Reproduction performance of Saanen bucks supplemented with selenium

By

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Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Animal Science

Faculty of Natural and Agricultural Sciences

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Declaration

I, Mamokou Margaret Mojapelo declare that the thesis/dissertation, which I hereby submit for the degree MSc (Agric) Animal Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature...…………………………..

Date.………………………………….

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Abbreviations

ACTH	Adrenocorticotropic hormone
AI	Artificial insemination
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AV	Artificial vagina
DM	Dry matter
EE	Electro-ejaculator
FSH	Follicle stimulating hormone
GLM	General linear model
GnRH	Gonadotropin releasing hormone
GSH-Px	Glutathione peroxidase
LH	Luteinising hormone
LPO	Lipid peroxidation
NAT	N-acetyl transferase
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
Se	Selenium
SE	Standard error
TB	Testicular breath
TL	Testicular length

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Abstract

Reproduction potential of farm animals is mainly determined by selection of appropriate strategies that benefit the reproductive performance. The aims of the study were first to evaluate the effect of selenium (Se) supplementation on attainment of puberty and reproductive performance of Saanen bucks and secondly, to evaluate the effect of selenium supplementation on semen yield and quality of Saanen bucks following two semen collection methods and induced stress. The study consisted of three experiments. For experiment 1, goats were allocated into two treatment groups: treatment and control. The treatment group were dosed with sodium selenite at three months interval. In experiment 2, the two groups from experiment one were further subdivided into two groups each based on semen collection method (artificial vagina - AV or electro ejaculator - EE) leading to four groups (Se + AV, Se +EE, Control + AV, Control + EE). For experiment 3, the major two groups (selenium supplemented and control) were subdivided into two groups each based on induced stress (injected with adrenocorticotropic hormone -ACTH) and not, leading to four groups (Se, Se + ACTH, ACTH and control). For the last two experiments, selenium supplementation was done at two months interval. ACTH was administered at three weeks interval. The phenotypic parameters were evaluated every two weeks. For all experiments semen was collected bi-weekly from bucks and evaluated for volume, colour, sperm motility, concentration, viability and morphology. Blood samples were collected at two weeks interval to determine selenium concentration and reproductive hormones. Data collected was analysed using GLM procedures of SAS (2012) and the means were separated by Duncan test. Supplementation with selenium significantly ($P < 0.05$) improved body weight, testicular measurements and decreased age at puberty. Selenium supplementation led to significantly ($P < 0.05$) greater ejaculate volume, semen colour and pH, high sperm motility, concentration and viability in the present study. There were lower ($P < 0.05$) percentages of morphological abnormal sperm from the selenium supplemented bucks than the control. There were also a lower percentage of primary sperm abnormalities from the selenium treated group, compared to the control. The electro-ejaculator method of semen collection had a greater ejaculate volume regardless of the selenium supplementation. The interaction of selenium supplementation and electro-ejaculator method of semen collection showed that when bucks are supplemented with selenium, the electro-ejaculator technique lead to a creamy semen appearance. The electro-ejaculator technique had an advantage of leading to a lower percentage of tertiary sperm abnormalities. Selenium supplementation also reduced the detrimental effects of stress on semen characteristics. Supplementation with selenium lessened the effects of induced stress, improved luteinising hormone and testosterone concentrations. Selenium supplementation enhanced serum blood glutathione peroxidase activity and lowered cortisol level in bucks. The results of the present study confirmed that male goat kids supplemented with selenium attain puberty with enhanced pubertal characteristics. It was observed that if selenium is

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supplemented and semen is collected frequently, both the electro-ejaculator and artificial vagina semen collection methods yield acceptable semen parameters and, consequently lead to improved semen quality of Saanen bucks.

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CHAPTER 1

INTRODUCTION

In South Africa, livestock production is regarded as an integral part of the agricultural systems (Schoeman *et al*., 2010). Small ruminants such as goats are kept for various purposes. In the rural areas goats are kept for food supply (meat and milk) and socio-economic purposes (Webb & Mamabolo, 2004). However, goats are poorly managed hence it affects the flock reproductive performance (Vatta & Lindberg, 2006). It is essential that appropriate management systems are implemented to increase production potential of the goats. Reproduction is considered as one of the production factors that contribute to the profitability of the farm animals. Several studies have documented that reproductive well-being and performance of farm animals is largely dependent on their nutritional status (Smith & Akinbamijo, 2000; Kheradmand *et al*., 2006; Chaurasia, 2013).

Adequate nutrition has profound effects on the development of reproductive and puberty traits in all animal species (Elhammali & Elsheikh, 2014). Jerysz & Lukaszewicz (2013) reported that mineral nutrients such as selenium (Se) are essential for many biochemical and physiological processes including reproduction. Se is known as an antioxidant that plays a vital role in maintaining and improving semen quality in animals (Ali *et al*., 2009). Additionally, it shields the spermatozoa against oxidative stress that may result from an increase in reactive oxygen species (ROS). An elevation in ROS has been reported that it damages the cellular membranes and organelles consequently decreasing fertility (Jerysz & Lukaszewicz, 2013). Therefore, Se increases the antioxidant glutathione peroxidase (GSH-Px) activity which decreases the ROS and preserves semen quality, subsequently fertility in male animals (El-Mokadem *et al*., 2012). Se also plays an important role in immunity, muscle development and function (Ali *et al*., 2009; Kumar *et al*., 2009). Growth development of the animals has an impact on the reproduction as it determines the age at attainment of puberty (Chentouf *et al*., 2011).

Young animals differ considerably on the age at which puberty is reached as it is modulated by the genetic type of the animals and management systems (Daramola *et al*., 2007). However, attainment of puberty is closely related to the level of nutrition and body weight (Bearden *et al*., 2004). Overfed animals reach puberty at a younger age. If body weight gain of the animals is slow as a consequent of suboptimal feeding, puberty is delayed (Adam *et al*., 1998). Addition of adequate level of Se has been found to accelerate body weight gain of the animals (Kumar *et al*., 2009).

Thus, attainment of puberty in young male goats can be enhanced through supplementation of essential dietary nutrients. Nutrients such as proteins, energy, vitamins and minerals have direct

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effects on sexual activity in sheep and goats (Haboby *et al*., 2004; Khalifa *et al*., 2013; Elhammali & Elsheikh, 2014). Additionally, minerals such as phosphorus (P), calcium (Ca), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), zinc (Zn) and selenium (Se) are essential for reproduction. These minerals also improve the quality of semen and fertility when supplied in correct amounts (Hafez & Hafez, 2000).

In order to assess semen quality, ejaculates are collected from goats (Suyadi, 2012). The most commonly used semen collection methods in goats are the artificial vagina (AV) and electro-ejaculator (EE) (Oyeyemi *et al*., 2001; Ramukhithi *et al*., 2011; Bopape *et al*., 2015). Both methods have achieved satisfactory semen quality and quantity (Kridli *et al*., 2005). The decision for using each is always based on circumstances e.g. where animals are handled regularly and trained, the AV is best suitable. On the other hand, under extensive production where goats are barely handled the EE is used, as it requires no training of the animals (Santiago-Moreno *et al*., 2009; Jiménez-Rabadán *et al*., 2012; Bopape *et al*., 2015). However, there is always a concern regarding the level of stress induced by the EE method. Ruminant spermatozoa are vulnerable to free radicals attack which can lead to loss in membrane and morphology integrity, impaired sperm motility and induction of sperm apoptosis (Bucak *et al*., 2010). Se has antioxidant effects that can reduce the oxidative stress due to over production of free radicals and therefore, might overcome the negative effects of EE induced stress during semen collection (Surai & Fisinin, 2015).

Stress has been implicated as a contributory factor to infertility of farm animals (Bansal & Bilaspuri, 2010). It was demonstrated that stress influences numerous mechanisms including the hypothalamic-pituitary-axis that in turn triggers the testicular endocrine function. Excessive stress induces the increase in the plasma concentration of the glucocorticoids which in turn supresses the LH receptors and consequently reduces testosterone secretion of the mammalian and farm animals (Brown *et al*., 1989; Van Lier *et al*., 2003). Animals exposed to stress were reported to have a reduced quality of semen and fertility as stress causes impairment of spermatogenesis (Hansen, 2009; Nargund, 2015). Previous reports showed that farm animals exposed to stress had reduced semen quality as a result of reduced sperm motility, sperm numbers and increased morphologically abnormal spermatozoa in the ejaculate (Mieusset *et al*., 1992; Hansen, 2009).

Many studies revealed that Se supplementation improves reproductive performance of animals (Ali *et al*., 2009; Marai *et al*., 2009, Shi *et al*., 2010). Haboby *et al*. (2004) reported that the addition of dietary Se increased male sexual activity that was observed through shortening the mating and ejaculation time and also increased the mating frequency. Se supplementation profoundly affects spermatogenesis (Ganabadi *et al*., 2010). Several factors such as diet, stress and physical activity affects sperm motility of the bucks. However, addition of Se enhances the motility of sperm (Behne *et al*., 1996). Experiments conducted in goats and rams indicated that semen quality, including an

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increase in the percentage of viable sperm and reductions in the percentages of dead abnormal sperm can be attained by the supplementation of selenium (Ali *et al*., 2009; Marai *et al*., 2009; Shi *et al*., 2010). Both the deficiency and excessive selenium has been reported to have adverse effects to normal spermatogenesis (Bindari *et al*., 2013). The requirements of dietary Se in goats are 0.2 mg Se/kg (NRC, 2007).

1.2. Research problem

Lack of supplementation of indispensable trace minerals such as Se has been found to delay growth rate and reproductive development of male goats, then ultimately, delay the onset of puberty. Se deficiency causes a decline in antioxidative defence thereby contributing to poor semen quality and quantity, and subsequently inability of the male goats to maintain fertility. However, semen quality and quantity of the goats can be affected semen collection methods. In goats, male fertility declines due to the stressful conditions which contribute to the impairment of spermatogenesis and a decline in reproductive hormones.

1.3. Aim of the study

The aims of this study were:

- To evaluate the effect of Se on attainment of puberty and reproductive performance of the young male Saanen goats.
- To determine whether Se can protect male Saanen goats against induced stress and preserve the semen quality and fertility.

1.4. Objectives

- To determine the effect of Se on attainment of puberty in male Saanen goats.
- To determine the effect of Se on body weight, scrotal circumference, testicular measurements and sexual behaviours of the male Saanen goats.
- To determine the effect of Se and collection methods on semen quality and quantity of the male Saanen goats.
- To determine the effect of Se supplementation and induced stress on reproductive hormones and semen quality of Saanen bucks.

1.5. Hypothesis

- Supplementation with Se will reduce time to attainment of puberty and improve the reproductive performance, semen quality and quantity of Saanen bucks.

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- Se supplementation will reduce the harmful effects of induced stress and consequently enhances fertility of Saanen bucks.
- Se supplementation will provide animals with antioxidant defence during semen collection.

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CHAPTER 2

LITERATURE REVIEW

2.1. Development of the male reproductive system from birth to puberty

The development of the male reproductive system involves a series of morphological changes with respect to growth and sexual development of the animal from birth to puberty (Hafez & Hafez, 2000). Bearden *et al*. (2004) reported that the endocrine system is responsible for the changes in body of animal that includes body conformation, increase in aggressiveness and sexual desire, rapid growth of the testis and separation of penis from prepuce to make it possible for extension of the penis. However, these changes occur before the presence of spermatozoa in the ejaculate.

In young growing goats sexual development occurs gradually and it is regulated by the interaction between pituitary gonadotropins and the gonadal steroid testosterone (Chakraboty *et al*., 1989). The sexual development of animals can be divided into three stages before attainment of puberty namely impubertal, pre-pubertal and pubertal stage (Rodrigues *et al*., 2012). The impubertal stage occurs between birth and the third month of life in goats. This stage is characterized by infrequent secretion of luteinising hormone (LH). It was observed that LH was 0.11 ng/mL from birth (Özsar *et al*., 1990) and showed to rise at 4 to 12 weeks (Chakraboty *et al*., 1989). The pre-pubertal stage occurs on the fourth month (Rodrigues *et al*., 2012). This stage is recognised by the fluctuation in gonadotropins. LH and follicle stimulating hormone (FSH) are involved in the regulation of sexual development and semen production (Bearden *et al*., 2004). Chakraboty *et al*. (1989) reported that there is an increase in the plasma LH and hypothalamic LH releasing factor until the age of puberty. The pituitary FSH declines after goats have reached post pubertal period. In the absence of these gonadotropins neither testicular development nor spermatogenesis would occur (Hafez & Hafez, 2000).

The pubertal stage is established after completion of first spermatogenic process, whereby the first released spermatozoid is found in the tubular lumen. This stage occurs up to 8 months in goats (Walkeden-Brown *et al*., 1994; Bezerra *et al*., 2009; Elsheikh *et al*., 2013). Thus, during the initiation of puberty gonocytes migrate to the periphery of the seminiferous tubules and produce two types of cells (Hafez & Hafez, 2000) namely: (i) Stem cells that give rise to spermatogonia and succeeding spermatocytes, spermatids and spermatozoa. (ii) Reversed stem cells that contribute to the significant increase in population of stem cells between puberty and adulthood. Bearden *et al*. (2004) reported that the spermatogonia in goats are present in the seminiferous tubules at 43 to 59 days.

The production and release of testosterone from the testes generally increases from birth to puberty. Testosterone is the most important male reproductive hormone, it is related to reproductive behaviour,

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initiation of spermatogenesis and secondary sex characteristics (Bearden *et al*., 2004). Goats are seasonal breeders thus testosterone level varies according to the season (Hussain, 2012). Bezerra *et al*. (2009) reported that testosterone level was higher during the breeding season however lower testosterone concentration was observed during the non-breeding season. It was also indicated that testosterone levels increases with age in the growing goats, however it lowers after the animals have reached puberty (Souza *et al*., 2011). The testosterone concentration decreased from 5.52 to 2.78 ng/ml, from the 18th to 28th week of age, respectively (Bezerra *et al*., 2009).

According to Amann & Schanbacher (1983) high peripheral concentrations of testosterone reduces the production of gonadotropin-releasing hormone (GnRH) through negative feedback in the hypothalamus. GnRH will in turn reduce the release of LH by pituitary through the cascade effect then, reduce the stimulus on the Leydig cells. A decrease in stimulus of the Leydig cells will then cause a decline in plasma concentration of testosterone.

2.2. The endocrine mechanism as it regulate the attainment of puberty

Bearden *et al*. (2004) reported that endocrine mechanism also regulate the onset of puberty. During the initiation of puberty, the concentration of circulating GnRH increases as a result to the rise of both amplitude and frequency of the periodic impulses of gonadotropins (Hafez & Hafez, 2000). The sex steroids are responsible for this process and albeit with the aid to maintain the increase in pulsatile GnRH release which stimulates the release of gonadotropins and in turn gonadal activity (Hafez & Hafez, 2000). The pattern in which GnRH is released during puberty is either regulated by the steroid or under steroid independent mechanisms (Valasi *et al*., 2012). Steroid-dependent mechanisms include sensitivity to gonadal steroid negative-feedback regulation of GnRH neurons (Foster, 1994). However, the immature rams and bucks are able to release high frequency of GnRH pulses that it is inherited at the early age. Though this is not fully expressed due to the presence of gonadal steroid and greater sensitivity to gonadal steroids decreases on the onset of puberty with the aid to allow GnRH frequency to increase. This remarkable change of the gonadal steroids clearly underlies the pubertal increase in GnRH/LH pulse frequency which is regarded as an important factor of reproductive function (Foster, 1994). During the onset of puberty, initiation of pulsatile discharges of LH takes place leading to the differentiation of the Leydig cells. FSH is also involved by helping up-regulate receptors for LH on Leydig cells. After the differentiation of Leydig cells, LH gradually induces an increase in concentration of testosterone. On the other hand this process lead to sustained development including that of secondary characteristics and sexual behaviours (Valasi *et al*., 2012).

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2.3. Factors that affect attainment of puberty in young male goat

2.3.1. Age and weight at puberty

Age at attainment of puberty can be defined according to the gender of the animal. In young male goats, puberty is defined as the age at which the male goat exhibit sexual behaviours that lead to mating and ejaculate that contains live and viable spermatozoa to impregnate a female goat (Bezerra *et al*., 2009). In females, it can be defined as the age at first behavioural oestrus (Bearden *et al*., 2004).

Hafez & Hafez (2000) reported that it is difficult to determine the exact time of attainment of puberty for males based on the occurrence of first differentiation of the spermatogenic cells that precedes the release of spermatozoa from seminiferous tubules, by a month or more. So, when puberty is reached, the testes are regulated by the increased levels of gonadotropins produced by the pituitary gland (Bearden *et al*., 2004).

The time taken to attain puberty in male goats differs within breeds based on body weight, genotype, nutrition, season and other environmental cues (Vilakazi, 2003). The onset of puberty is influenced more by the size and body weight than age at puberty (Hafez & Hafez, 2000). Male goats reach puberty at the age of 5 to 8 months at the body weight of 40 - 50% of mature body weight of bucks (Walkeden-Brown & Bocquire, 2000; Elhammali & Elsheikh, 2014). However, the pygmy breeds have been described to reach puberty as early as at 2-3 months (Chenoweth & Lorton, 2014). Elhammali *et al*. (2013) found that Nubian x Saanen cross buck kids reached puberty at 8 months with a satisfying body weight while on the other hand some of the bucks reached puberty at 7 months, 3 weeks of age. It is noteworthy that the difference in the attainment of puberty in bucks, differs within the breeds as a consequent of genotype and environmental factors (Souza *et al*., 2011).

2.3.2. Season of birth and photoperiod

Attainment of puberty in goats varies due to season of birth and photoperiodic changes (Hafez & Hafez, 2000). Both season of birth and photoperiod contributes considerably to the onset of spermatogenesis, then puberty in goat kids. Previous studies showed that goat kids born in spring achieved puberty between the ages of 5 to 6 months (Chenoweth & Lorton, 2014). In male Boer goats, kids born in rainy season had earlier development of the parameters evaluated for the attainment of puberty compared to that born during the dry season (Bezerra *et al*., 2009).

The animals born during the rainy season showed to have a heavier body weight, higher scrotal circumference and testosterone at puberty when are 5 months old. Hence, the animals born during the dry season managed to attain puberty with similar body weight, scrotal circumference and testosterone concentration at a later stage of 7 months (Bezerra *et al*., 2009). This study clearly shows the effect of season of birth on attainment of puberty in male goats.

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It has also been observed that male goats raised in the subtropical regions were affected by season of birth. It was observed that males born in May (summer) and October (autumn) attained puberty earlier than those born in January (spring). Although, both groups of males born in May and October had an ejaculate with immobile spermatozoa at the same age. The males born during May and October attained puberty with similar body weight and testicular measurements despite being born during increasing or decreasing day length (non-breeding or breeding season). However, these groups attained puberty earlier than those of January which were born during increasing days (non-breeding season). These findings revealed that the season of birth and photoperiod has an effect on the onset of puberty (Delgadillo *et al*., 2007).

The season of birth and photoperiod induces the testicular function of the animals (Hafez & Hafez, 2000). Chenoweth & Lorton (2014) reported that during the time of the onset of puberty in spring born goats from temperate climates, there is gradual increase in the testicular size that can be associated with changes in growth rate, more rapid phase of testicular growth occurring during the decreasing day length. At the same period, the GnRH induced LH secretion to drive the final maturation of testes including, stimulation of the secretion of testosterone.

2.3.3. Nutritional amounts of the animals

Nutrition has a significant impact on various reproduction functions including normal growth development, hormone production, reproduction and spermatogenesis (Garcia-Garcia, 2012). Bearden *et al*. (2004) reported that provision of adequate nutrition to the animals influenced normal growth rate. In case of balanced and optimal nutrition, bucks may reach puberty with a body weight of 40 to 50% of their adult weight (Van Tilburg *et al*., 2014).

Undernutrition has been proved to delay pubertal parameters such as growth development, body weight and testicular growth, penile development, first appearance of the spermatozoa and initiation of puberty in bucks (Adam *et al*., 1998). Low energy and protein deficiencies alter the rate of gain resulting in weight loss and also delaying the age at which puberty is reached. Abi Saab *et al*. (1997) reported that Baladi buck kids supplemented with a concentrate containing (18%) crude protein (CP) attained puberty earlier (22 weeks) at a lower body weight (23.8 kg) compared to bucks fed a concentrate containing 12% crude protein (31 weeks; 26.7 kg, respectively). The optimum CP is 12% which is considered adequate for maintenance and growth of the bucks (Abi Saab *et al*., 1997; Elhammali & Elsheikh, 2014). A study of the Nubian x Saanen cross goats supplemented with different protein types revealed that body weight of animals supplemented with 19% groundnut and 19% sesame seed cakes increased in pubertal body weight compared to that of 19% cotton cakes which was observed to reduce the body weight at puberty and increased percentage of abnormal sperm (Elhammali & Elsheikh, 2014).

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Nutritional levels were observed to modulate testicular measurements in growing goats. It has been reported that testicular measurements are excellent predictors of testicular size (Madani & Rahal, 1988) and the testicular size is considered as a good indicator for the semen production. Balanced nutrition increases testicular measurements and size of the seminiferous tubules which in turn contributes to the enhancement of semen quality (Abi Saab *et al*., 1996). Elhammali & Elsheikh (2014) found that adequate nutrition has led to improved semen quality at puberty with higher ejaculate volume, sperm motility, concentration, percentage of live sperm and reduced percentage abnormal sperm in the Nubian x Saanen goat kids.

Nutritional supplementation such as vitamins and minerals has profound effects on cellular metabolism, body growth and maintenance of spermatogenesis which in turn contribute to the onset of puberty in goats (Bearden *et al*., 2004). Kumar *et al*. (2009) reported that the ram lambs supplemented with Se showed to have an increased body weight than those in the control. Furthermore, previous study in mice showed that the supplementation of Se has led to an increase in body weight and testicular measurements (Wallace *et al*., 1983). Se supplementation has increased the testicular size in rams (Marai *et al*., 2009). Kolodziej & Jacyno (2005) found that the Se supplementation has led to increase in semen volume, higher sperm concentration and lower percentage of the abnormal sperm compared to that in the control.

Bucks that are on low plane of nutrition experience a decline in libido due to loss of weight. Previous studies showed that when the animals are fed higher diet resulted in higher mounts, libido and ejaculation than those fed low nutritional diets (Walkeden-Brown & Bocquier, 2000). Clearly, adequate nutrition is required for a successful mating in goats.

2.4. Methods of semen collection

The most common semen collection methods used in the bucks are artificial vagina (AV) and electro-ejaculator (EE) (Jiménez-Rabadán *et al*., 2012). The decision for using each method is always based on circumstances, availability and suitability of the experiment. Buck semen is mainly collected for various reasons including breeding soundness evaluation (fertility) and to be utilized during artificial insemination (AI) (Memon *et al*., 1986).

2.4.1. Semen collection using artificial vagina (AV)

The AV method is used for semen collection and is considered as a good imitation of the natural vagina (Hafez & Hafez, 2000). The AV consists of a rigid rubber tube for the inner lining that holds warm water (44 – 45 °C) and which is also used to place as an inner liner that is lubricated carefully with a surgical jelly.

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Before using the AV semen collection method, training of buck in the presence of a doe on oestrus is required. To collect semen, a female doe is restrained in a neck clamp and a male is allowed to mount. When a male mounts, a penis is deflected into the AV for ejaculation. In order to avoid contamination and spreading of venereal diseases from one buck to another, the rubber parts are cleaned thoroughly and rinsed with appropriate solutions (water, alcohol and distilled water) and allowed to dry (Bearden *et al*., 2004).

This method of semen collection is advantageous that the semen collected is similar to the natural ejaculation (Palmer *et al*., 2005). The semen collected with AV was reported to result in lower semen volume and higher sperm concentration (Malejane *et al*., 2014).

2.4.2. Electro-ejaculator (EE)

The EE is a technique that consists of the power source, transformer and a rectal probe. To collect semen using this technique the buck is restrained in a lateral position on the floor. Thus, the probe of the electro-ejaculator is inserted in the rectum to stimulate the prostate gland. Thus, the probe of the electro-ejaculator is inserted in the rectum to stimulate the prostate gland. However, the EE involves two important phase process namely, emission and ejaculatory phase. The emission phase involves the stimulation of the lumbar sympathetic nerves which form the hypogastric nerve and supply the ampullae and vasa deferentia. Then, the ejaculatory process includes the contraction of the urethral muscles, which are serviced by the sacral parasympathetic nerves forming the pelvic and pudendal nerves. However, semen collected with the EE may affect the semen quality by leading to the acidic semen pH that may have a negative effect on fertility of bucks (Bearden *et al*., 2004).

2.4.3. Comparison of the artificial vagina (AV) and electro-ejaculator (EE) methods of semen collection methods

Buck semen can be collected either using the AV or EE method to obtain the sample that can be evaluated (Sundararaman *et al*., 2007). Previous research showed that the results obtained with both methods may differ. Researchers found that semen samples collected with the aid of EE yield ejaculates with larger volume but lower sperm concentration compared to AV in bucks and rams (Memon *et al*., 1986; Sundararaman *et al*., 2007). The semen collected with EE has been showed to be slightly acidic in the previous studies ascribed to excessive accessory glands secretion as a result of electrical stimuli (Oritz-de-Montellano, *et al*., 2007). On the other hand, Bopape *et al*. (2015) stated that semen collected using AV resulted in a semen pH which is acceptable for the buck. The acceptable buck semen pH was reported to range between 7.0 - 7.8 (Prins, 1999).

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Previous studies also reported that semen collected with the EE resulted in acceptable semen pH, ejaculate volume, sperm concentration, motility and live sperm in the Markhoz and West African Dwarf goat bucks (Oyeyemi *et al*., 2001; Talebi *et al*., 2009).

2.5. Factors affecting sperm production and quality

2.5.1. Season and photoperiod

The term season refers to a change in a day light length observed throughout the year (spring, summer, autumn and winter) and it is characterized by weather and hours. Photoperiod is the duration of the animal's exposure to light (Chemineau *et al*., 1991). Malpaux *et al*. (2002) reported that the principal environmental cue that plays a key role in timing of seasonal reproduction is the annual photoperiodic variation.

Seasonality has profound effects on male reproductive activities. The sexual activities are influenced by many factors including latitude and seasonal breeding (Hafez & Hafez, 2000). But, the period of the breeding season differs according to the latitude where goats are raised. The breeding season increases when the latitude is decreasing (Talebi *et al*., 2009). Bucks of temperate latitudes show clear seasonality (Lebouf *et al*., 2000). Furthermore, bucks originating in hot climates (on the equator) are less affected by seasonality as compared to those which originate far from the equator. Perez & Mateos (1996) demonstrated that Spanish bucks (Verata and Malaguena) from two different latitudes resulted in different semen quality whereby bucks from lower latitude (37°N) resulted in greater ejaculate volume, sperm concentration, higher percentage of normal acrosome and lower percentage of abnormal sperm than bucks from higher latitude (40 °N).

During short days, the scrotal circumference, testicular growth, secretion of LH and testosterone level increases and lead to stimulation of sexual activities during breeding season (autumn) (La Falcia *et al*., 2002). It has been reported that the scrotal circumference in goats increases towards the end of the non-breeding season which in turn becomes beneficial during the breeding season as it leads to a high semen production and improved semen quality (Al-Ghaban *et al*., 2004). Furthermore, the semen volume, sperm motility and concentration were observed to be higher during the breeding season as compared to the non-breeding season (Jiménez-Rabadán *et al*., 2012). Moreover, in seasonal breeders such as Saanen bucks, the acceptable semen quality and quantity can be harvested when day light length is decreasing (breeding season) with the higher percentage of motile spermatozoa, sperm concentration and a decline in percentage of abnormal spermatozoa (Karagianindis *et al*., 1999).

Delgadillo *et al*. (1991) observed that the bucks (Saanen and Alpine) treated with photoperiod exhibited higher sperm production with increased semen volume and sperm motility than the bucks exposed to the natural photoperiodic changes.

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Reproductive responses to photoperiod are determined by degree of photo responsiveness and the type of photoperiodic stimulation. Photoperiod information conveys its effects on the reproductive system through hypothalamo-pituitary-axis via rhythmic diurnal secretion of the melatonin by the pineal gland (Arendt, 1986). Basically, melatonin is produced by the photoperiodically controlled cycle. Melatonin consists of a precursor serotonin. So, the serotonin is transformed to melatonin by a process that includes the enzyme N-acetyl transferase (NAT). The function of melatonin depends on photoperiodic stimulations, it can either be favourable or unfavourable depending on the day light length in seasonal breeders. Melatonin concentration increases when day light length decreases in sheep and goats. High concentration of melatonin stimulates reproductive activity, GnRH production thereby leading to stimulation of LH and subsequently, testosterone production (Hafez & Hafez, 2000). Melatonin increases scrotal circumference in short day breeders. Several studies conducted showed that high secretion of melatonin caused an increase in ejaculate volume during breeding season (Choe *et al*., 2000; Casao *et al*., 2010). In small stock and other mammals, the circulating levels of melatonin are high during the night and low during the day (breeding season) (Bearden & Faquay, 2004; Daramola *et al*., 2006). However, following long exposure to decreasing day light length, goats become photo refractory to the short-day stimulus and subsequently stop cyclic activity, unless if long light stimulation is considered (Hafez & Hafez, 2000).

Short days are categorised by high secretion of melatonin than long days. Previous studies showed the importance of prolonging the duration and amplitude of the presence of melatonin secretion in the blood during long days (Casao *et al*., 2010). Several studies conducted showed that treatment of bucks with melatonin during the non-breeding season accounted for increased sperm progressive motility and normal morphology of sperm cells (Kaya *et al*., 2000; Palacini *et al*., 2008). Melatonin treatment can be continually supplied to the animals through feeding, rumen bolus and subcutaneous implants. Daramola *et al*. (2006) reported that the interaction of light and melatonin plays an important role in semen production. Furthermore, the study conducted showed that light and melatonin treated bucks resulted in increased number of sperm cells and at the same time preserved the effectiveness of their spermatogenenic divisions at a higher rate. So, clearly, the melatonin and artificial lightning programmes enhance semen production and sperm cell parameters in small stock animals during the non-breeding seasons. Although, Delgadillo *et al*. (1992) reported that the reproductive performance in relation to the kidding rate of the semen produced by bucks exposed to natural photoperiod changes was higher than the semen produced by bucks under photoperiodic treatment. Thus, the lower sperm quality and prevention of occurrence of a restoration of the bucks during the non-breeding season can be corrected by using artificial photoperiodic treatments. Application of photoperiodic management in seasonal breeders can be used to prevent the problem of seasonality of sperm cell production and quality (Chemineau *et al*., 1999; Daramola *et al*., 2007).

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2.5.2. Nutritional effect on semen quality

Nutrition has a significant effect on the reproduction performance of goats. This intimate association depends on the farming systems used as goats can be maintained either in the extensive or semi extensive system where the availability of food is regulated by season. Nutrition has a link with the seasonal reproductive cycles. However, the response also depends on photoperiodic changes that also occur during times of food scarcity showing that season acts in juxtapose with nutrition (Zarazaga *et al*., 2009). Previous studies demonstrated that photoresponsive mature Australian cashmere bucks showed the importance of improving diet for the bucks during non-breeding season (spring) as it has led to a parallel increases in testicular size and body mass over the next 16 weeks.

Over and under nutrition in animals leads to a delay of first appearance of sperm cells in the ejaculate of growing animals (Brown, 1994). Animals that receive adequate nutrition have larger testis which can be associated with higher semen production and better quality of semen. It was reported that bucks fed poor nutrition diet has resulted in weight loss and also resulted in lower semen volume, sperm count per ejaculate volume, sperm motility, concentration, semen fructose, percentage live sperm and increase in sperm abnormalities (Hiroe & Tomizuka, 1965). While in the other study, it was demonstrated that young feral goat bucks fed low or high diets for 11 months has resulted in increased scrotal circumference and higher ejaculate volume in the breeding season (Bester *et al*., 2004).

Nutrition has direct effects on testicular growth and production of sperm in the absence of modifications of the gonadotrophin systems (Robinson, 1999). This has led to a development of a concept that both GnRH independent and dependent pathway are involved in nutritional effect on spermatogenesis.

Various nutrition components such as fatty acids, proteins, minerals and vitamins have an effect on semen quality. Nutritional components such as fatty acids play an important role in reproductive responses as a consequent of nutritional changes. Additionally, fatty acids are able to induce GnRH dependent pathways which initiate changes in the testicular function, and subsequently semen quality (Hafez & Hafez, 2000). Previous studies reported that the effect of protein on semen quality is confounding as high protein level in the ration revealed to increase the sperm concentration, although reduces ejaculate volume and sperm motility (Elhammalli & Elsheikh, 2014).

A deficiency in minerals and vitamins lead to a testicular degeneration which in turn affect spermatogenesis. Bucks that are deficient to vitamin A have soft testicles and produce poor semen quality. Minerals such as zinc, copper, and Se are also essential for reproductive function of the bucks (Kumar *et al*., 2014). In areas that Se is low, supplementation should be considered as it leads to a better semen quality (Rotruck *et al*., 1979).

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2.5.3. Age of the buck

Age is one of the factors that has pronounced effects on semen quality of bucks (Akpa *et al*., 2013). Previous reports showed that as the age increases the scrotal circumference increases in animals which in turn alters semen quality (Brito *et al*., 2002). This supports the findings of Al-Ghaban *et al*. (2004) who reported that mature bucks with larger scrotal circumference (2 – 4 years) had greater ejaculate volume, sperm concentration and lower percentage of abnormal sperm when compared to the yearlings. Furthermore, Bitto *et al*. (2012) reported that the pubertal bucks had higher percentage of dead sperm, lower sperm motility and ejaculate volume compared to the adult bucks.

2.5.4. Effect of breed

Variation in the semen quality and quantity in bucks occurs due to different type of breed and individual bucks of the same breed (Al-Ghaban *et al*., 2004). Furthermore, environment of the animal origin is also responsible for the difference in semen quality among breeds (Karagiannidis *et al*., 1999). These differences in semen quality in bucks can be observed during the breeding and non-breeding seasons (Jiménez-Rabadán *et al*., 2012). Saanen goats had a lower sperm concentration when compared to the Markhozi breeds during the breeding season signifying the difference in breeds.

2.5.5. Ambient temperature

Ambient temperature is one of the factors that affect reproduction in animals. Various physiological mechanisms play an important role in regulating testicular temperature (Hafez & Hafez, 2000). These mechanisms include scrotal sweat glands, regulation of the testis position in relation to the body through scrotal muscles and counter-current heat exchange in the vascular cone. Scrotal sac consists of several characteristics such as thin scrotal skin with minimal hair and extensive subcutaneous vasculature which assist with heat loss through radiation (Durairajanayagam *et al*., 2014). The neck of the scrotum is known as the warmest part, thus, it helps to reduce testicular temperature by increasing the area for radiation and enabling the testis to move away from the body. So, during the cold conditions the tunica dartos and cremaster muscles contract, in order to move the testes closer to the body and then, tunica dartos muscles also relaxes during hot conditions. The testis also comprises of the vascular cone which consist of highly coiled testicular artery surrounded by the pampiniform plexus known as a complex network. The testicular cone plays a significant role by facilitating counter-current heat exchanger thereby transferring heat from the artery to vein and contribute to testicular cooling (Hafez & Hafez, 2000).

When the ambient temperature is high, it may inhibit the fertilising capability of spermatozoa. A rise in ambient temperature can be harmful for spermatogenesis and also decreases testosterone level.

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High testicular temperature, leads to an increased metabolism and oxygen demand by the seminiferous epithelial cells (Machado *et al*., 2009). The abundant accumulation of oxygen damages the spermatocyte and spermatids (Hansen *et al*., 2009). Aguiar *et al*. (2013) reported a decline in semen quality during high temperatures in hot months. Furthermore, elevated body temperature leads to testicular degeneration and reduce percentage of normal and fertile spermatozoa in ejaculate (Hansen *et al*., 2009). Previous reports have demonstrated that high ambient temperatures decreased sperm motility and increased percentage of high sperm abnormalities (tailless, immature, coiled and deformed sperm cells) and dead sperm cells (Hamilton *et al*., 2015). High temperature negatively affects semen volume, sperm concentration, spermatogenesis and fertility and also a decrease in testicular activity. While during the cold season of winter months, sperm cells survival declines due to cold shock following semen collection and consequently leads to a high percentage of abnormal sperm (Daramola *et al*., 2006).

Exposure of the animals to harsh weather conditions such as high ambient temperature lead to a decline in testosterone and impaired spermatogenesis. Previous studies have reported that bucks found in hot climates had lower sperm production and extreme effects on sperm were observed in the epididymitis and destruction of the germinal epithelium (Elile *et al*., 2014). A high ambient temperature that is above 30°C during summer in hot climate areas is known to reduce libido and semen quality. Aguiar *et al*. (2013) reported that heat stress led to destruction in the sperm production which in turn caused reduction in sperm motility and an increase in the secondary sperm defects.

High ambient temperature that is above 40 °C affects the scrotum and causes sterility in bucks. Furthermore, high ambient temperatures also affect the ability to mate, sexual desire, sperm production, viability and fertilizing capacity of the ejaculated sperm (Marai *et al*., 2008).

2.6. Selenium (Se)

Se is an essential dietary trace element that it is required for many biochemical and physiological functions in humans and animals (Ganabadi *et al*., 2010). Se serves as a component of (GSH-Px) which complements the role of this element in the form of an antioxidant. Additionally, Se combines with the proteins such as selenocystine (SeCys) to form selenoproteins and function to prevent oxidative damage to body tissues (Rotruck *et al*., 1973). Se is mainly required in the testis as this organ plays a principal role in the male reproductive system (Behne *et al*., 1996).

2.6.1. Biological importance of selenium

Se is known to play a role in semen quality as an antioxidant form of GSH-Px. Se is known to stabilize oxidation, but a decline in this element allows high accumulation of oxidants. Se is known as the main selenoprotein in the testis expressed primarily in the sperm but expressed in epididymal

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epithelium. The GSH-Px develops in the spermatozoa during late spermatogenesis (Conrad *et al*., 2005). This enzyme protects cellular membranes and organelles from peroxidative damage (Surai & Fisinnin, 2015). GSH-Px was observed to protect the spermatozoa in the lumen of the epididymis against ROS during the maturation process and the entire process of spermatogenesis. It was indicated that the high antioxidant status of GSH-Px, lead to an increase in viable sperm and reduction in the percentage of dead and abnormal sperm, as well as decrease in the number of sperm with breaks in the flagellum and hence increase motility and the overall semen quality (El-Mokadem *et al*., 2012).

Se is also essential for the immune response in animals (Kumar *et al*., 2009). Oxidative stress affects health of animal, damaging the cells and tissues by free radicals or oxidants and if not controlled, it may impair the animal's immune system consequently lowering immunity of animals (Yatoo *et al*., 2013). Supplementation with Se can boost the immunity of the animals. Previous studies have demonstrated that the addition of Se in the diet improved both cell-mediated and humoral immune responses (Andrieu, 2008). It was reported that Se supplementation helps the immune system in the form of enhancing the neutrophil function and Se deficiency was observed to affect the neutrophils numbers in many species. Neutrophils produce superoxide derived radicals to take part in killing microbes. This system functions through providing protection to the neutrophils by killing invading organisms and also from attack of the radicals. It was also reported that neutrophils from Se deficient mice, rats and cattle are able to ingest pathogens but unable to kill the pathogens compound as compared to the animals with adequate Se (Boyne & Arthur, 1981). It was also observed that the animals with low Se were not strong regarding the immune responses to various diseases in comparison with the animals which are adequately supplied with Se (Boyne & Arthur, 1986).

Se is also closely related to some endocrine hormones (Lin *et al*., 2014). Adequate dietary Se is required for triiodothyronine (T_3) production. However, production of this hormone actually requires adequate supply of Se with sufficient iodine. Therefore, Se converts thyroxine (T_4) to triiodothyronine (T_3) regulated by thyroxin stimulating hormone (TSH). Thyroxine hormone is essential for controlling metabolic process in animals. Thyroxine hormone also influences testicular development and function (Wanger *et al*., 2008). In addition, T_3 regulate the maturation and growth of testis controlling the Sertoli cells and the Leydig cells proliferation, testicular development in rats and other mammal species (Mendis-Han-dagam & Ariyatne, 1994; Hosberger & Cooke, 2005).

2.6.2. Selenium supplementation

Se plays a vital role in male reproductive processes. A detection of high concentration in the testis and epididymis was considered as an indication that Se is important for the production and maturation of spermatozoa (Marin-Guzman *et al*. 1997). Several studies have reported that supplementation of Se in various farm species has led to an increase in sperm concentration, vitality, motility and lower

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morphological defects of spermatozoa (Liu *et al*., 1982; Marin-Guzman *et al*., 1997) thus leading to a good semen quality. In ganders for example, Se supplementation increased the semen volume and sperm concentration (Jerysz & Lukaszewicz, 2009).

Se toxicity is the state in which Se concentration is above requirement in the body of the animal. It was observed that toxicity of Se in rats led to a decline in body weight (Kaur & Kaur, 2000). While an extreme level of Se in the diet was observed to cause a decline in testicular and cauda-epididymal weight and number of spermatogenic cells. It was also observed that the seminiferous tubules diameter were reduced, lumen diameter, seminiferous epithelial height, and cauda-epididymal tubule diameter were caused by the excessive Se in rats (Kaur & Kaur, 2000). Shalini & Bansal (2008) reported that high concentration of Se in diets led to a decline in sperm motility, concentration and increased abnormalities of the mid piece, subsequently, fertility in mice (Surai *et al*., 1997; Shalini & Bansal, 2008).

Low levels of Se in diet of bucks leads to Se deficiency. In many parts of the world, plants lack Se and contain insufficient amounts to meet the dietary requirements of the animal (Hogan *et al*., 1993). Behne *et al*. (1996) reported that rats fed diets containing low Se had bilateral atrophy and delayed testis growth. Shi *et al*. (2010) reported that lower Se concentration in the testis has led to a decline in GSH-Px. It was also observed that Se deficiency in boars lead to decline in sperm motility, higher percentage of abnormal sperm and lower fertilisation rate of oocytes (Marin-Guzman *et al*., 1997). Furthermore, decline in Se concentration disrupts sperm maturation. In male goats, semen quality and fertility depend largely upon the sperm maturation process which means that abnormal process causes poor quality of semen and a decline in fertility (Ahsan *et al*., 2014). According to Marin-Guzman *et al*. (2000) the spermatozoa of boars fed Se-deficient diets had abnormalities in tail, midpiece, mitochondrial gaps and plasma membranes.

It was also demonstrated that Se deficiency impaired the responsiveness of Leydig cells to GnRH, thereby causing a reduction in secretion of testosterone in bucks. Testosterone production is depended on LH secretion. It was observed that Se deficiency leads to malfunctions in LH receptors mechanisms controlling storage and release of testosterone lesions in the biochemical system controlling steroid synthesis and damage to smooth endoplasmic reticulum (ER) of the Leydig cells where testosterone is synthesized. Usually this coincides with lower antioxidant status. Thus, Se deficiency leads to a decline in testosterone level consequently impair spermatogenesis (Elsissy *et al*., 2008).

2.6.3. Effect of selenium on male fertility

It has been documented that Se plays an important role in the male reproductive system (Ahangarai *et al*., 2013). Adequate levels of Se are required for normal spermatogenesis (Brown & Burk, 1973).

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Supplementation with Se has some intrinsic effects on the sperm production of animals (Kendal *et al*., 2000). However, Se deficiency has an effect on the morphology and motility of the spermatozoa (Scott *et al*., 1998). A decline in fertility is due to ROS production as it attacks the membranes of spermatozoa, decreasing their viability. In order to prevent such incidences, Se level should be increased and in turn the antioxidant status will increase to combat ROS then lead to improved male fertility (Hamilton *et al*., 2015).

Se supplementation enhances semen quality in animals (Kolodziej & Jacyno, 2005). Marai *et al*. (2009) documented that supplementation with sodium selenite improved semen quality in rams by increasing semen volume per ejaculate, spermatozoa motility and sperm concentration and a decrease in the percentage of dead spermatozoa, sperm abnormalities and a decline in acrosome damage. In mice, boars, sheep and goats diets supplemented with Se resulted in increased sperm concentration and the number of sperm cells (Marai *et al*., 2003; Ebeid *et al*., 2009; Shi *et al*., 2010; Speight *et al*., 2012).

In contrast, treatment with organic Se in boars did not affect semen volume, sperm concentration, total sperm in the ejaculate, sperm motility and morphology (Lovercamp *et al*., 2013). Injection of bulls with Se also had no effect on sperm production measures (Segerson *et al*., 1981).

Se is involved in many biological processes. It was observed in mammals that Se supplementation has caused an increase in male sexual activity manifested by significantly shortening mating and ejaculation times as well as increased mating frequency (Haboby *et al*., 2004). Rams and boars supplemented with sodium selenite had improved libido (Kolodziej & Jacyno, 2005; Marai *et al*., 2009).

2.7. The relationship between stress, male fertility and selenium

Stress influences reproduction in animals. Elevation of stress stimulates the hypothalamus, pituitary gland and gonads which causes a decline in GnRH secretion into the hypophyseal portal blood (Kumar *et al*., 2009). A decline in GnRH has major effect on production of androgen. It was observed that as the GnRH decreases, the LH pulse reduces as well and subsequently causes a decline in testosterone production. In farm animals, it was observed that a decline in testosterone is mainly caused by a failure of the responsiveness of the Leydig cells to gonadotropins. Generally, as the LH receptors mechanism malfunctions, the storage and release of testosterone also becomes affected (Hafez & Hafez, 2000).

In males incidences of reproductive hormone alterations are associated with deficiency in minerals and low antioxidant status (Behne e*t al*., 1996). A decrease in antioxidant status allows for increase in

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ROS. It has been reported that the antioxidant status can be improved by supplementing with antioxidants such as Se (Surai & Fisinin, 2015).

Se is a mineral element that is necessary for reproduction processes and improves fertility. Previous report has demonstrated that Se can be supplemented in farm animals in order to lessen the harmful effects caused by the oxidative stress (El-Mokadem *et al*., 2012). El-Sissy *et al*. (2008) reported that supplementation of Se provides the testis with protection from unfavourable effect of reactive oxygen species in the form of GSH-Px. Thus, Se has the ability to improve the testicular function ensuring normal operation of the Leydig cells and steroidogenic function in bucks.

2.8. Stress

The term stress can be defined as a reflex action revealed by the inability of an animal to cope with its environment which may lead to many unfavourable consequences ranging from discomfort to death (Kumar *et al*., 2012). However stress can be classified as physical, biological and physiological stress. Physiological and biological stress are considered as organism's reaction to the stimuli that may affect homeostasis. The biological and physiological stress responses are linked to the hypothalamo-pituitary-adrenal axis (Einarson *et al*., 2008).

2.8.1. Effect of stress on reproductive hormones of male animals

Stress influences reproductive functions in farm animals. Stress induces the hypothalamus, pituitary gland and gonads which in turn inhibit the GnRH secretion. Hence, the hypothalamo-pituitary-adrenal axis is triggered and produces corticotrophin-releasing factor (CRF) and arginine vasopressin (Kumar *et al*., 2012). Then, CRF activates the release of adrenocorticotrophic hormone (ACTH) and other propiomelanocorticortin (POMC) derived peptides, such as β-endorphin from the anterior lobe of the pituitary gland. ACTH acts on the adrenal glands to stimulate the secretion of glucocorticoids, for example cortisol (Einarson *et al*., 2008).

Cortisol has been classified as a stress hormone. Researchers have reported that if cortisol hormone is higher (above normal) it becomes detrimental, leading to the gonadal and sexual dysfunctional (Cowen, 2002). The circulating plasma concentration of cortisol increases rapidly in response to stress. The elevation of cortisol is detrimental to the testicles resulting in testicular dysfunction (Shukla *et al*., 2008). Cortisol also reduces the blood concentration of the testosterone in almost all mammalian species and rams (Fenske, 1997; van Lier, 1999).

Previous studies have demonstrated that stress is one of the factors that contributes to increase in blood concentration of glucocorticoids which in turn, reduces testosterone concentration in mammalian and farm animals (Fenske, 1997; Phogat, *et al*., 1999; van Lier *et al*., 2003). It was reported that glucocorticoids has a direct effect on Leydig cells which in turn causes a decrease in

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testosterone production (Briski *et al*., 1995). In a study conducted in humans, Cumming *et al*. (1983) evaluated the influence of cortisol on testosterone level. It was observed that testosterone levels decreased in response to cortisol administered but without altering LH levels (Cumming *et al*., 1983). Fenske *et al*. (1997) also demonstrated that ACTH injection to pigs increased cortisol which in turn led to a reduction in testosterone plasma concentration. However, it was found that the mechanism of action that causes a decrease in testosterone occurs independently of the LH plasma concentrations. Briefly, it was observed from *in vitro* experiment that cortisol cooperate with the Leydig cells thereby causing a delay in testicular 17α-hydroxylase and/or $C_{17,\ 20}$-lyase. Cortisol inhibit the function of the Leydig cells due to inhibition of testicular 17 α -hydroxylase and/or $C_{17,20}$-lyase. This occurs as a consequent of the influence of the elevated intracellular cortisol that is beyond the capacity of the 11α-hydroxysteroid dehydrogenase to inactivate it.

2.8.2. Effect of stress on semen quality

Stress has been implicated as a possible contributory factor to the quality of semen in farm animals, humans and mammals (Hamilton *et al*., 2015; Nargund, 2015). Elevated stress is associated with a decrease in androgens such as testosterone which play a principal role in spermatogenesis. Decline in testosterone was demonstrated to affect the Sertoli cells which are also involved in spermatogenesis. Testosterone links Leydig cells and Sertoli cells biochemically and in turn supports the mechanism of spermatogenesis. Elevated stress causes a decline in testosterone which in turn causes defective blood testis barrier and which in turn result in the germ cells not progressing beyond meiosis. Thus, during this condition, immature germ cells are prematurely displaced from Sertoli cells while on the other hand mature sperm cells cannot be released (Nargund, 2015). Thus, this explains the effect of stress on various semen parameters in animals.

Bulls exposed to stress have showed that stress condition leads to a decrease in semen quality (Waitse, 1970). Stressful conditions in mammals lead to harmful effects to semen quality such as reduced sperm motility and increased proportion of morphologically abnormal spermatozoa in ejaculates (Hansen, 2009). Eskiocak *et al*. (2006) reported that stress causes a reduction in sperm concentration and progressive motility. Several studies reported that farm animals exposed to stressful conditions had damaged spermatozoa and decreased sperm numbers (Moule & Waites, 1963; Mieusset *et al*., 1992). In mice, it was observed that stress causes damage in the epididymis and germ cells in the testis, and also the spermatocytes were affected. Exposure of bulls to stress has led to a decrease in motile spermatozoa and live sperm while the primary and secondary sperm abnormalities were increased (Barth & Bowman, 1994; Skinner & Louw, 1966). These observations confirm that stress has negative effects on semen quality.

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2.8.3. Oxidative stress as it affects fertility in animals

Oxidative stress is a state in which large amounts of prooxidative molecules are released to cause cellular damage as a consequence of lower antioxidant status (Hamilton *et al*., 2015). Oxidative stress is considered as one of the factors which contribute to poor semen quality in animals. The main cause of the oxidative stress is the increased production of ROS which is comprised of free radicals and peroxides (Bansal & Billanspuri, 2010).

The production of ROS by the spermatozoa is considered as a normal physiological process. ROS is involved in membrane fluidity and maintaining the fertilizing ability and acrosome reaction of the sperm (Aitken & Fischer, 1994). However, abundant production of ROS is toxic for the spermatozoa. ROS affects the structure and membrane of the spermatozoa, it increases the number of sperm with breaks in the flagellum and decreases sperm motility (Wu *et al*, 1979; Bansal & Billanspuri, 2010) subsequently reduces fertility. In infertile men, high production of ROS leads to sperm DNA fragmentation (Agwaral *et al*., 2005). In small ruminant, spermatozoa produce high levels of peroxide as a result of elevated amounts of polyunsaturated fatty acids (PUFA) (Bucak *et al*., 2010; Hamilton *et al*., 2015). In almost all mammals, high accumulation of PUFA induces ROS and free radicals which in turn induce high accumulation of lipid peroxidation (LPO). High accumulation of LPO causes plasma membrane to lose its ability to act as a permeability barrier, leading to loss of cytosolic enzymes and substrates hence, a decrease in sperm motility (Alverez & Storey, 1984). Clearly, in farm animals and humans, over production of ROS compromises fertility by causing damage to the sperm structure, morphology and functions accompanied by decreased sperm motility and viability (Atiken & Fischer, 1994; Bucak *et al*., 2010; Hamilton *et al*., 2015).

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CHAPTER 3

MATERIALS AND METHODS

3.1. Study location

This study was conducted at University of Pretoria experimental farm, Hatfield, Pretoria (latitude 25°45' South, longitude 28°16' East). The experimental farm is situated in the Highveld region of South Africa, at an altitude of 1 327 m above mean sea level (Mengistu *et al*., 2016).

3.2. Experimental animals

The study was approved by the University of Pretoria Animal Ethics Committee under the project number EC 10613 and was conducted using Saanen male goats. All the experimental animals were kept in an open pen from weaning throughout the experimental period of 11 months.

3.3. Animal management

All the animals were fed lucerne (*Medicago sativa*) *ad libitum* and also had free access to water throughout the study. Before the onset of the experiment, the lucerne sample was analysed for selenium (Se) concentration. Each time a new batch of lucerne was bought, the samples were tested for Se concentration. The lucerne had Se concentration of 0.003 mg/kg.

3.4. Experimental designs

The study was conducted in three experiments. Forty Saanen male goats were used for conducting all three experiments.

3.4.1. Experiment 1: Effect of selenium on attainment of puberty and semen quality of male Saanen goats.

The experiment was conducted from summer to autumn (December to April). At the onset of the experiment, the animals aged between 2 - 3 months with an average body weight of 15.7 ± 0.37 kg. The kids were weaned at the age of between 2 - 2 .5 months and fed pellets for a period of three days. Thereafter, the pellet diet was stopped two weeks before the onset of the experiment and the kids were then fed lucerne *ad libitum*.

The animals were grouped according to body weight then randomly allocated into two groups which consisted of the Se supplementation (n = 20) and control group (n = 20).

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The supplemented group had sodium selenite (ACE Chemicals®; South Africa), orally. From the supplemented group, animals received dosages of 0.34 mg Se/kg. Before Se supplementation, the blood samples were collected to determine Se level of the animals.

Se was administered at three months interval based on previous reports that it is carried in red blood cells (RBC) and therefore a decline in Se concentration will depend on the RBC life span (van Ryssen *et al*., 2002). In order to assess semen, attempts for semen collection were made when the kids were aged 4 - 5 months, however the animals responded at 5.5 months of age. One ejaculate was collected from each kid during the session. Semen was collected using an electro-ejaculator (Ramsem, South Africa). Semen was collected at two weeks interval throughout the experiment. Therefore, this experiment is comprised of five replicates of semen collection.

3.4.2. Experiment 2: Effect of selenium supplementation and semen collection methods on Saanen semen yield and quality.

The same animals used for the first experiment were used for this study under the same management. This experiment was conducted from autumn to winter (May to June). At the beginning of the trial the animals aged between 8 - 9 months, with an average body weight of 24.41 ± 0.37 kg. For this experiment, Se was supplemented at two months intervals as animals had below requirement level (74.27 ng/g) of Se concentration at the end of experiment 1.

The main groups (Se supplemented and control) were further subdivided into semen collection methods of artificial vagina (AV) and electro-ejaculator (EE). Semen was collected bi-weekly throughout the experimental period. This experiment is comprised of four replicates.

3.4.3. Experiment 3: Effect of selenium supplementation and induced stress on reproductive hormones and semen quality of Saanen goats.

The same animals used for the second experiment were utilized for this study under the same management. Se was also supplemented at two months interval for this experiment. In the present study, the work was carried out from winter to spring (July to October) for a period of 16 weeks. The age of the bucks ranged 11 - 12 months at the beginning of the study. For this experiment the two main groups (Se supplemented and control) were subdivided into adrenocorticotropic hormone (ACTH) injected, control, leading to four groups: selenium, ACTH, selenium + ACTH and control. The average body weight for the bucks of each treatment was 26.77 kg for Se supplemented, 25.7 kg for ACTH, 29.7 kg for Se + ACTH and 25.9 kg for control. Each group had 10 animals. Each treatment group was again subdivided into two groups according to the method of semen collection, AV (n = 5) and EE (n = 5). Semen was collected every two weeks throughout the experiment. This experiment is comprised of eight replicates.

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For the ACTH treatment, animals were injected with 0.3 mg ACTH/kg (Porcine, ACTH fragment 1-39, Sigma Aldrich®, USA) at three weeks. In order to induce stress, the ACTH was injected at 08H00 in the morning, then, after the injection animals were monitored for 24 hours while blood samples were being collected at 8 hours interval for evaluation of serum cortisol and reproductive hormones. For Se + ACTH treatment, the animals were administered Se as described above but also received ACTH injection. On the day that ACTH group was treated, the control group were also injected with 0.1 mg/kg saline solution at three weeks interval. The control group was neither supplemented with Se nor injected with ACTH.

3.5. Body weight and testicular measurements

Body weight (BW) and testicular measurements of experimental animals were recorded bi-weekly for experiment 1. The body weight (kg) of the animals was measured using electronic weighing balance [TAL-TEC®, South Africa, (Pty) (Ltd)]. The testicular length (TL) (Figure 3.1) and breath (TB) (Figure 3.2) were measured with the aid of a digital vernier calliper (Lasec®; South Africa) and were recorded in cm. Testicular length was measured from the top part of testis to the bottom part (on the tip) of the testis while the testicular breath was measured at the largest diameter of the testis. The scrotal circumference was measured using a flexible tape also at the widest scrotal diameter (cm).

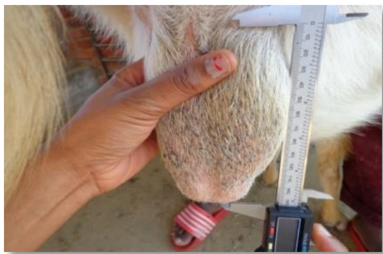

Figure 3.1. Measurement of testicular length (TL) of Saanen buck

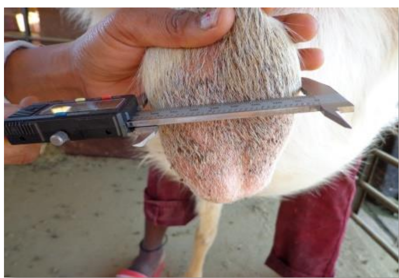

Figure 3.2. Measurement of testicular breath (TB) of Saanen buck

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3.6. Blood collection from Saanen goats

3.6.1. Blood collected for selenium status

Blood samples were collected from the bucks (Figure 3.3) using 4 mL BD vacutainer (lithium heparin) tubes (BD-Plymouth.PL6 7BP®, United Kingdom) from the jugular vein at the onset of the experiment and on monthly basis to determine the level of Se. The whole blood was stored at -20 °C until analysed for Se.

3.6.2. Blood collection for reproductive hormones evaluation

The blood samples were collected at two weeks interval using 4 mL BD sterile vacutainer tubes (BD-Plymouth.PL6 7BP®, United Kingdom) for testosterone and luteinising hormone (LH) assays. Following collection, the blood samples were centrifuged (Figure 3.4) at 3000 g for 10 min to recover serum. Serum was collected and stored immediately at -20 °C until testosterone and LH assay.

On the day of ACTH treatment (experiment 3), blood samples were collected for reproductive hormones, GSH-Px activity and cortisol. Blood samples were collected in 4 mL BD sterile vacutainer tubes through jugular vein. The blood samples were collected at 8 h intervals starting an hour following ACTH administration and continued for 24 h. After collection, the blood samples were centrifuged at 3000 g for 10 min to separate serum and recovered serum was stored immediately at -20 °C until assayed.

Figure 3.3. Collection of blood from Saanen buck

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Figure 3.4. Centrifugation of blood

3.7. Reproductive hormones, selenium, glutathione peroxidase and cortisol assays

For each assay, the manual was provided by the manufacturer and the procedures were followed accordingly. All the kits for reproductive hormones, glutathione peroxidase and cortisol were analysed using the micro plate reader multiskan FC (Thermo Scientific, United Kingdom). Glutathione peroxidase and cortisol hormone were analysed for experiment 3 only.

Testosterone and cortisol concentration in serum was measured using the ELISA kits (Demeditec, Germany). Briefly, 25 µL serum sample and manufacturer provided standards were pipetted into the wells that were precoated with antibody of a specific hormone tested. After the addition of the enzyme conjugate the wells were incubated at room temperature for 60 min. Following washing step with the provided buffer, a substrate solution was added to the wells and incubated for 15 min at room temperature. After that, the stop solution was added. The concentration of the hormone was determined using microplate reader set at an absorbency of 450 ± 10 nm.

For the luteinising hormone, a 25 µL serum sample and standards provided were pipetted into the wells that were precoated with antibody of a specific hormone tested. After the addition of the enzyme conjugate the wells were incubated at room temperature for 30 min. Following washing step with the provided buffer, a substrate solution was added to the wells and incubated for 10 min at room temperature. After that, the stop solution was added. The concentration of the luteinising hormone was determined using microplate reader set at an absorbency of 450 ± 10 nm.

Glutathione peroxidase activity in serum samples was measured using the glutathione peroxidase kit (Abcam, United Kingdom). A 50 µL of serum sample, a 100 µL of manufacturer provided standards were pipetted into the wells for the enzyme tested. After preparing reaction mix, (for assay buffer, 40 mM NADPH solution, GR solution, GSH solution), a 40 µL of reaction mix was added to the serum sample, positive controls and reagent control wells and the contents were incubated for 15 min. Following a 10 µL, the output (A1) was measured with a microplate reader at optical density (OD) of

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340 nm at T1. Then, the contents were incubated at 25 °C for 5 min. Then, output (A2) was measured with a microplate reader at OD 340 nm at T2.

Blood samples for Se concentration analyses were carried out by continuous hydride generation atomic absorption method (AOAC, 2000). Samples were read using Perkin-Elmer 2380 atomic absorption spectrophotometer (Perkin Elmer, United States of America) at an absorbency of 196 nm and lamp energy of 16 mA (Courtman, 2013).

3.8. Evaluation of sexual behaviour

Sexual behaviours exhibited by the animals were evaluated at weekly intervals for experiment 1. The animals were exposed to a teaser doe on oestrus for 15 min in the morning. Each behaviour elucidated by the kids towards the teaser doe was closely monitored and recorded. The sexual behaviours recorded included smelling, nudging, pawning, licking, bleating, flehmen and mounting.

3.9. Semen collection

Two methods of semen collection used were the AV and EE.

3.9.1. Preparation of artificial vagina

For semen collection with AV, goats were trained for a period of 8 weeks in the presence of a doe in oestrus before the onset of the study. The AV (Ramsem®, South Africa) was prepared as follows: The space between the outer and inner latex layer (Ramsem®, South Africa) of the AV was filled with warm water at 50 to 55 °C to maintain the temperature at 42 °C inside the AV. Soluble lubricating K-Y Jelly (Johnson & Johnson®, South Africa) was used to lubricate the latex of the AV and careful attention was paid to prevent excessive lubrication which might lead to contamination of the ejaculate. A graduated semen collecting tube (Ramsem®, South Africa) was connected on the other open side of the AV. After preparing the AV, a doe on heat was driven to the pen and it was restrained with a neck clamp. Thereafter, a buck was allowed to enter the pen for semen collection. Immediately when the buck mounted the doe, its penis was deflected into the AV for semen collection (Figure 3.5).

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Figure 3.5. Semen collection using artificial vagina

3.9.2. Ejaculate collection using electro-ejaculator

A buck was driven to an open pen and restrained in a lateral position on the floor. The sheath was cleaned by trimming the preputial hair. Thereafter, the sheath was cleaned with distilled water and wiped with a paper towel (Onderstepoort Pharmacy, South Africa). The sheath was then pushed back and the penis was gently grabbed using a sterile gauze swab (Afri Vet®, South Africa). The electro-ejaculator probe (Ramsem®, South Africa) was then lubricated with K-Y Jelly (Johnson & Johnson®, South Africa) and inserted rectally. A 15 mL graduated Falcon tube (Lasec®, South Africa) used to collect semen was held closely to the penis. The electrical stimulation was then applied at 4 - 5 seconds rest periods. The stimulation began with a low voltage being gradually increased (3 - 5 voltages) (Bearden *et al*., 2004). The rhythmic patterns were repeated four times, if the animal was unable to ejaculate, it was taken back to the pen without collection. Then it was collected again in the next collection with the rest of the group.

3.10. Laboratory semen evaluation

Immediately after semen collection, the samples were transported to the laboratory within 1 h for measurement of semen characteristics categorised into macroscopic (semen colour, volume and pH) and microscopic (sperm motility, concentration, morphology and viability) evaluations.

3.10.1. Evaluation of macroscopic semen characteristics

The volume of the ejaculate was recorded by reading the value on a 15 mL graduated falcon tube. The colour of the semen was evaluated using a score of 0 (clear) to 5 (thick creamy) as an indicator of sperm concentration (density) and the possibility of contamination (Hafez &Hafez, 2000).

The semen pH was evaluated using a pH meter (Labchem®, South Africa). On the day before analysis, the pH meter was calibrated. The pH was measured by dipping the probe in the semen and a

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value for each semen sample was recorded. Before and after each analysis of semen sample, the probe was rinsed with distilled water and wiped with a paper towel.

3.10.2. Evaluation of sperm mass and progressive motility

Sperm mass motility was assessed with the aid of a microscope (Olympus, Japan). A 5 µL of semen was placed on the pre-warmed microscope slide (Lasec®, South Africa) and evaluated using a score of 0 to 5 (scale for sperm waves) as it was indicated by Hafez & Hafez (2000). The sperm mass motility was evaluated under × 40 magnification.

Sperm progressive motility was determined by placing 5 µL of saline solution (Onderstepoort Pharmacy, South Africa) on the pre-warmed microscope slide placed on the microscope followed by a drop (5 µL) of semen and covered with a cover slip (Lasec®, South Africa). Then progressive sperm motility was evaluated under × 40 magnifications using a microscope according to Talebi *et al*. (2009).

3.10.3. Slide staining for sperm viability and morphology evaluation

The sperm viability was determined using eosin-nigrosine stain (Onderstepoort Pharmacy, South Africa). A mixture of 5 µL semen and 20 µL of eosin-nigrosine were prepared. Thereafter, a 5 µL of the mixture was placed on a clean warmed microscope slide and smeared using another slide. The slide was allowed to dry thereafter, a drop of immersion oil (Onderstepoort Pharmacy, South Africa) was placed on top of the slide. A cover slip was then placed on top of the slide and examined at × 1000 magnification using a microscope to determine the percentage live and dead sperm by counting a total of 100 sperm across the slide. The sperm that did not absorb stain (appeared as white or clear) were considered as live while those that absorbed stain and appeared pinkish were considered dead (Malejane *et al*., 2014).

The same slide used for analysis of sperm viability was also used to determine sperm morphology. The live spermatozoa were classified into normal and abnormal sperm. Sperm abnormalities were evaluated in two ways. The first method was for the evaluation of abnormalities that occurred at a specific location by observing the head, mid piece and tail of the sperm cell (Bearden *et al*., 2004). Then, the sperm abnormalities were further categorised into primary, secondary and tertiary abnormalities. The primary sperm abnormalities included abnormal acrosomes and elongated mid-piece of the sperm cells. The secondary sperm abnormalities observed were detached and loose heads as well as cytoplasmic droplets (Munyai, 2010). While the tertiary sperm abnormalities included bent and broken tails (Chenoweth & Lorton, 2014).

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3.10.4. Evaluation of sperm concentration

The sperm concentration was determined by counting sperm cells using an improved Neubauer haemocytometer (Lasec®, South Africa). Semen was diluted thoroughly and gently with distilled water (5 mL of water and 25 µL of semen) to kill the sperm cells according to Salisbury *et al*. (1978) and Mitchell & Doak (2004). Then, the diluted sample (10 µL) was loaded into the two sides of the chamber using a micro pipette (Merck, South Africa®) in the V-shape groove of the haemocytometer. The haemocytometer was covered with a coverslip then placed on the microscope (Nikon, Japan) and allowed the contents to settle for 5 min. A phase contrast microscope under a low magnification (× 10) was used. The sperm cells counted were multiplied with the dilution factor (201 for the current study) following the equation: Concentration/mL = (Dilution Factor) (Count in 5 squares) (0.05×10^6) to get the concentration of sperm cells in millions per mL semen (Matthews *et al*., 2003).

3.11. Data analysis

All data collected for pubertal traits, semen parameters and the blood components were analysed using PROC general linear model (GLM) of Statistical Analyses System (SAS, 2012). The analysis of variance (ANOVA) was used to test differences between the treatments. Means ± SE were separated using Duncan's test at a significant level of $P < 0.05$.

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CHAPTER 4

RESULTS

4.1. Selenium concentration in diet

The lucerne sample was tested for Se concentration two times for the current study due to stock purchased. The samples were Se deficient as they had 0.003 mg/kg Se.

4.2. Effect of selenium supplementation on attainment of puberty and reproductive performance of Saanen male kids (Experiment 1)

4.2.1. Body weight of Saanen goat treated with selenium

At the start of the experiment when the animals were 3 months of age, there was no significant difference in body weight between treatment groups. As the age of animals increased, the Se supplemented group continued to be heavier ($P < 0.05$) than the control (Figure 4.5). The average body weight was significantly ($P < 0.001$) higher in the Se supplemented group compared to the control (Table 4.2).

4.2.2. Testicular measurements of Saanen goat treated with selenium

Figure 4.6 presents the scrotal circumference values of Saanen bucks supplemented with Se. After 3 weeks of Se supplementation, there was no significant ($P > 0.05$) difference on scrotal circumference between the groups. The scrotal circumference increased significantly ($P < 0.05$) from the age of 5 months in Se supplemented compared to the control, as bucks continued to grow (Figure 4.6). Se supplemented goats had higher ($P < 0.001$) average scrotal circumference in comparison with control group (Table 4.2). The testicular width and length results are shown in Table 4.1. Results showed that the testicular width increased significantly ($P < 0.05$) from the age of 5.5 months until the end of the experiment, in Se supplemented compared to the control group. The testicular length increased significantly ($P < 0.05$) in Se supplemented from 4.5 months of age until the end of the experiment as compared to the control group. Se supplementation showed a significant ($P < 0.05$) effect on testicular measurements (Table 4.2). Goats supplemented with Se had significantly higher ($P < 0.001$) testicular width and length compared to control bucks.

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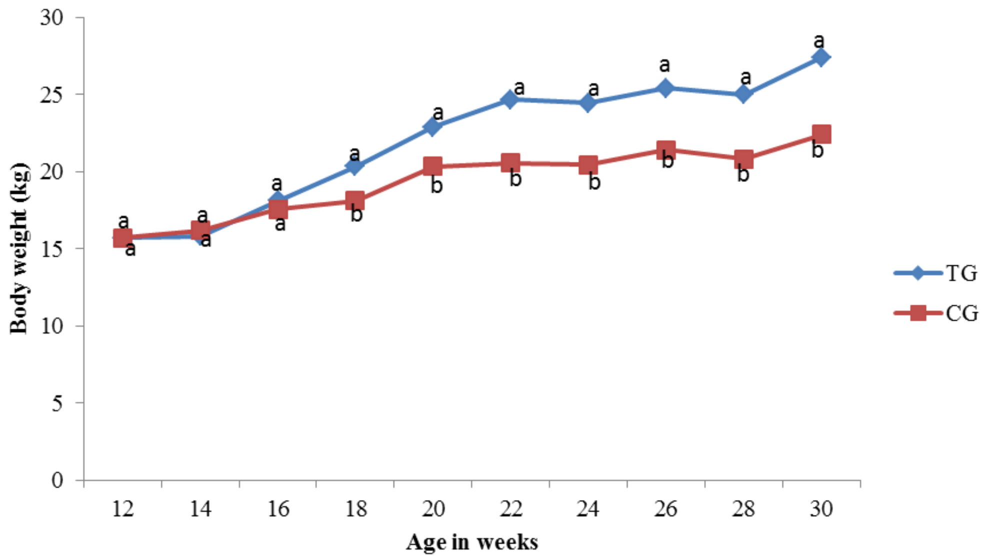

Figure 4.1. Effect of selenium on body weight (mean ± SE) of Saanen kids. Different superscripts differ significantly at P < 0.05. TG = Se treated; CG = Control group

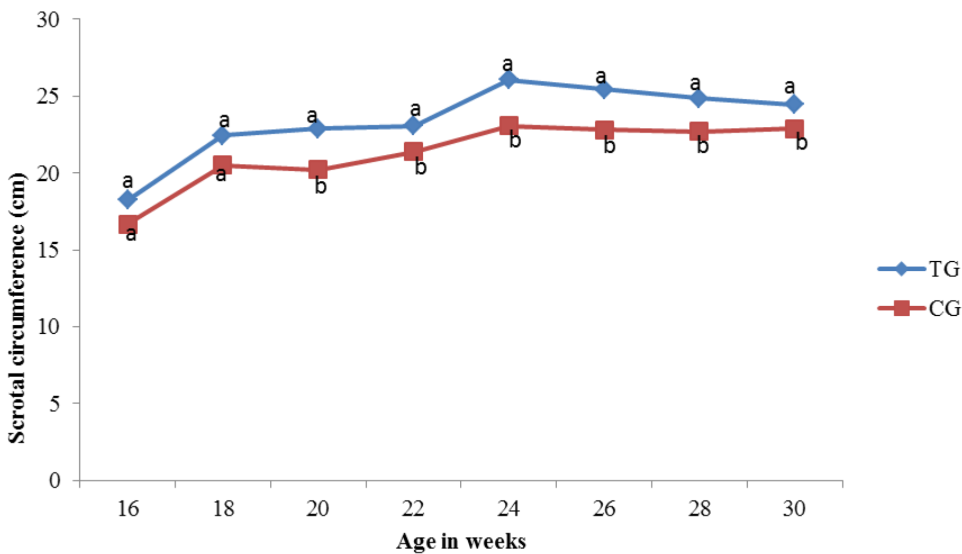

Figure 4.2. Effect of selenium on scrotal circumference (mean ± SE) of Saanen kids. Different superscripts differ significantly at P < 0.05. TG = Selenium treated, CG = Control group

4.2.3. Sexual behaviours of Saanen goat

The sexual behaviours (Table 4.2) particularly, nosing behaviour was observed at the age of 5.5 months in both groups, regardless of treatment and at this time the kids dispalyed interest to females in oestrus. The mounting behaviour in the control group was numerically expressed early (191.25 ± 15. 21 vs 199. 88 ± 37. 60 days) than the Se supplemented group without significant (P > 0.05) difference.

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4.2.4. Macroscopic semen characteristics of pubertal Saanen male goats

The significant increase ($P < 0.05$) in semen volume was recorded at the age of 5.5 months (Figure 4.7). At this age also, the Se supplemented group had the greatest (1.60 ± 0.19 mL) ejaculate volume compared to the control (0.74 ± 0.19 mL). The Se supplemented group had higher ($P < 0.05$) semen volume and continued to increase until the age of 7.5 months (the end of the experiment 1). The average semen volume was significantly ($P < 0.001$) higher in the Se supplemented group compared to the control (Table 4.2).

Figure 4.7 and table 2 shows that, Se supplementation improved ($P < 0.05$) the semen appearance resulting in creamy colour that was observed from the age of 5.5 to 7 months, as well as at the end of experiment.

The pH results presented in Fig 4.7 demonstrated that semen pH of the Se supplemented group differed significantly ($P < 0.05$) from the control group at the age of 5.5 months. For the rest of the experiment as the animals grow there were no significant differences observed.

It was observed that the Se supplemented group showed a significantly ($P < 0.05$) higher sperm concentration compared to the control at the age of 5.5 months and continued to increase at 7 months until the end of the experiment (Figure 4.7 and table 2).

4.2.5. Sperm motility of Saanen goat treated with selenium

Figure 4.8 represents the sperm mass and progressive motility of Saanen bucks treated with Se. Se supplementation showed a significant ($P < 0.05$) positive effect on sperm mass and progressive motility at the age of 5.5 to 6 months and also at the end of the experiment compared to the control. The average sperm mass and progressive motility of Se supplemented was significantly higher ($P < 0.05$) compared to the control.

4.2.6. Sperm viability and morphology of Saanen goat treated with selenium

The overall percentage live sperm differed significantly ($P < 0.001$) between Se and control groups (Table 4.2). It was also observed that the percentage of dead sperm declined as the age increased, regardless of the treatment. Semen from Se supplemented bucks had lower ($P < 0.05$) percentage of dead sperm compared to control group (Table 4.2).

The percentage of normal sperm were significantly ($P < 0.05$) higher in the Se supplemented than the control group (Table 4.2). In both experimental groups, the percentage of abnormal sperm declined with an increase in age. The percentage of abnormal sperm was significantly ($P < 0.05$) lower in the Se supplemented than the control group (Table 4.2). It was observed that the percentage of primary sperm abnormalities of Se treated group significantly ($P < 0.05$) declined throughout the study, compared to the control group. The Se supplemented had lower ($P < 0.05$) percentage of primary

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sperm abnormalities compared to the control (Table 4.2). There were no significant ($P > 0.05$) differences concerning the percentage of secondary sperm abnormalities between the Se supplemented and the control groups (Table 4.2). On the other hand, the percentage of tertiary sperm abnormalities was significantly ($P < 0.05$) lower in the Se supplemented than the control group (Table 4.2).

4.2.7. The concentration of selenium and reproductive hormones

Se concentration in whole blood of Saanen goats was significantly higher ($P < 0.05$) in the Se supplemented compared to the control group (Table 4.2). The Se supplemented group had a higher ($P < 0.05$) LH concentration compared to the control group at the age of 6 and 7.5 months (Figure 4.9).There was a significant ($P < 0.05$) effect of Se supplementation on the testosterone concentration at the age of 5.5 and 6 months (Figure 4.10). The mean overall testosterone was significantly ($P < 0.05$) higher in the Se treated than the control.

4.2.8. Correlations

Body weight was positively correlated to scrotal circumference (0.73) ($P < 0.01$), testicular width and length (0.74) ($P < 0.01$), semen volume (0.50) and semen colour (0.60) ($P < 0.01$). A positive correlation was also recorded between the testicular length and semen volume (0.53) ($P < 0.01$). There was a positive correlation recorded between luteinizing hormone with, testicular width (0.59) ($P < 0.01$) and length (0.59) ($P < 0.01$). There was also a positive correlation between mounting and nosing sexual behaviour (0.70) ($P < 0.05$). A significant positive correlation was noted for semen colour with sperm mass motility (0.51) ($P < 0.05$) and Se concentration (0.67) ($P < 0.05$).

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Table 4.1 Effect of selenium on testicular measurements (mean ± SE) of Saanen kids

Traits	Week 16	Week 18	Week 20	Week 22	Week 24	Week 26	Week 28	Week 30
TW								
TG	6.25 ± 0.66 ^{a}	7.45 ± 0.67 ^{a}	7.50 ± 0.89 ^{a}	8.14 ± 1.00 ^{a}	8.87 ± 0.83 ^{a}	8.58 ± 0.76 ^{a}	9.42 ± 1.07 ^{a}	9.20 ± 0.9 ^{a}
CG	5.50 ± 1.12 ^{a}	6.73 ± 1.28 ^{a}	7.24 ± 1.49 ^{a}	7.39 1.37 ^{b}	7.24 ± 1.41 ^{b}	7.94 ± 1.16 ^{b}	8.46 ± 1.44 ^{b}	8.38 ± 1.29 ^{b}
P-value	0.1011	0.1544	0.6631	0.0231	0.0214	0.0118	0.0313	0.0433
TL								
TG	7.20 ± 0.60 ^{a}	8.51 ± 0.62 ^{a}	8.29 ± 1.00 ^{a}	9.20 ± 1.10 ^{a}	9.83 ± 1.05^{a}	9.53 ± 0.90 ^{a}	10.71 ± 1.40 ^{a}	10.55 ± 1.12 ^{a}
CG	6.70 ± 1.20 ^{a}	7.37 ± 1.13 ^{b}	7.75 ± 1.11 ^{b}	8.59 ± 1.39 ^{b}	8.60 ± 1.09^{b}	8.88 ± 0.97 ^{b}	10.14 ± 1.02 ^{b}	9.58 ± 0.88 ^{b}
P-value	0.2800	0.0175	0.0294	0.0200	0.0135	0.0190	0.0284	0.0402

Different superscripts in the same column per parameter differ significantly at $P < 0.05$. TW = Testicular width, TL = Testicular length, TG = Selenium treated, CG = Control group

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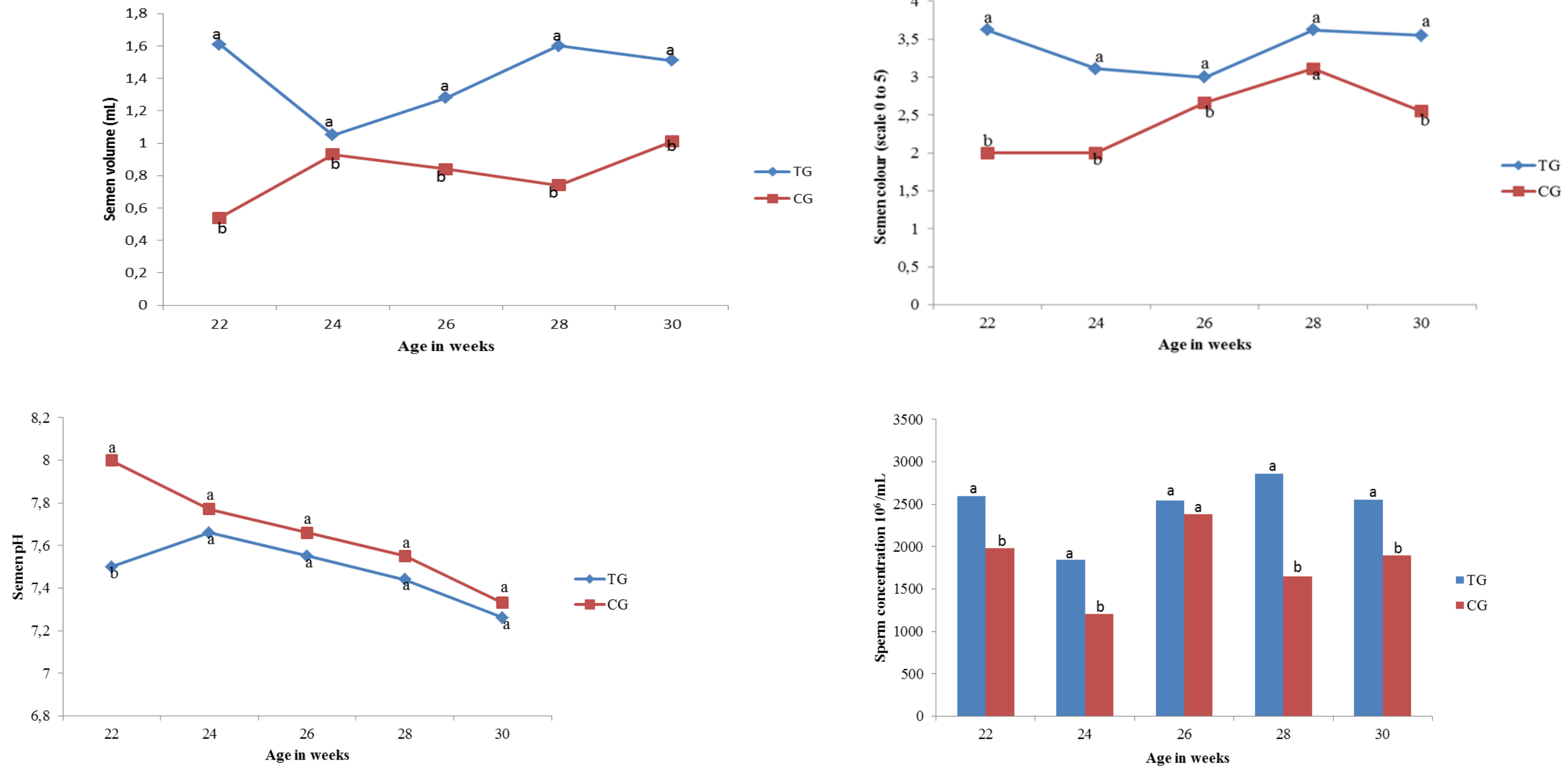

Figure 4.3. Effect of selenium on semen volume, colour, pH and sperm concentration (mean ± SE) observed in Saanen kids. Different superscripts differ significantly at P < 0.05. TG = Se treated, CG = Control

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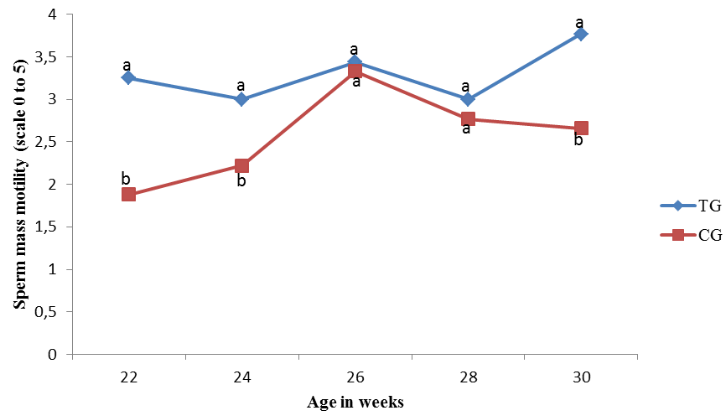

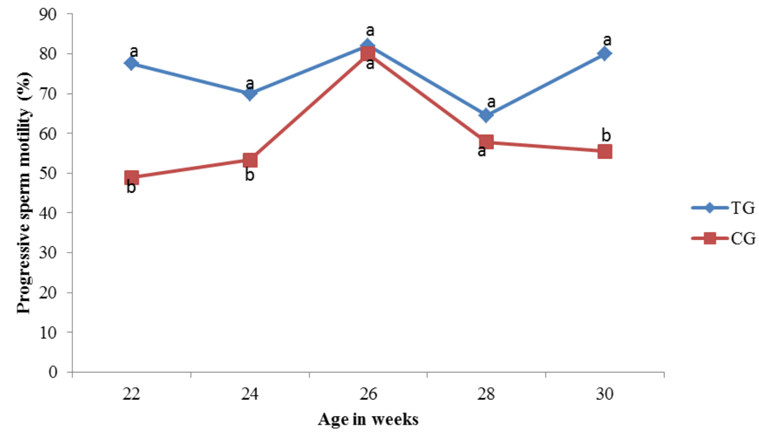

Figure 4.4. Effect of selenium on sperm mass and progressive motility (mean ± SE) observed in Saanen kids. Different superscripts differ significantly at (P < 0.05). TG = Se treated; CG = Control group

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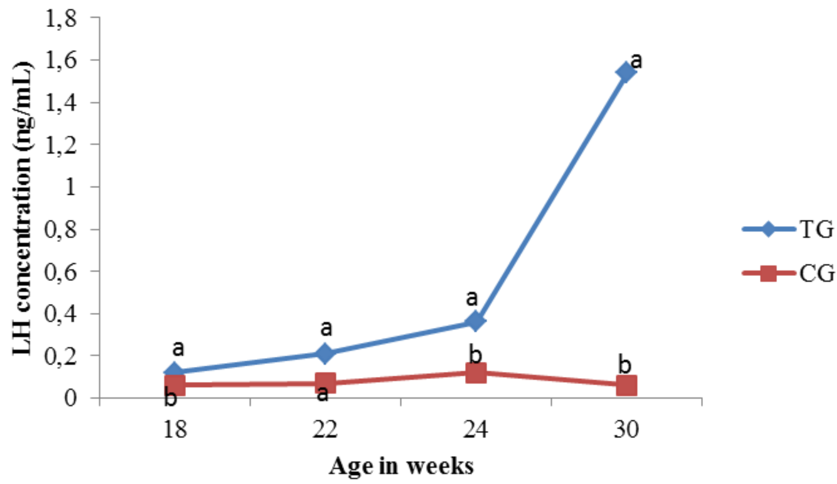

Figure 4.5. Effect of selenium on serum LH concentration (mean ± SE) of Saanen kids. Different superscripts differ significantly at P < 0.05. TG = Se treated; CG = Control group

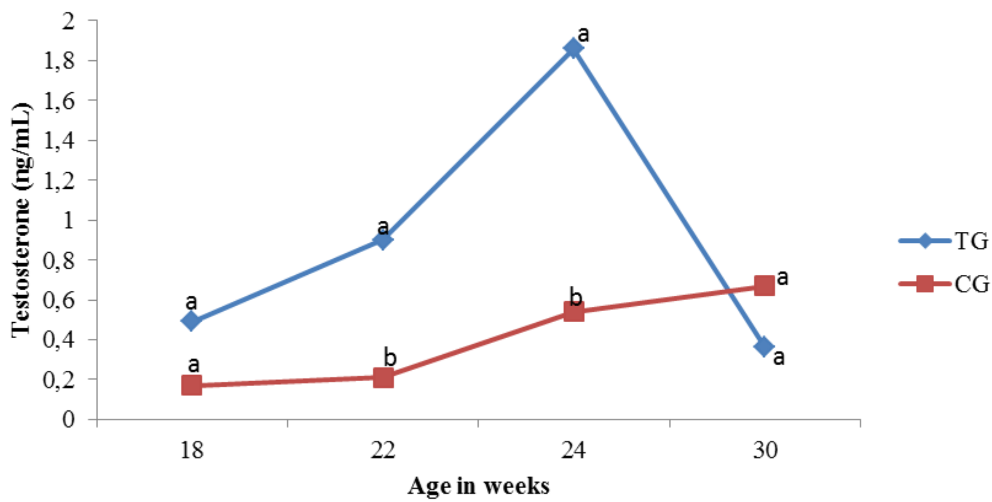

Figure 4.6. Effect of selenium on serum testosterone (mean ± SE) concentrations of Saanen kids. Different superscripts differ significantly at P < 0.05. TG = Se treated; CG = Control group

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Table 4.2 Effect of selenium supplementation on overall means of pubertal traits and semen parameters of Saanen kids (mean ± SE)

Traits	TG	CG	P-value
Body weight (kg)	23.41 ± 4.17^{A}	21.05 ± 4.09^{B}	<.0001
Scrotal circumference (cm)	23.42 ± 3.02^{A}	21.83 ± 3.70^{B}	<.0001
Testicular width (cm)	8.18 ± 1.29^{A}	7.47 ± 1.57^{B}	<.0001
Testicular length (cm)	9.35 ± 1.52^{A}	8.49 ± 1.47^{B}	<.0001
Nosing behaviour (days)	164.77 ± 8.36^{a}	164.33 ± 4.76^{a}	0.8916
Mounting behaviour (days)	199.88 ± 37.60^{a}	191.25 ± 15.21^{a}	0.5356
Semen volume (mL)	1.39 ± 1.17^{A}	0.94 ± 0.52^{B}	<.0001
Semen appearance (scale 0 to 5)	3.36 ± 0.89^{a}	2.46 ± 1.12^{b}	0.0024
Progressive sperm motility (%)	72.72 ± 29.11^{a}	59.11 ± 31.32^{b}	0.0328
Sperm mass motility (scale 0 to 5)	3.29 ± 1.40^{a}	2.57 ± 1.52^{b}	0.0217
pH	7.53 ± 0.48^{a}	7.66 ± 0.43^{a}	0.1402
Sperm concentration (10^{6}/mL)	2176.51 ± 1060.19^{a}	1947.9 ± 1049.52^{b}	0.0028
Live sperm (%)	83.04 ± 12.71^{a}	80.33 ± 12.71^{b}	0.0010
Dead sperm (%)	16.86 ± 12.85^{b}	19.77 ± 10.36^{a}	0.0008
Normal sperm (%)	86.38 ± 11.68^{a}	83.17 ± 13.70^{b}	0.0044
Abnormal sperm (%)	13.72 ± 13.34^{b}	16.83 ± 11.68^{a}	0.0044
Primary sperm abnormalities (%)	7.23 ± 8.63^{b}	8.42 ± 7.08^{a}	0.0249
Secondary sperm abnormalities (%)	8.47 ± 8.45^{a}	7.76 ± 8.28^{a}	0.4894
Tertiary sperm abnormalities (%)	3.31 ± 2.97^{b}	5.34 ± 2.77^{a}	0.0059
Selenium (ng/g)	180.03 ± 108.99^{A}	168.61 ± 138.78^{B}	0.0076
LH (ng/mL)	0.47 ± 0.69^{A}	0.08 ± 0.05^{B}	<.0001
Testosterone (ng/mL)	0.96 ± 1.06^{a}	0.31 ± 0.52^{b}	0.0047

Values with different superscripts in the same row per parameter differ significantly ($^{A, B}$ $P < 0.001$; $^{a, b}$ $P < 0.05$). TG = Se treated; CG = Control group

4.3. Effect of selenium and collection methods on Saanen semen yield and quality (Experiment 2)

4.3.1. Macroscopic semen characteristics of Saanen male goat

The effect of treatment and semen collection methods on semen colour, volume, pH are illustrated in Table 4.3. In Se supplemented group, semen appearance had a tendency of higher ($P = 0.0549$) colour score than the control. Semen collected with AV had creamy ($P < 0.05$) colour score compared to the EE collection method. There was a significant interaction between collection methods and Se

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supplementation, where semen from non-supplemented bucks collected with the EE had significantly ($P < 0.001$) watery appearance than the other groups.

The ejaculate volume was significantly ($P < 0.05$) higher from the Se supplemented group compared to the control group. The largest ($P < 0.001$) ejaculate volume was recorded from the EE than the AV method. No significant difference ($P > 0.05$) was observed in semen collected with the AV between Se supplemented and the control group. The interaction of Se x EE recorded a greater ($P < 0.001$) ejaculate volume compared to the interaction of control x EE. The semen pH did not differ between treatments and semen collection methods. Similarly, there was no significant ($P > 0.05$) difference recorded for the interaction between treatments and semen collection methods concerning pH.

4.3.2. Sperm motility and concentration of Saanen goat treated with selenium

The effect of Se treatment and semen collection methods on sperm motility and concentration is presented in Table 4.3. The highest ($P < 0.05$) sperm mass and progressive motility was recorded from the Se supplemented group as compared to the control. However, there was no significant difference between AV and EE techniques regarding both mass and progressive sperm motilities. The Se supplemented group had a tendency to increase ($P = 0.0895$) the sperm concentration. There was no significant ($P > 0.05$) difference recorded for the interaction between treatments and semen collection methods, concerning sperm concentration.

4.3.3. Sperm viability and morphology of the Saanen goat treated with selenium

In Table 4.4, the viability and morphology of the spermatozoa of Saanen goat are represented. The dead and abnormal sperm were significantly lower ($P < 0.001$) in the Se supplemented than the control. There were no significant ($P > 0.05$) differences concerning dead and abnormal sperm between the semen collection methods.

4.3.4. Morphological sperm abnormalities of Saanen goat treated with selenium

Table 4.5 shows the morphological sperm abnormalities of the Saanen goat. Se supplementation significantly ($P < 0.05$) lowered the percentage of primary sperm abnormalities. There were no significant differences in the percentage of primary sperm abnormalities between AV and EE semen collection methods. A significant ($P < 0.05$) difference regarding percentage of secondary sperm abnormalities were observed between Se supplemented and control groups. There was no significant ($P > 0.05$) difference in percentage of secondary sperm abnormalities between the AV and EE semen collection methods. The semen collected using the EE method from Se supplemented group had lower ($P < 0.05$) percentage of secondary sperm abnormalities. There was no significant ($P > 0.05$) difference concerning percentage of tertiary sperm abnormalities between Se supplemented and control groups. However, the EE semen collection method resulted in lower ($P < 0.05$) percentage of tertiary sperm abnormalities than the AV technique.

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Table 4.3 Effect of selenium on semen parameters of Saanen goats following semen collection with artificial vagina or electro ejaculator (mean ± SE)

Treatments	SAP (0-5 scale)	Volume (mL)	pH	MM (0- 5 scale)	PM (%)	SC ($\times 10^6$/ mL)
Selenium	3.72 ± 0.17[a]	1.19 ± 0.10[A]	7.25 ± 0.06[a]	3.63 ± 0.16[a]	73.88 ± 3.02[a]	2632.08 ± 386.27[a]
Control	3.24 ± 0.17[a]	0.83 ± 0.10[B]	7.25 ± 0.06[a]	3.08 ± 0.15[b]	62.97 ± 2.98[b]	1697.95 ± 381.01[a]
P-value	0.0549	0.0157	0.9388	0.0167	0.0124	0.0895
AV	3.87 ± 0.17[a]	0.63 ± 0.09[B]	7.24 ± 0.06[a]	3.57 ± 0.17[a]	71.51 ± 3.26[a]	2071.09 ± 411.54[a]
EE	3.15 ± 0.15[b]	1.32 ± 0.08[A]	7.26 ± 0.06[a]	3.17 ± 0.15[a]	65.75 ± 2.96[a]	2230.83 ± 373.80[a]
P-value	0.0031	<.0001	0.8735	0.0901	0.1956	0.7747
Se x AV	3.93 ± 0.19[a]	0.65 ± 0.04[C]	7.27 ± 0.74[a]	3.93 ± 0.11[a]	78.75 ± 2.21[a]	2440.14 ± 586.91[a]
Se x EE	3.55 ± 0.31[a]	1.62 ± 0.16[A]	7.27 ± 0.09[a]	3.40 ± 0.30[ba]	70.00 ± 5.28[a]	2785.63 ± 524.95[a]
Control x AV	3.82 ± 0.19[a]	0.61 ± 0.08[C]	7.22 ± 0.11[a]	3.23 ± 0.16[b]	64.70 ± 2.72[b]	1723.74 ± 569.39.[a]
Control x EE	2.75 ± 0.16[b]	1.02 ± 0.10[B]	7.28 ± 0.08[a]	2.95 ± 0.22[b]	61.50 ± 4.82[b]	1676.03 ± 524.95[a]
P-value	0.0017	<0.001	0.9598	0.0271	0.0367	0.3855

Values with different superscripts in the same column per parameter differ significantly ([A, B, C] $P < 0.001$; [a, b] $P < 0.05$). AV = Artificial vagina, EE = Electro-ejaculator, SAP = Semen appearance, MM = Sperm mass motility, PM = Progressive sperm motility, SC = Sperm concentration

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There was a significant (P < 0.05) interaction between semen collection methods and Se supplementation regarding percentage of tertiary sperm abnormalities, where semen collected with the EE from the control had higher (P < 0.05) percentage of tertiary sperm abnormalities.

Table 4.4 Effect of selenium on spermatozoa viability and morphology of Saanen goats following semen collection with artificial vagina or electro-ejaculator (mean ± SE)

Treatments	DS (%)	LS (%)	AS (%)	NS (%)
Selenium	14.30 ± 1.21^{B}	85.55 ± 1.19^{A}	13.63 ± 1.44^{b}	86.36 ± 1.44^{a}
Control	21.78 ± 1.19^{A}	77.94 ± 1.17^{B}	21.89 ± 1.42^{a}	77.83 ± 1.42^{b}
P-value	<.0001	<.0001	0.0001	<.0001
AV	16.78 ± 1.41^{a}	83.06 ± 1.39^{a}	18.21 ± 1.67^{a}	81.48 ± 1.68^{a}
EE	19.17 ± 1.28^{a}	80.57 ± 1.26^{a}	17.50 ± 1.51^{a}	82.50 ± 1.52^{a}
P-value	0.2147	0.1923	0.7532	0.6564
Se x AV	13.00 ± 0.95^{b}	86.68 ± 0.94^{a}	14.62 ± 2.18^{b}	85.37 ± 2.18^{a}
Se x EE	15.35 ± 1.78^{b}	84.65 ± 1.78^{a}	12.85 ± 1.95^{b}	87.15 ± 1.95^{a}
Control x AV	20.35 ± 1.05^{a}	79.64 ± 1.05^{b}	21.58 ± 2.11^{a}	77.82 ± 2.12^{b}
Control x EE	23.00 ± 2.24^{a}	76.50 ± 2.16^{b}	22.15 ± 1.95^{a}	77.85 ± 1.95^{b}
P-value	0.0003	0.0002	0.0018	0.0012

Values with different superscripts in the same column per parameter differ significantly ($^{A, B, C}$ P < 0.001; $^{a, b}$ P < 0.05). AV = Artificial vagina, EE = Electro-ejaculator, DS = Dead sperm, LS = Live sperm, AS = Abnormal sperm, NS = Normal sperm

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Table 4.5 Effect of selenium on morphological abnormalities of spermatozoa following semen collection with artificial vagina or electro-ejaculator in Saanen goats (mean ± SE)

Treatments	PA (%)	SA (%)	TA (%)
Selenium	5.72 ± 1.58[b]	3.80 ± 0.86[b]	3.91 ± 0.74[a]
Control	11.32 ± 1.56[a]	7.05 ± 0.85[a]	4.16 ± 0.73[a]
P-value	0.0142	0.0092	0.8156
AV	7.27 ± 1.71[a]	6.36 ± 0.93[a]	5.18 ± 0.75[a]
EE	9.62 ± 1.55[a]	4.70 ± 0.84[a]	3.10 ± 0.68[b]
P-value	0.3139	0.1922	0.0460
Se x AV	5.00 ± 0.93[b]	5.37 ± 1.17[a]	3.87 ± .81[ba]
Se x EE	6.30 ± 1.75[ba]	2.55 ± 0.75[b]	3.95 ± 1.23[ba]
Control x AV	9.41 ± 1.96[ba]	7.29 ± 1.25[a]	6.41 ± 0 .92[a]
Control x EE	12.95 ± 3.19[a]	6.85 ± 1.15[a]	2.25 ± 0.88[b]
P-value	0.0417	0.0246	0.0405

Values with different superscripts in the same column per parameter differ significantly ([a, b, ba] $P < 0.05$). AV = Artificial vagina, EE = Electro-ejaculator, PA = Primary sperm abnormalities, SA = Secondary sperm abnormalities, TA = Tertiary sperm abnormalities

4.3.5. The concentration of selenium and reproductive hormones of Saanen bucks

Table 4.6 shows Se and reproductive hormonal concentration of Saanen goats following Se supplementation. The Se supplemented bucks had a tendency ($P = 0.0801$) of higher testosterone concentration. There was no significant ($P > 0.05$) difference recorded for the interaction between treatments and semen collection methods for testosterone concentration. LH concentration was not affected by Se supplementation and semen collection methods. Se treated bucks had higher ($P < 0.05$) Se level compared to the control group. Furthermore, concerning the semen collection methods, bucks collected with the AV had significantly higher ($P < 0.05$) Se level compared to those collected with the EE technique. Regardless of semen collection method, the control group had lower Se concentration.

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Table 4.6 Selenium and reproductive hormonal concentrations of Saanen goats supplemented with selenium (mean ± SE)

Treatments	SE (ng/g)	TE (ng/mL)	LH (ng/mL)
Se supplemented	49.61 ± 3.55[a]	0.75 ± 0.08[a]	0.81 ± 0.12[a]
Control	28.21 ± 3.51[b]	0.53 ± 0.08[a]	0.87 ± 0.12[a]
P-value	0.0016	0.0801	0.7340
AV	40.06 ± 3.94[a]	0.69 ± 0.09[a]	0.90 ± 0.13[a]
EE	33.18 ± 3.58[b]	0.59 ± 0.08[a]	0.80 ± 0.12[a]
P-value	0.0008	0.4390	0.5638
Se x AV	49.02 ± 6.30[a]	0.88 ± 0.15[a]	0.81 ± 0.15[a]
Se x EE	41.08 ± 2.03[ba]	0.75 ± 0.17[a]	0.69 ± 0.13[a]
Control x AV	31.64 ± 7.61[bc]	0.91 ± 0.20[a]	0.57 ± 0.05[a]
Control x EE	25.29 ± 3.46[c]	0.84 ± 0.18[a]	0.49 ± 0.12[a]
P-value	0.0080	0.9275	0.2940

Values with different superscripts in the same column per parameter differ significantly ([a, b, ba, bc,c] $P < 0.001$; $P < 0.05$). AV = Artificial vagina, EE = Electro-ejaculator. LH = Luteinizing hormone, TE = Testosterone

The semen appearance was significantly and positively correlated to sperm progressive motility (0.57) ($P < 0.01$). Sperm mass motility was significantly and positively (0.80) ($P < 0.01$) correlated to sperm progressive motility.

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4.4. Effect of selenium supplementation and induced stress on reproductive hormones and semen quality (Experiment 3)

4.4.1. Macroscopic semen characteristics of Saanen goat following selenium supplementation and induced stress

The results for effect of Se supplementation on induced stress and semen collection methods on semen appearance, semen volume and semen pH are illustrated in Table 4.7. In Se supplemented group, the semen appearance was thinly creamy ($P < 0.001$) compared to the Se + ACTH, ACTH and control groups. However, in Se + ACTH group, semen appearance was more ($P < 0.001$) creamy compared to the control and ACTH groups. There were no significant ($P > 0.05$) differences in semen appearance between ACTH and control groups. There were also no significant ($P > 0.05$) differences on semen appearance between the AV and EE semen collection methods.

The mean ejaculate volume was significantly ($P < 0.05$) higher from the Se, Se + ACTH and ACTH groups than the control. There were no significant ($P > 0.05$) differences between Se + ACTH and ACTH groups for the ejaculate volume. The ejaculate volume was greater ($P < 0.001$) when semen was collected with EE compared to the AV method of semen collection. The interaction of Se x EE recorded a higher ($P < 0.05$) ejaculate volume compared to the interaction of control x EE and ACTH x EE. While the interaction of Se x ACTH x AV and Se x AV had higher ($P < 0.001$) ejaculate volume compared to ACTH x AV and control x AV.

The semen pH was approximately neutral (7.05 ± 0.05) ($P < 0.001$) in the Se supplemented group compared to other groups. There were no significant ($P > 0.05$) differences on semen pH between Se + ACTH, ACTH and control groups. There were no significant ($P > 0.05$) differences recorded for semen pH between the semen collection methods. There was also no significant ($P > 0.05$) interaction between collection methods and treatments.

4.4.2. Sperm motility and concentration of Saanen goat following selenium supplementation and induced stress

In Table 4.7, the effect of Se supplementation, induced stress and semen collection methods on sperm motility are presented. The highest ($P < 0.001$) sperm mass and progressive motility was recorded in the Se supplemented group compared to Se + ACTH, ACTH and control groups. From the Se + ACTH group, the sperm mass and progressive motility was higher ($P < 0.001$) and remained lower in the ACTH and control groups. On the other hand, the sperm mass and progressive motility was significantly lower ($P > 0.05$) in the ACTH than the control group. No significant ($P > 0.05$) difference in sperm mass and progressive motility was recorded between the semen collection methods. The interaction of ACTH x AV and control x AV had significantly ($P < 0.001$) lower mass

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and progressive motility compared to the Se + ACTH and Se treatments when semen was collected using AV technique.

The highest ($P < 0.001$) sperm concentration was recorded in Se supplemented compared to Se + ACTH, ACTH and control groups. ACTH reduced the sperm concentration but where Se was supplemented the sperm concentration was higher ($P < 0.001$). The sperm concentration was reduced due to ACTH administration as the sperm concentration was lower than in control. Ejaculates collected using AV semen collection method had greater ($P < 0.001$) sperm concentration compared to those collected by EE technique. The interaction of between Se x AV resulted in higher ($P < 0.001$) sperm concentration than the interaction of other groups when semen was collected using AV. The interaction of Se + ACTH x AV also had greater ($P < 0.001$) sperm concentration than the interaction of ACTH and control when AV technique was used. However, in the interaction of control x AV the sperm concentration was higher ($P < 0.05$) compared to the interaction of ACTH x AV.

4.4.3. Sperm viability and morphology of Saanen goat following selenium supplementation and induced stress

Table 4.8 presents the results of the effect of Se supplementation, induced stress and semen collection methods on the viability and morphology of spermatozoa. The highest ($P < 0.001$) percentage of live sperm was recorded in Se and Se + ACTH than the other groups. However, there were no significant ($P > 0.05$) differences concerning the percentage of live and dead sperm between the ACTH treated and control groups. There were also no significant ($P > 0.05$) differences in the percentage of live sperm between AV and EE semen collection methods. The interaction of Se x AV and Se x EE, Se + ACTH x AV and Se + ACTH x EE resulted in higher ($P < 0.001$) percentage of live sperm compared to ACTH x AV, ACTH x EE, control x AV and control x EE. However, the control x AV had significantly higher ($P < 0.001$) percentage of live sperm as compared to the ACTH x AV group.

The percentage of abnormal sperm was lower ($P < 0.001$) in the Se supplemented group compared to the other groups. Similarly, from Se + ACTH, the percentage of abnormal sperm was also lower ($P < 0.001$) than those in the ACTH and control groups. There were no significant ($P > 0.05$) differences concerning percentage of abnormal sperm between the ACTH and control groups. There were no significant ($P > 0.05$) differences in the percentage of abnormal sperm between AV and EE semen collection methods. The interaction of Se x AV led to lower ($P < 0.001$) percentage of abnormal sperm compared to control x AV and ACTH x AV. Also, the interaction of Se + ACTH x AV led to significantly lower percentage abnormal sperm ($P < 0.001$) compared to control x AV and ACTH x AV. It was observed that Se x EE and Se + ACTH x EE led to lower ($P < 0.001$) percentage of abnormal sperm compared to ACTH x EE and control x EE.

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Table 4.7 Effect of selenium supplementation and induced stress on semen parameters of Saanen bucks collected with artificial vagina or electro- ejaculator (mean ± SE)

Treatments	SAP (0-5 scale)	Volume (mL)	pH	MM (0- 5 scale)	PM (%)	SC (× 10^6/ mL)
Se + ACTH	3.90 ± 0.09^{B}	1.15 ± 0.08^{ba}	6.74 ± 0.05^{b}	4.16 ± 0.09^{B}	81.20 ± 1.67^{B}	2617.64 ± 73.60^{B}
Se	4.34 ± 0.09^{A}	1.30 ± 0.08^{a}	7.05 ± 0.05^{a}	4.66 ± 0.09^{A}	88.40 ± 1.67^{A}	3143.51 ± 73.60^{A}
ACTH	2.60 ± 0.13^{C}	1.10 ± 0.12^{ba}	6.83 ± 0.07^{b}	2.42 ± 0.09^{C}	50.40 ± 1.67^{D}	1683.64 ± 104.0^{D}
Control	2.60 ± 0.13$_{C}$	0.92 ± 0.08^{c}	6.76 ± 0.05^{b}	2.72 ± 0.13^{D}	64.80 ± 2.37^{C}	1971.02 ± 73.60^{C}
P-Value	**<.0001**	**0.0224**	**0.0004**	**<.0001**	**<.0001**	**<.0001**
AV	3.38 ± 0.10^{a}	0.60 ± 0.04^{B}	6.79 ± 0.04^{a}	3.45 ± 0.11^{a}	67.00 ± 2.02^{a}	2607.62 ± 69.50^{A}
EE	3.37 ± 0.10^{a}	1.45 ± 0.04^{A}	6.85 ± 0.04^{a}	3.48 ± 0.11^{a}	72.00 ± 2.02^{a}	2175.36 ± 69.50^{B}
P-Value	**0.9446**	**<.0001**	**0.3096**	**0.8575**	**0.0822**	**<.0001**
Se + ACTH x AV	3.75 ± 0.19^{B}	0.69 ± 0.09^{C}	6.79 ± 0.11^{ba}	4.16 ± 0.23^{A}	85.00 ± 3.86^{A}	2894.85 ± 138.16^{B}
Se + ACTH x EE	3.80 ± 0.17^{B}	1.50 ± 0.08^{BA}	6.85 ± 0.10^{ba}	4.13 ± 0.20^{A}	82.66 ± 3.45^{A}	2459.73 ± 123.58^{CD}
ACTH x AV	2.25 ± 0.19^{D}	0.34 ± 0.09^{E}	6.70 ± 0.11^{c}	2.25 ± 0.23^{C}	50.00 ± 3.86^{C}	2000.45 ± 138.16^{EF}
ACTH x EE	2.60 ± 0.17^{DC}	0.96 ± 0.08^{C}	6.91 ± 0.10^{ba}	3.13 ± 0.20^{B}	68.00 ± 3.45^{C}	1695.96 ± 123.58^{F}
Se x AV	4.75 ± 0.24^{A}	0.85 ± 0.12^{C}	7.03 ± 0.14^{ba}	4.62 ± 0.28^{A}	92.50 ± 4.73^{A}	3528.28 ± 169.21^{A}
Se x EE	4.30 ± 0.21^{BA}	1.71 ± 0.10^{A}	7.12 ± 0.13^{a}	4.50 ± 0.25^{A}	88.00 ± 4.23^{A}	2858.24 ± 151.35^{BC}
Control x AV	2.87 ± 0.24^{C}	0.43 ± 0.12^{DE}	6.76 ± 0.14^{ab}	2.50 ± 0.28^{CB}	45.00 ± 4.73^{C}	2269.48 ± 169.21^{CD}
Control x EE	3.00 ± 0.21^{C}	1.26 ± 0.10^{B}	6.74 ± 0.13^{ab}	2.40 ± 0.25^{CB}	52.00 ± 4.23^{C}	1689.80 ± 151.35^{F}
P-Value	**<.0001**	**<.0001**	**0.2670**	**<.0001**	**<.0001**	**<.0001**

Values with different superscripts in the same column differ significantly ($^{A, B, C, BA, C, D, DA, E, F, F}$ $P < 0.001$; $^{a, ab, b, ba, c}$, $P < 0.05$). AV = Artificial vagina, EE = Electro-ejaculator, SAP = Semen appearance, MM = Mass motility, PM = Progressive motility, SC = sperm concentration

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4.4.4. Morphological sperm abnormalities of Saanen goat following selenium supplementation and induced stress

Table 4.9 shows morphological sperm abnormalities of Saanen goats treated with Se and ACTH. The percentage of primary sperm abnormalities were lower ($P < 0.05$) in the Se supplemented and Se + ACTH than the ACTH and control groups. The percentage of primary sperm abnormalities were notably increased ($P < 0.05$) in the ACTH compared to the control group. Collecting semen with AV and EE technique did not influence ($P > 0.05$) the percentage of primary sperm abnormalities. There were no significant ($P > 0.05$) differences recorded for the interaction of the treatments and the semen collection methods concerning the percentage of primary sperm abnormalities.

The percentage of secondary sperm abnormalities were lower ($P < 0.05$) in the Se and Se + ACTH than ACTH and control groups. From the ACTH group, the percentage of secondary sperm abnormalities was higher ($P < 0.05$) than from of the control group. Both semen collection methods had no effect ($P > 0.05$) on the percentage of secondary sperm abnormalities. Regarding the interaction of both treatments and semen collection methods, the percentage of secondary sperm abnormalities decreased ($P < 0.001$) in the Se supplemented and Se + ACTH treated compared to the ACTH and control groups

The lowest ($P < 0.05$) percentage tertiary sperm abnormalities were recorded in the Se supplemented than the Se + ACTH, ACTH and control groups. There were no significant ($P > 0.05$) differences in percentage tertiary sperm abnormalities between the ACTH and control groups. There were no significant ($P > 0.05$) differences observed between the semen collection techniques in the percentage of tertiary sperm abnormalities. The percentage of tertiary sperm abnormalities decreased in the Se supplemented and Se + ACTH treated compared to the ACTH and control groups when both collection methods were used.

4.4.5. The concentration of selenium, reproductive hormones, glutathione peroxidase and cortisol of Saanen bucks

The blood Se concentration of the Saanen bucks following Se supplementation and induced stress is presented in Table 4.10. The Se concentration was greater ($P < 0.001$) in the Se and Se + ACTH than the ACTH and the control groups. There were no significant ($P > 0.05$) differences in the Se concentration between AV and EE semen collection methods.

Blood samples withdrawn from Se supplemented group, for cortisol and reproductive hormones were destroyed and the data was excluded from analysis. In Table 4.10, the levels of serum testosterone of the bucks are presented. The mean serum testosterone was highest ($P < 0.001$) in the Se supplemented compared to the Se + ACTH, ACTH and the control groups. Se + ACTH also had higher ($P < 0.001$)

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testosterone level than the ACTH and the control groups. There were no significant (P > 0.05) differences in the serum testosterone level between AV and EE semen collection methods.

The effect of ACTH and Se supplementation on serum testosterone levels is presented in Figure 4.7. Regardless of induced stress, it was observed that Se supplemented group had higher (P < 0.001) serum testosterone concentration at 1 h following ACTH administration and continued to be higher until the end of evaluation at 24 h, compared to testosterone level in the ACTH and control groups. There was no significant (P > 0.05) difference in the blood serum testosterone at 1, 8, 16 and 24 h following ACTH treatment. It was observed that the control group had lower (P > 0.05) testosterone level throughout the experiment.

The mean serum of LH was higher (P < 0.001) in the Se supplemented than the Se + ACTH, ACTH and control groups (Table 4.10). Se + ACTH group had a greater (P < 0.001) LH than the ACTH and control groups. There were no significant (P > 0.05) differences on serum LH levels between the two semen collection methods. In figure 4.8, the effect of induced stress on LH levels of Saanen bucks is presented. Se + ACTH treatment led to higher (P < 0.05) LH levels throughout the experiment compared to the ACTH and the control groups. ACTH and the control groups had similar (P > 0.05) LH concentration throughout the experiment.

The GSH-Px was notably higher (P < 0.001) from the Se and Se + ACTH and was lower in the ACTH and control groups (Table 4.10). There were no significant (P > 0.05) differences in the GSH-Px between the semen collection methods. There were no significant (P > 0.05) differences in the interaction of the treatments and the semen collection methods concerning GSH-Px.

Effect of ACTH on cortisol levels is illustrated in Figure 4.9. The lowest (P < 0.001) cortisol level was obtained in the bucks that received Se + ACTH compared to ACTH and control groups throughout the study. There were no significant (P > 0.05) differences in cortisol level between the ACTH and control groups at 1 and 8 h. At 24 h following ACTH administration, the cortisol level declined (P < 0.05) in the ACTH treated and remained higher in the control. Regarding the semen collection methods, no significant differences were recorded for the cortisol level.

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Table 4.8 Effect of selenium supplementation, induced stress and semen collection methods on sperm viability and morphology of Saanen goats (mean ± SE)

Treatments	DS (%)	LS (%)	AS (%)	NS (%)
Se	6.82 ± 0.78^{C}	93.14 ± 0.83^{A}	6.42 ± 0.62^{C}	93.58 ± 0.62^{A}
Se + ACTH	8.58 ± 0.78^{C}	91.22 ± 0.83^{A}	9.12 ± 0.62^{B}	90.78 ± 0.62^{B}
ACTH	35.28 ± 1.10^{A}	65.12 ± 1.18^{C}	27.44 ± 0.88^{A}	72.96 ± 0.88^{C}
Control	29.36 ± 0.78^{B}	71.36 ± 0.83^{B}	27.06 ± 0.62^{A}	72.42 ± 0.62^{C}
P-Value	**<.0001**	**<.0001**	**<.0001**	**<.0001**
AV	17.79 ± 1.29^{a}	82.67 ± 1.28^{a}	16.01 ± 1.07^{a}	83.68 ± 1.08^{a}
EE	20.65 ± 1.29^{a}	79.23 ± 1.28^{a}	18.92 ± 1.07^{a}	81.18 ± 1.08^{a}
P-Value	**0.1201**	**0.0593**	**0.0574**	**0.1042**
Se + ACTH x AV	8.33 ± 1.54^{C}	91.66 ± 1.58^{A}	8.66 ± 1.13^{D}	91.33 ± 1.21^{BA}
Se + ACTH x EE	8.93 ± 1.37^{C}	90.40 ± 1.41^{A}	9.86 ± 1.01^{D}	90.13 ± 1.08^{B}
Se x AV	6.75 ± 1.88^{C}	93.25 ± 1.94^{A}	4.87 ± 1.39^{E}	95.12 ± 1.49^{A}
Se x EE	5.80 ± 1.68^{C}	94.20 ± 1.73^{A}	6.30 ± 1.24^{DE}	93.70 ± 1.33^{BA}
ACTH x AV	31.41 ± 1.54^{A}	68.58 ± 1.58^{C}	28.66 ± 1.13^{BA}	71.33 ± 1.21^{D}
ACTH x EE	34.80 ± 1.37^{A}	65.20 ± 1.41^{C}	26.93 ± 1.01^{B}	73.06 ± 1.08^{DE}
Control x AV	24.62 ± 1.88^{B}	76.62 ± 1.94^{B}	22.00 ± 1.39^{C}	76.75 ± 1.49^{C}
Control x EE	33.70 ± 1.68^{A}	66.30 ± 1.73^{C}	31.80 ± 1.24^{A}	68.20 ± 1.33^{E}
P-Value	**<.0001**	**<.0001**	**<.0001**	**<.0001**

Values with different superscripts in the same column differ significantly ($^{A, B, BA, C, D, DE, E}$ $P < 0.001$; $^{a, b}$ $P < 0.05$). DS = Dead sperm, LS = Live sperm, AS = Abnormal sperm, NS = Normal sperm

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Table 4.9 Effect of selenium supplementation, induced stress and semen collection methods on morphological sperm abnormalities of Saanen bucks (mean ± SE)

Treatments	PA (%)	SA (%)	TA (%)
Se	3.46 ± 0.30^{C}	2.50 ± 0.25^{C}	1.94 ± 0.32^{C}
Se + ACTH	3.78 ± 0.30^{C}	3.24 ± 0.25^{C}	4.02 ± 0.32^{B}
ACTH	12.16 ± 0.43^{A}	13.08 ± 0.35^{A}	11.48 ± 0.46^{A}
Control	8.62 ± 0.30^{B}	12.28 ± 0.25^{B}	10.76 ± 0.32^{A}
P-Value	**<.0001**	**<.0001**	**<.0001**
AV	7.17 ± 0.44^{a}	7.32 ± 0.50^{a}	7.58 ± 0.46^{a}
EE	7.06 ± 0.44^{a}	7.72 ± 0.50^{a}	6.39 ± 0.46^{a}
P-Value	**0.8604**	**0.5763**	**0.0711**
Se + ACTH x AV	3.75 ± 0.67^{C}	3.33 ± 0.59^{B}	4.41 ± 0.50^{C}
Se + ACTH x EE	4.20 ± 0.60^{C}	3.33 ± 0.53^{B}	3.73 ± 0.45^{CD}
Se x AV	3.25 ± 0.82^{C}	2.00 ± 0.72^{B}	2.62 ± 0.62^{D}
Se x EE	3.40 ± 0.73^{C}	2.60 ± 0.65^{B}	0.60 ± 0.55^{E}
ACTH x AV	13.25 ± 0.67^{A}	11.25 ± 0.59^{A}	11.50 ± 0.50^{A}
ACTH x EE	12.33 ± 0.60^{A}	13.13 ± 0.53^{A}	11.80 ± 0.45^{A}
Control x AV	8.75 ± 0.82^{B}	12.75 ± 0.72^{A}	12.62 ± 0.62^{A}
Control x EE	10.00 ± 0.73^{B}	11.80 ± 0.65^{A}	8.80 ± 0.55^{B}
P-Value	**<.0001**	**<.0001**	**<.0001**

Values with different superscripts in the same column differ significantly ($^{A, B, C, CD, D}$ $P < 0.001$; $^{a, b}$ $P < 0.05$). AV = Artificial vagina, EE = Electro-ejaculator, PA = Primary abnormalities, SA = Secondary abnormalities, TA = Tertiary abnormalities

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Table 4.10 Effect of selenium supplementation and induced stress on the concentration of selenium, reproductive hormones, glutathione peroxidase and cortisol of Saanen bucks (mean ± SE)

Treatments	Se (ng/g)	Te (ng/mL)	LH (ng/mL)	GSH-Px (U/mL)	Cortisol (ng/mL)
Se	94.05 ± 4.98^{A}	3.47 ± 0.05^{A}	2.34 ± 0.04^{A}	5.96 ± 0.14^{A}	
Se + ACTH	86.09 ± 4.98^{A}	2.35 ± 0.05^{B}	1.40 ± 0.04^{B}	5.78 ± 0.14^{A}	1.30 ± 0.12^{C}
ACTH	16.64 ± 4.98^{B}	0.94 ± 0.05^{C}	0.61 ± 0.04^{C}	2.27 ± 0.14^{B}	5.52 ± 0.13^{A}
Control	11.41 ± 4.98^{B}	0.96 ± 0.05^{C}	0.56 ± 0.04^{C}	2.43 ± 0.16^{B}	4.97 ± 0.12^{B}
P-Value	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
AV	47.92 ± 6.80^{a}	1.83 ± 0.16^{a}	1.18 ± 0.11^{a}	3.28 ± 0.50^{a}	4.00 ± 0.57^{a}
EE	55.43 ± 6.15^{a}	2.01 ± 0.14^{a}	1.26 ± 0.10^{a}	3.79 ± 0.43^{a}	3.68 ± 0.24^{a}
P-Value	**0.4146**	**0.4008**	**0.6041**	**0.4468**	**0.6758**
Se x EE	93.94 ± 6.51^{A}	3.57 ± 0.05^{A}	2.19 ± 0.05^{B}	5.78 ± 0.22^{B}	
Se x AV	94.23 ± 7.98^{A}	3.32 ± 0.06^{B}	2.55 ± 0.07^{A}	5.84 ± 0.18^{B}	
Se + ACTH x AV	81.36 ± 7.98^{A}	2.14 ± 0.06^{D}	1.46 ± 0.07^{C}	5.63 ± 0.22^{A}	1.23 ± 0.19^{C}
Se + ACTH x EE	89.24 ± 6.51^{A}	2.49 ± 0.05^{D}	1.35 ± 0.05^{C}	5.89 ± 0.18^{A}	1.34 ± 0.16^{C}
ACTH x AV	22.71 ± 7.98^{B}	1.11 ± 0.06^{E}	0.53 ± 0.07^{D}	1.91 ± 0.22^{B}	5.22 ± 0.19^{B}
ACTH x EE	12.60 ± 6.51^{B}	0.83 ± 0.05^{F}	0.67 ± 0.05^{D}	2.51 ± 0.18^{B}	4.81 ± 0.16^{BA}
Control x AV	11.56 ± 6.51^{B}	1.10 ± 0.05^{E}	0.52 ± 0.05^{D}	2.29 ± 0.22^{B}	5.55 ± 0.19^{A}
Control x EE	11.20 ± 7.98^{B}	0.74 ± 0.06^{F}	0.63 ± 0.07^{D}	2.58 ± 0.22^{B}	5.49 ± 0.19^{A}
P-Value	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**

Values with different superscripts in the same column differ significantly $^{BA, C, D E, F}$ $P < 0.001$; $^{a, b}$ $P < 0.05$). AV = Artificial vagina, EE = Electro-ejaculator, Se = Selenium, Te = Testosterone, LH = Luteinising hormone, GSH-Px = Glutathione peroxidase

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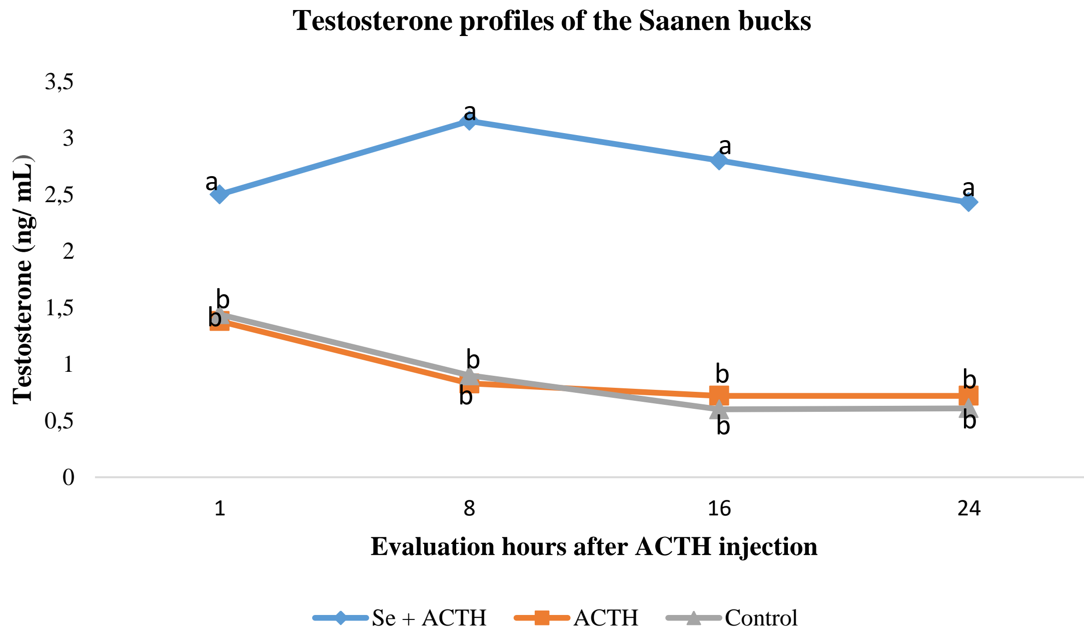

Figure 4.7. Effect of selenium supplementation and induced stress on serum testosterone levels of Saanen bucks.

Time 0 indicates time of ACTH administration

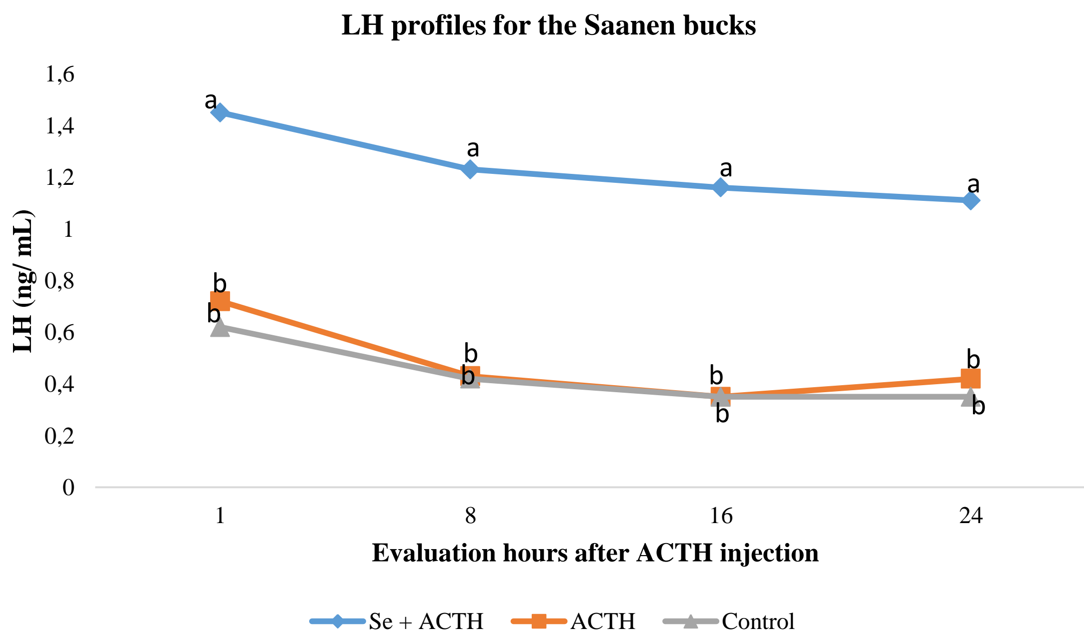

Figure 4.8. Effect of selenium supplementation and induced stress on serum luteinizing hormone concentration of Saanen bucks. Time 0 indicates time of ACTH administration

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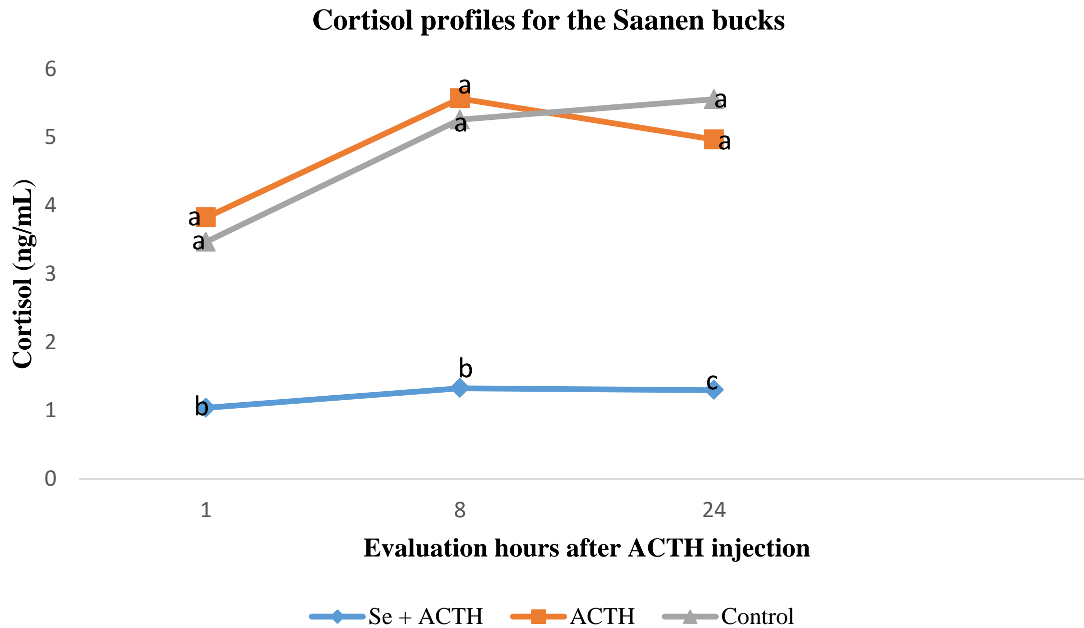

Figure 4.9. Effect of selenium supplementation and induced stress on serum cortisol levels of Saanen bucks. Time 0 indicates time of ACTH administration

4.4.6. Correlations

Semen appearance was positively correlated to sperm motility (0.562) (P < 0.01) and sperm concentration (0.558) (P < 0.01). It was observed that sperm motility was also positively correlated to sperm concentration (0.616) (P < 0.01), percentage of live sperm (0.691) (P < 0.01) and percentage of normal sperm (0.753) (P < 0.01). The sperm concentration was positively correlated to percentage of live sperm (0.651) (P < 0.01) and percentage of normal sperm (0.657) (P < 0.01). On the other hand the percentage of dead sperm was positively correlated to percentage of abnormal sperm (0.867) (P < 0.01), percentage of primary sperm (0.776) (P < 0.01), percentage of secondary sperm (0.843) (P < 0.01) and percentage of tertiary sperm (0.783) (P < 0.01). The results have showed that the percentage of live sperm was positively correlated with percentage of normal sperm (0.860) (P < 0.01).

Se concentration was positively correlated to sperm motility (0.631) (P < 0.01), semen appearance (0.660), sperm concentration (0.555) (P < 0.01), percentage of live sperm (0.729) (P < 0.01), percentage of normal sperm (0.760), testosterone (0.786) (P < 0.01) and LH (0.721) (P < 0.01) concentrations. Testosterone level was positively correlated to Se concentration (0.786) (P < 0.01), semen appearance, (0.718), sperm motility (0.671) (P < 0.01), sperm concentration (0.711) (P < 0.01), percentage of live sperm (0.858) (P < 0.01), percentage of normal sperm (0.859) (P < 0.01) and LH concentration (0.917) (P < 0.01). LH concentration was positively correlated to semen appearance

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(0.690) ($P < 0.01$), sperm motility (0.697), ($P < 0.01$), sperm concentration (0.689) ($P < 0.01$), percentage of live sperm, (0.736) ($P < 0.01$) and percentage of normal sperm (0.791) ($P < 0.01$).

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CHAPTER 5

DISCUSSION

5.1. Selenium status of lucerne hay sample during the investigation

It was found that the control bucks were deficient in Se. Both treatment groups were fed lucerne that was deficient in Se. The lucerne hay samples was deficient in Se as they contained 0.003 mg Se/kg DM. This value is lower than 0.05 mg/kg DM which is classified as Se deficient in plant material (Van Ryssen, 2001) and also compared to Se concentration of 0.1 and 0.3 mg/kg DM regarded as a requirement in animal feed (Plus, 1994).

5.2. Effect of selenium on attainment of puberty and reproductive hormones of Saanen goats (Experiment 1)

5.2.1. Effect of selenium on phenotypic pubertal characteristics of Saanen goat kids

In agreement to the results of the current study, previous researchers (Witchel *et al*., 1994; Kumar *et al*., 2009) indicated that Se supplementation had a positive growth response in lambs and heifers. The results of the current study proved that supplementing pre-pubertal male goat kids with Se increases body weight and this can reduce the time to onset of puberty.

The majority of animals attained puberty at 6 months of age as indicated by body weight (24.66 ± 1.30 kg for Se supplemented vs 20.44 ± 1.60kg for control group). At the same age (6 months), the animals in the Se supplemented group reached a relevant body weight of 40% of mature Saanen bucks that was reported to range between 60 to 70 kg (Van Tilburg *et al*., 2014) as it is required for the kids to attain puberty. However, at the age of 5.5 months, the first ejaculate containing motile spermatozoa was collected from 60% of the herd. But, 100% of the herd attained puberty at 6 months.

The age (5.5 to 6 months) of the attainment of puberty in Saanen bucks in the current study is in agreement with those reported in Baladi and Angora bucks (22. 23 kg at 5.5 months; 26 kg at 6 months, respectively) (Özsar *et al*., 1990 ; Abi Saab *et al*., 1997). It was also observed in previous study that Anglo-Nubian bucks reached puberty earlier with a body weight of 25.75 kg and 5 months (Souza *et al*., 2011). Although, Chakraborty *et al*. (1989) reported that age of puberty in Nubian bucks was 34 weeks with a body weight of 37.7 ± 3.3 kg which opposes the results of the current study. The differences on the onset of puberty emphasizes that attainment of puberty is influenced by season of birth, type of breed and management system (Ahmad & Noakes, 1996; Abi Saab *et al*., 1997).

Generally, it has been reported that the testis size is used as an indicator of fertility (Ahmed *et al*., 1997; Agga *et al*., 2011). The beneficial effect of larger scrotal circumference is higher sperm

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production. It was noticed that the scrotal circumference increased with age in both groups. These results are in agreement with the findings of Marai *et al*. (2009) who found higher scrotal circumference in rams supplemented with Se compared to the control group. The increase in scrotal circumference may be due to the beneficial effect of Se on the interstitial cells of the testis and protection of the testis cells from damage (Marai *et al*., 2009).

The animals attained puberty at 5.5 - 6 months of age with a scrotal circumference (23.05 ± 1.17 cm for Se supplemented vs 20.82 ± 1.31 cm, for control group) that is in line with those reported by previous researchers in Saanen goats (23 cm at 6.5 months of age) (Ahmed & Noakes, 1997). In contrast, different scrotal circumference was reported in pubertal Boer goats (26 cm at 8 months) and Nubian x Saanen cross (22 ± 0.8 cm) (Keith *et al*., 2009; Elhammali & Elsheikh, 2014). The difference in scrotal circumference obtained could be due to genotype and environment differences (Keith *et al*., 2009).

A positive significant effect of Se supplementation on the testis length was observed throughout the trial as the age of the animal advances. The results are in agreement with those reported by Mahmoud *et al*. (2013). However, these results contradict with those reported by Marai *et al*. (2009) who observed no significant difference on the testis length between Se supplemented and control in rams.

In the current study, the Saanen kids expressed nosing sexual behaviour a week before showing mounting behaviour. Nishimura *et al*. (2000) also reported that the Tokara male goats also showed the nosing behaviour first denoting that the sexual behaviours of the goats are regulated by vomeronasal system. Thus, this system assumes the considerable importance of the sexual behaviours of the pubertal goats (Darwish & Mahboub, 2011).

5.2.2. Effect of selenium supplementation on pubertal semen characteristics of Saanen goats

For the present study, semen volume was increased by Se supplementation. This is in agreement with previous reports in which the greatest ejaculate volume was obtained when supplemented with Se in bucks, boars and rams (Marin-Guzman *et al*., 1997; Kendal *et al*., 2000; Shi *et al*., 2010). The increase in semen volume of Se supplemented may be due to the report that Se stimulates growth and development of primary and secondary sex organs and prostate functions in many species (Cheah & Yang, 2011). Thus, the semen volume increased due to the increase in secretion of the secondary sex glands.

The semen appearance was dominantly thick creamy which showed enhancement of the Se supplementation in the treated group. Generally, thick creamy appearance is associated with high sperm concentration (Hafez & Hafez, 2000). Thus, it suggests that enhancement of the semen appearance was due to the antioxidant protection of Se to the sperm cells during spermatogenesis

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(Mahmoud *et al*., 2013). The overall semen appearance at puberty (thin creamy vs milky in the Se supplemented and control group, respectively) of the present study in the control group is in agreement with the reports in West Dwarf with a semen appearance of milky white colour (Bitto *et al*., 2012). Thus, it can be emphasized that at attainment of puberty semen appearance is thinly with milky but when animals are supplemented with Se, the semen quality in terms of semen appearance is improved by being creamy.

The bucks supplemented with Se produced higher sperm progressive motility than the control. These results are in agreement with those reported in goats, bulls and rams (Lee *et al*., 2000; Xu *et al*., 2003; Kumar *et al*., 2014). Se supplementation improves the sperm motility and function. The increase in the sperm motility shows that Se provided a sufficient protection to the spermatozoa as Se is known to reduce the number of sperm with broken flagellum which in turn enables the spermatozoa to move. In addition, the increase in sperm motility from Se supplemented bucks is also supported as it is known that Se plays a role as insulin mimetic (Stapleton, 2000). Se has been observed to stimulate the transport of glucose in the cell in a dose–dependent manner (Ezaki, 1990) and improves uptake of glucose together with increased rate of aerobic and anaerobic glycolysis (Furnisin, 1996). Thus, the improvement of the sperm motility in the present study can be associated to Se supplementation.

Se supplemented bucks produced higher sperm concentration than the control group. This observation is in agreement with earlier reports (Shi *et al*., 2010; Kumar *et al*., 2014) in bucks. The higher sperm concentration in the Se supplemented may be due to the antioxidant activity of Se that protects the sperm cells during spermatogenesis. The protection is provided by a GSH-Px that mediates the regulation of spermatogenesis (Shalini & Bansal, 2008). Furthermore, Se is necessary for the development of the spermatozoa (Mahmoud *et al*., 2013). Thus, Se provides more efficient protection to spermatozoa thereby ensuring the survivability and increasing the sperm concentration.

The percentage of live sperm in the Se treated group was higher than control group. These results are in agreement with previous studies that reported higher percentage of live sperm in Se supplemented bucks (Kamel, 2014; Kumar *et al*., 2014). The antioxidant protection of Se on cellular membrane and lipid containing organelle from oxidative damage has more explanation to this observation. This allowed more survival of sperm cells.

The sperm abnormalities observed during the period of the attainment of puberty were mostly the abnormal heads, cytoplasmic droplets and broken tails. These sperm abnormalities occur due to the destruction during spermatogenesis that leads to release of the immature sperm. However, due to Se supplementation, the percentage of primary sperm abnormalities (abnormal head, proximal cytoplasmic droplets and twin head) declined. It may be concluded that GSH-Px, an enzyme made up of Se prevents the lipid attack thereby reducing the proportion of the abnormal spermatozoa. Based on

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sperm abnormality scale according to Evans & Maxwell (1987), the abnormalities for the current study were lower (< 20%) and acceptable for both groups to be considered for a buck semen quality.

5.2.3. Effect of selenium supplementation on reproductive hormones of Saanen goats at puberty

In agreement to the results of the present study, Mahmoud *et al*. (2013) also observed that Se supplemented in rams increased serum LH and testosterone concentrations. Thus, it can be suggested that the increase in testosterone shows the responsiveness of the Leydig cells to gonadotropins which are stimulated by the functioning LH receptor mechanism that controls the storage and release of testosterone (Falana & Oyeyipo, 2012).

For the current study, animals in both groups had low levels of LH and testosterone at an early age of the sexual development. The LH and testosterone increased as the age advances in both treatments. Although for in Se supplemented group, it was observed that the animals attained puberty at the age of 6 months with the acceptable values of LH (0.31 ± 0.21 for Se supplemented vs 0.12 ± 0.04 ng/mL for control group) and testosterone (1.86 ± 0.81 for Se supplemented vs 0.54 ± 0.24 ng/mL for control group) which are satisfactory for the pubertal goats. As suggested in previous studies increase in testosterone levels from the age of 5.5 months as the animals continue to grow range between 0.9 and 1.81 ng/mL respectively, in the Saanen goats (Ahmad *et al*., 1996). This value is in agreement with the Se supplemented group, emphasizing the importance of Se supplementation for attainment of puberty. Moreover, Özsar *et al*. (1990) reported that the Angora goats had a testosterone level of 0.89 ± 0.72 ng/mL and LH levels that ranged between 0.11 and 0.42 ng/mL during the pubertal stage. Thus in the present study, kids supplemented with Se showed similar values at the age of 6 months which are considered acceptable for attainment of puberty.

5.2.4. Blood selenium concentration in pubertal Saanen goats

Se concentration in the whole blood was significantly higher in the Se supplemented than the control group. The availability of Se supplementation in the animal after supplementation is expected to 50 ng/g as considered to be marginal, and 80 ng/g showing to be adequate and greater than 300 ng/g being toxic levels (Puls, 1994). Thus, the average Se observed in the present study when Se was supplemented showed that as the bucks grow Se is used in abundance in the body. However, it was reported that Se is highly required and utilised mainly in the testis during the onset of puberty. This is due to the important role of Se in male reproductive system as it is an antioxidant and provides protection during spermatogenesis. Similar findings were reported in rats that Se concentration is beneficial for spermatogenesis (Behne *et al*., 1996). Thus, this confirms the acceptable results of the semen parameters obtained when Se was supplemented in the present study.

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5.3. Effect of selenium supplementation and semen collection methods on Saanen semen yield and quality (Experiment 2)

5.3.1. Effect of selenium and collection methods on Saanen goats semen parameters

The present study provided clear evidence that Se supplementation led to creamy semen appearance compared to the control. The creamy semen is considered normal and free from pathological changes or injury (Dhurvey *et al*., 2012). Generally, semen appearance is related to the sperm concentration in ruminant species (Hafez & Hafez, 2000). Thus, creamy appearance is an indication of high sperm concentration and this implies that Se supplementation can increase the number of spermatozoa (Ganabadi *et al*., 2010). The observation that the AV semen collection method led to creamy compared to thin creamy appearance from EE method, is a confirmation that lower semen volume usually obtained by the AV method lead to high sperm concentration. These results are in agreement with those reported by previous researchers (Greyling & Grobbelaar, 1988; Malejane *et al*., 2014) in farm animals.

Se supplementation increased semen volume in the current study. These results agree with previous studies in bucks and rams (Kendall *et al*., 2000; Shi *et al*., 2010). Se is known to stimulate growth and development of primary and secondary sex glands (Kolodziej & Jacyno, 2005), spermatogenesis (Ahsan *et al*., 2014) and mainly prostate function in many species. Therefore, the ejaculate volume increased due to the increase in secretions from the secondary sex glands and spermatogenesis (Kolodziej & Jacyno, 2005). The EE technique yielded higher semen volume than AV in the present study. Similar results were obtained by other studies (Malejane *et al*., 2014; Bopape *et al*., 2015) in rams and bucks. A greater ejaculate volume obtained when using EE technique is usually ascribed to excessive electrical stimulation of accessory glands (Mattner & Volgmeyer, 1962). As such, the AV is believed to obtain the volume that the animal would ejaculate under natural mating (Jiménez-Rabadán *et al*., 2012). Thus, it is noteworthy that the buck semen obtained in the present study is within the acceptable ranges (0.7 to 2.2 mL) for EE and (0.5 to 1.0 mL) for buck semen collected with AV (Hafez & Hafez, 2000).

The non-significant difference on semen pH between the Se supplemented and control groups shows that Se supplementation does not alter semen pH. Similar results were reported by previous studies (Marai *et al*., 2009; Shi *et al*., 2010) in rams and bucks, where the Se supplementation did not affect semen pH. The semen pH of the current study was within acceptable range for goats (Purdy *et al*., 2006).The semen collection methods did not alter semen pH of the present study. Semen pH of the current study when using AV was in agreement with those reported by Bopape *et al*. (2015). It is noteworthy that the semen pH found in the current study, was within the normal range of buck semen pH of 7.0 to 7.8 as reported by Prins (1999). The semen pH obtained using EE method was also

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within the acceptable range of the buck semen. Talebi *et al*. (2009) also reported similar results when EE was used to collect semen in bucks.

The increase in progressive sperm motility observed in the current study from the Se supplemented is in agreement with the previous studies in bucks and rams (Kumar *et al*., 2009; Mahmoud *et al*., 2013). It shows that Se supplementation was able to provide a sufficient protection to the spermatozoa (Mahmoud *et al*., 2013). It has been found that Se reduces the number of sperm with broken flagellum therefore, more spermatozoa are capable to move (Wu *et al*., 1979). Se also reduces the sperm membrane to lipid oxidation that can lead to plasma membrane to lose its barrier function and this is a major cause of decline in sperm motility due to leakage of cytosolic enzymes and substrates (Alvarez & Storey, 1984).

The semen collection methods did not alter the sperm motility obtained in the present study. This is an indication that the sperm motility is determined during sperm proliferation and maturation in the seminiferous tubules but not affected by ejaculation. The sperm motility obtained in the present study was lower than those reported by previous researchers (Ramukhithi *et al*., 2011; Jeminez-Rabadan *et al*., 2012; Souri & Mirmamoud, 2014) when the EE technique was used. The differences might be due to the effect of breed and season (Talebi *et al*., 2009). However, the sperm motility obtained with the aid of AV method is in line with that reported by Al-Ghaban *et al*. (2004) in bucks.

Se supplementation had a tendency to increase the sperm concentration. In agreement to the current study, other authors have reported that Se supplementation had a tendency to improve sperm concentration in farm animals (Marai *et al*., 2009; Lopez *et al*., 2010). Se is necessary for the development of spermatozoa and also contributes to increase in number of the germ cells in adult animals (Liu *et al*., 1982). In addition, Se is also important for the proliferation of Sertoli cells in the developing testes which in turn contribute to the health and nourishment of the germ cells leading to an increase in the number of sperm cells regarded as sperm concentration (Marin-Guzman *et al*., 2000). The findings that there was non-significant difference between the AV and EE semen collection methods on sperm concentration contradicts previous researchers in goats (Memon *et al*., 1986; Jiménez-Rabadán *et al*., 2012; Bopape *et al*., 2015). This is an indication that the two methods when used by experienced personnel can lead to similar semen quality.

Se supplementation contributed to a decline in the percentage of abnormal sperm in the current study. This is in agreement with the previous studies (Shi *et al*., 2010; Lovercamp *et al*., 2013) regarding the efficiency of Se supplementation in decreasing the percentage of abnormal sperm in farm animals. The observation might be a consequent of the role of Se, as is reported to form an integral part of GSH-Px (Rotruck *et al*., 1973). However, GSH-Px activity was not measured for the present study. It was reported that this enzyme provides protection for the sperm membrane against oxidative

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disintegration which causes sperm dysfunction, especially in terms of the loss of membrane fluidity or sperm membrane damage (Agwaral *et al*., 2003).

Semen collection methods had no effect on percentage of abnormal sperm in the present study. Greyling & Grobbelaar (1983) found no significant difference in the percentage of sperm abnormalities following semen collection with the AV and EE techniques in goats. However, the percentage abnormal sperm that range between 15 and 20% are considered as being normal and of good fertilizing ability in the small ruminants (Evans & Maxwell, 1987). Therefore, the percentage of abnormal sperm found in the current study when semen was collected with AV and EE methods is within the acceptable range. According to other researchers (Evans & Maxwell, 1987; Chemineau *et al*., 1991) the results obtained in the current study are regarded as good quality buck semen when AV and EE techniques are used.

The interaction of Se supplementation and semen collection methods in the present study regarding percentage of abnormal sperm, strongly suggests supplementation of Se, where semen is collected regularly for AI. There were higher percentages of abnormal sperm in semen collected from the control group than the Se supplemented for both semen collection methods. The results show that Se as an antioxidants can protect spermatozoa from damages that can lead to sperm abnormalities (Surai & Fisinin, 2015).

Se supplementation increased the percentage of live sperm. These findings are in agreement with those reported in farm animals (Lovercamp *et al*., 2013; Kumar *et al*., 2014). Se acts as an antioxidant which plays a major role protecting the sperm from morphological damage by preventing free radical oxygen from damaging sperm cells (Brezezinsk-Slebodinska *et al*., 1995; Marin-Guzman *et al*., 2000). It was also found that Se is involved in the formation of the normal structural development of sperm during spermiogenesis (Wanatabe & Endo, 1991).The findings of percentage of live sperm recorded in the present study are similar to other studies (Farshad *et al*., 2012; Ferdinand *et al*., 2012; Okupu *et al*., 2012; Aguiar *et al*., 2013) where AV and EE techniques were used. Regarding the percentage of live sperm, it can be concluded that both methods are suitable for being used for semen collection in goats.

The percentage of primary sperm abnormalities is associated with the head and mid-piece defects which generally arise as defects from spermatogenesis (Memon *et al*., 2012). So, for the present study it was observed that Se supplementation reduced the head and mid-piece sperm abnormalities. While semen from the control group had greater abnormalities of the altered spermatozoa head shape of the spermatozoa, headless and damaged mid-piece region of the tail. Similar results were observed in rats deficient in Se (Wanatabe & Endo, 1990; Behne *et al*., 1996; Maiorino *et al*., 2003). The percentage

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of primary sperm abnormalities was not changed by the collection methods in the present study. This was as expected since the primary sperm abnormalities occur before ejaculation (Barth & Oko, 1989).

The secondary sperm abnormalities usually occur during maturation phase in the epididymis (Salisbury *et al*., 1978). Such abnormalities lead to a decline in fertility (Ali *et al*., 2009). Thus, for the current study, semen from Se supplemented group had reduced proximal and distal cytoplasmic droplets. This suggests that Se was adequate in the epididymis to enhance proper sperm maturation as confirmed by semen from the control group that had high proximal and distal cytoplasmic droplets.

The semen collection methods did not contribute to the percentage of secondary abnormalities in the present study. These results clearly prove that secondary sperm abnormalities are related to the disturbances in sperm epididymal dysfunction (Roca *et al*., 1992) not what happens during ejaculation. However, when Se was supplemented the secondary sperm abnormalities declined even when semen was collected with EE technique. The consequence for the results obtained is unexplainable, as the secondary sperm abnormalities occur due to the disturbance of sperm maturation in the epididymis (Cheah & Yang, 2011).

Se supplementation did not affect the percentage of tertiary sperm abnormalities obtained in the current study. The percentage of tertiary sperm abnormalities is mainly caused by mishandling after ejaculation (Chenoweth & Lorton, 2014).

However, the AV technique led to higher percentage of tertiary sperm abnormalities. This implies that even though AV is regarded as a technique that leads to higher sperm quality, it somehow seems to lead to more tertiary sperm abnormalities. On the other hand, the EE technique had an advantage of leading to lower percentage of tertiary sperm abnormalities. These results confirm previous reports where the EE technique had no detrimental effects on tertiary abnormalities of the sperm tail (Adenji *et al*., 2010). Therefore, the EE method leads to better semen quality due to reduced tertiary sperm abnormalities.

5.3.2. Blood selenium concentration as it affects reproductive performance of Saanen goats

It was evident that the Se concentration increased significantly in the plasma of Se supplemented bucks, as expected. The findings are in agreement with previously published results by van Ryssen *et al*. (1999). In order for Se to be effective, it has to be in high concentration, acceptable for bucks as it was reflected in the present study. This observation is supported by the improved results obtained from semen parameters in Se supplemented bucks signifying acceptable semen quality since Se plays a major role in regulation of male reproductive process (Jacyno & Kolodziej, 2005).

The results obtained showed that Se concentration was lower in bucks collected semen using EE method while the Se concentration remained high in the bucks collected with AV method. It is well

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known that EE is associated with induction of stress during semen collection (Ortiz-de-Montellano *et al*., 2007). Thus, a decline in Se concentration from the bucks whose semen was collected overtime with the EE technique clearly shows that more Se was used, probably for production of GSH-Px to combat production of ROS during semen collection (Surai & Finnisin, 2015). Although not measured, the results obtained in the present study clearly show that more stress probability in the form of ROS is produced during EE semen collection, therefore more Se is utilised. The interaction of Se supplemented and semen collection methods in the current study regarding the Se level shows that Se should be supplemented when semen is collected in bucks using the EE method. Se concentration declined considerably in the control from the bucks collected semen with the EE technique. In this study, bucks that were supplemented with Se and collected semen with the EE method had better semen quality compared those without Se supplementation. Therefore, where semen is collected using the EE method, Se supplementation is necessary.

5.3.3. Effect of selenium on reproductive hormones of Saanen goats

Se supplementation had a tendency to increase testosterone levels but not significant in the present study. Previous studies (El-sissy *et al*., 2008; Camal *et al*., 2012) reported that Se supplementation caused a significant increase of testosterone level in goats. It was suggested that the increase in testosterone levels is due to the concomitant increase of GSH-Px activity that protects the testes from the unfavourable effect of ROS or its protection on the Leydig cells or the steroidegenic function as it was reported by El-sissy *et al*. (2008). Results obtained from the present study showed that the semen collection methods did not induce any change into testosterone levels. However, these results were expected, as it is well known that testosterone is produced by the Leydig cells in response from the LH that is secreted by the anterior pituitary gland, with no literature indicating Se effect to the anterior pituitary gland (Hafez & Hafez, 2000).

LH levels were also not affected by Se supplementation nor semen collection method. This shows that Se supplementation and semen collection methods did not interfere with the hypothalamo-pituitary-axis which regulate LH release (Behne *et al*., 1996). Similar results were reported by Kaur & Bansal (2004) that Se supplementation did not change LH levels in rats.

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5.4. Effect of selenium supplementation and induced stress on semen quality and reproductive hormones (Experiment 3)

5.4.1. Effect of selenium supplementation and induced stress on semen parameters of Saanen goats

Induced stress treatment with injection of ACTH decreased the semen appearance quality and led to a milky colour in the present study. This observation might be due to elevated stress as it induces free radicals and attacks the germ cells within the seminiferous tubules, which in turn, causes extensive apoptosis and the disruption of spermatogenesis (Atiken & Baker, 2013). A disruption in spermatogenesis causes a decline in concentration of sperm which then alters the semen appearance (Hafez & Hafez, 2000). Thus, a decline in the number of sperm produced was reflected by milky semen appearance in the stress induced group of the present study. The semen appearance was creamy in Se supplemented group, even when bucks were supplemented with Se and under induced stress. This observation indicated that Se can minimise stressful effects in semen.

Both semen collection methods led to creamy semen appearance with no signs of contamination. Thus, the results obtained show that both semen collection methods can be utilised in bucks without affecting semen quality (Hafez & Hafez, 2000).

In the current study, the semen volume was higher when stress was induced compared to the control group. Seminal plasma is a product of the accessory glands (Hafez & Hafez, 2000). Thus, it can be suggested under stressful conditions the accessory sex glands might be stimulated to produce more seminal plasma leading to high semen volume. Similar results were observed in pigs that high semen volume was ejaculated under stressful conditions (Ciereszko *et al*., 2000). During stressful conditions the sperm production and the androgens decline thereby failing to maintain the functionality of the accessory glands (Retana-Marquez, 2014). However, when Se was supplemented and stress induced in goats, the semen volume was similar to the Se and the induced stress groups, suggesting that Se was able to stimulate growth and development of primary and secondary sex glands (Kolodziej & Jacyno, 2005).

The ejaculate volume was reduced when EE technique was used in the stress induced bucks. However, when bucks were treated with Se and under induced stress, the ejaculate volume was higher, suggesting the improvement of ejaculate volume due to Se supplementation. When AV was used, ejaculate volume was also lower in the stress induced compared to the control group. However, when Se was supplemented the semen volume increased when AV was used to collect semen, signifying the importance of supplementing bucks with Se as an antioxidant (Surai & Fisinin, 2015).

The ejaculate volume declined when AV technique was used and increased when semen was collected using EE, as it was expected (Mattner & Volgmayer, 1961; Jiménez-Rabadán *et al*., 2012).

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The semen pH was slightly acidic in the control, induced stress and Se supplemented bucks with induced stress and neutral only in Se supplemented group. The slightly acidic semen pH might be due to low metabolic activity of the spermatozoa and also high accumulation of reactive oxygen species as it was reported that aerobic conditions alter semen pH (Anderson, 1952). However, it was observed that the supplementation of Se in bucks has a potential to maintain neutral semen pH.

The semen pH obtained in the Se supplemented was considered to be optimum 7.05 ± 0.05 as associated to the buck semen pH that ranges from 7.0 to 7.8 (Prins, 1999). Thus, this confirms that Se supplementation maintains semen pH.

The semen pH in both semen collection methods was slightly acidic. However, the semen pH reported when the AV was used contradicts with those reported by Barkawi *et al*. (2006) in goat bucks. While the semen pH obtained in the EE was also slightly acidic but in line with the findings of the previous studies (Ramukhithi *et al*., 2011; Bopape *et al*., 2015). The slightly acidic semen pH in the EE might be caused by the excessive accessory glands secretion due to electrical stimulation during semen collection (Oritz-de-Montellano *et al*., 2007). The slightly acidic semen pH is considered unacceptable as it may have a negative impact on fertility of the buck. It was previously reported that the acidic semen pH leads to reduced sperm motility as a consequence of the change in the metabolic activity and a disturbance in the cellular respiration of the sperm (Latif *et al*., 2005).

The sperm motility was decreased as a result of the induced stress compared to that of the control group. Previous studies also demonstrated that induced stress decreased the sperm motility in leopards (Brown *et al*., 1989). It is noteworthy that in the stress induced group, the GSH-Px declined in this study, which is an indication of a decline in the antioxidant status under stress conditions. It was reported that the lower antioxidant status is an indication of excessive production of ROS which in turn can impair sperm motility (Hamilton *et al*., 2015). It was also reported that ROS inhibit sperm motility through induction of loss of mitochondrial ATP (Adenosine triphosphate) production. The exposure of the spermatozoa to ROS, adversely attack the sperm plasma membrane, breaks the flagellum and leads to loss of spermatozoa motility. This also leads to a decline in the activity of ATPase and glucose transport (Surai & Fisinin, 2015). Se supplementation reduced the harmful effects of induced stress as reflected by high sperm motility in Se supplemented as well as where Se was supplemented in bucks under induced stress. These results are in agreement with the previous report that Se supplementation improves sperm motility in bucks (Shi *et al*., 2010).

The semen collection methods did not affect the sperm motility as this parameter is finalised in the epididymis during sperm maturation (Nas *et al*., 1990).

The sperm concentration of the bucks decreased under induced stress compared to that of the control group and even when Se was supplemented. The result of a decline in sperm concentration when

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stress was induced is in agreement with those obtained in rams (Alkas, 2009) and humans (Clarke *et al*., 1999). This is an indication that the semen ejaculated under stress in goats has high content of seminal plasma with low sperm concentration (Petrocelli *et al*., 2015). These results might be attributed to the destruction occurring in the sperm membrane resulting in impairment of membrane fluidity and permeability and damage of germ cells during spermatocytogenesis, as a result of stress (Zhu & Setchell, 2004). Thus, it can be suggested that failure of formation of germ cells led to a reduced formation of spermatozoa which in turn led to a decline in sperm production.

The observation that the sperm concentration increased when Se was supplemented is consistent with the previous reports in goats and rams (Kendall *et al*., 2000; Kumar *et al*., 2014).

The decrease in percentage live sperm observed in the ACTH group was attributed by the induced stress as the percentage live sperm was higher in the control group. Similar results have been reported previously that induced stress lead to reduced percentage of live sperm in bulls, rats and goats (Skinner & Louw, 1966; Zhu & Setchell, 2004). However, when Se was supplemented in goats which stress was induced, the percentage of live sperm was improved. The sperm viability response observed was in line with the report of Shi *et al*. (2010) when Se was supplemented in goats. Chen *et al*. (2012) suggested that Se play a major role as it is involved during maturation process of spermiogenesis for formation of live progressive motile spermatozoa, since the live spermatozoa are highly correlated to the sperm motility as immobile sperm are usually dead (Falana & Oyeyipo, 2012).

Both semen collection methods had no significant effect on percentage of live sperm obtained in the present study, suggesting the effectiveness of both AV and EE semen collection methods in goats (Talebi *et al*., 2009; Farshad *et al*., 2012).

Similar to the control group in this study, the induced stress led to higher percentages of abnormal sperm. The increased percentage of abnormal sperm when stress is induced and in the control group might be attributed to the lower GSH-Px activity which decreased in these groups in the current study. Previous report showed that lower GSH-Px activity can be associated with damage of the chromatin structure of the sperm in the epididymis thereby leading to increased number of abnormal sperm morphology (Hansen, 2009). Clearly reflected in this study, when Se was supplemented, the percentage of the abnormal sperm morphology was reduced. These results are in agreement with those reported by Kumar *et al*. (2014) following Se supplementation in goats.

The percentage of primary sperm abnormalities was higher in the induced stress group, while the percentage of primary sperm abnormalities was lower in the control group. The percentage of primary sperm abnormalities recorded was observed on the head and mid-piece of the spermatozoa and

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increased by induced stress. Similar results were observed in leopards under induced stress (Brown *et al*., 1989).

The percentage of primary sperm abnormalities was reduced when Se was supplemented and when bucks were under induced stress and also supplemented with Se. These results suggest that induced stress increase the percentage of primary sperm abnormalities but this condition could be minimised by Se supplementation. The reduced percentage of primary sperm abnormalities caused by Se supplementation may be due to high availability of GSH-Px observed in the present study, as high GSH-Px is critically required for protection of germ cells and spermatozoa membranes from oxidative stress during spermatogenesis (Shi *et al*., 2010).

The current study shows that the induced stress can lead to a significant increase in percentage of secondary abnormal sperm while the percentage of secondary abnormalities was lower in the control group. The percentage of secondary abnormalities is related to the disturbances in sperm maturation or epididymal dysfunction (Roca *et al*., 1992). The main abnormalities observed were presence of cytoplasmic droplet. The results of increased secondary abnormalities observed in the present study are in agreement with those reported by previous studies (Skinner & Louw, 1966; Brown *et al*., 1989; Zhu & Setchell, 2004) as a result of induced stress. The percentage of secondary sperm declined when Se was supplemented showing that Se has minimized the effects of stress.

The percentage of tertiary sperm abnormalities increased when stress was induced and this observation was similar to that of the control group. However, when Se was supplemented, the tertiary sperm abnormalities decreased. Thus, according to the results observed in the current study, it can be suggested that Se should be supplemented in bucks as it is essential during semen collection (Surai & Finisin, 2015) since it was reported that percentage of tertiary sperm abnormalities occur due to mishandling after ejaculate (Chenoweth & Lorton, 2014).

5.4.2. Blood selenium concentration as it affects reproductive performance of Saanen goats

Se concentration in the induced stress and control groups was low and beyond recommendations (Puls, 1994) as it was expected. Thus, the lower Se concentration in induced stress group gives a better explanation for observation of the poor semen quality and quantity, reduced testosterone level and GSH-Px activity obtained in the present study (Falana & Oyeyipo, 2012). However, the higher Se concentration in Se supplemented and also when Se was added in the induced stress group can be associated with the improved semen quality and quantity of bucks from the present study when Se was supplemented. This observation is supported by the role of Se as it regulates male reproductive process in male farm animals (Shi *et al*., 2010; El-mokadem *et al*., 2012; Surai & Finisin, 2015). Thus, it can be suggested that the blood Se concentration must be high in the testis and epididymides of goats in order to induce its physiological activities in sperm and semen (Surai & Finisin, 2015).

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Moreover, this is supported by the improved semen quality which shows the positive influence of Se in goats of the current study.

5.4.3. Effect of induced stress and selenium supplementation on reproductive hormones, cortisol and glutathione peroxidase activity of Saanen goats

The levels of blood serum LH of the control goats and induced stress group were similar. This suggests that stress has a regulatory effect on the hypothalamo-pituitary-axis, leading to destruction in GnRH pulses hence lowering the LH production (Clarke *et al*., 1999). On the other hand, when Se was supplemented in the induced stress group, the LH concentration increased. Although, the increase in LH concentration in Se supplemented groups is unexplainable since there is no literature indicating Se influence on LH secretion.

Induced stress decreased testosterone concentration compared to the control group. Similar results were reported in rams that, testosterone declines due to induced stress (Van Lier, 1999). It has been reported in the previous studies that, as cortisol level increased, the testosterone concentration decreased (Fenske, 1997) thus, similar results were observed in the present study that cortisol level increased and testosterone declined in the stress induced bucks. However, it was noted that the testosterone level increased in goats which were supplemented with Se in the induced stress group suggesting that, the addition of Se has lessened the stress effects. The increase in testosterone concentration might be attributed to the increased GSH-Px activity, as was observed in this study (Murakoshi *et al*., 1983). It has been reported that in the Leydig cells, the GSH-Px is required for metabolic pathway of testosterone biosynthesis as this enzyme is essential for protection against unfavourable effects of ROS attacks (El-Sheshtawy *et al*., 2002).

The current study has provided a clear observation that induced stress increases cortisol (a stress hormone) levels in blood serum of goats while in the control group, cortisol level remained lower. These results suggest that induced stress has interfered with the hypothalamo-pituitary-adrenal which regulates cortisol release (Collodel *et al*., 2008). These results are in agreement with the previous study which demonstrated that stress exposure can increase cortisol level in pigs (Fenske, 1997). However, the levels of blood serum cortisol decreased in the Se supplemented and also in the goats which were exposed to stress but also supplemented with Se. This observation denotes that supplementation of Se can reduce the detrimental effects of induced stress in goats.

Glutathione peroxidation (GSH-Px) activity in blood serum of goats decreased in both stress induced and control groups. A decrease in GSH-Px activity shows that there was lower Se concentration in both groups as it was expected. Since Se is an integral component of GSH-Px (Arthur *et al*., 2003), it can be suggested that a decrease in GSH-Px activity is an indication of the lower antioxidant status in the testis (Surai & Finisisn, 2015) of the goats in both induced stress and control groups. Thus, a

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decline in antioxidant suggests that accumulation of ROS was higher in the testis (Said *et al*., 2010), which in turn explains the poor semen quality obtained from the bucks in both induced stress and control groups from the present study.

It was observed that GSH-Px activity in blood serum of the goats was increased in Se supplemented group and even in goats treated with Se and also under induced stress. In agreement to the present study, it has been reported that Se supplementation increases GSH-Px (El-Sharaky *et al*., 2007). These results suggest that Se supplementation may reduce the effects of stress and enhance the testicular antioxidant status through increased GSH-Px activity (Said *et al*., 2010). Therefore, the increased GSH-Px activity can be associated with the improved semen yield and quality of the bucks when Se was supplemented in this study.

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CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

Conclusion

The results obtained in the present study showed that attainment of puberty in Saanen bucks can be characterised by the presence of motile and viable spermatozoa in ejaculate. In the present study, 60% of the experimental bucks attained puberty at 5.5 months based on the first collection of motile spermatozoa. At 6 months of age, 100% of the bucks attained puberty.

So, 100% of the Saanen bucks attained puberty at 6 months with a body weight of 24.66 ± 1.30 kg for selenium supplemented and 20.44 ± 1.60 kg for control group, with a scrotal circumference of 23.05 ± 1.17 cm for selenium supplemented vs 20.82 ± 1.31 cm for control group, while onset of mating behaviours was observed at 5.5 months in selenium supplemented and control groups. Supplementation of selenium enhanced reproductive hormones in Saanen bucks. At puberty, luteinising hormone concentration of the bucks was 0.36 ng/mL in Se supplemented and 0.12 ng/mL for control group while testosterone concentration was 1.86 ng/mL for Se supplemented and 0.54 ng/mL for control group. Selenium supplementation improved body weight, scrotal circumference, semen appearance, progressive sperm motility and sperm concentration and viability while, it reduced the percentage of abnormal sperm at attainment of puberty. The body weight was highly and positively correlated to the testicular size. The testicular length and body weight were positively correlated to the semen volume.

Regarding the effect of selenium supplementation and semen collection methods, both techniques were suitable for collecting semen from Saanen bucks. Supplementation with selenium led to greater ejaculate volume, high sperm motility, increased sperm concentration, high percentage of live sperm and decline in the percentage of abnormal sperm. The percentage of primary and secondary sperm abnormalities were lowered by selenium supplementation. The electro-ejaculator method of semen collection had a greater ejaculate volume regardless of the selenium supplementation. The interaction of selenium and semen collection methods showed that if semen is collected regularly and bucks are supplemented with selenium, the semen parameters were acceptable in both semen collection methods of electro-ejaculator and artificial vagina. The interaction of selenium supplementation and electro-ejaculator method of semen collection showed that when bucks are supplemented with selenium, the electro-ejaculator technique lead to a creamy semen appearance. Selenium concentration declined considerably in bucks semen collected with electro-ejaculator technique. The electro-ejaculator technique had an advantage of leading to a lower percentage of tertiary sperm abnormalities.

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Concerning the induced stress, selenium supplementation lessened the induced stress effects of adrenocorticotropin hormone, in bucks leading to improved semen characteristics, testosterone and luteinising hormone concentrations, glutathione peroxidase activity and a decline in cortisol level. These results clearly showed that it is imperative for the animals to be supplemented with selenium as it is an antioxidant that combat high production of reactive oxygen species during the reproductive processes and also if the animals are used for semen collection.

Recommendations

- Selenium supplementation is recommended in growing male goats in order to hasten the onset of puberty through enhancement of body weight, testis measurements, reproductive hormones and semen parameters.
- In parts of South Africa where selenium is deficient, selenium supplementation is recommended in order to enhance semen quality and quantity of goats.
- Selenium supplementation in male goats is also recommended where semen is collected regularly for protective roles against formation of free radicals. Selenium supplementation is even more important where semen is collected with the electro-ejaculator method.
- In South Africa, goats are generally under several stress factors, therefore it is important that are supplemented with selenium in order to enhance reproduction, reproductive hormones and semen quality.

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