Assessment of selenium supplementation on the attainment of puberty in Merino ram lambs

By

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Declaration

Candidate's Declaration

I acknowledge that I have read and understood the University's policies and rules applicable to postgraduate research, and I certify that I have, to the best of my knowledge and belief, complied with their requirements.

I, Ngelekanyo Elaine Makhado, declare that this dissertation is my original work and that the material submitted for examination has not been submitted, either in whole or in part, for a degree at this or any other university.

14/03/2023

Supervisor's declaration

I am satisfied that I have given the candidate the necessary supervision regarding this dissertation and that it meets the University's requirements for postgraduate research dissertations.

I have read and approved the final version of this dissertation, and it is submitted with my consent.

14/03/2023

Name: Khoboso Christina Lehloenya

Dedication

This work is dedicated to my mother, Nemadodzi Tshifhiwa.

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Above all, I would like to thank Almighty God for his grace, strength, guidance, protection, and wisdom to sail from start to completion.

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“Many are the plans in a person’s heart, but it is the LORD’s purpose that prevails” Proverbs 19: 21 (NIV)

Research output

Part of this dissertation was presented at the following scientific meetings:

- Makhado, N.E. & Lehloenya, K.C., 2021. Effect of selenium supplementation on semen quality of South African Merino ram lambs. 52nd Annual South African Society for Animal Science (SASAS) congress, 10–12 August 2021, Northern Branch, Pretoria, South Africa.
- Makhado, N.E. & Lehloenya, K.C., 2021. Effect of selenium supplementation on phenotypic parameters of South African Merino ram lambs. Faculty of Science, Agriculture and Engineering Symposium, University of Zululand, 20–21 October 2021, Empangeni, South Africa.

Abstract

In small ruminants, the attainment of puberty is influenced by different factors, such as season of birth, photoperiod, nutrition, breed type, management, genetics, endocrine regulation, body weight and the development of the reproductive system. This study was conducted to evaluate the effect of selenium (Se) supplementation on the attainment of puberty in South African Merino ram lambs. Forty South African Merino ram lambs were divided into two groups, Se-supplemented (n = 20) and control (n = 20). The treatment group received a dosage of 0.34 mg Se per kg in the form of sodium selenite at two-week intervals for an experimental period of 130 days. The phenotypic parameters were evaluated weekly. Semen was collected each week using an electroejaculator and was analysed for volume, pH, appearance, motility, viability, morphology and concentration. Blood samples were collected bi-weekly to determine the concentration of glutathione peroxidase (GSH-Px), cortisol and reproductive hormones. Collected data were analysed using analysis of variance, and the means were separated through the Student's t-test.

At 6 months, motile spermatozoa were collected from 80% of the supplemented group compared to 60% of the control. The Se treated group showed significantly higher semen quality in the form of improved spermatozoa motility, concentration, increased percentage of live spermatozoa, decreased percentage of dead spermatozoa and fewer spermatozoa abnormalities compared to the control group. Supplementation with Se had no effect ($P > 0.05$) on body weight and scrotum circumference. Selenium supplementation significantly ($P < 0.05$) improved testicular measurements and decreased the age to attain puberty. Supplementation with Se also increased luteinising hormone and testosterone concentrations ($P < 0.05$). However, Se supplementation did not affect glutathione peroxidase and cortisol concentrations ($P > 0.05$). The control group attained puberty at 6.5 months based on ejaculated semen with viable spermatozoa. Therefore, it was concluded that Se supplementation hastened the attainment of South African Merino ram lambs' puberty to 6 months. Treatment also improved testicular measurements, semen quality and reproductive hormones concentration of South African Merino ram lambs.

Key words: Selenium, Puberty, South African Merino ram lambs, Body weight, Semen quantity and quality, Testicular measurements

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List of Abbreviations

ANOVA	Analysis of variance
cm	Centimetre
ELISA	Enzyme-linked immunosorbent assay
et al.	And others
FSH	Follicle-stimulating hormone
GnRH	Gonadotrophin-releasing hormone
GSH-Px	Glutathione peroxide
HRP	Horseradish peroxidase
kg	Kilogram
LH	Luteinising hormone
mg	Milligram
mIU	Milli-international unit
mm	Millimetre
mL	Millilitre
µL	Microlitre
ng	Nanogram
r	Pearson correlation coefficient
s	Second
SC	Scrotum circumference
Se	Selenium
SE	Standard error
TB	Testicular breadth
TL	Testicular length
V	Volt

CHAPTER 1: INTRODUCTION

1.1. Introduction

This study assessed the effect of oral selenium (Se) supplementation on South African Merino ram lambs' attainment of puberty, phenotypic parameters and semen quality. This chapter provides the background to the study, followed by the problem statement, rationale, aim, objectives and hypotheses.

1.2. Background

The demand for livestock is escalating significantly due to an increase in the human population; it is projected that the world's population will be 9.5 billion by 2050 (Thornton, 2010). The increased demand for animal products places sheep production under pressure to comprise management practices that will improve production to produce more mutton to meet the increased demand (Langeveldt et al., 2016). Sheep production mainly depends upon the efficiency of reproduction. Therefore, using ram lambs in breeding at an early age can enhance their reproductive life (Kumar et al., 2021).

Reproductive success is a key component of economic production in ruminants, affecting animal productivity and genetic progress (Bauersachs et al., 2010). The reproductive cycle of the animal begins with puberty. The attainment of puberty in males is defined as the age when they first produce adequate viable spermatozoa capable of fertilisation (Ramukhithi et al., 2017). Rams attain puberty at 40–60% of mature body weight (Elhammali & Elsheikh, 2014). According to El-Zelaky et al. (2011), rams display sexual behaviour necessary for attaining puberty, such as sexual aggressiveness, penile development and good libido, at a young age. If ram lambs reach sexual maturity earlier, it can reduce the cost of management, thus accelerating the benefits of genetic selection and allowing earlier progeny testing (Moghaddam et al., 2019, Kumar et al., 2021).

In small ruminants, the attainment of puberty is influenced by different factors, such as season of birth, photoperiod, nutrition, breed type, management, genetics, endocrine regulation, body weight and development of the reproductive system (Mohamed et al., 2012; Arangasamy et al., 2018; Mojapelo & Lehloenya, 2019). Furthermore, it is recognised that the growth of the male reproductive system in all farm animals is

strongly affected by adequate nutrition (Elhammali & Elsheikh, 2014; Ghorbankhani et al., 2015). Likewise, nutrition is considered a vital factor affecting ram lambs' puberty onset and has a major influence on sexual maturity and breeding age (Hernandez et al., 2011). However, ram lambs are susceptible to nutrition constraints during the prepubertal period. Severe feed constraints can reduce gonadotropin levels, subsequently affecting the activity of the neuroendocrine axis, which is crucial for puberty attainment (Kastelic, 2013; Krishnan, 2014). Therefore, inadequate nutrition tends to delay puberty and results in hypogonadism in ram lambs (Sejian et al., 2010).

All animals require minerals, including macro and trace minerals, as they play a significant role in increasing the efficiency of reproduction and production. However, an imbalance of minerals may occur in ruminant animals, particularly those whose intake depends mainly on the concentration of minerals in the pasture and in the soil of the field where it grows (Lukusa & Lehloenya, 2017). Trace minerals, such as iron, copper, cobalt, iodine, zinc, manganese, chromium and Se, are important for various physiological processes, including growth, spermatogenesis, health and semen quality (Jerysz & Lukaszewicz, 2013). For example, Se is an essential trace element for male animals and is especially required for maintaining spermatogenesis and male fertility. In addition, Oluboyo et al. (2012) and Villaverde et al. (2014) have demonstrated that Se is necessary for testosterone biosynthesis and is positively correlated with the testosterone content in the blood. Moreover, Se has been identified as necessary for normal semen quality and reproductive function in sheep and goats (Pilarczyk et al., 2013, Lukusa & Lehloenya, 2017).

Selenium deficiency or low Se status is related to numerous reproductive disorders, including abnormal testicular morphology, poor semen quality and impaired spermatozoa structure (Ahsan et al., 2014). Plants supply inadequate Se to meet the dietary necessities of the animals in many areas of the world. Selenium deficiency in plants occurs mainly due to low Se concentration in the soil where they grow. Low soil pH and high concentrations of sulphur and phosphorus also lead to a decrease in Se availability for plants (Hall et al., 2013). According to Cloete et al. (1994) and Van Ryssen et al. (1992), subclinical Se deficiencies exist in South Africa, and supplementation of Se in animals in the KwaZulu-Natal midlands showed a production response.

Several animal studies have investigated the effects of oral Se supplementation. Selenium supplementation rapidly improved the testosterone level and semen quality in various animal species (Shi et al., 2010a; Ren et al., 2012). Furthermore, oral supplementation has been endorsed as a preferred way to rapidly improve male reproductive performance by reducing the level of oxidative damage (Barragry, 1994). Therefore, this study hypothesised that Se supplementation would hasten the attainment of puberty in South African Merino ram lambs.

1.3. Problem statement

The deficiency of trace minerals, such as Se, has been found to reduce growth, reproduction, immunity and semen quality (Mayasula et al., 2021). Furthermore, in male animals, Se deficiency leads to growth retardation and eventually delays the attainment of puberty (Mojapelo & Lehloenya, 2019). An extreme delay in reaching puberty, an important reproductive trait, will postpone ram lambs from being utilised for breeding (Esmailizadeh, 2015). However, marginal to acute Se deficiencies have been found in the midlands and mountainous regions of the KwaZulu-Natal province, South Africa. These areas have predominantly acidic soils and receive rainfall above 500 millimetres (mm), which leads to the depletion of the soil's nutrients by leaching (Van Ryssen, 2001). Therefore, evaluating the effect of Se supplementation on the attainment of puberty in South African Merino ram lambs in areas with acidic soils and high rainfall is necessary as these areas have a high possibility of nutrient deficiencies.

1.4. Rationale of the study

Minerals play many essential roles in the body; they are needed in varying amounts depending on animals' requirements. Furthermore, their deficiency results in poor animal health and reproduction (Overton & Yasui, 2014). For example, the lack of trace minerals in the diet can reduce animal reproduction performance by 20–30% (Farrag et al., 2021). Therefore, supplementing trace elements is essential to ensure rapid growth, boost reproductive performance and improve the immune response (Overton & Yasui, 2014). The trace mineral Se is an essential component of selenoproteins and plays a vital role in many biological functions, such as antioxidant defence, the formation of thyroid hormones, DNA synthesis, fertility and reproduction. In addition, Se is an essential trace element required for maintaining male fertility; it plays a role in regulating the process of spermatogenesis and spermatozoa motility (Erkekoglu et

al., 2012). The attainment of puberty is required for reproduction. Thus, lambs that reach puberty at a younger age are used for breeding sooner, enhancing reproduction productivity. Therefore, Se supplementation of ram lambs will not only hasten the attainment of puberty but also boost growth and the immune system and increase overall reproductive efficiency.

1.5. Aim of the study

The overall aim of the study was to evaluate the effect of Se supplementation on the attainment of puberty, phenotypic parameters and semen quality and quantity of South African Merino ram lambs.

1.6. Objectives

The following objectives were formulated to achieve the overarching aim:

- Determine the effect of Se supplementation on the attainment of puberty in South African Merino ram lambs
- Determine the effect of Se supplementation on the phenotypic parameters including body weight, testicular measurements and sexual behaviour of South African Merino ram lambs
- Determine the effect of Se supplementation on semen quality and quantity of South African Merino ram lambs

1.7. Hypotheses

The following hypotheses were formulated:

- **H_1**: Selenium supplementation reduces the time to attain puberty in South African Merino ram lambs.
- **H_1**: Selenium supplementation improves the phenotypic parameters of South African Merino ram lambs.
- **H_1**: Selenium supplementation improves the semen quality and quantity of ram South African Merino lambs.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

This study aimed to evaluate the effect of Se supplementation on the attainment of puberty, and its impact on phenotypic parameters and semen quality of South African Merino ram lambs. This chapter critically analyses the literature on the effects of Se supplementation on male animals and presents the male physiological activities, focusing on puberty. The following aspects are discussed: puberty, the endocrine regulation of puberty, factors affecting puberty attainment and spermatogenesis. In addition, this chapter reviews the biological importance of Se, and its deficiency, toxicity and plant concentration.

2.2. Background

In small ruminants, the attainment of puberty depends on nutritional status, genetics and environment conditions (Arangasamy et al., 2018). Supplementation of essential dietary nutrients can improve puberty attainment in young sheep. Nutrients, such as proteins, energy, vitamins and minerals, impact the onset of puberty in sheep and goats (Al-Haboby et al., 2004; Khalifa et al., 2013; Elhammali & Elsheikh, 2014). Dietary deficiencies and imbalances of minerals such as Se, zinc and copper can result in poor growth, reproductive failure and many other disorders. These minerals, if supplied in adequate amounts, play a role in improving the quality of spermatozoa and fertility in males (Hafez & Hafez, 2000). Arangasamy et al. (2018) found that male animals supplemented with trace mineral zinc and copper attained puberty earlier than those in the control group. These results support the study conducted by Mojapelo and Lehloenya (2019), in which bucks supplemented with trace mineral Se attained puberty earlier than the control group.

Trace mineral nutrition is critical; even small variations in the diet have an important influence on animals' reproduction performance and health (Qazi et al., 2019). Organic minerals are more efficiently utilized in the body for optimum productive function and have been suggested to improve semen production, spermatozoa motility and male fertility (Rowel et al., 2011). Among the trace minerals, Se is a dietary trace element that is essential for numerous functions required by the body, including normal growth, reproduction, immune system and protection of tissues (Pilarczyk et al., 2013).

Furthermore, Se is an antioxidant that plays an essential role in preserving and improving the quality of semen in male animals (Ali et al., 2009) and protects the spermatozoa against oxidative damage that could arise due to an increase in reactive oxygen species (ROS). Jerysz and Lukaszewicz (2013) found that an elevation in ROS decreased fertility due to damage to cellular membranes and organelles. Selenium increases glutathione peroxidase (GSH-Px) antioxidant activity by reducing ROS, thereby preserving semen quality and promoting fertility in male animals (El-Mokadem et al., 2012). The National Research Council (2007) suggest that sheep require 0.1–0.2 mg Se/kilogram (kg) dry matter (DM).

2.3. Puberty

The onset of puberty in small ruminant species with seasonal reproduction activity is difficult to define due to the complex mechanisms underlying gonad development and sexual maturity (Emsen, 2005; Moulla et al., 2018). The main factors influencing the attainment of puberty during the pre-pubertal stage are an interaction between body weight, testis growth (Martinez et al., 2012), testosterone secretion and spermatozoa production (Souza et al., 2010).

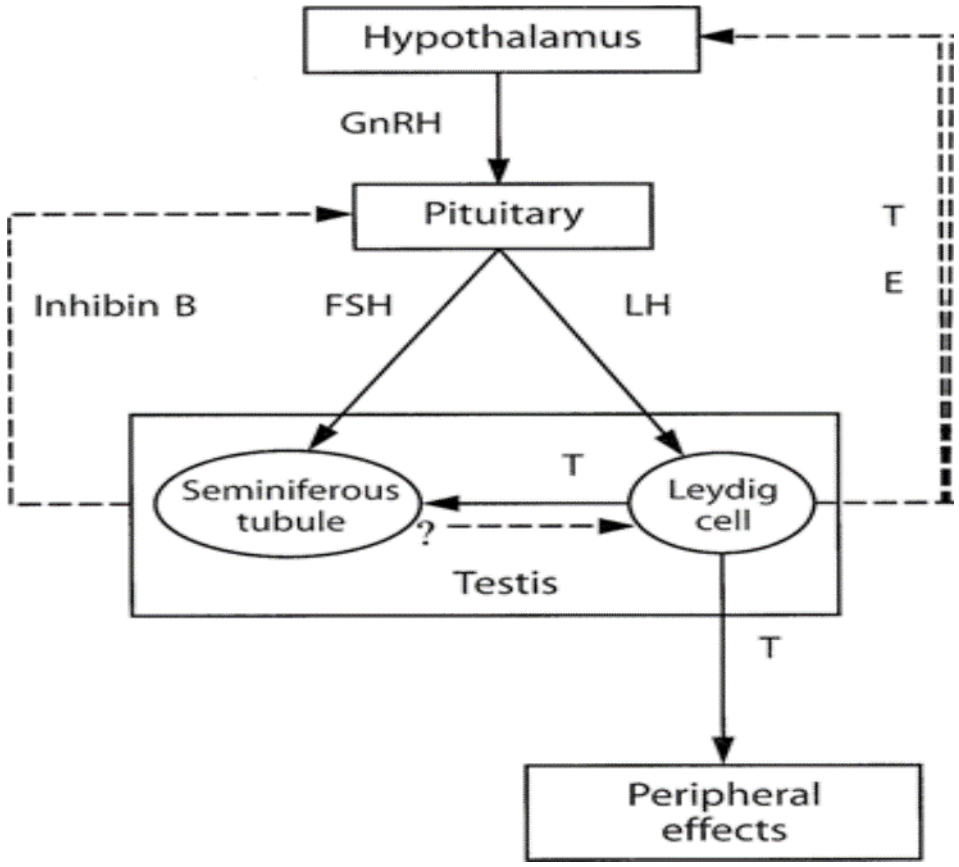
Hafez and Hafez (2000) defined the gradual change between increasing gonadotropic activity and the ability of gonads to simultaneously assume steroidogenesis and gametogenesis as *puberty*. During puberty, the hypothalamus stimulates an increase in the secretion of gonadotropin-releasing hormone (GnRH). This stimulates the release of the gonadotropins follicle-stimulating hormone (FSH) and luteinising hormone (LH), which in turn activates gonadal activity (Delemarre et al., 2008; Meza-Herrera, 2012). The initiation of spermatogenesis, a gradual increase in blood testosterone and changes in the LH secretion pattern are linked with a pubertal period, followed by rapid testicular growth (Geary et al., 2016).

Moreover, how the attainment of puberty in animals is defined depends on the sex. The age at which a young male animal displays mating behaviours that leads to breeding and ejaculation of semen containing a threshold number of live and viable spermatozoa adequate for fertilisation is defined as puberty (Bezerra et al., 2009; Valasi et al.,2012; Arangasamy et al., 2018). However, the exact time of males' puberty attainment is difficult to determine due to the complexity of testicular growth occurrence and the first differentiation of the spermatogenic cells that precede the

release of spermatozoa from the seminiferous tubules by a month or more (Hafez & Hafez, 2000).

2.3.1. Endocrine mechanism that regulates puberty attainment

Different physiological processes that occur in the animal body, including reproductive processes, are regulated by hormones. Figure 2.1 demonstrates the overall mechanism of the endocrine hormones regulating reproduction in rams. The hypothalamus, situated at the base of the brain, is responsible for releasing GnRH into the bloodstream. Gonadotropin-releasing hormone causes the pituitary gland to secrete FSH, also known as spermatogenic-stimulating hormone and LH, also known as interstitial cell-stimulating hormone in males (Hafez & Hafez, 2000).



Note. FSH: follicle-stimulating hormone, GnRH: gonadotropin-releasing hormone, LH: luteinising hormone, T: testosterone

Figure 2.1 *Endocrine hormone regulation in rams' reproduction (Letsoalo, 2017)*

Bester (2006) has illustrated that the level of FSH and LH in the testes rises during puberty attainment. Follicle stimulating hormone is essential for prepubertal Sertoli cell proliferation in several species (Oluwole et al., 2013). Furthermore, the number of

Sertoli cells in the testes determines its size and rate of spermatozoa production, and hence the males' reproductive capacity (Oluwole et al., 2013). Letsoalo (2017) established that the level of FSH at puberty reaches its peak and causes hypertrophy of the Sertoli cells and an increase in the diameter of the seminiferous tubules. Follicle stimulating hormone then stimulates the Sertoli cells to help convert spermatids to spermatozoa (Bester, 2006). Additionally, according to Hafez and Hafez (2000), FSH is critical in regulating spermiogenesis, the process that controls the formation of normal mature spermatozoa with fertilizing ability.

Pulsatile discharges of LH take place during the onset of puberty, leading to the differentiation of Leydig cells (Mojapelo, 2017). Luteinising hormone stimulates the testes' Leydig cells or interstitial cells, located outside the seminiferous tubules, to produce testosterone (Farshad et al., 2012). The testosterone then acts on the seminiferous tubules to initiate the onset of puberty and spermatozoa development (Bezerra et al., 2009). In the presence of testosterone, FSH stimulates spermiogenesis and the exhibition of mating behaviour activities, such as mounting, nosing, bleating, nudging, pawing, flehmen, licking, pelvic thrusts and penile erection, that are linked to sexual maturity (Nishimura et al., 2000; Farshad et al., 2012) and the maintenance of ideal conditions for spermiogenesis and semen ejaculation (Bester, 2006). Therefore, GnRH stimulates LH secretion towards the final maturation of the testes, including stimulation of the secretion of testosterone (Chenoweth & Lorton, 2014).

2.4. Factors affecting the attainment of puberty in sheep

2.4.1. Nutrition

Providing adequate nutrition is essential to accomplish the energy and nutrient requirements for animals' growth and reproduction. In addition, underfeeding of pre-pubertal animals may retard growth resulting in delayed puberty, primarily due to low LH secretion resulting from the altered secretion of GnRH (Polkowska et al., 2003; Valasi et al., 2012), and high-plane feeding may advance puberty in animals (Valasi et al., 2012).

Low reproductive performance has repeatedly been attributed to nutritional deficiencies. Weight loss and delayed puberty attainment result from low energy and protein shortages, which change the rate of weight gain. Nutritional deficiencies in males can hinder the onset of puberty; these deficiencies can also lower the

production of semen and semen characteristics (Arangasamy et al., 2018). Furthermore, severe nutritional restrictions can result in permanent damage to the gonads and neural tissues (Brown, 1994; Martinez et al., 2012).

Nutrition plays a major role in various reproduction functions, including normal growth development, hormone production and spermatogenesis (Garcia-Garcia, 2012). Balanced nutrition increases average daily growth rate and body weight and improves sexual maturity and testicular development. Over and underfeeding young male animals can delay the testes from descending to the scrotum, penile development and the first appearance of spermatozoa (Brown, 1994). Furthermore, young males exhibit retarded sexual development and delayed puberty during undernutrition; this is due to the suppression of the endocrine activity of the testes and, consequently, growth retardation and function of the male reproduction organs (Hafez & Hafez, 2000).

The reproductive functions in young animals seem to be more susceptible to restrictions in energy and protein than in adults. Post-weaning nutritional management strongly influences body weight increase, which has been associated with testicular growth and the onset of puberty in Menz breed rams (Mukasa-Mugerwa & Ezaz, 1992; Martinez et al., 2012). Ram lambs reach puberty with a body weight of 40 to 60% of their adult weight if provided with balanced and optimal nutrition (Van Tilburg et al., 2014). Larger quantities of proteins than those required for maintenance and growth improve the onset of lambs' puberty (Abi Saab et al., 1997). Moreover, Adam et al. (1998) have found that rams' delayed pubertal parameters, such as growth development, body weight, scrotal circumference, testicular growth, first ejaculation of semen and initiation of puberty, are a result of undernutrition. Additionally, testicular size and spermatozoa production may be affected by changes in protein ingestion, even when such changes exceed the maintenance requirements (Fernández et al., 2004).

2.4.2. Season of birth and photoperiod

Reproduction in sheep and goats is seasonal and mainly controlled by environmental factors, including the photoperiod that measures the length that animals are exposed to light in 24 hours (Chemineau et al., 1991). The season of the year and the photoperiod are environmental factors that also control seasonal reproduction in many other mammalian species. In seasonal breeders, which only breed during certain times

of the year, the photoperiod affects the reproductive functions and influences each animal's reproductive life by regulating puberty's onset (Chasles et al., 2019). Attainment of puberty only occurs during the breeding season in temperate (Chasles et al., 2019) and subtropical (Delgadillo et al., 2007) latitudes. The puberty age differs according to the season of birth and animals' exposure to different photoperiods, which depend on the time of the year they were born (Hafez & Hafez, 2000). For example, lambs born in autumn or spring reach puberty during the first autumn after birth, provided that food limitation does not affect their body weight (Papachristoforou et al., 2000). Abecia et al. (2021) found that ram lambs born in autumn attained puberty sooner than those born in spring. The absence of photoperiodic stimulation was related to changes in day length between 5.5 and 7 months of age and caused an earlier decline in young autumn-born rams testes' weight (Abecia et al., 2021). In comparison, during decreasing daylight length in spring-born animals from temperate climates, a steady increase in testicular size and growth was observed, leading to the onset of puberty (Camela et al., 2019).

The photoperiod and season of birth contribute significantly to the start of the spermatogenesis process in ram lambs (Maquivar et al., 2021). The spermatogenesis process increases with a decrease in daylight length, owing to the production of melatonin, which ultimately stimulates the release of the GnRH (Ridler et al., 2012). Ram lambs born in autumn are first exposed to long, followed by shorter daylight periods, which may result in faster sexual development (Hafez & Hafez, 2000). Young rams exposed to long days (16 hours light/day) for 2 months attain puberty early (Chemineau et al., 1992). Long days increase thyroid hormone availability in the basal hypothalamus by inducing increased type 2 iodothyronine deiodinase gene expression in sheep, leading to the conversion of thyroxine to the more active form, triiodothyronine, which in turn either suppresses or activates GnRH pulse release (Ebling, 2010). Parkinson et al. (1995) found that the thyroidectomy of 2-month-old ram-lambs increased the rate of testicular growth over 5 months; the authors concluded that the onset of puberty was thyroid hormone-dependent.

2.4.3. Age and body weight

Attainment of puberty in male sheep varies within breeds depending on age and body weight (Zarkawi & Darker, 2018). Generally, size and body weight impact puberty

attainment more than age (Hafez & Hafez, 2000). Rams attain puberty at 40–60% of the body weight of matured rams and between 5 to 8 months (Elhammali & Elsheikh, 2014). Jafari and Hashemi (2014) found that lambs with higher body weights tend to attain puberty earlier than those with lower body weights. Furthermore, it has been established that body weight is a better measure than age for ram lambs' puberty attainment (Al-kawmani et al., 2018), as sexual development is more closely related to body weight. In addition, males' live body weight and testicular size have been found to generally indicate the production of viable spermatozoa (Agga et al., 2011).

The highly significant correlation between testicular weight and age (approximately 80%) indicates that about 80% of the variation in testicular weight is associated with body weight in rams (Cloete et al., 2000). The testes usually start to increase in size at around 8 to 10 weeks and between 16 to 20 kg body weight (Kerketta et al., 2015). An increase in testicular size indicates the onset of active spermatogenesis, which coincides with the enlargement of the seminiferous tubules and the appearance of primary spermatocytes (Bester, 2006). Between 4 to 6 months of age and with a live weight of 40 to 60% of mature body weight, copulation with an ejaculation of viable spermatozoa can occur; however, there are breed differences (Ramukhithi et al., 2017).

Sexual maturation involves modifications in the effects of gonadal steroid and non-steroid factors on LH and FSH secretion. The increase in production of gonadotrophic hormones starts from 4 months of birth to puberty in male sheep. A functional negative feedback system exists between testosterone and LH before puberty in male sheep (Smith & Clarke, 2010). However, LH secretion begins much earlier in small ruminant males, at about 8–12 weeks of age, and initiates a gradual increase in testosterone secretion, leading to the sustained development of the testicular function, including the time to complete spermatogenesis (4–8 weeks) (Valasi et al., 2012). However, because of the length of the process, lambs are usually not capable of successful breeding until they are about 7–8 months or more of age (Foster, 1994; Valasi et al., 2012). The pubertal increase in testicular volume, which reflects increased spermatogenesis and steroidogenesis, begins at 3–5 months of age (Wood et al., 1991).

2.5. Spermatogenesis

Ram lambs' maturation and spermatogenesis occur at about 2–3 months old. Spermatogenesis transpires in the seminiferous tubules within the male animals' testes; the process is initiated at puberty as the result of stimulation by anterior pituitary gonadotrophic hormones and continues until death (Hafez & Hafez, 2000; Mohlomi, 2015). After spermatozoa formation in the seminiferous tubules, the spermatozoa are forced through the rete testis and vasa differentia into the epididymis, where they are stored while undergoing maturation changes that ensure the spermatozoa are capable of fertilisation (Hafez & Hafez, 2000; Munyai, 2012; Lestoalo, 2017). According to Barchi et al. (2019), the process consists of three main stages:

- Spermatocytogenesis, during which spermatogonia develop into spermatocytes
- Meiosis, which is the maturation division of spermatocytes that results in spermatids with a reduced (haploid) number of chromosomes
- Spermiogenesis, or the process of alteration of spermatids into spermatozoa

The spermatogenesis process typically takes 40–49 days to be completed in rams (Bester, 2006; Munyai, 2012).

2.6. Se

Selenium is an essential trace element which plays an important biological role in the antioxidant, reproductive, endocrine and immune systems of animals (Gresakova et al., 2013). In addition, Se plays a critical function in several biological processes, including fertility in both male and female animals (Ahsan et al., 2014). Furthermore, Se is a component of several enzymes, including GSH-Px, iodothyronine deiodinases and thioredoxin reductase (Birringer et al., 2002), which enhances its role as an antioxidant. Biological functions related to Se, which occurs mainly through selenoproteins P, W, R, T and N, include thyroid metabolism and immune and endocrine function; it also defends membranes from oxidative stress by free radicals (Bano et al., 2018).

2.6.1. Biological importance of Se

Selenium is required by livestock to maintain normal physiological functions. Furthermore, Se is an important source of dietary antioxidant defence (Sordillo, 2013)

and protects the body against oxidative damage (Bano et al., 2018). Oxidative stress, which harms cells and tissues, results from the production of highly unbalanced compounds, known as free radicals or oxidants (Gaur et al., 2021). If oxidative stress is not controlled, it can lower the immune system of animals (Yatoo et al., 2013). In addition, free radicals can damage the body's biological components by producing lipid peroxidation, protein carboxylation and DNA strand breakages, eventually leading to various clinical consequences (Gaur et al., 2021). However, Se protects the tissues against oxidative damage, which improves immune competence (Ziaei, 2015). Selenium also serves as a component of an enzyme of type I iodothyronine-5´-deiodinase (D1) that is essential for converting thyroxin into biologically active triiodothyronine (Yatoo et al., 2013).

Selenium is an essential component of GSH-Px, the most well-known selenoprotein and an antioxidant enzyme that protects tissues against oxidative damage (Rayman, 2000). Consequently, Se plays a significant role in scavenging free radicals as a component of GSH-Px, in addition to regulating prokaryotic and eukaryotic cell survival and maintaining the integrity of intracellular organelles (Hostetler et al. 2003). Furthermore, Se is reported to elevate GSH-Px's activity, which reduces ROS and conserves semen quality in rams (Kendall et al., 2000). An increase in male sexual activity expressed by shortening mating and ejaculation times and increased mating frequency has been reported in animals whose diet is supplemented with Se (Al-Haboby et al., 2004).

2.6.2. Se deficiency

Since Se is an essential mineral, deficiency can have far-reaching consequences. For example, Se deficiency stops the synthesis and functioning of GSH-Px, which protects against peroxides produced in the intermediate metabolism of the cells with the oxidation of fats and proteins of the membranes. Diets deficient in Se allow oxidative damage, increasing the risk of injury to physiological processes (Sadeghian et al., 2012).

Moreover, a deficiency of Se may lead to reproductive organ damage, such as degenerative spermatogonium, testicular damage and degeneration of the seminiferous tubules (Yue et al., 2010). Additionally, a low intake of Se can result in spermatozoa maturation disturbances. Ahsan et al. (2014) reported that an abnormal

spermatozoa process could result in poor semen quality, leading to male infertility. The deficiency of Se in the diet has been associated with spermatozoa having more gaps between successive mitochondria, mitochondrial swelling and loss, cytoplasmic droplets, unattached contact of a mitochondrial helix with the plasma membrane, outer dense fibre cleavage, and the presence of midpiece and principal piece inside a common plasma membrane (Shalini & Bansal, 2008). Selenium deficiency has also been related to several diseases in lambs, including white muscle disease and immunity repression (Alimohamady et al., 2013).

Selenium shortages can occur when the soil is poor in Se or contains high levels of other minerals competing for usage by pasture plants. Small amounts of Se, <0.5 milligram (mg)/kg in the soil or <0.1 mg/kg in plants, are considered deficient based on a minimum animal requirement of 100 µg Se/kg feed (Courtman et al., 2012). The availability of other minerals, such as sulphur, copper, arsenic and calcium, can restrict the use of Se by plants growing in soils with adequate levels of Se. In addition, the presence of these and similar minerals or polyunsaturated fat and nitrates in the diet can decrease Se absorption in the small intestine (Hefnawy & Perez, 2010). Therefore, Se intake must meet animals' needs as low concentrations in feed can lead to a deficiency (Hall et al., 2013).

Research has shown that Se deficiency can be reduced or prevented by supplementation with inorganic or organic sources. Still, excessive supplementation and consumption of Se-accumulating plants may lead to Se toxicity and animal poisoning (Saha et al., 2016).

2.6.3. Se toxicity

Dietary sources that contain >5 mg Se/kg DM may lead to toxicity in sheep (Wu et al., 2015). Selenium in an organic form, such as Se-enriched yeast, has proven to be more toxic than inorganic Se from sodium selenite (Pavlata et al., 2012). Selenium toxicity can be indicated by poor wool quality and growth, lameness, oedema of the coronary band, loss of appetite and a poor general condition in sheep (Zaki et al., 2018).

2.7. Plant Se concentration

Selenium is not an important element for the survivability of plants; however, it may still be incorporated into plant cells (Avci & Deveci, 2013). Cereals and forage crops

convert the Se they absorb from the soil mainly into selenomethionine (SeMet) and then into plant protein in place of methionine (Met); this is because the Met transfer ribonucleic acid (tRNA) does not differentiate between Met and SeMet (Schrauzer, 2000).

The concentration of Se in plants depends mainly on the soil's water-soluble or plant-available Se content. Both inorganic and organic forms of Se occur naturally in the soil (Adotey et al., 2011). There are three oxidation states in which inorganic Se is found, namely selenite, selenate and selenide. Plants obtain selenite or selenate from soil to synthesise Se-containing amino acids. Soils that lack Se or contain a form that is unavailable to plants and the presence of any substance in plants that binds Se may reduce its availability (Mehdi et al., 2013).

The concentrations of minerals in forages are influenced by soil type, the presence of antagonistic elements and contaminants, fertilisation, forage species, weather, season and plant maturity (Marijanušić et al., 2017). These factors may modify and stop the animals' ability to meet their trace mineral requirements (Fordyce, 2007). For example, volcanic soils have virtually no Se but high sulphur levels; sulphur competes with Se for absorption. Consequently, plants that grow in volcanic soil and animals that consume them suffer from Se deficiency (Gupta & Gupta, 2017).

Furthermore, the Se concentration of plants is determined by the soils' Se availability and concentration, pH and redox equilibrium and the plants' genetics (Haug et al., 2007). The Se concentration of the parent rock, the intensity of weathering and leaching, contamination of the soil and atmospheric deposition of Se also impact Se's availability in soil (Haug et al., 2007). Selenium occurs in different forms with various bio-availabilities, depending on the soil's redox potential. These forms include selenides, elemental Se, selenites, selenates and organic Se (Schiavon & Pilon-Smits, 2017).

Selenium is mainly available to plants growing in high rather than low pH soils, and Se deficiencies occur in areas receiving high rainfall and having low pH soils (Alfthan et al., 2015). Fertilisers play a role in soil pH and affect the Se content of plants, either through interaction with Se in the soil or through a dilution effect by increased plant DM yield (Alfthan et al., 2015).

Additionally, plants' Se concentration is influenced by genetics. Plants that grow on seleniferous soils seem to accumulate more Se (Courtman et al., 2012). Plants containing high Se concentrations are usually unpalatable and are only eaten if other forages are unavailable. Although the uptake of Se by plants does not constantly relate directly to the soil Se concentration, information on high and low soil Se areas is still beneficial to help identify areas which are likely to be toxic or deficient (Courtman et al., 2012). Supplementation in animal feed is essential in areas with low Se levels in soil.

2.8. Role of Se in semen quality

Male fertility depends on spermatozoa maturation, and an abnormal process may lead to poor quality semen and reduced fertility. Selenium is an essential trace element for maintaining spermatogenesis in male animals (Shi et al., 2020). In addition, Se is necessary for the biosynthesis of testosterone to maintain male fertility (Brown & Arthur, 2001). Slowińska et al. (2011) and Ahsan et al. (2014) found that Se deficiency or low Se status was associated with various reproductive disorders, including abnormal testicular morphology, poor semen quality and impaired spermatozoa structure. Moreover, Kleene (1993) described male hypogonadism in males bred on a low Se diet. In contrast, Se supplementation improved testosterone levels and semen quality in animal species, including sheep and goats (Shi et al., 2010b; Shi et al., 2017).

The presence of adequate Se in the male reproductive tract is vital for normal spermatogenesis and has a fundamental role in mammalian spermatozoa maturation (Ahsan et al., 2014). A lower or higher level of Se than required leads to disruption in spermatozoa maturation. Stefanov et al. (2018) observed that Se increased the maturation of spermatozoa in the epididymis and reduced the amount of spermatozoa with cytoplasmic droplets. Likewise, Mahmoud et al. (2013) reported that semen quality, including mass motility and percentage of live and dead spermatozoa and quantity (semen volume and concentration), increased with Se supplementation in Ossimi rams.

The production of ROS is beneficial and essential for hyperactivation, capacitation and the acrosome reaction. However, the overproduction of ROS can be harmful to spermatozoa (Lambrechts & Gulyas, 2019). Increasing the Se status increases GSH-

Px antioxidant activity, reducing ROS levels, which increases male fertility (Irvine, 1996).

2.9. Summary

This chapter critically analysed and provided an overview of the existing research, theory and evidence related to the effect of Se supplementation in male animals, more especially sheep. Nutrition is a major factor affecting the attainment of puberty in small ruminants. Nutrition restrictions reduce gonadotropin levels, which results in delaying the onset of puberty. Soils' Se content differs significantly depending on the soil type and pH. High concentrations of other minerals, such as sulphur and phosphorus, cause decreased Se availability for plants. Selenium is important for various physiological processes, including growth, immunity, spermatogenesis and semen quality. In addition, Se increases the body's antioxidant activity, decreasing ROS and preserving semen quality. Therefore, supplementation can benefit animals in areas with low levels of Se in soil and plants. Chapter 3 describes the research methodology adopted for this study.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

This chapter provides an overview of the research design and methodology used to determine the effect of Se supplementation on the attainment of puberty of South African Merino ram lambs. The experimental site and design are described; thereafter, the ethical considerations and experimental animals' management are discussed. The chapter outlines how the research was conducted, explaining the process used to select the animals into groups, the method used to collect semen, how body weight and testicular measurements were measured, and the approach used in blood collection and analysis.

3.2 Experimental site

The experiment was conducted at Kokstad Research Station, located in the Harry Gwala District municipality of the KwaZulu-Natal province, South Africa, latitude South 30° 31'16 72 and longitude East 29° 24' 30 38. The vegetation of this area is classified into two regions, Gs 12 East Griqualand Grassland and Gs 10 Drakensberg Foothill Moist Grassland (Mucina & Rutherford, 2006). This area receives a mean annual rainfall of 750 mm. The farm's mean maximum summer temperature is 25°C, and the mean minimum winter temperature is −1°C (Magawana et al., 2021).

3.3 Experimental animals and ethics clearance

The experimental procedures were approved by the University of Zululand Research Ethics Committee (UZREC); the ethical clearance number is UZREC 171110-030 PGM 2020/36 (Appendix A). A total of forty South African Merino ram lambs (Figure 3.1) born during the autumn lambing season (April 2019) were randomly selected and used in this experiment. Lambs were born on natural vegetation and weaned on planted pastures of ryegrass, chicory and oats; water was provided *ad libitum* throughout the study.

Figure 3.1 *Experimental South African Merino ram lambs*

After weaning at the age of 3 months, lambs were dosed with 4 millilitres (mL) of Valbazen® (Zoetis South Africa (Pty) Ltd., Sandton, RSA) and 2.5 mL of Vecoxan® (Elanco Animal Health Inc., Indiana, USA). At the age of 4 months, the lambs were vaccinated with Covexin®10 (Cooper Veterinary Products (Pty) Ltd., Johannesburg, RSA) for clostridial diseases and dosed with 6 mL of Nem-A-Rid® (Intervet South Africa (Pty) Ltd., Johannesburg, RSA). At 5 months, sheep were vaccinated for Bluetongue A viral disease (Product code 2013, Onderstepoort Biological Products, Ltd., Pretoria, RSA).

3.4. Experimental design

The experiment was conducted from winter to spring (July to November 2019). At the onset of the experiment, forty ram lambs aged 3.7 months were used. The animals were grouped according to their body weight and randomly allocated into two groups of 20 animals each, the treatment (Se-supplemented) and control (non-supplemented) groups. The average body weight was 24.00 ± 0.33 kg and 24.30 ± 0.33 kg for the Se-supplemented and control groups, respectively.

3.5. Se treatment

The treatment group had sodium selenite (Associated Chemical Enterprises (ACE) (Pty) Ltd., Johannesburg, RSA) administered orally at two-week intervals throughout the experimental period. Animals received a dose of 0.34 mg Se/kg body weight, as adjusted by Mojapelo and Lehloenya (2019), using a drenching gun (Figure 3.2).

Figure 3.2 *Se oral administration using a drenching gun*

3.6. Sexual behaviour evaluation

For sexual behaviour activities, the treatment and control rams were exposed weekly for 15 minutes in the morning to teaser ewes (Mojapelo & Lehloenya, 2019). The sexual behaviour activities were evaluated from the onset of the experiment until the rams reached puberty. Lambs were monitored closely for the following sexual behaviour activities: nosing, nudging, flehmen, protrusion of the penis and mounting, as described by Nishimura et al. (2000).

3.7. Body weight and testicular measurements

Body weight for the treatment and control groups was recorded weekly, starting from 3.7 months and continuing until the end of the experiment. An electronic scale (Model LS4, Libra Measuring Instruments (Pty) Ltd, Pretoria, RSA) attached to a crate of internal dimensions 1250 mm × 500 mm, was used to determine body weight (kg) (Figure 3.3).

Figure 3.3 *Body weight measurements*

The scrotum circumference (SC) was measured weekly in centimetres (cm) with the aid of a flexible measuring tape (Lasec® SA (Pty) Ltd., Midrand, RSA) at the widest part of the testes, after the testes had been firmly pushed into the scrotum (Figure 3.4) (Fourie et al., 2002).

Figure 3.4 *Scrotum circumference measurements*

Testicular measurements were recorded weekly in mm throughout the experiment using a vernier calliper (Lasec® SA (Pty) Ltd., Midrand, RSA). The testicular length (TL) was measured from the top part of the testis to the head of the epididymis (Figure 3.5).

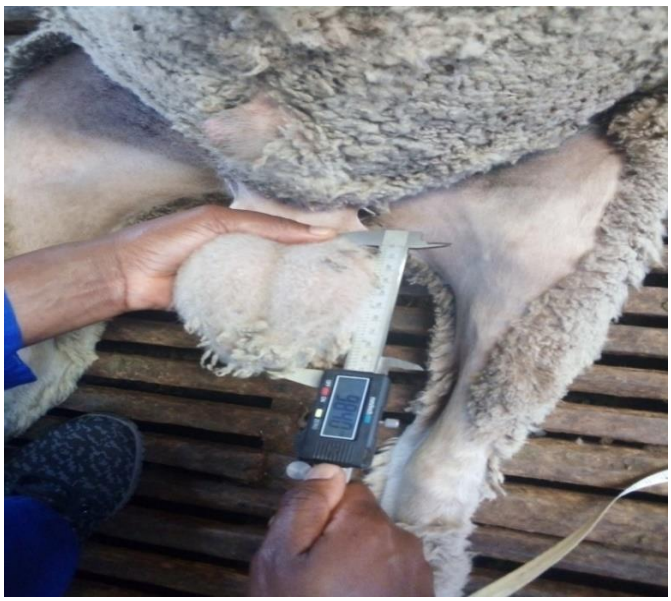


Figure 3.5 *Testicular length measurement*

The testicular breadth (TB) was measured at the largest diameter of the testis. All dimensions were done for the right and left testes (Mahmoud et al., 2013). Body weight, testicular measurements and SC were performed simultaneously, starting at the first oral administration of Se.

3.8. Enzyme activity, cortisol and reproductive hormones assays

3.8.1. Blood collection

Blood samples were collected from six ram lambs per treatment group for enzyme activity, cortisol and reproductive hormone determination (Figure 3.6). The animals were selected using similar procedures to allocating them into treatment groups. Blood samples were collected using a BD Vacutainer® needle (18G × 1.5” [1.2 × 38 mm], Becton, Dickinson & Company [BD], Plymouth, UK) and a 6 mL BD Vacutainer® clot activator tube (BD, Plymouth, UK) from the jugular vein. Blood samples were collected from the onset of the experiment and at two-week intervals throughout the experimental period.

Figure 3.6 *Blood collection from a South African Merino ram lamb*

After collection, blood samples were centrifuged in a Rotofix 32 A centrifuge (Product 1206, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 3000 rpm for 20 minutes to recover serum. Serum aliquots of 2 mL were harvested and stored at −20°C until assayed to determine the concentration of LH, testosterone and cortisol, and the GSH-Px activity.

3.8.2. Analysis of hormones and enzyme activity

Each assay was conducted following the manufacturers' procedures. Serum GSH-Px concentration was determined using a sheep GSH-Px assay kit (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd, Shanghai, China). The LH concentration was determined using sheep LH enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd, Shanghai, China). Testosterone and cortisol concentrations were determined using sheep testosterone and sheep cortisol ELISA kits supplied by the Shanghai Korain Biotech Co., Ltd, Shanghai, China.

Briefly, 40 microlitre (µL) of the serum samples and 50 µL of the standards were pipetted into wells coated with antibodies of the specific hormone or enzyme tested. Then 10 µL of anti-GSH, cortisol, testosterone, or LH antibody were added to the coated sample wells. Subsequently, 50 µL of streptavidin-horseradish peroxidase (HRP) was added to the sample and standard wells (Figure 3.7). After adding streptavidin-HRP, the plate was sealed and incubated for 60 minutes before washing the plate five times with the buffer provided. After that, 50 µL of the substrate solutions A and B were added to each well, followed by incubation for 10 minutes at room temperature in the dark. Then, 50 µL of stop solution was added to each well. A microplate reader was incubated for 10 minutes before optical density measurements were made. The optical density was determined using a Multiskan™ Go Microplate reader (Thermo Fischer Scientific Inc., Massachusetts, USA) set at an absorbency of 450 ± 10 nm. A standard curve was generated to determine the concentration of the specific hormone using the known absorbance and concentration of the standard samples. The standard curve range for GSH-Px, cortisol, testosterone and LH were 0.5–300 nanogram (ng)/mL, 0.5–60 ng/mL, 1– 400 ng/mL and 0.5–150 milli-international units (mIU)/mL, respectively.

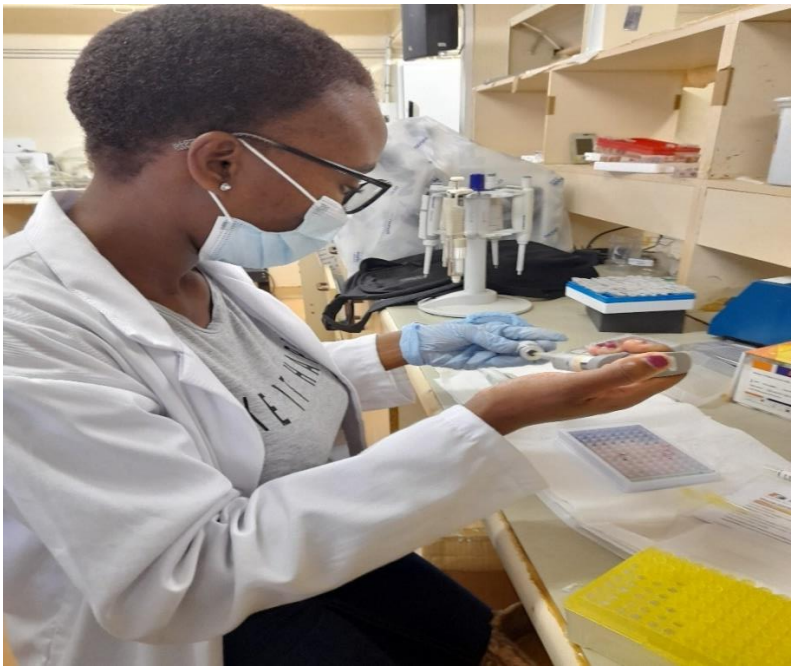


Figure 3.7 *Adding streptavidin-horseradish peroxidase to wells*

3.9. Semen collection and analysis

3.9.1. Semen collection

Semen was collected from only ten ram lambs per group, which were selected using a similar procedure to allocating them into treatment groups. The same twenty ram lambs were used for semen collection for the study's duration to ensure the consistency of the results. A Pulsator IV electroejaculator (EE) (Lane Manufacturing Inc., Denver, USA) was used for semen collection (Figure 3.8).

Figure 3.8 *Electroejaculator used for semen collection*

Semen was collected weekly during spring (October to November) for seven weeks (Nur et al., 2010) in the morning between 07:30 and 09:00. Attempts for semen collection started when ram lambs were 5.5 months old. This experiment consisted of seven replicates of semen collection.

3.9.2. Ejaculate collection using an electroejaculator

Before semen collection, the hair around the sheath was shaved using scissors, and the area was cleaned using water. After cleaning, the prepuce was wiped with a sterile paper towel (Kimberly-Clark Southern Africa (Holdings) (Pty) Ltd., Sandton, RSA) to prevent contamination. The sheath was then pushed back, and the penis was gently grabbed using a sterile gauze swab (Afrivet, a Bimeda® Company, Pretoria, RSA). A rectal probe was washed with water, wiped with a paper towel and lubricated with glycerol (ACE (Pty) Ltd., Johannesburg, RSA) before insertion in the rectum. The probe was inserted and placed in the rectum above the accessory sex glands (Dombo, 2002). Pre-warmed (37°C) 15 mL graduated Falcon™ tubes (Lasec® SA (Pty) Ltd.,

Midrand, RSA) were used to collect the semen samples. The electrical stimulation began with a low voltage (3–5 volts (V) for 3 seconds (s), at 6 s intervals); the rhythmic patterns were repeated until the animal ejaculated (Chella et al., 2017). If the ram did not ejaculate after five repetitions, it was released without semen collection; subsequently, semen was collected in the following collection with the rest of the group. The collected semen was stored at 37°C in a water bath, and the evaluation was performed within one hour of collection (Zamiri et al., 2010).

3.9.3. Laboratory semen evaluation

After collection, the samples were taken to a laboratory for the evaluation of semen characteristics, which were categorised into macroscopic (appearance, volume and pH) and microscopic (mass motility, progressive motility, concentration, morphology and viability) assessments.

Semen volume: The semen volume of the ejaculates was measured by reading the measurements on the 15 mL graduated Falcon™ tube (Lasec® SA (Pty) Ltd., Midrand, RSA). Semen volume was recorded in mL.

Semen appearance: Semen appearance was evaluated according to Hafez and Hafez (2000) on a scale of 0–5, with 0 being *clear* and 5 being *thick creamy* (Table 3.1).

Table 3.1 *Estimating spermatozoa concentration based on semen appearance*

Scale	Gross appearance	Approximate sperm concentration 10 sperm/mL
0	Clear to cloudy	0 to 200
1	Cloudy to milky	200 to 400
2	Milky	400 to 800
3	Thick milky	800 to 1200
4	Creamy	1200 to 1800
5	Thick creamy	1800+

Semen pH: Semen pH was determined using litmus pH papers (colour-fixed indicator strips) (MACHEREY-NAGEL Gmbh & Co. KG, Düran, Germany). A drop of 10 µL of semen was placed on the litmus paper, and the colour change was assessed after 15

minutes by comparing the colour between litmus pH paper and the suggested colour indicators.

Spermatozoa mass motility: Mass motility was determined using a mass motility score of 0 (*no swirl*) to 5 (*fast distinct swirl*) (Table 3.2) (Hafez & Hafez, 2000). A drop of (10 µL) semen was placed on a pre-warmed slide without a coverslip and examined using a Leica DM500 phase contrast microscope (Leica Microsystems, Wetzlar, Germany) at 40 × magnification (Malejane et al., 2014).

Table 3.2 *Mass motility rating scale*

Rating	Microscopic appearance
0	No – nil or sporadic oscillation of individual sperm
1	No swirl – generalised oscillation of individual sperm only
2	Very slow distinct swirl
3	Slow distinct swirl
4	Moderately fast distinct swirl
5	Fast distinct swirl – the appearance of good quality ram semen

Spermatozoa progressive motility: The spermatozoa progressive motility was examined according to the protocol of David et al. (2015) using a rating score of 0 (*no spermatozoa movement*) to 5 (*very rapid spermatozoa movement*) (Table 3.3). Spermatozoa progressive motility was evaluated by adding 10 µL each of semen and saline solution on a pre-warmed slide. The slide was then covered with a coverslip and examined under a phase contrast microscope at 40 × magnification.

Table 3.3 *Evaluating progressive spermatozoa motility*

Score	Microscopic appearance	Progressive sperm motility (%)
0	No sperm movement	No motility
1	Slight tail undulation without forward motion	20
2	Slow tail undulation with slow or stop and start forward motion	40
3	Forward progression at a moderate speed	60
4	Rapid forward progression	80

Score	Microscopic appearance	Progressive sperm motility (%)
5	Very rapid progression in which cells are difficult to follow visually	100

Spermatozoa viability: Live and dead spermatozoa were determined using eosin-nigrosin stain (Morphisto GmbH, Hessen, Germany). A mixture of semen (10 µL) and eosin- nigrosin (10 µL) was placed on a clean pre-warmed (37°C) slide and smeared using another slide. The smeared slide was then allowed to dry for 10 minutes before examination (Figure 3.9). A coverslip was then placed on top of the slide, and it was examined microscopically at 40 × magnification.

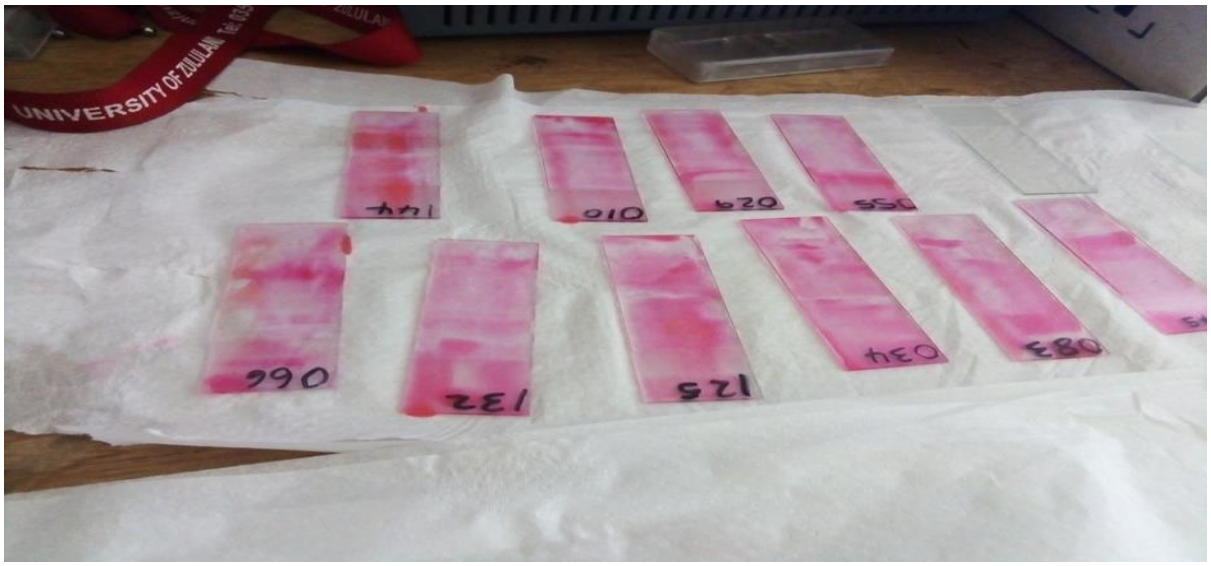


Figure 3.9 *Eosin nigrosin stained slides*

A total of 100 spermatozoa randomly selected from five microscopic fields were counted to determine the percentage of live and dead spermatozoa. The spermatozoa that did not absorb stain and appeared white or clear were considered live; those that absorbed stain and appeared pink were considered dead (Akpa et al., 2012; Malejane et al., 2014).

Spermatozoa morphology: The slides prepared to examine spermatozoa viability were also used to evaluate spermatozoa morphology. A total of 100 spermatozoa randomly selected from five microscopic fields were counted. The live spermatozoa were categorised into normal and abnormal spermatozoa. Spermatozoa abnormalities were evaluated in two ways. First, abnormalities occurring at a particular location were

observed at the head, midpiece and tail of the spermatozoa (Bearden et al., 2004; Jothipriya et al., 2014) (Figure 3.10). Then, the spermatozoa abnormalities were further categorised into primary, secondary and tertiary abnormalities. The primary spermatozoa abnormalities included abnormal acrosomes and an elongated midpiece (Mojapelo, 2017). The secondary spermatozoa abnormalities included detached and loose heads (Munyai, 2012). Finally, the tertiary spermatozoa abnormalities included bent and broken tails (Chenoweth & Lorton, 2014).

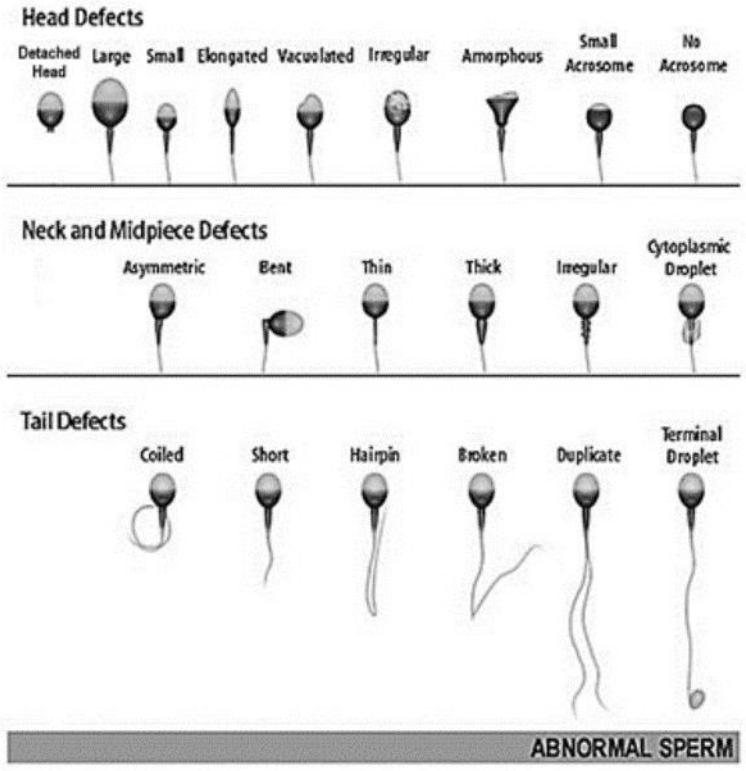


Figure 3.10 *Schematic diagram of spermatozoa abnormalities (Jothipriya et al., 2014)*

Spermatozoa concentration: The spermatozoa concentration was estimated by diluting 10 µL semen into 100 µl water. Then a 10 µL solution was pipetted into the chamber of a Neubauer haemocytometer (Hauser Scientific, Horsham, Pennsylvania, USA) (Karagiannidis et al., 2000; Kheradmand et al., 2006). The haemocytometer was then covered with a coverslip and placed on a Leica DM500 light microscope (Leica Microsystems, Wetzlar, Germany), and the contents were allowed to settle for four minutes. The light microscope was used under 40 × magnification to count the

spermatozoa. The following equation (Lukusa & Lehloenya, 2017) was used to calculate spermatozoa concentration:

$$\text{Concentration} = (\text{number of spermatozoa counted} \times \text{dilution}) / (\text{number of squares} \times 4) \text{ in million/mL}$$

3.10. Data analysis

A randomized complete design experiment was used. Analyses of variance (ANOVA) was performed using repeated measurements for pubertal traits, semen characteristics, reproductive hormones, cortisol and GSH-Px concentrations. The standardised residuals were tested for deviations from normality using the Shapiro-Wilk test. In cases where significant deviation from normality occurred due to skewness, outliers were removed until a normal or symmetrical distribution was obtained. The mean ± standard error (SE) were compared using the Student's t-test and least significant difference (LSD) test at a 5% significance level. All data analyses were performed using Statistical Analysis Systems® (SAS®) software (Version 9.4, SAS Institute Inc., 2012). The scores were subjected to a 1:1 frequency table, and a Chi-Square (χ^2) test was performed to test for equal proportions of sexual behaviour activities. Contingency (RxC) frequency tables were constructed to determine the association between the treatments. Pearson's correlation coefficient (r) was calculated to show the relationships between pubertal traits, semen characteristics and reproductive hormones.

CHAPTER 4: RESULTS

4.1. Introduction

This chapter presents the data analysis and results to test the hypotheses (Section 1.7) and achieve the overarching aim (Section 1.5) and objectives (Section 1.6), which was to evaluate the effect of Se supplementation on the attainment of puberty, phenotypic parameters and semen quality of South African Merino ram lambs. The results are presented in the form tables, diagrams and descriptions.

4.2. Effect of Se supplementation on the attainment of puberty

At the age of 5.5 months, none of the South African Merino ram lambs ejaculated semen. However, 80% of the treatment group ejaculated semen containing live spermatozoa at six months compared to 60% in the control group. At 6.5 months, motile spermatozoa were collected in 100% of the experimental South African Merino ram lambs. At the age of 6.5 months, ram lambs reached puberty at a body weight of 40.80 ± 0.33 and 41.45 ± 0.33 kg for the Se supplementation and control groups, respectively.

4.3. Effect of Se supplementation on phenotypic pubertal parameters

4.3.1. Body weight

The effect of Se supplementation on body weight revealed no significant difference ($P > 0.05$) between the groups. However, the body weight of all the lambs increased with age. Furthermore, the results showed no significant difference ($P > 0.05$) in the effect of Se supplementation on the SC between the two groups (Table 4.1).

Table 4.1 *The overall effect of 5 months of Se supplementation on pubertal traits*

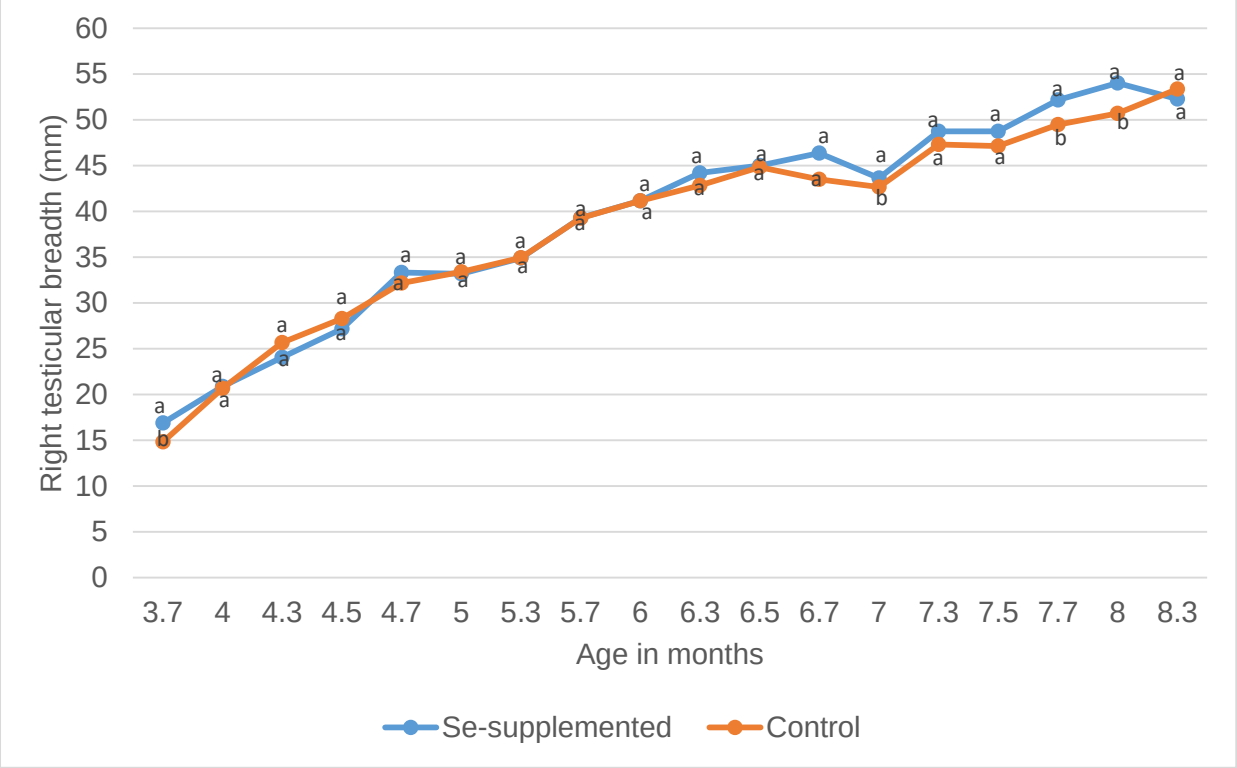
Traits	Se supp	CG	P-value
Body weight (kg)	37.27 ± 0.08	37.45 ± 0.08	0.0985
Scrotum circumference (cm)	25.67 ± 0.06	25.65 ± 0.06	0.7865
Right testes breadth (mm)	39.38 ± 0.17	38.52 ± 0.17	0.0004
Left testes breadth (mm)	38.58 ± 0.17	37.69 ± 0.17	0.0003
Right testes length (mm)	73.99 ± 0.25	72.62 ± 0.25	0.0001
Left testes length (mm)	73.24 ± 0.25	71.85 ± 0.25	<.0001
Nosing (age in days)	147.55	148.35	0.0616

Traits	Se supp	CG	P-value
Flehmen (age in days)	175.55	169.35	<.0001
Mounting (age in days)	196.55	197.35	0.6206

Se supp: Selenium supplemented group, CG: control group.

4.3.2. Testicular measurements

The results showed that there was a significant difference (P < 0.05) at the beginning of the experiment between the treatment and control groups on the right testicular breadth (Figure 4.1).

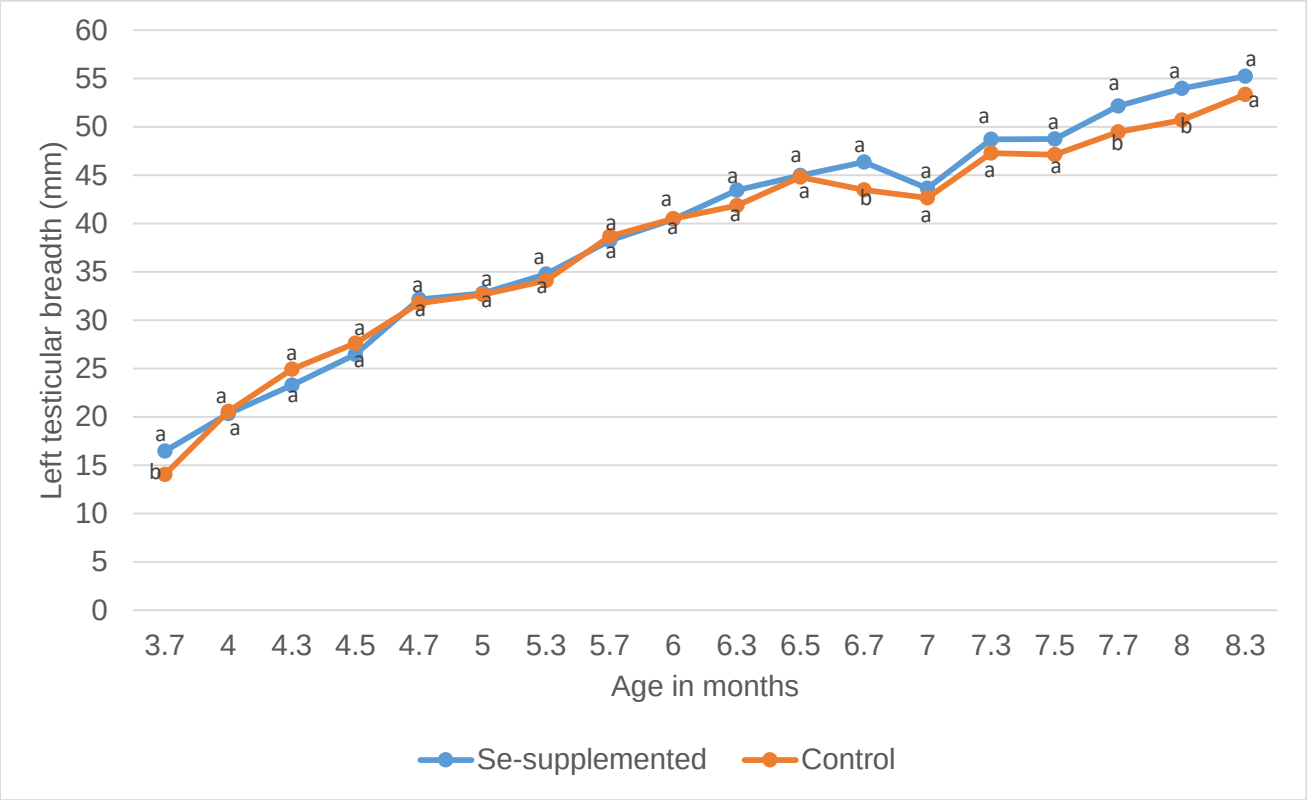


[a, b] Different superscripts differ significantly at P < 0.05 between groups.

Figure 4.1 *Effect of Se supplementation on the right testicular breadth*

At 7 months, the Se-supplemented group differed significantly on the right TB (P < 0.05) compared to the control group. At 7.3 months, there was no significant difference (P > 0.05) between the Se-supplemented and control groups until 7.7 months. The right TB of ram lambs supplemented with Se increased significantly (P < 0.05) from 7.7 months to 8 months compared to the control group. South African Merino ram lambs supplemented with Se had higher (P < 0.05) overall right TB compared to the control group (Table 4.1).

At the start of the experiment, the Se-supplemented group had significantly larger left TB ($P < 0.05$) compared to the control group (Figure 4.2). However, from the age of 4 to 6.5 months, no significant difference was observed between the groups ($P > 0.05$). The left TB increased significantly ($P < 0.05$) at 6.7 months in the Se-supplemented group compared to the control group. However, at 7 months, there was no significant difference ($P > 0.05$) between the Se-supplemented and control groups until 7.5 months. The left TB of ram lambs supplemented with Se increased significantly ($P < 0.05$) from 7.7 to 8 months compared to the control group. In general, the South African Merino ram lambs supplemented with Se had higher overall left TB ($P < 0.05$) than the control group (Table 4.1).

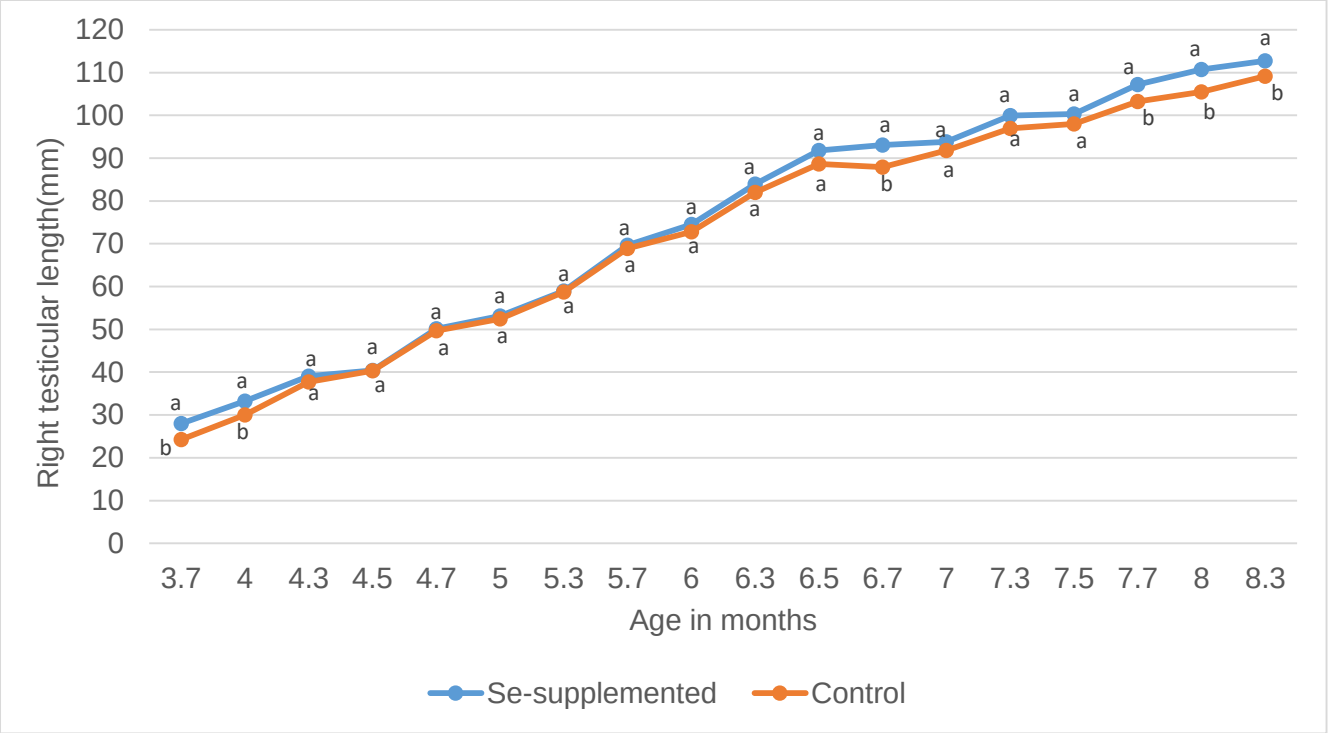


[a, b] Different superscripts differ significantly at $P < 0.05$ between groups.

Figure 4.2 *Effect of Se supplementation on the left testicular breadth*

The results revealed that right TL was significantly different ($P < 0.05$) from the start of the experiment until 4 months old in the Se-supplemented group compared to the control group (Figure 4.3). However, from 4.3 to 6.5 months, there was no significant difference ($P > 0.05$) between the groups. The right TL of the South African Merino ram lambs supplemented with Se increased significantly ($P < 0.05$) from 6.7 and 7.3 months. Furthermore, the results showed that right TL increased significantly ($P <$

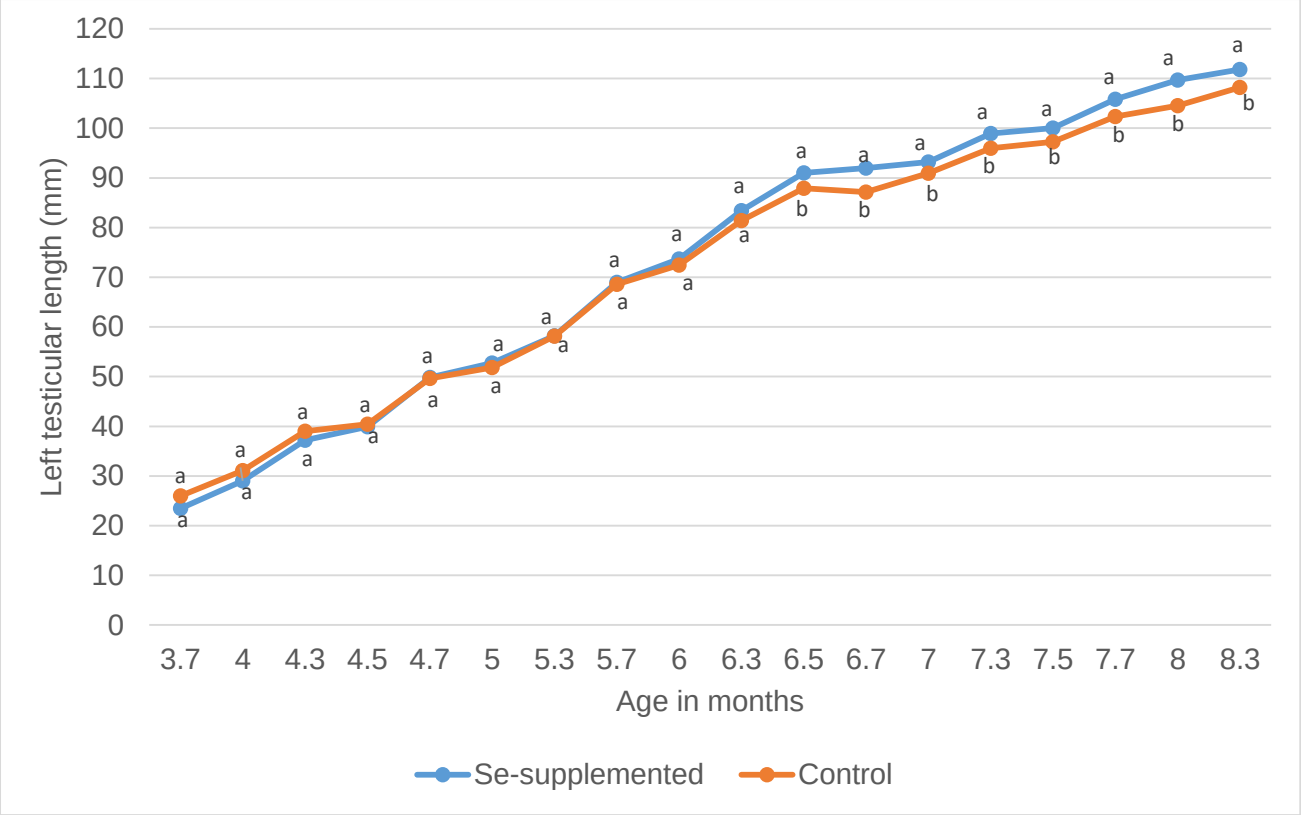
0.05) from 7.7 months until the end of the experiment in the Se-supplemented compared to the control group. Overall, the Se-supplemented South African Merino ram lambs had higher right TL ($P < 0.05$) (Table 4.1).



[a, b] Different superscripts differ significantly at $P < 0.05$ between groups.

Figure 4.3 *Effect of Se supplementation on right testicular length*

Figure 4.4 presents the results of the left TL of South African Merino ram lambs supplemented with Se. After 10 weeks of Se supplementation, there was no significant difference ($P > 0.05$) in the left TL among the groups. Results showed that at the age of 6.5 months, left TL increased significantly ($P < 0.05$) in the Se-supplemented group compared to the control group until the age of 6.7 months. Thereafter, there was a significant difference ($P < 0.05$) in the left TL from 7.3 months until the end of the experiment. South African Merino ram lambs supplemented with Se had higher ($P < 0.0001$) overall left TL (Table 4.1).



[a, b] Different superscripts differ significantly at P < 0.05 between groups.

Figure 4.4 *Effect of Se supplementation on left testicular length*

4.4. Effect of Se supplementation on sexual behaviour

The sexual behaviour activities results are summarised in Table 4.1 and show the behaviours displayed by 50% of the experimental animals at a certain age. The results demonstrate that South African Merino ram lambs exhibited nosing behaviour at 5.5 months, irrespective of Se supplementation. At 6 months, ram lambs started to display flehmen behaviour in the presence of ewes. Furthermore, there was a significant difference (P < 0.05) between the two groups' exhibition of flehmen behaviour. Compared to the control group, the ram lambs supplemented with Se displayed greater flehmen behaviour. The ram lambs started to display mounting behaviour at 7 months; Se supplementation did not affect the age at which they displayed this behaviour. The results revealed that mounting behaviour accompanied by protrusion of the penis was evident when ram lambs were 7.5 months old, regardless of Se supplementation.

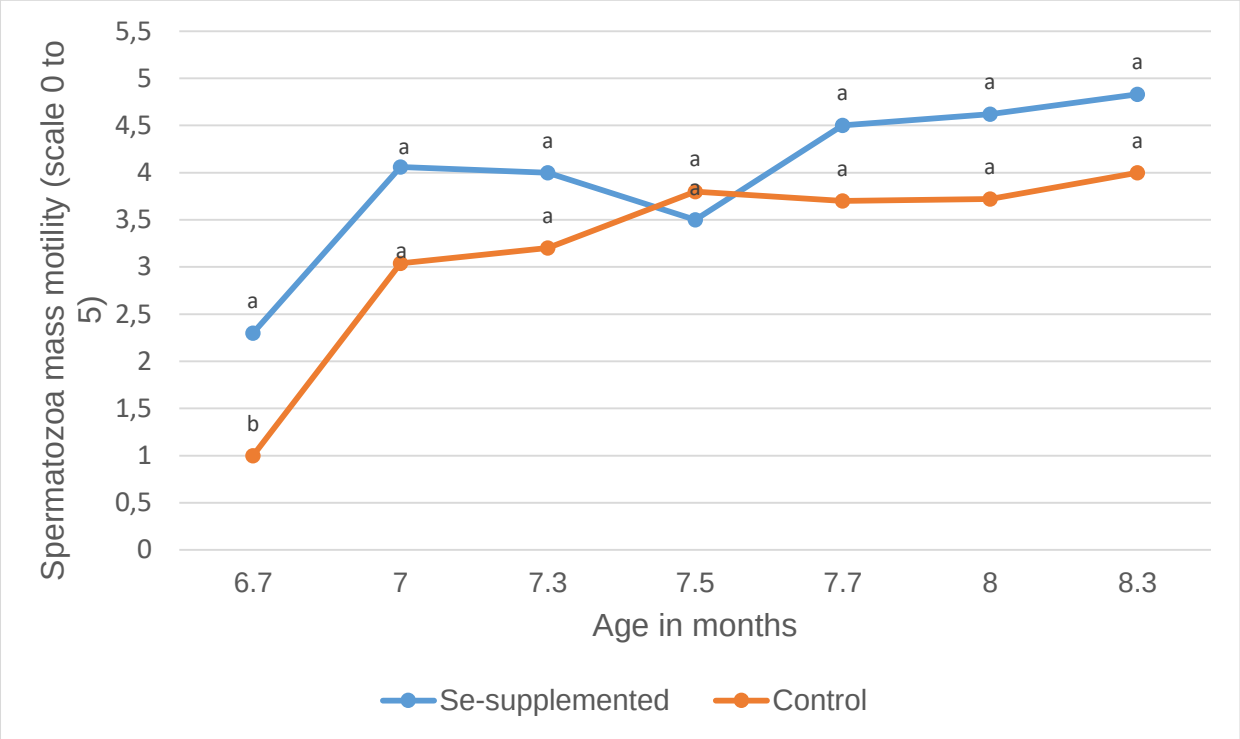
4.5. Macroscopic semen characteristics after Se supplementation

Supplementation of ram lambs' with Se had no effect (P > 0.05) on semen volume (Table 4.6). Furthermore, semen appearance did not differ significantly (P > 0.05) between the Se-supplemented and control groups. In addition, results showed that there was no significant difference (P > 0.05) in semen pH between the treatment and control groups.

4.6. Microscopic semen characteristics after Se supplementation

4.6.1. Spermatozoa mass motility

The Se-supplemented group had significantly different (P < 0.05) spermatozoa mass motility at 6.7 months (Figure 4.5).



[a, b] Different superscripts differ significantly at P < 0.05 between groups.

Figure 4.5 *Effect of Se supplementation on spermatozoa mass motility*

However, for the rest of the study, there was no significant difference (P > 0.05) between the groups (Table 4.2). However, South African Merino ram lambs supplemented with Se had higher (P < 0.0001) overall spermatozoa mass motility (Table 4.2).

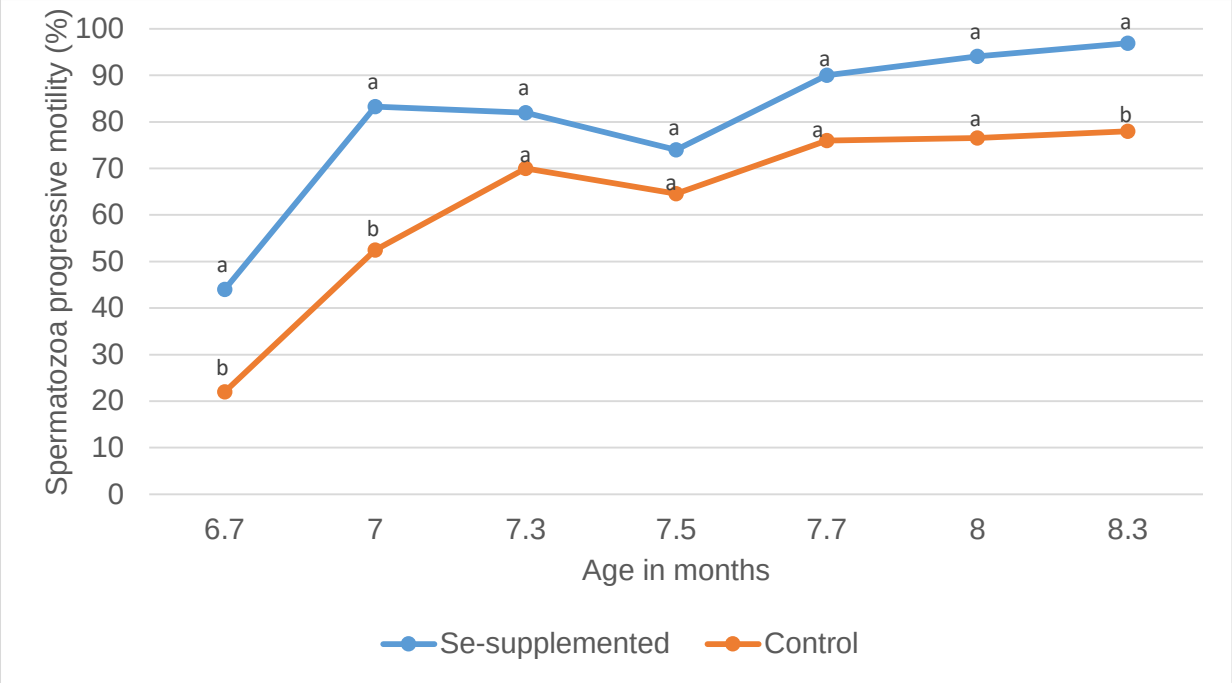
Table 4.2 *The overall effect of 5 months of Se supplementation on semen characteristics (mean ± SE)*

Traits	Se supp	CG	P-value
Volume	1.38 ± 0.07	1.29 ± 0.07	0.3783
pH	7.09 ± 0.13	7.23 ± 0.12	0.1036
Appearance (Rating scale 0 to 5)	2.38 ± 0.11	2.12 ± 0.11	0.0752
Spermatozoa mass motility (Rating scale 0 to 5)	3.97 ± 0.13	3.21 ± 0.13	< .0001
Progressive motility (%)	80.61 ± 2.46	62.79 ± 2.45	< .0001
Live spermatozoa (%)	78. 43 ± 1.95	67.50 ± 1.94	0.0001
Dead spermatozoa (%)	21.57 ± 1.95	32.49 ± 1.94	0.0001
Normal spermatozoa (%)	72.74 ± 1.89	59.12 ± 1.88	< .0001
Abnormal spermatozoa (%)	5.69 ± 0.39	8.38 ±0.39	< .0001
Primary abnormalities (%)	2.10 ± 0.18	2.72 ± 0.18	0.0180
Secondary abnormalities (%)	2.02 ± 0.18	2.79 ± 0.18	0.0025
Tertiary abnormalities (%)	1.58 ± 0.20	2.85 ± 0.20	<.0001
Spermatozoa concentration ($mL/10^6$)	3027.39 ±164.02	2171.54 ±162.11	0.0003

Se supp: Se-supplemented group, CG: control group.

4.6.2. Spermatozoa progressive motility

Figure 4.6 shows the effect of Se supplementation on South African Merino ram lambs' spermatozoa progressive motility. The Se-supplemented group had a significantly higher ($P < 0.05$) spermatozoa progressive motility at 6.7 to 7 months and 8.3 months compared to the control group. The overall average spermatozoa progressive motility was significantly higher ($P < 0.0001$) in ram lambs supplemented with Se compared to the control group (Table 4.2).

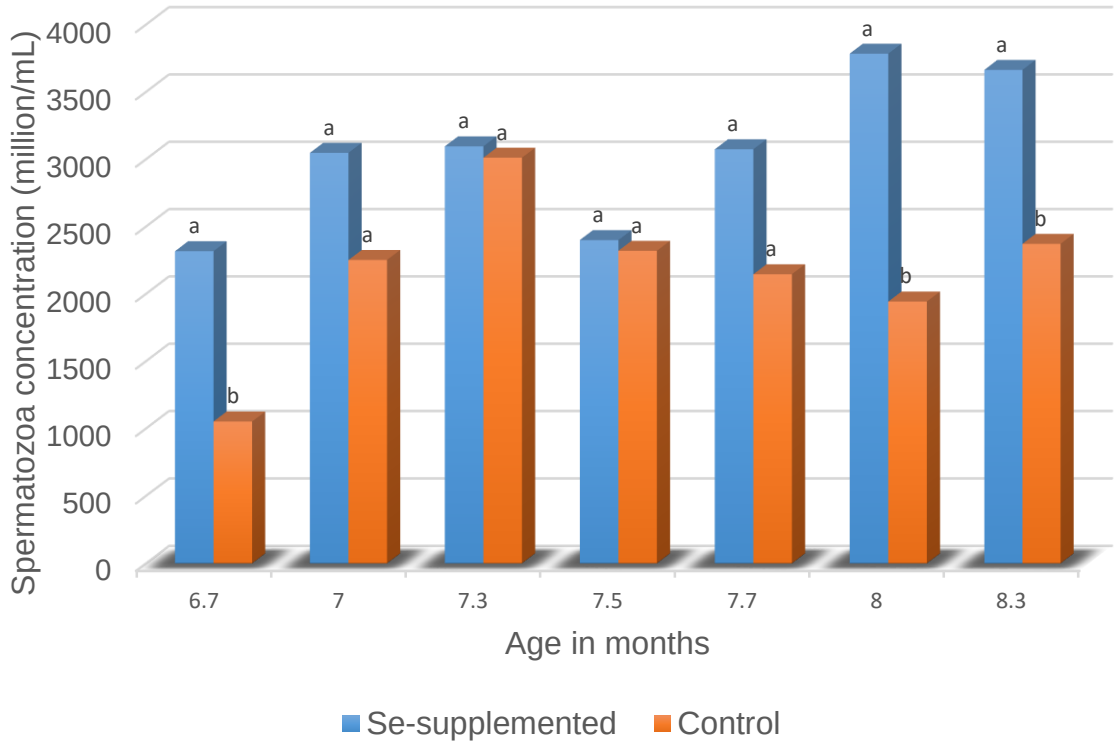


[a, b] Different superscripts differ significantly at P < 0.05 between groups.

Figure 4.6 *Effect of Se supplementation on spermatozoa progressive motility*

4.6.3. Spermatozoa concentration

Selenium supplementation had a significant effect (P < 0.05) on spermatozoa concentration of the experimental South African Merino ram lambs. The Se-supplemented group had a higher spermatozoa concentration at 6.7 months (2316.50 ± 416.85 mL/10^6) than the control group (1051.50 ± 416.85 mL/10^6). The spermatozoa concentration increased significantly (P < 0.05) at 8 months until the end of the experiment in the Se-supplemented group compared to the control group (Figure 4.7). The overall average spermatozoa concentration of South African Merino ram lambs supplemented with Se was significantly higher (P < 0.05) than the control group (Table 4.2).



[a, b] Different superscripts differ significantly at P < 0.05 between groups.

Figure 4.7 *Effect of Se supplementation on spermatozoa concentration*

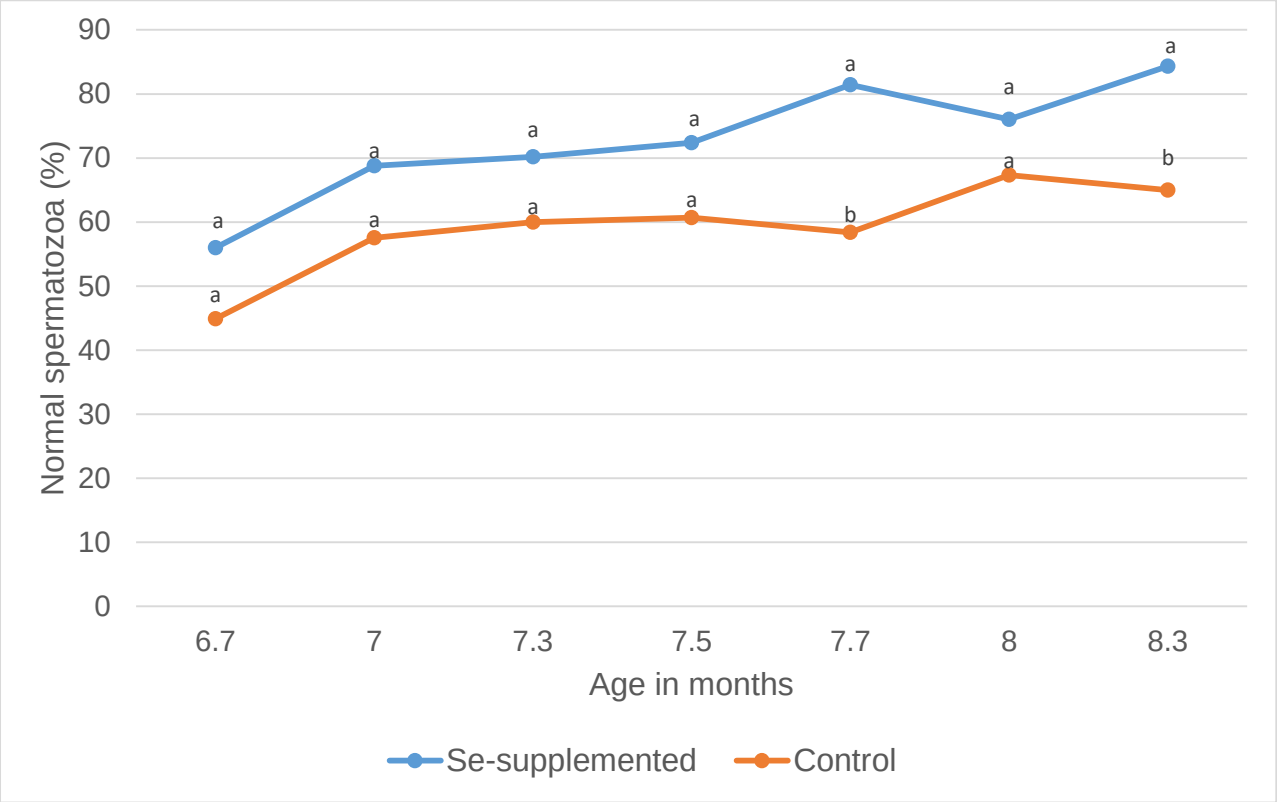
4.6.4. Spermatozoa viability

There was a significant difference (P < 0.05) in live spermatozoa percentage between the South African Merino ram lambs' groups. The percentage of live spermatozoa increased with age, irrespective of the supplementation of Se. Furthermore, the live spermatozoa percentage increased significantly (P < 0.05) at 7.7 months until the end of the experiment in the Se-supplemented group compared to the control group. The Se-supplemented group had a higher (P < 0.05) overall percentage of live spermatozoa than the control group (Table 4.2).

The dead spermatozoa percentage differed significantly (P < 0.05) in the South African Merino ram lamb groups. Results showed that the dead spermatozoa percentage decreased as the animal's age increased. The dead spermatozoa percentage decreased significantly (P < 0.05) from 7.7 months until the end of the experiment in the Se-supplemented group compared to the control group. South African Merino ram lambs supplemented with Se had an overall lower (P < 0.05) average percentage of dead spermatozoa in comparison with the control group (Table 4.2).

4.6.5. Spermatozoa morphology

The results revealed a significant difference ($P < 0.05$) in the normal spermatozoa percentage between the Se-supplemented and control groups. Furthermore, the percentage of normal spermatozoa increased as the age of the South African Merino ram lambs increased. The percentage of normal spermatozoa increased significantly ($P < 0.05$) at 7.7 and 8.3 months in the Se-supplemented group than the control group (Figure 4.8). The Se-supplemented group had a higher ($P < 0.0001$) overall average percentage of normal spermatozoa in comparison with the control group (Table 4.2).

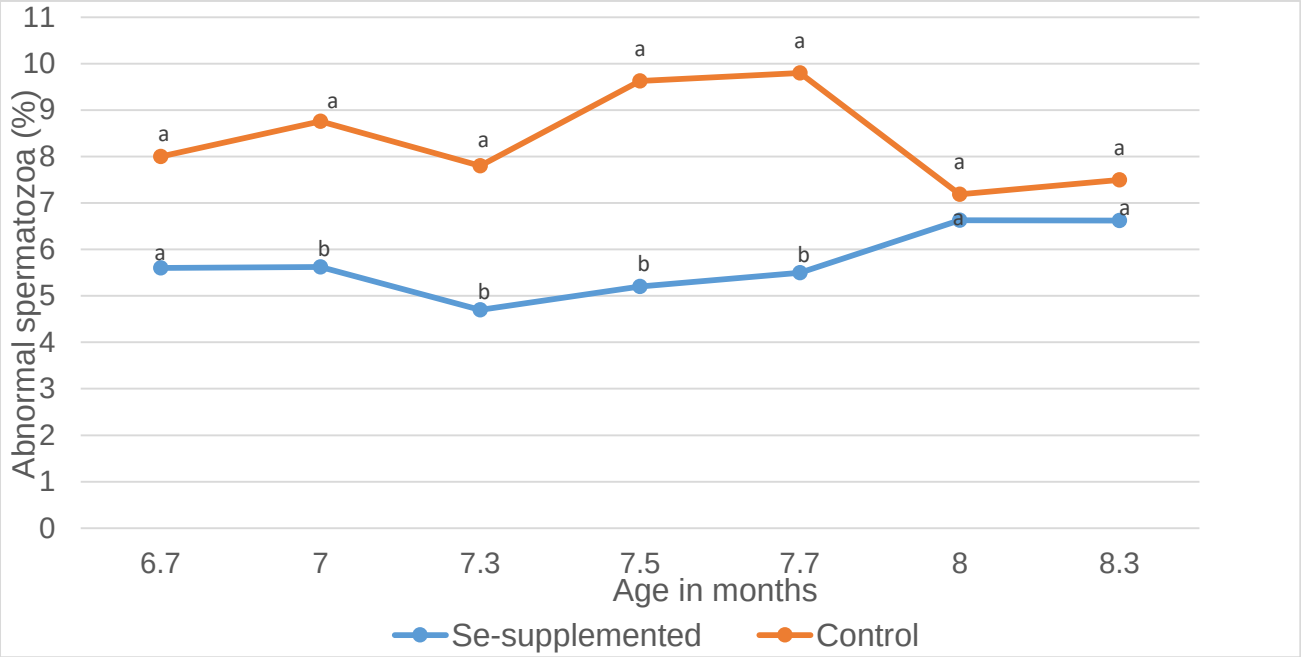


[a, b] Different superscripts differ significantly at $P < 0.05$ between groups.

Figure 4.8 *Effect of Se supplementation on normal spermatozoa*

The results showed that the percentage of abnormal spermatozoa was significantly ($P < 0.05$) lower in the Se-supplemented group compared to the control group. The abnormal spermatozoa percentage declined as the age of South African Merino ram lambs increased in both groups, irrespective of Se supplementation. The percentage of abnormal spermatozoa decreased significantly ($P < 0.05$) at 7 to 7.7 months in the Se-supplemented group compared to the control group (Figure 4.9). Ram lambs

supplemented with Se had a lower (P < 0.0001) overall percentage of abnormal spermatozoa compared to the control group (Table 4.2).



[a, b] Different superscripts differ significantly at P < 0.05 between groups.

Figure 4.9 *Effect of Se supplementation on abnormal spermatozoa*

The overall mean percentage of primary and secondary spermatozoa abnormalities were significantly lower (P < 0.05) in the Se-supplemented group compared to the control group (Table 4.2). Furthermore, the Se-supplemented group had a significantly lower (P <.0001) percentage of tertiary abnormalities compared to the control group.

4.7. GSH-Px, cortisol and reproductive hormones levels

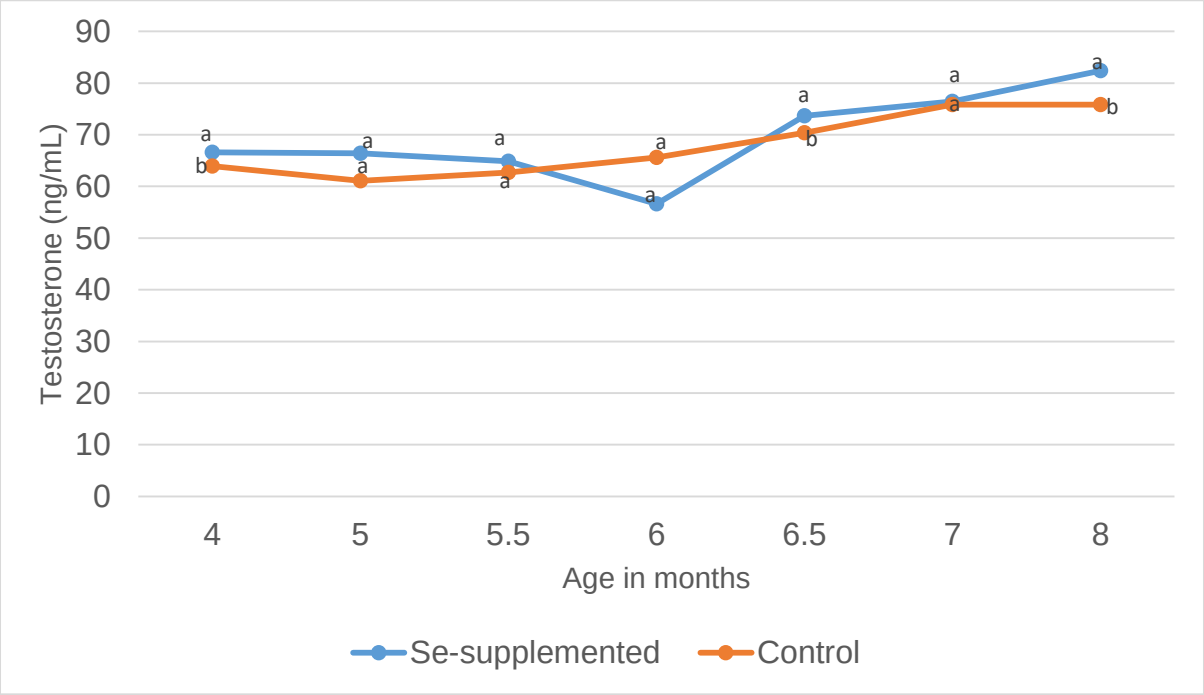
Table 4.3 summarises the hormones and enzyme concentration results for GSH-Px (ng/mL), cortisol (ng/mL), LH (mIU/mL) and testosterone (ng/mL). Selenium supplementation had no effect (P > 0.05) on the GSH-Px concentration in the two groups. The cortisol concentration decreased numerically without a significant difference (p > 0.05) in the Se-treated group compared to the control group (Table 4.3).

Table 4.3 *Overall effect (mean ± SE) of 5 months of Se supplementation on GSH-Px, cortisol and reproductive hormones*

Hormone	Se supp	CG	P-value
GSH-Px (ng/mL)	15.01 ± 1.24	14.27 ± 1.24	0.6379
Cortisol (ng/mL)	3.32 ± 0.74	4.13 ± 0.74	0.3032
LH (mIU/mL)	43.06 ± 1.94	34.98 ± 1.94	0.0065
Testosterone (ng/mL)	74.66 ± 2.98	68.46 ± 2.98	0.0431

GSH-Px: glutathione peroxidase, LH: luteinising hormone, Se supp: Se-supplemented group, CG: control group.

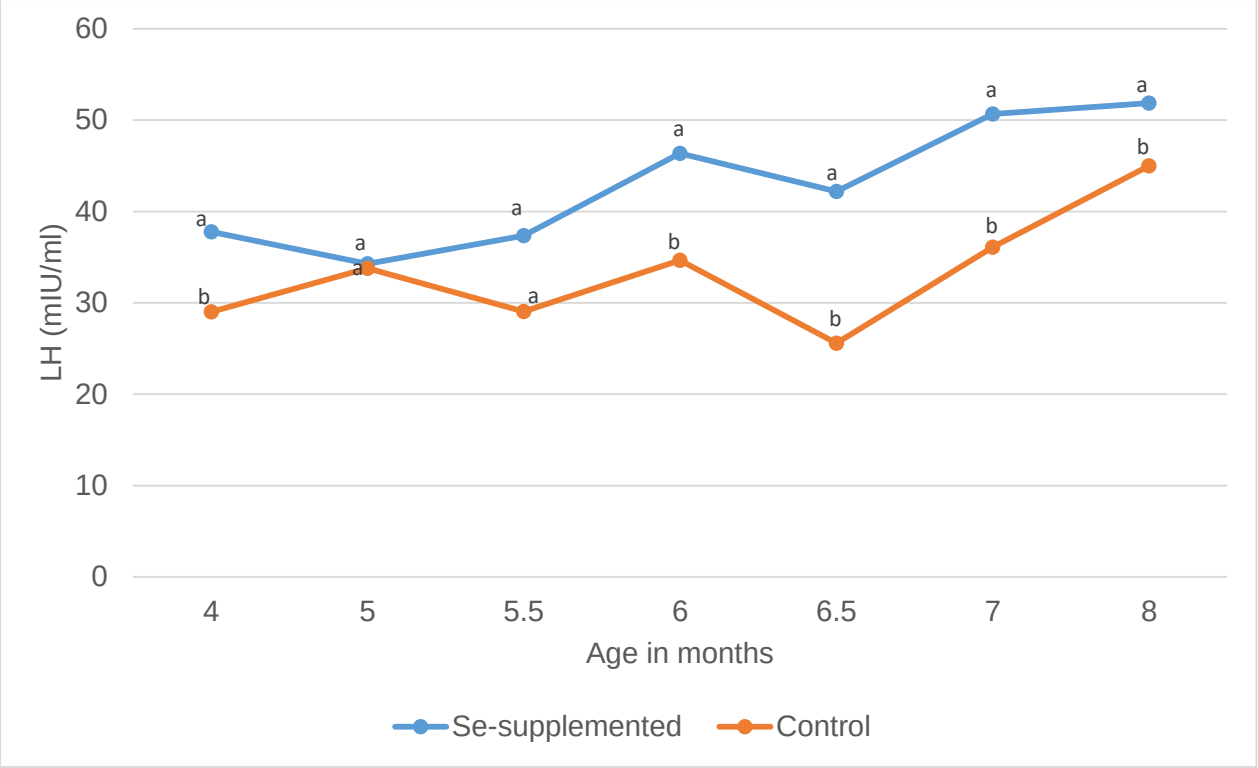
The Se-supplemented group had a higher testosterone concentration ($P < 0.05$) than the control group at 4, 6.5 and 8 months (Figure 4.10). The testosterone concentration declined at 6 months in the Se-supplemented group. However, the testosterone concentration gradually increased in the control group at the same age.



[a, b] Different superscripts differ significantly at $P < 0.05$ between groups.

Figure 4.10 *Effect of Se supplementation on testosterone levels*

Selenium supplementation of South African Merino ram lambs significantly increased the LH concentration ($P < 0.05$) at 4, 6, 6.5, 7 and 8 months (Figure 4.11).



[a, b] Different superscripts differ significantly at P < 0.05 between groups.

Figure 4.11 *Effect of Se supplementation on LH*

4.8. Pearson correlation coefficient for semen characteristics

Tables 4.4 and 4.5 summarise the results for macro and microscopic semen characteristics and phenotypic parameter traits. Semen volume was significantly correlated with semen appearance (r = 0.456, P < 0.0001) (Table 4.4). A significant and positive correlation was observed between spermatozoa mass and progressive motility (r = 0.916, P < 0.001) (Table 4.4). Spermatozoa mass motility was significantly and positively correlated with semen appearance (r = 0.538, P < 0.001) and spermatozoa concentration (r = 0.581, P < 0.001) (Table 4.4).

Table 4.4 *Pearson correlation coefficient (r) of semen characteristics and phenotypic parameters*

	Progressive motility	**Mass motility**	**Appearance**	**Spermatozoa concentration**	**Secondary abnormalities**	**Tertiary abnormalities**
Volume	0.207*	0.211*	0.456**	0.294*		0.022*
Mass motility	0.916**	1.000	0.538**	0.581**	0.079	
PM	1.000	0.916**	0.575**	0.575**	0.050	0.027
Live %	0.799**	0.737**	0.569**	0.572**		
Normal %	0.788**	0.729**	0.567**	0.574**		
Abnormal %	0.019	0.006			0.758**	0.737**
Primary Ab					0.364**	0.268**
Secondary Ab	0.050	0.079	0.088	0.009	1.000	0.314**
Tertiary Ab	0.027				0.314*	1.000
Spermatozoa concentration	0.575**	0.581**	0.713**	1.000	0.009	
Body weight	0.129	0.166	0.042		0.076	0.128
SC	0.292*	0.278*	0.106			0.003
Right TB	0.283*	0.286*	0.104			
Left TB	0.270*	0.239*	0.095		0.011	
Right TL	0.281*	0.261*	0.116		0.052	0.009
Left TL	0.558**	0.547**	0.136	0.107	0.014	0.029

Ab: abnormalities, MM: mass motility, PM: progressive motility, SC: scrotum circumference, TB: testicular breadth, TL: testicular length.

*P < 0.05, **P < 0.0001.

Table 4.5 *Pearson correlation coefficient (r) for semen characteristics and phenotypic parameters*

	Live %	**Normal %**	**Abnormal %**	**Dead %**	**Primary Abnormalities**
Volume	0.291*	0.306*			
Progressive motility	0.799**	0.788**	0.019		
Mass motility	0.737**	0.729**	0.006		
Normal %	0.984**	1.000			
Abnormal %	0.039		1.00		0.715**
pH			0.031	0.355**	0.119
Primary Ab			0.715**		1.00
Secondary Ab			0.758 **	0.004	0.364**
Tertiary Ab	0.053		0.737 **		0.268*
Live %	1.00	0.984**	0.039		0.031
Body weight			0.102	0.028	0.014
SC	0.389	0.407			
Right TB	0.381	0.111			
Left TB	0.431	0.097			
Right TL	0.436	0.086	0.025		
Left TL	0.342*	0.325*			

Ab: abnormalities, SC: scrotum circumference, TB: testicular breadth, TL: testicular length.

*P < 0.05 **P < 0.0001.

The results showed significant and positive correlation between spermatozoa progressive mass motility with semen appearance (r = 0.575, P < 0.0001) and spermatozoa concentration (r = 0.575, P < 0.001) (Table 4.4). The percentage of live spermatozoa was significantly and positively correlated to spermatozoa progressive motility (r = 0.799, P < 0.0001) and spermatozoa mass motility (r = 0.737, P < 0.0001) (Table 4.4). In addition, there was a significant and positive correlation between the live spermatozoa percentage and spermatozoa concentration (r = 0.572) and semen appearance (r = 0.569, P < 0.0001) (Table 4.4).

Furthermore, the percentage of normal spermatozoa was significantly and positively correlated to spermatozoa progressive motility (r = 0.788, P < 0.0001) (Tables 4.4 & 4.5), live spermatozoa percentage (r = 0.984, P < 0.0001) (Table 4.5) and spermatozoa mass motility (r = 0.737, P < 0.0001) (Tables 4.4 & 4.5). The percentage of normal spermatozoa was highly and positive correlated to semen appearance and spermatozoa concentration (r = 0.569 and r = 0.572, respectively, P < 0.0001) (Table 4.5). In addition, spermatozoa concentration was significantly and positively correlated to semen appearance (r = 0.713, P < 0.0001) (Table 4.4).

The results showed no significant correlation between body weight and semen characteristics. However, the results showed that the TL was significantly and positively correlated with spermatozoa progressive motility (r = 0.558, P < 0.001) and spermatozoa mass motility (r = 0.547, P < 0.001) (Table 4.4).

4.9. Pearson correlation coefficient for physical parameters

Table 4.6 summarises the r values for the physical parameters and shows a significant and highly positive correlation between body weight and SC (r = 0.913, P < 0.001).

Table 4.6 Pearson correlation coefficient (r) for physical parameters

	SC	**Right TB**	**Left TB**	**Right TL**	**Left TL**
BW	0.913**	0.888**	0.886**	0.896**	0.897**
SC	1.000	0.951**	0.949**	0.962**	0.931**
Right TB				0.996**	0.943**
Left TB				0.923**	0.953**

BW: body weight, SC: scrotum circumference, TB: testicular breadth, TL: testicular length. **P < 0.0001.

In addition, body weight was significantly and positively correlated with the right TB (r = 0.888, P < 0.001) and left TB (r = 0.886, P < 0.001) (Table 4.6). Furthermore, the results showed that the SC was significantly and positively correlated to the right TB (r = 0.951, P < 0.0001) and left TB (r = 0.949, P< 0.0001) (Table 4.6). There was also a significant and positive correlation between body weight and the right and left TL (r = 0.896, P < 0.001 and r = 0.897, P< 0.001, respectively) (Table 4.6). The SC was significantly and positively correlated with right TL (r = 0.962, P < 0.001) and left TL (r = 0.931, P < 0.001). There was a significant and highly positive correlation between the right TB and the right TL (r = 0.996, P < 0.001) and left TB (r = 0.943). In addition, there was a significant and high positive correlation between the left TB with the left TL (r = 0.953, P < 0.001) and right TL (r = 0.923, P < 0.001) (Table 4.6).

4.10. Pearson correlation coefficient of reproductive hormones, cortisol and GSH- Px with physical parameters

Table 4.7 reports the r values for cortisol, LH and testosterone concentration with the phenotypic parameters.

	BW	Left TB	Right TB	Left TL	Right TL	SC	GSH-Px	Cortisol
Cortisol							0.642**	
LH	0.257*	0.261*	0.265*	0.268*	0.256*	0.207*		0.432**
Testosterone		0.208*	0.260*	0.263*	0.216*			0.420*

BW: body weight, GSH-Px: glutathione peroxidase, LH: luteinising hormone, SC: scrotum circumference, TB: testicular breadth, TL: testicular length. *P < 0.05 **P < 0.0001.

The cortisol concentration had no significant correlation with the phenotypic parameters (Table 4.7). The cortisol concentration (r = 0.642, P < 0.001) was significantly and positively correlated to the GSH-Px concentration. Furthermore, the results showed no significant correlation between the GSH-Px concentration with the phenotypic parameters and semen characteristics. However, the testosterone concentration was significantly and moderately correlated to the cortisol concentration (r = 0.420, P < 0.001).

In addition, Table 4.7 shows a significant moderate positive correlated was recorded between cortisol and LH concentration ($r = 0.432$, $P < 0.0001$). Body weight ($r = 0.257$, $P< 0.05$) and SC ($r = 0.207$, $P < 0.05$) were significantly and weakly correlated to the LH concentration (Table 4.7). There was a significant and weak positive correlation between the LH concentration and left ($r = 0.261$, $P < 0.05$) and right TB ($r = 0.265$, $P < 0.05$). A significant weak positive correlation was noted for LH concentration with the left and right TL ($r = 0.268$, $P < 0.05$ and $r = 0.256$, $P < 0.05$, respectively) (Table 4.7).

4.11. Summary

This chapter critically analysed and provided an overview of the results. From the findings, conclusions can be drawn to achieve the overarching aim and objectives of the study (Sections 1.5 and 1.6). The results indicate that Se supplementation hastens the attainment of puberty and improves semen characteristics and testicular measurements of South African Merino ram lambs. The findings also showed that oral Se supplementation had no significant effect on South African Merino ram lambs' body weight, SC and semen volume and appearance. Furthermore, oral supplementation with Se increased LH and testosterone concentrations. However, Se supplementation did not affect GSH-Px and cortisol concentrations. Chapter 5 discusses the study's findings reported in this chapter.

CHAPTER 5: DISCUSSION

5.1. Introduction

This chapter discusses the results reported in Chapter 4 with reference to the aim and objectives of the study (Sections 1.5 and 1.6), which was to evaluate the effect of Se supplementation on the attainment of puberty, phenotypic parameters and semen quality of South African Merino ram lambs. The findings are discussed in the light of previous research findings and available literature to identify similarities and differences between the findings of this and previous studies.

5.2. Effect of Se supplementation on phenotypic pubertal parameters

5.2.1. Body weight

In the present study, the body weight of South African Merino ram lambs increased in both treatment groups regardless of Se supplementation. This result aligns with similar studies' findings that Se supplementation did not affect body weight in ram lambs (Baimony et al., 2009; Sushma et al., 2015). The effect of Se supplementation on body weight is possibly overshadowed by lambs' fast growth rate towards the attainment of puberty. It has been reported that insulin stimulates body weight gain by promoting the synthesis of proteins and fats (Mohammed & Mutassim, 2016). There is also high secretion of hormone, which increases the sensitivity of tissues to insulin, thereby increasing the growth rate. Furthermore, growth hormone promotes protein retention towards the attainment of puberty (Mohammed & Mutassim, 2016).

South African Merino ram lambs attained puberty at 40–60% of their mature body weight. In South Africa, typical mature South African Merino rams weigh 90 kg, which means that 40% of the mature body weight is 36 kg (Cloete et al., 1998). The Se-supplemented South African Merino ram lambs attained puberty at 6 months compared to 6.5 months for the control group. Therefore, Se supplementation hastened puberty attainment at 40% of the mature body weight. However, all the experimental ram lambs (100%) had reached 60% of their mature body weight (40.80 ± 0.33 kg and 41.45 ± 0.33 kg for the Se-supplemented and control groups, respectively) at 6.5 months, regardless of Se supplementation. Therefore, it can be concluded that South African Merino ram lambs reach 40% of mature body weight at 6–6.5 months as an indication of the attainment of puberty.

This study's finding that the attainment of puberty based on South African Merino ram lambs' body weight occurs at 6–6.5 months; is consistent with those of Kumar et al. (2010). These authors found that Malpura ram lambs attained puberty at 6.5 months and a body weight of 32.15 kg. Similar results were obtained in Pelibuey ram lambs, which attained puberty at the same age as this study but at a lower body weight of 23 kg (Martinez et al., 2012). In contrast, Jafariahangari et al. (2012) found that Cheviot and Suffolk ram lambs attained puberty earlier at 5.5 months with lower body weight (34.3 kg).

Other studies report later puberty attainment in sheep than in this study. For example, Najdi and Ghezel ram lambs attained puberty at 8–9 months and a body weight of 40 and 42 kg, respectively. In addition, Tazegzwat ram lambs attained puberty at 11 months and a body weight of 43.2 ± 4.6 kg (Al-kawmani et al., 2014; Nazari-Zenouz et al., 2016; Moulla et al., 2018). These studies' findings support the strong effect of breed on the attainment of puberty in terms of body weight. This study's results suggest that South African Merino ram lambs attain puberty at 6–6.5 months, regardless of Se supplementation.

5.2.2. Testicular measurements

Scrotum circumference is a good indicator of the breeding ability of rams. Elbaz and Abdel Razek (2019) reported that the selection of rams for breeding should be made through testicular measurements because it is highly heritable and correlated with semen quality and spermatozoa production. This study found that Se supplementation did not affect the SC, which increased in both treatment groups. This finding is supported by the results of Mahmoud et al. (2013) and Ghorbani et al. (2018). The effect of Se supplementation on the SC is probably overshadowed by the rapid growth rate of ram lambs towards the attainment of puberty. It has been reported that during animals' growth development, metabolic hormones, such as leptin, adiponectin, insulin and insulin-like growth factor-1, signal the nutritional status of the animal to the hypothalamus via receptors in the arcuate, neuropeptide Y and pro-opiomelanocortin nuclei; this regulates the secretion of LH and FSH, moderated through the action of GnRH (Byrne et al., 2017). These hormones are required for testicular and SC development, which determines the age at which puberty is attained (Amstalden et al., 2014).

South African Merino ram lambs ejaculated semen with viable spermatozoa at 6.5 months with a SC of 29.21 ± 0.27 cm. South African Merino ram lambs achieve this SC size at an earlier age than Barki mature rams (29.81 ± 0.53 cm), which only reach this size SC at 2–2.5 years when supplemented with Se (Elbaz & Abdel Razek., 2019). Therefore, the differences indicate that SC is highly affected by breed (Akpa et al., 2013). For example, Serbia Meat Institute sheep showed larger SC (31.10 ± 0.49 cm) at 6 months (Maksimović et al., 2016).

Contrary to this study's finding of no effect of Se supplementation on the SC of South African Merino lamb rams, the TB and TL increased significantly following supplementation. In support of these findings, Abd El-Hafez et al. (2016) reported that Sohagi ram lambs supplemented with different sources of Se had significantly increased testicular measurements. However, injected Se had no effect on Barki Egyptian rams' testes measurements, including length and breadth (Elbaz & Abdel Razek., 2019).

5.3. Sexual behaviour activities

The literature reviewed suggests that nosing is the first sexual behaviour shown in animals, followed by flehmen behaviour (Nishimura, 2000). The present study revealed that South African Merino ram lambs displayed nosing at 5.5 months, and flehmen sexual behaviour was exhibited at 6 months. These results are supported by Bousta et al. (2020), who reported that Rembi ram lambs started to exhibit nosing sexual behaviour activities at 5.5 months and showed flehmen behaviour at 6 months.

Animals usually attain puberty before exhibiting mounting sexual behaviour (Nishimura et al., 2000). Based on this study's results, South African Merino ram lambs ejaculated semen with viable spermatozoa at 6 months and displayed mounting behaviour a month later at 7 months. This result is supported by Damián et al. (2015), who found that Polwarth ram lambs displayed mounting sexual behaviour at 7 months.

5.4. Effect of Se supplementation on semen quality and quantity

5.4.1. Semen volume

Selenium supplementation did not affect semen volume in South African Merino ram lambs. A similar observation has been reported in studies that used supplementation of organic or inorganic Se or when Se was combined with vitamin E (Mahmoud et al.,

2014; Piagentini et al., 2017; Stefanov et al., 2018; Baker et al., 2021). The effect of Se supplementation on semen volume was possibly overshadowed by the method of semen collection, which increased semen volume in both treatment groups. The higher semen volume in both treatment groups could be due to the electroejaculation method used for semen collection, which stimulates the accessory gland to secrete more seminal plasma, resulting in higher semen volume (Bopape et al., 2015; Lukusa & Lehloenya, 2017).

5.4.2. Semen pH

Semen pH plays an important role in sustaining spermatozoa viability and ensuring fertilisation and is recognised as a factor that influences semen quality in rams (Zhou et al., 2015). The semen pH in both experimental groups in this study was within small ruminants' normal range of 7.0 and 7.2 (Maina et al., 2006). Therefore, Se supplementation did not affect South African Merino ram lambs' semen pH. These results agree with a previous study by Stefanov et al. (2018); they found that organic and inorganic Se did not lead to any significant change in the semen pH of Bulgarian Merino rams. Contrary to these results, Marai et al. (2009) reported that inorganic Se supplementation led to an acidic semen pH in Egyptian Suffolk rams.

5.4.3. Semen appearance

Semen appearance is an indicator of spermatozoa concentration (Mojapelo & Lehloenya, 2019). The results of this study indicated that South African Merino ram lambs' oral supplementation with Se did not alter semen appearance. The milky semen appearance (2.38 ± 0.11) for Se-supplemented and (2.12 ± 0.11) for control groups in this study (Table 4.2) suggests an increase in free radicals and attack of the germ cells within the seminiferous tubules. This, in turn, causes extensive apoptosis and the disruption of spermatogenesis (Aitken & Baker, 2013). Disruption of spermatogenesis leads to decreased spermatozoa concentration, which changes the appearance of semen (Mojapelo et al., 2021).

This study's results were surprising, as semen appearance also indicates spermatozoa concentration (Jha et al., 2018). Although Se supplementation did not affect semen appearance, the present study confirmed previously reported findings that semen appearance was positively correlated with spermatozoa concentration (Chella et al., 2017).

5.4.4. Spermatozoa mass motility

Spermatozoa motility is considered the main factor affecting semen quality (David et al., 2015). Selenium supplementation in South African Merino ram lambs' improved spermatozoa mass motility. Similar results have been reported in numerous studies where rams supplemented with Se produced semen with higher spermatozoa mass motility (Mahmoud et al., 2013; Ghorbani et al., 2018; Alkhashab & Hameed, 2020). Therefore, the increase in spermatozoa motility found in this study was expected; Se maintains mitochondrial structural integrity, which is reflected by an increase in spermatozoa's adenosine triphosphate and, therefore, greater spermatozoa motility (Lukusa & Lehloenya, 2017). However, contrary to this study's results, it has been reported that Se supplementation did not improve spermatozoa mass motility in sheep breeds (Mahmoud et al., 2014; Piagentini et al., 2017).

5.4.5. Spermatozoa progressive motility

This study found that Se supplementation increased spermatozoa progressive motility in South African Merino ram lambs. Similarly, Ghorbani et al. (2018) reported that Sanjabi rams supplemented with Se produced semen with higher spermatozoa progressive motility. The increase in spermatozoa progressive motility indicates that Se supplementation plays a crucial role in protecting the spermatozoa membrane against lipid peroxidation (Mahmoud et al., 2013). A high accumulation of lipid peroxidation causes the plasma membrane to lose its ability to act as a permeability barrier, decreasing spermatozoa progressive motility (Nowicka-Bauer & Nixon, 2020). On the contrary, the results of Piagentini et al. (2017) indicate that oral supplementation of Se did not affect Brazil rams' spermatozoa progressive motility; this could be because the rams received other mineral supplements in addition to Se, which may have overshadowed the effects of Se supplementation.

5.4.6. Spermatozoa concentration

Supplementation with Se increased South African Merino ram lambs' spermatozoa concentration in this study. These results agree with previous studies that reported that rams supplemented with Se had a higher total spermatozoa concentration (Mahmoud et al., 2013, 2014; Ghorbani et al., 2018; Alkashab & Hameed, 2020). The higher spermatozoa concentration in the Se-supplemented group is attributed to its antioxidant activity, which protects spermatozoa during spermatogenesis and

improves spermatozoa production. Furthermore, Se is essential for the development of germ cells in the testes (Mahmoud et al., 2013).

5.4.7. Spermatozoa viability

This study found that Se supplementation increased the percentage of live spermatozoa in the semen of South African Merino ram lambs. This finding is supported by previous studies that report that rams supplemented with Se had a higher percentage of live spermatozoa (Stefanov et al., 2018; Dolník et al., 2019; Alkashab & Hameed, 2020). In contrast, Ghorbani et al. (2018) found that supplementation with Se in Sanjabi mature rams did not affect the percentage of live spermatozoa. This discrepancy suggests that the type of Se used for supplementation has an effect. In Ghorbani et al. (2018)'s study, the rams were supplemented with organic Se. Based on the literature reviewed (Stefanov et al., 2018; Dolník et al., 2019; Alkashab & Hameed, 2020), this study used inorganic Se sources for supplementation.

Selenium supplementation also reduced the percentage of dead spermatozoa in the semen of South African Merino ram lambs. The observed decrease in dead spermatozoa percentage is in agreement with Alkashab and Hameed (2020), who reported a significantly lower percentage of dead spermatozoa following Se supplementation.

5.4.8. Spermatozoa morphology

As expected, the Se-supplemented group produced semen with a high percentage of normal spermatozoa. The increase in normal spermatozoa percentage is ascribed to Se being a component of GSH-Px, which acts as an antioxidant during spermatogenesis. In addition, GSP-Px presence reduces the high incidence of head, tail and midpiece deformations in spermatozoa (Piagentini et al., 2017). As a result, the semen of South African Merino ram lambs supplemented with Se in this study had a lower percentage of abnormal spermatozoa (abnormal, detached and loose heads, elongated midpieces, bent and broken tails and twin heads and tails). The observation has been previously reported in other sheep breeds supplemented with Se (Ghorbani et al., 2018, Alkashab & Hameed, 2020). Spermatozoa abnormalities observed in this study were lower than 20% for both treatment groups; this is considered acceptable and good semen quality (Evans & Maxwell, 1987).

5.5. Effect of Se supplementation on reproductive hormones, GSH-Px and cortisol concentrations

In this study, Se supplementation increased serum testosterone and LH concentrations. This result supports Elbaz and Abdel Razek (2019), who reported that Se supplementation in Barki rams increased serum testosterone concentration. Furthermore, high LH concentrations in the Se-supplemented ram's blood has been observed previously (Mahmoud et al., 2013; Elbaz & Razek, 2019). The increased LH concentration following Se supplementation is linked to the observation that Se accumulates in the anterior pituitary, activating the GnRH receptors in the anterior pituitary gonadotrophs to produce more LH (Lukusa & Lehloenya, 2017).

This study found that South African Merino ram lambs had low testosterone and LH levels at the early stage of sexual development. Testosterone and LH concentrations increased as the age of ram lambs advanced in both treatment groups. However, testosterone concentration decreased at 6 months of age. Testosterone is an integral component of the hypothalamic-pituitary-gonadal axis and modulates the release of gonadotropins by the anterior pituitary gland (De Souza et al., 2011). Therefore, this study's observation might be due to high sensitivity to the negative feedback by gonadal steroids – including testosterone – following puberty in the Se-supplemented group. The control groups' testosterone concentration continued to increase at 6 months, possibly due to the observation that the control ram lambs reached puberty later than the Se-supplemented ram lambs at 6.5 months of age.

The serum testosterone concentration (74.66 ng/mL) recorded in this study at the puberty attainment of South African Merino ram lambs is consistent with that reported in Awassi ram lambs at the attainment of puberty (Saeed & Zaid, 2019). However, in other species, such as Ghezel ram lambs, a very low testosterone concentration (1.69 ng/mL) was reported at the attainment of puberty (Nazari-Zenouz et al., 2016).

Selenium is an integral part of the antioxidant enzyme GSH-Px. Therefore, GSH-Px activity is directly proportional to Se intake, and it is the first enzyme affected by a Se deficiency (Vural et al., 2017). However, in this study, Se supplementation did not influence the South African Merino ram lambs' GSH-Px concentration; this supports the findings of similar studies (Baiomy et al., 2009; Paiva et al., 2019). In a study where GSH-PX concentration was elevated after Se supplementation, rams were

supplemented twice a week (Mahmoud et al., 2013). In contrast, this study supplemented the ram lambs with Se at two-week intervals. In this study, the decreased GSH-Px concentration suggested that the ram lambs were under stress. Furthermore, the stress can be related to many factors, including the environment and handling, for animal husbandry, semen and blood collection (Mojapelo et al., 2021). Additionally, the decreased GSH-Px activity indicated lower antioxidant levels in both treatment groups.

The release of cortisol indicates the activation of the hypothalamic-pituitary-adrenal axis during stressful conditions (Ihsanullah et al., 2017). However, Se supplementation did not affect the South African Merino ram lambs' cortisol concentration in this study. Similarly, Shakirullah et al. (2017) reported that Se supplementation did not affect cortisol levels in Balkhi sheep.

5.6. Correlation of phenotypic parameters and semen characteristics

This study's results indicated that progressive spermatozoa motility increased with increased spermatozoa mass motility; this is supported by previous studies' findings of a positive correlation between spermatozoa mass motility and spermatozoa progressive motility in rams (Chella et al., 2017; Al-kawmani et al., 2020). Therefore, it can be concluded that spermatozoa mass motility is directly proportional to spermatozoa progressive motility. In addition, this study confirmed that high spermatozoa motility is directly proportional to the percentage of live spermatozoa. Therefore, motility depends on live spermatozoa percentage as only live spermatozoa can create motility. A positive correlation has been previously reported between rams' live spermatozoa percentage and spermatozoa mass and progressive motility (Belkadi et al., 2017; Chella et al., 2017; Badi et al., 2018).

South African Merino ram lambs' spermatozoa mass and progressive motility increased with an increase in spermatozoa concentration. A similar observation has been reported in ram breeds in Saudi Arabia (Al-kawmani et al., 2020). The positive correlation between South African Merino rams' semen volume and spermatozoa concentration observed in this study aligns with the findings of Ahmed et al. (2021). Furthermore, this observation suggests that an increase in spermatogenesis leads to an increase in spermatozoa concentration and semen volume.

There was a positive correlation between body weight and SC in the South African Merino lamb ram treatment groups; this finding is aligned with that of previous studies (Maksimovic et al., 2016; Omar, 2016; Belkadi et al., 2017), which reported that Karadi ram lambs' body weight positively correlated to SC. Likewise, this study found that body weight positively correlated with testicular measurements. The strong correlation between body weight and testicular measurements suggests that body measurements can be used to determine the attainment of puberty and the selection of ram lambs for breeding programs (Omar, 2016). Furthermore, the TL and TB were positively correlated with serum testosterone concentration; previously, Jafariahangari et al. (2012) reported that the left and right TL and TB were positively correlated with the testosterone level. Testosterone concentration increases testicular growth, which stimulates the Leydig cells to produce more testosterone and, eventually, stimulates spermatogenesis, leading to puberty attainment (Ghorbani et al., 2018).

5.7. Summary

This chapter discussed the results to achieve the study's aim of demonstrating the effects of oral Se supplementation of South African Merino ram lambs in specific areas of the KwaZulu-Natal province, South Africa. The findings emphasise the need for Se supplementation in the midland and mountainous regions of the province, where soil Se is reported to be deficient (Van Ryssen, 2001), as lamb rams' testes require higher Se concentrations at puberty (Ahsan et al., 2014).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Introduction

The study aimed to determine the effects of Se supplementation on South African Merino ram lambs in some regions of the Kwazulu-Natal province, South Africa. This chapter draws conclusions based on the study's findings to achieve the study's overarching aim (Section 1.5) and objectives (Section 1.6) and test the hypotheses (Section 1.7). The implications of these findings are described, together with recommendations for ram lambs' Se supplementation. Furthermore, the study's limitations and suggestions for future research are outlined.

6.2. Conclusions

The current study proposes the presence of motile and viable spermatozoa in ejaculates as the primary indicator of the attainment of puberty in South African Merino ram lambs. The ram lambs attained puberty at 6–6.5 months. Eighty percent of the Se-supplemented ram lambs had the first collection of viable spermatozoa at 6 months and; 100% of South African Merino ram lambs attained puberty at 6.5 months, regardless of the treatment. Based on the phenotypic parameters, South African Merino ram lambs reached puberty at 6.5 months and 60% of mature body weight, 40.80 ± 0.33 kg and 41.45 ± 0.33 kg for Se-supplemented and control groups, respectively and a SC of 29.21 ± 0.27 cm and 29.33 ± 0.27 cm for the Se-supplemented and control groups, respectively. South African Merino ram lambs also exhibited sexual behaviour (nosing and flehmen) at 5.5 months prior to the onset of puberty.

Oral supplementation with sodium selenite improved the South African Merino ram lambs' testicular measurements, and semen characteristics, including spermatozoa mass and progressive motility, viability and concentration. In addition, Se supplementation reduced the percentage of dead and abnormal spermatozoa at the attainment of puberty. Furthermore, Se supplementation increased the LH and testosterone concentrations in pubertal South African Merino ram lambs. However, Se supplementation in pubertal ram lambs did not affect body weight, SC, cortisol, or GSH-Px concentrations.

6.3. Recommendations

- In parts of Kwazulu-Natal midlands where Se is deficient, inorganic Se supplementation is recommended in South African Merino ram lambs to hasten the attainment of puberty through the enhancement of body weight, testicular measurements, reproductive hormones synthesis and semen characteristics.
- In South Africa, sheep are raised under an extensive production system. Therefore, Se supplementation is recommended to enhance reproduction and semen quality.

6.4. Limitations of the study and future research

- The duration of the research and the interval between Se supplementation might have contributed to the lack of a significant difference in some of the parameters assessed. Therefore, it is recommended that Se supplementation at weekly intervals is evaluated in areas where Se is reported to be deficient to determine its effect.
- Several antioxidants, such as vitamins E, C and Zn, can be combined with Se supplementation to improve antioxidant activity. This study only evaluated the effects of Se supplementation. Therefore, it is recommended that Se supplementation, in combination with other antioxidants, is assessed to determine if there is an improvement in the semen characteristics and antioxidant activity of South African Merino ram lambs.
- The concentration of the Se in the pasture was unknown during this experiment. Pasture samples should have been collected before the experiment to analyse the concentration of Se before supplementation; this would have allowed a more informed Se supplement dosage to be administered to meet the lamb rams' recommended Se intake. Therefore, future studies should analyse the pasture Se concentration before the commencement of the study.

References

Abd El-Hafez, G.A., Solouma, G.M.A., Kassab, A.Y. & Ali, A.S., 2016. Some reproductive performance of male lambs and feeding values of rations as affected by supplementation of different selenium sources. *Egyptian Journal Nutrition and Feeds*, 19(1), 103–114. https://dx.doi.org/10.21608/ejnf.2016.74873

Abecia, J.A., Gave, M., García, A I., Casao, A., Carvaja-lSerna, M., Palacious, C., Keller, M., Chemineau, P. & Delgadillo J.A., 2021. Long days in winter or the presence of adult sexually active rams did not influence the timing of puberty of autumn-born Rasa Aragonesa ram-lambs. *Biological Rhythm Research*, 52(3), 462–473. https://doi.org/10.1080/09291016.2019.1613321

Abi Saab, S., Sleiman, F.T., Nassar, K.H., Chermaly, I. & El-Skaff, R., 1997. Implications of high and low protein levels on puberty and sexual maturity of growing male goat kids. *Small Ruminant Research*, 25(1), 17–22. https://doi.org/10.1016/S0921-4488 (96)00972-8

Adam, C.L., Findlay, P.A., Kyle, C.E. & Young, P.E., 1998. Effect of restricted nutrition on timing of puberty in female Soay Sheep. *Journal of Reproduction and Fertility*, 112(1), 31–37. https://doi.org/10.1530/jrf.0.1120031

Adotey, D.K., Stibilj, V., Serfor-Armah, Y., Nyarko, B.J.B., & Jacimovic, R., 2011. Dietary supply of selenium for adolescents in three residential care orphanages in Southern Ghana. *Science of the Total Environment*, 410, 72–79. https://doi.org/10.1016/j.scitotenv.2011.09.039

Agga, G.E, Udala, U., Regassa, F. & Wudie, A., 2011. Body measurements of bucks of three goat breeds in Ethiopia and their correlation to breed, age and testicular measurements. *Small Ruminant Research*, 95(2–3), 133–138. https://doi.org/10.1016/j.smallrumres.2010.09.011

Ahmed, O., Lehloenya, K., Mphaphathi, M. & Hassen, A., 2021. Effect of *Acacia mearnsii* tannin extract supplementation on reproductive performance and oxidative status of South African mutton Merino rams. *Animals*, 11(11), 3266. https://doi.org/10.3390/ani11113266

Ahsan, U., Kamran, Z., Raza, I., Ahmad, S., Babar, W. & Riaz, M.H., 2014. Role of selenium in male reproduction – a review. *Animal Reproduction Science*, 146 (1–2), 55–62. https://doi.org/10.1016/j.anireprosci.2014.01.009

Aitken, R.J. & Baker, M.A., 2013. Causes and consequences of apoptosis in spermatozoa; contributions to infertility and impacts on development. *International Journal of Developmental Biology*, 27(2–4), 265–272. https://doi.org/10.1387/ijdb.130146ja

Akpa, G.N., Ambali, A.L. & Suleiman, I.O., 2013. Body conformation, testicular and semen characteristics as influenced by age, hair type and body condition of Red Sokoto goat. *New York Science Journal*, 6(7), 44–58. http://www.sciencepub.net/newyork/ny0607/009_18357ny0607_44_58.pdf

Akpa, G.N., Suleiman, I.O., Alphonsus, C., 2012. Relationships between body and scrotal measurements, semen, and characteristics in Yankasa ram. *Continental Journal of Animal and Veterinary*, 4(1), 7–10. https://doi.org/10.5707/cjavres.2012.4.1.7.10

Alfthan, G., Eurola, M., Ekholm, P., Venäläinen, E.R., Root, T., Korkalainen, K., Hartikainen, H., Salminen, P., Hietaniemi, V., Aspila, P., Aro, A., 2015. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. *Journal of Trace Elements in Medicine and Biology,* 31, 142–147. https://doi.org/10.1016/j.jtemb.2014.04.009

Al-Haboby, A.H., Hamra, A.H., Al-Tamemmy, M.J. & Al-Rawi, T.S., 2004. Effect of vitamin E and selenium on semen quality, sexual activity, and some blood parameters of Awassi Rams. *Journal of Agriculture*, 2, 58–62.

Ali, A.B., BomBoi, G. & Floris, B., 2009. Does vitamin E or vitamin E pus selenium improve reproductive performance of rams during hot weather. *Italian Journal of Animal Science*, 8(4), 743–754. https://doi.org/10.4081/ijas.2009.743

Alimohamady, R., Aliarabi, H., Bahari, A. & Dezfoulian, A.H., 2013. Influence of different amounts and sources of selenium supplementation on performance, some blood parameters, and nutrient digestibility in lambs. *Biological Trace Element Research*, 154(1), 45–54. https://doi.org/10.1007/s12011-013-9698-4

Al-kawmani, A.A., Farah, M.A., Nahdi, S., Harrath, A.H. & Al-Jawdah, H., 2020. A comparative study of semen characteristics and testosterone concentration in ram breeds of Saudi Arabia. *Indian Journal of Animal Research*, 54(12), 1476–1480. https://doi.org/10.18805/ijar.B-1239

Al-kawmani, A.A., Mansour, M.A, Faisal, M.A.-T., Alodan, M.A., & Farah, M.A., 2014. Developmental changes in testicular interstitium in the Najdi ram lambs. *Saudi Journal of Biological Sciences,* 21(2), 133–137. http://doi.org/10.1016/j.sjbs.2013.09.001

Al-kawmani, A.A., Mansour, M.A., Kandeal, S.A, Farah, M.A. & Alanazi, K.M., 2018. Pubertal changes in testicular parameters and secretion of testosterone in Najdi and Naemi ram lambs under desert conditions. *Indian Journal of Animal Research*, 52(2), 212–219. https://doi.org/10.18805/ijar.v0iOF.9133

Alkhashab, A.T.H.M. & Hameed, M.M., 2020. Effect of the injection of vitamin E and selenium on semen characteristics and some testicular measurements on Karadi rams. *Plant Archives*, 20(2), 999–1004. http://www.plantarchives.org/SPL%20ISSUE%2020-2/151__999-1004_.pdf

Amstalden, M., Cardoso, R., Alves, B.R. & Williams, G.L., 2014. Reproduction symposium: Hypothalamic neuropeptides and the nutritional programming of puberty in heifers. *Journal of Animal Science*, 92(8), 3211–3222. https://doi.org/10.2527/jas.2014-7808

Arangasamy, A., Venkata Krishnaiah, M., Manohar, N., Selvaraju, S., Guvvala, P.R., Soren, N.M, Reddy, I.J., Roy, K.S. & Ravindra, J.P., 2018. Advancement of puberty and enhancement of seminal characteristics by supplementation of trace minerals to bucks. *Theriogenology*, 110, 182–191. https://doi.org/10.1016/j.theriogenology.2018.01.008

Avci, H. & Deveci, T., 2013. Assessment of trace element concentrations in soil and plants from cropland irrigated with wastewater. *Ecotoxicology and Environmental Safety*, 98, 283–291. https://doi.org/10.1016/j.ecoenv.2013.08.013

Baiomy, A.A., Mohamed, A.E.A. & Mottelib, A.A., 2009. Effect of dietary selenium and vitamin E supplementation on productive and reproductive performance in rams. *Beni-Suef Veterinary Medical Journal*, 19(1), 39–43. https://jvmr.journals.ekb.eg/article_77807_847cfcdcdbec765f36838dec58b7fb99.pdf

Baker, A.G., Allsadi, S.A.R.A. & Mohammad, A.K., 2021. The effect of selenium and vitamin E addition on semen quality of Awassi rams. *Zanco Journal of Pure and Applied Sciences*, 33(s1)127–134. https://doi.org/10.21271/ZJPAS.33.s1.14

Bano, I., Malhi, M., Soomro, S.A., Kandhro, S., Muhammad, A., Seema, B., Perveen, S. & Sajjad, H., 2018. Effect of dietary selenium supplementation on morphology and antioxidant status in testes of goat. *Journal of Basic & Applied Sciences*, 14, 53–61. https://doi.org/10.6000/1927-5129.2018.14.08

Barchi, M., Innocenzi, E., Giannattasio, T., Dolci, S., Rossi, P. & Grimaldi, P., 2019. Cannabinoid receptors signaling in the development, epigenetics, and tumours of male germ cells. *International Journal of Molecular Sciences*, 21(1), 25. https://doi.org/10.3390/ijms21010025

Barragry, T.B., 1994. *Veterinary drug therapy*. Lea and Febiger, USA.

Bauersachs, S., Blum, H., Krebs, S., Fröhlich, T., Arnold, G.J. & Wolf, E., 2010. Creating new knowledge for ruminant reproduction for rapidly expanding and evolving scientific databases. *Society of Reproduction*

Bearden, H.J., Fuquay, J.W. & Willard, S.T., 2004. *Applied animal reproduction* (6th ed.). Pearson Prentice Hall, USA.

Belkadi, S., Safsaf, B., Heleili, N., Tlidjane, M., Belkacem. L. & Oucheriah, Y., 2017. Seasonal influence on sperm parameters, scrotal measurements, and serum testosterone in Ouled Djellal breed rams in Algeria. *Veterinary World*, 10(12), 1486–1492. https://doi.org/10.14202/vetworld.2017.1486-1492

Bester, N., 2006. *Effect of different dietary energy levels on productive and reproductive traits in Dorper rams* [MSc Dissertation, University of Free State, Bloemfontein,SA]. https://scholar.ufs.ac.za:8080/bitstream/handle/11660/1342/BesterN.pdf?sequence=1&isAllowed=y

Bezerra, F.Q.G., Aguiar Filho, C.R., Freitas Neto, L.M., Santos Junior, E.R., Chaves, R.M., Azevedo, E.M.P., Santos, M.H.B., Lima, P.F. & Oliveira, M.A.L., 2009. Body weight, scrotal circumference and testosterone concentration in young Boer goat

males born during the dry or rainy seasons. *South African Journal of Animal Science*, 39 (4), 301–306. https://hdl.handle.net/10520/EJC94632

Birringer, M., Pilawa, S. & Flohé, L., 2002. Trends in selenium biochemistry. *Natural Products Reports*, 19(6), 693–718. https://doi.org/10.1039/b205802m

Bopape, M.A., Lehloenya, K.C., Chokoe, T.C. & Nedambale, T.L., 2015. Comparison of electroejaculator and artificial vagina on semen collection from South African Indigenous goat following assessment by computer aided sperm analysis. *Open Journal of Animal Science*, 5(2), 210–218. https://doi.org/10.4236/ojas.2015.52023

Bousta, O., Noui, A., & Boucif, A., 2020. Onset of puberty in Rembi ram lambs; "Clinical and biochemical follow-up". *Livestock Research for Rural Development*, 32(6), 1–5. http://www.lrrd.org/lrrd32/6/abouc32092.html

Brown, B.W., 1994. A review of nutritional influences on reproduction in boars, bulls and rams. *Reproduction Nutrition and Development*, 34(2), 89–114. https://doi.org/10.1051/rnd:19940201

Brown, K.M. & Arthur, J.R., 2001. Selenium, selenoproteins and human health: a review. *Public Health Nutrition*, 4(2B), 593–599. https://doi.org/10.1079/phn2001143

Byrne, C.J., Fair, S., English, A.M., Urh, C., Sauerwein, H., Crowe, M.A., Lonergan, P. & Kenny, D.A. 2017. Effect of breed, plane of nutrition and age on growth, scrotal development, metabolite concentrations and on systemic gonadotropin and testosterone concentrations following a GnRH challenge in young dairy bulls. *Theriogenology*, 96, 58–68. https://doi.org/10.1016/j.theriogenology.2017.04.002

Camela, E.S.C., Nociti, R.P., Santos, V.J.C., Macente B.I., Murawski, M., Vicente, W.R., Bartlewski, P.M. & Oliveira, M.E.F., 2019. Changes in testicular size, echotexture, and arterial blood flow associated with the attainment of puberty in Dorper rams raised in a subtropical climate. *Reproduction in Domestic Animals*, 54(2), 131–137. https://doi.org/10.1111/rda.13213

Chasles, M., Chesneau, D., Moussu, C., Abecia, J.A., Delgadillo, J.A., Chemineau, P. & Keller, M., 2019. Highly precocious activation of reproductive function in autumn-born goats (Capra hircus) by exposure to sexually active bucks. *Domestic Animal Endocrinology,* 68, 100–105. https://doi.org/10.1016/j.domaniend.2019.01.004

Chella, L., Kunene N. & Lehloenya K., 2017. A comparative study on the quality of semen from Zulu rams at various ages and during different seasons in KwaZulu-Natal, South Africa. *Small Ruminant Research*, 151,104–109. https://doi.org/10.1016/j.smallrumres.2017.04.003

Chemineau, P., Cagnie, Y., Guerin, Y., Orgeur, P. & Vallet, J.C., 1991. *Training manual on artificial insemination in sheep and goat*. Food and Agricultural Organization of the United Nations, Rome.

Chemineau, P., Malpaux, B., Delgadillo, J.A., Guérin , Y., Ravault, J., P., Thimoniier, J. & Pelleteir. J., 1992. Control of sheep and goat reproduction: Use of light and melatonin. *Animal Reproduction Science*, 30(1–3), 157–184. https://doi.org/10.1016/0378-4320(92)90010-B

Chenoweth, P.J. & Lorton, S.P. (Eds)., 2014. *Animal andrology: Theories and applications*. CABI, UK. https://cabidigitallibrary.org/doi/book/10.1079/9781780643168.0000

Cloete, S.W.P., Olivier, J.J., Snyman, M.A. & du Toit, E., 1998. Genetic parameters and trends in a selection experiment for increased clean fleece weight involving South African Merinos. *Australian Journal of Experimental Agriculture*, 38(5), 427–432. https://doi.org/10.1071/EA97071

Cloete, S.W.P., Snyman, M.A. & Herselman, M.J., 2000. Productive performance of Dorper sheep. *Small Ruminant Research*, 36(2), 119–135. https://doi.org/10.1016/S0921-4488(99)00156-X

Cloete, S.W.P., Van Niekerk, F.E., Kritzinger N.M., van der Merwe, G.D., Heine, E.W.P. & Scholtz, A.J., 1994. Production responses of sheep supplemented with copper, cobalt and selenium on kikuyu ryegrass pastures. *Journal of the South African Veterinary Association*, 65(2), 52–58. https://hdl.handle.net/10520/AJA00382809_1525

Courtman, C., Van Ryssen, J.B.J & Oelofse, A., 2012. Selenium concentration of maize grain in South Africa and possible factors influencing the concentration. *South African Journal of Animal Science*, 42(5), Suppl. 1. https://doi.org/10.4314/sajas.v42i5.2

Damián, J.P., Beracochea, F., Hötzel, M.J., Banchero, G. R. & Ungerfeld, R., 2015. Reproductive and sexual behaviour development of dam or artificially reared male lambs. *Physiology & Behavior*, 147, 47–53. https://doi.org/10.1016/j.physbeh.2015.04.004

David, I., Kohnke, P., Lagriffoul, G., Praudf, O., Plouarbouéf, F., Degondg, P. & Druart, X., 2015. Mass sperm motility is associated with fertility in sheep. *Animal Reproduction Science*, 161, 75–81. https://doi.org/10.1016/j.anireprosci.2015.08.006

De Souza, L.E.B., De Cruz, J.F., Neto, M.R.T., Nunes, R,C.S. & Cruz, M.H.C., 2011. Puberty and sexual maturity in Anglo-Nubian male goats raised in semi-intensive system. *Revista Brasileira de Zootecnia*, 40(7), 1533–1539. https://doi.org/10.1590/s1516- 35982011000700019

Delemarre, E.M., Felius, B. & Delemarre-van de Waal, H.A., 2008. Inducing puberty. *European Journal of Endocrinology*, 159, Suppl. 1, S9–S15. https://doi.org/10.1530/EJE-08-0314

Delgadillo, J.A., De Santiago- Miramonte, M.A. & Carrillo, E., 2007. Season of birth modifies puberty in female and male goats raised under subtropical conditions. *Animal,* 1(6), 858–864. https://doi.org/10.1017/S1751731107000080

Dolník, M., D. Mudroňová, D., Lazar, & Mudroň, P., 2019. Effect of selenium on oxidative stress and viability of the ram spermatozoa during the spermatogenesis. *The Animal Biology*, 21(2), 16–20. https://doi.org/10.15407/animbiol21.02.016

Dombo, M.H., 2002. *Seasonal effect on semen quality of Gorno Altai and South African indigenous goats* [MSc Dissertation, University of Pretoria, Pretoria, SA]. https://repository.up.ac.za/handle/2263/28586?show=full

Ebling, J.F.P., 2010. Photoperiodic regulation of puberty in seasonal species. *Molecular and Cellular Endocrinology*, 324(1–2), 95–101. https://doi.org/10.1016/j.mce.2010.03.018

Elbaz, H.T & Abdel Razek, E.M., 2019. Effect of vitamin E and selenium injections on the testes and accessory sex glands of Barki rams during non-breeding season. *Advances in Animal and Veterinary Sciences*, 7(6), 434–440. https://doi.org/10.17582/JOURNAL.AAVS%2F2019%2F7.6.434.440

Elhammali, N.S. & Elsheikh, A.S., 2014. Pubertal traits of male goats kept on rations supplemented with different protein types. *Journal of Agriculture and Veterinary Science*, 7, 18–21. https://www.iosrjournals.org/iosr-javs/papers/vol7-issue4/Version-1/D07411821.pdf

El-Mokadem, M.Y., Taha, T.A., Samak, M.A. & Yassen, A.M., 2012. Alleviation of reproductive toxicity of gossypol using selenium supplementation in rams. Journal of Animal Science .90, 3274-3285. https://doi.org/10.2527/jas.2011-4545

El-Zelaky, O.A., Khalifa, E.I., Mohamed, A.H., Bahera, K.M. & Hussein, A. M., 2011. Productive and reproductive performance of Rahmani male lambs fed rations containing Jatropha cake. *Egyptian Journal of Sheep and Goat Sciences*, 6(2), 15–24. https://dx.doi.org/10.21608/ejsgs.2011.27026

Emsen, E., 2005. Testicular development and body weight gain from birth to 1 year of age of Awassi and Redkaraman sheep and their reciprocal crosses. *Small Ruminant Research*, 59(1), 79–82. https://doi.org/10.1016/j.smallrumres.2004.11.012

Erkekoglu, P., Zeybek, N.D., Giray, B., Asan, E. & Hincal, F., 2012. The effects of di (2-ethylhexyl) phthalate exposure and selenium nutrition on sertoli cell vimentin structure and germ-cell apoptosis in rat testis. *Archives of Environmental Contamination and Toxicology*, 62(3), 539–547. https://doi.org/10.1007/s00244-011-9712-9

Esmailizadeh, A.K., 2015. Detection of chromosomal segments underlying scrotal circumference in ram lambs and age at onset of puberty in ewe lambs. *Animal Production Science*, 55(8), 1018–1024. https://doi.org/10.1071/AN14008

Evans, G. & Maxwell, W.M.C., 1987. Handling and examination semen. In: Evans, G. & Maxwell, W.M.C. (Eds.), *Salamon's artificial insemination of sheep and goat*, pp. 93–106. Butterworths, Sydney, Australia.

Farrag, B., El-Hamid, I.S.A., El-Rayes, M.A.H., Shedeed, H.A. & Younis, F.E., 2021. Effect of some organic and mineral salts supplementation on hematological, biochemical, immunological constituents and reproduction in Hassani goats under arid conditions. *Advances in Animal and Veterinary Sciences*, 9(12), 2036–2046. http://dx.doi.org/10.17582/journal.aavs/2021/9.12.2036.2046

Farshad, A., Yousefi, A., Moghaddam, A. & Khalili, B., 2012. Seasonal changes in serum testosterone, LDH concentration and semen characteristics in Markhoz goats. *Asian Australasian Journal of Animal Sciences*, 25(2), 189–193. https://doi.org/10.5713/ajas.2011.11179

Fernández, M., Giráldez, F. J., Frutos, P., Lavín, P. & Mantecón, A. R., 2004. Effect of undegradable protein supply on testicular size, spermiogram parameters and sexual behavior of mature Assaf rams. *Theriogenology*, 62(1–2), 299–310. https://doi.org/10.1016/j.theriogenology.2003.10.003

Fordyce, F., 2007. Selenium geochemistry and health. *AMBIO, A Journal of the Human Environment*, 36(1), 94–97. https://doi.org/10.1579/0044-7447(2007)36[94:sgah]2.0.co;2

Foster, D.L., 1994. Puberty in the female sheep. In: Knobil, E. & Neill, J.D. (Eds.), *The physiology of reproduction*, Vol. 1, pp. 411–447. Raven Press, New York, USA.

Fourie, P.J., Neser, F.W.C., Olivier, J.J. & Westhuizen, C., 2002. Relationship between production performance, visual appraisal and body measurements of young Dorper rams. *South African Journal of Animal Science*, 32 (4), 256–262. https://www.sasas.co.za/wp-content/uploads/2012/09/fourievol32no4_0.pdf

Garcia-Garcia, R.M., 2012. Integrative control of energy balance and reproduction in females. *International Scholarly Research Network*, *Veterinary Science*, Article 121389. https://doi.org/10.5402/2012/121389

Gaur, P., Prasad, S., Kumar, B., Sharma, S.K. & Vats, P., 2021. High-altitude hypoxia induced reactive oxygen species generation, signaling, and mitigation approaches. *International Journal of Biometeorology*, 65, 601–615. https://doi.org/10.1007/s00484-020-02037-1

Geary, T.W., Kelly, W.L., Spickard, D.S., Larson, C.K., Grings, E.E. & Ansotegui, R.P., 2016. Effect of supplemental trace mineral level and form on peripubertal bulls. *Animal Reproduction Science*, 168, 1–9. https://doi.org/10.1016/j.anireprosci.2016.02.018

Ghorbani, A., Moeini, M.M., Souri, M. & Hajarian, H., 2018. Influences of dietary selenium, zinc and their combination on semen characteristics and testosterone

concentration in mature rams during breeding season. *Journal of Applied Animal Research*, 46(1), 813–819. https://doi.org/10.1080/09712119.2017.1406858

Ghorbankhani, F., Souri, M., Moeini, M.M. & Mirmahmoudi, R., 2015. Effect of nutritional state on semen characteristics, testicular size and serum testosterone concentration in Sanjabi ram lambs during the natural breeding season. *Animal Reproduction Science*,153, 22–28. https://doi.org/10.1016/j.anireprosci.2014.12.006

Gresakova, L., Cobanova, K. & Faix, S., 2013. Selenium retention in lambs fed diets supplemented with selenium from inorganic or organic sources. *Small Ruminant Research.* 111, 76–82. http://dx.doi.org/10.1016/j.smallrumres.2012.10.009

Gupta, M. & Gupta, S., 2017. An overview of selenium uptake, metabolism, and toxicity in plants. *Frontiers in Plant Science*, 7, Article 2074. https://doi.org/10.3389/fpls.2016.02074

Hafez, B. & Hafez, E.S.E., 2000. Reproduction in farm animals (7th ed.). Lippincott Williams and Wilkins, USA.

Hall, J.A., Bobe, G., Hunter, J.K., Vorachek, W.R., Stewart, W.C., Vanegas, J.A., Estill, C.T., Mosher, W.D. & Pirelli, G.J., 2013. Effect of feeding selenium-fertilized alfalfa hay on performance of weaned beef calves. *PLoS ONE*, 8(3), e58188. https://doi.org/10.1371/journal.pone.0058188

Haug, A., Graham, R.D., Christophersen, O.A. & Lyons, G.H., 2007. How to use the world's scare selenium resources efficiently to increase the selenium concentration in food. *Microbial Ecology in Health and Disease*, 19(4), 209–228. https://doi.org/10.1080/08910600701698986

Hefnawy, A. E. G. & Pérez, J. L., 2010. The importance of selenium and the effects of its deficiency in animal health. *Small Ruminant Research*, 89(2–3), 185–192. https://doi.org/10.1016/j.smallrumres.2009.12.042

Hernandez, F., Elvira, L., Gonzalez-Martin, J. V., Gonzalez-Bulnes, A. & Astiz, S., 2011. Influence of age at first lambing on reproductive and productive performance of Lacaune dairy sheep under an intensive management system. *Journal of Dairy Research*, 78(2), 160–167. https://doi.org/10.1017/S0022029911000033

Hostetler, C., Kinkaid, R.L. & Mirando, M.M., 2003. The role of essential trace elements in embryonic and fetal development in livestock. *The Veterinary Journal (London, UK:1997)*, 166(2), 125–139. https://doi.org/10.1016/s1090-0233(02)00310-6

Ihsanullah, S.P., Qureshi, M.S., Suhail, S.M., Akhtar, S. & Khan, R.U., 2017. Postpartum ovarian activities, blood metabolites and milk yield are influenced by changing levels of thermal stress in crossbred dairy cows. *International Journal of Biometeorology*, 61(9), 1561–1569. https://doi.org/10.1007/s00484-017-1335-z

Irvine, D.S., 1996. Glutathione as a treatment for male infertility. *Reviews of Reproduction*, 1(1), 6–12. https://doi.org/10.1530/ror.0.0010006

Jafari, S. & Hashemi, A., 2014. Estimation of genetic parameters for body measurements and their association with yearling live weight in the Makuie sheep breed. *South African Journal of Animal Science*, 44(2). http://dx.doi.org/10.4314/sajas.v44i2.6

Jafariahangari, Y., Smith, S., Sharma, R.K., Zerehdaran, S. & Blair, H., 2012. The effect of pre-natal maternal environment on live weight, reproductive and semen characteristics in ram lambs. *Small Ruminant Research*, 103(2–3), 200–204. https://doi.org/10.1016/j.smallrumres.2011.08.010

Jerysz, A. & Lukaszewicz, E., 2013. Effect of dietary selenium and vitamin E on ganders' response to semen collection and ejaculate characteristics. *Biological Trace Element Research*,153(1–3), 196–204. https://doi.org/10.1007/s12011-013-9652-5

Jha, P.K., Alam, G.S., Mansur, A.A., Islam, T. & Bari, F.Y., 2018. Selection of breeding rams by evaluating semen quality. *Journal of Applied Animal Science*, 11(1), 9–20. https://www.cabdirect.org/cabdirect/abstract/20183184608

Jothipriya, R., Sasikumar, S., Madhankumar, E., Pranetha, A. & Kalaiselvi, S., 2014. A study of hypo osmotic swelling test in human spermatozoa. *International Journal Current Research and Academic Review*, 2(12), 47–63. http://www.ijcrar.com/vol-2-12/R.Jothipriya,%20et%20al.pdf

Karagiannidis, A., Varsakeli, S., Alexopoulos, C. & Amarantidis, I., 2000. Seasonal variation in semen characteristics of Chios and Friesian rams in Greece. *Small*

Ruminant Research, 37(1–2), 125–130. https://doi.org/10.1016/s0921-4488(99)00143-1

Kastelic, J.P., 2013. Male involvement in fertility and factors affecting semen quality in bulls. *Animal Frontiers*, 3(4), 20–25. https://doi.org/10.2527/af.2013-0029

Kendall, N. R., McMullen, S., Green, A. & Rodway, R.G., 2000. The effect of zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs. *Animal Reproduction Science*, 62(4), 277–283. https://doi.org/10.1016/s0378-4320(00)00120-2

Kerketta, S., Singh, M., Patel, B.H.M., Patel, T., Dutt, T., Upadhyay, D., Bharti, P.K., Sahu, S. & Kama, R., 2015. Relationships between age, body measurements, testicular measurements and total ejaculation of semen in local goat of Rohilkhand region. *Small Ruminant Research.* 130, 193–196. https://doi.org/10.1016/j.smallrumres.2015.07.006

Khalifa, E.I., Ahmend, M.E., Hafez, Y.H., El- Zolaky, O.A., Bahera, K.M. & Abido, A.A., 2013. Age at puberty and fertility of Rahmani sheep fed on biological inoculated corn silage. *Annals of Agricultural Sciences*, 58(2), 163–172. https://doi.org/10.1016/j.aoas.2013.07.003

Kheradmand, A., Babaei, H. & Batavani, R.A., 2006. Effect of improved diet on semen quality and scrotal circumference in the ram. *Veterinarski Archiva*, 76(4), 333–341. https://core.ac.uk/download/pdf/14379387.pdf

Kleene, K.C., 1993. The mitochondrial capsule selenoprotein—a structural protein in the mitochondrial capsule of mammalian sperm. In: Burk, R.F. (Ed.), *Selenium in biology and human health*, Vol. 7, pp. 133–149. Springer- Verlag, New York, USA.

Krishnan, R.U., 2014. Influence of nutrition on livestock reproduction [Online]. https://en.engormix.com/dairy-cattle/articles/influence-nutrition-livestock-reproduction-t36126.htm

Kumar, D., Bhatt, R.S., Balaganur, K., De, K., Mahla, A.S. &. Sahoo, A., 2021. Milk replacer and linseed supplementation promotes puberty and semen quality in growing male lambs. *Small Ruminant Research*, 202, 106457. https://doi.org/10.1016/j.smallrumres.2021.106457

Kumar, D., Joshi, A. & Naqvi, S.M.K., 2010. Objective assessment of sperm motion characteristics of Malpura ram lambs raised under intensive management system in semiarid tropical environment. *Tropical Animal Health and Production*, 42(4),653–658. https://doi.org/10.1007/s11250-009-9472-y

Lambrechts, H. & Gulyas, G.L., 2019, June 10–12. *The potential of diterpenes to preserve sheep sperm viability and morphological integrity during heat stress conditions* [Conference presentation]. 51st South African Society of Animal Science Congress, University of Free State, Bloemfontein, SA.

Langeveldt, J. Lambrechts, H. & S.W.P Cloete., 2016, July 3–6. *The influence of ram breed and experience on the ease of training for semen collection purposes* [Conference presentation]. 49th South African Society of Animal Science Congress, Stellenbosch, SA.

Letsoalo, P.T., 2017. Characterisation and cryopreservation of semen from the indigenous Namaqua Afrikaner sheep breed, in comparison with the Dorper and Dohne Merino breeds. University of Fort Hare, Alice, SA.

Lukusa, K., & Lehloenya, K.C., 2017. Selenium supplementation improves testicular characteristics and semen quality of Saanen bucks. *Small Ruminant Research*, 151, 52–58. https://doi.org/10.1016/j.smallrumres.2017.04.016

Magawana, M., Dugmore, T.J., de Villiers, J.F. & Gcumisa, S.T., 2021. Effect of lambing season, year, sex and birth status on weaning and post-weaning growth performance of Merino lambs. *Applied Animal Husbandry & Rural Development*, 14, 61–68.https://www.sasas.co.za/wp-content/uploads/2021/12/Magawana-M_2021-Vol-14-1.pdf

Mahmoud, G. B., Abdel-Raheemb, S.M. & Hussein, H.A., 2013. Effect of combination of vitamin E and selenium injections on reproductive performance and blood parameters of Ossimi rams. *Small Ruminant Research*, 113(1), 103–08. https://doi.org/10.1016/j.smallrumres.2012.12.006

Mahmoud, G.B., Abdel-Raheem, S.M.& Hussein, H.A., 2014. Reproductive and physiological traits of Ossimi rams as affected by vitamin E and selenium injection. *Egyptian Society of Animal Production*, 51(2),99–05. https://doi.org/10.21608/ejap.2014.93656

Maina, V.A., Chaudhari, S.U.R., Mshelia, G.D. & Williams, A., 2006. Influence of season on semen characteristics of Sahel bucks in Borno state. *Journal of Applied Sciences*, 6(2), 353−356. https://dx.doi.org/10.3923/jas.2006.353.356

Maksimović, N., Hristov, S., Stankovik, B., Petrovic, M.P., Mekic, C., Ruzic-Muslic, D. & Caro-Petrovic, V., 2016. Investigation of serum testosterone level, scrotal circumference, body mass, semen characteristics, and their correlations in developing MIS lambs. *Turkish Journal of Veterinary Animal Science*, 40(1), 53–59. http://dx.doi.org/10.3906/vet-1505-61

Malejane, C.M., Greyling, J.P.C. & Raito, M.B., 2014. Seasonal variation in semen quality of Dorper rams using different collection techniques. *South African Journal of Animal Science*, 44(1), *27–32.* https://doi.org/10.4314/sajas.v44i1.4

Maquivar, M.G., Smith, S.M. & Busboom, J.R., 2021. Reproductive management of rams and ram lambs during the pre-breeding season in US sheep farms. *Animals,* 11, 2503. https://doi.org/10.3390/ani11092503

Marai, I.F.M., El-Darawany, A.-H.A., Ismail, El.-S.A.-F. & Abdel-Hafez, M.A.M., 2009. Reproductive and physiological traits of Egyptian Suffolk rams as affected by selenium dietary supplementation and housing heat radiation effects during winter of the sub-tropical environment of Egypt (Short Communication). *Archives Animal Breeding*, 52(4), 402–409. https://doi.org/10.5194/aab-52-402-2009

Marijanušić, K., Manojlović, M., Bogdanović, D., Čabilovski, R. & Lombnaes, P., 2017. Mineral composition of forage crops in respect to dairy cow nutrition. *Bulgarian Journal of Agricultural Science*, 23(2), 204–212. https://open.uns.ac.rs/handle/123456789/3303

Martinez, J.M., Dominguez, B., Barrientos, M., Canseco, R., Ortega, E. & Lamothe, C., 2012. Biometry and testicular growth influenced by nutrition on prepubertal Pelibuey lambs. *Online Journal of Animal and Feed Research*, 2(3), 314–321. http://www.ojafr.ir

Mayasula, V.K., Arunachalam, A., Babatunde, S.A., Naidu, S.J., Sellappana, S., Krishnan, B.B., Rajendran, U.S., Janardhan, R.I. & Bhatta, R., 2021. Trace minerals for improved performance: A review of Zn and Cu supplementation effects on male

reproduction in goats. *Tropical Animal Health and Production*, 53(5), 491. https://doi.org/10.1007/s11250-021-02943-5

Mehdi, Y., Hornick, J. L., Istasse, L. & Dufrasne, I., 2013. Selenium in the environment, metabolism and involvement in body functions. *Molecules*, 18(3), 3292–3311. https://doi.org/10.3390/molecules18033292

Meza-Herrera, C.A. 2012. Puberty, kisspeptin and glutamate: A ceaseless golden braid. In: Benhardt, L.V (Ed.), *Advances in medicine and biology*, Vol. 52, pp. 97–124. Nova Science Publishers, NY, USA.

Moghaddam, A., Panah, M. & Souri, M., 2019. Improved early postnatal nutrition and its effect on histomorphological parameters in the testes of Sanjabi ram lambs. *Tropical Animal Health and Production*, 51(6), 1539–1544. https://doi.org/10.1007/s11250-019-01842-0

Mohamed, S.S., Abdelatif, A.M. & Adam, A.A.G., 2012. Effects of exposure to solar radiation on thermoregulation and semen characteristics of Sudanese desert rams (Ovis aries). *Global Veterinaria*, 9(4), 502–507. https://doi.org/10.5829/idosi.gv.2012.9.4.65180

Mohammed, M.Q. & Mutassim, M. A., 2016. Role of insulin and other related hormones in energy metabolism—A review. *Cogent Food & Agriculture*, 2(1), 1267691. https://doi.org/10.1080/23311932.2016.1267691

Mohlomi, M.H., 2015. *Evaluation of cryopreserved ram semen following fertilization in vitro* [MSc Dissertation, University of Free State, Bloemfontein, SA]. https://scholar.ufs.ac.za:8080/bitstream/handle/11660/4218/MohlomiMH.pdf?sequence=1&isAllowed=y

Mojapelo, M.M. & Lehloenya, K.C., 2019. Effect of selenium supplementation on attainment of puberty in Saanen male goat kids. *Theriogenology*, 138, 9–15. https://doi.org/10.1016/j.theriogenology.2019.06.044

Mojapelo, M.M., 2017. Reproduction performance of Saanen bucks supplemented with selenium [MSc dissertation, University of Pretoria, Pretoria]. https://repository.up.ac.za/handle/2263/63294

Mojapelo, M.M., Van Ryssen, J.B.J. & Lehloenya, K.C., 2021. Selenium supplementation reduces induced stress, enhances semen quality and reproductive hormones in Saanen bucks. *Small Ruminant Research*, 201, 106443. https://doi.org/10.1016/j.smallrumres.2021.106443

Moulla, F., El-Bouyahiaoui, R., Nazih, R., Abdelaziz, N., Zerrouki, N. & Iguer-Ouada M., 2018. Characterization of the onset of puberty in Tazegzawt lambs, an endangered Algerian sheep: Body weight, thoracic perimeter, testicular growth and seminal parameters. *Veterinary World*, 11(7), 889–894. https://doi.org/10.14202/vetworld.2018.889-894

Mucina, L. & Rutherford, M.C. (Eds.), 2006. *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute, Pretoria, SA. http://biodiversityadvisor.sanbi.org/wp-content/uploads/2015/12/Strelitzia_19_2006_Part_1.pdf

Mukasa- Mugerwa, E. & Ezaz, Z., 1992. Relationship of testicular growth and size to age, body weight and onset of puberty in Menz ram lambs. *Theriogenology*, 38(5), 979–988. https://doi.org/10.1016/0093-691x(92)90172-n

Munyai, P.H., 2012. *Cryopreservation of South African indigenous ram* [MSc. dissertation, University of Free State, Bloemfontein, SA]. https://scholar.ufs.ac.za:8080/bitstream/handle/11660/1697/MunyaiPH.pdf?sequence=1&isAllowed=y

National Research Council, 2007. *Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids* (6th ed.). The National Academies Press. Washington, DC, USA. https://doi.org/10.17226/11654

Nazari-Zenouz, F., Moghaddam, Gh., Hamidian, Gh., Ashrafic, J., Rafata, S.A. & Qademi-panahi, B., 2016. Postnatal testicular development and testosterone changes in Ghezel ram lambs. *Small Ruminant Research*, 141, 70–76. https://doi.org/10.1016/j.smallrumres.2016.07.001

Nishimura, S., Okano, K., Yasukouchi, K., Gotoh, T., Tabata, S. & Iwamoto, H.K., 2000. Testis developments and puberty in the male Tokara (Japanese native) goat. *Animal Reproduction Science*, 64(1–2), 127–131. https://doi.org/10.1016/s0378-4320(00)00197-4

Nowicka-Bauer, K. & Nixon, B., 2020. Molecular changes induced by oxidative stress that impair human sperm motility. *Antioxidants*, 9(2), 134. https://doi.org/10.3390/antiox9020134

Nur, Z., Zik, B., Ustuner, B., Sagirkaya, H. & Ozguden, C.G., 2010. Effects of different cryoprotective agents on ram sperm morphology and DNA integrity. *Theriogenology*, 73(9), 1267–1275. https://doi.org/10.1016/j.theriogenology.2009.12.007

Oluboyo, A.O., Adijeh, R.U., Onyenekwe, C.C., Oluboyo, B.O., Mbaeri, T.C., Odiegwu, C.N., Chukwuma, G.O. & Onwuasoanya, U.F., 2012. Relationship between serum levels of testosterone, zinc and selenium in infertile males attending fertility clinic in Nnewi, south east Nigeria. *African Journal of Medical Science*, 41 Suppl., 51–54.

Oluwole, O.A., Bartlewski, P.M. & Hahnel, A., 2013. Relationships of serum thyroid hormones and follicle-stimulating hormone concentrations to Sertoli cell differentiation during the first wave of spermatogenesis in euthyroid ram lambs. *Reproductive Biology*, 13(2), 150–160. https://doi.org/10.1016/j.repbio.2013.04.001

Omar, C.A., 2016. Study of some testicular dimensions and their relationship to body weight in Karadi ram lambs. *Assiut Veterinary Medical Journal*. 62(150), 31–38. https://dx.doi.org/10.21608/avmj.2016.169986

Overton, T.R. & Yasui, T., 2014. Practical applications of trace minerals for dairy cattle. *Journal of Animal Science*, 92(2), 416–426. https://doi.org/10.2527/jas.2013-7145

Paiva, F.A., Saran Netto, A., Corrêa, L.B., Silva, T.H., Guimarães, I.C.S.B., Del Claro, G.R., Cunha, J.A. & Zanetti, M.A., 2019. Organic selenium supplementation increases muscle selenium content in growing lambs compared to inorganic source. *Small Ruminant Research*, 175, 57–64. https://doi.org/10.1016/j.smallrumres.2019.04.008

Papachristoforou, C., Koumas, A. & Photiou, C., 2000. Seasonal effects on puberty and reproductive characteristics of female Chios sheep and Damascus goats born in autumn or in February. *Small Ruminant Research*, 38(1), 9–15. https://doi.org/10.1016/s0921-4488(00)00143-7

Parkinson, T.J., Douthwaite, J.A. & Follett, B.K., 1995. Responses of prepubertal and mature rams to thyroidectomy. *Journal of Reproduction and Fertility*, 104(1), 51–56. https://doi.org/10.1530/jrf.0.1040051

Pavlata, L., Misurova, L., Pechova, A., Husakova, T. & Dvorak, R., 2012. Direct and indirect assessment of selenium status in sheep – a comparison. *Veterinary Medicine*, 57(5), 219–223. https://doi.org/10.17221/5951-VETMED

Piagentini, M., Silva, D.C., Dell'Aqua, C.P.F., Moya-Araujo, C.F., Codognoto, V.M., Ramos, A.A. & Oba, E., 2017. Effect of selenium supplementation on semen characteristics of Brazil's ram. *Reproduction in Domestic Animals*, 52(3), 355–358. https://doi.org/10.1111/rda.12903

Pilarczyk, B., Tomza-Marciniak, A., Dobrzański, Z., Szewczuk, M., Stankiewicz, T., Gaczarzewicz, D. & Lachowski, W., 2013. The effect of selenized yeast supplementation on some performance parameters in sheep. *Turkish Journal of Veterinary and Animal Science,* 37(1), 61–67. https://doi.org/10.3906/ vet-1110-23

Polkowska, J., Lerrant, Y., Wańkowska, M., Wójcik-Gładysz, A., Starzec, A. & Counis, R., 2003. The effect of dietary protein restriction on the secretion of LH and FSH in pre-pubertal female lambs. *Animal Reproduction Science*, 76(1–2), 53–66. https://doi.org/10.1016/s0378-4320(02)00237-3

Qazi, I.H., Angel, C., Yang, H., Zoidis, E., Pan, B., Wu, Z., Ming, Z., Zeng, C.J., Meng, Q., Han, H. & Zhou, G., 2019. Role of selenium and selenoproteins in male reproductive function: A review of past and present evidences. *Antioxidants*, 8(8), 268. https://doi.org/10.3390/antiox8080268

Ramukhithi, F.V., Nephawe, K.A., Chokoe. T.C., Matabane, M.B., Mphaphathi, M.L., Lehloenya, K.C., Nedambale, T.L., 2017. Attainment of puberty in South African unimproved indigenous bucks. *Small Ruminant Research*, 153, 57–61. https://doi.org/10.1016/j.smallrumres.2017.05.009

Rayman, M.P., 2000. The importance of selenium to human health. *Lancet*, 356(9225), 233–241. https://doi.org/10.1016/S0140-6736(00)02490-9

Ren, X.M., Wang, G.G., Xu, D.Q., Luo, K., Liu, Y.X., Zhong, Y.H. & Cai, Y.Q., 2012. The protection of selenium on cadmium-induced inhibition of spermatogenesis via activating testosterone synthesis in mice. *Food and Chemical Toxicology*, 50(10), 3521– 3529. https://doi.org/10.1016/j.fct.2012.07.021

Ridler, A.L., Smith, S.L. & West, D.M., 2012. Ram and buck management. *Animal Reproduction Science*, 130(3–4), 180–183. https://doi.org/10.1016/j.anireprosci.2012.01.012

Rowel, M.P., Powell, J.G., Kegley, E.B., Lester, T.D., Williams, C.L., Page, R.J. & Rorie, R.W., 2011. Influence of organic versus inorganic trace mineral supplementation on bull semen quality. In: Kreider, D.L. (Ed.), *Arkansas Animal Science Department Report 2011*, pp. 11–13. Arkansas Agricultural Experiment Station Research Series. https://scholarworks.uark.edu/aaesser/56

Sadeghian, S., Kojouri, G.A. & Mohebbi, A., 2012. Nanoparticles of selenium as species with stronger physiological effects in sheep in comparison with sodium selenite. *Biological Trace Element Research*, 146(3), 302–308. https://doi.org/10.1007/s12011-011-9266-8

Saeed, F.F. & Zaid, N.W., 2019. Serum and testicular testosterone levels of ram lamb during puberty. *Advances in Animal and Veterinary Sciences*, 7(2), 92–95. http://dx.doi.org/10.17582/journal.aavs/2019/7.2.92.95

Saha, U., Fayiga, A., Hancock, D. & Sonon, L., 2016. Selenium in animal nutrition: Deficiencies in soils and forages, requirements, supplementation and toxicity. *International Journal of Applied Agricultural Sciences*, 2(6), 112–125. https://doi.org/10.11648/j.ijaas.20160206.15

SAS Institute Inc., 2012. Statistical Analysis Systems® (SAS®). Cary, NC, USA. https://support.sas.com/software/93/

Schiavon, M., & Pilon-Smits, E.A., 2017. The fascinating facets of plant selenium accumulation–biochemistry, physiology, evolution and ecology. *New Phytologist,* 213(4), 1582–1596. https://doi.org/10.1111/nph.14378

Schrauzer, G.N., 2000. Selenomethionine: A review of its nutritional significance, metabolism and toxicity. *Journal of Nutrition*, 130(7), 1653–1656. https://doi.org/10.1093/jn/130.7.1653

Sejian, V., Maurya, V.P., Naqvi, S.M., Kumar, D., & Joshi, A., 2010. Effect of induced body condition score differences on physiological response, productive and reproductive performance of Malpura ewes kept in a hot, semi-arid environment.

Journal of Animal Physiology and Animal Nutrition, 94(2), 154–161. https://doi.org/10.1111/j.1439-0396.2008.00896.x

Shakirullah, Q.M.S., Akhtar, S. & Khan, R.U., 2017. The effect of vitamin E and selenium on physiological, hormonal and antioxidant status of Damani and Balkhi sheep submitted to heat stress. *Applied Biological Chemistry*, 60(6), 585–590. https://doi.org/10.1007/s13765-017-0313-9

Shalini, S. & Bansal, M.P., 2008. Dietary selenium deficiency as well as excess supplementation induces multiple defects in epididymal spermatozoa: understanding the role of selenium in male fertility. *International Journal of Andrology*, 31(4), 438 – 449. https://doi.org/10.1111/j.1365-2605.2007.00789.x

Shi, L., Duan, Y., Yao, X., Song, R. &, Ren, Y., 2020. Effects of selenium on the proliferation and apoptosis of sheep spermatogonial stem cells in vitro. *Animal Reproduction Science*, 215, 106330. https://doi.org/10.1016/j.anireprosci.2020.106330

Shi, L., Song, R., Yao, X. & Ren, Y., 2017. Effects of selenium on the proliferation, apoptosis and testosterone production of sheep Leydig cells in vitro. *Theriogenology*, 93, 24–32. http://dx.doi.org/10.1016/j.theriogenology.2017.01.022

Shi, L., Zhang, C., Yue, W., Shi, L., Zhu, X. & Lei, F., 2010b. Short-term effect of dietary selenium-enriched yeast on semen parameters, antioxidant status and Se concentration in goat seminal plasma. *Animal Feed Science and Technology*, 157(1–2), 104–108. https://doi.org/10.1016/j.anifeedsci.2010.02.006

Shi, L.G, Yang, R.J, Yue, W.B, Xun, W.J, Zhang, C.X, Ren, Y.S, Shi, L. & Lei, F.L, 2010a. Effect of elemental nano-selenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats. *Animal Reproduction Science*, 118 (2–4), 248–254. https://doi.org/10.1016/j.anireprosci.2009.10.003

Slowińska, M., Jankowski, J., Dietrich, G.J., Karol, H., Liszewska, E., Glogowski, J., Kozłowski, K., Sartowska, K. & Ciereszko, A., 2011. Effect of organic and inorganic forms of selenium in diets on turkey semen quality. *Poultry Science*, 90(1), 181–190. https://doi.org/10.3382/ps.2010-00956

Smith, T.J., & Clarke, I.J., 2010. Seasonal breeding as a neuroendocrine model for puberty in sheep. *Molecular and Cellular Endocrinology*, 324(1–2), 102–109. https://doi.org/10.1016/j.mce.2010.03.007

Sordillo, L.M., 2013. Selenium dependent regulation of oxidative stress and immunity in peripaturm dairy cattle. *Veterinary Medicine International*, Article 1540045. https://doi.org/10.1155/2013/154045

Souza, C.E.A., Araújo, A.A., Oliveira, J.T.A., Lima Souza, A.C., Neiva, J.N.M. & Moura, A.A., 2010. Reproductive development of Santa Inês rams during the first year of life: Body and testis growth, testosterone concentrations, sperm parameters, age at puberty and seminal plasma proteins. *Reproduction in Domestic Animal*, 45(4), 644–653. https://doi.org/10.1111/j.1439-0531.2008.01322.x

Stefanov, R., Chervenkov, M., Anev, G., Maksimović, N., Andreeva, M., Ivanova, T. & Milovanović, A., 2018. Effect of supplementation with inorganic and organic selenium on sperm quality and quantity in north-east Bulgarian merino rams. *Biotechnology in Animal Husbandry*, 34(1), 69–81. https://doi.org/10.2298/BAH1801069S

Sushma, K., Reddy. Y.R., Kumari, N.N., Reddy. P.B., Raghunandan, T. & Sridhar, K., 2015. Effect of selenium supplementation on performance, cost economics, and biochemical profile of Nellore ram lambs. *Veterinary World*, 8(9), 1150–1155. https://doi.org/10.14202/vetworld.2015.1150-1155

Thornton, P.K., 2010. Livestock production: Recent trends, future prospects. *Philosophical Transactions of the Royal Society B*, 365, 2853–2867. https://doi.org/10.1098/rstb.2010.0134

Valasi, I., Chadio, S., Fthenakis, G.C. & Amiridis, G.S., 2012. Management of pre-pubertal small ruminants: Physiological basis and clinical approach. *Animal Reproduction Science*, 130(3–44), 126–134. https://doi.org/10.1016/j.anireprosci.2012.01.005

Van Ryssen, J.B.J., 2001. Geographical distribution of the selenium status of herbivores in South Africa. *South African Journal of Animal Science*, 31(1), 1-8. https://doi.org/10.4314/sajas.v31i1.3840

Van Ryssen, J.B.J., Bradfield, G.D., van Malsen, S. & De Villiers, J.F., 1992. Response to selenium supplementation of sheep grazing cultivated pastures in the Natal Midlands. *Journal of South African Veterinary Association*, 63(4), 148–155. https://journals.co.za/doi/pdf/10.10520/AJA00382809_2969

Van Tilburg, M.F. Salles, M.G.F., Silva M.M., Moreira, R.A., Moreno, F.B., Monteiro-Moreira, A.C.O. Martins, J.A.M., Cândido , M.J.D. Arulijo, A.A. E. & Moura, A.A.A., 2014. Semen variables and sperm membrane protein profile of Saanen bucks (Capra hircus) in dry and rainy seasons of the northeastern Brazil (3°S). *International Journal of Biometeorology*, 59(5), 561–573. https://doi.org/10.1007/s00484-014-0869-6

Villaverde, A.I.S.B., Fioratti, E.G., Ramos, R.S., Neves, R.C.F., Ferreira, J.C.P., Cardoso, G.S., Padilha, P. M. & Lopes, M. D., 2014. Blood and seminal plasma concentration of selenium, zinc and testosterone and their relationship to sperm quality and testicular biometry in domestic cats. *Animal Reproduction Science*, 150(1–2), 50–55. https://doi.org/10.1016/j.anireprosci.2014.08.004

Vural, P., Kabaca, G., Firat, R.D. & Degirmecioglu, S., 2017. Administration of selenium decreases lipid peroxidation and increases vascular endothelial growth factor in streptozotocin induced diabetes mellitus. *Cell Journal*, 19(3), 452–460. https://doi.org/10.22074/cellj.2017.4161

Wood, R.I., Ebling, F.J.P., l'Anson, H. & Foster, D.L., 1991. The timing of neuroendocrine sexual maturity in the male lamb by photoperiod. *Biology of Reproduction*, 45(1), 82–88. https://doi.org/10.1095/biolreprod45.1.82

Wu, Z., Bañuelos, G.S., Lin, Z.Q., Liu, Y., Yuan, L., Yin, X. & Li, M., 2015. Biofortification and phytoremediation of selenium in China. *Frontiers in Plant Science*, 6, 136. https://doi.org/10.3389/fpls.2015.00136

Yatoo, M.I., Saxena, A., Deepa, P.M., Habeab, B.P., Devi, S., Jatav, R.S. & Dimri, U., 2013. Role of Trace elements in animals: A review. *Veterinary World*, 6(12), 963–967. https://doi.org/10.14202/vetworld.2013.963-967

Yue, D., Yan, L., Luo, H., Xu, X. & Jin, X., 2010. Effect of vitamin E supplementation on semen quality and testicular cell membrane and mitochondrial antioxidant abilities in Aohan fine-wool sheep. *Animal Reproduction Science.* 118(2–4), 217–222. https://doi.org/10.1016/j.anireprosci.2009.08.004

Zaki, M.S., Hammam, A.M, Olfat, M.F. & Refat A.Y., 2018. Clinicopathological and Biochemical Study on Selenium toxicity in sheep. *Journal of Advanced Pharmacy Education & Research*, 8(3), 20–23. https://www.cabdirect.org/cabdirect/abstract/20193345322

Zamiri, M.J., Khalili, B., Jafaroghli, M. & Farshad, A., 2010. Seasonal variation in seminal parameters, testicular size, and plasma testosterone concentration in Iranian Moghani rams. *Small Ruminant Research*, 94(1–3), 132–136. https://doi.org/10.1016/j.smallrumres.2010.07.013

Zarkawi, M. & Darker, M.B., 2018. Productive and reproductive parameters in high and low growing Syrian Awassi lambs. *Acta Scientiarum, Animal Sciences*, 40, 379–383. https://doi.org/10.4025/actascianimsci.v40i1.37983

Zhou, J., Chen, L., Li, J., Li, H., Hong, Z., Xie, M., Chen, S.H. & Yao, B., 2015. The semen pH affects sperm motility and capacitation. *PLoS ONE.* 10(7), e0132974. https://doi.org/10.1371/journal.pone.0132974

Ziaei, N., 2015. Effect of selenium and vitamin E supplementation on reproductive indices and biochemical metabolites in Raieni goats. *Journal of Applied Animal Research*, 43(4), 426–430. https://doi.org/10.1080/09712119.2014.980415

Appendix A: Ethical Clearance certificate

UNIVERSITY OF ZULULAND
RESEARCH ETHICS COMMITTEE
(Reg No: UZREC 171110-030)

RESEARCH & INNOVATION
Website: http://www.unizulu.ac.za
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ETHICAL CLEARANCE CERTIFICATE

Certificate Number	**UZREC 171110-030 PGM 2020/36**							
Project Title	**Assessment of selenium supplementation on the attainment of puberty in Merino ram lambs**							
Principal Researcher/ Investigator	N.E Makhado							
Supervisor and Co-supervisor	Prof K.C Lehloenya							
Department	Agriculture							
Faculty	Science and Agriculture							
Type of Risk	Low Risk- Desktop, fieldwork or laboratory							
Nature of Project	Honours/4th Year		**Master's**	x	Doctoral		Departmental	

The University of Zululand's Research Ethics Committee (UZREC) hereby gives ethical renewal approval in respect of the undertakings contained in the above-mentioned project. This approval is extended for another 1 year. The Researcher may therefore continue with data collection as from the date of this Certificate, using the certificate number indicated above.

SPECIAL CONDITIONS: **(1) This certificate is valid for 1 year from the date of issue.**
(2) Principal researcher must provide an annual report to the UZREC in the prescribed format [due date- 25 May 2023]
(3) The UZREC must be informed immediately of any material change in the conditions or undertakings mentioned in the documents that were presented to the meeting.
(4) Under the Protection of Personal Information Act, 04 of 2013 ("POPIA"), researchers have a general legal duty to protect information they process. They must ensure the security and protection of any personal information processed through the research and provide a compliant and consistent approach to data protection. The information collected via interviews must be for research purposes only. No personal information such as opinions, views and academic background may be linked to the respondents' identity or shared with anyone for marketing purposes or otherwise.

The UZREC wishes the researcher well in conducting research.

Prof. Nokuthula Kunene
Chairperson: University Research Ethics Committee
Deputy Vice-Chancellor: Research & Innovation
25 May 2022

CHAIRPERSON
UNIVERSITY OF ZULULAND RESEARCH
ETHICS COMMITTEE (UZREC)
REG NO: UZREC 171110-030
25 -05- 2022
RESEARCH & INNOVATION OFFICE