred to the venous blood. As the arterial blood is cooled by passage through the vascular cone, the transfer of heat is reduced as the temperature differential decreases. Thinner arterial walls and a shorter arterial-venous distance at the bottom of the vascular cone may compensate for a smaller arterial-venous temperature differential, and may facilitate the transfer of heat from the arterial to venous blood. Thinner arterial walls at the base of the vascular cone may also be more elastic and help to reduce both the pulse and blood pres-

sure. These researchers showed the dorsal part of the vascular cone and the skin distance to increase with age. The tissue between the vascular cone and the skin is assumed to be primarily fat, with low echogenicity which can easily be compressed. Fat in the scrotum, particularly over the testicular cone (neck) reduces heat radiation from the testicular artery, as heat must be conducted over a greater distance against an increased resistance to heat transfer (Cook *et al.* 1994). It appears as if the tail of the epididymis must also be maintained at a cooler temperature than the head and body of the epididymis – reflecting the function of the tail as a storage area for spermatozoa. It was concluded from this study that an increased temperature gradient results in larger epididymal sperm reserves and greater progressive motility.

It is thus agreed that the scrotum is a thermo-regulatory organ and one of its chief functions is to maintain the testicles at an optimum temperature for spermatogenesis. Sufficient temporary insulation applied to the scrotum of the ram raising the temperature of the testicles 2 to 2.5 ºC above the optimum temperature would cause an increase in the occurrence of abnormal sperm and if continued would result in testicular damage (Burkhart, 1973). Lunstra and Coulter (1996) demonstrated that bulls exhibiting abnormal high scrotal temperature patterns recorded lower percentages of sperm exhibiting normal head, tail and acrosome morphology and had a higher percentage of sperm with proximal droplets than bulls with normal scrotal temperatures. It has been stated that abnormal testicular thermoregulation can be associated with bulls with a small increase in testis size. Scrotal circumference, as a measure of potential sperm production, appears to make a significant contribution to the variation of in bull fertility (Coulter & Kozub, 1984). Coulter

and Keller (1982) indicated scrotal circumference appears to be an excellent technique to quickly, accurately, and inexpensively estimate testicular size – which in turn appears to be related to a bull's potential as a successful breeder. Similarly, a high correlation in rams was recorded between body weight and scrotal circumference (Burfening & Rossi, 1992). Coulter *et al.* (1997) also suggested that part of the increase in scrotal circumference in bulls fed high-energy diets may be a result of additional scrotal fat deposition. Insulation of the scrotal neck (simulating fat deposition within the scrotal neck) resulted in a decrease of morphologically normal sperm in bulls. This seemed to be the result of elevated subcutaneous scrotal and intra-testicular temperatures (Coulter *et al.,* 1997). It would seem as if the thermoregulation mechanisms aim at maintaining the testes at ideal temperatures may be camouflaged by increased scrotal insulation (either artificially applied or via increased local fat deposits), resulting in decreased seminal quality. There was also a significant increase in the number of head and mid-piece defects, indicating that these parts are very susceptible to damage caused by an increase in scrotal/testicular temperature (Kastelic *et al.,* 1996). Similar effects were reported by Schwalbach *et al.* (2003) in Bonsmara bulls with high scrotal fat accumulation.

2.8.3 Seasonality

Hafez and Hafez (2000) indicated that the ram does not show a definite restricted mating season, but the sexual activity is highest during the autumn and declines in late winter, spring and summer. Cole and Cupps (1969) also reported that spermatogenic activities tended to decrease as the daylight duration increased from 8 to 16 hours. The reason for this increased sexual activity during autumn is due to the decrease in daylight length that

stimulates the secretion of FSH, LH and testosterone in rams – while increased daylight length has the opposite effect. There are breed differences in mature rams in the concentration of serum gonadotropin and testosterone secretion in response to daylight length changes – especially during short days when the hypothalamic-pituitary-testis axis is most active. Melatonin (N-acetyl-5mehoxytrpt-amine) is synthesized in the pineal gland and this synthesis and secretion of melatonin is greatly elevated during darkness. The daylight length stimulates the retina and via the hypothalamus to stimulate melatonin secretion. Long daily periods of elevated melatonin secretion results in secretion of gonadotropin releasing hormone (GnRH) which are probably responsible for the induction of ovarian cycles in ewes. The pineal parechymal cells take up the amino acid, tryptophan, from the circulation and convert it to serotonin. Two steps in the metabolism of serotonin are under neural control (Hafez & Hafez, 2000). Fitzgerald and Stellflug (1991) reported melatonin to be more effective in switching the reproductive activity after testicular regression, than in preventing regression after peak activity has occurred. Further photoperiodic management (short light periods) of rams may improve the semen quality and increase fertility of the flock bred out of season. In a study done by Chemineau *et al.*, (1992) it was demonstrated that by decreasing the photoperiod or melatonin treatment was very effective in advancing puberty in rams and causing a marked increase in sperm production. Rams show seasonal fluctuations in semen qualities and scrotal circumference. The magnitude of these seasonal effects is not so marked as to prevent rams from being used for breeding purposes throughout the year. Semen of superior quality and quantity has however been collected in late summer and throughout autumn (Kafi *et al.*, 2004). Cole and Cupps (1969) reported high summer temperatures to decrease fertility in

sheep and the metabolism of spermatocytes is severely influenced when the scrotal temperature is artificially increased to 41 ºC for 3 hours. However, no relationship between rainfall or ambient temperature and breeding soundness or testicular consistency has been recorded in bulls. It was suggested that the negative effect of environmental temperature on scrotal circumference is a direct consequence of reduced spermatogenesis (i.e. testicular degeneration), as seen in bulls exposed to hot environments. A decrease in the scrotal circumference was not reflected by a significant increase in the frequency of abnormal sperm (heads and midpieces) in the ejaculate (as an indicator of testicular degeneration) or in reduction of testicular consistence at palpation (Chacòn *et al.,* 1999).

In a study carried out by Amir *et al.* (1986) in the Northern hemisphere it was demonstrated that the effect of season on sperm quality was via a significant increase in the percentage abnormal cells and a decrease in motility in the ejaculates in March (Spring) and by an increase number of ejaculated sperm cells in November – December (Autumn). It was also found that the fertility of the ejaculates, assessed by the lambing rates of ewes inseminated, either naturally or artificially, was not affected by the frequency of 5 daily ejaculations over 17 consecutive days – in spite of the significant regression coefficient of the number of sperm in both the 1st and the 5th ejaculates on the day of semen collection

2.8.4 Nutrition

The onset of puberty, expression of libido, testicular function and endocrinology are all affected by both the energy and the protein content of the diet. Normal development of ram lambs depend on an adequate plane of nutrition. The rate of sexual development is

highly dependent on the growth rate of the animal. Body weight appears to be a better indicator of the onset of puberty in rams than age (James, 1968). In overfed calves, puberty seems to occur at earlier stages, than in calves fed normal diets. Depending on the breed and sensitivity to photoperiodic stimulation, the body weight at puberty may vary from 40 to 70% of adult body weight. Dietary changes have a smaller effect on the reproductive organs of the adult male than in the growing immature animal. However Rae *et al.* (2002) concluded pre-natal under-nutrition in sheep to have effect on male reproductive development and adult function – but it resulted in a reduced ovulation rate in the female progeny. Severe feed restrictions may even result in permanent damage to gonadal and neural tissue. A reduction of androgen secretion and semen quality can be caused by a restriction of feed intake, and such effects are temporary, as re-feeding previously underfed adult animals usually restores the reproductive function. Certain components of the diet can be affected differently by the production and/or release of LH and FSH, as some nutritional regimes imposed on animals can alter the volume of ejaculates and androgen activity without necessarily affecting spermatogenesis. Hafez and Hafez (2000) suggested the effects of nutritional restrictions on fertility to be more notable in the female than in the male animal. As previously discussed, nutritional deficiencies delay the onset of puberty in the male and can also depress production and the semen characteristics. Under-nutrition or sub-nutrition has been found to delay the onset of puberty and sexual maturity in the bovine, with the sperm concentration and motility being subnormal. Under-nutrition may not merely results in morphological changes, but also functional changes in the male sex organs. In rats, a low energy intake was found to result in a decrease of secretary function of the accessory sex glands in the same order of magni-

tude as that caused by castration. The influence of underfeeding on the chemical composition of semen in bulls was also elucidated, notwithstanding the fact that the spermatogenic function of the testes seemed to remain normal (Cole & Cupps, 1969).

Despite the ability of a mature male to maintain sperm production and testosterone secretion under low levels of nutrition, the young male exhibits retarded sexual development and delayed puberty. This is caused by the suppression of endocrine activity of the testes and, consequently, retardation of growth and secretary function of the male reproduction organs. Libido and testosterone production will be affected much earlier than semen characteristics when the rams are fed low energy diets for prolonged periods of time. The effect of under-nutrition may be corrected successfully in mature animals, but it is less successfully corrected in young animals because of the permanent damage caused to the germinal epithelium of the testis (Hafez & Hafez, 2000). In general, most of the nutritional-induced changes to reproductive function in adult rams are temporary, but the severity can vary from slight effect on seminal characteristics and/or libido to infertility (Brown, 1994).

Common nutritional factors affecting spermatogenesis include caloric (energy), protein, and vitamin deficiencies, but minerals or toxic agents may also be important. Robinson (1996) reported there to be a 6 to 7 week delay in the response of sperm numbers to diet in mature rams; reflecting the time it takes for the developing spherical spermatids in the germinal epithelium to fully mature into spermatozoa in the distal *cauda epididymis*. Evidence of direct effects of nutrition on testis growth and the production of spermatozoa

in the absence of changes in the GnRH-gonadotropin system have developed the concept that both GnRH independent and dependent pathways are involved in the nutritional effects on spermatogenesis.

2.8.4.1 Energy content of the diet

Shadnoush *et al.* (2003) found that feeding ram lambs 10 % less energy than the recommended level resulted in no significantly difference in carcass weight, eye muscle area, wholesale cuts and chemical composition, compared to lambs that received a standard level of energy. However, Nel *et al*. (2004) reported dietary energy level to have a significant effect on the growth performance and carcass characteristics of Dorper sheep. Similarly, Woody *et al.* (1983) reported that the diet any energy levels influence the carcass composition, especially total fat, backfat thickness and dressing percentage in cattle, while maturity, marbling, quality grade, rib eye, and kidney, heart and pelvic fat were not influenced by dietary grain level. Chestnutt (1994) demonstrated that lambs fed restricted diets lost more fat subcutaneously than in any other parts of the body. In a study performed by Drouillard *et al.* (1991), it was concluded that the protein mass of energy-restricted lambs was unchanged, but losses in fat and water were notable. This suggests that when energy-restricted lambs are provided with sufficient protein, they are capable of maintaining body protein and that body fat and dietary protein are utilized as energy substrates.

The intensive feeding (high energy diets) of young Dorper rams increase testicular development, but seem to have detrimental effects on semen quality – as a result of excessive scrotal fat deposition that may interfere with the testicular thermoregulatory mechanisms, essential for optimal spermatogenesis. Therefore selection of breeding rams based only on scrotal and testicular measurements is not sufficient. If rams are intensively fed, their testicular measurement may seem acceptable, but the ram's reproductive potential may be hampered by excessive scrotal fat deposition, particularly in the neck of the scrotum (Fourie *et al.,* 2003). Obesity and overfeeding reduces libido and sexual activity in rams, particularly during warm weather. A change from a high to low feed intake terminates fat deposition and helps to restore sexual interest (Salisbury *et al.,* 1978). In general, the feeding of high-energy diets to young beef bulls, irrespective of age, seems to be advantageous to their productive performances (growth, feed efficiency and carcass characteristics). It seems however to also have a detrimental effect on their potential fertility (semen quantity and quality). It was concluded that the feeding of high-energy diets to young bulls results in fat accumulation in different parts of the body, including the scrotum – negatively affecting spermatogenesis (Labuschagnè, 2001). The feeding of high energy diets to bulls resulted in fat deposition in the neck of the scrotum and mimicked the insulation effect on semen quality of a covered scrotum (Coulter & Bailey, 1988). It was also found that sperm reserves of bulls fed on a medium rather than on a high energy diet to be superior. The proportion of progressively motile spermatozoa being lower in high energy fed bulls (Coulter *et al.*, 1997). Mwansa and Makarechian (1991) reported that bulls fed a high-energy diet throughout the performance testing period, resulted in reduced semen quality. The feeding of high-energy diets however in either half of the

performance testing period, did not decrease the semen quality and may help in preventing the detrimental effects of prolonged high energy diets on semen quality. These results indicated that in general, high energy diets increase scrotal circumference and prolonged high energy diets may have a negative effect on the semen quality. Cameron *et al.* (1988) observed dietary changes in rams to cause a rapid response in the semen volume collected daily. A reason for this phenomenor can be the changes in the level of testosterone secretion. Changes in testicular size can begin as soon as 3 weeks after the change in diets. A high level of energy was found to have no detrimental effect on semen quality or serving capacity of beef bulls. Pruitt and Corah (1985) concluded that within normal range of energy fed to beef bulls from weaning to the beginning of the breeding season, as yearlings, it may be more likely to underfeed large mature size breeds than to overfeed medium frame British breeds. Pruitt and Corah (1986) and Fourie *et al.* (2003) reported the nutritional effects on scrotal circumference need to be considered when evaluating bulls and rams for reproductive soundness, or when using scrotal circumference as a selection criteria.

2.8.4.2 Protein content of the diet

Lindsay *et al.*, (1984) demonstrated that the feeding of high levels of protein increase testicular weight, diameter and volume, whereas low levels of protein had little or no effect on these testicular parameters. Hafez and Hafez (2000) concluded that diets high in protein were not essential for optimal sperm production in the ram.

Nevertheless, extreme protein underfeeding (typical of poor veld during the winter) of young bulls and rams was found to reduce the feed intake of the animal and to impair the

production of semen. Thus under-nutrition was reported to have a significant detrimental effect on testicular development and semen quality in young Boer goat bucks (Schwalbach *et al.,* 2000). Irrespective of feeding levels and protein intake, the fertility of semen seemed to be identical – although the concentration of spermatozoa in semen was highest for bulls that received the highest levels of protein (Cole & Cupps 1969).

2.8.4.3 Vitamin content of the diet

Dietary vitamin A or ß-carotene deficiency leads to testicular degeneration in all farm animals as the release of pituitary gonadotropins is suppressed by a vitamin A deficiency (Hafez & Hafez, 2000). Cole and Cupps (1969) showed the deficiency of vitamin A to result in a gradual loss of germinal cells in the seminiferous tubules (spermatogonia), with the primary spermatocytes apparently being most susceptible. An avitaminosis A generally results in reduced growth rate and impaired development of the accessory sex glands and a pronounced vitamin A deficiency in bulls delays the onset of sexual maturity and suppressed libido. Vitamin A deficiency in bulls does not result in severe disturbances in spermatogenesis, until such a deficiency has progressed to an advanced stage.

In addition, testicular degeneration is responsible for reduced production and inferior quality sperm. However, in rams vitamin A deficiency may retard the development of the testes and impair the production of spermatozoa. Vitamin A deficiency induces lesions in the testes that are reversible. Injections of gonadotropic hormones or vitamin A will restore spermatogenesis, except if there is permanent damage to the testis. Vitamin

E is also important for normal reproduction, but its role in the fertility of male farm animals is poorly studied (Hafez & Hafez, 2000).

2.8.4.4 Mineral content of the diet

Hafez and Hafez (2000) observed an improvement in sperm production and fertility following supplementary feeding of Cu, Co, Zn and Mn. The concentration of Cu in the semen is known to be rather high. In the spermatozoa the Cu is localised mainly in the tail and the middle piece, but its biochemical importance remains to be clarified. However, function of testes seems to remain normal under conditions of moderate hypocuprosis (Cole & Cupps, 1969).

The role of Zn in the reproductive processes of male animals has been recognised for many years. The Zn concentration in the accessory sex glands was found to be reduced after castration and could be normalised by the administration of testosterone. Zn deficiency is known to inhibit the spermatogenic and endocrine testicular functions – a feature that may find its explanation in the concomitant inanition. Zn presumably is a functional component of certain hypophyseal hormones. Kendall *et al.* (2000) investigated the effect of Zn, Co and Se soluble glass bolus, on the trace element status and semen quality of ram lambs grazing on pastures that were not considered to be deficient in any of the either elements. A significant increase in erythrocyte glutathione peroxidase activity in seminal fluid and sperm motility, the proportion of live sperm and the proportion of intact sperm membranes was found. Plant oestrogens exert adverse effects on the male accessory sex organs. Many chemicals, rare earth salts, and ionising radiations interfere with

spermatogenesis in a variety of mammalian species, but their contribution to male infertility remain to be clarified. Cole and Cupps (1969) found hypothyrosis due to iodine deficiency to reduce the reproductive capacity of farm animals and that thyroidectomy inhibits the development of reproductive organs.

2.9 Post-ejaculation viability of sperm cells and sperm preservation methods

2.9.1 Factors affecting the viability of post - ejaculation sperm cells

The rate of sperm cell metabolism is the rate at which spermatozoa utilise their energy substrates. This can be monitored under aerobic conditions by measuring the oxygen consumption, the amount of carbon dioxide liberated, or by methylene blue reduction per unit volume of semen. While under anaerobic conditions, the rate of reduction of pH, or, chemical determinations of lactic-acid build-up, and/or, fructose reduction can be used as indicators of metabolic rate. The control of metabolic rate in sperm cells is important, as a reduction in metabolic rate, achievable by a number of factors, is necessary to extend the storage life span of sperm cells in the epididymis (Bearden & Fuquay, 1980). Spermatozoa may remain fertile in the epididymis for up to 60 days, however, in a fresh ejaculate of semen; sperm will only be fertile for a few hours if steps are not taken to reduce their metabolic rate. The measures used must be reversible, without injury to the spermatozoa if they are to be practical for semen preservation (Evans & Maxwell, 1987).

2.9.1.1 Temperature

Evans and Maxwell (1987) stated that short term semen preservation for up to 24 hours can be achieved at 5 ºC. If the semen is chilled to 15 ºC, the time it can be kept viable is

only 6 to 12 hours. However semen can be preserved frozen for a long term, using liquid nitrogen (-196 ºC) in plastic straws or by using dry ice (solid carbon dioxide at -79 ºC) in a pellet form.

The metabolic rate increases and the life span of spermatozoa decrease as the temperature of the semen increases (Bearden & Fuquay, 1980). Spermatozoa suffer an irreversible loss in motility when the temperature increases above 50 ºC. At body temperature spermatozoa will survive for only a few hours – due to either exhaustion of the available energy substrates, decrease in pH due to build-up of lactic acid, or a combination of these factors. Reducing the temperature of the semen will slow down the metabolic rate and extend the fertile life of spermatozoa, if precautions are taken to protect it against cold shock (Evans & Maxwell, 1987; Pérez-Pé *et al.*, 2001). Reducing the temperature of semen to lower its metabolic rate has been the most successful means used of extending the fertile life of sperm, as it allows the restoration of the metabolic rate before insemination. In bull semen, the metabolic activity is reduced to less than 0.02% of the metabolic rate at body temperature when frozen in liquid nitrogen at –196 ºC. Fertility of sperm can be therefore maintained for decades (Bearden & Fuquay, 1980). Spermatozoa in all species are susceptible to cold shock if cooled too quickly. Irreversible loss of motility is the most obvious indication of cold shock (Bearden & Fuquay, 1980). The most critical range for cold shock occurs when semen temperature is reduced from 15 to 0 ºC. The addition of an egg yolk or milk diluents protects ram semen from cold shock if the cooling process is slow. Both egg yolk and milk contain lecithin and lipoproteins, which protect the sperm against cold shock (Salamon & Maxwell, 2000). The second challenge when

reducing the metabolic rate and extending the fertile life of semen, is to freeze the semen. The disruption of the sperm cell membrane is the apparent reason why spermatozoa may be killed during the freezing and/or thawing processes (Söderquist *et al.,* 1997). Bearden and Fuquay (1980) observed that adequate protection is provided for bull semen by equilibration in diluents containing glycerol. Although a percentage of sperm cells are killed by freezing, the fertility of the semen will not be severely affected if sufficient motile spermatozoa are placed in the semen dose. Glycerol offers some cryoprotection when ram semen is frozen – however it is less effective than in bull semen. Van Wagtendonk-de Leeuw *et al.* (2000) disagreed with the statement that the fertility of the surviving semen is unaffected by cryopreservation and found that the post thawed live sperm's quality is negatively affected by the freezing and thawing processes.

2.9.1.2 Semen pH

The optimum range of functioning for most of the enzymes in spermatozoa is a pH of approximately 7.0. A higher metabolic rate is therefore expected when the pH of semen is maintained near neutrality. The metabolic rate will be reduced if the pH of semen deviates towards alkalinity or acidity. The practicability of altering the pH of semen to extend its life is therefore limited in a narrow range, without permanently reducing its viability. Research in this area has established the importance of diluting semen in a buffered medium that accommodates changes in pH – so that maximum fertility of the semen can be maintained (Bearden & Fuquay, 1980).

2.9.1.3 Osmotic pressure

Maximum metabolic activity is maintained by semen when diluted with an isotonic extender and spermatozoa remain motile longest when suspended in isotonic media. The sperm membrane is semi-permeable, therefore both hypotonic and hypertonic extenders will alter the transfer of water through this membrane and disrupt the integrity of the cell. Thus it is important that only isotonic diluters are used (Bearden & Fuquay, 1980).

2.9.1.4 Concentration of spermatozoa

Increasing the concentration of spermatozoa above that found in the normal ejaculate will decrease the metabolic rate. The potassium to sodium ratio in the semen will be increased by an increase in the cellular concentration. Potassium is a natural metabolic inhibitor and by increasing its concentration will also reduce the metabolic activity of the spermatozoa. Moderate dilution of semen in a buffered isotonic medium containing fructose will not greatly alter the metabolic rate, but it will extend the lifespan of the spermatozoa. Such dilution is usually done before lowering the temperature of the semen sample. However some caution must be taken when diluting semen (Bearden & Fuquay, 1980).

2.9.1.5 Sex hormones

Testosterone and other androgens can depress the metabolic rate of the sperm, but at the concentrations found in the male reproductive system no permanent effect is caused. The female's reproductive tract fluids on the other hand increase the metabolic activity of the

spermatozoa. It is thought that oestrogen is primarily involved, but other unidentified factors may also be involved. The increased metabolic activity of sperm in the female tract is likely to increase motility, which increases the frequency of collisions between spermatozoa and the oocyte in the oviduct and favours fertilization (Bearden & Fuquay, 1980).

2.9.1.6 Gasses

Bearden and Fuquay (1980) have reported the aerobic metabolism of spermatozoa to be stimulated by low concentration of carbon dioxide. If the partial pressure of carbon dioxide exceeds 5 to 10%, the metabolic rate is depressed. Carbon dioxide has also been identified as an important factor in regulating the metabolic rate in the epididymis, while oxygen is necessary for aerobic metabolism. If the level of oxygen is too high, it is toxic to the spermatozoa and will depress the metabolic rate. This is unlikely to occur in the laboratory unless oxygen or air is being bubbled through the semen. Anaerobic metabolism can proceed under nitrogen, hydrogen or helium gasses with no effect on the metabolic rate.

2.9.1.7 Light

Light intensities that are normally found in the laboratory can decrease the metabolic rate, motility and fertility of spermatozoa, if semen is in contact with oxygen. The enzyme catalayse will prevent the harmful effect of light – which suggests that light causes a photochemical reaction in the semen, resulting in the production of hydrogen peroxide. Thus

semen should be protected from light and never be exposed to direct sunlight (Bearden & Fuquay, 1980).

2.9.1.8 Antimicrobial agents

Penicillin and dihydrostreptomycin or neomycin is often added to semen during processing for cryopreservation to control microbial growth. None of the above agents has a definite effect on the metabolic rate. Antimicrobial agents may also extend the fertile life of the semen by controlling microbes, thus saving energy substrates for the spermatozoa (Bearden & Fuquay, 1980).

2.10 Long term preservation of sperm cells

2.10.1 History of artificial insemination (AI)

Yoshida (2000) stated the sperm cell to be a highly polarized and specialized cell with a tripartite structure of a head, mid-piece and tail. During the final phase of spermatogenesis it loses the ability of biosynthesis, repair, growth and cell division. In an effort to prolong the sperm cells' life, preservation of the sperm generally requires a reduction or arrest in metabolism. Investigations regarding artificial insemination (AI) of sheep were initiated at the beginning of the 20th century by Ivanov (1907). These studies were centred on diluting media that led to the development and the practical application of AI in farm animals. Artificial insemination with fresh, diluted semen was again used on a large

scale in sheep breeding programmes by the early 1930s under the leadership of Milovanov in the old Soviet Union. Research on storage of spermatozoa under artificial conditions was encouraged by the necessity to use rams over extended periods, distances and at different times of the year. To achieve this, methods to reduce or arrest the metabolism of sperm and thereby prolong their fertile life were to be developed. Researchers thus examined the storage of semen in (i) a liquid (unfrozen) state, using reduced temperatures and other means to lower sperm metabolism; and (ii) in a frozen state which involved the preservation at sub-zero temperatures (cryopreservation) (Salamon & Maxwell, 2000).

2.10.2 Liquid storage of semen

The main methods of storage of semen in a liquid state are the storage at low (0-5 or 10-15 ºC) and ambient temperatures by reversible inactivation of spermatozoa (Salamon & Maxwell, 2000).

2.10.2.1 Storage at reduced temperatures

Spermatozoa must not be subjected to cold shock during storage at low temperatures. When sperm cells are cooled to temperatures close to 0 ºC, irreversible effects can occur (Salamon & Maxwell, 2000). By gradual cooling of semen from room temperature, or by addition of lipids to the diluent, the damaging effects of cold shock can be overcome (Milovanov, 1951). Salamon and Maxwell (2000) found egg yolk (and more likely its high

molecular weight low-density lipoprotein fraction) to have the ability to reduce the loss of acrosomal enzymes, prevent degenerative changes in the acrosome and provide protection against cold shock during liquid storage.

Most researchers use glucose and fructose as the sugar components in semen diluents. Some other sugars (e.g. sucrose and lactose) only function to maintain the extra-cellular osmotic pressure of the diluent and membrane integrity of the sperm cells during storage. Several studies during the early and mid-1970s, focused on the use of organic buffers. This was done, as organic buffers have a higher buffering capacity than phosphate or citrate – being relatively non-toxic to living cells, and being able to penetrate the sperm cell – thereby promoting an intercellular increase in monovalent cations. Another important function of organic buffers is to increase the overall tonicity of the diluents, which is critical when the semen is stored in liquid form at lower temperatures (Salamon & Maxwell, 2000).

Visser and Salamon (1974) examined diluents containing tris (hydroxymethal) aminomethane (Tris), as the main component, for storage of semen from the bull, boar and ram. Buffers with efficient hydrogen ion buffering capacity have a "sperm tolerance" range in pH of 6.5 to 7.5, e.g. Tes [N-Tris(hydroxymethyl)-methylaminoethane-sulfonic acid], Hepes [N-2-hydroxyethylpiperazine-N'-2-ethanesul-fonic acid], Mops [3-(N-morpholino)propane sulfonic acid], Mes [2(N-morpholine) ethane sulfonic acid] and Pipes (piperazine-N,N-bis) (2-ethane sulphonic acid), require more research.

Currently, the tris-based media are recommended as extenders for ram semen, while whole, skimmed or reconstituted milk has also been used as extenders for ram semen for many years (Evans & Maxwell, 1987). Salamon and Maxwell (2000) reported milk to be a successful semen diluent, because of its protein fraction, which may act as a buffer against changes in pH and because it can function as a chelating agent against any heavy metals present. Cow milk is preferred above the milk of other species. Commercially available ultra-heat-treated (UHT) "long-life" skim milk has proved to be a satisfactory diluent for fresh semen. An advantage of UHT milk is that it is sterile, and thus does not require heating and may be used directly as a diluent, without further treatment. A freshly opened carton of UHT milk is used for each day.

2.10.3 Cryoperservation of semen

The first recorded attempts to freeze ram semen at -21 °C, was performed by Bernstein and Petropavlovsky (1937). These researchers successfully used a glycerol solution (1 M; 9.2%) for the storage of mammalian (rabbit, guinea pig, ram, bull, boar, stallion) and avian (fowl and duck) spermatozoa. However, glycerol, at a concentration of 18 %, has a toxic effect on spermatozoa (Salamon & Maxwell, 2000).

The storage of frozen ram semen has undergone considerable evaluation (Yoshida, 2000). Poor fertility results – approximately 5% lambing rate (Blackshaw & Emmens, 1953; Dauzier, 1956) – were initially obtained using the same method used for freezing bull semen. Salamon and Maxwell (2000) stated the "bull method" to have obvious limitations when used for ram semen. A wealth of information has been accumulated over the

years of research on the aspects of cryopreservation of ram semen – e.g. various diluents, cryoprotective agents, processing, freezing and thawing methods, cryogenic damage and post-thaw fertility of ram spermatozoa have been investigated.

2.10.3.1 Diluents

The diluents used for cryopreservation of ram semen, as for other species, generally should have the correct pH and buffering capacity, suitable osmolality, contain an energy source and should protect spermatozoa from cryogenic injury. The following diluents are the most commonly used: citrate-sugar, skim milk, lactose, saccharose, raffinose and Tris (Salamon & Maxwell, 2000).

2.10.3.2 Cryoprotective agents

2.10.3.2.1 Glycerol

Salamon and Maxwell (2000) reported glycerol to be the most commonly used cryoprotective substance in diluents for freezing ram semen. For semen frozen by the slow "conventional" method, using mainly hypertonic diluents, most researchers found the optimal glycerol concentration to be within the range of 6 to 8 % (Maxwell & Watson, 1996). Spermatozoa frozen rapidly by the pellet method, survived better with a 3 to 4 % glycerol in the diluent and Graham *et al.* (1978) concluded that glycerol levels of above 6 % are detrimental to post-thawing survival of spermatozoa. The permeability of glycerol on ram and bull spermatozoa seems to be different, as it penetrates easier in ram spermatozoa (Nauk *et al.*, 1970). The level of glycerol included in diluents for the frozen storage of ram semen is ultimately limited by its toxicity (Fahy, 1986) – which in turn depends

on the cooling and freezing rates, diluent composition, and method of addition to the semen. Evaluation of the combined effects of glycerol concentration and cooling rates (0 – 8 % and pelleting on dry ice or into liquid nitrogen) (Visser & Salamon, 1974); (0 – 16 % and cooling at 1 – 100 ºC / min) (Fiser & Fairfull, 1984), showed that the higher the cooling rate, the lower the optimum glycerol concentration. The best post-thaw sperm survival rates were observed at 4 to 6 % glycerol and a freezing rate of 10 to 100 ºC / min. Apart from the cooling rate, the optimum glycerol concentration also depends on the composition of the diluent and in particular its osmotic pressure (Salamon, 1968; Lightfoot & Salamon, 1969). Furthermore, the egg yolk level in the diluent may influence the optimal glycerol concentration. An increase in concentration of egg yolk may reduce the required concentration of glycerol (Watson & Martin, 1974). Salamon and Maxwell (2000) noted that glycerol could be added to ram semen in separate diluent fractions (two-step dilution), or by a single addition of the diluent containing glycerol (one-step dilution).

While glycerol offers cryoprotection to spermatozoa, it may also cause structural damage during the pre-freezing process. Consequently, it was suggested that glycerol should be added not earlier than 20 to 30 min before the freezing of the semen. Effective cryoprotection after short (5 to 10 s) periods of contact with glycerol has been demonstrated for bull and boar semen, and also for ram semen (0 to 5 min) – which supports the earlier view that the penetration of glycerol into the cell is not essential for cryoprotection. Removal of glycerol from thawed semen by centrifugation or by dialysis had no effect on the lambing percentage following AI in sheep (Salamon & Maxwell, 2000). Several a-

gents (dimethylsulfoxide [DMSO], ethylene, glycol, albumin, low molecular weight polyols, polymeric compounds, surfactants, high concentrations of sugar of various types, compatible solutes and anti-freeze proteins from polar fish) other than glycerol have also been evaluated for their cryoprotective action on ram spermatozoa. El-Alamy and Foote (2001) regarded glycerol as a superior cryoprotectant, as it penetrates the sperm membrane rapidly.

2.10.3.2.2 Egg yolk

Egg yolk is a common constituent of semen diluents that protects the spermatozoa against cold shock and confers protection during the freezing and thawing process. It is believed that egg yolk acts at the level of the cell membrane, and has a bigger effect on bull than on ram spermatozoa. A wide range of egg yolk concentrations have been evaluated in diluents for the cryoperservation of ram semen (Salamon & Maxwell, 2000). For ampoule freezing, egg yolk concentrations of 3 to 6 % seem to be sufficient, but for pellet freezing, 15 % was the optimum concentration suggested – although the concentration depends on the composition of the diluent (Salamon & Lightfoot, 1969). Salamon and Maxwell (2000) observed that egg yolk, although sometimes partially replaced by substance(s) with similar activity, is still likely to remain an important component of diluents used for the freezing of ram semen – particularly due to its protective effect on the plasma membrane. Watson and Martin (1976) also found evidence to suggest that egg yolk can delay the aging of ram spermatozoa.

2.10.3.3 Processing and freezing of ram semen

In general all semen cryopreservation protocols have a number of potentially damaging stresses. Firstly, the change in temperature, secondly, the osmotic and toxic stresses presented by exposure to molar concentrations of cryoprotectants and thirdly, the formation and dissolution of ice in the extra-cellular environment (Watson, 2000). Holt (2000) suggested that cytoplasmic disruption through intracellular ice formation may be further compounded by the growth of ice crystals during thawing (recrystallization).

Salamon and Maxwell (2000) reported the success of cryoperservation depends to a notable degree on the rate of dilution of the semen. Originally, semen was diluted to protect spermatozoa during cooling, freezing and thawing, but the rate of dilution was often changed for technical reasons – such as to increase the number of females which could be inseminated with each ejaculate (insemination dose), or to standardise the number of spermatozoa in each dose of frozen/thawed semen. For the freezing of ram semen nowadays, generally a 2- to 5-fold (v/v) pre-freezing dilution rate is used, with the diluent composition being adjusted for the rate of dilution (Salamon, 1976; Evans & Maxwell, 1987).

After dilution, semen should be cooled slowly to a temperature close to 0 ºC. Cooling allows for a period of adaptation (equilibration) of the spermatozoa to a reduced metabolism. Traditionally, equilibration has been regarded as the total time spermatozoa remain in contact with glycerol before freezing – during which it penetrates into the sperm cell to establish a balanced intracellular and extra-cellular concentration. Although these may be overlooked in the light of the other favourable cryogenic properties of glycerol, it

should also be noted that this medium may, to a certain extent, adversely affect fertility (Salamon & Maxwell, 2000). With the two-step method of dilution the contact with glycerol begins at 2 to 5 ºC. However, when a one-step dilution is adopted, glycerolisation begins at 30 ºC. In both methods, a period of cooling to 2 to 5 ºC of between 3 – 4h is required. Rapid cooling of extended semen from 30 ºC to about 15 ºC may have no effect on the survival of spermatozoa, but fast cooling from 30 ºC to 10 ºC, 5 ºC or 0 ºC decreases the post-thaw motility of spermatozoa (Fiser & Fairfull, 1986).

The one-step method of dilution with a glycerolated medium is generally used and it is recommended to freeze ram semen after a 1.5 to 2h cooling or equilibration period to 5 ºC (Salamon 1976, Evans & Maxwell, 1987). Salamon and Maxwell (2000) reported that there seems to be no convincing evidence supporting either a longer cooling/equilibration period, or the need for the more complicated two-step dilution procedure. The cooling rate of diluted semen from room temperatures to just above 0 ºC can significantly influence the survival of spermatozoa after freezing (post-thawing). Ram semen frozen in ampoules is generally cooled at the slow "conventional" rates applied for bull semen. This method is seldom used today, and freezing is done in PVC straws, minitubes or in a pellet form.

Cryopreservation of semen in straws is performed by suspension of the straws in liquid nitrogen vapour, and the tempo of cooling is regulated by the distance of the straws from the surface of the liquid nitrogen. Ram spermatozoa tolerate a range of freezing rates in straws. Suspension of straws in liquid nitrogen vapour between –75 ºC and –125 ºC had

no effect on the sperm, but vapour at –55 ºC reduced the survival rate of spermatozoa (Colas, 1975). Although the cooling rate was found to interact with the glycerol concentration, cooling rates from 10 to 0 ºC / min had a smaller effect on the survival of spermatozoa than glycerol concentration (Visser & Salamon, 1974; Fiser & Fairfull, 1984).

The shape of the freezing curve may also be important when freezing semen in straws. Instead of a linear decrease in temperature, it is better to cool the semen according to a parabola-shaped curve, which can be achieved by suspending the straws 4 to 6 cm above the liquid nitrogen surface. Maxwell *et al.* (1995) stated a height of 6 cm above liquid nitrogen to be optimal for the 0.25 ml and 0.5 ml straws – whereas 4 cm was optimal for 0.25 ml minitubes. It was further found that both post-thaw recovery of motility and fertility of spermatozoa frozen in PVC straws, pellets or minitubes are maintained, although fertility was better after freezing spermatozoa in pellets and minitubes than in the other forms of packaging. Therefore minitubes provide an useful alternative to pellets as a storage form for ram spermatozoa, and allow for individual dose identification and easier storage – while maintaining fertility indistinguishable from that obtained with pellet-frozen semen.

Dropping the semen straws directly into liquid nitrogen has resulted in poor post-thaw motility, or death of all spermatozoa. The reason for this may be that liquid nitrogen is not a good medium for very fast freezing of sperm as it is a liquid at its boiling point, and has a calefactory effect, which delays the thermal contact with the semen (Rey, 1957). However, when semen droplets (0.03 ml) were pipetted into liquid nitrogen, or vials with

semen (1.5 to 2.5 ml) were immersed into liquid nitrogen for 5 to 7 s and then elevated and held in liquid nitrogen vapour for a few minutes before re-immersion into liquid nitrogen, the post-thawing motility of pelleted spermatozoa was similar to that of semen pelleted onto dry ice (Visser & Salamon, 1974) and resulted in satisfactory pregnancy rates after AI (51 to 69 %) (Kasymov & Ashimov, 1975). Bag *et al.* (2002) recorded the best survival rate of ram spermatozoa when straws were cooled under controlled conditions and the initial freezing temperature was –125 ºC.

When a solution is cooled below freezing point, ice crystals are nucleated and pure water crystallises out as ice. The solutes are dissolved in the remaining liquid water fraction and the osmotic strength of the solution rises. The proportion of the water crystallising out as ice, and hence the osmotic strength of the remaining solution, depends on the temperature – the lower the temperature, the smaller the unfrozen fraction and hence the higher the osmotic strength of the solution. The cooling rate must be slow enough to allow water to leave the cells by osmosis, preventing intracellular ice crystals formation which is lethal. Sperm cells are generally frozen at rapid rates in the range of 15 to 60 ºC/min – which has been empirically confirmed as giving the best survival rates (Watson, 2000).

2.10.3.4 Thawing of semen

Salamon and Maxwell (2000) suggested that in the freeze-thaw procedure, the warming phase (thawing) is just as important to the survival of the spermatozoa as the cooling phase. Spermatozoa that survived cooling to -196 ºC still face the challenge of warming and thawing, and must thus traverse the critical temperature zone twice (-15 ºC to -60 ºC).

The rates of cooling and thawing exert an effect on the survival of spermatozoa (Mazur, 1985). These effects depend on whether the rate of cooling is high enough to induce intracellular crystal freezing, or low enough to produce cell dehydration. In the former, fast thawing is required to prevent re-crystallisation of any intracellular ice present in the spermatozoa. Spermatozoa thawed at a fast rate may also be exposed for a shorter period of time to the concentrated solute and cryoprotectant (glycerol). The restoration of the intracellular and extra-cellular equilibrium is more rapid than for slow thawing (Salamon & Maxwell, 2000).

Salamon and Maxwell (2000) observed no agreement between early researchers regarding on the optimum temperature for thawing of semen frozen in ampoules. Certain researchers found ram spermatozoa cooled slowly in ampoules by "the bull method," to be revived better, when thawed at 40 to 43 ºC, than slowly "by reversing the cooling procedures". Other workers thawed ampoules in ice water, (at 2 to 5 ºC, 6 to 8 ºC or at room temperature, 18 to 20 ºC), with contradicting results regarding the recovery rate of spermatozoa. In the majority of reports, ampoule-frozen ram semen was thawed in a water bath at 37 ºC. Ram semen frozen in straws has been thawed by most researchers at 38 to 42 ºC. Some workers reported thawing at high temperatures (60 to 75 ºC) for shorter periods of time to be comparable with that at 38 to 42 ºC – in terms of post-thaw motility, acrosome integrity and fertilising capacity of the spermatozoa. Söderquist *et al.* (1997) found spermatozoa thawed at 35 ºC not only to showed the highest incidence of membrane damage, but also the most pronounced morphological changes. Significantly higher

values for post-thaw motility were also recorded for straws thawed at 70 ºC (5 seconds), compared with those thawed at 35 ºC (12 seconds).

Moses *et al.* (1997) reported a 40 to 45 % post-thaw motility, while Van Wagtendonk-de Leeuw *et al.* (2000) recorded a 50 % mortality rate. Half the cells during cryopreservation and the quality of the remaining population being negatively affected in bulls – this is in agreement with Vishwanath (2003). The reasons for this mortality rate can be that sperm undergo volumetric changes, oxidation of the membrane lipids and experience damage to the selective permeability mechanism of its membrane. Similarly Watson (2000) recorded only 40 to 50% of the sperm population to survive cryoperservation – even with optimised protocols. When comparisons are made on the basis of similar numbers of motile spermatozoa (assumed viable), results are still generally poorer than with fresh semen – indicating that the viable sub-population after cryoperservation is compromised.

2.10.3.5 Other factors affecting the survival of post-thawed semen.

The tolerance to the freezing/thawing protocols varies between rams and ejaculates. Windsor (1997) found no guarantee that a ram whose semen showed high post-thaw fertility, was also a productive ram. It was concluded that consistent between-ram variation is likely to have a large genetic component, while variation between ejaculates within rams will be the result of environmental differences.

Chapter 3

General materials and methods

3.1 Study area and period

This study was conducted on the campus of the University of the Free State in Bloemfontein, South Africa, situated at a latitude of 29.10º South, longitude of 26.29º East and an altitude of 1351m above sea level. The study was undertaken between February and September 2003 (the end of summer and the onset of spring), in two phases. The first phase lasted 127 days and was conducted between 19 February 2003 and 25 June 2003 and the second phase lasted 90 days and was conducted between 26 June 2003 and 22 September 2003.

3.2 Experimental animals

Sixty Dorper rams aged between 9 and 10 months, previously managed extensively on natural pastures (veld) of the Brakveld area on a farm located in the Luckhoff district in the South-Western Free State, at a latitude of 29.46º South and a longitude of 24.39º East, and an altitude of 1143 m above se level were initially selected for the study. The vegeta-

tion on the farm of origin is typical Appearance Karoo, which is the transition between the Great Karoo and the grasslands to the North (Acocks, 1988). The rams had previously been vaccinated against enterotoxeamia (pulpy kidney; alum) at 3 months of age and weaned at 5 months of age. At 6 months of age the rams received a booster vaccination against enterotoxaemia (oil), and blue tongue disease and at 4 months were vaccinated against *Brucella ovis* (Rev 1). The external sexual organs of all rams (testes), were examined for palpable anatomical soundness by a veterinarian. Only rams with two symmetrical, well developed testes, of normal consistency and absence of any palpable anatomical abnormalities were selected for the initial group of 60 rams. The rams were maintained in one group, under extensive conditions (veld, mid-summer), while being trained for semen collection with the aid of the artificial vagina (AV). The AV was filled with water at 54 ºC and the rams were trained from 4 weeks prior to the onset of the study, using a ewe in oestrus, induced by an intramuscular injection of ECP (Pharmacia Animal Health) and restrained in a neck clamp. The ECP containing 2mg estradiolcypionate and 0.54 % anhydrous chlorobutanol in 1 ml a dose of 1mg/24kg and was administrated intramuscularly every third day.

Forty-two rams from the initial group of 60 that had been successfully trained for semen collection with the aid of an AV were selected and taken to the University of the Free State Campus in Bloemfontein and housed in individual metabolic cages. A 30 days adaptation period to the new environment, feeding and housing conditions was incorporated before the onset of the study. During this period, semen was collected twice weekly from each ram using the same procedures described above. The rams were dosed on ar-

rival against internal parasites with a broad-spectrum remedy (Valbantel, Pfizer Animal Health), at a dose of 1ml/5kg body weight. Thirty-six (11 to 12 months of age) rams that adapted well to the new environment were finally selected from the group of 42 rams and randomly allocated to one of the three similar experimental groups; (n=12 per group). The mean body weight (±se) of the rams (following an overnight fasting period) at the onset of the trial was 42.3 ± 0.9, 42.8 ± 0.8 and 42.2 ± 1.1 kg for the 3 groups respectively.

3.3 Housing

All rams (n=36) were housed in individual metabolic cages in a closed, well-ventilated building. The metabolic cages had square mesh (2x2cm) floors, and the rams were separated from each other by partitions of steel tubing. The cages were approximately 1.8m long, with adjustable width (Max = 1m). The width of each cage was adjusted according to the animal's size, preventing the ram from being able to turn around in the cage. Each cage was fitted with a feed and water trough. The cages were designed to prevent water from the drinking troughs contaminating the feed troughs. Fresh clean water was available ad libitum throughout the entire trial period. The cages were placed in 3 rows of 12 cages each (next to each other), with 4 rams from each experimental group in each row during phase 1 of the study. During the second phase 2, rows of 12 cages with 3 rams from each group/row were used. The cages were placed, in such a way that the rams were visible to one another.

3.4 Experimental diets

3.4.1 Feeding of the animals

One of the 3 experimental diets was randomly assigned to a group (n=12) and fed at an ad libitum basis. In an effort to minimise the wastage of the diets by the rams, animals were fed 3 times daily (8h00, 12h00 and 16h00). Each animal received 1 to 2kg feed at each feeding, depending on how much feed was left in the feed trough from the previous feeding. All the spillage was collected throughout the day and re-fed to the rams. The feed intake of each ram was calculated weekly as the difference between the total feed offered and the total feed refused at the end of the week.

3.4.2 Composition of the experimental diets

The compositions of the formulated diets are set out in Table 3.1.

Table 3.1 Physical and chemical composition (DM basis) of the 3 diets fed to the experimental rams

Item	Le	Me	He
Physical composition			
Maize (%)	0	23.8	51.05
Cotton seed oilcake (%)	0	4.8	6.0
Lucerne hay (%)	33.0	23.0	13.7
Eragrostis curvula hay (%)	65.7	47.0	27.3
Urea (%)	0.8	0.4	0.45
Feed lime (%)	0	0.5	1.0
Salt (%)	0.5	0.5	0.5
Chemical composition [1]			
Crude protein (%)	13.58	13.59	13.59
Degradable protein (%)	8.73	8.3	8.52
Calcium (%)	0.65	0.64	0.64
Phosphorus (%)	0.17	0.23	0.25
Energy (MJ ME/kg DM)	8.23	9.77	11.32

[1] Van der Merwe & Smith (1991), Erasmus *et al* (1988;1990)
Le – Low energy; Me – Medium energy; He – High energy

A standard medium energy (Me) diet was formulated according to the NRC requirements (1985) to serve as the control and to provide the nutrient requirements for growing re-

placement ram lambs (NRC, 1985). The metabolisable (ME) energy content of this diet (Me) was increased by 15 % for the high energy (He) and decreased by 15 % for the low energy (Le) diets, respectively. The 3 diets were formulated on a crude protein, degradable protein, calcium and phosphorus equivalent basis. The chemical composition of the feeds as set out by Van der Merwe and Smith (1991) and Erasmus *et al.* (1988; 1990) were used to formulate the 3 experimental diets.

3.4.3 Preparation of the experimental diets

The lucerne *(Medicava sativa)* and the hay (*Eragrostis curvula)* were ground through a 25 mm grid using a Drosky hammer mill, and the maize to be used in the diets was crushed in the same mill. The urea, feed lime and salt were dissolved/suspended prior to mixing in a small quantity of water. The feed components of the individual diets (± 500 kg per batch) were accurately weighed and thoroughly mixed with the aid of a mechanical mixer. The water suspension containing urea, feed lime and salt was mixed with each feed batch.

3.5 Experimental design

3.5.1 Trial phases

This study was conducted in 2 phases (exact periods described in 1).

3.5.1.1 Phase 1

During the 1st phase, the 36 rams were randomly allocated to 3 groups of 12 rams each and each group randomly received one of the experimental diets (Le, Me or He) as described in Table 3.1 for a period of 127 days. The experimental layout was a complete

random design with 12 replications for growth performance and reproductive characteristics data, 6 replications for carcass traits and 5 replications per group for the feed digestibility data. At the end of the first phase, 6 rams from each group were randomly chosen, slaughtered at a commercial abattoir and the carcasses, as well as scrotal and testicular characteristics, recorded.

3.5.1.2 Phase 2

The 18 remaining rams (n=6 per group) from phase 1, were maintained for a further period of 90 days on the Le diet. During this phase, the Le group served as the control group, while the energy level of both the Me and the He groups were reduced, to simulate the situation when rams previously conditioned for sale or for show are placed back on natural pastures (veld) or a maintenance diet. At the end of this phase, all rams were slaughtered at a commercial abattoir and carcasses, as well as scrotal and testicular characteristics recorded. The experimental layout was a completely random design with 6 replications for the growth performance, the reproductive characteristics as well as the carcass characteristics data.

3.6 Semen collection and evaluation

During phase 1, semen was collected fortnightly from every ram (from week 4 until the end of the trial), early in the morning (7h30 – 9h30), over 3 consecutive days (12 rams/day), with the aid of an AV and an ewe restrained in a neck clamp. On each semen collection day, samples from 4 rams from each treatment group were collected. During

phase 2, semen was collected every 3 weeks over two consecutive days using the same procedures as described in phase 1.

The volume of the ejaculates collected was recorded directly from the calibrated test tube used at the end of the AV, before being placed in a water bath (32 ºC). A volume of 10µl of semen was diluted with 990µl pre-warmed (32 ºC) TL-Hepes solution (99 TL-Hepes:1 semen). The diluted semen sample was evaluated on a pre-warmed (35 ºC) glass slide under a compound microscope (x400 magnification) within 2 minutes of collection. Microscopic evaluation of forward progression (%) and overall motility on a scale of 0 (no movement) to 5 (very fast forward movement) was performed by scoring 100 sperm cells individually (Loskutoff & Crichton, 2001).

The sperm cell concentration per ejaculate was calculated, using 10µl of the previously diluted semen sample after being refrigerated for 24 h at 4 to 5 ºC when all sperm cells were dead, with the aid of an improved Newbawer haemocytometer. The sperm cells lying inside the 5 blocks that formed a diagonal line on the grid were counted and the total multiplied by 5 to obtain the concentration of sperm cells in the semen sample in x 10^6 per ml (Loskutoff & Crichton, 2001).

Two thin semen smears were made of each fresh semen sample on a pre-warmed (35 ºC) glass slide. One slide was stained with eosin/nigrosin (60µl eosin/nigrosin and 6µl semen) and the other with eosin B-fast green (60µl eosin B-fast green and 6µl semen). One hundred individual sperm cells from different areas on each slide were evaluated

microscopically (x1000 magnification). The eosin/nigosin stained smear was evaluated for vitality (dead or live sperm) and morphology (sperm abnormalities). The sperm cells evaluated were classified as dead or live and the abnormalities were classified as head, mid-piece or tail abnormalities. An eosin B-fast green stained semen smear was used to evaluate sperm vitality (live/dead) and acrosome reaction. The sperm cells evaluated were classified into 4 groups: live/acrosome intact, live/acrosome reacted, dead/acrosome intact and dead/acrosome reacted (Loskutoff & Crichton, 2001).

3.7 Semen cryopreservation

At each semen collection session, 0.5ml semen from each ram was diluted with a semen extender containing 5 % glycerol as a cryoprotectant. All semen extender used in the trial, (all the ingredients, except the egg yolk) was prepared at the onset of the trial as described in Table 3.2 and preserved frozen in small bottles (100ml). One bottle was thawed on the day prior to semen collection in a refrigerator (4 to 5 ºC). Fresh egg yolk was added to the semen extender (1:6 parts) on the semen collection day (just prior to collection). The extender solution was placed in the water bath (32 ºC). Pre-warmed (32 ºC) semen extender (2.0ml) was added drop by drop to the 5 ml plastic screw top tube, containing semen (0.5ml), within 10 minutes following semen collection.

Table 3.2 Composition of the semen extender used for the one-step dilution and cryoperservation of ram semen

Ingredients	Dilution rate (1+4)
Tris(hydrowymethyl)amino-methane (g)	3.634
Glucose (g)	0.500
Citric acid (monohydrate) (g)	1.990
Egg yolk (ml)	15
Glycerol (ml)	5

Penicillin (IU)	100.000
Streptomycin (mg)	100
Distilled water (ml)	100

Source: Evans and Maxwell (1987)

The 5ml tube containing the extended semen (2.5ml) was gently agitated, tightly closed and placed into a 50ml plastic screw top tube containing water from the warm bath (32 ºC). This was then placed in a cool box (4 to 5 ºC) for equilibration. This procedure slows down the rate of cooling and therefore protects the sperm cells from cold shock (Loskutoff & Crichton, 2001). The coolbox was equipped with a thermometer and ice blocks to aid the cooling down of the extended semen sample to 4 or 5 ºC. In the meantime the rest of the semen samples were being collected from the other rams. After semen samples were collected from all 12 rams for the day, the coolbox containing the extended semen was taken into and opened inside a walk-in refrigerator (4 to 5 ºC). The 50ml plastic screw top tubes containing water and the 5ml plastic screw top tubes with 2.5ml of extended semen samples were removed from the coolbox, and placed in a plastic rack.

After a total equilibration period of 4 hours (in the coolbox and in the walk-in-refrigerator), the extended semen was packed into pre-labelled 0.25ml plastic straws (6 straws/animal) and sealed with PVP straw sealing powder. The filled straws were then placed in liquid nitrogen (LN^2) vapour, approximately 4cm (-70 ºC) above the LN^2 level, using a polystyrene boat, floating on a plastic box filled with LN^2 for 10 minutes – before being plunged into the LN^2 (-196 ºC). The frozen straws were then transferred into goblets and stored in a LN^2 flask (Loskutoff & Crichton, 2001).

3.8 Post-thaw evaluation of the semen

One week after freezing, one straw from each ram, was thawed in water (38 ºC) for 30 seconds and evaluated for overall motility and forward progression. Two thin smears were stained (nigrosin + eosin and eosin B-fast green) and evaluated for vitality, acrosome integrity, as well as sperm morphology using the same criteria and procedures used for fresh semen and previously described (Salamon & Maxwell 2000; Loskutoff & Crichton, 2001).

3.9 Body weight

The initial and final body weights of the rams (phase 1 and 2) were recorded (between 7h00 and 7h30) with the aid of an electronic scale, fitted into a weighing cage after overnight fasting of the rams. During the experimental period the body weight of the individual rams was recorded weekly at the same time (7h00). Overnight fasting was not implemented during the trial period to prevent possible digestive disorders and interference with the experimental procedures.

3.10 Slaughtering, grading and carcass evaluation of the rams

At the end of phases 1 and 2, 6 rams per group were slaughtered at a commercial abattoir and their carcasses, scrotal and testicular characteristics evaluated and recorded. The body weight (empty stomach) and the scrotal circumference (SC) of each ram at the end of each trial phase were determined prior to slaughter. The SC was measured at the widest part of the scrotum with the aid of a measuring tape. Following slaughtering, the carcas-

ses were skinned and eviscerated. The warm carcass weight and the carcass grades were recorded according to the official methods practised in South Africa (SAMIC, 2004). According to this grading system a code of 1 represents a very lean carcass, while 6 represents an over-fat carcass, as summarised in Table 3.3. Following slaughter, the scrota were excised from the carcasses by severing the scrotal sac at the base and as close to the body as possible, preserved by means of refrigeration (4 to 5 ºC) for 24 hours and then weighed and dissected into scrotal skin, testes, fat, epididymis and *pampiniform plexus*.

Table 3.3 Official sheep carcass classification system used in South Africa

Age description (Teeth)	Age class code	Fat description	Grade	Backfat mm
0	A	No fat	0	0
1-2	AB	Very Lean	1	< 1
3-6	B	Lean	2	1 – 3
>6	C	Lean	3	3 – 5
		Fat	4	5 – 7
		Over fat	5	7 –10
		Excessively over fat	6	>10

Source: Government notice no. R 1748, 26 June 1992.

Scrotal skin thickness was measured with the aid of a calliper at the widest part of the scrotum in the same region where the SC was measured. The weight of each trait was recorded and the total volume of the testicles and epididymis was estimated separately by measuring the volume of water displaced by gently plunging the structures into a volumetric measuring cylinder filled with water to the brim.

Carcass measurements were then taken 24h after refrigeration according to the methods described by Fisher and De Boer (1993) and Ramsay *et al.,* (1991). The external length of the carcass, the circumference of the brisket and the circumference of the buttock were measured after 24h of refrigeration. The carcass was then halved along the vertebral column with the aid of an electrical saw. The backfat thickness was measured on the left half of the carcass between the 12th and 13th rib of the *M. longissimus thoracis* (at 3 points) (Edwards *et al.,* 1989). The pH of the muscle was determined with the aid of a pH meter (Hanna HI8915) 24 hours post mortem by inserting the probe approximately 5 cm into the muscle. The kidney fat was dissected and weighed. The marbling was estimated by scoring the 3 rib cut (ribs 11, 12 and 13)**,** according to the amount of fat present in the eye muscle (score out of 5). The eye muscle area was measured by the rib eye dot grid method as the length x width of the eye muscle between the 9th and 10th thoracic vertebrae (Edwards *et al.,* 1989; Strydom, 1998).

3.11 The digestibility trial

A 7 day digestibility trial was conducted during week 14 of the 1st phase of the trial. Five rams from each group were randomly allocated and used in this trial. Each ram was fitted with a faeces collecting bag. The amount of feed offered to each animal was determined as 130% of the average daily intake during the previous 5 days (Koster, 1995). The feed refused from each ram was collected each day and placed in a marked bag. The faeces collection bag of each ram was emptied daily, the faeces thoroughly mixed, weighed and a 10% sample (weight basis) placed in an oven (80 ºC) to dry for 12 hours. At the end of the week the total amount of oven dried faeces from each ram was weighed and the total

faecal excretion calculated. A representative sample of each of the 3 diets was also taken daily, and mixed thoroughly at the end of the 7 days with the feed samples collected daily. The diets, refusals and faeces were milled through a 0.8mm screen with the aid of a laboratory mill. A representative sample of each was taken using the quartering method and sealed in clean, dry flasks for later analysis.

3.11.1 Chemical analyses

Milled diet samples, refused feed and dried faeces were analysed in duplicate for dry matter (DM), crude protein (CP) and gross energy (GE) content using standard laboratory procedures.

3.11.1.1 Dry matter determination (DM)

Approximately 1g of each sample (diet, refused feed and oven dried faeces) was weighted accurately in a 30ml porcelain crucible and dried in an oven at 100 ℃ for a minimum period of 16 hours (overnight) to a constant mass (AOAC, 1984)

Calculation method used:

$$\% \text{ Dry matter (DM)} = \frac{\text{MCDS} - \text{CM}}{\text{MOCS-CM}} \times \frac{100}{1}$$

Where CM = crucible mass

MOCS = crucible mass plus sample

MCDS = crucible mass plus dried sample

% Moisture = 100 - % DM

3.11.1.2 Crude protein determination (CP)

Crude protein was determined using the Dumas method of combustion with a LECO FP 2000 machine (Randall, 1993). A sample was combusted in an atmosphere of oxygen to produce the oxide of nitrogen and other gasses.

An oven dried sample (feed, refusal or faeces) of approximately 1g was accurately weighed into a re-usable ceramic boat and placed into a purge chamber of the horizontal furnace. The boat was placed into the furnace, O_2 allowed to flow directly onto the sample and combustion initiated at 950 ºC. The resulting gaseous products were passed through a thermo-electric cooler, removing most of the moisture and the gasses collected in a ballast chamber. Nitrogen (N) was measured using a thermal conductivity detector against a background of pure helium (He). The detector signal was transmitted to a computer via a microprocessor and the data analysed to determine the nitrogen content of the sample. Crude protein (CP, g/100g DM) was calculated as N (g/100g DM) x 6.25 (Scholtz, 2001).

3.11.1.3 Gross Energy (GE)

Gross energy was determined using a Gallencamp adiabatic bomb calorimeter. The bomb was standardised using benzoic acid. A sample of approximately 0.5g of feed or faeces was accurately weighed and approximately 5cm platinum wire was connected to the electrodes of the bomb. The bomb was then tightly closed and filled with oxygen to 3 000KPa and placed into a calorimeter to equilibrate the temperature between the outer container and the internal calorimeter container and then ignited. An automatic heating unit and the circulation of cold water created an adiabatic environment. The final temperature was determined by an electronic microprocessor (CP 400: Digital Data Systems).

Calculation used to determine the gross energy of the samples was as follows:

$$\text{Gross energy (MJ/kg)} = \frac{A}{B}$$

A = Micro-processor reading

B = Weight of sample

3.12 Statistical analysis

Data was statistically analysed using PROC GLM procedures of SAS (1995). Repeated measures analysis was performed to determine the effect of different dietary energy levels, time (weeks) and the interaction on the semen parameters considered in this study. The one-way ANOVA was used to compare treatments for carcass, scrotal, testicular and semen traits at the end of each phase in the trial. When treatment means differed significantly for a specific trait, Tukey's method for multiple comparisons was used to determine which means differed significantly.

Chapter 4

The effect of different energy levels on fat accumulation and distribution in young replacement Dorper rams

4.1 Introduction

The male breeding animal makes an important contribution to the reproductive performance and genetic improvement in a herd/flock. It is therefore important to pay attention to the factors which may influence male fertility such as libido, mating ability and semen quality. According to Brown (1994) sire reproductive capacity can be detrimentally affected by overfeeding. Labuschagné *et al*. (2002) found a detrimental effect of high energy diets on fertility of young bulls. Both semen quantity and quality were significantly and negatively affected by the high energy content of the diet. These findings were supported by those of Fourie *et al.* (2003) in young Dorper rams. An endeavour should be made to produce replacement ram lambs with good growth rates, but not to allow them to become overfat.

The NRC (1985) nutritional requirements for rams do not make provision for different fat maturing types of rams. Dorper rams could be classified as an early maturing type. Feeding them according to the NRC (1985) standards could result in the accumulation of fat and the subsequent detrimental effects on fertility. Therefore the suitability of the NRC standards for young Dorper replacement ram lambs needs further investigation.

The aims of this study were: (i) to evaluate the efficiency of different dietary energy levels on the body and carcass development and especially fat accumulation and distribution in growing replacement Dorper ram lambs; and (ii) to evaluate the NRC (1985) nutritional requirements for replacement Dorper rams.

4.2 Materials and Methods

Thirty-six, 11 to 12 months old Dorper rams with a mean initial body weight of (42 ± 0.52kg) were randomly allocated to 3 treatment groups (12 per group). The rams (n = 36) were housed in individual metabolic cages (each one fitted with a feed and a water trough) in a closed, well-ventilated building during the total feeding period of 18 weeks (127 days; phase 1 of the study). The rams in each group received randomly one of the following 3 experimental diets (on an ad libitum, basis) containing: (i) a low (Le), (ii) a medium (Me) or (iii) a high (He) energy level respectively. The composition of the experimental diets is set out in Table 4.1.

A standard medium energy (Me) diet was formulated according to the NRC requirements to serve as a norm (control) of the energy requirements for growing replacement ram

lambs (NRC, 1985). Two extreme energy diets, namely a Le diet (high roughage) with a 15% lower energy content and a He diet (finishing diet) with 15% higher energy content relative to the standard diet (NRC, 1985), were formulated. All 3 diets were formulated on a crude protein, degradable protein, calcium and phosphorus equivalent basis. The chemical composition (Table 4.1) of feeds as set out by Van der Merwe and Smith (1991) and Erasmus *et al* (1988; 1990) were used to formulate (balance) the diets.

Table 4.1 Physical and chemical composition (DM basis) of the 3 diets fed to the experimental rams

Parameter	Le	Me	He
Physical composition			
Maize (%)	0.00	23.80	51.05
Cotton seed oilcake (%)	0.00	4.80	6.00
Lucerne hay (%)	33.00	23.00	13.70
Eragrostis curvula hay (%)	65.70	47.00	27.30
Urea (%)	0.80	0.40	0.45
Feed lime (%)	0.00	0.50	1.00
Salt (%)	0.50	0.50	0.50
Chemical composition [1]			
Crude protein (%)	13.58	13.59	13.59
Degradable protein (%)	8.73	8.30	8.52
Calcium (%)	0.65	0.64	0.64
Phosphorus (%)	0.17	0.23	0.25
Energy (MJ ME/kg DM)	8.23	9.77	11.32

[1] Feed values according to Van der Merwe & Smith (1991), Erasmus *et al.* (1988; 1990); Le – Low energy; Me – Medium energy; He – High energy

The lucerne (*Medicava sativa*) and *Eragrostis curvula* hay were milled through a 25mm grid using a Drosky hammer mill, and the maize crushed before being used in the diets. The urea, feed lime and salt were dissolved prior to mixing, in a small quantity of water. The feed components of the individual diets (± 500kg per batch) were accurately weighed and thoroughly mixed by means of a mechanical mixer. In an effort to minimise the was-

tage of the diets by the rams, animals were fed 3 times daily (8h00, 12h00 and 16h00). All the edible spillage was collected throughout the day and re-fed to the rams. The feed intake of each ram was calculated weekly as the difference between the total feed offered and the total feed refused at the end of the week. Rams were weighed at the onset and the end of the feeding period (after an overnight fasting period) and once a week during the trial early in the morning (7h00).

Table 4.2 Official sheep carcass classification system used in South Africa

Age description (Teeth)	Age class code	Fat description	Grade	Backfat mm
0	A	No fat	0	0
1-2	AB	Very Lean	1	< 1
3-6	B	Lean	2	1 – 3
>6	C	Lean	3	3 – 5
		Fat	4	5 – 7
		Over fat	5	7 –10
		Excessively over fat	6	>10

(Source: Government notice no. R 1748, 26 June 1992; SAMIC, 2004)

At the end of the 127 day feeding period, 6 randomly sellected rams from each group were slaughtered at a commercial abattoir and the carcasses characteristics evaluated and recorded. The body weight (empty stomach) of each ram was determined prior to slaughter and after skinning and evisceration the warm carcass weight and the carcass grades were recorded according to the official methods practised in South Africa (SAMIC, 2004). According to this grading system, 1 represents a very lean carcass, while 6 represents an overfat carcass as summarised in Table 4.2.

Carcass measurements were recorded according to the methods described by Fisher and De Boer (1993) and Ramsay *et al.* (1991). The external length of the carcass, the circum-

ference of the brisket and the circumference of the buttock were measured after 24h of refrigeration. Thereafter the carcass was split in two halves along the vertebral column with the aid of an electrical saw. Backfat thickness was measured on the left half of the carcass between the 12th and 13th rib of the *M. longissimus thoracis* (at 3 points;Edwards *et al.,* 1989). The kidney fat was dissected and weighed and the marbling was estimated by scoring the 3 rib cut (ribs 11,12 and 13) according to the amount of fat present in the eye muscle (score out of 5). The eye muscle area was measured by the rib eye dot grid method, as the length x width of the eye muscle between the 9th and 10th thoracic vertebrae (Edwards *et al.,* 1989; Strydom, 1998).

A 7 day digestibility trial (collection period) was carried out with 5 randomly chosen rams from each group during week 14 of the growth study. Representative samples from each of the diets, refused feed and faeces were analysed in duplicate for dry matter (DM) (AOAC, 1984). Crude protein (CP) was determined by means of the Dumas method of combustion with a LECO FP 2000 (Randall, 1993) and gross energy (GE) by means of bomb calorimetry.

Data was statistically analysed using PROC GLM procedures of SAS (1995). The one-way ANOVA was used to compare treatments for feed intake, growth, feed conversion ratio (FCR) and carcass traits. When treatment means differed significantly for a specific trait, the Tukey's method for multiple comparisons was used to determine which means differed significantly.

4.3 Results and Discussion

The chemical composition of the experimental diets, dry matter intake and digestibility data are summarised in Table 4.3. It is evident that the crude protein (CP) and the metabolisable energy (ME) values of the experimental diets fed to the rams were lower than the calculated values (Table 4.1). These findings could probably be attributed to the low quality (high fibre) roughages used. The actual ME content of the He diet compared well with the ME requirements (9.6 MJ ME/kg), recommended by the NRC (1985) for replacement ram lambs with an average weight of 50kg. The actual CP content of the experimental diets used compared well with the NRC (1985) recommended level for this weight group of ram lambs.

Table 4.3 The mean (±se) chemical composition of the experimental diets, dry matter intake and digestibility data for Dorper ram lambs

Parameter	Le	Me	He
Chemical composition of diets			
Dry matter (%)	91.65	92.03	92.08
Crude protein (%)	12.23	12.83	12.88
Dry matter intake (kg/ram/day)	$1.43^{a} \pm 0.08$	$1.55^{b} \pm 0.10$	$1.65^{b} \pm 0.08$
Apparent digestibility coefficients:	n = 5	n = 5	n = 5
Gross energy (%)	$43.98^{a} \pm 3.62$	$56.41^{b} \pm 3.73$	$63.73^{b} \pm 1.21$
Crude protein (%)	$60.23^{a} \pm 1.50$	$65.37^{b} \pm 0.35$	$61.02^{ab} \pm 1.54$
Digestible crude protein (%)	$7.54^{a} \pm 0.24$	$8.64^{b} \pm 0.07$	$7.79^{a} \pm 0.17$
Digestible energy(MJ/kg)	$8.14^{a} \pm 0.69$	$10.11^{b} \pm 0.24$	$11.74^{b} \pm 0.23$
Metabolisable energy (MJ/kg)[1]	$6.52^{a} \pm 0.55$	$8.09^{b} \pm 0.20$	$9.39^{b} \pm 0.18$

[a,b,c] Means in rows with different superscripts differ significantly (P < 0.05) [1] Digestible energy x 80% (McDonald 2002); Le – Low energy; Me – Medium energy; He – High energy

From Table 4.3 it further seemed that the apparent digestibility of GE, digestible energy and the ME-content of the experimental diets increased significantly (P<0.05) from the

Le to the Me diet, thereafter a non significant (P<0.05) increase occurred to the He diet. There was thus a significant difference (P<0.05) between the Le and the He diet, but not between the He and the intermediate diet (Me) in terms of the gross energy (GE) content. The apparent CP digestibility of the Le was significantly (P<0.05) lower than the Me diet (Table 4.3). The higher cotton seed oilcake and maize content of the Me diet could have contributed to these results. According the Van der Merwe and Smith (1991) the digestibility of CP in these concentrates is higher than those in lucerne and *Eragrostis curvula* hay. This explanation is however not supported by the lower digestibility CP content of the He diet (however non significant; P>0.05). The reasons for the higher CP digestibility and digestible CP content of the Me diet is therefore unclear.

The growth performance of the Dorper ram lambs during the 127 day trial period is despicted in Table 4.4. In accordance with energy content (Table 4.3) the DM, CP and ME-intake of the rams increased significantly (P<0.05) from the Le (6.52 MJ ME/kg) to the Me (8.09 MJ ME/kg) diet. A further increase in energy content of the He diet (9.39 MJ ME/kg) did not result in a significant higher DM, CP and ME-intake. The ADG and the FCR of rams however significantly (P<0.05) improved with each increment of energy content of the diet. A more efficient utilisation of the ME (less heat production and especially fermentation heat) by rams fed the He diet probably contributed to these findings. McDonald *et al.* (2002) stated that the work of digestion is the cause of the poor utilisation of ME from low quality forages. Ely *et al.* (1979) found that lambs slaughtered at 40 kg converted feed more efficiently than those slaughtered at 50 kg body weight.

The dry matter intake (DMI) of rams fed the He diet (equivalent to NRC standards) was 13.6 % lower, when compared to the recommendations of the NRC (1985) nutrient requirement standards for replacement ram lambs. The CP and ME intake, as well as ADG of the replacement rams on the He diet were respectively 9.8 %, 16 % and 30 % lower compared to NRC (1985) feeding standards. However, these ME-intakes were in line with those reported by Marais (1988) for young growing Dorper ram lambs.

Table 4.4 Feed and nutrient intake and average daily gain (ADG) of yearling Dorper rams fed diets with different energy levels.

Parameter	**Le**	**Me**	**He**
Dry matter intake (kg/ram/day)	$1.62^{a} \pm 0.04$	$1.85^{b} \pm 0.03$	$1.88^{b} \pm 0.04$
Dry matter intake g/kg $W^{0.75}$	$18.60^{a} \pm 0.33$	$19.97^{b} \pm 0.24$	$20.66^{b} \pm 0.28$
Crude protein intake (g/ram/day)	$198^{a} \pm 5$	$226^{b} \pm 4$	$230^{b} \pm 5$
Digestible crude protein intake (g/ram/day)	$121.93^{a} \pm 2.88$	$159.78^{c} \pm 2.72$	$146.79^{b} \pm 3.22$
Metabolisable energy intake (MJ/ram/day)	$10.54^{a} \pm 0.25$	$14.96^{b} \pm 0.26$	$17.70^{b} \pm 0.39$
Initial body weight (kg)	$42.25^{a} \pm 0.89$	$42.82^{a} \pm 0.79$	$42.24^{a} \pm 1.05$
Final body weight (kg)	$56.46^{a} \pm 1.52$	$65.50^{ab} \pm 1.09$	$71.04^{b} \pm 1.40$
ADG (g/ram/day)	$112^{a} \pm 7$	$180^{b} \pm 5$	$229^{c} \pm 12$
FCR (kg feed/kg weight gain)	$14.76^{a} \pm 0.78$	$10.29^{b} \pm 0.24$	$8.43^{c} \pm 0.43$
MJ ME/kg weight gain	$96.61^{a} \pm 4.93$	$83.53^{ab} \pm 1.79$	$79.56^{b} \pm 3.96$

[a,b,c] Means in rows with different superscripts differ significantly ($P < 0.05$); Le – Low energy; Me – Medium energy; He – High energy

Factors like breed (fat maturity), physical form of the roughage, quality of the roughage (fibre content and composition), degradability of the protein, moisture content of the diet, individual feeding and ambient temperature could have also contributed to these differences. The Dorper is an early maturing breed with a higher fat content in the body at a specific weight when compared to other sheep breeds, which results in a lower weight gain

(Cloete *et al.,* 2000). Therefore, the energy requirements for replacement ram lambs should be different between various fat maturing breeds.

The effect of the different dietary energy levels on the carcass characteristics of Dorper rams are set out in Table 4.5. The cold carcass weight, dressing percentage and shoulder circumference of the experimental rams increased significantly (P<0.05) with an increase in dietary energy levels. These results are in accordance with the growth performance results of the lambs, already discussed (Table 4.4). The Le group showed a significantly lower (P<0.05) mean carcass grade, buttock circumference and backfat thickness than the Me and He groups – with no significant differences between the latter groups. Ely *et al.* (1979) recorded higher dressing percentages, higher quality grades and lower yield grades for lambs slaughtered at 50kg compared to 40kg.

Table 4.5 The mean (± se) carcass characteristics of young rams fed at 3 different energy levels

Parameter	Le	Me	He
Cold carcass weight (kg)	$22.17^{a} \pm 1.14$	$28.00^{b} \pm 0.72$	$35.50^{c} \pm 1.56$
Dressing percentage (%)	$39.77^{a} \pm 0.62$	$44.25^{b} \pm 0.29$	$49.73^{c} \pm 0.86$
Carcass grade (0 – 6)	$3.0^{a} \pm 0.4$	$5.2^{b} \pm 0.4$	$6.0^{b} \pm 0.0$
External carcass length (cm)	$63.3^{a} \pm 1.08$	$64.0^{ab} \pm 0.9$	$66.8^{b} \pm 0.7$
Shoulder circumference (cm)	$79.6^{a} \pm 1.3$	$85.4^{b} \pm 0.8$	$91.3^{c} \pm 1.3$
Buttock circumference (cm)	$76.1^{a} \pm 1.4$	$79.7^{b} \pm 1.3$	$83.2^{b} \pm 0.5$
Backfat thickness (mm)	$2.31^{a} \pm 0.55$	$4.02^{b} \pm 0.43$	$6.00^{b} \pm 0.89$
Eye muscle area (cm^2)	$20.46^{a} \pm 1.45$	$20.85^{a} \pm 1.26$	$23.23^{a} \pm 1.12$
Marbling (0-5)	$1.33^{a} \pm 0.21$	$1.67^{a} \pm 0.33$	$2.67^{b} \pm 0.21$
Kidney fat (g)	$245^{a} \pm 19$	$420^{a} \pm 78$	$1083^{b} \pm 154$
Scrotal fat (g)	$106^{a} \pm 16$	$180^{a} \pm 14$	$280^{b} \pm 28$

^{a,b,c} Means in rows with different superscripts differ significantly (P < 0.05); Le – Low energy; Me – Medium energy; He – High energy

From Table 4.5 it would further seem that external carcass length of rams increased significantly ($P<0.05$) from the Le to the He diet, while the Me diet did not differ significantly from the other two diets. The Me diet did also not differ significantly in terms of eye muscle area, marbeling, kidney fat and scrotal fat from the Le group. However, a statistically significantly higher ($P<0.05$) accumulation of fat occurred in the eye muscle (marbling), scrota and around the kidneys of rams fed the He (9.39 MJ ME/kg) diet.

These parameters could be regarded as good indicators of undesirable fat accumalation in the body of ram lambs. Accordingly this is a clear indication that the He diet caused the over fattening of rams. The amount of fat deposited around the kidneys and in the scrotal and eye muscle areas seems to be related to the level of energy in the diet. Therefore, the He diet which was equivalent to the energy requirement recommendations of the NRC (1985) feeding standards, seems to be unsuitable for Dorper replacement ram lambs (early maturing rams) with an average live weight between 40-70kg.

As already indicated, the Me diet, with the exception of subcutaneous fat, did not result in the same undesirable fat accumulation in the body of ram lambs. This diet would probably provide the best in terms of energy requirements for replacement ram lambs in the weight range used in the current study.

4.4 Conclusions

It seems from the results of the present study that the energy requirement as recommended by the NRC (1985) is not applicable for early maturing Dorper replacement ram

lambs. The provision of this recommended energy level (9.6 MJ ME/kg dietary DM or 21 MJ ME/ram/day) will result in an undesirable fat accumulation and distribution in the body of growing replacement Dorper ram lambs. This unnecessary and costly fat accumulation could influence the reproductive performance of rams negatively. The Me diet seems to be more appropriate for the body and carcass development, while preventing undesirable fat accumulation and distribution of replacement Dorper ram lambs. The results of the present study suggested that 8 MJ ME/kg dietary dry matter or 15 MJ ME/day is needed by replacement Dorper (early mature) ram lambs at a weight range of 40 – 65kg. The precise energy requirements of replacement Dorper ram lambs at different live body weights need further investigation. Accordingly the observed excessive scrotal fat deposition in rams fed the He diet on semen quantity and quality warrants urgent investigation.

Chapter 5

The effect of dietary energy levels on the carcass, scrotal, testicular, fresh and cryopreserved semen characteristics in young Dorper rams

5.1 Introduction

It is a common practice in South Africa to feed high energy diets to young replacement rams. This is usually done for performance testing trials and in preparation for shows and sales – as conditioned (fat) rams often realize better prices (Fourie *et al.,* 2004). Very little is however known regarding the effect of such diets on subsequent ram fertility. Some farmers and animal scientists object to the fattening of breeding rams, as it is believed that this practice reduces their fertility. According to Brown (1994) overfeeding of sires (boars, bulls and rams) can have detrimental effects on their reproductive capacity. There are however, very few studies regarding the effect of high energy diets on ram fertility in the literature. In a study on sperm production, in which two levels of dietary energy and/or protein were fed to mature Merino rams, Braden *et al*. (1974) found the dietary energy levels to have a significant effect on the testes weight and daily sperm production. Significantly larger testes and seminal vesicles were recorded in rams fed high energy diets. Increasing the energy intake of the ram had a greater effect on the daily

sperm production, compared to increasing the protein intake. These results indicate that diet has a marked effect on the rate of sperm production in rams. Furthermore, it was suggested that energy intake probably has no direct affect on spermatogenesis or on testosterone production, but may influence these parameters indirectly via an effect on gonadotropin secretion.

In recent studies Coulter *et al.* (1997) and Labuschagné *et al.* (2002), have demonstrated the detrimental effects of high energy diets on young bull's fertility. Both semen quantity and quality in high energy fed bulls were of inferior standard when compared to those of medium energy fed bulls. This was probably a result of impaired testicular thermoregulation, due to scrotal fat accumulation – particularly in the neck region of the scrotum over the *pampiniform plexus*. Similar findings were reported by Fourie *et al*. (2004) in young Dorper rams.

There are no reports in the literature on the effect of nutrition on the resistance/tolerance of ram semen to cryopreservation. Furthermore, freezing of semen has not been as successful in rams as in other livestock species (e.g. cattle). There is most certainly a species specific and genetic basis for the decreased tolerance of ram sperm to cryopreservation, but there are also differences between breeds, individuals and ejaculates of the same ram (Evans & Maxwell, 1987). There is some anecdotal evidence suggesting that diet and particularly the energy content, may also play an important role on the sperm tolerance to cryopreservation. Animal scientists and veterinarians working at AI stations in South A-

frica frequently blame the over-conditioning (obesity) of rams for the poor quality and freezability of their semen.

The aims of this study were: (i) to evaluate the effects of different dietary energy levels on the carcass, scrotal, testicular and semen characteristics of young Dorper rams and (ii) to evaluate the effect of dietary energy level on the sperm cell tolerance to cryopreservation in these rams.

5.2 Materials and Methods

Thirty-six, 11 to 12 months old Dorper rams with a mean initial body weight of 42kg ± 0.52kg) were randomly allocated to 3 treatment groups (12 per group). All rams had previously been managed extensively on natural pastures (veld) and successfully trained for semen collection with the aid of an artificial vagina (AV). The rams were randomly allocated to 3 experimental diets groups (n=12 per group). The rams in each group received one of the 3 experimental diets (randomly selected), formulated on a crude protein, degradable protein, calcium and phosphorus equivalent basis. These diets containing low, medium or high energy levels, respectively were fed, on an ad libitum basis for a period of 127 days, between the end of summer and the onset of winter (natural sheep breeding season – autumn).

Table 5.1 Physical and chemical composition (DM basis) of the 3 diets fed to the experimental rams

Diet composition	**Diet**		
Physical composition	**Le**	**Me**	**He**
Maize (%)	-	23.8	51.1
Cotton seed oilcake (%)	-	4.8	6.0

Lucerne hay (%)	33.0	23.0	13.7
Eragrostis curvula hay (%)	65.7	47.0	27.3
Urea (%)	0.8	0.4	0.5
Feed lime (%)	-	0.5	1.0
Salt (%)	0.5	0.5	0.5
Chemical composition [1]			
Crude protein (%)	13.58	13.59	13.59
Degradable protein (%)	8.73	8.30	8.52
Calcium (%)	0.65	0.64	0.64
Phosphorus (%)	0.17	0.23	0.25
Energy (MJ ME/kg DM)	8.23	9.77	11.32

[1] Van der Merwe & Smith (1991), Erasmus *et al* (1988; 1990)**;** Le – Low energy; Me – Medium energy; He – High energy

The composition of the formulated diets is set out in Table 5.1.A standard medium energy diet was formulated to serve as the control diet, and to provide all the nutrient requirements for growing replacement ram lambs (NRC, 1985). The metabolisable energy content of this diet (Me) was increased by 15% for the high energy (He) and decreased by 15% for the low energy (Le) diets, respectively. All rams (n=36) were housed in individual metabolic cages in a closed, well-ventilated building on the campus of the University of the Free State. Fresh, clean water and feed were available ad libitum throughout the trial period.

A semen sample was collected fortnightly from every ram (from week 4 to the end of the trial), early in the morning (7h30 – 9h30), over 3 consecutive days (12 rams/day), with the aid of an AV. On each semen collection day, samples from 4 rams from each treatment group were collected. The volume of the ejaculates collected was recorded directly from the calibrated test tube used, before being placed in a water bath (32 ºC). A volume of 10µl of semen was diluted with 990µl of a pre-warmed (32 ºC) TL-Hepes solution (99 TL-Hepes:1 semen). The diluted semen samples were then evaluated on a pre-warmed

(35 ºC) glass slide under a compound microscope (x400 magnification) within 2 minutes of collection. Forward sperm progression (%) and overall motility on a scale of 0 (no movement) to 5 (very fast forward movement) were evaluated by scoring 100 sperm cells individually (Loskutoff & Crichton, 2001). For the determination of the ejaculate concentration, 10µl of fresh semen was diluted in 990µl water. Sperm cell concentration was calculated, using 10µl of this semen solution placed on an improved Newbawer haemocytometer. The sperm cells lying inside the five blocks that formed a diagonal line on the grid were counted and the total multiplied by 5 to obtain the concentration of sperm cells in the semen sample in (x 10^6/ ml) (Loskutoff & Crichton, 2001).

Table 5.2 Composition of the semen extender used for the one-step dilution and cryoperservation of ram semen

Ingredients	Dilution rate (1+4)
Tris(hydrowymethyl)amino-methane (g)	3.634
Glucose (g)	0.500
Citric acid (monohydrate) (g)	1.990
Egg yolk (ml)	15
Glycerol (ml)	5
Penicillin (IU)	100,000
Streptomycin (mg)	100
Distilled water to (ml)	100

Source: Evans and Maxwell (1987)

Two thin semen smears were made from each fresh semen sample on pre-warmed (35 ºC) glass slides. One slide was stained with eosin/nigrosin (60µl eosin/nigrosin and 6µl semen) and the other with eosin B-fast green (60µl eosin B-fast green and 6µl semen). One hundred individual sperm cells from different fields on each slide were evaluated microscopically (x1000 magnification) and the eosin/nigrosin stained smear was

evaluated for vitality (dead or live) and morphology. The sperm cells observed were classified as dead or live and the abnormalities were classified as head, mid-piece and tail abnormalities (Salamon & Maxwell, 2000; Loskutoff & Crichton, 2001). The eosin B-fast green stained semen smears were used to evaluate sperm vitality (live/dead) and acrosome reaction. The sperm cells observed were classified into 4 groups: live/acrosome intact, live/acrosome reacted, dead/acrosome intact and dead/acrosome reacted (Loskutoff & Crichton, 2001).

From each ejaculate, 0.5ml of semen was placed in a 5ml plastic tube in a water bath (32 ºC), to which 2.0ml of pre-warmed (32 ºC) semen extender was added drop by drop within 10 minutes following semen collection. The composition of the semen extender used, is as set out in Table 5.2.

The 5ml plastic tube containing the extended semen (2.5ml) was gently agitated, tightly closed and placed into a 50ml plastic screw top tube containing water from a warm bath (32 ºC). This was then placed in a cool box (4 to 5 ºC) for equilibration. This procedure slows down the rate of cooling and therefore protects the sperm cells from cold shock (Loskutoff & Crichton, 2001). The coolbox was equipped with a thermometer and ice blocks for cooling down the collected extended semen samples to 4 ºC, while the rest of the semen samples were being collected from the other rams. After collecting semen from all 12 rams for the day, the coolbox, containing the extended semen, was taken into and opened inside a walk-in refrigerator (4 to 5 ºC). The 50ml plastic screw top tubes

containing water and the 5ml plastic screw top tubes with 2.5ml of extended semen samples were removed from the coolbox.

After a total equilibration period of 4 hours (in the coolbox and in the walk-in-refrigerator) the extended semen was packed into pre-labelled 0.25ml plastic straws (6 straws/animal) and sealed with PVP straw sealing powder. The filled straws were then placed in liquid nitrogen (LN^2) vapour, approximately 4cm (-70 ºC), above the LN^2 level using a polystyrene boat, floating in a plastic box filled with LN^2 for 10 minutes, before being plunged into the LN^2 (-196 ºC) (Loskutoff & Crichton, 2001). The frozen straws were then transferred into goblets and stored in a LN^2 flask. One week after freezing, one straw from each ram was thawed, in water (38 ºC) for 30 seconds and evaluated for overall motility, forward progression. Two thin smears were stained (nigrosin + eosin and eosin B-fast green) and evaluated for vitality, acrosome integrity and sperm morphology, using the same criteria and procedures described earlier for fresh semen (Salamon & Maxwell, 2000; Loskutoff & Crischton, 2001). These results were later statistically compared between groups.

At the end of the trial period (127 days), 6 rams from each group were randomly selected and slaughtered at a commercial abattoir and the carcasses, scrotal and testicular characteristics evaluated and recorded. The body weight (empty stomach) and the scrotal circumference (SC) of each ram were determined prior to slaughter. The SC was measured at the widest part of the scrotum with the aid of a measuring tape. Following slaughter, the carcasses were skinned and eviscerated. The warm carcass weight, and the dressing

percentage were calculated and carcasses graded according to the official methods practised in South Africa (SAMIC, 2004). According to this grading system, 1 is a very lean carcass, while 6 represents an over-fat carcass. The backfat thickness was measured on the left half of the carcass between the 12th and 13th rib of the *M. longissimus thoracis* (at 3points) (Edwards *et al.*, 1989) (For more details on the methodology used, refer to Chapter 3 [General Materials and Methods]).

The scrota were excised from the carcasses, by severing the scrotum at the base, as close to the body as possible, preserved by means of refrigeration (4 to 5 ºC) for 24h and then weighted and dissected into scrotal skin, testis, fat, epididymis and *pampiniform plexus*. The scrotal skin thickness was measured with the aid of a caliper at the widest part of the scrotum – in the same region where the SC was measured (Fourie *et al,* 2004). The weight of each component was recorded and the total volume of the testicles and epididymis were recorded separately by measuring the volume of water displaced after gently plunging these structures into a container filled to the brim with water.

Data was statistically analysed using PROC GLM procedures of SAS (1995). Repeated measures analysis was performed to determine the effect of different dietary energy levels, time (weeks) and the interaction on the semen parameters considered in this study. The one-way ANOVA was used to compare treatments for carcass, scrotal, testicular and semen traits recorded during the trial. When treatment means differed significantly for a specific trait, the Tukey's method for multiple comparisons was used to determine which means differed significantly.

5.3 Results and Discussion

The influence of dietary energy level on body weight and carcass characteristics has been discussed in Chapter 4. Therefore the final body weight and the most important carcass characteristics of the young Dorper rams evaluated at the end of the trial are summarised in Table 5.3 and briefly discussed. In general, these results indicate significant differences at the end of the trial for most parameters considered between the two nutritional extremes (Le and He groups), with a higher values recorded from the He group.

The Le group recorded significantly lower (P<0.05) mean final body weight, mean carcass weight and grade than the Me and He groups, respectively – with no significant differences between the latter groups. As expected, both the mean warm carcass weight and the dressing percentage increased significantly with increasing dietary energy levels. The back fat thickness, as well as the kidney fat, showed the same trend and were significantly (P<0.05) higher in the He group when compared to the Le group. Although the Le group recorded higher values for the same parameters, these differences were not significantly (P>0.05) different. The actual ME content of the the Le, Me and He diets were 6.52, 8.09 and 9.39 MJ/kg respectively. For practical reasons it must be said that the actual ME content of the He diet was similar to that recommended by the NRC for growing rams - the formulated Me diet. Despite this fact, this diet (He) caused considerable fattening of the rams.

The scrotal and testicular characteristics of the young replacement Dorper rams at the end of this trial (127 days) are summarised in Table 5.4. All the scrotal and testicular parameters considered in this study, namely the total scrotal weight and circumference, testes

weight and volume, scrotal fat and scrotal skin weight, as well as the weight and volume of the epididimys, recorded in the Le group were significantly ($P<0.05$) lower than in the He group. Although the He group recorded higher values than the Me group, these differences were not significant. It further seems from Table 5.4 that significant ($P<0.05$) more fat accumulated in the scrota of rams fed the He compared to the Le and Me diets, respectively. Almost all the scrotal fat deposits dissected out were located in the neck region of the scrotum, over the *pampiniform plexus*. The amount of fat deposited in the scrotum was directly related to the level of energy in the diet, although no significant differences were recorded between the Le and Me groups. As concluded in Chapter 4 it thus seems safe to feed the Me diet to replacement Dorper rams (early maturing) as this diet did not result in significantly higher scrotal fat accumulation when compared to the Le diet.

Table 5.3 The mean (±se) final body weight and carcass characteristics of young Dorper rams fed diets with different energy levels.

Parameter	Dietary energy level		
	Le	Me	He
Final body weight (kg)	56.5 ^{a} ± 1.5	65.5 ^{b} ± 1.1	71.0 ^{b} ± 1.4
Carcass grade (0-6)	3.0 ^{a} ± 0.4	5.2 ^{b} ± 0.4	6.0 ^{b} ± 0.0
Warm carcass weight (kg)	22.8 ^{a} ± 1.2	28.8 ^{b} ± 0.8	36.0 ^{c} ± 1.5
Dressing percentage (%)	39.8 ^{a} ± 0.6	44.3 ^{b} ± 0.3	49.7 ^{c} ± 0.9
Back fat thickness (mm)	2.3 ^{a} ± 0.6	4.0 ^{ab} ± 0.4	6.0 ^{b} ± 0.9
Kidney fat (g)	245 ^{a} ± 19	420 ^{a} ± 78	1083 ^{b} ± 154

^{a,b,c} Means in rows with different superscripts differ significantly ($P<0.05$); Le - Low energy: Me - Medium energy; He - High energy

Negative effects on semen quality of high energy diets have been reported by Labuschagné *et al.* (2002) and Fourie *et al*. (2004) in young Bonsmara bulls and young

Dorper rams, respectively. Table 5.5 depicts the most important fresh semen characteristics of young Dorper rams fed 3 equivalent diets with different energy levels, at the end of the trial period (127 days).

Table 5.4 The mean (±se) scrotal and testicular characteristics of young Dorper rams fed on diets with different energy levels at the end of the trial period (127 days).

Parameter	Dietary energy level		
	Le	Me	He
Total scrotal weight (g)	646 ^{a} ± 50	920 ^{b} ± 54	1062 ^{b} ± 53
Scrotal circumference (cm)	29.5 ^{a} ± 1.1	34.5 ^{b} ± 0.9	35.5 ^{b} ± 0.7
Scrotal skin weight (g)	161 ^{a} ± 14	198 ^{b} ± 6	221 ^{b} ± 12
Scrotal fat (g)	106 ^{a} ± 16	180 ^{a} ± 14	280 ^{b} ± 28
Testes volume (mm^3)	246 ^{a} ± 30	378 ^{b} ± 44	384 ^{b} ± 21
Testes weight (g)	262 ^{a} ± 27	406 ^{b} ± 40	398 ^{b} ± 24
Epidydimis weight (g)	27.00 ^{a} ± 0.94	33.85 ^{b} ± 2.15	34.27 ^{b} ± 2.22
Epidydimis volume (mm^3)	22.33 ^{a} ± 2.09	26.67 ^{a} ± 3.42	30.00 ^{a} ± 2.80

^{a,b,c} Means in rows with different superscripts differ significantly (P<0.05); Le – Low energy; Me – Medium energy; He – High energy

Table 5.5 The mean (±se) fresh semen parameters of young Dorper rams fed different energy levels at the end of the trial period (127 days).

Parameter	Dietary energy level		
	Le	Me	He
Semen volume (ml)^{ns}	0.8 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Overall motility (%)^{ns}	84 ± 2	86 ± 2	77 ± 3
Forward progression (0-5)^{ns}	2.8 ± 0.1	3.0 ± 0.1	2.7 ± 0.2
Sperm concentration (x 10^6/ml)^{ns}	1851 ± 121	1678 ± 172	1834 ± 169
Live sperm (%)^{ns}	70 ± 2	69 ± 4	64 ± 3
Normal sperm (%)^{ns}	96 ± 2	91 ± 4	91 ± 4

^{ns} No significant difference between means (P<0.05); Le – Low energy; Me – Medium energy; He – High energy

Despite the significant differences recorded for the scrotal and testicular characteristics, no significant differences were recorded between the 3 nutritional groups regarding the different quantitative and qualitative semen parameters evaluated, namely semen volume, sperm cell concentration, overall motility, forward progression, percentage live and normal sperm cells. The results in terms of sperm concentration and motility, contradicted those recorded by Fourie *et al.* (2004), who reported a high energy diet to have a detrimental effect on sperm concentration and motility in Dorper rams. It was expected that using a diet (He) with a ME level of 9.39 MJ ME/kg DM, similar to the diet used by Fourie *et al.* (2004) (9.5 MJ ME/kg DM), for a longer period (127 vs 49 days), would induce greater differences in the seminal parameters between the groups. Possible reasons for these conflicting results may be the difference in age of the rams, the fact that those authors made no use of diets balanced on an equivalent basis, as well as season and semen collection techniques used in the two studies. Fourie *et al.* (2004) conducted the study during winter (out of the natural breeding season), and collected semen by means electro-ejaculation (EE). These differences in the experimental design may account in part for the discrepancies in the results obtained. According to Matthews *et al.* (2003), ram semen collected with the aid of an AV, is more concentrated than that collected by EE. With the latter method (EE), the semen sample obtained usually contains more seminal plasma (more diluted), and is often contaminated with urine.

The results obtained in this trial in terms of semen quantity and quality are also contradictory to those reported by Coulter *et al.* (1997) and Labuschagnè *et al.* (2002) who clearly demonstrated a detrimental effect of a high energy diet on the semen characteristics in

young bulls. Nevertheless, the results of this trial in terms of volume of ejaculate, percentage live and normal sperm cells are supported by those of Fourie *et al.* (2004), who also recorded no significant effect of high dietary energy levels on these parameters in young Dorper rams. Despite the considerable amount of fat accumulated in the scrotum (mainly in the neck region over the *pampiniform plexus* as a result of a higher dietary energy level), no detrimental effects were observed in the semen. It may be possible that spermatogenesis in rams is less affected by scrotal fat deposition than in bulls.

The results (P-values) of the repeated measures analysis of the means for the different seminal parameters recorded during the 16 weeks of this study are set out in Table 5.6. As it can be observed in Table 5.6, there were very few weeks in which significant differences were recorded between the different treatment groups regarding some of the semen characteristics considered in this study (mainly overall sperm motility and forward sperm progression). Significant differences (P<0.05) in terms of mean sperm cell motility were recorded during the first 3 collections (weeks 4-6 of the trial), but thereafter no more significant differences between groups were recorded, for this parameter. Forward progression also recorded significant differences (P<0.05), but only in the first 2 semen collections (weeks 4-6 of the trial). The dead/acrosome intact sperm evaluation indicated a significant difference between the means of the 3 nutritional treatment groups at the third collection (week 8), while mean tail abnormalities were significantly different during the first collection (week 4).

As can be seen in Table 5.6, there were no clear trends over time (weeks) in terms of the different semen characteristics considered in this trial. These results further indicate that the significant differences recorded were probably not as a result of the factors (independent variables) considered in this trial (diet, weeks into the trial and their interaction), but due to other confounding factors accounted for but not considered in the model.

Table 5.6 P-values for the different semen parameters evaluated fortnightly in Dorper rams fed at 3 different energy levels during the 127 day trial period

Y value	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16
Semen volume	0.3031	0.2228	0.1720	0.1004	0.1848	0.1323	0.0342
Semen concentration	0.4234	0.9620	0.8614	0.8143	0.4224	0.7751	0.8785
Sperm motility	0.0321**	0.0076**	0.0505**	0.2075	0.6102	0.8021	0.0731
Forward progression	0.04**	0.0124**	0.2407	0.1116	0.7108	0.8534	0.2114
Live/acrosome intact	0.1492	0.1384	0.1137	0.7561	0.6748	0.9108	0.1740
Dead/acrosome intact	0.9184	0.2640	0.0038**	0.6955	0.5333	0.2911	0.4798
Dead/acrosome react	0.0740	0.4586	0.8052	0.0877	0.8886	0.4873	0.1471
Live sperm	0.2662	0.2915	0.4035	0.0802	0.1291	0.7366	0.4978
Dead sperm	0.2662	0.2915	0.4035	0.0802	0.1291	0.7366	0.4978
Head abnormalities	0.2036	0.0518	0.8066	0.4546	0.1406	0.3785	0.2826
Tail abnormalities	0.0117**	0.0871	0.2622	0.2025	0.2657	0.2186	0.5952

** Significant differences ($P<0.05$) between groups were recorded on the specific collection week.

The results of the post-thawed, cryopreserved semen samples at the end of the trial period are set out in Table 5.7. From Table 5.7 it is clear that there were no significant differences in terms of the main characteristics of cryopreserved semen evaluated post-thawing between the 3 treatment groups – namely, sperm motility, forward progression, percentage live and normal sperm cells. These results were expected as no significant differences between these parameters were recorded in the fresh semen. In general, the

post-thawing semen quality is relatively lower than that recorded for the fresh semen (viability and motility), due to cell stress and injuries induced by the freezing and thawing processes. Nevertheless these post-thawing results are well above the minimum of 40% live sperm cells considered acceptable, which indicates that the freeze-thawing processes were acceptably performed (Evans & Maxwell, 1987).

Table 5.7 The mean (±se) post-thawing semen parameters in young Dorper rams fed different energy levels at the end of the trial period (127 days).

Parameter	Dietary energy level		
	Le	Me	He
Overall motility (%)^{ns}	60 ± 3	63 ± 2	57 ± 5
Forward progression (0-5) ^{ns}	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.2
Sperm concentration (x 10^6/ml)^{ns}	1851 ± 121	1678 ± 172	1834 ± 169
Live sperm (%)^{ns}	60 ± 2	54 ± 4	52 ± 3
Normal sperm (%)^{ns}	95 ± 2	92 ± 4	93 ± 2

^{ns} No significant difference between treatment (P<0.05); Le – Low energy (6.52 MJ ME/kg DM); Me – Medium energy; He – High energy

Unfortunately the real ME content of the He diet was not as high as intended by formulation. Nevertheless, considerable fat deposition was recorded in the body and in the scrotum of rams. So the intended scrotal fat deposition was achieved with the He diet used. Despite the significant differences recorded for the growth performance, carcass, scrotal and semen characteristics between the treatment groups, the results indicate that the high energy diet had no adverse effect on the semen quality of the 12 rams. From these results, it also seems that it is safe to condition yearling rams previously raised on veld, using a diet containing 9.6 MJ ME/kg DM, for a period of 4 months. A possible reason for this can be that the He diet energy content was 9.39 MJ ME/kg DM and the energy con-

tent of the Me diet was 8.09 MJ ME/kg. Thus there was a relatively small difference between the Me and the He diet in terms of ME content. Perhaps the rams on the He diet did not accumulate enough fat around the *pampiniform plexus* to sufficiently impair the thermoregulation of the in testis and have a negative effect on the semen quality and cryotolerance.

5.4 Conclusions

Despite the significant differences in fat accumulation induced by the different ME content of the experimental diets, no detrimental effects were recorded in both fresh and frozen (post-thawed) semen characteristics of young Dorper rams. Of the 3 independent variables considered in the model (diet, time and interaction), only the diet (ME level) had a significant effect on certain semen parameters considered in some weeks. However, no clear trend was recorded, suggesting that other unaccountable (confounding) factors may have been responsible for these changes.

It remains to be confirmed whether rams are less susceptible to the effect of high energy diets than bulls. Further research on the effect of scrotal fat accumulation as a result of high energy diets and/or longer feeding periods at different ages of rams should be executed.

Chapter 6

Effects of reducing dietary energy levels on the scrotal, testicular and semen characteristics of young replacement Dorper rams previously fed on high energy diets

6.1 Introduction

The effects of nutrition on livestock fertility have been relatively poorly studied in males compared to females (Schwalbach *et al.*, 2003). It is common practice in South Africa to feed high energy diets to young replacement rams for performance testing and in preparation for sales, as conditioned (fat) rams often realize better prices (Fourie *et al.,* 2004). Very little is known regarding the effects of such diets on subsequent (short and long term) ram fertility. This information is important to evaluate the common feeding practices used by sheep farmers in South Africa. Some farmers and animal scientists are against the over-conditioning of breeding rams, as it is believed that this reduces fertility. According to Brown (1994) overfeeding of sires can have detrimental effects on their reproductive capacity. In recent studies, Coulter *et al.* (1997) and Labuschagné *et al.* (2002) observed the same phenomena in bulls and suggested that scrotal fat deposition, particularly in the neck region of the scrotum around the *pampiniform plexus,* to impair testicular thermoregulation by reducing the amount of heat that can be radiated from the vascular cone in the scrotal neck, thereby increasing the temperature of the testis. Similar findings were reported by Fourie *et al*. (2004) in young Dorper rams.

The short term detrimental effects of high energy diets on bull and ram fertility seems to be gaining some awareness. In Chapter 5 considerable fat accumulation in the body and scrotum of Dorper ram lambs over a 127 day period resulted in no detrimental effect on semen quantity and quality. It was hypothesized that perhaps not enough scrotal fat deposition was achieved in the He diet to affect spermatogenesis. What is not known, are the long-term effects and the reversibility of the detrimental effects of high energy diets on the scrotal, testicular and subsequent influence on semen characteristics in males. There are no previous reports in the literature on the mobilisation of scrotal fat deposits during subsequent periods of dietary energy restrictions in males. Skinner (1971), observed no significant reduction in scrotal neck fat deposits after reducing the energy level of young bulls previously fed on a high energy diet and speculated it may be extremely difficult for a bull to rid itself of residual testicular fat, once it has been deposited.

The aims of this study were: (i) to determine the effects of reducing the level of dietary energy on the scrotal, testicular and semen characteristics, and (ii) to determine if scrotal fat mobilization occurs in Dorper rams previously fed high energy diets, during subsequent periods of moderate dietary energy restrictions.

6.2 Materials and Methods

In this part of the study, (phase 2) the remaining rams in each group (n=6) that were not slaughtered at the end of phase 1 (Chapter 5), were further maintained on the Le diet for a period of 90 days, before also being slaughtered. The initial Le group acted as the control group, while the energy level of both the Me and the He groups were reduced to the Le

level to simulate the situation when rams previously conditioned for sale or shows are placed back on natural pastures (veld) or a maintenance diet. (For more details regarding the experimental diets and management practices used during phase 1 and 2 of this study, refer to Chapter 3 [General Material and Methods] and Chapter 5 [Phase 1 of this study]).

All rams were housed in individual metabolic cages in a closed, well-ventilated building on the campus of the University of the Free State. Fresh, clean water was available ad libitum throughout the entire trial period. Semen was collected every 3rd week (21 days) from each ram, early in the morning (7h30 – 9h30), over 2 consecutive days, with the aid of an artificial vagina. On each semen collection day, samples from 3 rams from each treatment group were collected. The volume of the ejaculates collected was recorded directly from the calibrated test tube used, before being placed in a water bath (32 ºC).

A volume of 10µl semen was diluted with 990µl pre-warmed (32 ºC) TL-Hepes solution (99 TL-Hepes:1 semen). The diluted semen sample was evaluated on a pre-warmed (35 ºC) glass slide under a compound microscope (x400 magnification) within 2 minutes of collection for forward progression (%) and overall motility – on a scale of 0 (no movement) to 5 (very fast forward movement) by scoring 100 sperm cells individually (Loskutoff & Crichton, 2001). Sperm cell concentration per ejaculate was calculated, using 10µl of the previously described diluted semen sample after being refrigerated for 24h at 4-5 ºC and all sperm cells being dead (immobile), with the aid of an improved Newbawer haemocytometer. The sperm cells lying inside the 5 blocks that formed a diagonal line

on the grid were counted and the total multiplied by 5 to obtain the concentration of sperm cells in the semen sample (x 10^6) per ml (Loskutoff & Crichton, 2001).

Two thin semen smears were made from each fresh semen sample on pre-warmed (35 ºC) glass slides. One slide was stained with eosin/nigrosin (60µl eosin/nigrosin and 6µl semen) and the other with eosin B-fast green (60µl eosin B-fast green and 6µl semen). One hundred individual sperm cells from different fields on each slide were evaluated microscopically (x1000 magnification). The eosin/nigosin stained smear was evaluated for vitality (dead or live sperm) and morphology (sperm abnormalities). The sperm cells observed were classified as dead or live and the abnormalities were classified as head, mid-piece and tail abnormalities (Salamon & Maxwell, 2000; Loskutoff & Crichton, 2001). The eosin B-fast green stained semen smear was used to evaluate the sperm vitality (live/dead) and acrosome reaction. The sperm cells observed were classified into 4 groups, namely: live/acrosome intact, live/acrosome reacted, dead/acrosome intact and dead/acrosome reacted (Loskutoff & Crichton, 2001). The rams were slaughtered after 90 days at a commercial abattoir. (The procedure is set out in Chapter 5 regarding the methods used).

At the end of this trial period (90 days), all 6 rams from each group were slaughtered at a commercial abattoir and their carcasses, scrotal and testicular characteristics evaluated and recorded, using the same procedures as described in the previous chapter (Chapter 5). The body weight (empty stomach) and the scrotal circumference (SC) of each ram were determined prior to slaughter. The SC was measured at the widest part of the scrotum

with the aid of a measuring tape. Following slaughter, the carcasses were skinned and eviscerated, the warm carcass weight and grades were recorded and the dressing percentage calculated (SAMIC, 2004). According to this grading system, 1 is a very lean carcass, while 6 represents an over-fat carcass (For more details on the methodology used, refer to Chapter 3 – General Materials and Methods]). The scrota were excised from the carcasses, by severing at the base, as close to the body as possible, preserved by means of refrigeration (4 ºC) for 24h and later the scrota and then weighed and dissected into the scrotal skin, testis, fat, epididymis and *pampiniform plexus*. The scrotal skin thickness was measured with the aid of a caliper at the widest part of the scrotum in the same region where the SC was measured. The weight of each component was recorded and the total volume of the testicles and epididymis were recorded separately by measuring the volume of water displaced after gently plunging these structures into a container filled to the brim with water.

Data was statistically analysed using PROC GLM procedures of SAS (1995). Repeated measures analysis was performed to determine the effect of different dietary energy levels, time (weeks) and the interaction on the semen parameters considered in this study. The one-way ANOVA was used to compare treatments for carcass, scrotal, testicular and semen traits recorded during this trial. When treatment means differed significantly for a specific trait, Tukey's method for multiple comparisons was used to determine which means differed significantly.

6.3 Results and Discussion

Table 6.1 The mean (±se) body weight, carcass, scrotal and testicular characteristics of young Dorper rams at the beginning and end of this phase.

Parameter	Group	Beginning (n=12/group)	End (n=6/group)	Difference
Body weight (kg)	Le	$56.45^{a} \pm 1.50$	$62.70^{b} \pm 2.00$	6.25^{**}
	Me	$65.50^{a} \pm 1.09$	$70.37^{b} \pm 0.66$	4.87^{**}
	He	$71.04^{a} \pm 1.40$	$70.70^{a} \pm 1.11$	-0.70^{ns}
Warm carcass weight (kg)	Le	$22.83^{a} \pm 1.19$	$26.58^{a} \pm 0.89$	3.75^{ns}
	Me	$28.82^{a} \pm 0.80$	$32.75^{b} \pm 0.51$	3.93^{**}
	He	$36.00^{a} \pm 1.49$	$34.00^{a} \pm 0.32$	-2.00^{ns}
Carcass grade (0-6)	Le	$3.00^{a} \pm 0.37$	$2.50^{a} \pm 0.22$	-0.50^{ns}
	Me	$5.17^{a} \pm 0.40$	$4.17^{a} \pm 0.48$	-1.00^{ns}
	He	$6.00^{a} \pm 0$	$3.66^{b} \pm 0.33$	-2.34^{**}
Scrotal circumference (cm)	Le	$9.52^{a} \pm 1.05$	$32.17^{a} \pm 1.22$	2.65^{ns}
	Me	$34.45^{a} \pm 0.94$	$33.50^{a} \pm 0.62$	-0.95^{ns}
	He	$35.53^{a} \pm 0.74$	$34.67^{a} \pm 0.40$	-0.86^{ns}
Total scrotal weight (g)	Le	$646^{a} \pm 50$	$738^{a} \pm 36$	92^{ns}
	Me	$920^{a} \pm 54$	$864^{a} \pm 37$	-56^{ns}
	He	$1062^{a} \pm 53$	$877^{b} \pm 38$	-185^{**}
Scrotal fat (g)	Le	$106^{a} \pm 16$	$122^{a} \pm 22$	16^{ns}
	Me	$180^{a} \pm 14$	$172^{a} \pm 21$	-8^{ns}
	He	$280^{a} \pm 28$	$194^{b} \pm 25$	-86^{**}
Scrotal skin weight (g)	Le	$161^{a} \pm 14$	$163^{a} \pm 13$	2^{ns}
	Me	$198^{a} \pm 6$	$175^{a} \pm 9$	-23^{ns}
	He	$221^{a} \pm 12$	$181^{b} \pm 8$	-40^{**}
Testes weight (g)	Le	$263^{a} \pm 27$	$337^{a} \pm 32$	74^{ns}
	Me	$408^{a} \pm 40$	$373^{a} \pm 17$	-34^{ns}
	He	$399^{a} \pm 24$	$359^{a} \pm 13$	-40^{ns}
Epididymis weight (g)	Le	$54^{a} \pm 1$	$60^{a} \pm 1$	6^{ns}
	Me	$68^{a} \pm 2$	$66^{a} \pm 2$	-2^{ns}
	He	$68^{a} \pm 2$	$60^{a} \pm 1$	-8^{ns}
Testes volume (ml)	Le	$246^{a} \pm 30$	$324^{a} \pm 32$	39^{ns}
	Me	$380^{a} \pm 44$	$357^{a} \pm 16$	-11^{ns}
	He	$384^{a} \pm 21$	$348^{a} \pm 10$	-18^{ns}

^{a,b} Means in rows with different superscripts differ significantly ($P < 0.05$)
^{ns} Non significant differences; ** Significant difference ($P < 0.05$); Le – Low energy; Me – Medium energy; He – High energy

The body weights as well as the most important carcass, scrotal and testicular characteristics in young Dorper rams at the onset and end of this 2nd phase of the trial are summarised in Table 6.1.

The results at the beginning of this 2nd phase of the study (end Table 6.1 of phase 1), are presented as base-line reference values. In fact, these represent the recorded values for each parameter considered at the beginning of this trial phase. During this 2nd phase of this study, animals in both the Le and the Me groups gained some weight ($P<0.05$), however there were no significant changes in carcass grade, scrotal and testicular characteristics as set out in Table 6.1. These results indicate that the ME content of the Le diet was above the actual nutritional maintenance level required by the rams. Nevertheless, lowering the dietary energy level of the He group resulted in a significant ($P<0.05$) reduction in carcass grade, scrotal weight, scrotal fat and scrotal skin weight – without any significant changes in body and carcass weights, as well as scrotal circumference, testes weight, testes volume and epididymis weight.

These results indicate that once the dietary energy level of rams previously fed a high energy diet are restricted, these animals mobilise body fat deposits to maintain their body weight. Adipose tissue from various parts of the body, including scrotal fat (deposited mainly around the *pampiniform plexus* over the vascular cone in the neck region of the scrotum) was mobilised, as indicated by the significant reduction of total scrotal weight and scrotal fat (Table 6.1). This occurred despite the fact that the animals in this group (He) only lost approximately 1 % of their body weight (no significant weight reduction).

It is generally speculated that once deposited, scrotal fat cannot be remobilized again. T-hese fat deposits, contrary to what was suggested by Skinner (1981), seem to be mobili-zed (at least partially) during periods of dietary energy restriction. Consequently the t-hermoregulatory mechanisms of the testis in rams previously fed on high energy diets, may improve. What remains to be evaluated and clarified is whether the degeneration of the seminiferous tubules, reported by Schwalbach *et al*. (2003), as a result of scrotal fat accumulation and consequent thermo-insulation of the testes in bulls, is reversible or not.

The results of the semen characteristics recorded in each group at the beginning and the end of the 90 day feeding period on the Le diet (phase 2 of the study) are depicted in Table 6.2.

No significant differences were recorded between the 3 experimental nutritional groups regarding any of the semen parameters considered during this phase of the trial, when rams previously fed He and Me diets, were fed the Le diet for 90 days. The results obtained at the end of this trial (phase 2 of the study) were similar to those at the beginning of the trial (end of phase 1). It is possible that the lack of any detrimental effect recorded at the end of the previous phase of the study (Chapter 5), have to a large extend determined the outcome in this phase of the study. In other words, in this trial the semen quality was unaffected by the diets used. A possible reason for this can be the fact that the actual energy content of the He diet fed to the rams and/or feeding period was not

high enough and/or not fed for long enough period to cause significant differences in the semen parameters considered between the 3 feeding groups during phase 1 of the study.

Table 6.2 The mean (±se) semen characteristics in young Dorper rams fed a Le diet following a 90 day trial period.

Parameter	Group	Beginning N=12/group	End n=6/group	Difference ns
Semen volume (ml)	Le	0.8 ± 0.1	0.8 ± 0.1	0 ^{ns}
	Me	1.1 ± 0.1	0.1 ± 0.1	-0.1 ^{ns}
	He	1.2 ± 0.1	1.3 ± 0.3	0.1 ^{ns}
Overall motily (%)	Le	84 ± 2	82 ± 2	-2 ^{ns}
	Me	86 ± 2	84 ± 3	-2 ^{ns}
	He	77 ± 3	76 ± 3	-1 ^{ns}
Forward progression (0-5)	Le	2.8 ± 0.1	2.5 ± 0.2	-0.3 ^{ns}
	Me	3.0 ± 0.1	2.8 ± 0.2	-0.2 ^{ns}
	He	2.7 ± 0.2	2.7 ± 0.2	0 ^{ns}
Sperm concentration	Le	1851 ± 121	1850 ± 172	-1 ^{ns}
(x 10^6/ml)	Me	1678 ± 172	1144 ± 185	-534 ^{ns}
	He	1834 ± 169	1640 ± 260	-194 ^{ns}
Live sperm (%)	Le	70 ± 2	82 ± 2	12 ^{ns}
	Me	69 ± 4	80 ± 3	11 ^{ns}
	He	64 ± 3	77 ± 5	13 ^{ns}
Normal sperm (%)	Le	96 ± 2	88 ± 4	-8 ^{ns}
	Me	91 ± 4	87 ± 6	-4 ^{ns}
	He	91 ± 4	96 ± 1	5 ^{ns}
Live/ acrosome intact	Le	72.36 ± 1.50	70.33 ± 3.98	-2.03 ^{ns}
	Me	69.35 ±1.66	76.67 ± 3.53	7.32 ^{ns}
	He	65.23 ±1.66	67.33 ± 6.28	2.1 ^{ns}
Dead/ acrosome intact	Le	17.71 ± 1.01	17.33 ± 1.67	-0.38 ^{ns}
	Me	18.42 ± 1.07	10.83 ± 2.50	-7.59 ^{ns}
	He	20.98 ± 1.20	15.83 ± 2.54	-5.15 ^{ns}
Dead/ acrosome reacted	Le	9.98 ± 0.93	11.00 ± 2.24	1.02 ^{ns}
	Me	11.49 ± 1.02	12.50 ± 3.91	1.01 ^{ns}
	He	13.48 ± 1.29	16.83 ± 5.38	3.35 ^{ns}

^{ns} Non significant difference between means; Le – Low energy; Me – Medium energy; He – High energy

These results may have undermined those in phase 2 of the study. However, animals in the Le and Me group gained ($P<0.05$) weight during the 90 days of the trial period on the Le diet. This clearly indicates that the actual ME content of the Le diet was slightly higher than the maintenance level for Dorper rams (11-12 month old).

The results (P-values) of the repeated measures analysis of the means of the different semen parameters during this trial are set out in table 6.3. As observed (Table 6.3), there were no significant differences recorded between the different treatment groups regarding the semen characteristics.

Table 6.3 P-values for different semen parameters evaluated in Dorper rams fed a low energy diet (90 day trial period)

Y value	Week 3	Week 6	Week 9	Week 12
Volume ^{ns}	0.1379	0.3287	0.2874	0.2348
Concentration ^{ns}	0.7164	0.5495	0.4038	0.2602
Motility ^{ns}	0.8943	0.2759	0.9938	0.1829
Forward progression ^{ns}	0.7618	0.2273	0.9786	0.3849
Live/acrosome intact ^{ns}	0.7758	0.3837	0.8438	0.1471
Dead/acrosome intact ^{ns}	0.3873	0.2579	0.6033	0.1053
Dead/acrosome react ^{ns}	0.9318	0.7143	0.4918	0.3482
E/N Live ^{ns}	0.8838	0.9327	0.3651	0.5466
E/N Dead ^{ns}	0.8838	0.9327	0.3651	0.5306
Head abnormalities ^{ns}	0.3952	0.4607	0.4081	0.6142
Tail abnormalities ^{ns}	0.7406	0.6838	0.2342	0.6548

^{ns} No significant differences ($P<0.05$)

6.4 Conclusions

The layout of the experiment, particularly, the lower actual ME content of the He fed during the previous phase of this study did not allow a meaningful evaluation of the reversi-

bility of the hypothesized detrimental effects of the He diets on semen quality and quantity. In fact, no detrimental effects were recorded on the semen parameters that could be reversed in this second phase of the study. Nevertheless, these results clearly indicate that the scrotal fat deposits can be mobilized (at least partially) in rams previously fed He diets during subsequent periods of dietary energy restrictions. This is the first time that such a phenomenon has been reported.

Further research on the effect of He diets on subsequent ram fertility (short and long term) as well as on their reversibility are warranted to evaluate the common practice of over-conditioning young replacement breeding rams.

Chapter 7

General Conclusions and Recommendations

7.1 General Conclusions

In general, an increase in the dietary energy level, resulted in higher growth rates, better feed conversion rates, larger carcasses, higher dressing percentages, greater fat deposition in the body (in the carcass and around the kidneys) and in the scrotum, particularly over the vascular testicular cone (scrotal neck) of the rams. Testicular growth (scrotal circumference, testes volume and weight) also increased by feeding higher energy levels. At the end of the trial period (phase 1) significant differences were recorded in most production traits considered in the study between the 2 nutritional extremes (He and Le), but not between the He and the intermediate dietary energy level (Me). It is clear from the results of the present study that the feeding of young replacement ram lambs for 127 days according to NRC (1985) feeding standards, will result in over fat Dorper ram lambs (weight range 40 to 70kg). This energy level (9.6 MJ ME/kg dietary DM or 21 MJ ME/day) will result in excessive subcutaneous, kidney, intermuscular (marbeling) and scrotal fat accumulation. Under these circumstances, an energy level of 8 MJ ME/kg DM or 15 MJ ME/ram/day seems to be more appropriate for young replacement Dorper rams.

Despite the significant differences induced by the different dietary ME contents and excessive fat accumulation recorded in Dorper ram lambs at the highest dietary energy level, no detrimental effects occurred in their semen quantity, quality and cryotolerance. Of the 3 independent variables (factors) considered in the model (diet, week in trial and

their interaction), only the diet (ME level) had a significant effect on certain semen parameters considered in some weeks. However, no clear trend was recorded, suggesting that other unaccountable (confounding) factors may have been responsible for these changes.

The layout of the experiment, particularly, the absence of the hypothesized detrimental effects of scrotal fat deposition on semen quality and quantity, probably due to an insufficiently high ME content of the He diet fed during the first phase of the study, did not allow a meaningful evaluation of the reversibility of the hypothesized detrimental effects of the He diets on semen quality and quantity. No evident detrimental effects were recorded on the semen parameters that could possibly be reversed in the second phase of the study. Nevertheless, the results of the second phase of the study clearly indicated that the scrotal fat deposits could be mobilized (at least partially) in rams previously fed He diets – during subsequent periods of dietary energy restrictions. This is the first time that such a phenomenon has been reported. It is generally speculated that once deposited, scrotal fat cannot be mobilized again but this is not completely true. Semen quality was not detrimentally influenced by dietary levels; age of the males and the nutritional period could have been restricting factors.

Further research is warranted on the effects of high energy diets, associated with many other management factors commonly used on subsequent ram fertility (short and long term) as well as on their reversibility, in order to fully evaluate the common practice of feeding high energy diets to young replacement rams.

7.2 Recommendations

For future studies on the possible effect of high energy diets on scrotal, testicular and semen characteristics (quantity, quality and cryotolerance) in rams, the following aspects can be recommended:

1. The age of the animals used may also may have had an effect on the outcome of the trial. It is thus recommended that rams of different age groups (growing to fully grown) are compared – in order to evaluate the effect of age at which high energy diets are fed, on the scrotal, testicular and semen characteristics. Breeds i.e. woolled vs. meat producing breeds may respond differently due to the partioning of nutrients

2. In future trials, it may be important to increase the number of experimental animals in each experimental group considered. The scrotal, testicular and especially the semen characteristics of males tend to record great variation (variance), which limits the probability of finding significant differences between treatments. Lager numbers of experimental animals may allow more useful statistical comparisons. Seasonality is an aspect not to be underestimated.

3. Different cryodiluents and freezing protocols commonly used for the cryopreservation of ram semen and new cryodiluents and protocols should be evaluated to identify alternative and perhaps better diluents and freezing protocols especially for rams on different (energy/protein) diets. However the cryopreservation of semen adds other variables that could or could not be correlated with the dietary level.

4. Furthermore, the ME differences between experimental groups should be increased and more extreme diets should be used (higher and lower ME content than those used in this study), to induce definite response differences.

Abstract

Effect of different dietary energy levels on productive and reproductive traits in Dorper rams

by

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A study was conducted to evaluate the effects of different dietary energy levels on the productive (ADG, FCR, body weight, carcass) and reproductive (scrotum, testes, semen) characteristics of young Dorper rams. The study was carried out in two phases at the University of the Free State campus in Bloemfontein, South Africa. Out of a group of 60 rams, 36 animals that were successfully trained for semen collection with the aid of an artificial vagina (AV), were selected to be part of this study and housed in individual metabolic cages.

During phase 1 of this study, 36 (11 to 12 months old) Dorper rams with a mean initial body weight of 42.0 ± 0.52kg were randomly allocated to 3 groups (n=12 per group). Each group was randomly assigned to one of 3 experimental diets (treatments), formulated on a crude protein, degradable protein, calcium and phosphorus equivalent basis. A medium energy (Me) diet was formulated according to the National Research Council standards (NRC) for young growing rams to serve as the control diet. The metabolisable energy (ME) content of this diet (Me) was increased by 15 % for the high energy (He) and decreased by 15% for the low energy (Le) diets respectively. The actual ME levels for the Le, Me, He: were 6.52, 8.09 and 9.39 MJ/kg, respectively. The rams received the diets and fresh water ad libitum during a 127 day trial period and were weighed once a week. Their ADG, FCR and ME/kg weight gain calculated and compared amongst groups.

During this trial, a digestibility trial was conducted for 7 days (during week 14 of the trial), using 5 randomly selected animals from each treatment group. The amount of feed offered and consumed by each animal as well as the feaces excreted daily were recorded. The chemical composition of the diets and the feaces (dry matter, energy, crude and degradable protein) were determined.

Semen was collected from each ram, every forthnight for 3 consecutive days, with the aid of an AV and evaluated for volume, overall sperm motility, forward progression, sperm concentration, % live sperm and % normal sperm. The semen samples were then diluted (using a one-step dilution method with a cryodiluent containing 5% glycerol), packed in

0.25ml plastic straws, equilibrated for 4 hours and cryoperserved (frozen) in liquid nitrogen vapour (-70 ºC).

One week after freezing, the semen was thawed (38 ºC for 30 seconds) and evaluated for the same qualitative parameters as the fresh semen. The results were compared amongst groups for each collection time, using ANOVA for repeated measures analysis procedures of SAS. The energy level of the diet and the collection week as well as their interaction was used as independent variables, while the different semen parameters considered were the dependent variables in the model. When means differed significantly, the Tukey method was used to compare means.

At the end of this trial period, 6 rams per group were randomly selected and slaughtered at a commercial abbatoir. After slaughtering the carcass characteristics (cold carcass weight, dressing percentage, carcass grade, shoulder circumference, buttock circumference, backfat thickness, eye muscle area, marbeling and kidney fat) were recorded and compared amongst groups. The scrotum (total weight, skin weight, fat and circumference), testes (volume and weight) and epidydimis (weight and volume) characteristics were also recorded and compared between groups, using ANOVA procedures of SAS. When means differed significantly the Tukey method was used to compare means.

From the independent variables considered in the model, only the diet (ME level) had a significant effect on the parameters considered. In general, an increase in dietary energy level resulted in higher growth rates, better feed conversion rates, heavier carcasses, hig-

her dressing percentages and greater fat deposition, both in the carcass, around the kidneys and in the scrotum (particularly in the neck region, over the testicular vascular cone). It seems that the energy requirements recommended by the NRC (1985) are not applicable for early mature Dorper replacement ram lambs, as those in the He group, (real ME level similar to NRC recommendations) deposited excessive fat in their body. Despite the significant differences induced by the He on the growth performance, carcass characteristics as well as on the scrotal and testicular characteristics of Dorper rams, no detrimental effects were recorded in their semen quantity, quality and cryotolerance.

During the following 90 days (phase 2), the remaining rams (n=6 per group) were further maintained on the Le diet for 90 days. This was done to simulate the situation when overconditioned (fat) rams bought at auctions are placed on low energy diets (veld). Other objectives of this trial phase were to evaluate if the fat deposits accumulated in the scrota of rams fed on high energy diets are mobilized during subsequent periods of moderate nutritional restriction and if these nutritional restrictions induce any changes on the semen characteristics of rams.

During this phase of the trial, semen was collected, with the aid of an AV, every 3 weeks during 2 consecutive days. The semen was evaluated using the same procedures described for phase 1 of this study. The rams were slaughtered at the end of this trial period (90 days) at a commercial abattoir. After slaughtering, the carcass characteristics (cold carcass weight, dressing percentage, carcass grade, shoulder circumference, buttock circumference, backfat thickness, eye muscle area, marbeling and kidney fat), the testicular

(volume and weight), the scrotal (weight, circumference, skin weight and fat) and the e-pididymis characteristics (weight and volume) were also recorded and compared amongst groups. Data was analysed statistically using the same procedures as described for phase 1.

The results of this trial clearly indicated that the scrotal fat deposits of rams previously fed on the He diet are mobilized (at least partially) during subsequent periods of moderate dietary energy restrictions without adverse effects on semen quantity and quality. This is the first time that such finding is reported.

Further research on the effect of high energy diets on subsequent ram fertility (short and long terms), as well as their reversibility are warranted. Different age groups, feeding regimes and other management practices must also be associated to high energy diets in order to fully evaluate the effect of these common practices on subsequent ram fertility.

Opsomming

Effek van verskillende dieet vlakke op die produksie en reproduksie kenmerke van Dorper ramme

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Die studie is uitgevoer om die effekte van verskillende energiepeile in diëte op die produksie - (GDT, VOV, liggaamsgewig en karkaseienskappe) en reproduksie - (skrotum, testes en semen) eienskappe in jong Dorperramme te bepaal. Die studie is in 2 fases by die Universiteit van die Vrystaat kampus in Bloemfontein, Suid-Afrika uitgevoer.

Uit 'n groep van 60 ramme, is 36 suksesvol afgerig vir semenkolleksie mbv 'n kunsvagina. Hulle is vir die studie opgeneem en gehuisves in individuele metaboliese hokke. Gedurende fase 1 van die studie is 36 Dorperramme (wat wissel in ouderdom van 11 tot 12

maande) en met ‘n gemiddelde aanvangs- liggaamsgewig van 42.0 ± 0.52kg, lukraak aan 3 behandelingsgroepe (n=12 per groep) toegewys.

Elke groep het een van 3 eksperimentele diëte, behandelings, (geformuleer op ekwivalente ru-protein, degradeerbare proteïen, kalsium en fosfor basis) ontvang. ‘n Medium-energie (Me) dieet is volgens die NRC-riglyne vir jong, groeiende ramme geformuleer en het as ‘n kontrole dieet gedien. Die metaboliese energie (ME) inhoud van hierdie dieet (Me) is met 15 % verhoog om ‘n hoë-energie (He) dieet daar te stel en met 15 % verminder vir ‘n lae energie (Le) dieet. Die werklike ME vlak vir die Le, Me en He was 6.52, 8.09 en 9.39 MJ/kg onderskeidelik. Die ramme het hierdie diëte en vars water ad libitum vir ‘n 127 dae proefperiode ontvang. Die diere is weekliks geweeg en die GDT, VOV en ME/kg gewigstoename is bepaal en tussen groepe vergelyk.

Tydens hierdie tydperk van die proef is ‘n verteerbaarheidsstudie vir ‘n periode van 7 dae gedurende week 14 van die proefperiode uitgevoer, deur 5 diere lukraak van elke behandelingsgroep te kies. Die hoeveelheid voer verskaf en geweier deur elke dier sowel as die faeces uitgeskei, is bepaal. Die chemiese samestelling van die dieet en faeces (ruproteïen, degradeerbare proteïen en energie) is bereken.

Semen is mbv ‘n kunsvagina elke 2 weke (3 opeenvolgende dae) gekollekteer vir semenvolume, algehele spermmotiliteit, progressiewe beweging, spermkonsentrasie, % lewendige sperme en % normale sperme geëvalueer. Deur gebruik te maak van ‘n een-stap verdunningsmetode, is die semen met ‘n krioverdunningsmiddel (5 % gliserol) verdun,

in 0.25ml plastiese strooitjie verpak en vir 4 ure geëkwilibreer en vervolgens in vloeibare stikstof dampe (-70 ºC) gekriopresiveer.

Die semen is 'n week na bevriesing ontdooi (38 ºC vir 30 sekondes) en geëvalueer vir dieselfde kwantatiewe kenmerke as vir vars semen. Die resultate is vergelyk tussen groepe vir elke kolleksie deur gebruik te maak van die ANOVA vir herhaalde meting prosedures van SAS. Die energievlak van die dieet sowel as die interaksie van die energievlakke, is as onafhanklike veranderlikes in die model gebruik, terwyl die verskillende semenparameters in ag geneem is ook as onafhanklike veranderlikes. Waar die gemiddeldes betekenisvol verskil het, is die Tukey-metode gebruik om die gemiddeldes te vergelyk.

Aan die einde van hierdie proefperiode is 6 ramme per groep gekies en by 'n kommersiële slagpale geslag. Na slagting is die karkaseienskappe (koue karkasgewig, uitslagpersentasie, karkasgradering, karkasgraad, skoueromtrek, boudomtrek, rugvetdikte, oogspierarea, marmering, niervet en skrotale vet) bepaal en tussen groepe vergelyk. Die volgende eienskappe; skrotum (totale gewig, vel gewig, vet en omtrek); testes (volume en gewig) en epididimis (gewig en volume), is bepaal en tussen groepe vergelyk, deur gebruik te maak van die ANOVA prosedures van SAS. Waar die gemiddeldes betekenisvol verskil het, is die Tukey-metode gebruik om die gemiddeldes te vergelyk. Van die onafhanklike veranderlikes wat in die model in ag geneem is, het slegs dieet (ME vlak) 'n betekenisvolle effek op die parameters gehad.

Oor die algemeen, het 'n toename in dieët-energievlakke 'n hoër groeitempo, beide tov die karkas en vetneerlegging om die niere en in die skrotum (veral in die nek area, oor die testikulêre keël), tot gevolg gehad. Dit blyk of die energiebehoeftes wat deur die NRC (1985) voorgestel is nie vir vroegryp Dorper vervangingsramslammers gebruik kan word nie omdat die karkasse van die ramme in die He groep (ware ME vlak dieselfde as NRC se aanbevelings) te veel vet aangesit het.

Alhoewel betekenisvolle verskille veroorsaak is deur die He dieet op groeitempo, karkas-eienskappe sowel as op skrotale and testikulêre eienskappe van Dorper ramme, is geen betekenisvolle effekte in die semen kwantiteit, kwaliteit en krioperservering waargeneem nie.

Tydens die volgende 90 dae (fase 2) is al die oorblywende ramme (n=6 per groep) die Le dieet gevoer. Die rede hiervoor was om die situasie te simuleer waar oorvet ramme gekoop word en op lae energie diëte (veld) geplaas word. 'n Ander doelwit van die studie was om te bepaal of die vetdeponering wat in die skrota akkumuleer, tydens gemiddelde periodes van voedingbeperkings gemobiliseer word en of hierdie voeding veranderings enige verandering in die semeneienskappe van ramme tot gevolg het.

Gedurende hierdie fase is semen mbv 'n KV, elke 3 weke (oor 2 opeenvolgende dae) gekollekteer. Die semen is geëvalueer deur gebruik te maak van dieselfde prosedures as die wat in fase 1 van dié studie beskryf is. Al die ramme is aan die einde van hierdie proeftydperk by 'n kommersiële slagpale geslag. Na slagting is die karkaseienskappe

(koue karkasmassa, uitslagpersentasie, karkasgradering, skoueromtrek, boudomtrek, rugvetdikte, oogspierarea, marmering, niervet en skrotale vet) bepaal en tussen groepe vergelyk. Die skrotum - (totale gewig, vel gewig, vet en omtrek), testes - (volume en gewig) en epididimis eienskappe (gewig en volume) is bepaal en tussen groepe vergelyk. Data is statisties geanaliseer deur gebruik te maak van dieselfde prosedures soos beskryf in fase 1.

Resultate in die studie wys duidelik dat die skrotale gedeponeerde vet in ramme wat voorheen 'n He dieet gevoer is, gedurende opvolgende periodes van gemiddelde energiebeperkings gemobiliseer (of ten minste gedeeltelik) word. Hierdie periodes van middelmatige energiebeperkings het egter geen negatiewe effek op semenkwantiteit en -kwaliteit veroorsaak nie.

Verdere navorsing oor die effek van hoë-energie diëte op daaropvolgende vrugbaarheid in ramme (kort- en langtermyn), sowel as die omkeerbaarheid van vetneerlegging, is geregverdig. Verskillende ouderdomsgroepe, skaaprasse, periodes van behandeling, voerprogramme and ander bestuurspraktyke geassosieer met hoë-energie diëte om die effek van hierdie algemene praktyke op ram vrugbaarheid ten volle te evalueer, moet ook aandag geniet.

Chapter 9

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Chapter 10

Annexure A

10.1 Annexure to chapter 5

Semen volume, concentration, motility and forward progression during of phase 1 of the trial (phase 1)

	Treatment	Volume (ml)	Concentration (x 10^6/ml)	Motility (%)	Forward progression (0-5)
Week 4	Le	$1.02^{a} \pm 0.19$	$1201^{a} \pm 157$	$69.70^{a} \pm 4.93$	$1.91^{a} \pm 0.28$
	Me	$0.95^{a} \pm 0.13$	$1153^{a} \pm 183$	$63.89^{a} \pm 5.94$	$1.46^{a} \pm 0.26$
	He	$1.02^{a} \pm 0.09$	$1030^{a} \pm 210$	$67.22^{a} \pm 7.46$	$2.06^{a} \pm 0.21$
Week 6	Le	$0.07^{a} \pm 0.07$	$1156^{a} \pm 158$	$83.09^{a} \pm 1.80$	$2.35^{a} \pm 0.13$
	Me	$0.88^{a} \pm 0.09$	$1200^{a} \pm 116$	$73.64^{a} \pm 3.19$	$2.07^{a} \pm 0.17$
	He	$0.81^{a} \pm 0.09$	$1462^{a} \pm 208$	$74.50^{a} \pm 3.54$	$2.03^{a} \pm 0.16$
Week 8	Le	$0.83^{a} \pm 0.07$	$1025^{a} \pm 130$	$77.33^{a*} \pm 2.14$	$1.85^{a*} \pm 0.11$
	Me	$0.91^{a} \pm 0.09$	$867^{a} \pm 102$	$63.67^{b*} \pm 4.69$	$1.39^{b*} \pm 0.15$
	He	$1.08^{a} \pm 0.11$	$1054^{a} \pm 164$	$68.00^{ab*} \pm 2.42$	$1.59^{ab*} \pm 0.12$
Week 10	Le	$0.90^{a} \pm 0.06$	$1129^{a} \pm 167$	$83.45^{a*} \pm 2.97$	$2.28^{a*} \pm 0.18$
	Me	$1.05^{a} \pm 0.08$	$1154^{a} \pm 137$	$68.67^{b*} \pm 3.73$	$1.71^{ab*} \pm 0.17$
	He	$1.12^{a} \pm 0.08$	$1160^{a} \pm 119$	$67.17^{b*} \pm 4.21$	$1.55^{b*} \pm 0.14$
Week 12	Le	$0.92^{a} \pm 0.08$	$1577^{a} \pm 108$	$74.17^{a*} \pm 3.93$	$1.79^{a} \pm 0.19$
	Me	$1.13^{a} \pm 0.09$	$1657^{a} \pm 127$	$70.67^{ab*} \pm 3.94$	$1.83^{a} \pm 0.22$
	He	$1.07^{a} \pm 0.07$	$1619^{a} \pm 134$	$62.27^{b*} \pm 4.50$	$1.44^{a} \pm 0.19$
Week 14	Le	$0.88^{a} \pm 0.07$	$1417^{a} \pm 127$	$77.33^{a} \pm 3.11$	$2.31^{a} \pm 0.16$
	Me	$1.06^{a} \pm 0.12$	$1495^{a} \pm 100$	$75.67^{a} \pm 2.14$	$2.06^{a} \pm 0.07$
	He	$1.19^{a} \pm 0.12$	$1489^{a} \pm 64$	$71.67^{a} \pm 3.51$	$2.01^{a} \pm 0.18$
Week 16	Le	$0.88^{a} \pm 0.07$	$1760^{a} \pm 118$	$80.33^{a} \pm 2.66$	$2.56^{a} \pm 0.18$
	Me	$1.06^{a} \pm 0.11$	$1930^{a} \pm 107$	$78.83^{a} \pm 3.26$	$2.49^{a} \pm 0.15$
	He	$1.67^{a} \pm 0.08$	$1892^{a} \pm 103$	$79.17^{a} \pm 2.46$	$2.62^{a} \pm 0.15$
Week 18	Le	$0.93^{a} \pm 0.08$	$1848^{a} \pm 94$	$77.82^{a} \pm 4.13$	$2.59^{a} \pm 0.26$
	Me	$0.92^{a} \pm 0.09$	$1844^{a} \pm 113$	$75.17^{a} \pm 2.50$	$2.48^{a} \pm 0.11$
	He	$1.21^{a} \pm 0.11$	$2011^{a} \pm 170$	$78.5^{a} \pm 3.28$	$2.60^{a} \pm 0.16$
Week 20	Le	$0.79^{a} \pm 0.07$	$1851^{a} \pm 121$	$83.5^{a} \pm 2.34$	$2.82^{a} \pm 0.13$
	Me	$1.08^{ab} \pm 0.11$	$1678^{a} \pm 172$	$85.67^{a} \pm 3.89$	$3.03^{a} \pm 0.13$
	He	$1.15^{b} \pm 0.09$	$1834^{a} \pm 168$	$77.17^{a} \pm 2.01$	$2.65^{a} \pm 0.18$

10.1 Annexure to chapter 5

Sperm alive/dead (eosin/nigrosin) and acrosome evaluation (eosin B-fast green) during of phase 1 of the trial

	Treatment	Live/AI (%)	Dead/AI (%)	Live/AR (%)	Dead/AR (%)
Week 4	Le	73.09 [a] ± 3.99	18.7 [a] ± 2.80	0	9.3 [a] ± 4.06
	Me	57.2 [a] ± 4.35	27.1 [a] ± 4.43	0	11.5 [a] ± 3.32
	He	60.8 [a] ± 3.46	27.55 [a] ± 3.31	0	13.45 [a] ± 4.36
Week 6	Le	86.00 [a] ± 1.69	11.92 [a] ± 1.53	0	2.00 [a] ± 0.49
	Me	73.75 [a] ± 5.42	15.08 [a] ± 2.22	0	6.45 [a] ± 1.85
	He	69.3 [a] ± 5.94	20.1 [a] ± 3.06	0	10.7 [a] ± 4.61
Week 8	Le	75.42 [a] ± 3.17	13.42 [a] ± 2.21	0	11.00 [a] ± 2.22
	Me	75.67 [a] ± 3.54	13.75 [a] ± 2.16	0	9.83 [a] ± 2.59
	He	66.92 [a] ± 5.88	12.75 [a] ± 2.50	0	20.58 [a] ± 6.48
Week 10	Le	80.36 [a] ± 2.74	12.9 [a] ± 1.95	0	8.00 [a] ± 1.53
	Me	77.92 [a] ± 2.70	13.25 [a] ± 1.75	0	8.42 [a] ± 2.06
	He	73.25 [a] ± 1.44	15.83 [a] ± 1.27	0	10.92 [a] ± 1.29
Week 12	Le	72.25 [a] ± 3.90	15.08 [a] ± 1.89	0	12.92 [a*] ± 2.58
	Me	74.82 [a] ± 4.42	15.18 [a] ± 2.63	0	10.00 [a*] ± 2.55
	He	62.36 [a] ± 5.30	24.45 [a] ± 2.68	0	9.82 [a*] ± 2.27
Week 14	Le	80.00 [a] ± 2.71	14.17 [a] ± 2.37	0	5.08 [a] ± 0.96
	Me	80.5 [a] ± 2.47	10.27 [a] ± 1.17	0	9.42 [a] ± 2.37
	He	78.08 [a] ± 2.80	13.67 [a] ± 2.92	0	8.75 [a] ± 1.30
Week 16	Le	69.17 [a] ± 3.36	19 [a] ± 2.63	0	11.92 [a] ± 1.98
	Me	62.33 [a] ± 4.38	25.67 [a] ± 2.83	0	12.08 [a] ± 2.36
	He	64.17 [a] ± 5.05	22.92 [a] ± 3.64	0	12.92 [a] ± 2.67
Week 18	Le	52.91 [a] ± 53.77	28.73 [a] ± 4.59	0	18.45 [a] ± 4.98
	Me	50.67 [a] ± 5.34	21.25 [a] ± 2.63	0	25.75 [a] ± 4.46
	He	57.58 [a] ± 4.40	27.17 [a] ± 4.49	0	15.00 [a] ± 2.82
Week 20	Le	61.92 [a] ± 4.14	25.75 [a] ± 2.84	0	11.58 [a] ± 2.02
	Me	66.08 [a] ± 5.22	24.75 [a] ± 4.37	0	9.42 [a] ± 1.77
	He	56.67 [a] ± 6.01	25.58 [a] ± 4.66	0	18.08 [a] ± 5.28

[a,b,c] Means in rows with different superscripts differ significantly ($P<0.05$) Le – Low energy; Me – Medium energy; He – High energy

10.1 Annexure to chapter 5

Sperm acrosome reaction (eosin B-fast green) and abnormalities during of phase 1 of the trial

	Treatment	Live (%)	Dead (%)	Abnormalities (%)
Week 4	Le	66.92 [a] ± 6.04	32.91 [a] ± 6.61	5.58 [a] ± 1.63
	Me	68.36 [a] ± 2.73	30.73 [a] ± 2.84	11.42 [a] ± 3.01
	He	66.91 [a] ± 3.61	31.64 [a] ± 3.62	7.33 [a] ± 2.57
Week 6	Le	81.42 [a] ± 2.78	18.58 [a] ± 2.78	4.67 [a] ± 0.77
	Me	71.58 [a] ± 4.05	29.25 [a] ± 4.18	11.17 [a] ± 3.74
	He	65.00 [a] ± 5.36	35.00 [a] ± 5.36	10.42 [a] ± 3.26
Week 8	Le	82.25 [a] ± 3.33	17.75 [a] ± 3.33	9.00 [a] ± 1.74
	Me	85.42 [a] ± 1.27	14.08 [a] ± 1.35	25.25 [a] ± 4.33
	He	78.25 [a] ± 3.30	21.75 [a] ± 3.30	14.5 [a] ± 3.95
Week 10	Le	88.72 [a] ± 0.65	11.18 [a] ± 0.70	9.18 [a] ± 2.14
	Me	87.25 [a] ± 1.92	12.50 [a] ± 1.97	23.92 [a] ± 5.02
	He	86.03 [a] ± 2.09	13.25 [a] ± 2.07	15.42 [a] ± 5.13
Week 12	Le	77.25 [a] ± 3.78	20 [a] ± 3.16	9.00 [a] ± 1.83
	Me	74.92 [a] ± 4.33	24.83 [a] ± 4.31	15.33 [a] ± 3.81
	He	74.27 [a] ± 4.08	24.63 [a] ± 3.95	16.08 [a] ± 3.48
Week 14	Le	80.83 [a] ± 2.80	18.92 [a] ± 2.81	6.75 [a] ± 1.32
	Me	78.67 [a] ± 3.43	21.33 [a] ± 3.41	14.58 [a] ± 3.72
	He	71.75 [a] ± 4.71	24.25 [a] ± 3.06	13.5 [a] ± 3.41
Week 16	Le	70.5 [a] ± 2.52	28.25 [a] ± 2.56	10.75 [a] ± 2.37
	Me	69.75 [a] ± 3.12	29.33 [a] ± 3.37	14.25 [a] ± 4.66
	He	64.92 [a] ± 3.17	33.25 [a] ± 3.03	7.5 [a] ± 1.44
Week 18	Le	64.82 [a] ± 3.56	34.80 [a] ± 3.42	14.58 [a] ± 3.08
	Me	61.00 [a] ± 3.14	37.83 [a] ± 3.06	10.50 [a] ± 2.42
	He	62.45 [a] ± 3.74	32.45 [a] ± 2.94	10.50 [a] ± 2.42
Week 20	Le	68.73 [a] ± 2.49	30.36 [a] ± 2.59	4.62 [a] ± 1.74
	Me	69.45 [a] ± 4.12	28.55 [a] ± 4.28	8.67 [a] ± 3.97
	He	61.08 [a] ± 3.69	33.92 [a] ± 2.81	6.83 [a] ± 1.57

[a,b,c] Means in rows with different superscripts differ significantly ($P<0.05$)Le – Low energy; Me – Medium energy; He – High energy

10.2 Annexure to chapter 6

Semen volume, concentration, motility and forward progression during phase 2 of the trial (phase 2)

	Treat-ment	Volume (ml)	Concentration (x 10^6)	Motility (%)	Forward progression (1 – 5)
Week 3	Le	0.80 [a] ± 0.15	1786.00 [a] ± 109.22	77.20 [a] ± 2.87	2.66 [a] ± 0.13
	Me	1.29 [a] ± 0.16	1858.33 [a] ± 305.57	74.67 [a] ± 2.95	2.52 [a] ± 2.95
	He	0.08 [a] ± 00.17	2027.50 [a] ± 129.23	75.67 [a] ± 4.72	2.45 [a] ± 0.24
Week 6	Le	0.92 [a] ± 0.08	2020.00 [a] ± 343.60	83.33 [a] ± 3.71	2.95 [a] ± 0.27
	Me	1.33 [a] ± 0.22	1453.33 [a] ± 160.45	70.67 [a] ± 7.69	2.62 [a] ± 0.46
	He	0.96 [a] ± 0.26	1880.00 [a] ± 221.89	85.33 [a] ± 3.71	2.98 [a] ± 0.70
Week 9	Le	0.77 [a] ± 0.08	1559.17 [a] ± 94.48	67.67 [a] ± 10.26	2.36 [a] ± 0.43
	Me	1.38 [a] ± 0.29	1591.67 [a] ± 194.73	50.33 [a] ± 11.48	1.74 [a] ± 0.41
	He	1.38 [a] ± 0.35	1815.83 [a] ± 157.25	72.67 [a] ± 5.05	2.71 [a] ± 0.23
Week 12	Le	0.92 [a] ± 0.17	1286.67 [a] ± 57.59	74.67 [a] ± 6.57	2.66 [a] ± 0.35
	Me	1.04 [a] ± 0.14	1492.50 [a] ± 132.29	74.67 [a] ± 4.52	2.65 [a] ± 0.20
	He	0.41 [a] ± 0.18	1640.83 [a] ± 260.69	73.67 [a] ± 9.60	0.85 [a] ± 0.46
Week 15	Le	0.79 [a] ± 0.10	1580.83 [a] ± 172.08	82.67 [a] ± 2.29	2.74 [a] ± 0.21
	Me	1.04 [a] ± 0.12	1144.17 [a] ± 185.31	84.00 [a] ± 3.22	2.80 [a] ± 0.22
	He	1.29 [a] ± 0.31	1640.83 [a] ± 260.69	76.33 [a] ± 3.15	2.50 [a] ± 0.20

10.2 Annexure to chapter 6

Sperm alive/dead (eosin/nigrosin) and acrosome reaction (eosin B-fast green) during phase 2 of the trial

	Treatment	Live/AI	Dead/AI	Live/AR	Dead/AR
Week 3	Le	61.20 ^{a} ± 10.30	20.00 ^{a} ± 1.00	0	18.80 ^{a} ± 9.85
	Me	54.17 ^{a} ± 8.85	28.50 ^{a} ± 9.42	0	17.33 ^{a} ± 6.12
	He	63.00 ^{a} ± 9.10	15.60 ^{a} ± 4.23	0	21.40 ^{a} ± 7.46
Week 6	Le	79.00 ^{a} ± 8.54	17.00 ^{a} ± 8.02	0	4.00 ^{a} ± 0.58
	Me	54.33 ^{a} ± 7.26	21 ^{a} ± 3.21	0	24.67 ^{a} ± 10.40
	He	76.67 ^{a} ± 3.84	13.00 ^{a} ± 1.53	0	10.33 ^{a} ± 5.33
Week 9	Le	51.83 ^{a} ± 10.45	38.33 ^{a} ± 12.26	0	9.33 ^{a} ± 3.71
	Me	70.50 ^{a} ± 4.17	16.00 ^{a} ± 2.67	0	13.33 ^{a} ± 2.64
	He	62.17 ^{a} ± 9.66	22.50 ^{a} ± 7.71	0	13.67 ^{a} ± 7.09
Week 12	Le	75.50 ^{a} ± 5.37	16.17 ^{a} ± 2.97	0	8.33 ^{a} ± 2.72
	Me	72.00 ^{a} ± 6.80	13.33 ^{a} ± 2.01	0	15.00 ^{a} ± 5.55
	He	70.67 ^{a} ± 5.67	13.33 ^{a} ± 2.78	0	15.83 ^{a} ± 4.21
Week 15	Le	70.33 ^{a} ± 3.98	17.33 ^{a} ± 1.67	0	11.00 ^{a} ± 2.36
	Me	76.67 ^{a} ± 3.53	10.83 ^{a} ± 2.50	0	12.5 ^{a} ± 3.91
	He	67.33 ^{a} ± 6.28	15.83 ^{a} ± 2.54	0	16.83 ^{a} ± 5.37

10.2 Annexure to chapter 6

Sperm acrosome reaction (eosin B-fast green) and abnormalities during the trial (phase 1)

	Treatment	E/N Live	E/N dead	Abnormalities
Week 3	Le	61.33 ^{a} ± 7.63	38.67 ^{a} ± 7.63	0.60 ^{a} ± 0.40
	Me	64.73 ^{a} ± 5.52	35.27 ^{a} ± 5.52	5.83 ^{a} ± 5.44
	He	64.82 ^{a} ± 3.07	34.18 ^{a} ± 3.07	11.83 ^{a} ± 7.20
Week 6	Le	72.33 ^{a} ± 11.05	27.67 ^{a} ± 11.05	0
	Me	69.13 ^{a} ± 2.69	30.87 ^{a} ± 2.69	5.33 ^{a} ± 4.48
	He	79.79 ^{a} ± 11.71	20.21 ^{a} ± 11.71	2.00 ^{a} ± 2.00
Week 9	Le	76.39 ^{a} ± 2.77	23.61 ^{a} ± 2.77	2.50 ^{a} ± 2.11
	Me	78.78 ^{a} ± 4.05	21.21 ^{a} ± 4.05	1.33 ^{a} ± 0.67
	He	80.56 ^{a} ± 5.65	19.43 ^{a} ± 5.65	8.50 ^{a} ± 6.55
Week 12	Le	79.89 ^{a} ± 3.44	20.11 ^{a} ± 3.44	1.33 ^{a} ± 1.14
	Me	78.48 ^{a} ± 4.64	21.52 ^{a} ± 4.64	3.67 ^{a} ± 1.80
	He	70.54 ^{a} ± 5.37	29.46 ^{a} ± 5.37	6.33 ^{a} ± 4.67
Week 15	Le	82.08 ^{a} ± 1.74	17.92 ^{a} ± 1.74	0.50 ^{a} ± 0.34
	Me	82.70 ^{a} ± 3.39	17.30 ^{a} ± 3.39	1.17 ^{a} ±0.31
	He	78.92 ^{a} ± 2.47	21.08 ^{a} ± 2.47	3.50 ^{a} ± 3.50

Papers submitted for publication

Nel, A.J., Hugo, A.S., Bester, N. & Schwalbach, LM.J., 2004. The effects of different dietary energy levels on the carcass characteristics of Dorper sheep. Proceedings of the 2nd Joint Congress of the Grassland Society of Southern Africa and South African Society of Animal Science. Goudini, 28 June – 1 July 2004. pp 20 (abstr).

Schwalbach, L.M.J., Bester, van der Merwe, H.J. & Greyling, J.P.C., 2006. Effects of high dietary energy level on the cryotolerance of ram semen. Proceedings of the 41st Congress of the South African Society for Animal Science. Bloemfontein, 3 – 6 April. pp 31 (abstr)

Papers published from this study

Articles are included.