THE EFFECT OF EXOGENOUS ANABOLIC STEROIDS ON CERTAIN BODY PARAMETERS AND SEMINAL CHARACTERISTICS OF BOER GOATS

by

Thapelo Makae

Submitted in partial fulfillment of the requirements for the degree

Magister Scientae Agriculturae

in the

Department of Animal, Wildlife and Grassland Sciences

Faculty of Natural and Agricultural Sciences

University of the Free State

Bloemfontein

Supervisor: Prof. J.P.C. Greyling

Co-supervisor: Dr. L.M.J. Schwalbach

May 2011

Acknowledgements

- To GOD Almighty, thank you for always shining your light on me and for leading my path.

- My supervisor Prof. J.P.C. Greyling (UFS) and co-supervisor and greatest mentor Dr L.M.J. Schwalbach for their guidance, motivation and patience with me and my writing during the past several months.

- Boer Goat Breed Society of South Africa for the donation of the bucks.

- Mr. M.D. Fair for always being patient and willing to help with the data analysis. Without you, I don't know what students will do. Thank you Sir.

- To the ever hardworking friend and colleague Mr M.B. Raito and Papa Joseph Mojakisane for always standing by me through the hot summer days and coldest winter months to get the job done, thank you Magents!

- Mev H. Linde, for your patience and dedication through thick and thin and for always willing to help me.

To all my colleagues in the Department of Animal, Wildlife and Grassland Science for your valuable inputs through this not so easy journey.

Thank you every one.

Dedications

- I dedicate this to my loving and supportive family, my wife (Dr Nokufa Mnguni-Makae), my daughters (Bokang Minenhle, Rorisang Langelihle and Tlolo Abongile Makae)

- My parents (Pule and Matauli Makae) and siblings (Tumelo, Teboho, Thato, Tsenolo Makae and Tsepo Dolo and her family)

I want to take this opportunity to thank each and every one of you for supporting me through the toughest and most challenging times in my life, and for believing in me.

Declaration

I hereby declare that this dissertation submitted by me to the University of the Free State for the degree, **Magister Scientae 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.

.....

Thapelo Makae

May 2011

Table of contents

List of Tables

List of Figures

List of Plates

Chapter 1

General Introduction

The main difference between commercial and stud or breeder farmers is that the former sell mainly livestock products and animals to be slaughtered for meat, while the latter raise and sell genetically superior animals for breeding purposes. Due to the completely different farming goals, small and large stock in these two types of production systems (stud and commercial) should be reared differently. Farm animals intended for breeding should generally grow at a slower rate and remain leaner than those for slaughter.

Different husbandry practices and production systems have been developed over the years to assist these two types of livestock producers in achieving their goals and to assist in improving the productivity of their animals.

So for example, different growth enhancing substances, also known as growth promoters or growth stimulants, have been developed to be used in the meat producing industry (Cecava & Hancock, 1994; Dhanda *et al*., 2003). Growth stimulants are then commonly used in the feedlot industry in South Africa, where a large number of animals are rounded off under intensive feeding conditions, prior to slaughter. These agents can be divided into natural or synthetic; hormonal or non-hormonal agents. Amongst the growth stimulants, anabolic steroids (sex hormones) are commonly used in cattle and sheep feedlots in South Africa – to improve the feed

conversion efficiency, growth rate and carcass composition and grading (Macgregor, 1988; Van Niekerk *et al*., 1988).

Anabolic steroids increase the protein synthesis and hence reduce fat deposition in the body, resulting in a more efficient feed utilization and conversion rate, a more masculine appearance of animals and larger and leaner carcasses (Heitzman, 1976). Ultimately, the use of anabolic steroids to round off farm animals, result in higher profits for the meat producer.

On the other hand, stud farmers who produce and sell genetically superior animals for breeding purposes, select animals at a younger age, based on their growth performance (faster growth rate and more favourable feed conversion ratio) and body conformation (i.e. masculinity in males). These characteristics will then hopefully be passed onto their offspring (hereditary). As growth performance and body conformation can be manipulated using anabolic steroids, some unethical stud farmers have been tempted in the past or may be tempted in the future to use these agents to gain an unfair advantage with the animals at shows and sales. There are actually rumors that anabolic steroids are being used in the stud industry in South Africa (Greyling & Schwalbach, 2009).

The use of anabolic steroids is generally contra-indicated for breeding animals due to its side effects on the hypothalamic-hypophysis- gonads axis, which ultimately reduce the fertility of the animals (Hafez & Hafez, 2000). There are however relatively few studies in the literature of the effects of anabolic steroids on farm animals intended for breeding, and making this information available to livestock breeders.

This study will thus focus mainly on the effects of anabolic steroids on the growth performance, body parameters and particularly on testicular development and semen characteristics of yearling Boer goat bucks (although in principle this practice could apply to all livestock species).

Chapter 2

Literature Review

The effect of exogenous anabolic steroids on certain body parameters and seminal characteristics of Boer goats

The most important factors affecting growth, testicular characteristics and semen quality in bucks are reviewed in this chapter. Focus being concentrated on the effects of exogenous anabolic steroids (testosterone) on these parameters.

2.1 Hormones

i) Hormones are chemical substances secreted in the body fluids by a cell or group of cells (glands). This group of cells can then exert a physiological effect on other cells or groups of cells or an entire organ by releasing specific hormones. Some hormones exert a local effect (immediate environment), while some end up in the blood stream (endocrine) after being secreted and exert an action on certain target organs. These secretory organs are also known as endocrine glands, and include the anterior pituitary gland (adenohypophysis) and the posterior pituitary gland located at the base of the brain (in a depression the sella turcica). The hormones released by the anterior pituitary gland include, growth hormone or somatotrophic hormone (GH or STH), adreno-corticotrophic hormone (ACTH), thyrotrophin or thyroid stimulating hormone (TSH) and the gonadotrophins or gonadotrophic stimulating hormones. The sex stimulating or gonadotrophic hormones can again be sub-divided into FSH

(follicle stimulating hormone), LH (luteinizing hormone) and LTH (luteotrophic hormone). Similarly the posterior pituitary gland releases hormones such as oxytocin and anti-diuretic hormone (ADH). Finally, there is also the pineal gland which produces melatonin, which has an effect on the activity of the hypothalamus/pituitary glands via the optic nerve (Hafez & Hafez, 2000).

2.2 Steroid hormones and their chemical structure

i) Hormones can be classified according to their source and function, and are also described based on their chemical composition. There are generally three chemical types of hormones (Hafez & Hafez, 2000).

ii) Protein or polypeptide hormones which consist of a string of amino acids. So for example, the anterior pituitary gland only produces protein hormones - all of which have different functions e.g. the gonadotrophic hormones and adrenocorticotrophic hormone that act on the gonads or the adrenal cortex.

iii) Some hormones are relatively simple amino acid derivatives, such as e.g. the thyroid hormones, which are derived from thyrosine and cause the thyroid cells to enlarge and increase - resulting in an increase in the secretion of the hormone thyroxine (involved in the cell metabolism).

iv) The steroid hormones are however non-polar, lipid-soluble cholesterol derivatives (Figure 2.2 and 2.3). These hormones are produced by the gonads, the adrenal glands and the placenta. Examples of these complex molecules include the steroids e.g. oestrogens, progesterone and testosterone, the

adrenal steroids, etc. The estrogens then have several specific functions e.g. to induce behavioral estrus in the female, to act on the uterus to increase both the amplitude and frequency of contractions by complementing the effects of oxytocin and prostaglandins. Progesterone on the other hand prepares the endometrium of the uterus for implantation and the maintenance of pregnancy. Testosterone again stimulates the late stages of spermatogenesis and prolongs the life of the epididymal sperm, while it also maintains the secondary sex characteristics and sexual behavior or libido in the male (Hafez & Hafez, 2000).

Of all the hormones mentioned, the aim of this study is to focus on the effects of anabolic steroids (testosterone), on the reproductive potential of young Boer goat bucks, treated for a prolonged period of time.

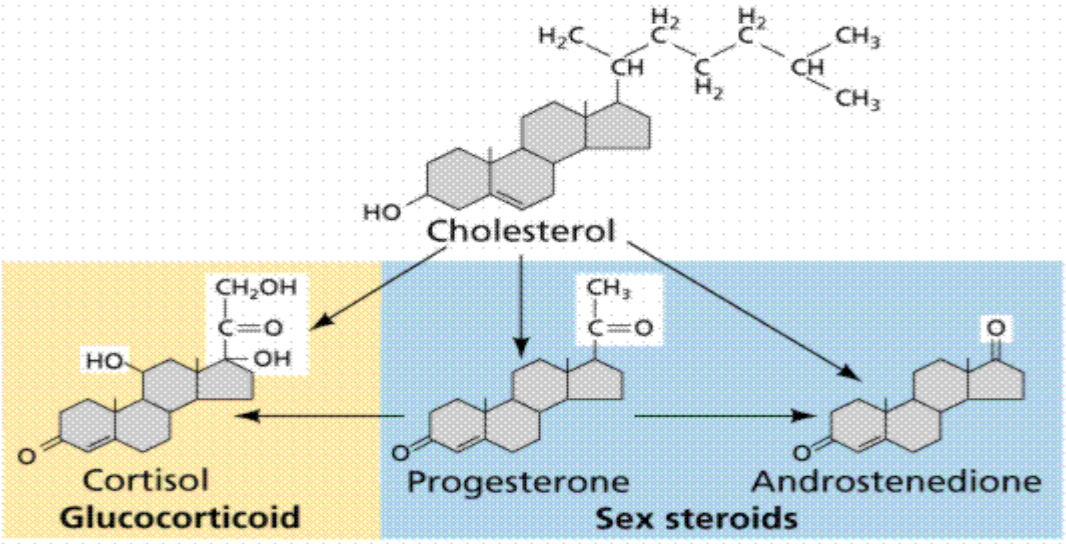


Figure 2.1 **Different chemical structures of the steroid hormones (www.emc.maricopa.edu)**

2.3 Exogenous steroid hormones

The exogenous steroid hormones are generally synthetic hormones or natural analogues which are artificially manufactured and have a similar chemical structure and induce a response similar to those hormones that are naturally produced endogenously in the animal. These exogenous steroid hormones may however also have an adverse effect in both the male and female animal. Chronically high levels of steroid hormones have been shown to be disadvantageous so e.g. high levels of corticosterone over an extended period of time have been shown to reduce or impair reproductive function in animals (Brown *et al.,* 2005). The continuous administration of exogenous bovine growth hormone (bGH or BST) to dairy cows has also been implemented to increase milk production over both the short and long term (Peel *et al.,* 1983). In this specific study, the effect of daily injections of GH during early and late lactation revealed a significant increase in milk yield, fat and lactose content. On the other hand a decline in feed intake was also recorded. It should be mentioned that the long term usage of this hormone could have a detrimental effect on both the milk yield quantity and quality. Little is however known regarding the consequences of very low levels of these steroid hormones (Brown *et al.,* 2005).

2.4 Effect of exogenous anabolic steroids on animals

Exogenous anabolic steroids are generally synthetic derivatives of oestrogen and progesterone which are the primary female sex hormones and testosterone, the primary male sex hormone. In practice anabolic steroids are generally used (growth stimulants) to enhance growth performance (muscle deposition) and the general body conformation of the animal. These hormones may however also have an adverse effect on the liver, serum lipids, general behavior and the reproductive system. So for example, steroid use in clinical trials and in laboratory studies have been associated

with numerous acute deleterious changes associated with cardiovascular diseases, liver tumors, infertility, and the physiology of various organs and body systems. Suggesting a potential of steroids for inducing health problems (Brown *et al.,* 2005).

Other areas of concern following steroid treatment include behavioral disorders, cardiomyopathy, coronary artery disease, cerebrovascular diseases, prostate and immune function changes in humans. The liver structure and function is also said to be altered by the administration of anabolic steroids and these changes may induce conditions such as cholestatic jaundice and peliosis hepatis, to mention but a few. As mentioned previously, the effects are still unclear regarding anabolic steroid treatment in animals, as very little intensive research has been done in the past – however it is expected that these health problems which are induced in humans, may also be relevant to farm animals (Bahrke & Yesalis*.,* 2004).

2.5 Endogenous steroid hormones

Endogenous hormones are released within the body of an animal, by different endocrine glands as messages to communicate and facilitate functions between organs in the body. Endogenous steroid hormones can be classified according to their chemical structure and function. The sex or gonadotrophic hormones such as testosterone are released by the testes, while oestrogen and progesterone are produced by the ovaries. These are good examples of endogenous steroid hormones. Chronically high levels of steroid hormone secretion can however be deleterious, and lead to certain reproductive abnormalities. High concentrations of testosterone may have a suppressive effect on the immune system of the animal, making males more susceptible to harmful pathogens and parasites (Brown *et al.,* 2005).

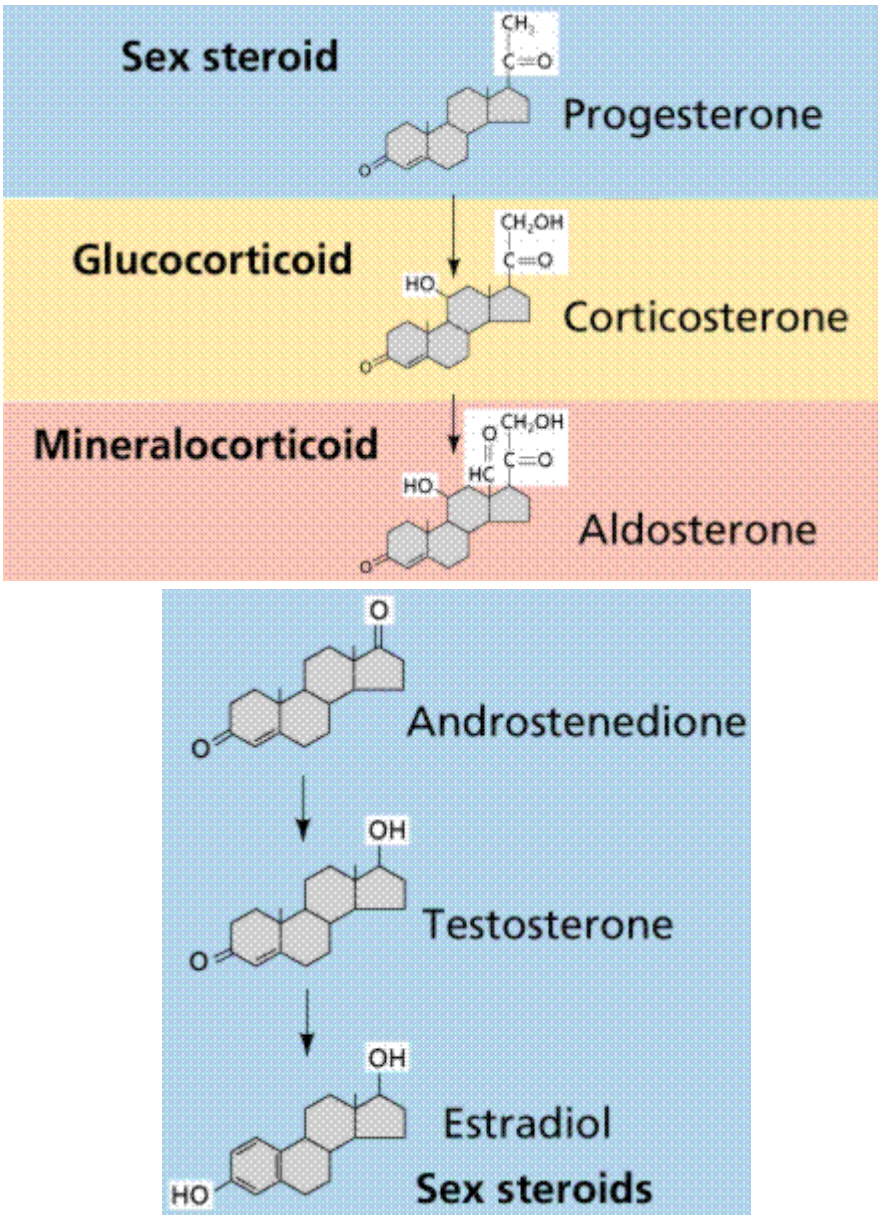


Figure 2.2 Cholesterol as the backbone chemical structure of the steroid hormones (www.emc.maricopa.edu)

2.6 Spermatogenesis

In the lining of the seminiferous tubules, where spermatogenesis is initiated, two basic cell types are found, namely the Sertoli cells and the developing germ cells or spermatogonia. These germ cells undergo a continuous series of cellular divisions (spermacytogenesis) and developmental changes, and divide several times before eventually forming spermatocytes (Hafez & Hafez, 2000). Goat spermatogonia initially

contain the full diploid number of chromosomes (n=54) at puberty, the spermatogonia begin to divide mitotically, and undergo several divisions to produce more diploid spermatogonia. The primary spermatocytes then undergo two meiotic divisions (spermiogenesis), first forming secondary spermatocytes, and then finally haploid spermatids (Bester, 2006).

Spermatogenesis (Figure 2.3) thus consists of two main phases – spermacytogenesis and spermiogenesis (Salisbury *et al.*, 1978). During the first phase or spermatocytogenesis the gonocytes undergo differentiation (before puberty), leading to different types of spermatogonia being formed - namely type AO and type B. From the type AO different types of germ cells will develop. The type A1 spermatogonia then progressively divide to form type A2, A3 and type A4 spermatogonia. The type B spermatogonia divides at least once or twice to form the primary spermatocytes. It is at this stage when further meiotic divisions occur to form the secondary spermatocytes and later haploid cells, the spermatids. Briefly, spermacytogenesis is divided into three phases, a Proliferation, Growth and Maturation phase. During the proliferation phase the type AB spermatogonia are reproduced, forming the preleptotene spermatocytes. The growth phase includes a lengthened prophase and in the maturation phase the primary spermatocytes undergo two guide successive meiotic divisions to form the spermatids. This spermatid then undergo a transformation (spermiogenesis) to form the sperm (Bester, 2006).

Spermiogenesis or the second stage of spermatogenesis is where structural changes from spermatids to the haploid stage and development to complete spermazoa occur (Hafez & Hafez, 2000).

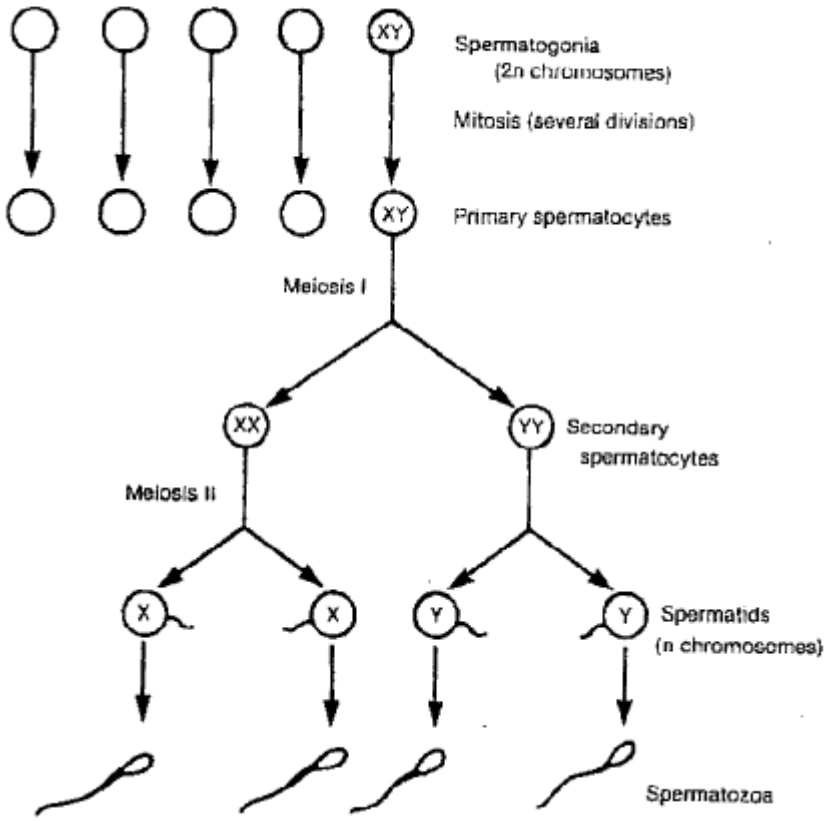


Figure 2.3 Schematic representation of spermatogenesis (Evans & Maxwell, 1987)

During spermiogenesis as such, there are four subdivisions or phases which characterize the transformation of the spermatids to sperm, namely the Golgi, cap, acrosomal, and maturation phase. During the Golgi phase there is formation of pro-acrosomal granules within the Golgi apparatus, while during the cap phase, the acrosomal granules are spread over the surface of the nucleus of the spermatid. The acrosomal phase is again characterized by changes in the nucleus, the acrosome and the tail of the developing spermatid. All these developmental changes are facilitated by the rotation of each spermatid, so that the acrosome is directed toward the basement or outer wall of the seminiferous tubule and the tail toward the lumen. The maturation phase is the final stage of spermiogenesis during which the transformation

of the spermatids into sperm cells is concluded and sperm are released into the lumen of the seminiferous tubule (Bester, 2006). These sperm cells, are then capable of fertilizing an ovum after a series of maturation or morphological changes over a period of time. The duration of the entire process of spermatogenesis is approximately 50 to 60 days, with another 2 weeks in the epididymis (Hafez & Hafez, 2000).

Various potential sperm cell types within any cross section of the seminiferous epithelium define the well-defined cellular stages that undergo continuous changes. A complete, time-dependent cycle of epithelial stages, known as the cycle of the seminiferous epithelium, can be thus defined as “a series of changes in a given area of the seminiferous epithelium, between two appearances of the cellular associations or developmental stages”. The duration of this cycle is approximately 10 days in a buck. Depending on the specie, 4 to nearly 5 epithelial cycles are required for a type A spermatogonia from the first cycle to complete the metamorphosis of spermiogenesis (Hafez & Hafez, 2000). It thus takes approximately 40 days in bucks from the activation of the stem cell to the release of a free sperm. As mentioned, it takes 10 to 21 days for the sperm to move through the epididymis - thus ultimately taking 50 to 54 days for the formation of mature, viable sperm (Evans & Maxwell, 1987). If the spermatogenesis cycle is interrupted by disease or any other stress factor, normal fertility will not be restored until a full spermatogenic cycle has been completed. This could imply that temporary sterility in the male may persist for several weeks. This aspect is of utmost importance when selecting males for the breeding season (Bester, 2006).

2.7 Factors affecting spermatogenesis

The formation of sperm or spermatogenesis in the testis is the critical process in the reproduction of the male, and this process can be negatively affected by various factors such as age of the male, nutritional regime, environmental factors (photoperiod and ambient temperature), hormonal abnormalities, work load of the animals (e.g. percentage males in the breeding herd), frequency of semen collection or natural mating and disease (Hafez & Hafez, 2000).

2.7.1 Major factors affecting spermatogenesis

Spermatogenesis in males is generally affected by several major factors, such as the environment, seasonality, hormonal levels, nutritional regime of the animals, inherent genetic factors and the age of the animals (Evans & Maxwell, 1987).

2.7.1.1 The environment and seasonality

Seasonal effects in small stock are induced by several factors, such as e.g. ambient temperature, humidity, rainfall and daylight length (Mathevon *et al.,* 1998). Goats and sheep are known to be seasonal breeders (short day breeders), which implies that these animals and their sexual activities are mainly affected by daylight length or photoperiod. In the southern hemisphere the short days are known to begin in March and last until the end of May (autumn), while the long days are experienced during the summer months (December to February). Seasonality as such, is thus considered to be a major limiting factor in goat reproduction, affecting semen production and quality. So for example, seasonal variation in sexual activity and semen quality of the buck has been shown to be ascribed to changes in daylight length throughout the year (Chemineau *et al*., 1992; Perez & Mateos, 1996). Thusfar photoperiod has been shown to be the major environmental cue in the regulation of testosterone secretion

by the buck, during the year (Delgadillo *et al*., 2004). The effect of other climatic factors on semen quality traits, particularly ambient temperature, which is closely associated with seasonal weather patterns, is said to be immediate (Colas, 1983; Rege *et al*., 2000). Elevated testicular temperatures resulting from the incomplete descent of the testes (cryptorchidism), high environmental temperatures, fever or inflammation are known to be detrimental to the process of spermatogenesis in all males. Cold ambient temperatures appear to be innocuous, unless actual freezing of the tissue occurs (Foote, 1978).

2.7.1.2 Hormonal factors

Photoperiod is accepted to be the main cue responsible for initiating the testicular cycle, performed primarily by altering the pituitary secretions of follicle stimulating hormone (FSH) and luteinizing hormone (LH), and consequently, the secretion of testosterone by the testes (Dickson & Sanford, 2005). FSH (spermatogenesis stimulating hormone or SSH in the male) and testosterone are key hormonal regulators of spermatogenesis, and thus germ cell mass and testicular size, in adult rams (Dickson & Sanford, 2005). Each of the gonadotrophic hormones play a unique role in the differentiation and proliferation of the spermatogonia, after which testosterone promotes the formation and differentiation of spermatocytes into round and elongated spermatids (Courot *et al*., 1979; Kilgour *et al*., 1994; Dickson & Sanford, 2005). Gonadotrophic endocrine hormonal profiles in the post pubertal ram may thus be important predictors of adult sperm production capacity (Langford *et al*., 1998). It has been reported that the concentrations of circulating LH (interstial cell stimulating hormone or ICSH in the male), the frequency of the LH pulses and the blood FSH concentrations are higher during the natural breeding season (autumn in the southern

hemisphere) than during the non-breeding season. All these changes in circulating hormonal concentrations primarily occur due to the changes in length of the photoperiod (Bremner *et al*., 1988; Tillbrook *et al.,* 1999). In rams or bucks the secretion of LH is regulated by the negative feedback effect of testosterone and/or its primary metabolites (Tillbrook & Clark, 1995), while FSH secretion is regulated by the negative feedback effect of testosterone and inhibin (Tillbrook *et al.,* 1999).

2.7.1.3 Nutritional status of animals

Normal development of young farm animals, depend on the supply of adequate, nutritionally balanced diets. So for example in well-fed calves, the occurrence of puberty was found to occur at an earlier stage, compared to calves fed restricted diets. Depending on the breed of small stock and the sensitivity to photoperiodic stimulation, the attainment of body weight at puberty may vary between 40 and 70% of the adult body weight (Bester, 2006). The pre-natal under-nutrition of ewes or does was found to have an effect on the male reproductive development of the offspring and the adult function - also a reduced ovulation rate in female progeny. Severe feed restrictions may even result in permanent damage to the gonadal and neural tissue. Thus a reduction in androgen secretion and semen quality can be induced by a restriction in feed intake. However such effects are temporary (Rae et al., 2002). General under-nutrition has been found to delay the onset of puberty and sexual maturity in bulls, with the sperm concentration and motility being below normal. This effect of under-nutrition may however be corrected successfully in mature animals, while it is not so easily rectified in young animals - because of the permanent damage caused to the germinal epithelium cells of the testes (Hafez & Hafez, 2000). In sexually mature animals, the effects of nutrition can be classified as short-term effects - that act mainly on the neuro-

endocrine system, controlling testicular activity (Martin *et al.,* 1994) or long-term effects, that act on testicular growth and sperm production (Blache *et al.,* 2000). The general nutritional factors affecting spermatogenesis include the energy, protein and vitamin content of the diet (Lindsay *et al*., 1984; Hafez & Hafez, 2000; Shadnoush *et al*., 2003).

2.7.1.4 Genetic factors

Spermatocytogenesis involves mitotic cell divisions to increase the yield of spermatogenesis and to produce stem cells and primary spermatocytes. Meiosis on the other hand involves the duplication and exchange of genetic material and two cell divisions that reduce the chromosome number to the haploid number and yield 4 spermatids. In general, the daily sperm production of the male is related to the rate of germ cell degeneration, the rate of pubertal development, season of the year, breed and the aging of the animal. The number of Sertoli cells and the quantity smooth endoplasmic reticulum of the Leydig cells and Leydig cell numbers are again all related to semen production. The seminiferous epithelium is generally sensitive to elevated temperatures, dietary deficiencies, androgenic drugs (e.g. anabolic steroids), metals (cadmium and lead), x-ray radiation, dioxine, alcohol, and certain infectious diseases. However, these different external factors may elicit the same temporary or permanent response in that degeneration of the germ cells - becoming more frequent by forming multi-nucleate giant germ cells following the coalescence of the spermatocytes or spermatids, the ratio of the germ cells to Sertoli cells being reduced, and sperm production ultimately being negatively affected. In short, spermatogenesis involves both mitotic and meiotic cell divisions, and is an unsurpassed example of cell differentiation in the production of the sperm cell. Several extrinsic factors can thus

influence this process of spermatogenesis and cause a similar degenerative response of the seminiferous epithelium and reduce fertility (Bester, 2006). Semen quality of different breeds of rams, have been reported to differ together with the secretory patterns, however the correlation with fertility still has to be established (Hafez & Hafez, 2000; Kumar *et al*., 2010).

2.7.1.5 Age of the Male

Together with other factors mentioned earlier, age of the male is one of the major factors contributing to differences in scrotal circumference and semen characteristics - with testicular size being highly correlated to total semen output (Al-Ghalban *et al*., 2004). Semen traits such as ejaculate volume, sperm concentration, and the total number of sperm per ejaculate have been shown to be significantly affected by the age of the animal, and by an interaction between age and the interval between semen collections. In addition, age of the male has been shown to have a significant interaction with season of the year, the volume of the ejaculate and the percentage of motile sperm. An important factor that determines the total number of sperm produced, being the size of the testes or the amount of testicular tissue. The effect of age of a male at semen collection is also important in young animals, mainly due to the physiological changes that occur as the young animals grow to sexual maturity (Mathevon *et al*., 1998).

2.7.2 Minor factors affecting spermatogenesis

There are certain factors that may also affect the process of spermatogenesis, but are considered to be less important than the factors discussed previously. In actual fact,

these factors are just as important, but are considered minor, as they can either be controlled or manipulated. The frequency of semen collection is important with regard to spermatogenesis, as it is to be remembered that animals need to be rested (mature sperm) - in order to make sure that males perform satisfactory in terms of fertility (Hafez & Hafez, 2000). Breed (genetic) differences may also have an impact on the process of spermatogenesis (Kumar *et al*., 2010). These two factors (frequency of semen collection and breed) will be discussed in more detail later.

2.7.2.1 Frequency of semen collection

Ejaculate volume, sperm concentration and the total number of sperm per ejaculate have been found to significantly decline in consecutive collections of ejaculates in rams (Kaya *et al*., 2002). Similarly, a marked decline in semen volume and sperm concentration was recorded in Angora goats subjected to a frequent ejaculation regime (Ritar *et al*., 1992). Kaya *et al. (*2002) also reported an osmotic resistance in the acrosomal membrane of the sperm cell, due to insufficient maturation of the sperm cell in the epididymis, resulting from too frequent ejaculation in boars. Semen collection and the frequency thereof has thus been reported to be an important factor influencing sperm quality and also the fertilizing capacity (Foote, 1978).

2.7.2.2 Breed differences

This is also a genetic factor. While Greyling and Grobbelaar (1983) reported no significant seasonal variation in Boer goat semen quality (collected by artificial vagina), although the percentage of live sperm differed significantly between certain months, the Angora goat was recorded to be more seasonal than the Boer goat, with a definite

breeding season (Loubser *et* al., 1983). Ejaculate volume and semen pH were also significantly lower, and sperm concentration and motility significantly higher, when collected from bucks of different breeds by means of the artificial vagina, compared to the electro-ejaculator. Semen from the Angora goat recorded a significantly higher sperm motility and sperm concentration, and a significantly lower semen volume, compared to semen collected from electro-ejaculated Boer goats (Greyling and Grobbelaar, 1983).

2.8 Effect of anabolic steroids on growth rate

Body growth is generally related to an increase in cell number and cell volume of the individual. Growth in any trait can be related to the genetic potential of the individual and genetic x environment interaction (Eisen, 1976). There are several factors that can be affected by body growth, of which the nutritional and genetic factors are critical. During growth of goats (Figure 2.4), Tatar *et al.* (2009) reported that in goats, the fastest growth rate occurs between 2 and 5 months of age, and thereafter it slows down and ultimately growth will reach a plateau at 11 to 12 months of age. Thus the age will have a definite effect on the growth rate achieved.

In most instances livestock producers need to accelerate the growth process or average daily gain (ADG) – saving time and thus money. In many countries anabolic steroids or growth stimulants have thus successfully been used to increase the growth rate in farm animals (Heitzman, 1976). The administration of these anabolic agents to certain farm animals may thus be performed in three ways: (i) The anabolic agent may be administered orally in the feed directly into the mouth, (ii) via a slow release anabolic implant administered under the skin and then also, (iii) by the administration

of the steroids by repeated intramuscular injections. These anabolic steroids or growth promoters are generally used to improve the growth rate and also increase the feed efficiency of growth and the finishing of feedlot animals (Cecava and Hancock, 1994).

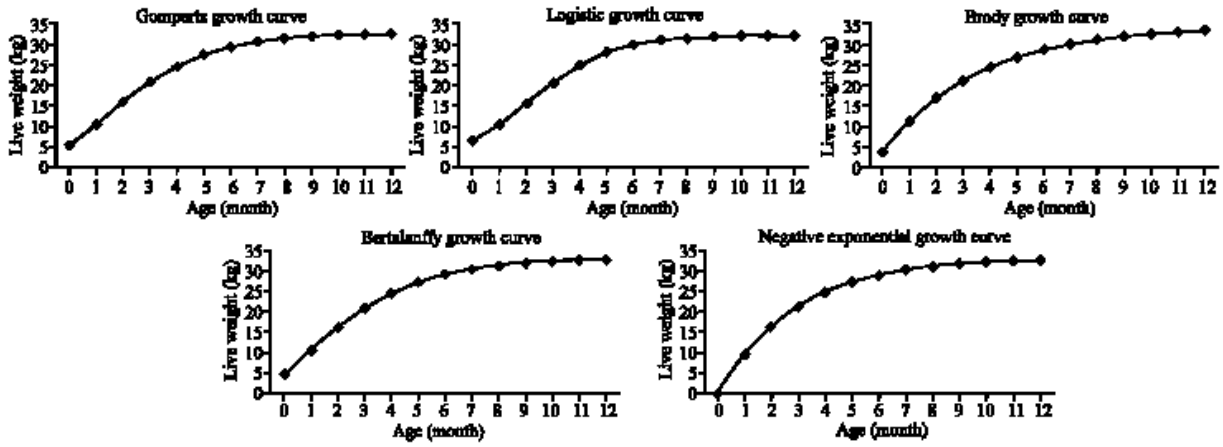


Figure 2.4 Growth curves of young goats estimated with various growth models (Tatar *et al.,* 2009).

It is important to note that these anabolic agents or growth stimulants have an increased effect on muscle or protein deposition, and the total body or carcass protein deposition is thus enhanced e.g. in ruminants by implantation of these anabolic steroids. This anabolic steroid-induced increase in muscle protein accretion can be attributed to a decrease in muscle protein degradation, together with a less extensive reduction in protein synthesis (Hayden *et al.*, 1992).

Oestrogenic and testosterone anabolic agents can increase the net protein accretion and N retention - resulting in greater lean tissue deposition (Cecava and Hancock,

1994). Not only may anabolic steroids have an adverse effect on the skeleto-muscular protein retention, but the visceral organs may also be affected, when using these implants or stimulants. In a study conducted by Hutcheson *et al.* (1997), it was shown that goat kids implanted with a combination of growth implants recorded a smaller gastro-intestinal tract (GIT), compared to the control kids. Other organs which were also affected included the liver and spleen following treatment. The body condition score (BCS) and body weight of treated animals were also found to be higher and heavier than those that were not treated with anabolic steroids. Anabolic implants thus generally increase the growth rate by accelerating nutrient deposition as protein, but not at the expense of fat deposition (Hutcheson *et al.,* 1997).

2.9 Semen Quality (Male Breeding Soundness)

The major contributor to the variation in semen quality is the environment (ambient temperature, disease and feed availability). These environmental effects may be either temporary or permanent of nature. Elevated testicular temperatures resulting from the incomplete descent of the testes (cryptochidism), high environmental temperatures or inflammation have been found to be detrimental to spermatogenesis in all scrotal mammals. Cold temperatures appear to be innocuous, unless actual freezing of the tissue occurs (Foote, 1978).

There are however several other factors which can also contribute to the quality of the semen being acceptable, or of poor quality. So for example, nutrition has been identified as one of the factors of utmost importance in the expression of the reproduction parameters (Almeida *et al.,* 2007). Another important factor which also affects the quality of semen negatively is the age of the male. It is known that, animals

which have not reached puberty cannot be utilized in a breeding program. This is due to the fact that semen production is generally low and the quality of the ejaculate is poor (Bester, 2006).

What is the definition of poor semen quality? Generally semen samples that result in high fertility in cows, without transmitting diseases to such cows, are generally classified as being of good quality. Poor semen quality on the other hand, may originate during any of the different phases of spermatogenesis i.e. spermatocytogenesis, spermiogenesis, epididymal passage and the sperm storage. Ejaculation as such, could contribute to poor semen being produced due to e.g. contamination with exudates from the vasiculitis, or urine during electro-ejaculation or dirt, toxins, water, etc. Nöthling and Annandale (2007) described other causes of poor semen quality to be due to the process of cryopreservation, thawing and even insemination of the semen. In the microscopic evaluation of semen, sperm parameters such as motility, the percentage live and even sperm abnormalities may also serve as an indication of semen quality (Evans & Maxwell, 1987).

2.10 Factors affecting the quality of semen

Different processing aspects of semen need to be considered.

- extension of the ejaculate (different diluents available for fresh and cryopreservation of semen)
- cryopreservation of the ejaculate and the different techniques currently being used (with varying success)

- storage of the frozen semen (in straws or pellets, long and short term)

Generally it is accepted that the contamination of a semen sample with urine or dirt, collection of semen in dirty or toxic apparatus, incubating of the unextended ejaculate with a high concentration of sperm at temperatures near body temperature, all have a negative effect on semen quality. A definition of a good semen sample is generally that the semen sample must support the conclusion that the testes, epididymides and accessory sex glands are healthy and functioning normally, and the male does not carry any heritable causes for poor semen quality. Nöthling and Annandale (2007) also goes on to explain that the semen sample as such, must also provide no hint of the occurrence of oligospermia.

A breeding soundness examination is an array of tests relating to reproductive well-being of the animal, and in this case the male. This soundness examination is generally designed to also detect those morphological abnormalities which frequently cause infertility or sub-fertility. This examination not only focuses on the fertility of an animal as such, but also examines the ability of an animal to mate. Clinical examinations can be performed, which generally include three different types of evaluation, namely a general, a more detailed clinical examination and a clinical examination of the genital system. The general clinical examinations of bucks include a visual inspection of general health status of the animal, an assessment of the body condition, including the allocation of a body condition score (BCS), plus visual inspection of the eyes, bite, back, legs, gait and conformational defects. When a male is unable to mate, and pain causes the animal to spend more time lying down, this may amongst others, compromise the thermoregulatory ability of the testes (with a

resultant decrease in semen quality and quantity). During a detailed clinical examination the digestive system should also be evaluated by means of the assessment of the teeth, rumen movements, ballotment, simultaneous percussions and auscultation, rectal palpation and physical examination of the texture of the faeces. Clinical examination of the genital system include visual inspection and palpation of the scrotal contents and penis and preputium, the determination of the scrotal circumference and rectal palpation of the internal genitalia and associated structures (Irons and Bertschinger, 2007).

2.11 Semen collection and sperm evaluation techniques

2.11.1 Semen collection

There are two main methods of collecting semen in small ruminants: (i) The artificial vagina (AV) and (ii) the electro-ejaculator (electro-stimulation). When using the AV, the temperature of the water used is critical, as the AV has to mimic the conditions of the natural vagina and the place of semen deposition. It should also be kept in mind that spermatogenesis and sperm maturation normally takes place at a temperature of 4 to 7°C under body temperature (39°C). The pressure in the AV also has to be such that the animals will be tactilely stimulated to ejaculate. The other vital point to remember in the setting up of the AV, is the lubrication of the AV. These factors all contribute to making the AV method of semen collection, providing an ejaculate similar to that naturally produced when mating. With the electro-stimulation or EE method of semen collection, there are several advantages and disadvantages regarding the procedure. The main advantage is that this technique of semen collection is not dependent on the libido of the animal. What this implies, is that a semen sample can

be collected from animals at any time of the year (not season dependent) and no prior training of the animal is required. The disadvantage of this technique of semen collection is however that often a poor or less dense semen sample is obtained from an animal. This method may also cause some degree of discomfort to the animals, caused by the electric current and it is essential that the animal is firmly restrained (Greyling & Grobbelaar, 1988).

2.11.2 Assessment of semen quality in goats

The examination of semen entails a standard method of evaluating the potential fertility of breeding males, other than directly evaluating their ability to induce a pregnancy (Hafez & Hafez, 2000). The quantity of the sperm produced per ejaculate is thus dependent on the volume and the concentration of the semen sample. Further tests (macroscopic and microscopic) are however required to evaluate the quality of the ejaculate (Evans & Maxwell, 1987).

2.12 The macroscopic evaluation of buck semen

The colour of a buck semen sample is firstly macroscopically evaluated to test for the presence of any abnormal colour of the semen, which could indicate contamination of the sample so e.g. a red colour is indication of blood, most likely from an injury of the penis; a yellow colour indicates the presence of urine, this which is most likely induced when the electro-ejaculator is used and the animals may be stimulated to urinate and a grey colour is due to the presence of pus, because of an infection in the male reproductive tract. The semen colour of the ejaculate could then also serve as an indicator of the density of the semen sample – varying from thick creamy to a watery colour (Table 2.1). The pH of the semen sample is another parameter that could be

monitored, with the normal semen pH varying between 6.4 and 6.7. So for example, large variations in semen pH could be indicative of an abnormality e.g. if the semen is too acid (lower pH), it could be as a result of too little secondary gland fluid being secreted, or if semen is too alkali (pH above 7.6) it could be indicative of inflammation in the reproductive tract (Hafez & Hafez, 2000).

Table 2.1 The average buck semen concentration, as scored by visual colour assessment

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	Insignificant	Insignificant

Source: Hafez & Hafez, 2000

2.12.1 Semen concentration

The actual sperm density (sperm/ml) in a semen sample can be measured with the aid of a hemacytometer, colorimeter or spectrophotometer. The hemacytometer (traditionally used to perform red blood cell counts) is a microscope slide calibrated with precisely scored chambers and the number of sperm per chamber is then manually counted. This technique is very time - consuming, however, very accurate. This method can be bypassed or replaced with the use of either a spectrophotometer or colorimeter that has been calibrated with the aid of a hemacytometer. However, though initially time-consuming in the setting up of a graph, illustrated the absorbance at certain densities. These facilities have the advantage of being accurate and fast.

The normal concentration of a buck ejaculate varies between 3.5 and 6 x 10^9 sperm/ml (Hafez & Hafez, 2000).

2.12.2 Sperm motility and percentage live sperm

Sperm motility assessment involves the subjective evaluation of sperm viability by estimating the degree of sperm motility. Generally the evaluation of raw semen using sperm motility is an indicator of sperm performance in its own accessory gland fluid (Hafez & Hafez, 2000). Normally the semen of small stock exhibit a wave-like motion when examined for sperm motility under the microscope (X10 magnification). An estimate of this sperm motility is made, based on the vigour of the wave motion, or on the overall sperm activity if wave motion is not present. The sperm motility is assessed using a scoring system of 0 to 5 - were 5 is excellent sperm wave motion and 0 being no sperm movement. Sperm motility is thus classified into 6 grades or scores (Bester, 2006).

For the evaluation of the percentage live sperm and abnormal sperm in the ejaculate, a semen smear was made using a drop (approximately 20µl) of an eosin-nigrosin mixture and a drop (approximately 20µl) of semen. It is often difficult to determine the quantity of eosin-nigrosin and the quantity of semen to be used, as the ratio often depends on the concentration of the sperm in the ejaculate. In the stain, dead sperm colour red, while sperm abnormalities and live sperm do not stain and remain transparent (Nöthling and Annandale, 2007).

Table 2.2 The criteria used for motility scoring semen in goats (Nöthling and Annandale, 2007)

- 5 - Very strong progressive black waves. 90^{+}% live sperm;
- 4 - Strong progressive waves. 70-85% live sperm;
- 3 - Weak waves. 50-65% live sperm;
- 2 - Few, weak, non-progressive waves. 30-45% live sperm;
- 1 - No waves. 5-25% live sperm;
- 0 - All sperm dead – no movement.

2.12.3 Sperm morphology

Morphological sperm abnormalities have been found to have a strong correlation with fertility in livestock - with heat stress causing a high percentage of damage or injury to the sperm. Periods of high ambient temperature, combined with high humidity may render a male sterile for up to 6 weeks and many abnormal sperm cells may occur in the ejaculates collected during this period. All ejaculates generally contain a proportion abnormal sperm. However, when 20% or more sperm are abnormal, the ram's fertility becomes questionable. Semen with more than 15% abnormal sperm should generally not be used for AI. An eosin-negrosin stain as described earlier can be used to evaluate the sperm morphology as such. Stained (nigrosin-eosin) thin semen smear microscope slides are microscopically examined with the aid of a high magnification (X100) (Bester, 2006).

Table 2.3 Abnormal goat sperm are classified into 5 morphological categories (Hafez & Hafez, 2000)

- Loose sperm heads (without tails)
- Abnormal sperm heads and abnormal sperm tail formations (different shape of head and tail configurations)
- Abnormal tail formations (any defect from normal)

- Abnormal tail formations with proximal cytoplasmic droplets (generally identified in young or sexually overworked males)
- Abnormal tail formations with a distal droplet (also a sign of immaturity)

Abnormalities of the sperm can thus occur on any part of the sperm cell. According to Salisbury *et al*. (1978) sperm abnormalities occur in the head, neck, mid-piece, tail, or any combination of these regions of the sperm cell. The most common sperm abnormalities include those of the mid-piece, and can range from bent, broken, short and enlarged or a thickened mid-piece and the main abnormalities of the sperm tail being described as coiled, twin, broken, crooked, kinky and truncated (Bester, 2006). Loskutoff and Crichton (2001) went as far as classifying sperm cell abnormalities into the following categories: Firstly primary abnormalities (those occurring during spermatogenesis in the seminiferous tubules of the testis) including the following:

Sperm head:

- Microcephalic (small heads)
- Macrocephalic (large/swollen heads)
- Double head formation or placement
- Abnormal acrosome

Mid-piece of the sperm cell:

- Swollen
- Elongated
- Abaxial

Tail of the sperm:

- Double, short
- Absent

Secondary abnormalities (those occurring during maturation in the epydidymis) including the following:

Sperm head:

- Detached heads
- Loose/damaged acrosomes

Mid-piece of the sperm cell:

- Bent mid-piece
- Protoplasmic droplets

Tail of the sperm cell:

- Bent
- Shoe-hook
- Protoplasmic droplets

Tertiary abnormalities (those resulting from poor handling of the semen – post ejaculation) which including the following:

- Detached (dead) acrosomes (often due to technical error in the preparation of the nigrosin-eosin smear
- Coiled sperm tails

Chapter 3

General material and methods

3.1 Study location and period

The trial was conducted at the University of the Free State on the Bloemfontein campus, South Africa, situated at a latitude of 29.10° South, longitude of 26.29° East, and an altitude of 1351m above sea level. The study was undertaken between April and October 2009 (mid-autumn to mid-spring), in two phases. The first phase was conducted between April and July 2009 (treatment phase) and the second phase was conducted between August and October 2009 (recovery phase) – following an initial adaptation period.

This project was approved by the University of the Free State's Animal Ethical Committee (project no: 23/08). Care and handling of all the experimental animals were performed strictly in accordance to the prescribed guidelines (SANS, 2002).

3.2 Experimental animals

Initially 20 Boer goat bucks (between 14 and12 months of age, with a mean body weight of 47 kg) which had been previously managed extensively on natural pastures were vaccinated for enterotoxaemia (pulpy kidney), and examined for breeding soundness by a veterinarian and allotted to two treatment groups. The majority of the bucks were successfully trained (during adaptation period) for semen collection with the aid of an AV and all the animals were adapted to their new environment for a period

of a month (March – onset of autumn) and dosed for internal parasites prior to the onset of the trial.

The animals (n=20) were randomly divided into two groups, one group (n=10) was treated weekly with an intramuscular injection of 25 mg (1 ml) Nandrolone Deca-Durabolin (Adcock Ingram, The Netherlands), while the second group of bucks (n=10), served as a control. All animals were individually housed in 4 by 3 pens and provided with fresh water and a pelleted diet daily, and allowed to exercise twice weekly. A total treatment period of 4 months or 16 weeks (April to July), and a 3 month or 16 week (August to October) withdrawal period (no treatment) was implemented. During the entire period bucks received a balanced maintenance pelleted diet (8.5 MJ ME/kg), lucerne hay (13% protein) and fresh water ad lib (Greyling *et al.,* 1999).

Plate 3.1 Water troughs

Plate 3.2 Feed troughs

Plate 3.3 Individual crates for animals

The following body and other parameters were recorded for each buck at weekly intervals: (1) Testicular size, by measuring scrotal circumference (cm) with the aid of a measuring tape and testis volume (ml) using a water displacement method (Knight, 1977; Madgwick *et al*., 2008). (2) Semen was collected by a means of an artificial vagina, and different sperm analyses for, evaluating the semen quantity, sperm concentration, sperm motility and sperm morphology, percentage alive or dead sperm were performed while semen pH using pH-indicator strips purchased from Merk (pH 5.0-10.0 Neutralit®) was also recorded. (3) Weekly body weights were recorded, following a 12h fasting period at a fixed time (Greyling *et al.,* 1999; Fourie *et al*., 2004), (4) Body length was determined with the aid of a measuring tape, by measuring the distance from the sternum (Manubrium) to the aitch-bone (Tuber ischiadicum) of the animal. (5) Shoulder height (cm) was also measured dorsally from the Thoracic vertebrae to the floor (Greyling *et al.,* 1999; Fourie *et al*., 2004). (6) Shoulder width (cm) was determined with the aid of special caliper, as the distance between the processus on the left shoulder blade (Tuber spinae) and that on the right shoulder blade (Fourie *et al*., 2005). (7) The circumference (cm) (tape) and diameter (cm) (caliper) of the cannon bone (Metacarpus) of the right fore-limb was measured to monitor bone development (Greyling *et al.,* 1999; Fourie *et al*., 2004). (8) Weekly feed intake (kg) was recorded for each animal to determine the feed conversion ratio (FCR).

3.3 Feed intake measurements

Animals were allocated feed buckets in which a quantity of feed (kg) was measured for each animal, every Monday (once a week, 8:00). The feed intake was determined by subtracting the amount of feed refused by each animal in the feeding troughs from

the feed offered to the animal over the period of time. Feed offered (kg) to the animals was always adjusted, according to the intake (kg), so avoid wastage.

3.4 Body parameters

As mentioned previously, the different body parameters, such as body weight, body width and length, shoulder height and width, metacarpal diameter and circumference, scrotal diameter, circumference and volume were recorded every Tuesday (8:00).

3.4.1 Body weight and other body measurements

Similarly during the experimental period, the body weight of the individual bucks was also recorded weekly at the same time (8:00). All animals were fasted for a period 12h prior to weighing. The body weights of the animals were recorded with the aid of an electronic scale, fitted to a weighing cage.

Plate 3.4 Weighing cage

All the individual body parameters were measured once the animal had been weighed by the same technical people, to avoid human error. The body width of the animals were measured using a pair of calipers (cm) between sinister lateral scapula and dexter lateral scapula (Tuber spinae). The body length was measured by measuring

the distance from sternum (Manubrium) to the aitch-bone (Tuber ischiadicum) (cm) and shoulder height, measured dorsally from the thoracic vertebrae to the floor using a measuring tape (cm). The metacarpal bone was measured using a caliper for the diameter and a measuring tape for the circumference (cm). Scrotal diameter was measured in a similar manner as the metacarpus at the broadest part of the scrotum, while scrotal volume was measured using a technique described as the water displacement method. Here a bucket was filled with water (32°C) to the brim and the scrotum of the animal submerged into the water. All water that spilled was collected due to displacement of water by the scrotum, and this volume was measured using a measuring cylinder (ml).

3.5 Semen collection and evaluation

During the trial, semen was collected every Wednesday morning (8:00 – 10:00), with the aid of an AV, filled with water at 41 to 46°C, and a doe restrained in a neck clamp. During semen collection, the buck mounted the doe and the penis was gently guided into the AV. The volume of ejaculated semen was recorded immediately after collection. To minimize stress and maximize the quality of semen of ejaculation, the collection procedure was always carried out under the same conditions i.e. by the same person, at the same time of the day, in the same pen, using the same equipment. Immediately following collection, visual and microscopic evaluation of semen was performed.

Plate 3.5 The vagina used for semen collection

Plate 3. 6 A buck mounting

3.5.1 Semen evaluation

Immediate macroscopic semen evaluation was performed after collection, by checking the ejaculate with regard to colour, semen pH and ejaculate volume, while the microscopic sperm motility was also evaluated. Microscopic sperm evaluation included the characteristics of sperm motility, morphology, viability (percentage live or dead sperm) and semen concentration ($X10^6$sperm/ml).

3.5.1.1 Macroscopic evaluation

The collected semen sample was checked immediately after collection for any colour abnormalities e.g. a brown (dirt), yellow (urine) or red (blood) colour contamination, respectively. The pH of the semen sample was also recorded using semen pH strips (Merk pH indicator strips) - based on colour changes as described by Bester (2006). The volume of the Boer goat ejaculates collected were recorded by directly reading the volume from the calibrated test tube, at the end of the AV - before placing the sample in a water bath (32°C). The semen sample was then immediately evaluated on a pre-warmed (35°C) glass slide, under a microscope (X40 magnification). The microscopic evaluation of forward sperm 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 (Table 2.2) (Loskutoff & Crichton, 2001).

3.5.1.2 Microscopic evaluation

A volume of 10µl of semen was diluted with 990µl water. The sperm cell concentration per ejaculate was then calculated, using the diluted semen sample. After being refrigerated for 24h at 4 to 5°C (to kill or immobilise the sperm), the sperm cell concentration was determined with an aid of a Newbauer haemocytometer. The sperm cells lying within 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 (x10^6 per ml) (Evans & Maxwell, 1987).

Plate 3.7 Microscopic semen analysis

Thin semen smears were made of the fresh semen sample on a pre-warmed (35°C) glass slide. The smears were prepared using 60µl eosin-nigrosin stain and 6µl semen. One hundred individual sperm cells from different areas on the slide were evaluated microscopically (x100 magnification). The eosin-nigrosin stained smears were evaluated for sperm viability (live or dead) and morphology (sperm abnormalities). All

dead sperm cells coloured red following staining. The sperm abnormalities were then classified as either head, mid-piece or tail abnormalities (Table 2.3) (Bester, 2006).

3.6 Statistical analysis

All data collected in the 3 experimental phases were statistically analysed and the means between groups compared using ANOVA procedures for repeated measure analyses of SAS (2010), at the 5% level of confidence.

Chapter 4

The effect of anabolic steroids on growth performance and certain body parameters in yearling Boer goat bucks

4.1 Introduction

In animals, growth rate (GR), average daily gain (ADG) and feed conversion ratio (FCR), like many other productive and reproductive traits, results from complex interactions between genetic and environmental factors (Unruh, 1986; Hossner, 2005). The genetic make-up of an animal subsequently determines its productive potential, while environmental factors - of which nutrition is the most important, could thus ultimately determine how much of the genetic potential will realize. From a physiological point of view growth as such, is regulated by several hormones secreted by different endocrine glands. These then regulate the activity of the different tissues in the body and hence the growth of the animal. An understanding of these interactions and the activity of the hormones involved in growth regulation could thus allow the manipulation of this physiological process, with the aid of exogenous hormones (Hossner, 2005).

Exogenous hormones or hormones applied (which could either be natural or synthetic of origin) can be used to manipulate the physiological processes regulating growth in farm animals. Therefore the manipulation of growth with the aid of hormones i.e. anabolic steroids can stimulate protein synthesis (anabolism) as such, and afford a practical way of increasing the efficiency of meat production (Lough *et al*., 1993). Anabolic steroids are thus agents or exogenous hormones that are able to accelerate

the growth rate and improve the feed conversion rate of animals and generally include a wide range of agents - with distinct and often complex modes of action (Unruh, 1986; Hayden *et al*., 1992).

So for example anabolic steroids, which mimic the actions of the natural steroid hormones e.g. estrogen, progesterone or testosterone, are generally used extensively as growth stimulants in the South African feedlot industry (mainly beef cattle and sheep) - to increase the growth rate and protein (muscle) deposition, improve the ADG, FCR and also improve the carcass quality of the animals, with significant economical benefits (Hutcheson *et al*., 1997). In addition to these anabolic effects, exogenous anabolic steroids have also been found to have an androgenic effect, resulting in certain negative side-effects on reproduction and fertility in farm animals (Unruh, 1986).

These side-effects are due to the negative endocrine feedback mechanisms induced on the hypothalamus, following the administration of exogenous anabolic steroids resulting in decreased gonadotrophin production, which then lowers the overall fertility of both males and females. For this reason anabolic steroid treatment is not recommended for animals intended to be used for breeding purposes (Hafez & Hafez, 2000).

However, in South Africa, there are rumors regarding the unethical abuse of certain anabolic steroids by some animal breeders (including goat breeders), to enhance the growth rate and improve the feed conversion rate, body conformation and masculinity of breeding males which are intended for breeding at an early age. It is alleged that

anabolic steroids are used, especially in young males (in this case Boer goat bucks), to obtain the same desired effect as in athletes (although illegal) – for increased muscle deposition and enhanced masculinity. This alleged practice seems to be done e.g. especially when breeders are preparing young rams and bucks for auctions or shows – to obtain a higher body weight, a better body conformation and enhanced masculinity at a younger age and thus obtain a higher price (Greyling & Taylor, 1999). Very little is known on the effects of using anabolic steroids on body parameters (e.g. growth performance), as well as on the feed efficiency i.e ADG and FCR of young Boer goat bucks. In addition it has been reported that certain body measurements and specific body conformations in e.g. sheep are correlated phenotypically with certain growth characteristics (Lopez-Carlos *et al*., 2010). Similarly positive relationships between body conformation, body measurements and growth traits have also been reported in cattle and sheep (De Haas *et al*., 2007; Vatankhah & Talebi, 2008).

The aim of this part of the study was to evaluate the anabolic and androgenic effects of a prolonged exogenous Deca-Durabolin (a testosterone analogue) treatment on the feed intake, ADG and FCR, as well as its effects on certain body parameters in yearling Boer goat bucks intended for breeding bucks. An additional aim was to evaluate the possible recovery of animals from any of these possible effects (if any) following the termination of such a prolonged anabolic steroid treatment.

4.2 Materials and methods

The trial was conducted at the campus of the University of the Free State South Africa, located at 28.57° South longitude and 25.89° East latitude, at an altitude of 1304m

above sea level. The mean minimum and maximum ambient temperatures recorded in winter are 13.5°C to 21.8°C and 21°C to 37.9°C in summer, with an average annual rainfall in the area of 559mm (precipitation being predominantly in the summer).

Nineteen Boer goat bucks (12 months of age, with a mean initial body weight of 47 ± 3.8 kg) were individually housed (4 x 3 m pens) and fed a maintenance pelleted diet (8.5MJ ME/kg and 13% CP), and received water *ad lib* for a total period of 31 weeks from March (onset of autumn) to October (mid-spring). Following an adaptation phase of 3 weeks, all bucks were randomly allocated to two treatment groups. Bucks in the first group (n=9) received a weekly i.m. injection of 1ml (25mg) of the anabolic steroid Deca-durabolin (nandrolone decanoate, N.V. Oregon, The Netherlands) for a total period of 16 weeks. The remaining 10 bucks acted as controls and received a weekly i.m. injection of 1ml physiological saline (no steroid treatment - placebo) on the same day as the steroid treated animals. At the end of the 16 week treatment phase, a recovery phase of 12 weeks (withdrawal or recovery period) in which no further anabolic steroid treatment was administered, followed. The body parameters recorded weekly during the total observation period of 31 weeks in all animals (during the adaptation, treatment and recovery phases), included the following:

i. Body weight (kg) following a 12 h fasting period to determine the average daily gain (ADG) during the different phases of the trial.

ii. Scrotal circumference (cm) measured at the broadest part of the scrotum and scrotal volume (ml) using the water displacement technique (Knight, 1977; Madgwick *et al*., 2008).

iii. Body length (cm) measured by determining the distance from the sternum (*Manubrium*) to the aitch-bone (*Tuber ischiadicum) (*Fourie *et al*., 2004).

iv. Shoulder height (cm), measured dorsally from the tip of the processus spinosum of the thoracic vertebrae to the ground, using a measuring stick *(*Fourie *et al*., 2004).

v. Shoulder width (cm) measured using a pair of measuring calipers (outside spring calipers), between both tuber spinae scapulae (left and right) *(*Fourie *et al*., 2004)

vi. Canon bone diameter (cm) and circumference (cm) as measured on the left metacarpus (middle of the diaphysis), using a measuring caliper and a measuring tape *(*Fourie *et al*., 2004).

vii. Feed intake was recorded weekly over the entire trial (adaptation, treatment and recovery phases), the average daily gain (ADG) and the feed conversion rate (FCR) were calculated for each buck using the weekly weights and feed intake.

For more details on the methodology used, please refer to Chapter 3 - General Materials and Methods. All data collected in the 3 experimental phases were statistically analysed and the means between groups compared using ANOVA procedures for repeated measure analyses of SAS (2010), at the 5% level of confidence.

4.3 Results and discussion

In Table 4.1, all body parameters (measurements) considered in this study, as well as the feed intake, ADG and FCR at the end of the adaptation period (3 weeks), steroid treatment (16 weeks) and recovery or withdrawal (12 weeks) phases respectively, are set out.

Table 4.1 Mean (±SE) body measurements, feed intake, average daily gain (ADG) and feed conversion ratios (FCR) of yearling Boer goat bucks after treatment with an anabolic steroid (25mg Deca-durabolin/week)

Parameter	Adaptation Phase (3 weeks)		Treatment Phase (16 weeks)		Recovery Phase (12 weeks)	
	Treated group (n=9)	Control group (n=10)	Treated group (n=9)	Control group (n=10)	Treated group (n=9)	Control group (n=10)
Body Weight (kg)	56±4.2[a]	55.7±5.0[a]	70.3±3.7[a]	70.1±5.5[a]	76±4.4[a]	78.1±5.3[a]
Body Length (cm)	64.2±2.5[a]	64.8±2.4[a]	67.6±2.2[a]	66.2±2.3[a]	68.6±2.3[a]	66.8±2.5[a]
Shoulder Height (cm)	66.5±3.0[a]	63.9±3.6[a]	70.3±2.3[a]	68.7±3.5[a]	72.3±2.6[a]	67.4±3.8[a]
Shoulder Width (cm)	21.9±1.5[a]	23.6±1.4[a]	22.8±2.0[a]	24.9±1.8[a]	25.1±1.2[a]	25.7±1.0[a]
Canon bone Circumference (cm)	12.1±0.5[a]	12.5±1.3[a]	12.2±0.6[a]	12.3±0.7[a]	12.9±0.5[a]	12.5±1.8[a]
Scrotal Circumference (cm)	29.4±2.3[a]	30.8±2.1[a]	26.1±1.9[a]	31.2±2.0[b]	27.9±2.1[a]	32.2±1.6[b]
Scrotal Volume (ml)	472.3±79.5[a]	496±66.8[a]	397±83.3[a]	530±61.6[b]	584.4±87.3[a]	640±81[b]
Total feed Intake (kg)	50.5±4.3[a]	50±5.0[a]	53.8±1.8[a]	54.3±1.4[a]	47.3±1.1[a]	45.5±6.6[a]
ADG(g/d)	147±2.8[a]	150±3.0[a]	153±12.1[a]	155±11.2[a]	144±10.6[a]	147±14.6[a]
FCR (weight gained/feed consumed)	14.6±2.3[a]	15.8±2.0[a]	17.4±1.6[a]	18.3±2.4[a]	18.1±1.9[a]	18.3±2.2[a]

[a-b]Values with different superscripts within the same row for the same trial phase differ significantly at $P<0.05$

No significant differences were recorded during the entire period of the study (31 weeks) regarding the mean body weight, ADG and FCR of the bucks in the treatment group and those in the control group (Table 4.1 and Figure 4.1). The ADG was similar for both experimental groups due to the fact that the animals were in an advanced phase of the growth curve (Eisen, 1976; Tatar, 2009). During the last half of the treatment phase and the entire recovery phase, the treated bucks tended to be relatively heavier than those of the control group. However these numerical differences were not statistically significant. Similarly, no significant differences between the two groups (treated and control groups) were recorded for all other body measurements (e.g. canon bone diameter and circumference, body length, shoulder width and shoulder height) considered in this study, with the exception of the scrotal circumference (SC) and volume (SV) during the entire study period (31 weeks).

Research for example, has shown phenotypic (P<0.05) correlations of r=0.44 (P<0.05), r=0.46, r=0.13 and r=0.37 (P<0.05) for shoulder height, body length, shoulder width and canon bone circumference respectively, with body weight in Dorper ram lambs (Lopez-Carlos *et* al., 2010). Similar data is unfortunately not available for goats. Thus it would seem that there exist significant correlations between certain body parameters and growth traits, irrespective of the specie involved. It would thus seem to make sense to record the changes in certain body parameters – which then relate to the growth performance.

Body conformation and certain parameters have been described using measurements and visual assessment. Some of these body parameters relate to the functioning of the individual and hence its production potential (Maiwashe, 2000). Body weight is a frequently recorded variable in animal research and most frequently used to evaluate growth (Otte *et al*., 1992). Although an important indicator of growth, body weight fails to describe the conformation of the animal. Thus other parameters used include wither (shoulder) height and body length, while scrotal circumference has also been shown to be an indicator of testes size (Hoogenboezem, 1995; Benyi, 1997). Shoulder height has been regarded as a good indicator of frame size as these parameters are moderately correlated (r=0.59) (Vargas *et al*., 1998; Fourie, 2002). Wercinko *et al*. (1994) also reported a significant correlation between scrotal circumference and the period when conception occurred, with a larger SC in rams being associated with early conception in the breeding season (r=0.28) and also with the number of lambs born (r=0.27). Thus indicating the importance of monitoring these parameters. Scrotal circumference was positively and significantly (P<0.05) correlated with body weight (r=0.38), shoulder height (r=0.22), and shoulder width (r=0.27), but not significantly

correlated with canon bone circumference (r=0.14) and serum testosterone concentration in Dorper ram lambs (Fourie, 2002). A highly positive correlation between metacarpus (canon bone) length and mature body size has been reported by Meyer (1995).

Body size and animal shape or conformation can be described using measurements and visual assessment. How these measurements relate to the performance of the individual, is of paramount importance in livestock production (Maiwashe, 2000).

Plate 4.1 Body conformation of experimental animals (left: treated with anabolic steroids and right: control)

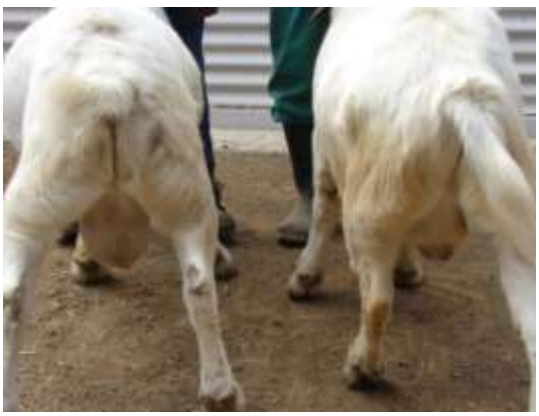
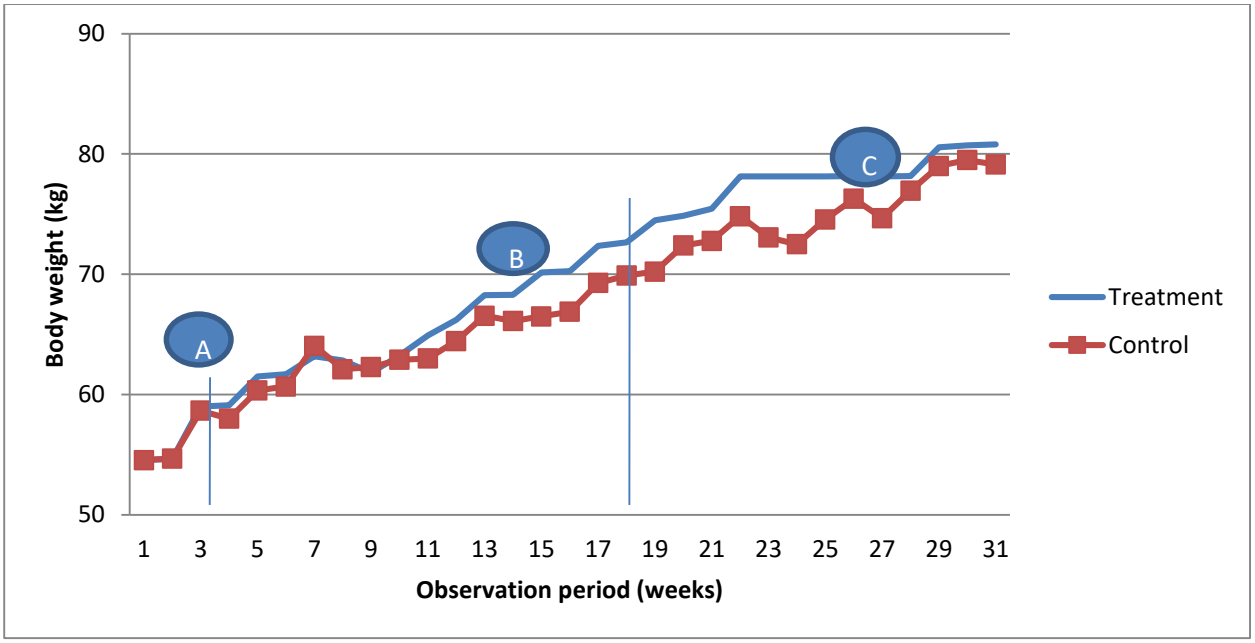


Plate 4.2 Difference in testicular size (left: control and right treated)

A graphical representation of the mean body weight changes during the entire trial period for the Boer goat yearling bucks in the treated and control groups is set out in Figure 4.1



A – Adaptation B – Treatment Phase C – Recovery Phase

Figure 4.1 Body weight changes in Boer goat bucks with or without anabolic steroid (25mg Deca-durabolin/weekly) treatment over an observation period of 31 weeks

The anabolic effect of Deca - durabolin (exogenous anabolic steroid) on body weight (Figure 4.1) and ADG (Table 4.1) were not significant (P>0.05) in this trial. The treated bucks tended to weigh more than the control bucks from week 10 of the trial (5 weeks after the onset of anabolic steroid treatment), until the termination of the trial. The greatest body weight difference was recorded during week 24 – which was already in the recovery phase (Figure 4.1). However these recorded differences were not significant (P>0.05). It would thus seem as if the exogenous anabolic steroid treatment given to the bucks had no significant effect on their growth rate. This could be possibly ascribed to one or more of the following 3 factors or a combination of these factors: (i) the drug and the dosage used (and the interaction between these factors) were not potent and high enough to induce additional protein deposition (ii) the dietary energy content of the diet was not sufficiently high to induce a significant response – with accelerated growth, a corresponding intake of sufficient energy is essential (iii) the bucks where relatively matured (12 months of age) or relatively too old and in a more

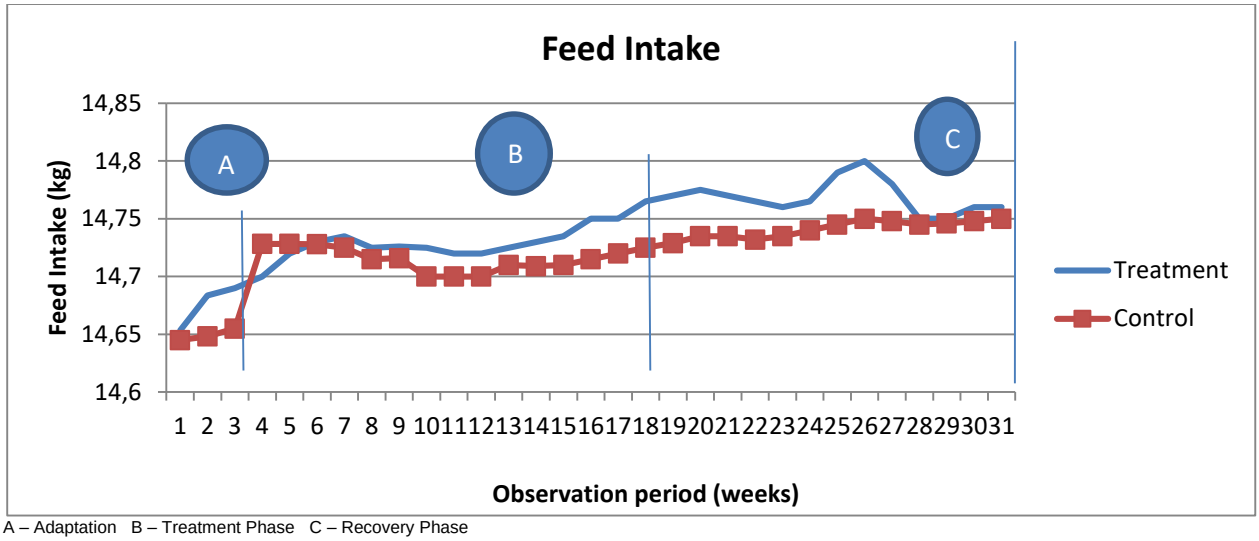
advanced stage (plateau) of their growth curve to respond to the anabolic steroid stimulation. It could however be mentioned that the dosage used was in fact double that recommended by the manufacturer in an attempt to ensure an anabolic effect.

Unfortunately due to practical reasons, the animals used in this trial were already 12 months of age, at the onset of the trial and approximately 20 months at the end of the trial, and thus relatively mature and at a slower growth rate phase of their growth curve. The growth curve generally being defined as the changes in body weight over time (or age), due to the interaction of the genetic potential and environmental (nutrition, ambient temperature, age, gender, etc.) factors (Eisen, 1976). Average post-weaning growth rates of 227 g/day have been reported in Boer goats by Naude and Hofmeyr, (1981) – this ADG was however not realized in this study – reflecting the energy in the diet, as indicated for breeding bucks being lacking. In the present study, the bucks grew on average at approximately 150g/day during the adaptation and treatment phases. In the growth and development curve of goats, it is evident that the fastest growth rate is experienced by the animals from 2 to 5 months of age, thereafter the growth curve flattens and growth almost ceases at 12 months of age (Tatar *et al.,* 2009). Season (in this case winter) when a large part of the trial was performed, could also have played a role, as animals utilize more energy to keep warm under low environmental temperatures (Maree & Casey, 1993; Hafez & Hafez, 2000).

The anabolic treatment in this study thus failed to increase the ADG of yearling Boer goat bucks significantly. In a study conducted by Dhanda *et al.* (2003) in crossbreed of Boer and Spanish goats, the ADG was found to decrease significantly with an increase in age and body weight. It was thus evident that despite 16 weeks of

treatment with the anabolic steroid Deca-durabolin (25mg/ml) weekly, it was not possible to induce the desired anabolic effect in 12 to 14 month old Boer goat bucks. Similarly no significant differences were recorded between experimental groups in terms of feed intake, ADG and FCR. A graphical representation of the mean weekly feed intake of the bucks from the treatment and control groups is shown in Figure 4.2.

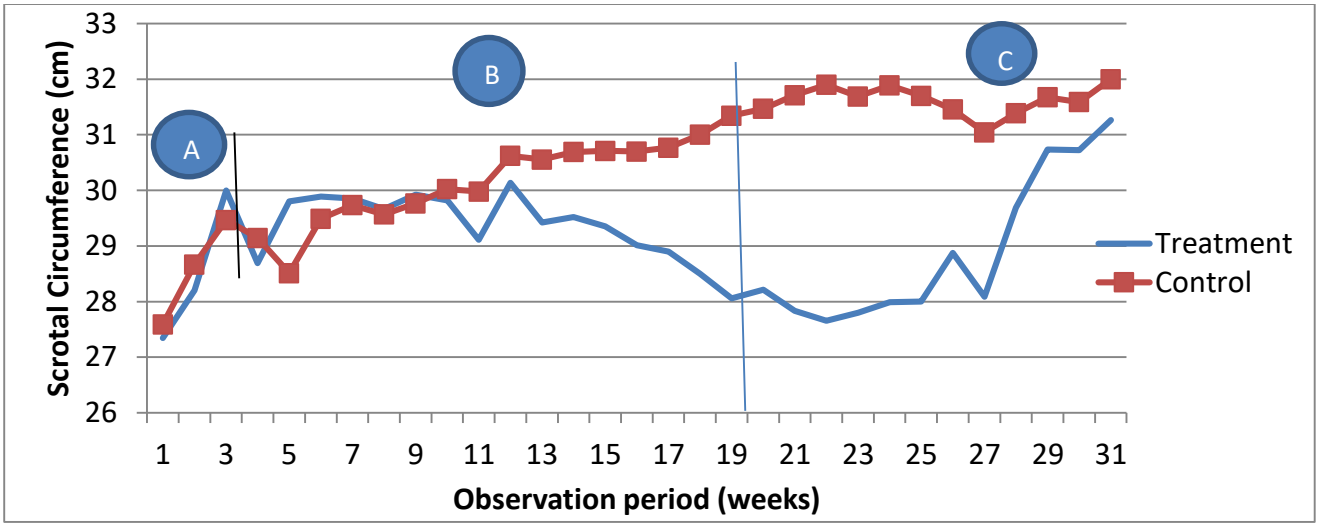
From Figure 4.2 a trend is observed that the treated bucks recorded a relatively higher feed intake over the entire observation period, more particularly during the treatment and recovery phases, compared to those bucks in the control group. However, these intake differences between the two groups were not significant ($P>0.05$). Almost universally, live weight gains (ADG) are most economical when the rate of gain is maximal. A high rate of gain invariably requires a higher feed intake. In general certain nutrients are essential to maintain certain body functions and only nutrients in excess of maintenance levels can be utilized for body weight gain. Feed intake can also be affected by odour, flavour, the level of forage and grain and the degree of processing, as well as the dryness and texture of the diet. Generally cold stress (winter) will increase feed intake (Maree & Casey, 1993), but it cannot be said that this tendency was observed for the duration of the trial.



A – Adaptation B – Treatment Phase C – Recovery Phase

Figure 4.2 **The average weekly feed intake of Boer goat bucks treated (or not) with an anabolic steroid (25mg Deca-Durabolin/week), over an observation period of 31 weeks**

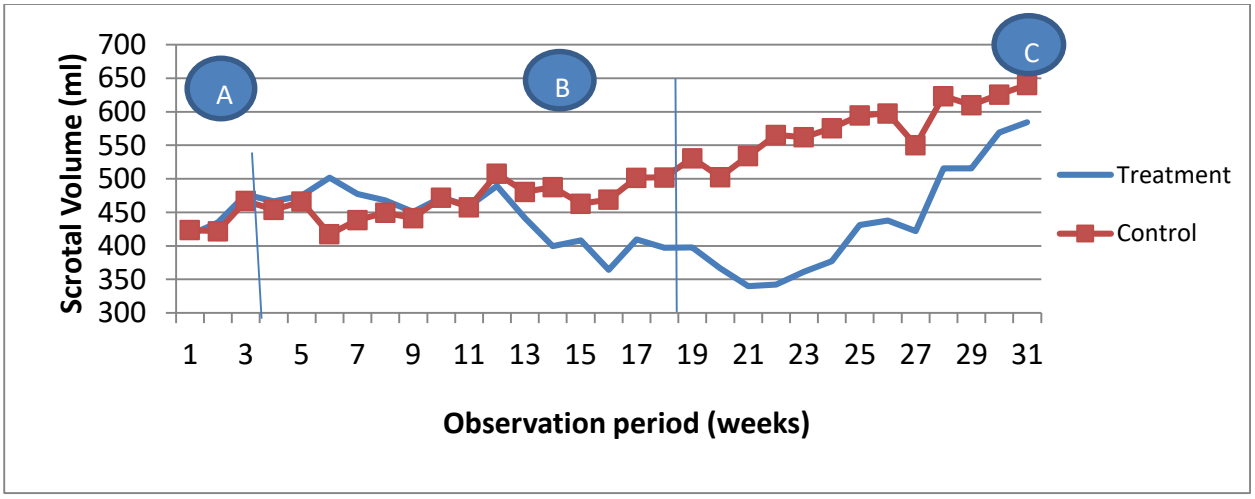
The only significant differences (P<0.05) recorded between treatment groups were with regard to the scrotal measurements (scrotal circumference and volume) - most probably as a result of different rates of testicular development. So for example, the treated Boer goat bucks (Figure 4.3) recorded a significantly (P<0.05) lower scrotal circumference and volume, during the last part of the treatment phase (from week 14) and even during the most part of the recovery or withdrawal phase of the trial. A graphical representation of the mean scrotal circumference of the bucks in the treatment and control groups during the entire observation period is depicted in Figure 4.3, while a representation of the mean scrotal volume is demonstrated in Figure 4.4.



A – Adaptation B – Treatment Phase C – Recovery Phase

Figure 4.3 **Scrotal circumference changes in Boer goat bucks treated (or not) with an anabolic steroid (25mg Deca-Durabolin/week), over an observation period of 31 weeks**

The same trends for the scrotal circumference were also recorded in terms of the scrotal volume for the anabolic steroid treated animals (Figure 4.4.)



A – Adaptation B – Treatment phase C – Recovery phase

Figure 4.4 **Scrotal volume changes in Boer goat bucks treated (or not) with an anabolic steroid (25mg Deca-Durabolin/week), over an observation period of 31 weeks**

From Figures 4.3 and 4.4 it is evident that between weeks 16 to 19 of the treatment phase of the trial, these differences in the scrotal parameters were relatively high and statistical analyses found these to be significant ($P<0.05$) - with the treated bucks recording lower SC and SV measurements. This degeneration of the scrotal measurements can be ascribed to the negative feedback induced by the exogenous anabolic steroid treatment on the gonads (Greyling & Taylor, 1999). This trend continued into the first part of the recovery phase, 6 to 8 weeks after termination of the exogenous anabolic steroid treatment. From week 8 of the recovery phase, fast, but gradual recovery in the SC and SV was recorded in the previously steroid treated bucks (Figure 4.3 and Figure 4.4). This could possibly be explained by the termination of the effect of the exogenous testosterone, and an increase in endogenous testosterone secretion by the cells of Leydig of the testes, once this negative feedback was terminated. According to Hunt *et al.* (1991), circulating serum testosterone concentrations decrease following the use of anabolic implants in cattle and increase again to reach normal levels 60 days following treatment. However it is evident in Figures 4.3 and 4.4 that the treated bucks never fully recovered during the 12 weeks of recovery period. With a longer time observation period following treatment, a stage may be reached when the difference in scrotal measurements of the treated and control bucks are almost non-existent. Similar results to those obtained in the present study have been reported by Greyling and Taylor (1999), who found the most obvious body measurement differences recorded in weaner Dorper rams to be the SC and SV of rams treated with anabolic steroids (nandrolone laurate; Laurabolin, Intervet). In the current trial, by the end of the recovery period, the differences in SC and SV between the buck in both experimental groups had decreased to approximately only

7% - with the treated animals still recording a significantly lower ($P<0.05$) scrotal volume than the bucks in the control group (584.4 ± 87 ml vs 640 ± 81 ml, respectively).

Although the detrimental effects of the anabolic steroids on the scrotal development had partially recovered following anabolic treatment during the withdrawal phase, 12 weeks after the termination of the treatment, these negative effects were still evident. These results seem to indicate the long term effect of the exogenous anabolic steroid treatment on the development of the gonads. This can ultimately also be seen as the main disadvantage (androgenic effect) following treatment of bucks intended to be used for breeding purposes, with exogenous anabolic steroids. There generally seems to exist a high correlation between the scrotal circumference and the sperm reserves in rams. Thus animals with larger scrotal circumference have higher sperm reserves (Ugwu, 2009). Whether the testes of the anabolic steroid treated bucks will ever fully recover and their activity return to normal, is still unclear - although there exists an expectancy of a possible inclination towards full recovery – given sufficient time. How long this process will take, is however also unclear. These questions are not possible to answer from the findings in this trial.

Regarding the age of the animals, maybe a better response could have been induced if younger animals, of approximately 3 to 4 month of age post weaning, being in a more active growth phase of their life when used in the trial.

4.4 Conclusions

Although the anabolic steroid treatment could not produce the expected anabolic effects on the yearling Boer goat bucks in terms of ADG, FCR and the most important

body size and conformation parameters considered, such as body weight, body length, shoulder width and height cannon bone circumference and diameter. An androgenic effect was induced and its effects observed in the testes. This was emphasized by a significant reduction in scrotal circumference and volume in the Boer goat bucks treated with the anabolic steroid (25mg Deca-durabolin/week). This androgenic effect continued for some weeks after the cessation of the steroid treatment. The scrotal circumference and volume then recovered slowly after termination of the anabolic treatment, but had not completely recovered after 12 weeks. The androgenic effects on testicular function (spermatogenesis and endogenous testosterone production) are all aspects still to be investigated. Further studies are thus warranted to determine the effect of age of the animals, dosage and duration of anabolic treatment, as well as dietary energy level on the parameters considered in this study.

Chapter 5

The effect of long term exogenous anabolic steroid treatment on the semen characteristics in yearling Boer goat bucks

5.1 Introduction

The Boer goat breed is a world renowned meat producing goat breed, extensively utilized across the world in breeding and crossbreeding programmes. This is mainly due to its meat producing characteristics such as e.g. a high growth rate, superior carcass quality, good body conformation of the male (masculinity), general adaptability and resistance to diseases (McGregor, 1985). The use of anabolic steroids may ultimately lead to the enhancement of some of these productive traits in bucks (i.e. growth rate, ADG, FCR, body conformation and masculinity). For this reason certain breeders may be tempted to use these exogenous hormones to give an unfair advantage to the young bucks intended for shows or to be sold at auctions. In South Africa there have been unconfirmed rumors regarding the use of anabolic steroids in the small stock industry, including in Boer goats by certain unethical breeders.

As discussed in the previous chapter, the anabolic steroid (Deca-durabolin; NV Oregon The Netherlands) has a negative androgenic effect on yearling Boer goat bucks, in which it reduces the testicular size (scrotal circumference-SC and scrotal volume-SV). This may then lead to a poor reproductive performance due to poor sperm (quantity and quality) production. Even though no permanent inhibition of sperm production may be induced in the short term, the prolonged use of this anabolic steroid in relatively high doses could lead to hypogonadotrophic gonadism, with a

resultant decrease in the secretion of LH, FSH and ultimately testosterone production (Kuipers, 1998). All these gonadotrophic hormones are directly or indirectly involved in spermatogenesis of bucks or any male for that matter. Spermatogenesis is influenced by many factors, these factors include e.g. breed, nutrition, age, environment (daylight length, ambient temperature and humidity) and management - resulting in variations of the semen characteristics obtained (Folch, 1983).

The quantity and quality of sperm and semen, irrespective of the animal species or breed, remain of utmost importance for fertility when natural mating and even when assisted reproductive technologies such as AI, MOET, sperm cryopreservation and IVEP programmes are implemented in livestock. It is thus essential to evaluate the effect of prolonged usage of anabolic steroids on the semen characteristics of males, in order to evaluate its effects on potential fertility in the male.

The aim of this study was thus to evaluate the effect of long-term exogenous anabolic steroid treatment (Deca-durabolin; nandrolone decanoate) on the semen quality and quantity of yearling Boer goat bucks.

5.2 Materials and methods

The trial was conducted at the campus of the University of the Free State South Africa, located at 28.57° South longitude and 25.89° East latitude, at an altitude of 1304m above sea level. The mean minimum and maximum ambient temperatures recorded in winter are 13.5°C to 21.8°C and 21°C to 37.9°C in summer, with an average annual rainfall in the area of 559mm (precipitation occurring predominantly in the summer).

Nineteen Boer goat bucks (12 months of age, mean body weight of 47 ± 3.8 kg) were individually housed (4 x 3 m pens) and fed a maintenance pelleted diet (8.5MJ ME/kg and 13% CP), plus water *ad lib* for a total observation period of 31 weeks from March (onset of autumn) to October (mid-spring), were used in the trial. Following an adaptation phase of 3 weeks, all bucks were randomly allocated to two treatment groups. Bucks in the first group (n=9) received a weekly i.m. injection of 1ml (25mg) of the anabolic steroid Deca-durabolin (nandrolone decanoate, N.V. Oregon, The Netherlands), for a total period of 16 weeks. The remaining 10 bucks acted as control animals, and received a weekly i.m. injection of 1ml physiological saline (no steroid treatment) on the same days as the treated animals. At the end of the 16 week treatment phase a recovery phase of 12 weeks (seen as a withdrawal phase) in which no further anabolic steroid treatment was implemented, followed. During this period all parameters were still recorded.

Bucks were trained to mount and ejaculate into an artificial vagina (AV), before the onset of the study during the adaptation phase. Training for semen collection by the AV method was conducted daily for a period of 3 weeks, using a doe in oestrus as a teaser female. Briefly, a Boer goat doe female was detected in oestrus and restrained in a neck clamp before the introduction of the buck into the testing arena or pen (Bester, 2006). In order to improve the libido of the bucks, all animals were placed in a pen adjacent to the semen collection arena, prior to semen collection. Thus the awaiting bucks were able to observe other bucks mounting the restrained doe, in the presence of a collector in the collection arena (Price *et al*., 1984; Silvestre *et al.*, 2004). Bucks were allowed a 5 minute period to attempt to mount and ejaculate in the AV. After ejaculation or a period of 5 minutes, whichever occurred first, the buck was

moved to an adjacent pen and after 10 to 15 minutes, males that did not ejaculate at their previous attempt, were again placed in the collection area. Training was considered to be successful when males mounted and ejaculated successfully into the AV at regular intervals i.e. 4 consecutive days when presented to any restrained female as a teaser (even if the doe was not in oestrus), in the presence of the same semen collector (Silvestre *et al*., 2004).

The trained bucks were thus initially adapted to the holding pens, diet and management during the 3 week period. The anabolic steroid treatment period of 16 weeks was followed by a 12 week recovery period, or a withdrawal phase (with no further anabolic steroid treatment).

During the trial, semen was collected, weekly every Wednesday morning (8:00 – 10:00), with the aid of an artificial vagina (AV), filled with water at 41 to 46°C, and a doe restrained in a neck clamp. During semen collection, when a buck mounted the doe, the penis was gently guided into the AV. To minimize stress and maximize the quality of semen of the ejaculate, the collection procedure was always carried out under the same conditions i.e. by the same person, at the same time of the day, in the same pen, using the same equipment. Immediately following semen collection, macroscopic and microscopic evaluations of the semen were performed.

The ejaculated semen volume (ml), colour and pH were recorded immediately following collection. The semen volume was measured directly from the graduated collection tubes, while the semen pH was recorded with the aid of Neutralit® pH-indicator strips (Merck KGaA, 64271 Darmstadt, Germany) - by pipetting a drop (10

µl) of fresh semen onto the strip and spreading it gently. The resultant colour of the strip was then compared to the colour code of the graduated pH scale to obtain a pH reading.

The different analyses for evaluating the semen quality i.e. sperm concentration, sperm mass motility and sperm morphology and percentage live or dead sperm were performed (as described in the General Material and Methods – Chapter 3).

To determine the ejaculate concentration, a volume of 10µl semen was diluted with 990µl distilled water. After being refrigerated for 24h at 4 to 5°C (to kill the sperm), the sperm cell count or concentration was calculated with an aid of Newbauer haemocytometer (Evans & Maxwell, 1987).

Thin semen smears were made of the fresh semen sample on a pre-warmed (35°C) glass slide to determine the percentage live sperm and sperm morphology. Briefly the smears were prepared using a 60µl eosin-nigrosin stain and 6µl semen. One hundred individual sperm cells from different areas on the slide were evaluated microscopically (X100 magnification) and the percentages determined. The live sperm were identified as the transparent (clear) sperm which did not absorb the stain, while the sperm morphology was assessed, as described by Bester (2006). For more details on the methodology used, please refer to Chapter 3 (General Material and Methods).

All data collected in the 3 experimental phases were statistically analysed and the means between groups compared using the ANOVA procedures of SAS (2010) for

repeated measure analyses. Differences with a confidence level of $P<0.05$ was considered to be significant.

5.3 Results and discussion

The semen quantitative and qualitative parameters recorded for the steroid treated and control animals are set out in Table 5.1.

Table 5.1 Mean (± SE) semen volume and pH, sperm concentration and percentage live and normal sperm of young Boer goat bucks treated (or not) with an anabolic steroid

	Adaptation Phase (3 weeks)		Treatment Phase (16 weeks)		Recovery Phase (12 weeks)	
Semen Parameter	Treated* (n=9)	Control* (n=10)	Treated* (n=9)	Control* (n=10)	Treated* (n=9)	Control* (n=10)
Volume (ml)	1.4±0.5	1.5±0.6	1.3±0.4	1.6±0.5	1.3±0.7	1.4±0.6
pH	7.2±0.3	7.3±0.2	7.2±0.2	7.2±0.2	7.3±0.2	7.6±0.21
Mass Motility (0-5)	4.0±0.7	4.1±0.7	3.6±0.7	3.8±0.7	3.6±0.6	3.9±0.6
Sperm Conc ($\times 10^9$/ml)	3.1±0.6	3.2±0.7	2.6±0.72	2.8±0.5	2.5±0.49	2.7±0.44
% Live sperm	67.3±14.5	69.6±15.6	60.7±15.4	62.9±15.5	70.0±21.5	68.0±13.4
% Normal sperm	90.0±5.0	89.4±6.9	87.0±5.2	85.0±10.5	81.0±8.3	83.0±5.2

*No Significant differences between means for the same parameters within treatment phases $P>0.05$

The semen parameters depicted on Table 5.1 indicate that the Boer goat bucks of both experimental groups produced on average, semen of good quality during the entire trial period. High quality ram semen has been classified as semen with a sperm motility of higher than 85%, and containing less than 10% abnormal sperm (Evans & Maxwell, 1987; Hafez & Hafez, 2000; Gil *et al*., 2003). The semen collection technique using the AV was also satisfactory, as evaluated by the relatively high semen volume and sperm concentration (relatively low standard deviation), with a constant semen pH being recorded. Table 5.1 indicates the administration of the anabolic steroid (25mg Deca-durabolin/week) to the yearling Boer goat bucks for a period of 16 weeks,

between 12-18 months of age, did not have a significant effect on the semen quantity and quality of the bucks. Throughout the entire trial, the semen quality of the treatment group and the control group were similar (P>0.05) for the two experimental groups. Despite a significant reduction in the testicular size, as indicated by the significantly (P<0.05) lower scrotal circumference and scrotal volume (Chapter 4) following exogenous testosterone treatment, none of the semen parameters measured in this trial (i.e. sperm mass motility, semen volume, semen pH, sperm concentration, percentage live and percentage normal sperm) were affected by the steroid treatment. Greyling *et al*. (1999) reported similar findings in a trial conducted on young Dorper rams under intensive feeding conditions. These researchers also found that although testicular development was retarded during treatment with anabolic steroids, the semen production (quantity and quality) was not affected by the anabolic steroid treatment. From the current results recorded, there was an insignificant decline in sperm concentration, % live and % normal-these could possibly be attributed to a change in season. Greyling and Schwalbach (2002) also reported a decline in the % live sperm due to seasonality. The % normal sperm decreased by 7-8% during the observation period and approximately 60% of the abnormalities could be attributed to tail abnormalities, with both mid-piece and sperm head abnormalities attributing 30% and 10%, respectively. In a study conducted by Squires *et al*. (1982) using an anabolic steroid on stallions, it was found that the semen volumes that had decreased during the study were not because of steroid treatment, but rather due to the change in season. Season, especially in small stock is an aspect that must always be considered when semen characteristics and sexual activity are evaluated. The mean semen pH and sperm concentration obtained in this study were lower than that recorded in older Boer goat bucks by Greyling and Grobbelaar (1983).

It would thus seem as if the inhibitory effect of the exogenous anabolic steroid hormone treatment on testicular development, did not significantly affect spermatogenesis in the yearling Boer goat bucks. This effect on testicular development however seems to be only temporary, and the males may recover, without any apparent loss in potential fertility, as measured by semen quantity and quality (Hunt *et al*., 1991, Wolfe *et al*., 1991). The doses of anabolic steroids and potency of the agents used unethically by certain breeders, are not known and these will definitely have an effect on the response obtained in the treated animals.

5.4 Conclusions

The results which were generated in this trial show that anabolic steroids did not have a significant effect on semen quality and quantity of yearling Boer goat bucks treated with exogenous testosterone. More research on the effects of anabolic steroids on bucks is needed in order to evaluate the effect of type, dosage and duration of anabolic treatment. Age of the bucks, nutritional level and season may also have an effect on the semen characteristics of bucks.

Chapter 6

General conclusions and recommendations

6.1 General conclusions

Anabolic steroids are generally sex hormones used as growth stimulants that increase protein synthesis, reduce fat deposition in the body and result in a more efficient feed utilization and conversion rate, together with a more masculine appearance of the animals and larger and leaner carcasses. These anabolic agents are then mainly used to round off farm animals under intensive feeding conditions (e.g. feedlots), prior to slaughter and potentially result in higher profits for the meat producer. Certain stud breeders have however been tempted to unethically use anabolic steroids to manipulate the growth performance and body conformation of their stud animals to gain an unfair advantage (i.e. higher prices) in the animals at shows and sales.

In this study, it was evident that the anabolic steroid treatment used did not have a significant anabolic effect on the growth performance and body parameters of yearling Boer goat bucks, except for the degeneration in testicular development. Despite this androgenic effect observed in the bucks treated with anabolic steroids (expressed by a significantly lower scrotal circumference and volume when compared with the control bucks), no significant negative effects were recorded regarding the semen characteristics of these yearling Boer goat bucks.

For the duration of the trial, there was a relatively small decline in semen characteristics such as sperm concentration, percentage normal and live sperm in both experimental groups, but this could be most probably attributed to seasonal factors. More research on the effects of anabolic steroids on fertility and sexual activity of farm animals is warranted.

6.2 Recommendations

There are many uncertainties concerning the alleged unethical usage of exogenous anabolic steroids in the stud industry by some breeders, particularly to prepare young males for sales and auctions. Therefore it is extremely difficult to set up experimental designs to scientifically evaluate these practices. There are also many unknown and potentially confounding factors such as type, dosage and frequency of anabolic steroid administration; age at onset and duration of treatment, nutritional value of the diets used to feed the animals, amongst others. Further research on the effects of anabolic steroids in young males intended to be used for breeding purposes is warranted and the following aspects should be taken into consideration:

- Drug type, dosage, regimen and duration of treatment
- Age of males at the onset of treatment (starting at younger ages e.g. at weaning)
- Nutritional value (particularly energy) of the diet and feeding regimen
- Seasonal effects (e.g. photoperiod, ambient temperature, etc)

In addition to measuring growth performance, body parameters and semen characteristics, researchers should also consider measuring blood levels of certain endocrine hormones (e.g. testosterone, growth hormone, thyroid hormones, etc), as well as blood nutrient levels.

ABSTRACT

THE EFFECT OF EXOGENOUS ANABOLIC STEROIDS ON CERTAIN BODY PARAMETERS AND SEMINAL CHARACTERISTICS OF BOER GOATS

by

Thapelo Makae

Supervisor: Prof. J.P.C. Greyling
Co-Supervisor: Dr. L.M.J. Schwalbach

Department Animal, Wildlife and Grassland Sciences
University of the Free State
Bloemfontein

Degree: M.Sc. (Agric)

Initially 20 yearling Boer goat bucks (between 14 and12 months of age, with a mean body weight of 47 kg) were successfully trained for semen collection with the aid of an AV. Animals were randomly divided into two groups, one group (n=9) was treated weekly with an intramuscular injection of 25 mg (1 ml) Nandrolone Deca-Durabolin (Adcock Ingram, The Netherlands), while the second group of bucks (n=10), served as a control.

The following body and other parameters were recorded for each buck at weekly intervals: Testicular size and testis volume (ml), weekly body weights (kg), body length (cm), shoulder height (cm), shoulder width (cm) and the canon bone circumference (cm). Weekly feed intake (kg) was also recorded for each animal to determine the feed conversion ratio (FCR).

No significant differences were recorded during the entire period of the study period, regarding the mean body weight gain, ADG and FCR of the bucks in the treatment group and those in the control group. The ADG was similar for both experimental

groups, due to the fact that the animals were in an advanced phase of the growth curve. During the last half of the treatment phase and the entire recovery phase, the treated bucks tended to be relatively heavier than those of the control group, however these numerical differences were not statistically significant. Similarly, no significant differences between the two groups (treated and control groups) were recorded for all the other body measurements (e.g. canon bone circumference, body length, shoulder width and shoulder height) considered in this study, with the exception of the scrotal circumference (SC) and volume (SV) during the study period. All these changes in certain body parameters were recorded which ultimately then relate to the growth performance.

Semen was collected by a means of an artificial vagina, and different sperm analyses for, evaluating the semen quantity, sperm concentration, sperm motility and sperm morphology, percentage alive or dead sperm were performed, while semen pH using pH-indicator strips was also recorded.

Thin semen smears were stained with eosin/nigrosin and evaluated under a microscope for overall viability (percentage live), morphology (percentage normal or abnormal), percentage mass motility and sperm concentration during the two experimental (treatment and recovery) phases.

Throughout the entire (31 week period) trial, the semen quality of the treatment group and the control group were similar ($P>0.05$) for the two experimental groups. Despite a significant reduction in the testicular size, as indicated by the significantly ($P<0.05$) lower scrotal circumference and scrotal volume following exogenous testosterone treatment, none of the semen parameters measured in this trial (i.e. sperm mass motility, semen volume, semen pH, sperm concentration, percentage live and percentage normal sperm) were significantly affected by the steroid treatment. From the current results recorded, there tended to be an insignificant decline in sperm concentration, % live and % normal sperm changes - these could be attributed to changes in seasonality. The % normal sperm decreased by 7-8% during the observation period and approximately 60% of the abnormalities could be attributed to tail abnormalities - with both the mid-piece and sperm head abnormalities attributing 30% and 10% respectively.

From the data generated, it would seem that a lack in significant differences of the body parameters in the treated animal could possibly be ascribed to the dose of hormone used, the age of the animals, the duration of treatment and the energy content of the diet fed.

REFERENCES

Al-Ghalban, A.M., Tabbaa, M.J. & Kridli, R.T., 2004. Factors affecting semen characteristics and scrotal circumference in Damascus bucks. Small Rumin. Res. 53, 141-149.

Almeida, A.M., Schwalbach, L.M.J., Cardoso, L.A. & Greyling, J.P.C., 2007. Scrotal, testicular and semen characteristics of young Boer bucks fed winter veld hay: The effect of nutritional supplementation. Small Rumin. Res. 73, 216-220.

Bahrke, M.S. & Yesalis, C.E., 2004. Abuse of anabolic androgenic steroids and related substances in sport and exercise. Current Opinion in Pharm. 4, 614-620.

Benyi, K., 1997. Estimation of live weight from chest girth in pure and crossbred West African goats. Trop. Anim. Hlth. Prod. 29, 124-128.

Bester, N., 2006. Effect of different dietary energy levels on productive and reproductive traits in dorper rams. MSc Agric. University of the Free State, South Africa.

Blache, D., Chagas, L.M., Blackberry, M.A., Vercoe, P.E. & Martin, G.B.,2000. Metaqbolic factors affecting the reproductive axis in male sheep. J. Reprod. Fertil. 120, 1-11

Bremner, W.J., Matsumoto, A.M., Burger, H.G., de Kretser, D.M., & McLachlan, R.I., 1988. Relative roles of follicle-stimulating hormone and luteinizing hormone in the control of inhibin secretion in normal men. J. Clin. Invest. 82, 880–884.

Brown, C.R., Bomberger Brown, M., Raouf, S.A., Smith, L.C., & Wingfield, J.C., 2005. Effects of Endogenous steroid hormone levels on annual survival in cliff swallows. Eco. 4, 1034-1046.

Casey, N.H. & van Niekerk, W.A., 1988. Growth, Nutrient Requirements, Carcass and Meat Quality. Small Rumin. Res. 4, 355-368.

Cecava, M.J. & Hancock, D.L., 1994. Effects of anabolic steroids on nitrogen metabolism and growth of steers fed corn silage and corn-based diets supplemented with urea or combination of soybean meal and feathermeal. J. Anim. Sci. 72, 515-522.

Chemineau, P., Malpaux, B., Guerin, Y., Maurice, F., Daveau, A. & Pelletier, J., 1992. Light and melatonin in the control of sheep and goat reproduction. Maitrise de la reproduction animale, CRTII-ISIS. 41 (34), 247-261.

Colas, G., 1975. Effect of initial freezing temperature, addition of glycerol anddilution on the survival and fertilizing ability of deep-frozen ram semen. J. Reprod. Fertil. 42, 277-285.

Dhanda, J.S., Taylor, D.S. & Murray, P.J., 2003. Part 1. Growth, carcass and meat quality parameters of male goats: effects of genotype and liveweight at slaughter. Small Rumin. Res. 50, 57-66.

De Haas, Y., Janss, L.L. & Kadarmideen, H.N., 2007. Genetic and phenotypic parameters for conformation and yield traits in three Swiss dairy cattle breeds. J. Anim. Breed. Gen. 124, 12-19.

Delgadillo, J.A., Fitz-Rodriguez, G., Duarte, G., Véliz, F.G., Carrillo, E., Flores, J.A., Vielma, J., Hernandez, H. & Malpaux, B., 2004. Management of photoperiod to control caprine reproduction in the subtropics. Reprod. Fert. Dev. 16, 471-478.

Dickson, K.A. & Sanford, L.M., 2005. Breed diversity in FSH, LH and testosterone regulation of testicular function and libido of young adult rams on the southeastern Canadian prairies. Small Rumin. Res. 56, 189-203.

Eisen, E.J., 1976. Result of growth curve analysis in mice and rats. J. Anim. Sci., 42, 1008-1023.

Evans, G. & Maxwell, W.M.C., 1987. Solomon's Artificial Insemination of sheep and goats. Butterworths, Sydney. 194 pp.

Folch, J., 1983. Influence of age, photoperiodism and temperature on semen production of rams. In: Courot, M. (Ed), The male in farm animal reproduction. Martinus Nijhoff Publishers, Amsterdam. pp. 201.

Foote, R.H., 1978. Factors influencing the quality of semen harvested from bulls, rams, boars and stallions. J. Anim. Sci. 47, Suppl 2:1-11.

Fourie, P.J., 2002. Selected factors influencing production and reproduction in Dorper sheep. D. Tech. thesis, Free State University of Technology, Bloemfontein, South Africa.

Fourie, P.J., Schwalbach, L.M.J., Neser, F.W.C. & Van der Westhuizen, C., 2004. Scrotal, testicular and semen characteristics of young Dorper rams managed under intensive and extensive conditions. Small. Rumin. Res. 54, 53-59.

Gil, J., Lundeheim, N., Söderquist, L. & Rodriguez-Martinez, H., 2003. Influence of extender, temperature and addition of glycerol on post-thaw sperm parameters in ram semen. Theriogenology 59, 1241-1255.

Greyling, J.P.C. & Grobbelaar, J.A.N., 1983. Seasonal variation in semen quality of Boer and Angora goat rams using different collection techniques. S.A. J. Anim. Sci. 13, 250–252.

Greyling, J.P.C. & Grobbelaar, J.A.N., 1988. Artificial insemination in sheep. Mutton sheep B.7/1988. Farming in South Africa. Dept. Agriculture and Water Supply, Directorate of Agricultural Information, Private Bag X144, Pretoria, 0001.

Greyling, J.P.C. & Taylor, G.J., 1999. The effect of the anabolic agent, nandrolone laurate, on certain production and reproduction parameters in ram lambs, under intensive and extensive feeding regimes. S.A. J. Anim. Sci. 29, 179-187.

Greyling, J.P.C. & Schwalbach, L.M.J., 2009. Anabolic steroids in the small stock industry. Boer Goat News (18)140-142.

Greyling, J.P.C. & Schwalbach, L.M.J., 2002. Seasonal variation in goat semen using different collection techniques. 9th International Symposium on Spermatology, Cape Town, South Africa, October 6-11

Hafez, B. & Hafez, E.S.E., 2000. Reproduction in farm animals, 7th edition. Lippincott Wilkins, Philadelphia, 509 pp.

Hayden, J.M., Bergen, W.G. & Merkel, R.A., 1992. Skeletal muscle protein metabolism and serum growth hormone, insulin and cortisol concentration in growing steers implanted with estradiol-17- beta, trenbolone acetate, or estradiol-17 beta plus trenbolone acetate. J. Anim. Sci. 70, 2109-2119.

Heitzman, R.J., 1976. The effectiveness of anabolic agents in increasing rate of growth in farm animals; report on experiments in cattle. Environ. Qual. Saf. Suppl. 5, 89-98.

Hossner, K.L., 2005. Hormonal regulation of farm animal growth. CABI publishing. pp 231

Hoogenboezem, J.M., 1995. Zootechnological aspects of bull fertility evaluation. M Sc (Agric) dissertation, University of the Free State, Bloemfontein, South Africa.

Hunt, D.W., Hendricks, D.M., Skelley, G.C. & Grimes, L.W., 1991. Use of Trebolone Acetate and Estradiol in inact and castrate male cattle: Effects on growth, serum hormones, and carcass characteristics. J. Anim. Sci. 69, 2452-2462.

Hutcheson, J.P., Johnson, D.E., Gerken, C.L., Morgan, J.B. & Tatum, J.D., 1997. Anabolic implant effects on visceral organ mass, chemical body composition, and estimated energetic efficiency in cloned (genetically identical) beef steers. J. Anim. Sci. 75, 2620-2626.

Irons, P. & Bertschinger, H., 2007. Breeding Soundness Certification of Bulls: A practitioner's Manual. Evaluation of bulls for breeding soundness, 58-67.

Kaya, A. Aksoy, M., & Tekeli, T., 2002. Influence of ejaculation frequency of sperm characteristics, ionic composition and enzymatic activity of seminal plasma in rams. Small. Rumin. Res. 44, 153-158.

Kilgour, M., Courot, M., Pisselet, C., Dobois, M.P & Sairam, M.R., 1994. Inhibition of FSH affects spermatogenesis in the mature rams. Anim. Prod. Sci. 32, 213-225

Knight, T.W., 1977. Methods for the indirect estimation of testes weight and sperm numbers in Merino and Romney rams. N.Z.J. Agric. Res., 20: 291-296

Kuipers, H., 1998. Anabolic steroids: Side effects. Encyclopedia of Sports Medicine and Science. 10 pp.

Kumar, D., Joshi, A. & Naqvi, S.M.K., 2010. Comparative semen evaluation of Malpura and Bharat Merino rams by computer-aided sperm analysis technique under semi-arid tropical environment. Int. J. Anim. Vet. Advance 2(1), 26-30.

Langford, K.S. & Miell, J.P., 1998. Insulin-like growth factor-1/binding protein axis: physiology, pathology and therapeutic manipulation. Euro J. Clin. Invest. 23, 503-516

Leboeuf, B., Restall, B. & Salamon, S., 2000. Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci. 62, 113-141.

Lindsay, D.R., Pelletier, J., Pisselet, C. & Couret, M., 1984. Changes in photoperiod and nutrition and their effect in testicular growth of rams. J. Reprod. Fertil. 71, 351-356.

Lopez-Carlos, M.A., Ramirez, R.G., Aguilera-Soto, J.I., Arechiga, C.F. & Rodriguez, H., 2010. Size and shape analyses in hair sheep ram lambs and its relationships with growth performance. Livest. Sci. 131, 203-211.

Loskutoff, N.M. & Crichton, E.G., 2001. Standard operating procedures for genome resource banking. The Bill and Bernience Grewcock Centre for Conservation and Research. Omaha's Henry Doorly Zoo. pp. 1-16.

Loubser, P.G., Van Niekerk, C.H. & Botha, L.J.J., 1983. Seasonal changes in sexual activity and semen quality in the Angora ram. 1. Libido and male hormone concentrations. S.A. J. Anim. Sci. 13(2), 131-133.

Lough, D.S., Kahl, S., Solomon, M.B. & Rumsey, T.S., 1993. The effect of trenbolone acetate on performance, plasma lipids, and carcass characteristics of growing ram and ewe lambs. J. Anim. Sci, 71, 2659-2665.

Madgwick, S., Bagu, E.T., Duggavathi, R., Bartlewski, P.M., Barrett, D.M.W., Huchkowsky, S., Cook, S.J., Beard, A.P. & Rawlings, N.C., 2008. Effects of treatment with GnRH from 4 to 8 weeks of age on the attainment of sexual maturity in bull calves. Anim. Reprod. Sci. 104, 177-188

Maiwashe, A.N., 2000. The value of recording body measurements in beef cattle. M Sc (Agric) dissertation. University of the Free State, Bloemfontein, South Africa.

Malan, S.W., 2000. The improved Boer goat. Small. Rumin. Res. 36, 165-170.

Maree, C. & Casey, N.H., 1993. Livestock production systems. Agri Development Foundation, South Africa.

Martin, G.B., Tjondronegoro, S. & Blackberry, M.A., 1994. Effects of nutrition on testicular size and the concentrations of gonadotrophins, testosterone andinhibin in plasma of mature male sheep. J. Reprod. Fertil. 101, 121-128

Mathevon, M., Buhr, M.M. & Dekkers, J.C.M., 1998. Environmental, Management, and Genetic Factors Affecting Semen Production in Holstein Bulls. J. Dairy Sci. 81, 3321-3330.

McGregor, B.A., 1988. Growth, development and carcass composition of goats: A review. In: J.W. Copland (Ed.). Goat production and research in the tropics. University of Queensland, Brisbane: ACIAR. pp. 82-90.

Meyer, K., 1995. Estimates of genetic parameters for mature weight of Australian beef cows and its relationship to early growth and skeletal measures. Livest. Prod. Sci. 44, 125-137.

National Department of Agriculture, 2010. Pocket Guide to South Africa 2009/10 Agriculture, Forestry and Fisheries. pp. 1-8.

Naude, R.T. & Hofmeyr, H.S., 1981. Meat Production. In: C. Gall (Editor), Goat Production, Chapter 9. Academic Press, London, England. pp. 285-307.

Nöthling, J.O. & Annandale, H., 2007. Semen evaluation as part of the evaluation of bulls for breeding soundness. Short Course: University of Pretoria; Evaluation of bulls for breeding soundness. pp. 12-27.

Otte, M.J., Woods, A.J. & Abuabara, Y., 1992. Live weight estimation of cattle by scale and tape-A method comparison study. Trop. Anim. Hlth. Prod. 24, 109-114.

Peel, C.J., Fronk, T.J., Bauman, D.E. & Gorewit, R.C., 1983. Effect of exogenous growth hormone in early and late lactation on lactational performance of dairy cows. J. Dairy Sci. 66, 776-782.

Price, E.O., Smith, V.M.,& Katz, L.S., 1984. Sexual stimulation of male dairy goats. App.Anim.Behav.Sci. 13, 83-92

Rae, M.T., Kyle, C.E., Miller, D.W., Hammond, A.J., Brooks, A. & Rhind, S.M., 2002. The effects of undernutrition in utero, on reproductive function in adult male and female sheep. Anim. Reprod. Sci. 72(1-2), 63-71.

Rege, J.E.O., Toe, F., Mukasa-Mugerwa, E., Tembely, S., Anindo, D., Baker, R.L. & Lahlou Kassi, A., 2000. Reproductive characteristics of Ethiopian highland sheep II. Genetic parameters of semen characteristics and their relationship with testicular measurements in ram lambs. Small Rumin. Res. 37, 173-187.

Ritar, A.J., Mendoza, G., Salamon, S., White, I.G., 1992. Frequent semen collection and sperm reserves of the male Angora goat _*Capra hircus*.. J. Reprod. Fertil. 95, 97–102.

Salisbury, G.W., Van Demark, N.L. & Lodge, J.R., 1978. Physiology of reproduction and artificial insemination of cattle. (2nd ed.) San Francisco. W.H. Freeman and company. USA. pp. 798.

SAS Institute Inc., SAS® Systems for Regression, Cary, NC: SAS Institute Inc., 2010.

Shadnoush, G.H., Ghorbani, G.R. & Edris, M.A., 2003. Effect of different energy levels in feed and slaughter weights on carcass and chemical composition of Lori-Bakhtiari ram lambs. Small Rumin. Res. 51, 243-249.

Silvestre, M.A., Salvador, I., Sanchez, J.P. & Gomez, E.A., 2004. Effect of changingfemale stimulus on intensive semen collection in young Murciano-Granadina male goats. J. Anim. Sci. 82, 1641-1645.

South African National Standards, 2002. SANS 10386:200X, 29, 38.

Squires, E.L., Todter, G.E., Berndtson, W.E. & Pickett, B.W., 1982. Effect of Anabolic steroids on reproductive functions of young Stallions. J. Anim. Sci, 52, 576-582.

Tatar, A.M., Tekel, N., Özkan, M., Baritci, I. & Dellal, G., 2009. The determination of growth function in young hair goat. J. Anim. Vet. Adv. 8, 213-216.

Tillbrook, A.J., de Krester, D.M. & Clarke, I.J., 1999. Seasonal changes in the negative feedback regulation of the secretion of the gonadotrophins by testosterone and inhibin in rams. J. Endocrinol. 160, 155-157.

Wolfe, D.F., Carson, R.L., Mysinger, P.W., Duran, B.S. & Rahe, H.S., 1991. Effects of the anabolic steroid, boldenone undecylenate, on certain reproductive parameters in bulls. Theriogenology 35, 893-906.

Ugwu, S.O.C., 2009. Relationship between scrotal circumference, *in situ* testicular measurements and sperm reserves in the West African dwarf bucks. Afr. J. Biotechnol. 8, 1354-1357.

Unruh, J.A., 1986. Effects of Endogenous and Exogenous growth-promoting compounds on carcass composition, meat quality and meat nutritional value. J. Anim. Sci. 62, 1441-1448.

Van Niekerk, W.A. & Casey, N.H., 1988. Growth, Nutrient Requirements, Carcass and Meat Quality. Small Rumin. Res. 1, 355-368.

Vargas, C.A., Elzo, M.A., Chase, C.C., Chenoweth, P.J. & Olson, T.A., 1998. Estimation of genetic parameters for scrotal circumference at puberty in heifers and hip height in Brahman cattle. J. Anim. Sci. 76, 2536-2541.

Vatankhah, M. & Talebi, M.A., 2008. Genetic parameters of body weight and fat-tail measurements in lambs. Small Rumin. Res. 75, 1-6.

Wercinko, D., Nowakowski, P. & Cwikla, A., 1994. The relationship between the reproductiveness of ewes and testicle size of related rams. J. Anim. Feed Sci. 3, 171-180.