Attainment of puberty in South African indigenous ram lambs

by

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‘We’re all smart. Distinguish yourself by being kind.’ – Anne Galloway

Declaration

I, Rimbilana N'wa-Nghondzweni Shingange, declare that this dissertation which I hereby submit for the degree MScAgric Animal Science Production Physiology and Product Quality at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:  Date: 06 August 2021

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List of Abbreviations

ANOVA	Analysis of Variance
ARC	Arcuate nucleus
BST	Blood serum testosterone
CASA	Computer aided semen analysis
EIA	Enzyme immunoassay
FAO	Food and Agriculture Organisation
FSH	Follicle stimulating hormone
GnRH	Gonadotrophic releasing hormone
HOS	Hyperosmotic swelling test
IM	Immotile
LH	Luteinizing hormone
LIN	Linearity index
LSmean	Least square mean
NP	Non-progressive progression
PR	Progressive progression
SC	Scrotal circumference
SDG	Sustainable Development Goal
SE	Standard error
STR	Straightness index
TM	Total motility
VAP	Average value
VCL	Curve speed
VSL	Linear speed
WOB	Oscillation index

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"Alone we can do so little; together we can do so much."
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Abstract

This study aimed to evaluate the attainment of puberty in indigenous sheep breeds: 7 ram lambs of the Bapedi, Namaqua-Afrikaner and Zulu sheep breeds were studied. Data was collected biweekly: Scrotal circumference was measured using a measuring tape; blood samples were collected from the jugular vein and analysed using a competitive enzyme immunoassay (EIA); semen was collected using an electro-ejaculator and semen samples were evaluated for macroscopic and microscopic characteristics; sexual behaviour activities recorded were nudging, pawing, bleating, licking, Flehmen's response, pelvic thrust, mounting, penile erection, and nosing; and body weight was measured using a livestock scale. Neither of the three breeds displayed sexual behaviour activities at 3 months, with Namaqua-Afrikaner ram lambs only displaying sexual behaviour at 4.5 months (1 month later than Bapedi and Zulu ram lambs) and being classified as late-maturing. The most observed sexual behaviour activity was nosing. In addition, Bapedi, Namaqua-Afrikaner and Zulu ram lambs had similar bodyweights for 66.6% of the study period and similar blood serum testosterone levels for 88.9% of the study period, showing the homogeneity of these breeds. However, Bapedi, Namaqua-Afrikaner and Zulu ram lambs had significantly different scrotal circumferences for the entire study period ($P<0.05$). This was also the case for most studied semen parameters, except for semen volume, medium velocity, viability, membrane integrity and mid-piece abnormalities - Bapedi and Zulu rams had significantly different sperm cell concentrations at 7 and 8 months ($1.4 \pm 0.61 \times 10^9$ / mL and $4.3 \pm 0.52 \times 10^9$ / mL, respectively for Bapedi ram lambs) and there was also a significant difference in the progressive progression of Bapedi ram lamb semen compared to Zulu ram lamb semen at 7 months (34.4 ± 9.3% and 73.9 ± 4.5% respectively), as well as in other semen characteristics. All three breeds produced satisfactory semen at 7 months of age. For all studied parameters, Pearson correlations were performed, and the strongest correlation was 0.60 between body weight and scrotal circumference. In conclusion, this study indicated that South African indigenous sheep attained puberty at the age of 7 months. The current study also recommended that there should be more research on indigenous sheep breeds for improved reproductive performance and conservation strategies.

Keywords:

Puberty, indigenous, sheep, conservation, ram lamb

Chapter 1: General Introduction

Genetic diversity loss is not only detrimental to the propagation of the breed or species itself, but also to humans. This is particularly true for domestic livestock like sheep. Sheep in general and indigenous breeds in particular have served as sources of livelihood and income generation for smallholder farmers, livestock keepers and consuming families (Kunene, 2010). Through economic and social development, many indigenous breeds positively influence the improvement of livelihoods of resource-poor farmers (Rege *et al.*, 2000). However, breed characterisation in an accurate and robust manner are necessary towards effective conservation in the future (Qwabe *et al.*, 2012) and this breed characterisation is lacking for many indigenous breeds.

A breed's characteristics are important to document comprehensively, to ease effective conservation, utilisation and improvement of such species (Groeneveld *et al.*, 2010; Kunene, 2010). Hitherto, breeds of European origin have been extensively characterised with just a paucity of our indigenous African breeds having been characterised (Qwabe *et al.*, 2012). Hence, necessary studies of our sub-Saharan indigenous breeds (which grow under conditions of heat, ticks and gastro-intestinal nematodes (Maqhashu *et al.*, 2019)) are required in order to reduce a plight situation of European breed replacement (Rege *et al*., 2000).

Due to their robustness and hardiness, these indigenous breeds are more likely to be used by smallholder farmers and livestock keepers in South Africa than European breeds due to their adaptation to the specific pressures of the region. Smallholder farmers are those farmers that depend solely on familial labour; those that own small plots of land where they grow subsistence crops; and those that farm one or two 'cash crops' - crops that are used less by the farmer and more for their commercial value (Department of Agriculture, Forestry and Fisheries, 2017) and livestock keepers are custodians of animal genetic resources (FAO, 2009).

Three of South Africa's oldest indigenous sheep breeds are the Bapedi, Namaqua-Afrikaner and Zulu sheep: The Bapedi sheep have small frames, they are polled, and have fat-tails along with long legs and a shallow body. They are hardy and disease tolerant (Maqhashu *et al.*, 2019). Similar in general phenotype but differing in colour, the Namaqua-Afrikaner is considered to be South Africa's oldest indigenous sheep breed (Snyman *et al.*, 1993; Ramsay *et al.*, 2001). It is used primarily in smallholder

farming systems (Qwabe *et al.*, 2012) and also has a fat tail. They are adapted to the South African Karoo's environmental conditions (Snyman and Herselman, 2005). It is endangered: the number of breeding females ranges from a hundred to one thousand and the number of breeding males ranges between six and twenty (FAO, 2009). Thus, intervention to conserve this breed is necessary and urgent. The Nguni sheep of Zululand, Zulu sheep, are generally dark colored (sometimes with spots), also with fat tails and long legs and shallow bodies. They are classified as insecure (Kunene, 2010) because of the limited available information on their characteristics. The Zulu sheep are, however, heat, humidity, and tick tolerant (Kunene, 2010).

The Bapedi, Namaqua-Afrikaner and Zulu sheep have barely been characterised except in very few scientific studies by authors like Maqhashu (2019) for Bapedi sheep; there is still a sore lack of scientific information about these breeds, particularly on their puberty characteristics. In addition, their semen characteristics have rarely been analysed using computer aided sperm analysis, as is done in this study. It is with the consideration of climate change and the SDGs (Sustainable Development Goals): a commitment to goals like Zero Hunger, No Poverty and Life on Land established by the United Nations' General Assembly, that there is an urgency to conserve our indigenous animals and their genetics, and a part of this conservation is characterisation – genotypic, phenotypic as well as reproductive characterisation. Indigenous breeds also have a role to play in society in the wake of the COVID-19 pandemic: Due to the restriction of movement and limitations on trade, indigenous breeds can be a home-grown source of sustenance and income.

The first chapter of this dissertation introduces the study with a brief introduction while the second chapter of this dissertation provides a broad literature review with incorporated motivations for the study. In the third chapter, the materials and methods used in this research are explained. Chapters four and five then explore the results and discussion of this study, respectively. Finally, a general conclusion and recommendations based on this work are discussed in the sixth chapter.

1.1. Problem statement

Genetic diversity's loss is not only detrimental to the propagation of the breed or species itself, but also to humans (Molotsi *et al.*, 2019). In the case of sheep, particularly indigenous sheep, a source of livelihood and income may be lost for smallholder farmers and livestock keepers and their families. However, a breed can only be conserved if it is characterized accurately and robustly (Groeneveld *et al.*, 2010; Kunene, 2010). Moreover, farmers are increasingly showing preference for the use of high-output

exotic breeds such as Merino sheep in their production systems over indigenous sheep like the Bapedi, Namaqua-Afrikaner or Zulu which are characterised as slow growing, hardier, disease and heat tolerant (Maqhashu, 2019), but albeit less productive. Most government and private organizations are making efforts in terms of general advocacy for exotic breeds (Molotsi *et al.*, 2019; Mendelsohn, 2003). For the aforementioned smallholder farmers and livestock keepers, this strategy places them in jeopardy and places indigenous breeds at risk of extinction - 30% of indigenous animal breeds will become extinct before they are characterised or documented (Muigai, 2002). Thus, this study aims to contribute to the characterisation of these indigenous breeds for their effective conservation and subsequent propagation and availability to the wider livestock market.

1.2. Objectives

Aim:

This study sought to characterise the attainment of puberty in some South African indigenous sheep breeds: the Zulu, Namaqua-Afrikaner and Bapedi sheep. This was specifically achieved via characterizing anthropometric differences between those indigenous rams that have attained puberty and those rams that have not through gonadal measurements, hormonal profiles, semen evaluation, sexual behaviour and live body weights.

Specific objectives:

- To characterise the sexual behaviour of South African indigenous sheep.
- To evaluate gonadal development and hormonal profiles in blood serum of South African indigenous sheep.
- To evaluate when South African indigenous sheep start to produce good quality semen.
- To correlate blood serum testosterone, body weight, scrotal circumference and semen characteristics.

1.3. Hypotheses

A: H_0 = As they age, ram lambs will display no sexual behaviour.

B_1: H_0 = As they age, ram lambs will have static gonadal and body weight measurements.

B_2: H_0 = As they age, ram lambs will have static blood serum testosterone concentrations.

C: H_0 = As they age, ram lambs will have static semen characteristics.

D: H_0 = There will not exist a correlation between testosterone blood serum, body weight, scrotal circumference, and semen characteristics.

Chapter 2: Scientific background and literature review

2.1. Introduction

On the African continent, it is particularly important to have sustainable livestock production as it aids in alleviating poverty while increasing household food security (Webb *et al*., 2018). This sustainable model can be achieved through sound and robust animal reproductive management to ensure proliferation and propagation of certain breeds like the multi-purpose sheep: The sheep is the second animal to be domesticated, after dogs, by humans (Ryder and Bridbury, 1984). The sheep was and continues to be prized for its multi-purposefulness: The sheep produces wool, meat and milk and can adapt to various environmental conditions.

Even in sheep, as with other domestic animals, there exists complex of mechanisms that underly gonadal development and sexual maturity (Moulla *et al.*, 2018). This reflects the difficulty of accurately defining the start of puberty, especially in species with seasonal reproduction activity like sheep (Moulla *et al.*, 2018). In addition, male reproductive endocrinology has not been studied as frequently or extensively as it has been in the female (Bearden *et al.*, 2004).

Several claims have been demonstrated in literature with respect to puberty attainment: According to Hafez and Hafez (2016), puberty attainment occurs as a result of gonadotropic activity that increases due to a simultaneous assumption of gametogenesis and steroidogenesis by the gonads. On the other hand, Smith and Clarke (2010) postulated that it occurs due to awakening of the hypothalamic-pituitary-gonadal axis. However, the puberty process is vital to commercial and small holder farmers (not forgetting consumers) as it is a prerequisite for later sexual maturity- the capability of an organism to reproduce. Without the reproduction of offspring, genetic propagation of a species ceases and so does production and any livelihood or nourishment that was gained from it.

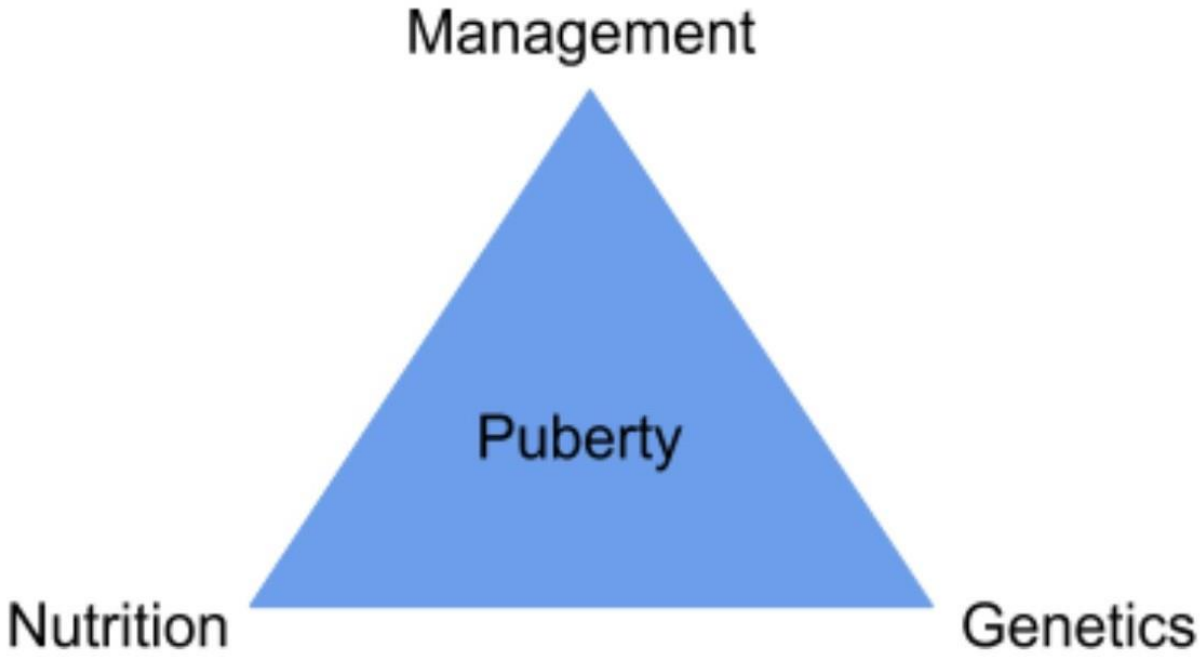

Figure 2.1: Summation of the factors influencing puberty

As pictured above (Figure 2.1), there are various factors, both intrinsic to the animal like genetics and genotype and extrinsic to the animal like the environment that affect puberty and its onset. Foster and Hileman (2015) stated that "the attainment of puberty relies on the integration of information from a wide variety of internal and external sources." Various studies have shown that there exists a relationship between genetic origin, birth date, photoperiod length, nutrition and puberty. Puberty seems to be more dependent on body weight in sheep (Haynes and Schanbacher, 1983; Dyrmundsson and Lees, 1972). This is assumed true even for the studied indigenous breeds.

2.2. Sheep in South Africa

The Department of Agriculture, Forestry and Fisheries (2017) reported that sheep and goats take up 50% of land suitable for extensive and subsistence farming: The Eastern Cape houses 30% of the national sheep population, followed by the Northern Cape at 25%, the Free State at 20%, the Western Cape at 11%, Mpumalanga at 7%, the North West at 3% and Limpopo at 1%. These percentages include a proportion of exotic breeds like the Merino and a proportion of indigenous breeds like the Bapedi, Namaqua-Afrikaner and Zulu sheep.

In the Southern African Development Community (SADC), indigenous sheep play an integral role in the income and sustenance of smallholder farmers and livestock keepers (Molotsi *et al.*, 2019). This is partially due to the arid/semi-arid environment of sub-Saharan Africa, to which sheep are adapted. Indigenous breeds like the Bapedi, Namaqua-Afrikaner and Zulu are particularly suited to South African environmental conditions due to their indigenousness and continued adaptation. Despite their adaptation, however, indigenous breeds are continuously being replaced by foreign breeds like the Merino due to foreign breeds' better production (Snyman and Herselman, 2005). This problem is exacerbated by there being no or slow genetic progress for the indigenous genetic resources used by livestock keepers and small holder farmers (Molotsi *et al.*, 2019). Indigenous breeds are important and their genetic erosion is problematic because of the loss of their adaptability (Molotsi *et al.*, 2019): When indiscriminately cross-bred with exotic/foreign breeds and not maintained as pure breeds, the loss of genetic diversity occurs. This is important particularly with regards to indigenous South African breeds because they are adapted to the environment. With climate change an ever-growing concern in sub-Saharan Africa, it is thus important to maintain breeds that are adapted. For example, higher temperatures may have led to a decrease in tsetse flies that cause trypanosomiasis in Zimbabwe's Zambezi valley, these flies have now migrated to cooler regions (Lord *et al.*, 2018). In addition, another effect that the loss of indigenous animals has is on the remaining animals: They are not adapted to heat and heat stress, which negatively affects their homeostatic mechanisms and subsequently affects growth, performance and reproduction (Marai *et al.*, 2007).

South Africa houses 46 of the 109 different sheep breeds in sub-Saharan Africa (African Union, 2009); it also houses 19.9 million of the 39 million sheep in the SADC region (Department of Agriculture, Forestry and Fisheries, 2019). Three of South Africa's oldest sheep breeds are the Bapedi, Namaqua-Afrikaner and Zulu sheep: The Bapedi sheep are a fat tailed breed that is small-framed, hornless and has long legs and a shallow body. They are indigenous to South Africa (Maqhashu, 2019) are well adapted for the semi-arid bushveld. However, this breed is at risk of disappearing through gradual replacement by imported European sheep breeds due to non-selective cross-breeding and irregular mating (Maqhashu *et al.*, 2019). Their tail is usually long and straight and they are hardy and disease tolerant (Maqhashu *et al.*, 2019). Bapedi sheep's colour differs from uniform brown to white to black and white patterns; the most common colour is white with a red or brown head. When the Bapedi people migrated to the Northern Province from southern Africa, into what is now known as the Limpopo Province, they brought Bapedi sheep with them. This migration occurred between 200 and 400 AD. Many years later, in the mid-1980s, flocks of Bapedi sheep were established and maintained in Sekhukhuneland and Roedtan in Limpopo.

Figure 2.2: Bapedi ram

Similarly, standing tall with a narrow body and with long and lean legs, the Namaqua-Afrikaner is also a South African indigenous sheep breed with fat tails where a maximum of 38% of their body fat can be stored (Snyman *et al.*, 1993). Namaqua-Afrikaners are not favored for slaughter lamb production because, firstly, their body build is unique and their conformation different to other mutton breeds; and secondly, a noteworthy amount of their subcutaneous fat is stored in their tails which means that over the rest of the body there is only a thin layer of fat (Snyman *et al.*, 1993). Namaqua-Afrikaner sheep are primarily found in the north-west Cape of South Africa, as well as Southern Namibia (Snyman, 2014). It is interesting to note that Namaqua-Afrikaner ewes are ideal for accelerated lambing systems because they can be mated throughout the year (Snyman, 2014).

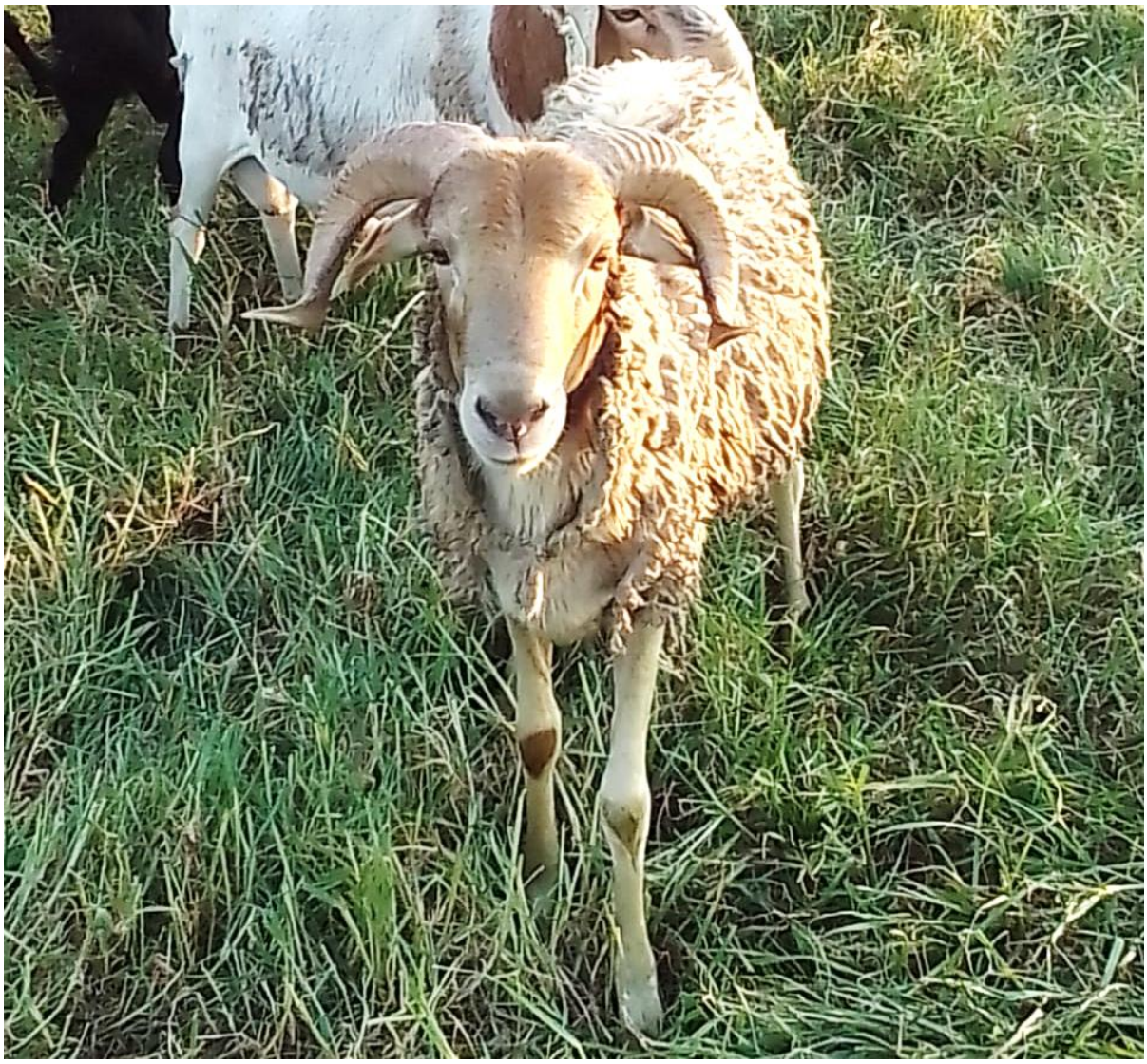

Figure 2.3: Namaqua-Afrikaner ram

Lastly, the Nguni sheep of Zululand, the Zulu sheep, are said to be adapted to the heat and humidity of Kwa-Zulu Natal in South Africa (Kunene, 2010). The sheep are multi-colored with a brown and white combination being most dominant (Kruger, 2001). Interestingly, sheep that are brown as adults are born as black lambs and gradually change colour with age (Kunene, 2010). Ramsey *et al.* (2000) described the breed as “small to medium”. Zulu sheep have four ear types: mouse; small; medium and large, with large ears being the most common (Kunene and Fossey, 2006) as pictured in Figure 2.4. Haigh (2008) speculates that mouse ears may be an adapted defensive mechanism against picking up ticks.

Figure 2.4: Zulu ram

For males of a given breed, a valuable tool for selection may be the characterisation of early sexual development and puberty (Khalifa *et al.*, 2013). This, however, logically requires sufficient academic research into the characterization of their reproductive physiology. This tool may achieve the cessation of poverty, which relies heavily on food security and can be improved by the proper identification of puberty and use of younger ram lambs. Progeny testing and libido testing may be done earlier if younger ram lambs are used; in addition, using younger ram lambs may accelerate the benefits of genetic selection and even reduce production costs (Khalifa *et al.*, 2013). These rams can be considered as genetically superior animals, and their identification and selection for breeding purposes is the only way to achieve long term improvement of animal production efficiency (Casey and Maree, 1993). In addition, practical strategies to decrease the generational interval, to synchronise market demand with lambing, and to increase the overall production of food and fibre can be achieved by an understanding of how puberty is timed (Foster and Hileman, 2015).

The cessation of poverty can also be accelerated through the empowerment of smallholder farmers, who mostly farm with indigenous breeds: Smallholder farmers are those farmers that depend solely on familial labour; those that own small plots of land where they grow subsistence crops; and those that farm

one or two ‘cash crops’ - crops that are used less by the farmer and more for their commercial value (Department of Agriculture, Forestry and Fisheries, 2017). For example, smallholder farmers own 1 - 15 head of cattle and 1-10 sheep or goats (Lehohla, 2011). Small stock plays a foundational role in the economic security and food security of millions in sub-Saharan Africa, with small scale and communal farmers owning 12% of sheep (Meissner *et al.*, 2014). Small ruminants possess an important role in the continuously growing world population mainly for the harsh climatic conditions or sub-fertile lands in economies of developing countries (Amiridis and Cseh, 2012). The productivity and proliferation of small stock is, however, affected by their reproductive efficiency. This reproductive efficiency can be improved and accelerated through robust and accurate characterisation of the reproductive traits and processes of indigenous breeds, which is important not only for income and livelihood gained from these animals, but also for their conservation.

2.3. Conservation

The United Nation’s Food and Agriculture Organisation (FAO) in 2009 indicated that in order to determine unique traits like fitness and performance, it is important to perform comprehensive characterisation of indigenous breeds in their habitats, in order to design conservation strategies. In addition, the Sustainable Development Goals (SDGs) are an example of the international acknowledgement of the importance of indigenous knowledge; they were endorsed in 2015 by Nation Member States. Farmers around the world are abandoning indigenous breeds that have been locally adapted for thousands of years for exotic albeit more productive breeds. An example of this is seen in the South African mutton industry, wherein exotic breeds like the Merino, of European lineage, are preferred for use in feedlots and on larger, more commercial farms (Molotsi *et al.*, 2019) over indigenous breeds like the Bapedi or Zulu sheep. Mendelsohn (2003) points out that especially in developing countries, the replacement of indigenous breeds raises many concerns for conservation.

If market forces and economies dictate to farmers that they are to farm with more profitable species for the sake of their livelihoods and productivity, what worth do indigenous breeds have? Mendelsohn (2003) highlights four reasons to support conservation: firstly, genetic stock value, secondly, environmental/landscape effects, thirdly, maintaining traditional lifestyles, and lastly, existence value. With regards to genetic stock value, even if individual indigenous breeds are no longer seen as valuable or viable by farmers, they may be instrumental in the creation of future breeds - breeds may contain certain characteristics that may be useful for future breeds, like the Booroola gene (a major prolificacy gene that leads to twinning in sheep). However, environmental/landscape effects simply outline that indigenous

breeds may be useful for societal aesthetics. The maintenance of traditional lifestyles is also linked to society, as society may want to maintain historic activities and traditional livelihoods. Lastly, existence value is the notion that a species or breed may be valuable simply by virtue of its existence – and that they must be preserved for future posterity.

It is important to explicitly state the benefits of conservation. This is because conservation tends to be a term that many people are desensitized to. In addition, these benefits can be quantified so that society (and governments) have a better sense of the true value of indigenous breeds and determine how much they would like to invest in conservation efforts. Lastly, those in charge of conservation are better able to identify their aims.

Economically, it makes sense that indigenous breeds are declining: Farmers are ultimately driven by profit and despite theories that they are simply following farming trends by using exotic breeds, these exotic breeds have continuously proven to be more profitable and these 'trends' would not be sustained. Economics may be a factor in the decline of indigenous species, however a more restrictive factor, particularly in developing countries, is government policies: Governments very often attempt to gain market share by subsiding intensive animal husbandry that very often uses exotic breeds; and they may be subsiding capital-intensive agriculture, which also very often uses exotic species, without analysis as to whether that form of agriculture is beneficial for the local economy and livelihoods. Despite these government inefficiencies, sperm biotechnologies require the generation of knowledge on reproductive physiology. This is a prerequisite for future promoting and conservation strategies (Moulla *et al.*, 2018).

Conservation ultimately aims to maintain the contribution of animals towards food production (Hanotte *et al.*, 2000) in one of two ways: *ex situ* or *in situ* conservation. *In situ* conservation, also coined 'on-farm conservation' aims to conserve animals in their production system or in the area where the breed developed its characteristics (Kunene, 2010). Research into the genetic uniqueness of a breed is enabled as breeds adapt continuously to fluctuating environmental pressures. In addition, this method is ideal for resource-poor farmers because it is inexpensive and convenient. Contrastingly, *ex situ* conservation conserves animals outside of their habitat, very often using methods like semen or ova freezing (Collins *et al.*, 2001) and even extends to the keeping of animals in zoos or experimental farms.

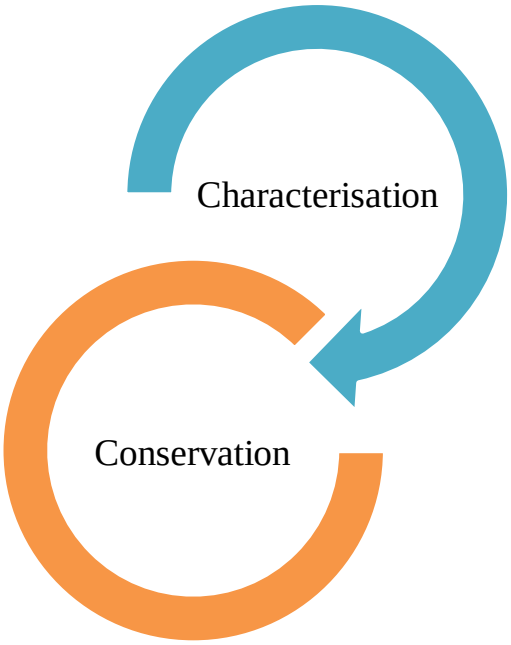

Figure 2.5: Characterisation and conservation (adapted from Kunene, 2010)

Ultimately, this study will contribute to the much-needed *in situ* conservation of the Bapedi, Namaqua-Afrikaner and Zulu sheep breeds through the characterisation of their attainment of puberty. This is because the findings of this study will contribute to decision-making policy for the conservation, breeding and improvement programmes of indigenous sheep' genetic resources in South Africa. Moreover, information obtained in this study will be disseminated to researchers, sheep producers and emerging farmers in South Africa through publications, workshops and conferences.

2.3.1. *In situ* vs *Ex situ* conservation

The *in situ* method is the conservation of live animals with utilization in their production system, or in the area where the breed developed its characteristics and is commonly referred to as 'on-farm conservation.' This contrasts with the *ex situ* conservation method wherein animals are conserved outside of their habitat and can commonly be achieved using zoos or experimental or show farms (Maqhashu, 2019).

2.4. Puberty defined

The challenge with accurately and concisely defining 'puberty' is that it firstly involves an acceleration of developmental processes that have begun much earlier (Bronson and Rissman, 1986); secondly, it varies widely between sexes and species; and lastly, from a scientific perspective, different authors have different motivations for studying puberty and thus see and characterise it very differently. To determine puberty in males is more difficult as the production of sperm (an endpoint for the attainment of puberty) is the culmination of a process that began approximately 60 days earlier (Foster and Hileman, 2015). In males, the ram included, puberty may be defined in various ways. Smith and Clarke (2010) refer to puberty as the initiation of the hypothalamic-pituitary axis (similar to gonadarche) while Foster and Hileman (2015) and Bearden *et al.* (2004) also make reference to the fertilizing ability or spermarche of the ram. Similarly, Edmondson (2012) defined puberty in males as the age at which a ram develops a sexual interest in ewes and produces spermatozoa in numbers sufficient to cause pregnancy and Bearden *et al.* (2004) and Plant (2015) concur.

Overall, in all definitions of puberty, sensitivity to negative feedback on the testes caused by testosterone decreases over time and allows circulating gonadotropins (FSH and LH) to rise to the point wherein they can stimulate testicular development and differentiation (Ramirez and McCann, 1963). Therefore, this study will consolidate the seminal and testicular developments and milestones of the ram lamb with a concise definition for the attainment of puberty.

2.5. Factors affecting the attainment of puberty

There are several signals, as depicted above (Figure 2.1) that pace the timing of when the GnRH neurosecretory system increases its activity from pre- to post-puberty (Foster and Hileman, 2015). The signals work to provide information as to when the organism must begin to reproduce – through metabolic cues, whether a sufficient body size has been attained; through environmental cues, whether conditions are optimal; through social cues, whether beneficial relationships exist with other individuals.

Foster *et al.* (1986) gives an interesting analogy to describe puberty and its attainment: "Using a clock analogy, the alarm, but not the clock itself, is species-specific and may be modified genetically to reflect race or breed differences. Expression of the alarm (when it rings) is determined by the integration of the alarm with multiple permissive cues. Thus, for example, while the clock determines the year in which

an individual will produce the pubertal GnRH rise, it is the permissive cues that ultimately determine the month in which one or more genes that control the regulation of GnRH secretion are activated. Such a long-running developmental clock (days, months, or years depending upon species) awaits an anatomical–biochemical description, as does the mechanism by which permissive cues are integrated with the clock."

Puberty and the age at which it is attained depends on various factors, two of which are season born and breed. Interestingly, there exists an analogue of the ram effect in ewes which will be referred to as the 'cycling ewe' effect: The ram effect is the stimulation of estrus and ovulation in ewes following exposure to a ram. The 'cycling ewe' effect can then be considered as the triggering of puberty in rams exposed to cycling ewes periodically, who will then reach puberty earlier (Edmondson, 2012).

The attainment of puberty depends on the endocrine system: Weeks before puberty, LH surges commence and causes differentiation of the Leydig cells that subsequently produce the androgen, androstenedione (Bearden *et al.*, 2004). Differentiation continues and LH stimulates an increasing concentration of testosterone. There then occurs a synergy between testosterone and FSH to cause development of the Sertoli cells (which then produce androgen-binding protein) and preparation for the production of spermatozoa by the seminiferous tubules. However, there are various other factors that may interact with the neuroendocrine system:

2.5.1. Body weight, composition and leptin

Growth and nutrition are a prerequisite to begin reproduction in almost all living organisms (Foster and Hileman, 2015). Kennedy and Mitra (1963) hypothesized that one of the cues for the attainment of puberty is changes in energy availability as the energy demands of growth and thermoregulation take priority over that for reproductive function.

The newborn lamb has virtually no body fat (Webster, 1979) and thus milk from the ewe acts as a continuous source of energy. The lack of body fat indicates the lack of energy excess, which outlines that the priority for nonessential processes like reproduction is low. In addition, the newborn lamb has high energy expenditure (Foster and Hileman, 2015). The greatest energetic cost is thermoregulation and cellular maintenance, and the rest of energy intake is dedicated to muscular activity and growth (Foster and Hileman, 2015) – at this point, the lamb is hypogonadotropic. However, as body mass becomes larger with growth and relative surface area decreases, metabolic rate decreases which allows energy to be available for other physiological demands. This does not occur with slow or restricted growth and the attainment of

puberty is delayed: the frequency of GnRH pulses in the pituitary portal vasculature is reduced in growth retarded lambs (I'Anson *et al.*, 2000) but GnRH concentrations in the terminals and cell bodies is not markedly reduced in growth-retarded lambs (Ebling *et al.*, 1990). This suggests that the central mechanisms controlling GnRH release are limiting LH secretion when the attainment of puberty is delayed by growth retardation, rather than being limited by the synthesis of GnRH or LH. In order for excess energy reserves to be available to support reproductive processes, sufficient somatic development must have occurred to complete sexual maturation. It is important to note that the postnatal period of hypogonadotropism may be prolonged in sheep with retarded growth.

When considering the ontogeny of the sheep in a flock of lambs, it is notable that the frequency of LH pulses that increases in the neonatal period has a greater correlation with body weight than with age (Foster and Hileman, 2015) – reducing the caloric daily intake to suckling lambs fed on artificial milk retards the increase in LH pulse frequency. In addition, short-term fasting of lambs gradually depresses LH secretion and rapidly increases with realimentation, suggesting that even on a moment-to-moment basis, mechanisms controlling GnRH secretion are highly sensitive to changes in metabolic fuel.

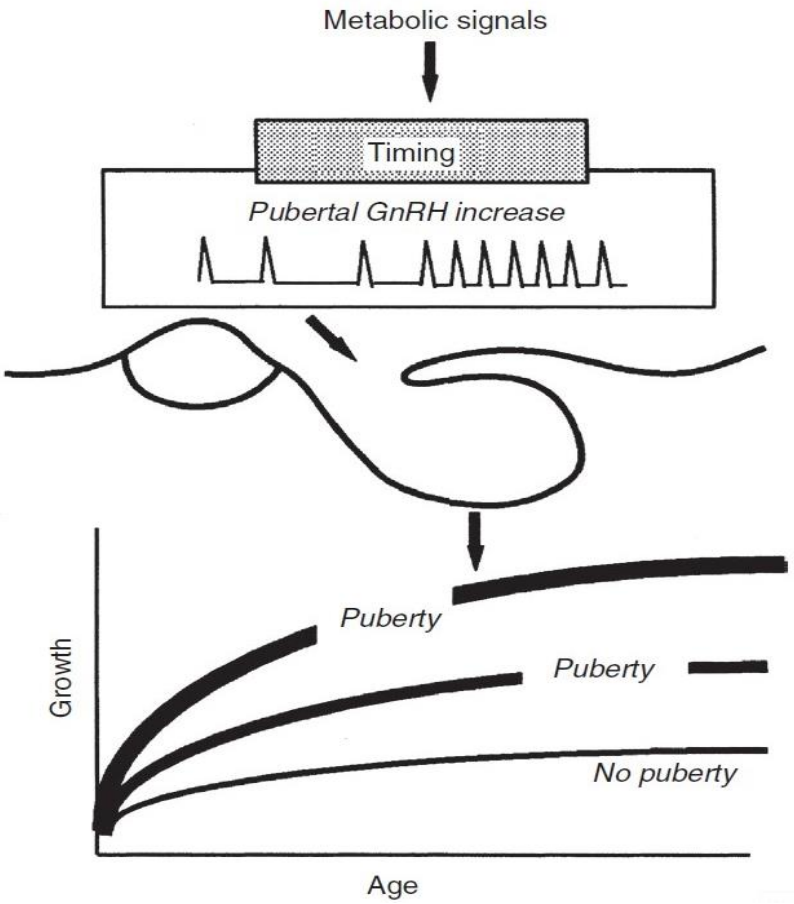

Figure 2.6: Growth and the timing of puberty (Foster and Hileman, 2015)

Frisch (1991) hypothesized that in order to start reproductive cycles, it is important to achieve a definite level of fatness. Until the discovery of adipocyte-derived hormone leptin, there existed no mechanism for how fatness could affect GnRH neurons. In the hypothalamus, there is a protein called leptin that functions in appetite suppression and is secreted primarily by adipocytes (Halaas *et al.*, 1995). The function of leptin was determined by studies in mice wherein obese, hypogonadal nice were injected with leptin and subsequently had a suppressed appetite, reduced body weight, and established fertility (Foster and Hileman, 2015).

For leptin to function as a metabolic timing signal for the attainment of puberty and its associated GnRH secretion increase, it must satisfy the following criteria (Foster and Hileman, 2015):

- Puberty must be related to a pattern of peripheral leptin secretion.
- Exogenous/administered leptin must stimulate or advance the attainment of puberty in prepubertal animals.

In sheep, the pattern of leptin depends on nutrition and sex: During the pre-ruminant period when the suckling reflex is still active, plasma leptin levels are controlled by level of nutrition; During the prepubertal phase, plasma concentrations of leptin correlate with the frequency of LH pulses (Foster and Hileman, 2015). With regards to the second criteria, in developing rams the ability of leptin to stimulate the attainment of puberty depends on the stage of development and energy availability – LH pulse frequency increases after leptin treatment in some rams, but there is no clear evidence that GnRH or LH secretion is stimulated by artificial leptin treatment in the ewe lamb. This suggests that there are sex differences. For example, the energetic cues may be qualitatively similar but different sexes may require quantitatively different levels to sustain the high level of GnRH secretion required for gonadotrophin secretion to the gonads (Foster *et al.*, 1986). Lastly, it is interesting to note that although body weight, body composition and leptin may indicate that the attainment of puberty is optimal, other factors may counteract these signals: For example, with season and the environment, even if growth indicates that adequate size or sufficient energy balance for puberty has been attained, if environmental conditions indicate that the season for puberty is not optimal then the expression of high frequency GnRH pulses will not be permitted (Foster and Hileman, 2015).

2.5.2. Kisspeptin

Kisspeptin (a family of neuropeptides critical for initiating puberty (Hu *et al.*, 2018)) is important for puberty and the seasonal shift in reproduction as determined by day-length in sheep (Smith and Clarke, 2010). Kisspeptins are products of the Kiss1 gene and stimulate GnRH secretion (Gottsch *et al.*, 2004). As an example of comparison, in rodents there exists two populations of kisspeptin producing cells – in the arcuate nucleus (ARC) and the anteroventral periventricular area (AVPV) (Smith and Clarke, 2010). Similarly, kisspeptins are located in the arcuate nucleus (ARC) in the ovine brain (Estrada *et al.*, 2006). These ARC cells transmit feedback from sex-steroids to the GnRH neurons (both positive and negative feedback) (Smith and Clarke, 2010). In addition, in the ARC of the ewe there is a greater number of kisspeptin cells that mediate the positive and negative feedback effects of estrogen (Smith and Clarke, 2010).

Injection of kisspeptin has stimulated LH surges in various species including mice, rats, humans, cattle and sheep and its effect seems to be primarily due to actions on GnRH neurons (Foster and Hileman, 2015). Kisspeptin expression in rodents is reduced by food restriction (Quennell *et al.*, 2011; Luque *et al.*,

2007; Kalamatianos *et al.*, 2008; Roa *et al.*, 2009) and notably, ARC produced kisspeptin co-express the receptor for leptin as well as insulin (discussed below) (Qiu *et al.*, 2013) – Deleting kisspeptin insulin receptors from kisspeptin neurons delays the onset of puberty (Qiu *et al.*, 2013). It is thus important to note that the loss of function mutations in either kisspeptin or its receptor (KISS1R) result in a failure to reach puberty (Foster and Hileman, 2015). In addition, KISS1 mRNA expression in ewe lambs that are fed to be lean compared to ewe lambs fed to a normal body weight in lower (Backholer *et al.*, 2010).

Thus, in ram lambs being studied, kisspeptin will play an active and important role in the attainment of puberty. Unfortunately, kisspeptin is a neuropeptide not found in blood serum and cannot be tested for using the materials and methods in this study – It would be interesting to note whether there is a correlation between the secretion patterns of GnRH and LH and kisspeptin.

2.5.3. Gender

In the figure below, (Figure 2.7), the rise in circulating LH concentrations overlaps with the onset of ovarian cycles and spermatogenesis initiation in gonadal-intact lambs (shaded areas). When comparing ewes to rams, ovulations begin at approximately 30 weeks of age in response to the LH surge that drives the first follicular phase rise in estradiol to trigger the preovulatory GnRH surge. This is then followed by ovulatory cycles that are repetitive and each have a 3-day follicular phase and 14-day luteal phase. In rams, however, the prepubertal LH rise begins much earlier (spermatozoa are formed in the seminiferous tubules many weeks before appearing in ejaculate (Bearden *et al.*, 2004)) and initiates a gradual increase in testosterone secretion. Thus, testicular function is slow in its development and requires 2 months to complete and sustain spermatogenesis (Foster and Hileman, 2015).

It is interesting to consider that the earlier LH surges in the male are in order to coincide the readiness of fertile sperm with ovulations and the onset of ovulations; as well as to gradually develop secondary sex characteristics that allow competition with other rams.

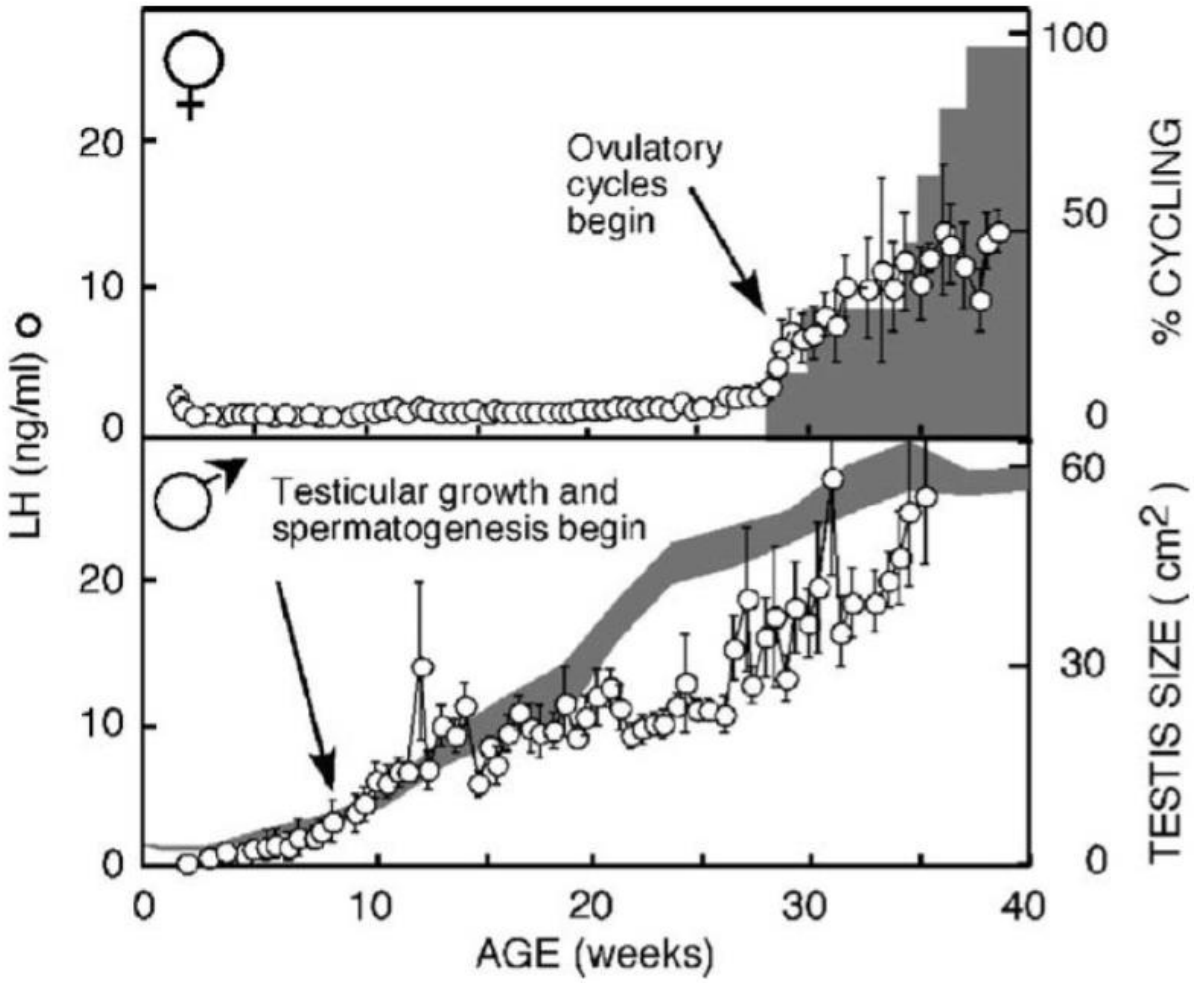

Figure 2.7: The pubertal increase in luteinizing hormone in gonadectomized females and male lambs in which a subcutaneous implant was used to maintain circulating estradiol (Foster and Hileman, 2015).

2.5.4. Comparing the sheep as a species

In order to understand puberty in the sheep, in rams specifically, one must be willing to study and compare other species and compare their mechanisms of puberty attainment to establish a model for sheep. Because sheep are precocial, many maturation stages are achieved prenatally in sheep (Foster and Hileman, 2015).

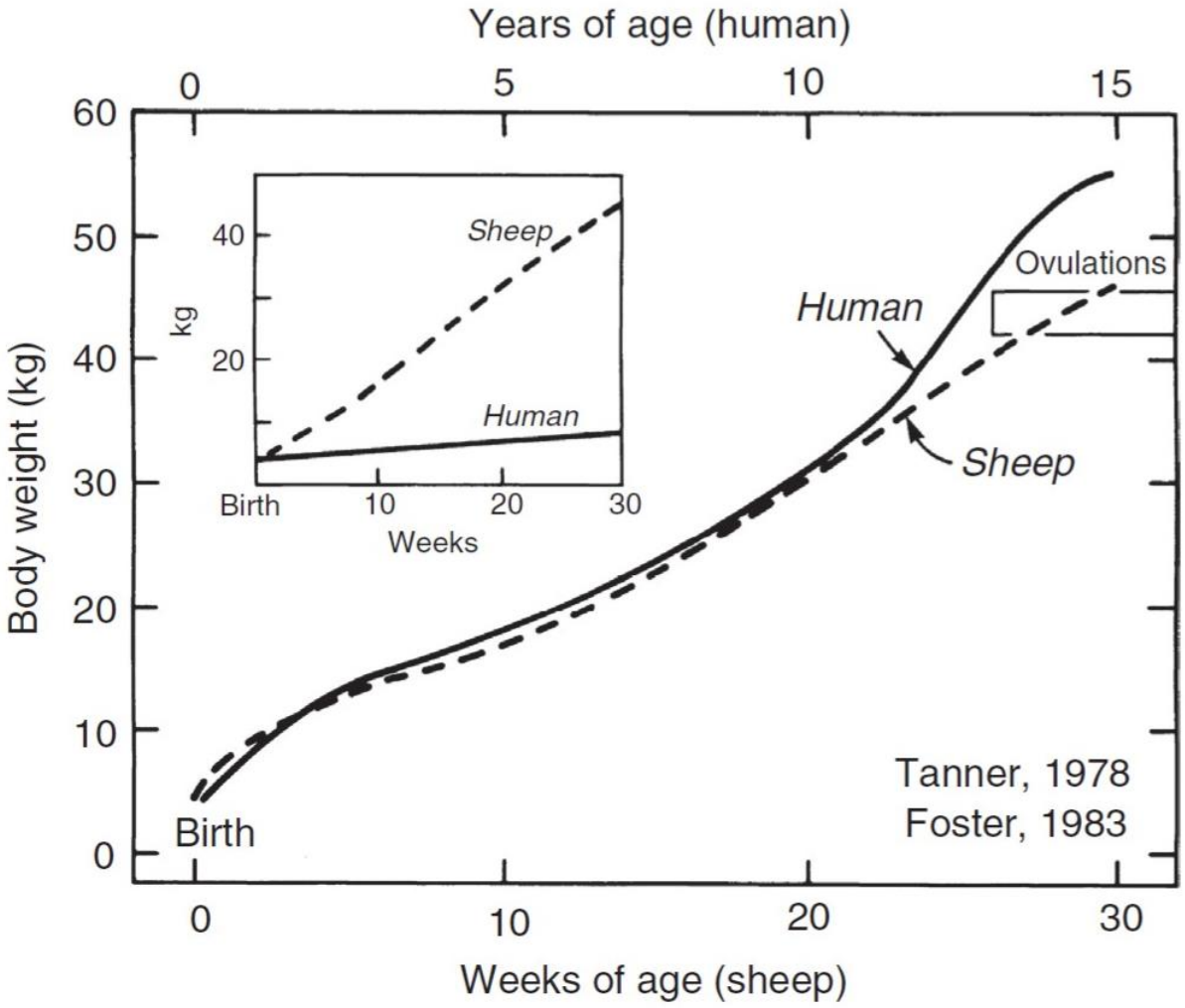

Figure 2.8: Growth curves illustrating the precocial versus altricial species concept (Foster *et al.*, 1985)

The figure above (Figure 2.8) illustrates the precocial versus altricial species concept: Even though the lamb and human are both at similar birth weights, the lamb grows faster. For example, at 30 weeks of age human infant's weight has only doubled whereas that of the lamb has decupled and reproductive cycles have begun (Foster and Hileman, 2015). Thus, the lamb serves as a model for many mammalian species and although their cycles are merely accelerated, they are nonetheless comparable.

It is interesting to note that, firstly, humans deviate from the normal mammalian pattern of seasonal reproduction, and it may be considered that the expression of sexual traits in the human is less complex than in the mammal. Secondly, the lamb has the ability to spontaneously produce hourly LH surges (under the removal of the ovaries and subsequent estradiol negative feedback) thus the ability to produce high

frequency gonadotropin releasing hormone (GnRH) and LH surges like those that initiate the follicular phase exists from a few weeks after birth (Foster and Hileman, 2015). This also suggests that the pituitary is highly responsive to GnRH.

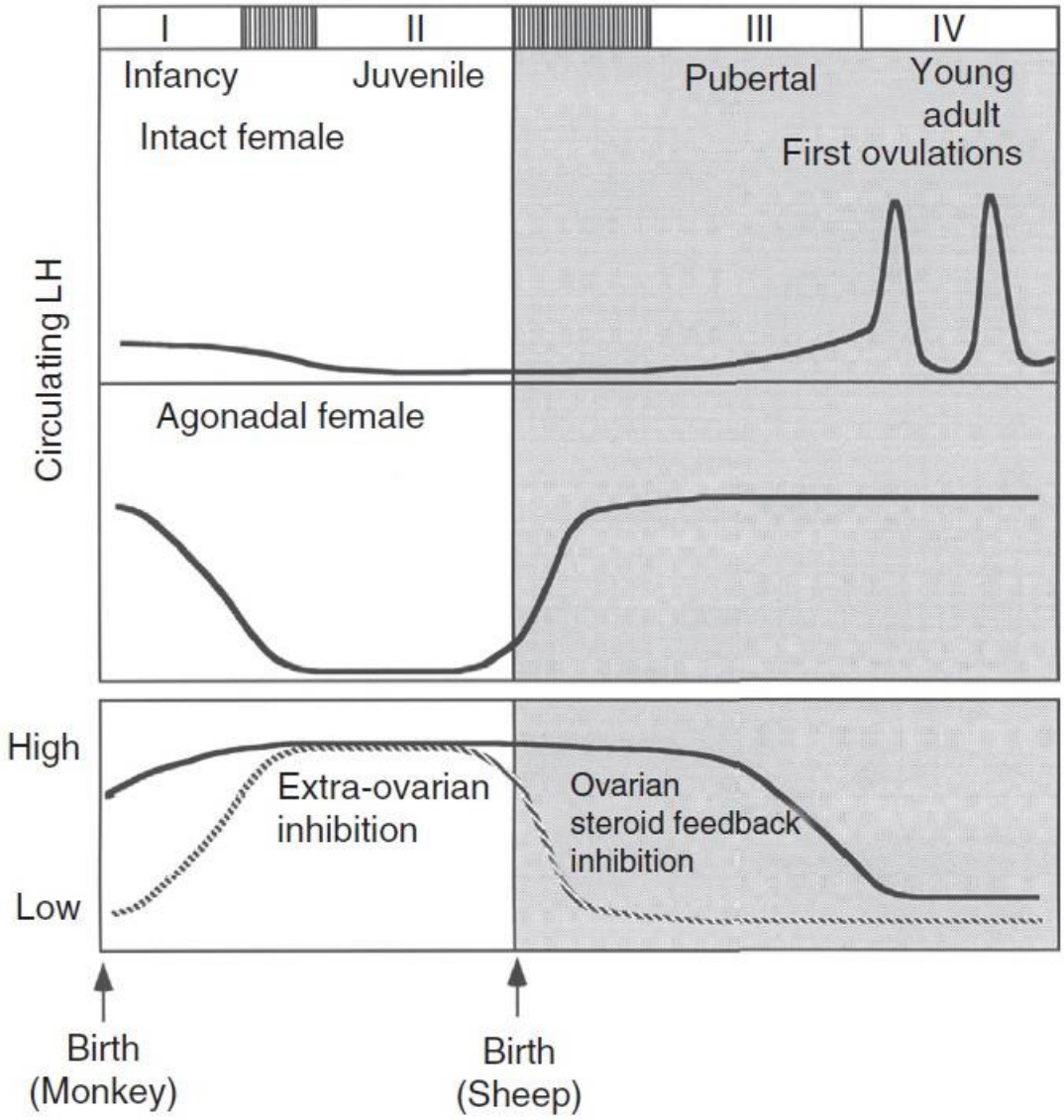

Figure 2.9: Phases of sexual maturity in the monkey and sheep (Foster *et al.*, 1986)

As shown in the figure above (Figure 2.9), the precocial sheep has developed the hypothalamo-hypophyseal-ovarian axis necessary for puberty and is at Stage III before birth. However, the ability to secrete GnRH and subsequently LH at high frequency and amplitude is dimmed in postnatal sheep through estradiol hyperresponsiveness that is dominant from birth until the attainment of puberty at approximately 7 months of age (Foster and Hileman, 2015). In contrast, the monkey's period of sensitivity to steroid

feedback inhibition is only the final 25-30% of its postnatal development. Because testosterone is essential for the development and maintenance of secondary sex characteristics, sperm production and the regulation of sexual characteristics (Saaed and Zaid, 2018), this study will profile the blood serum testosterone levels of ram lambs in order to gauge the point of puberty attainment and effect of rising testosterone levels.

2.6. Bibliographic comparison of the attainment of puberty in ram lambs of different breeds

In this section, reference to puberty in ram lambs of other sheep breeds is given in order to provide a scope of the variation that exists.

Table 2.1: Bibliographic comparison of the attainment of puberty in ram lambs of different breeds (adapted from Bousta *et al.* (2020))

Country	Breed	Number	Attainment of puberty (months)	Body weight (kg)	Scrotal circumference (cm)	Blood serum testosterone concentration (ng/ml)	References
Algeria	Rembi	18	7.3 ± 0.2	36.3 ± 3.0	24.3 ± 0.5	3.5 ± 0.4	Bousta *et al. (*2020)
Algeria	Ouled Djellal	10	7.5 ± 0.2	40.4 ± 1.2	21.8 ± 0.8	4.0 ± 1.7	Boussena *et al*. (2016)
Algeria	Tazegzawt	10	7.7	38.0 ± 5.0	25.8 ± 3.7	-	Moulla *et al.* (2018)
Greece	Friesland	20	5.9 ± 0.4	49.8 ± 3.7	44.5 ± 0.9	-	Belibasaki and Kouimzis (2000)
	Karangouniki		6.2 ± 0.3	44.9 ± 3.2	27.5 ± 2.0	-	
	Chios		6.3 ± 0.4	50.2 ± 2.6	28.9 ± 2.9	-	
	Serres		6.9 ± 0.2	44.5 ± 3.9	27.0 ± 1.1	-	
Iran	Ghezel	36	7.9	42.39	25.8	2.0	Nazari-Zenouz *et al.* (2016)
Jordan	Awassi	13	8.0 ± 0.2	41.3 ± 1.7	24.6 ± 1.1	2.0	Kridli and Al-Yakoub (2006)
Morocco	D'Man	16	4.8 to 5.4	16.7 ± 19.7	21.4 ± 2.0	2.1 ± 1.0	Derqaoui *et al.* (2009)
Saudi Arabia	Nadji	24	6.4	38.6 ± 0.6	25.0 ± 0.3	3.4 ± 1.4	Swelum *et al.* (2017)

2.7. Neuroendocrine and endocrine regulators in reproduction

Hypogonadotropism underlies the anovulatory condition of prepubertal sheep (Foster and Hileman, 2015). This statement is better understood once a characterisation of the neuroendocrine and endocrine regulators in reproduction is performed: The glands most integral to reproduction and subsequently puberty in the sheep are the hypothalamus, the anterior pituitary, and the testes in males. In addition, it is noteworthy that sheep, in general, are a precocial (born in advanced state or able to feed itself almost immediately) species that attains puberty within a year after birth (Foster *et al.*, 1985).

The glands produce the following reproductive hormones (Bearden *et al.*, 2004): The hypothalamus produces gonadotropin-releasing hormone (GnRH), a decapeptide that functions in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) release; the anterior pituitary produces FSH (that functions in spermatogenesis) and LH (that functions in the formation and release of testosterone); and inhibin (that functions to prevent the release of FSH); the testes in males produce androgens / testosterone (that function in male mating behaviour, spermatogenesis and maintenance of the male duct system) and inhibin (that functions to prevent the release of FSH). Thus, luteinising hormone is used by the brain to control the gonads. Through the linking pathways between the hypothalamus and anterior pituitary, the brain maintains absolute control over the frequency of the GnRH and LH and FSH pulses and only partial control over their amplitude.

With regards to the pituitary and hypothalamus, they are inaccessible *in vivo* (Foster and Hileman, 2015). This, plus the very small amounts of neuropeptide that are secreted into the pituitary circulation, make GnRH's secretion pattern and its effect on the pituitary difficult to determine. However, a technique to collect hypophyseal blood from an unanaesthetised sheep was developed in the 1980s: This technique showed that each LH pulse was preceded by a GnRH pulse and that each GnRH pulse resulted in an LH pulse (Foster and Hileman, 2015). Subsequently, the 'gonadostat' hypothesis was developed to explain why, in the prepubertal lamb, GnRH pulses of high frequency are not expressed (Foster and Hileman, 2015): The prepubertal ram is sensitive to estrogen induced inhibition of GnRH secretion because testosterone is aromatised to estradiol.

In males, as depicted below (Figure 2.10), the process is similar: GnRH from the hypothalamus acts on the pituitary gland to stimulate the release of FSH and LH. Follicle stimulating hormone and LH then have different effects when they reach the testes through circulation. LH stimulates the Leydig cells to produce testosterone. Subsequently, there exists a negative feedback loop in which high concentrations of

testosterone inhibit the production of GnRH from the hypothalamus and LH and FSH from the anterior pituitary. FSH stimulates the testes' Sertoli cells to produce inhibin and androgen-binding protein - Inhibin has a negative feedback loop to FSH release and androgen binding protein binds testosterone in the seminiferous tubules to ensure a continuous supply for spermatogenesis.

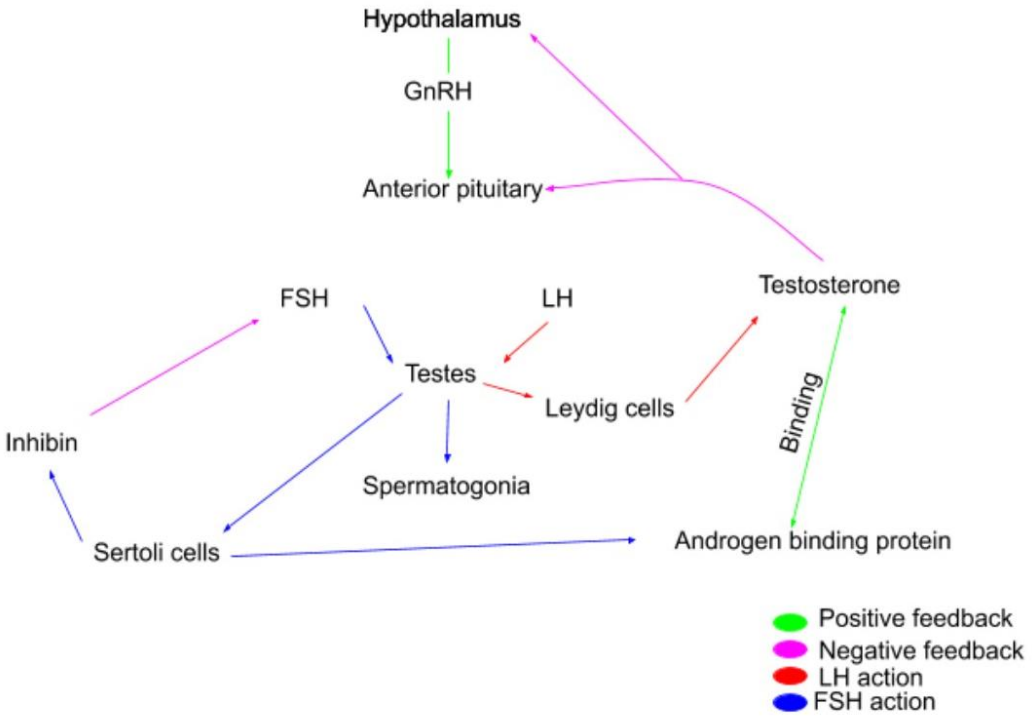

Figure 2.10: Negative and positive neuroendocrine feedback loops of male reproduction (adapted from Bearden, 2004)

Despite the role that each hormone plays in the reproductive system of the ram lamb, testosterone is considered the most fundamental. This is because testosterone is important for the maintenance and initiation of sexual behaviour in rams (Saaed and Zaid, 2018). Blood serum concentrations of testosterone also increase with increasing testicular weight and size (Khalifa *et al.*, 2013) and testosterone is responsible for the regulation of sexual behaviour, secondary sex characteristics and sperm production (Perkins and Roselli, 2007). This over-arching effect on the ram's reproductive performance and behaviour is the primary reason this study will test for blood serum testosterone concentrations.

2.7.1. Seasonality of sheep breeding

Mammals have, over centuries, evolved strategies, both physiological and anatomical, that ensure that their offspring have optimal chances of survival – including the timing of their birth through seasonal breeding. Sheep are seasonal breeders: They have an annual reproductive cycle that is controlled by environmental cues. During the breeding season, there is an increase in melatonin (a hormone secreted from the pineal gland). Melatonin is responsible for many of the physiological changes that occur with decreasing daylight: Courtship behaviour is initiated and occurs more frequently, and there occurs a Flehmen response (the exposure of the pheromone by the animal inhaling with the upper lip curled and mouth open).

Sheep are short day breeders and they mate during the long days of winter and autumn and deliver their offspring in summer and spring. This is compared to long day breeders that complete their reproductive cycles during the summer. This reproductive mechanism allows the use of high-quality forage/feed for both lactation of the ewe and the nutrient requirements of the postweaning lamb. In the northern hemisphere, spring lambing is particularly advantageous as postnatal development occurs during spring and summer and so that puberty can occur in the same year (the first Autumn after birth).

It is interesting to note the similarities between breeding season and the attainment of puberty: For example, sheep have a 5-month gestation period and for gestation to occur in the spring, puberty must be attained during the previous autumn. These spring-born lambs will attain puberty at approximately 4-6 months old (Bearden *et al.*, 2004), which coincides with autumn, the breeding season. Subsequently, the changes attributed to the breeding season's start and to puberty can be attributed to the activation of the hypothalamus and the reduced effectiveness of the negative feedback loop that testosterone has on gonadotropin-releasing hormone (GnRH).

Environmental cues cause differences in the pulsatile secretion of GnRH as well as LH (Smith and Clarke, 2010) in different seasons, for different species. A variety of environmental factors affect the attainment of puberty, but changes in daylength are the dominant cue. Ebling (2010) hypothesised that this may be due to the unwavering consistency of changing seasons, which is of note for species with gestations that span many months. Ultimately, rams must attain puberty in order to become sexually mature. However, they must coordinate the timing of puberty with environmental cues in order for their first mating to occur at the same time as sexual activity in adults during the annual breeding season. It is important to note that despite the negative and positive feedback loops of the ram and ewe, puberty must necessarily occur during the breeding season (Foster *et al.*, 1986; Hammond, 1994) thus the seasonal breeder must be able to determine when he or she is sufficiently mature for the initiation of reproductive cycles. One must also consider the types of environmental changes that may affects seasonal breeding: Food availability,

temperature, rainfall etc. may link to determine the start of the breeding season and of puberty. In addition, in many seasonal species the hypothalamo-pituitary-gonadotropic axis downregulates for part of the year during the non-breeding season, so the seasonal onset of fertility is a 'reoccurrence' of puberty (Ebling, 2010).

Males can mate throughout the year but have semen quality (fructose concentrations) and anatomy (weight of testes) that varies with the seasons; this includes sexual behaviour (Thimonier, 1981). Rams can thus be considered as seasonal breeders because their reproductive characteristics are affected by increased periods of darkness (Edmonson, 2012). In addition, rams can be considered seasonal breeders because of the change in testicular circumference. Testicular circumference increases by 1 to 2 cm during the breeding season (Edmonson, 2012). In addition, it is interesting to consider that seasonal changes in spermatozoa production may be as a result of ambient temperature changes, in addition to due to photoperiod.

2.8. The male reproductive system

2.8.1. Anatomy of the male reproductive system

As shown in figure 2.11, the male reproductive system consists of the scrotum, spermatic chords, testes, accessory glands, penis, prepuce and male duct system which includes the vasa efferentia located within the testes along the epididymis, vas deferens and urethra external to the testes. The testes functions to produce spermatozoa and androgens. The scrotum functions to support the testis, in temperature control the testes and to protect the testes. The spermatic cord functions to support the testis as well, and to temperature control the testes. The epididymis functions to concentrate spermatozoa, and to store, mature and transport spermatozoa. The vas deferens functions to transport spermatozoa. The urethra functions to transport semen. The vesicular gland functions to contribute fluid, energy substrates and buffers to semen. The prostate gland functions to contribute fluid and inorganic ions to semen. The bulbourethral gland functions to flush urine residue from urethra. The penis functions as the male organ of copulation. The prepuce functions to enclose the free end of the penis.

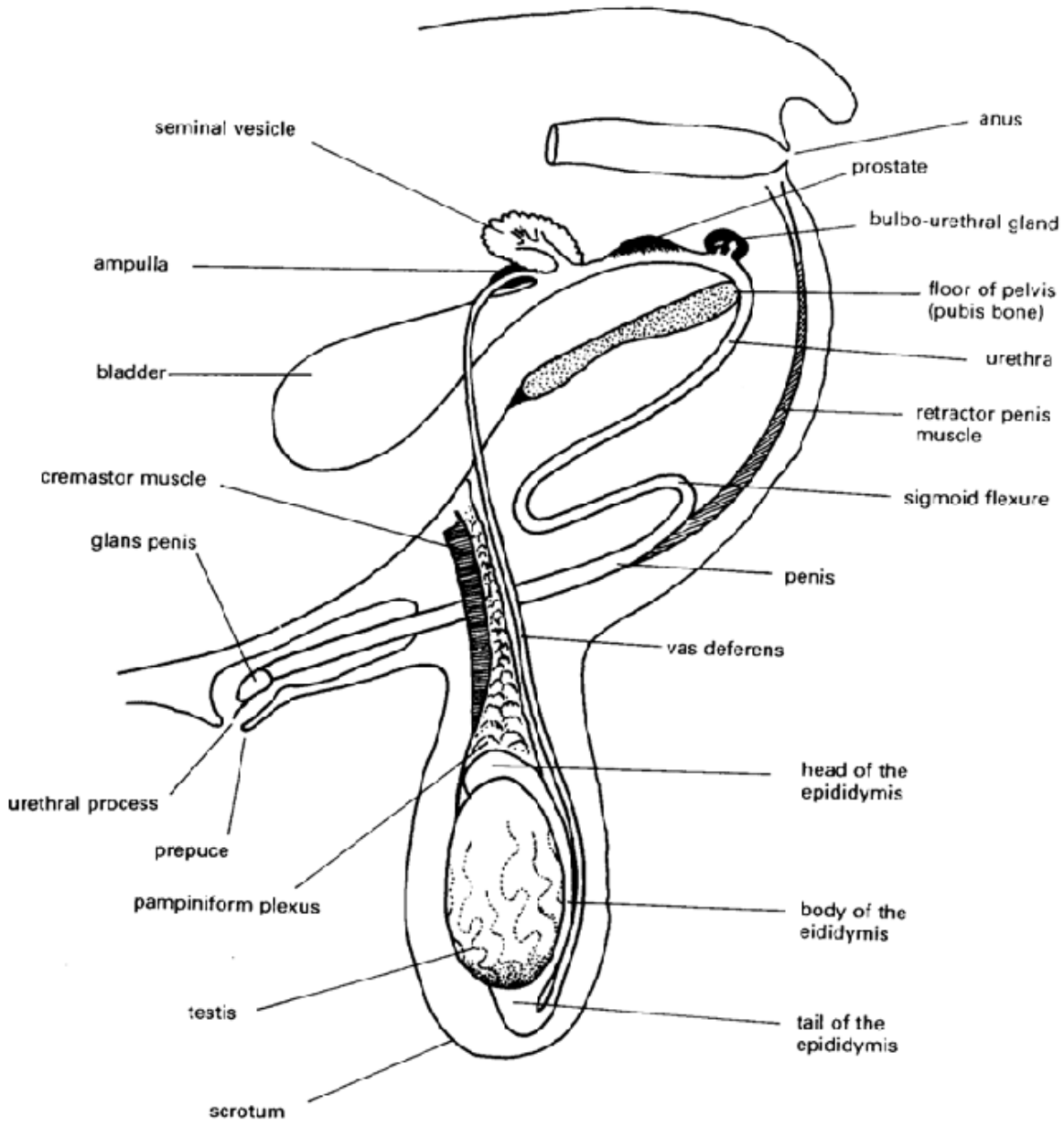

Figure 2.11: The ram reproductive system (Miller, 1991)

2.8.2. Spermatogenesis and maturation of spermatozoa

Spermatogenesis is the process by which haploid spermatozoa are developed from germ cells in the testes' seminiferous tubules (de Kretser *et al.*, 1998) and occurs continually in the male after puberty (Bearden *et al.*, 2004). Semen is a liquid with cellular suspension which consists of sperm cells from seminiferous tubules and secretions (seminal plasma) from the accessory glands (seminal vesicles, bulbourethral and prostate gland) of the male reproductive tract (Ramukhithi, 2011). Spermatozoa are

formed in the seminiferous tubules, then forced through the rete testis and vasa efferentia into the epididymis where they undergo maturation changes to gain the ability to fertilise.

In rams there occurs three to seven LH surges per day and subsequent testosterone surges (Bearden *et al.*, 2004). It is important to re-emphasise that the principal role of LH in spermatogenesis is to act on the Leydig cells to stimulate the release of testosterone. Subsequently, testosterone and FSH act on the seminiferous tubules to stimulate spermatogenesis. The first stage of spermatogenesis is spermatocytogenesis; mitotic division takes place in this stage i.e., spermatogonia form primary spermatocytes. The second stage is a meiotic division i.e., the primary spermatocytes go through reduction division, forming rounded spermatids with haploid nuclei. The third stage is spermiogenesis; during this stage the spermatids undergo a metamorphosis, forming elongated sperm cell (Bearden *et al.*, 2004), as pictured in figure 2.12. Testosterone has high concentrations (100 to 300 times higher) in the fluids that bathe the seminiferous tubules than in peripheral plasma (Bearden *et al.*, 2004). Thus, there are high concentrations of testosterone in the rete testis, vas efferentia, epididymis and the seminiferous tubules. This is maintained by the binding action of testosterone to androgen-binding protein (secreted by Sertoli cells upon stimulation by FSH).

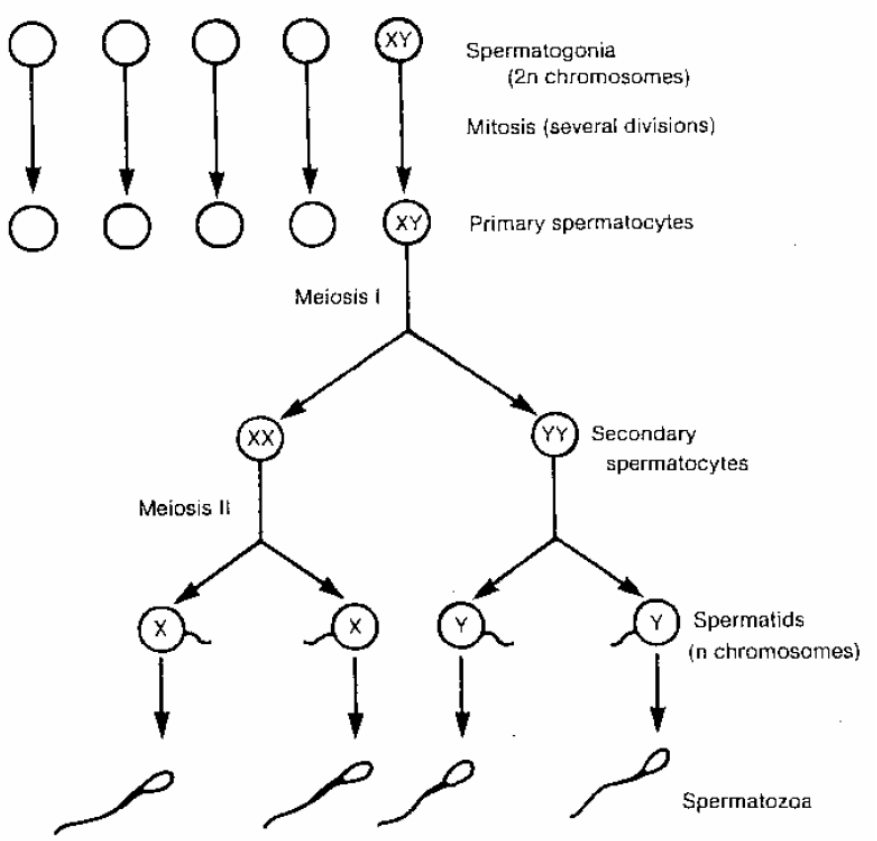

Figure 2.12: Schematic representation of spermatogenesis (Bearden *et al.*, 2004)

2.9. Sexual behaviour

The primary objective of mating behaviour is copulation (animal sexual behaviour in which a male introduces sperm into a female's body) (Bearden *et al.*, 2004; Kent, 2000) – This process requires the following: A female that is firstly fertile, and secondly sexually receptive (in estrus) and a male that is also fertile and has the desire and ability to mate. This first parameter is particularly linked to puberty as it is defined by Bearden *et al.* (2004) as they claim puberty is only attained when fertile spermatozoa form a part of the ejaculate. Similarly, puberty is associated with the desire to mate – with the young ram showing behavioural signs like mounting and aggression or dominance prior to and at puberty in natural mating. In addition, mating behaviour is particularly sequential in males and can be divided into the sexual arousal, courtship, erection, mounting, intromission, ejaculation and dismount stages as in Figure 2.13:

Figure 2.13: Mating behaviour in males (adapted from Bearden *et al.*, 2004)

The autonomic system controls a process called 'erection': With sexual excitement/arousal in the pre-copulatory stage, there is an increase in arterial blood flow followed by a dilation of the corporal sinusoids and restriction of venous outflow from the corpus cavernosum and spongiosum penis. This causes an increase in intra-penile pressure with the relaxation of the retractor penis muscle. This process is then followed by the copulatory stage: Intromission, sensory stimulation of the glans penis, sudden contraction of penile muscles by a series of peristaltic contractions in the urethra, vas differentia and epididymis, and actual ejaculation. Ejaculation is the ejection of semen from the body (Bearden *et al.*, 2004). This ejaculate contains spermatozoa from the vas differentia and fluid from the accessory glands.

Testosterone has a dominant role in the expression of sexual behaviour but interacts with estradiol (Bearden *et al.*, 2004): In an interesting experiment, male red deer were castrated after puberty and regained sexual aggressiveness with injection with testosterone or estradiol. It thus seems that estradiol is involved in the restoration of elements of the central nervous system but androgens are necessary for the peripheral elements related to tactile and penile stimulation and erection. This suggests that the aromatisation of testosterone to estradiol may be necessary in the regulation of normal mating behaviour. Thus, high serum levels of estradiol may not be surprising in the pre-pubertal and pubertal ram lamb.

In terms of mating behaviour, pheromones (chemical factors that are excreted by one individual of a species in order to trigger a reaction or response, social or physiological, in members of the same species) serve as sexual attractants to the opposite sex. In sheep particularly, there exists specialised pheromone responses:

- The ram effect: the stimulation of estrus and ovulation in ewes following exposure to a ram
- The cycling ewe effect: the triggering of puberty in rams periodically exposed to cycling ewes, who will then reach puberty earlier (Edmondson, 2012)

These responses are triggered by pheromones located dorsally and medially from the horns (Bearden *et al.*, 2004), and represent the olfactory effects on mating behaviour.

In terms of the other senses (vision, audition and tactility), it is difficult to discount their effect, even though these senses are strongly linked to olfaction and pheromones. For example, in rams, sight of a mountable, immobile object will illicit the mounting response (Bearden *et al.*, 2004). It is interesting to note that males that are blind from birth reach puberty and subsequently sexual maturity later than males with normal sight (Bearden *et al.*, 2004). Similarly, tactility like biting, nudging and licking and rubbing are part of courtship before copulation (Bearden *et al.*, 2004). In addition, the temperature to which the ram and his penis are exposed to is sensitive (notably a variable during artificial insemination). Audition is particularly important in rams, with grunting being noticeable (Bearden *et al.*, 2004).

The way that senses affect mating behaviour may indirectly affect puberty and its attainment: Many of the signs of mating behaviour are similar to those that indicate the approach or attainment of puberty. This may make the separation of observed behaviours difficult i.e., is the behaviour due to puberty attainment or in response to a more mature, cycling animal of the opposite sex?

2.10. Semen evaluation

Semen evaluation is important for the evaluation of male fertility as male fertility accounts for 45% of infertility problems (Brooks and Keel, 1979). Even macroscopic examination methods like semen volume and semen colour are important in an assessment of male fertility, coupled with microscopic evaluation like sperm cell concentration using a spectrophotometer and motility and kinetics like computer assisted sperm analyzer technology (CASA).

The Sperm Class Analyzer © is a program of CASA. CASA is useful for the following reasons: It is time saving and provides objective analysis (Ramukhithi, 2011). Although originally developed for humans, CASA has been adapted for various species, including sheep, goat and elephants (Saikhun *et al.*, 2007) and provides information on semen motility, progression, velocity and progressivity, average values of speed, amplitude of lateral head and beat frequency, many of which cannot be measured by eye.

In terms of semen motility, it is an important and useful characteristic of sperm cells (Fahrig, 2003) and helps to assess semen quality and fertility (Verstegen *et al.*, 2002). In addition, semen volume is read immediately after collection on graduated tubes (Saikhun *et al.*, 2007) and varies according to collection

method and season and age – young animals are reported to produced low semen volume compared older animals (David *et al.*, 2007). Also, Dombo (2002) reported that due to a larger site for semen production and storage, small testes produce a smaller volume of semen than big testes.

Sperm cell morphology concerns itself with the form of sperm cells: How they look in comparison to other 'normal' sperm cells. Abnormalities can be grouped into abnormalities that occur on the head, tail or midpiece (Ramukhithi *et al.*, 2017) and can be viewed under a stained microscope slide wherein 100 to 500 sperm cells must be examined per slide in order to give reliable results (Bester, 2006). Abnormalities can be induced by factors like temperature differences or be caused by spermatogenesis in the testes; maturation in the epididymis; or poor handling of the semen after the collection (Bester, 2006). Live or dead sperm cells are seen under a stained microscopic slide: Live cells appear white/clear as they do not absorb the stain, and dead cells appear pink or purple, depending on the colour of the stain used (Samper, 2000).

The goal of semen evaluation is to cheaply but accurately and robustly predict the fertility potential of a semen sample (Ramukhithi, 2011) however the prediction of possible fertility using a single sperm characteristic is unreliable. Thus, it is important to analyze various parameters as in this study: The most important semen characteristics to be evaluated are semen volume, motility, morphology, viability and concentration (Hafez and Hafez, 2016).

2.11. Conclusion

The characterisation of the attainment of puberty in the Bapedi, Namaqua-Afrikaner and Zulu sheep is significant for conservation, food security and reproductive physiology, in terms of the definition of puberty, neuroendocrine and endocrine regulators of puberty, factors affecting the attainment of puberty, anatomy of the male reproductive system, and mating behaviour. This is particularly important for these under-studied and under-characterised indigenous breeds.

Many South African indigenous breeds are reported to be under threat. This is particularly unfortunate for smallholder farmers and livestock keepers in sub-Saharan Africa because these breeds, like the Bapedi, Namaqua-Afrikaner and Zulu breeds, are adapted to the subtropical climate of the region and are tolerant to the parasites that are prevalent.

This study chose to focus on rams, instead of both rams and ewes, because the survival of a species is heavily dependent on male fertility when that population is at risk or endangered. This is especially true because although rams may contribute a small physical proportion of the flock, their genetics are amplified in breeding as they breed with various ewes. It is thus important to understand how the process of sexual maturity begins and when it is initiated.

Chapter 3: Materials and Methods

3.1. Ethical approval

This study received ethical approval from the Agricultural Research Council Animal Production, Irene: APAEC 2020/07, as well as from the University of Pretoria: NAS207/2020. In addition, the study received Department of Agriculture, Forestry and Fisheries Section 20 approval.

3.2. Study design

In this study, one primary trial was conducted: A quantitative longitudinal observational study (a type of study in which animals are observed and certain outcomes measured as repeated observations over a period of time. No attempt was made to affect the outcome (for example, no treatment is given)) that was designed to observe the sexual behaviour activities, and change in body weight, blood serum testosterone and scrotal circumference of South African indigenous ram lambs, as well as their semen characteristics.

3.3. Study area

The study was conducted at the small stock section of the Agricultural Research Council in Irene, Pretoria. The site is situated at 25°55' South and 28°12' East and is found on the Highveld at an altitude of 1525m above sea level (Ramukhithi, 2011).

3.4. Animals and their management

In this study, 21 immature ram lambs at 3 months of age (7 Bapedi, 7 Namaqua-Afrikaner and 7 Zulu) were used. The chosen ram lambs were randomly selected from respective flocks of pure-bred animals typical of their breeds, with carefully recorded genealogies. All animals were kept under the same conditions in the same camp of approximately 5000 m² and grazed together on natural pasture and supplemented with pellets, while water was provided *ad libitum* in a metal drinking trough. The first observation was taken when the ram lambs were 3 months of age, in February and the last in June, when ram lambs were 8 months of age – In South Africa, the seasons are as follows (Thoithi *et al*., 2021):

- Summer months are December to March,

- Autumn is April to May,
- Winter is June to August, and
- Spring is September to November.

Thus, this study was conducted from summer to winter, which overlaps with autumn, the natural breeding season of sheep in the southern hemisphere (Department of Agriculture, Forestry and Fisheries, 2008).

3.5. Sexual behaviour activities

To determine sexual behaviour activities as per the first specific objective to characterise the sexual behaviour of South African indigenous sheep, visual observation was used. The rams were exposed for 20 minutes to teaser ewes of their own breed every second week from 3 months of age (during the month of February i.e., summer season) at a ratio of 6 rams: 1 ewe. The parameters observed were:

- Nudging - To push against gently especially in order to gain attention or give a signal
- Pawing – To hit the ground several times with a hoof (usually a front hoof)
- Bleating – Cry of a goat or sheep
- Licking – The action of passing the tongue over a surface
- Flehmen's response – The animal inhales with the mouth open and upper lip curled to facilitate exposure of the pheromone into the vomeronasal organ for detection by the accessory olfactory system.
- Pelvic thrust – Thrusting motion of the pelvic region
- Penile erection – The penis becomes engorged, firmer and enlarged
- Nosing – Smelling (Ramukhithi *et al*., 2017)

Figure 3.1: Namaqua-Afrikaner ewes for teasing

3.6. Body weights

To determine body weights, animals were placed in the crush and individually led into the electronic livestock scale and their weight recorded in kilograms (kg) (Ramukhithi *et al.*, 2017; Maqhashu, 2019). These weighings were done on a bi-weekly basis from 3 months of age (from February, i.e., summer season).

3.7. Scrotal circumference

To determine the scrotal circumference and achieve the second specific objective of evaluating gonadal development of South African indigenous sheep from 3 months until puberty, both of the ram's testes were pulled down ventrally into the scrotum and measured at its largest circumference and recorded, using a tape measure marked in centimeters (cm) (Maqhashu, 2019). These measurements were recorded from when the ram lambs were 4 months of age, in March (i.e., summer season) until they were 8 months of age, in June (i.e., winter season).

3.8. Blood serum testosterone concentrations

To determine blood serum concentrations of testosterone to achieve the second specific objective of evaluating hormonal profiles in blood serum of South African indigenous sheep, 10 mL of blood samples were collected bi-weekly from 4 months of age in March (i.e., summer season) from the jugular vein using a 21-gauge needle into a red-cap vacutainer until 8 months of age in June (i.e., winter season). Following collection, this blood serum was harvested by pipetting and stored in 2 mL tubes at -20°C until analysed.

Blood serum testosterone levels were obtained using a competitive microtiterplate enzyme immunoassay (EIA) whereby antibodies were used to detect the presence of antigens. A colourless molecule called a 'chromogen' was used as substrate. Aliquots of blood serum were pipetted in duplicate into the microtiterplate wells. Then 50µl of label and antiserum were added and the plates incubated overnight at 4°C. Following incubation, the plates were washed four times and 150µl of streptavidin-peroxidase was added to each well. Following incubation in the dark for 30 min, plates were washed again before 150µl peroxidase substrate solution was added, and plates were further incubated for 30–60 min. The reaction was terminated by adding 50 µl of 4N H2SO4 and the absorbance was measured at 450 nm in absorbance units (Au) (Ganswindt *et al.*, 2002).

3.9. Semen collection

Once the rams started to show sexual behaviour activities, to achieve the third specific objective of determining when South African indigenous sheep start to produce good quality semen, an electro-ejaculator was used to collect semen samples: Hair from around the sheath was shaved and the prepuce cleaned with sterile paper towel containing 70% ethanol for the prevention of contamination, and the electrode wiped clean with the same solution and lubricated. The lubricated electrode was inserted and positioned over the accessory glands (seminal vesicles, prostate and bulbourethral glands) while the ram lay on its side (Ramukhithi *et al.*, 2017). The electroejaculator which was used consisted of a bipolar electrode and variable source of electric current which varied from 0 to 30 volts and 0.5 to 1.0 amps (Dombo, 2002). Semen was collected on a bi-weekly basis (figure 3.2) between 08h30 and 10h30 in the morning into pre-warmed (37 ^{o}C) 15 mL graduated tubes before being analysed. These collections began at 7 months of age in May (i.e., autumn season) until 8 months of age in June (i.e., winter season).

Figure 3.2: Semen collection

3.10. Laboratory evaluation of semen characteristics

3.10.1. Semen volume

To determine semen volume, the measurements on the 15 mL calibrated collection tubes were read (before dilution) in millilitres (mL) (Yamashiro *et al.*, 2006) as in figure 3.3.

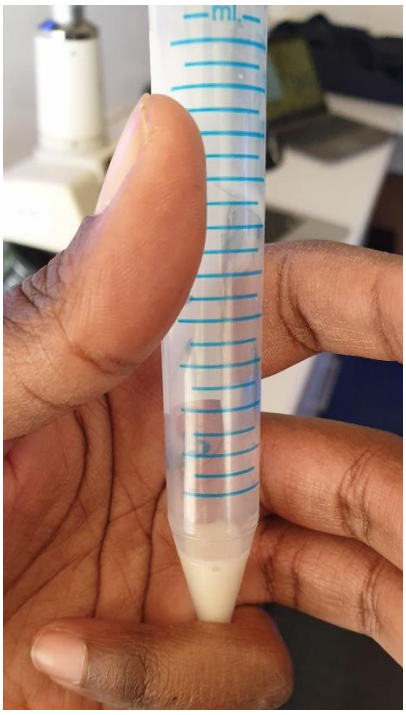

Figure 3.3: Measurement of semen volume

3.10.2. Semen motility

To determine semen motility, a Sperm Class Analyser © (version 6.3) (Microptic ®, Spain) was used whereby 500 µL of Tris and 10 µL of semen were mixed in a 1 mL graduated tube and incubated for 5 minutes at 37 ℃. After incubation, 10 µL of extended semen was placed on a pre-warmed microscopic slide (37 °C), mounted with a cover slip and examined at x10 magnification under a phase contrast microscope (Ramukhithi *et al.*, 2017).

The evaluated semen characteristics are defined as follows:

- Total motility (TM) – the sum of PR and NP
- Progressive progression (PR) – sperm cells that are moving forward
- Non-progressive progression (NP) – sperm cells that are not moving forward, but are moving
- Immotile (IM) – sperm cells that are not moving
- Rapid velocity – sperm cells moving at an average of 81 – 180 µm/s
- Medium velocity – sperm cells moving at an average of 51 – 80 µm/s
- Slow velocity – sperm cells moving at an average less than 50 µm/s
- Curve speed (VCL) – average velocity along a sperm cell's actual path

- Average value (VAP) – average velocity of the smoothed cell path
- Linear speed (VSL) – average velocity of a sperm cell's path from beginning to end
- Straightness index (STR) – ratio of VSL:VAP
- Linearity index (LIN) – ratio of VSL:VCL
- Oscillation index (WOB) – ratio of VAP:VCL (Ramukhithi, 2016)

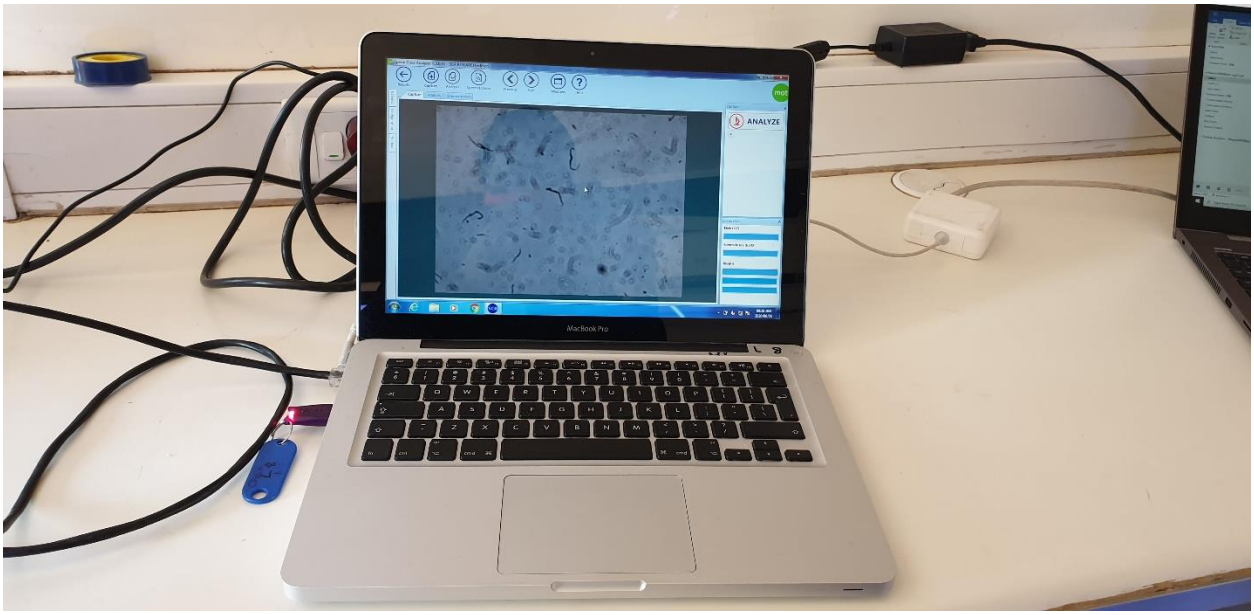

Figure 3.4: Sperm Class Analyser

3.10.3. Sperm cell concentration

To determine sperm cell concentration, a spectrophotometer was used (Jenway, United Kingdom). A square cuvette was filled with 3 mL of sodium citrate solution and placed in the spectrophotometer for at least 30 s. Raw semen (20 µL) was added in a square cuvette containing the sodium citrate solution, again placed in the spectrophotometer in order to read the absorbance. The absorbance read was used to determine the final sperm cell concentration with the aid of a formula: 151 × (25.97 X absorbance − 0.3) where 151 is the dilution factor. The final sperm cell concentration was recorded in millions per millilitre (Ramukhithi *et al.*, 2017).

3.10.4. Sperm cell morphology and abnormalities

To evaluate morphology and abnormalities of sperm cells, samples were stained with nigrosine-eosin stain and evaluated under a fluorescent microscope (Olympus BX51 with lens UPlanFLN 100x at phase 2) and 200 sperm cells per slide counted (Ramukhithi *et al.*, 2017). In an Eppendorf tube, 20 µL of nigrosine-eosin stain was mixed with 7 µL of semen, and 5 µL of that mixture was smeared onto a microscopic slide and allowed to dry before evaluation.

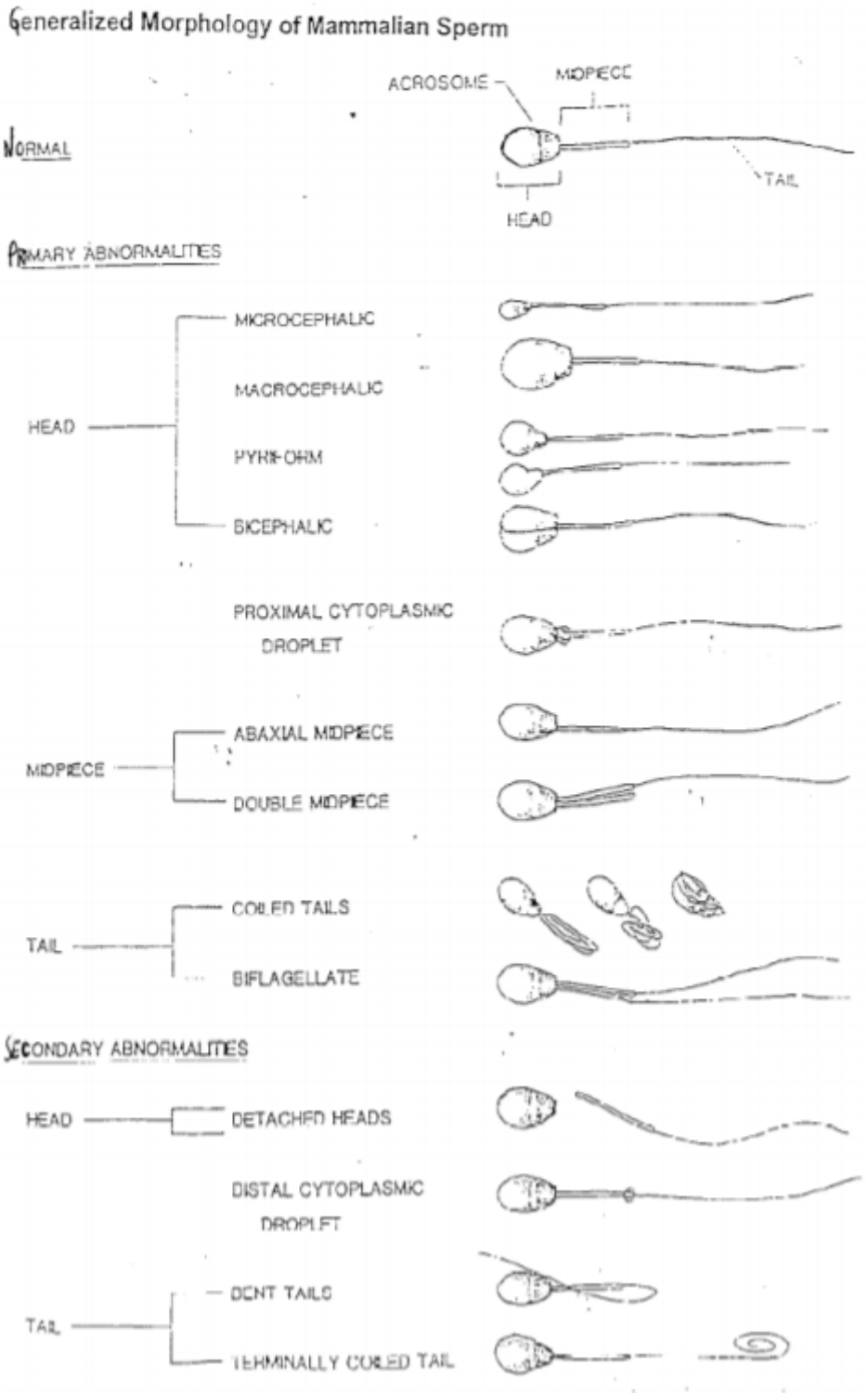

Figure 3.5: Schematic representation of sperm abnormalities (Matshaba, 2010)

As per figure 3.5, abnormalities/defects are those in which there exists a deviation in morphology from a 'normal' sperm cell. These defects were counted and reported as a proportion of the 200 counted sperm cells and categorised as either head, tail or midpiece abnormalities (Matshaba, 2010).

3.10.5. Sperm cell membrane integrity

In order to evaluate sperm cell membrane integrity, a hyperosmotic osmotic swelling test (HOS) was performed. The samples were evaluated under a phase contrast microscope (400x) and 200 sperm cells per slide were counted. Sperm cells with swollen and coiled tails were considered intact (Naing *et al.*, 2010). The HOS test predicts membrane integrity by determining the ability of the sperm membrane to maintain equilibrium between the sperm cell and its environment. Influx of the fluid due to hypo-osmotic stress causes the sperm tail to coil and balloon or "swell" (Ramu and Jeyendran, 2013).

3.11. Statistical analysis

Data was analysed using Statistical Analysis Software (SAS University Edition, 2020) at a 95% confidence limit. One-way ANOVA and N-Way ANOVA were used to generate least square means, standard errors and significance levels using PROC GLM with the STDERR statement. In addition, the PROC FREQ procedure with the CHISQ FISHER statement was used to analyse differences between sexual behaviour activities – This statement produced Fisher's Exact Test of Independence P-values. Significant differences are important because a significant difference quantifies that a result is not due to chance and is rather due to some factor of interest; for this study, a significance level of $P<0.05$ was used.

The dependant variable was the variable being tested and measured in the experiment, with the breed and collection day being classes/categorical variables. In addition, Tukey-Kramer adjustments were used for multiple comparisons.

Pearson correlations were also performed to determine the linear correlation between variables. The Pearson correlation coefficient ranges from -1 to +1. A value of +1 is total positive linear correlation, 0 is no linear correlation, and −1 is total negative linear correlation. Pearson correlations thus indicate proportionality: A positive value indicates direct proportionality i.e., as x increases, so does y, and a negative value indicates inverse proportionality i.e., as x increases, y decreases.

Chapter 4: Results

4.1. Sexual behaviour activities of South African indigenous ram lambs

The sexual behaviour activities of South African indigenous ram lambs are shown in table 4.1: No sexual behaviour activities for any of the three breeds (Bapedi, Namaqua-Afrikaner or Zulu) were observed at 3 months of age, however, at 3.5 months of age, Bapedi and Zulu ram lambs began showing nosing; this contrasts with Namaqua-Afrikaner ram lambs that only began showing sexual behaviour activities (Flehmen's response and nosing) at 4.5 months of age.

Similarly, at 4 months Bapedi and Zulu still only displayed nosing, but at 4.5 months when Namaqua-Afrikaner ram lambs showed nosing and Flehmen's response, Bapedi ram lambs also showed nosing and Flehmen's response while Zulu ram lambs showed pawing, bleating, mounting, and nosing. At 5 months, it was observed that Namaqua-Afrikaner ram lambs only showed nosing; however, Bapedi ram lambs displayed licking, Flehmen's response, penile erection and nosing; and Zulu ram lambs showed pawing, licking, Flehmen's response and nosing. At 5.5 months, when Bapedi ram lambs showed pawing, licking, Flehmen's response, mounting, penile erections and nosing, Zulu and Namaqua-Afrikaner only showed nosing. At 6 months, however, Namaqua-Afrikaner sheep showed licking, Flehmen's response, mounting and nosing, and Zulu ram lambs showed pawing, Flehmen's response and nosing; Bapedi ram lambs at 6 months showed pawing, bleating, Flehmen's response, mounting and nosing. Lastly, at 6.5 months, Bapedi ram lambs displayed nudging (the only breed to display this sexual behaviour), licking, mounting, penile erection and nosing, and Namaqua-Afrikaner and Zulu ram lambs only displayed Flehmen's response and nosing.

Thus, for Bapedi sheep, sexual behaviour steadily increased from 3.5 months of age to 6.5 months of age. Similarly, the sexual behaviour for Bapedi with the highest percentage of observed animals was nosing (79%) and that with the lowest percentage of observed animals was bleating and nudging (2%) with there being no observations of pelvic thrusts (0%). For Namaqua-Afrikaner sheep, sexual behaviour only began at 4.5 months, and increased from 4.5 months to 6.5 months of age. The activity with the highest percentage of observed animals was nosing (55%), as with Bapedi sheep, and the activities with the lowest observed animals were licking and mounting (2%) with nudging, pawing, bleating, pelvic thrust and penile erection having no observations (0%). Lastly, for Zulu sheep, sexual behaviour also steadily increased from 3.5 months to 6.5 months. The sexual behaviour for Zulu sheep with the highest percentage of observed

animals was nosing (80%) and that with the lowest percentage of observed animals was bleating, licking, and mounting (2%) with nudging, pelvic thrust and penile erection having no observations (0%).

It is also important to note that, as per table 4.1b, a Chi-Square Test of Independence may have been performed in order to explain the differences found in table 4.1a. However, a Chi-Square Test of Independence was not used for this data set as at least 50% of the cells have expected counts less than 5 (SAS University Edition, 2020) meaning that the Fisher's Exact Test of Independence, which accounts for smaller sample numbers, was more appropriate (Kim, 2017). The null hypothesis for the Fischer's Exact Test of Independence is that the two categories, breed and sexual behaviour, are independent. The null hypothesis is only rejected ($P<0.05$) at the following instances: Nosing at 3.5 and 4 months, Mounting at 6 months; and Penile erection at 6.5 months. This indicates that in the majority of instances, the null hypothesis is accepted ($P>0.05$) and it can be assumed that breed is independent of sexual behaviour activity, and there is not enough evidence to say that there is an association between breed and sexual behaviour activity. However, importantly, it is noticeable that many of the P-Values obtained from the Fisher's Test, are null – This is because those cells have a row or column sum zero and no statistics can be computed for those cells (SAS University Edition, 2020).

Table 4.1a: Sexual behaviour activities of South African indigenous ram lambs (%)

Breed	Sexual behaviour activities	Age								
		3 months	**3.5 months**	**4 months**	**4.5 months**	**5 months**	**5.5 months**	**6 months**	**6.5 months**	**Average**
Bapedi	Nudging	0	0	0	0	0	0	0	14	**2**
	Pawing	0	0	0	0	0	14	14	0	**4**
	Bleating	0	0	0	0	0	0	14	0	**2**
	Licking	0	0	0	0	29	14	0	29	**9**
	Flehmen's response	0	0	0	29	29	43	57	0	**20**
	Pelvic thrust	0	0	0	0	0	0	0	0	**0**
	Mounting	0	0	0	0	0	14	29	43	**11**
	Penile erection	0	0	0	0	14	14	0	57	**11**
	Nosing	0	100	71	86	86	100	86	100	**79**
Namaqua-Afrikaner	Nudging	0	0	0	0	0	0	0	0	**0**
	Pawing	0	0	0	0	0	0	0	0	**0**
	Bleating	0	0	0	0	0	0	0	0	**0**
	Licking	0	0	0	0	0	0	14	0	**2**
	Flehmen's response	0	0	0	14	0	0	29	29	**9**
	Pelvic thrust	0	0	0	0	0	0	0	0	**0**
	Mounting	0	0	0	0	0	0	14	0	**2**
	Penile erection	0	0	0	0	0	0	0	0	**0**
	Nosing	0	0	0	57	100	86	100	100	**55**
Zulu	Nudging	0	0	0	0	0	0	0	0	**0**
	Pawing	0	0	0	14	14	0	14	0	**5**
	Bleating	0	0	0	14	0	0	0	0	**2**
	Licking	0	0	0	0	14	0	0	0	**2**
	Flehmen's response	0	0	0	0	29	0	29	14	**9**
	Pelvic thrust	0	0	0	0	0	0	0	0	**0**
	Mounting	0	0	0	14	0	0	0	0	**2**
	Penile erection	0	0	0	0	0	0	0	0	**0**
	Nosing	0	71	100	86	100	100	86	100	**80**

Table 4.1b: Fisher's Exact Test of Independence for sexual behaviour activities of South African indigenous ram lambs (P-Value)

Breed	Age	Fisher's Exact Test of Independence (P-Value)								
		Sexual behaviour activities								
		Nudging	Pawing	Bleating	Licking	Flehmen's Response	Pelvic thrust	Mounting	Penile erection	Nosing
Bapedi Namaqua-Afrikaner Zulu	3 months	-	-	-	-	-	-	-	-	-
Bapedi Namaqua-Afrikaner Zulu	3.5 months	-	-	-	-	-	-	1.00	-	0.00
Bapedi Namaqua-Afrikaner Zulu	4 months	-	-	-	-	-	-	-	-	<.0001
Bapedi Namaqua-Afrikaner Zulu	4.5 months	-	1.00	1.00	-	0.42	-	1.00	-	-
Bapedi Namaqua-Afrikaner Zulu	5 months	-	1.00	-	0.26	0.39	-	-	0.30	-
Bapedi Namaqua-Afrikaner Zulu	5.5 months	-	1.00	-	1.00	0.08	-	1.00	1.00	-
Bapedi Namaqua-Afrikaner Zulu	6 months	-	0.51	0.63	1.00	0.51	-	0.04	-	1.00
Bapedi Namaqua-Afrikaner Zulu	6.5 months	1.00	-	-	0.30	0.74	-	0.08	0.02	-

4.2. Body weights of South African indigenous ram lambs

The body weights of South Africa indigenous ram lambs are reported in table 4.2: At 4.5 months of age, Bapedi ram lambs had a significantly higher bodyweight than Zulu ram lambs ($P<0.05$); this is also the case for Bapedi and Namaqua-Afrikaner ram lambs at 6 months of age, for Bapedi and Zulu ram lambs at 6 months of age, and for Bapedi and Zulu ram lambs at 8 months of age. Thus, at the rest of the ages all breeds had similar body weights ($P>0.05$). In addition, the body weight for Bapedi ram lambs ranged from 21.5 ± 2.4 kg to 27.3 ± 2.4 kg; that of Namaqua-Afrikaner ram lambs from 17.9 ± 1.7 kg to 24.2 ± 1.7 kg; and that of Zulu ram lambs from 17.3 ± 1.3 kg to 23.1 ± 1.3 kg – The ages of the ram lambs were 4 months to 8 months of age.

Table 4.2: Body weight of South African indigenous ram lambs (LSmean ± SE) (kg)

Breed	Age (months)								
	4	4.5	5	5.5	6	6.5	7	7.5	8
Bapedi	21.5 ± 2.4	22.6 ± 2.6[a]	22.8 ± 2.6	23.4 ± 2.4	24.5 ± 2.6[a]	24.3 ± 2.4	25.6 ± 2.4	27.2 ± 2.4	27.3 ± 2.4[a]
Namaqua-Afrikaner	18.1 ± 1.7	19.7 ± 2.3	17.9 ± 1.7	18.9 ± 1.8	19.1 ± 1.7[b]	20.6 ± 1.7	22.3 ± 1.7	23.6 ± 1.7	24.2 ± 1.7
Zulu	17.3 ± 1.3	17.4 ± 1.4[b]	18.0 ± 1.3	18.8 ± 1.3	19.4 ± 1.3[b]	21.1 ± 1.3	22.0 ± 1.3	23.1 ± 1.3	22.4 ± 1.3[b]

[a, b] Means with different superscripts within the same column differ significantly ($P<0.05$)

4.3. Scrotal circumferences of South African indigenous ram lambs

The scrotal circumferences (SC) of South African indigenous ram lambs are reported in table 4.3: The age range of the lambs was 4 months to 8 months of age. There existed a significant difference between the scrotal circumference of Zulu ram lambs at 4 months of age and 7.5 and 8 months of age (15.7 ± 1.0, 20.7 ± 1.0 and 20.7 ± 1.0, respectively) ($P<0.05$). However, there also existed between-breed significant differences ($P<0.05$): At 4 months of age, Bapedi ram lambs had higher scrotal circumferences than Namaqua-Afrikaner and Zulu ram lambs (23.5 ± 1.8, 12.5 ± 3.6 and 15.7 ± 1.0 cm, respectively); at 4.5 months of age, Bapedi ram lambs also had higher scrotal circumferences than Zulu ram lambs; at 5 months, Bapedi sheep had higher scrotal circumferences than Namaqua-Afrikaner sheep and Zulu ram lambs had higher SC than Namaqua-Afrikaner ram lambs; and at 6 to 8 months, Bapedi ram lambs had higher scrotal circumferences than Namaqua-Afrikaner ram lambs while Zulu ram lambs also had higher scrotal circumferences than Namaqua-Afrikaner ram lambs. In addition, the SC of Bapedi ram lambs ranged from 19.8 ± 1.7 cm to 23.5 ± 1.8 cm; that of Namaqua-Afrikaner ram lambs from 8.5 ± 3.9 cm to 16.2 ± 4.7 cm; and that of Zulu ram lambs from 15.7 ± 1.0 cm to 20.7 ± 1.0 cm.

Table 4.3: Scrotal circumferences of South African indigenous ram lambs (LSmean ± SE) (cm)

Breed	Age (months)								
	4	4.5	5	5.5	6	6.5	7	7.5	8
Bapedi	23.5 ± 1.8[1]	21.8 ± 1.7[1]	19.8 ± 1.7[1]	20.3 ± 1.5[1]	22.3 ± 1.7[1]	21.5 ± 1.5[1]	21.6 ± 1.5[1]	19.9 ± 1.5[1]	22.1 ± 1.5[1]
Namaqua-Afrikaner	12.5 ± 3.6[2]	16.2 ± 4.7	9.7 ± 3.6[2]	8.5 ± 3.9[2]	10.4 ± 3.6[2]	12.0 ± 3.6[2]	10.3 ± 3.6[2]	12.0 ± 3.6[2]	14.3 ± 3.6[2]
Zulu	15.7 ± 1.0[a, 2]	16.2 ± 1.1[2]	18.4 ± 1.0[1]	18.7 ± 1.0[1]	19.5 ± 1.1[1]	19.8 ± 1.0[1]	20.5 ± 1.0[1]	20.7 ± 1.0[b, 1]	20.7 ± 1.0[b, 1]

[a, b] Means with different superscripts within the same row differ significantly (P<0.05); [1, 2] Means with different superscripts within the same column differ significantly (P<0.05)

4.4. Blood serum testosterone concentrations of South African indigenous ram lambs

The blood serum testosterone (BST) concentration of South African indigenous ram lambs are reported in table 4.4: There was a significant difference between the BST concentration of Bapedi and Namaqua-Afrikaner ram lambs at 6 months of age (6.0 ± 1.1 and 3.2 ± 0.6 ng/ml, respectively). The ages of the ram lambs were 4 months to 8 months of age, and the blood serum testosterone ranges of Bapedi sheep were 3.9 ± 1.0 ng/ml to 6.3 ± 1.0 ng/ml; that of Namaqua-Afrikaner sheep were 3.1 ± 0.6 ng/ml to 4.6 ± 0.6 ng/ml; and that of Zulu sheep were 3.2 ± 0.9 ng/ml to 6.2 ± 0.9 ng/ml.

Table 4.4: Blood serum testosterone concentration of South African indigenous ram lambs (LSmean ± SE) (ng/ml)

Breed	**Age (months)**								
	4	4.5	5	5.5	6	6.5	7	7.5	8
Bapedi	3.9 ± 1.0	4.6 ± 1.0	6.1 ± 1.0	5.8 ± 1.1	6.0 ± 1.1[a]	4.2 ± 1.0	4.7 ± 1.0	6.3 ± 1.0	4.4 ± 1.0
Namaqua-Afrikaner	4.0 ± 0.6	3.4 ± 0.6	3.7 ± 0.6	3.6 ± 0.6	3.2 ± 0.6[b]	3.1 ± 0.6	3.3 ± 0.6	4.6 ± 0.6	3.4 ± 0.6
Zulu	3.9 ± 0.9	5.2 ± 0.9	3.6 ± 0.9	5.9 ± 0.9	4.1 ± 0.9	5.2 ± 0.9	5.0 ± 0.9	6.2 ± 0.9	3.2 ± 0.9

[a, b] Means with different superscripts within the same column differ significantly ($P<0.05$)

4.5. Semen characteristics of South African indigenous ram lambs

The semen characteristics of Namaqua-Afrikaner, Bapedi and Zulu ram lambs are compared in tables 4.5a and 4.5b: In table 4.5a, at ages 7, 7.5 and 8 months of age, there was no significant difference between the semen volumes of any of the three breeds ($P > 0.05$). In addition, the semen volumes ranged from 0.3 ± 0.1 mL to 0.6 ± 0.1 mL.

Contrastingly, there was a significant difference ($P<0.05$) between the sperm cell concentrations of Bapedi sheep at 7 and 8 months of age (1.4 ± 0.6 $x10^9$/mL and 4.3 ± 0.5 $x10^9$/mL, respectively); and of Zulu ram lambs between 7 and 8 months (1.5 ± 0.5 $x10^9$/mL and 5.4 ± 0.5 $x10^9$/mL, respectively), and between 7.5 and 8 months (2.3 ± 0.5 $x10^9$/mL and 5.4 ± 0.5 $x10^9$/mL, respectively).

Progression: Total motility ranged from 70.5 ± 15.1 % to 97.1 ± 2.1 %; there was a significant difference ($P < 0.05$) in the total motilities between Bapedi and Namaqua-Afrikaner ram lambs at 8 months of age (90.1 ± 3.3 % and 70.5 ± 15.1 %, respectively) and between Namaqua-Afrikaner and Zulu ram lambs at 8 months of age (70.5 ± 15.1 % and 93.0 ± 2.1 %, respectively). There was a significant difference for progressive progression (PR) between Bapedi and Zulu ram lambs at 7 months of age (34.4 ± 9.3 % and 73.9 ± 4.5 %, respectively), between Zulu ram lambs at 7 and 7.5 months of age (73.9 ± 4.5 % and 48.3 ± 4.9 %, respectively) ($P< 0.05$), between Namaqua-Afrikaner and Zulu ram lambs at 7 months of age (49.0 ± 14.6 % and 73.9 ± 4.5 %, respectively), and between Bapedi and Namaqua-Afrikaner ram lambs at 8 months of age and Namaqua-Afrikaner and Zulu ram lambs, also at 8 months of age. For non-progressive progression (NP): There was a significant difference ($P<0.05$) between the NP of Zulu ram lambs at 7 and 7.5 months of age (23.1 ± 3.5 % and 44.4 ± 3.8 %, respectively); between the NP of Bapedi ram lambs at 7 and 8 months of age (55.3 ± 5.2 % and 30.9 ± 4.6 %, respectively). In addition, for between breed differences, there was a significant difference ($P<0.05$) between the NP of Bapedi and Zulu ram lambs at 7 months, between Namaqua-Afrikaner and Zulu ram lambs at 7 months, between Namaqua-Afrikaner and Zulu ram lambs at 7.5 months, between Bapedi and Namaqua-Afrikaner ram lambs at 8 months and between Namaqua-Afrikaner and Zulu ram lambs, also at 8 months of age. However, for immotile (IM) progression, there was only a statistically significant difference ($P<0.05$) between Namaqua-Afrikaner and Zulu ram lambs at 8 months (29.5 ± 15.1 % and 6.9 ± 2.1 %, respectively).

Velocity: There was a significant difference between the percentage of rapid sperm cells of Bapedi and Zulu ram lambs at 7 months (17.5 ± 9.3 % and 58.4 ± 5.5 %, respectively); between Namaqua-Afrikaner and Zulu ram lambs at 7 months (29.0 ± 11.5 % and 58.4 ± 5.5 %, respectively); between Bapedi and

Namaqua-Afrikaner ram lambs at 8 months (46.8 ± 8.3 % and 18.9 ± 11.5 %, respectively); and between Namaqua-Afrikaner and Zulu ram lambs at 8 months (18.9 ± 11.5 % and 47.9 ± 5.5 %); as well as within-breed between the rapid percentage of Zulu ram lambs at 7 and 7.5 months (58.4 ± 5.5 % and 31.7 ± 6.1 %) ($P<0.05$). Bapedi, Namaqua-Afrikaner and Zulu ram lambs had no statistically significant differences in percentage of medium sperm cells at all studied ages ($P>0.05$). However, there was a significant difference between the slow percentage of Bapedi ram lambs at 7 and 8 months of age (40.2 ± 5.6 % and 14.9 ± 5.0 %, respectively); a significant difference between the slow percentage of Zulu ram lambs at 7 and 7.5 months (9.0 ± 2.8 % and 27.2 ± 3.1%, respectively), and at 7.5 and 8 months (27.2 ± 3.1 % and 14.6 ± 2.8 %, respectively); and a significant between-breed difference ($P<0.05$) between Bapedi and Zulu ram lambs at 7 months of age, Namaqua-Afrikaner and Zulu ram lambs at 7 months of age, Namaqua-Afrikaner and Zulu ram lambs at 7.5 months of age and 8 months of age. However, for IM velocity, there was a statistically significant difference ($P<0.05$) between Namaqua-Afrikaner and Zulu ram lambs at 8 months (29.5 ± 15.1 % and 6.9 ± 2.1 %, respectively).

Average values of speed: There was a significant difference ($P<0.05$) between the VCL of Bapedi and Zulu ram lambs at 7 months of age (72.9 ± 18.5 μm/s and 144.6 ± 10.4 μm/s, respectively); between Namaqua-Afrikaner and Zulu ram lambs at 7 months of age (81.3 ± 26.4 μm/s and 144.6 ± 10.4 μm/s, respectively); between Bapedi and Namaqua-Afrikaner ram lambs at 8 months of age (125.6 ± 16.6 μm/s and 68.9 ± 26.4 μm/s); between Namaqua-Afrikaner and Zulu ram lambs at 8 months of age (68.9 ± 26.4 μm/s and 126.4 ± 10.4 μm/s); and between the VCL of Zulu ram lams at 7 and 7.5 months of age (144.6 ± 10.4 μm/s and 101.6 ± 11.4 μm/s). Similarly, there was a significant difference ($P<0.05$) between the VAP of Zulu ram lambs at 7 and 7.5 months (105.6 ± 7.7 μm /s and 64.6 ± 8.5 μm/s); and between the VAP of Bapedi and Zulu ram lambs at 7 months; Namaqua-Afrikaner and Zulu ram lambs at 7 months and at 7.5 months; Bapedi and Namaqua-Afrikaner ram lambs at 8 months; and between Namaqua-Afrikaner and Zulu ram lambs at 8 months of age. There was also a significant difference ($P<0.05$) within-breed between the VSL of Zulu ram lambs at 7 and 7.5 months (83.0 ± 6.6 μm/s and 48.7 ± 7.3 μm/s) and at 7 and 8 months (83.0 ± 6.6 μm/s and 57.6 ± 6.6 μm/s); between-breed between the VSL of Bapedi and Zulu ram lambs at 7 months; Namaqua-Afrikaner and Zulu ram lambs at 7 months; Namaqua-Afrikaner and Zulu ram lambs at 7.5 months; Bapedi and Namaqua-Afrikaner ram lambs at 8 months; and between Namaqua-Afrikaner and Zulu ram lambs at 8 months. There was also a significant difference ($P<0.05$) between the STR percentage of Zulu ram lambs at 7 and 7.5 months (70.5 ± 2.2 % and 60.5 ± 2.4 %) and at 7 and 8 months (70.5 ± 2.2 % and 58.9 ± 2.2 %), as well as between Bapedi and Zulu ram lambs at 7 months. There was also a significant difference ($P<0.05$)

between the LIN of Zulu ram lambs at 7 and 8 months (52.2 ± 3.2 % and 38.8 ± 3.2 %) and between the LIN of Bapedi and Zulu ram lambs at 7 months; and Bapedi and Namaqua-Afrikaner ram lambs at 8 months. Lastly, there was a significant difference ($P<0.05$) between the WOB of Zulu ram lambs at 7 and 7.5 months (69.0 ± 2.8 % and 57.5 ± 3.0 %), and at 7 and 8 months (69.0 ± 2.8 % and 57.4 ± 2.8 %), as well as between Bapedi and Zulu ram lambs at 7 months of age.

Table 4.5a: Semen characteristics of South African indigenous ram lambs (LSmean ± SE)

Breed		**Bapedi**			**Namaqua-Afrikaner**			**Zulu**		
Age (months)		**7**	**7.5**	**8**	**7**	**7.5**	**8**	**7**	**7.5**	**8**
Semen volume (mL)		0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Sperm cell concentration (x10^9/mL)		1.4 ± 0.6^{1}	2.3 ± 0.5	4.3 ± 0.5^{2}	1.1 ± 0.9	2.4 ± 0.9	4.9 ± 0.9	1.5 ± 0.5^{1}	2.3 ± 0.5^{1}	5.4 ± 0.5^{2}
Progression (%)	TM	89.8 ± 4.8	94.4 ± 4.0	90.1 ± 3.3^{a}	95.4 ± 15.1	95.3 ± 15.1	70.5 ± 15.1^{b}	97.1 ± 2.1	92.7 ± 2.3	93.0 ± 2.1^{a}
	PR	34.4 ± 9.3^{a}	55.9 ± 7.6	59.2 ± 8.3^{a,b,c}	49.0 ± 14.6^{a}	62.4 ± 14.6	28.4 ± 14.6^{a,b,d}	73.9 ± 4.5^{1,b}	48.3 ± 4.9^{2}	59.8 ± 4.5^{a,b,c}
	NP	55.3 ± 5.2^{1,a}	38.4 ± 4.2	30.9 ± 4.6^{2,a,b,c,d,f}	46.4 ± 3.6^{a}	32.8 ± 3.6^{a,b,c}	42.0 ± 3.6^{a,b,c,d,e}	23.1 ± 3.5^{1,b}	44.4 ± 3.8^{2,a,b,d}	33.2 ± 3.5^{a,b,c,d,f}
	IM	10.1 ± 4.8	5.6 ± 4.0	9.8 ± 4.3	4.5 ± 15.1	4.6 ± 15.1	29.5 ± 15.1^{a}	2.8 ± 2.1	7.2 ± 2.3	6.9 ± 2.1^{b}
Velocity (%)	Rapid	17.5 ± 9.3^{a}	43.6 ± 7.6	46.8 ± 8.3^{a,b,c}	29.0 ± 11.5^{a}	49.5 ± 11.5	18.9 ± 11.5^{a,b,d}	58.4 ± 5.5^{1,b}	31.7 ± 6.1^{2}	47.9 ± 5.5^{a,b,c}
	Medium	32.0 ± 4.5	27.8 ± 3.7	28.4 ± 4.1	38.4 ± 9.7	30.3 ± 9.7	24.5 ± 9.7	29.7 ± 3.6	33.7 ± 4.0	30.4 ± 3.6
	Slow	40.2 ± 5.6^{1,a}	23.0 ± 4.6	14.9 ± 5.0^{2}	27.9 ± 6.4^{a}	15.4 ± 6.4^{a,b,c}	27.0 ± 6.4^{a,b,c,d,e}	9.0 ± 2.8^{1,b}	27.2 ± 3.1^{2,a,b,d}	14.6 ± 2.8^{1,a,b,c,d,f}
	IM	10.1 ± 4.8	5.6 ± 4.0	9.8 ± 4.3	4.5 ± 15.1	4.6 ± 15.1	29.5 ± 15.1^{a}	2.8 ± 2.1	7.2 ± 2.3	6.9 ± 2.1^{b}
Average values of speed	VCL (μm/s)	72.9 ± 18.5^{a}	121.7 ± 15.1	125.6 ± 16.6^{a,b,c}	81.3 ± 26.4^{a}	126.2 ± 26.4	68.9 ± 26.4^{a,b,d}	144.6 ± 10.4^{1,b}	101.6 ± 11.4^{2}	126.4 ± 10.4^{a,b,c}
	VAP (μm/s)	49.9 ± 19.3^{a}	87.2 ± 15.8	94.3 ± 17.3^{a,b,c,d,e}	60.2 ± 17.7^{a}	92.6 ± 17.7^{a,b,c}	42.3 ± 17.7^{a,b,c,d,f}	105.6 ± 7.7^{1,b}	64.6 ± 8.5^{2,a,b,d}	79.8 ± 7.7^{a,b,c,d,e}
	VSL (μm/s)	38.6 ± 16.8^{a}	68.4 ± 13.7	74.4 ± 15.0^{a,b,c,d,e}	48.8 ± 13.6^{a}	71.2 ± 13.6^{a,b,c}	30.9 ± 13.6^{a,b,c,d,f}	83.0 ± 6.6^{1,b}	48.7 ± 7.3^{2,a,b,d}	57.6 ± 6.6^{2,a,b,c,d,e}
	STR (%)	57.0 ± 4.0^{a}	60.4 ± 3.2	63.4 ± 3.5	64.1 ± 6.5	67.7 ± 6.5	53.0 ± 6.5	70.5 ± 2.2^{1,b}	60.5 ± 2.4^{2}	58.9 ± 2.2^{2}
	LIN (%)	40.1 ± 5.9^{a}	42.5 ± 4.8	46.7 ± 5.3^{a,b,c}	47.0 ± 8.1	50.2 ± 8.1	32.0 ± 8.1^{a,b,d}	52.2 ± 3.2^{1,b}	40.2 ± 3.5	38.8 ± 3.2^{2}
	WOB (%)	58.7 ± 6.3^{a}	60.1 ± 5.1	64.6 ± 5.6	64.4 ± 7.8	68.5 ± 7.8	50.4 ± 7.8	69.0 ± 2.8^{1,b}	57.7 ± 3.0^{2}	57.4 ± 2.8^{2}

$^{a, b}$ Means with different superscripts within the same row differ significantly (P<0.05); $^{1, 2}$ Means with different superscripts within the same cell in the same row differ significantly (P<0.05); TM = Total motility, PR = Progressive progression, NP = Non-progressive progression, IM = Immotile, VCL = Curve speed, VAP = Average value, VSL = Linear speed, STR = Straightness index, LIN = Linearity index, WOB = Oscillation index

In table 4.5b the semen characteristics of South African indigenous ram lambs are continued: All three breeds had similar intact and non-intact percentages under membrane integrity ($P>0.05$). This is the same for live and dead percentages under Viability.

Lastly, in terms of abnormalities: There was a significant difference ($P<0.05$) between the number of head abnormalities of Bapedi sheep at 7 and 8 months (1.2 ± 0.5 and 0.3 ± 0.4). Similarly, for Zulu ram lambs, there was a significant difference between the tail abnormalities at 7 and 8 months of age (4.1 ± 0.7 and 1.0 ± 0.7) ($P<0.05$). However, in terms of midpiece abnormalities, Bapedi, Namaqua-Afrikaner and Zulu ram lambs had similar midpiece abnormalities to each other across ages 7 to 8 months ($P>0.05$).

Table 4.5b: Semen characteristics of South African indigenous ram lambs (LSmean ± SE)

Breed		***Bapedi***			***Namaqua-Afrikaner***			***Zulu***		
Age (months)		**7**	**7.5**	**8**	**7**	**7.5**	**8**	**7**	**7.5**	**8**
Membrane integrity (%)	Intact	78.8 ± 5.1	81.6 ± 4.1	84.0 ± 4.1	81.0 ± 7.3	80.7 ± 7.3	84.5 ± 7.3	79.5 ± 4.1	85.1 ± 4.6	86.4 ± 4.1
	Non-intact	21.1 ± 4.8	18.3 ± 3.9	15.8 ± 3.9	19.0 ± 6.8	16.7 ± 6.8	15.5 ± 6.8	21.3 ± 3.9	14.9 ± 4.3	13.5 ± 3.9
Viability (%)	Live	79.0 ± 5.3	82.9 ± 4.3	85.3 ± 4.3	76.5 ± 11.4	80.5 ± 11.4	83.7 ± 11.4	82.8 ± 7.7	77.7 ± 8.5	86.0 ± 7.7
	Dead	21.0 ± 5.3	17.0 ± 4.3	14.6 ± 4.3	23.5 ± 11.4	19.5 ± 11.4	16.2 ± 11.4	17.1 ± 7.7	22.3 ± 8.5	14.0 ± 7.7
Abnormalities (n)	Head	1.2 ± 0.5[1]	0.5 ± 0.4	0.3 ± 0.4[2]	2.0 ± 0.7	1.0 ± 0.7	0.5 ± 0.7	1.3 ± 0.4	0.8 ± 0.5	0.2 ± 0.4
	Mid-piece	0.0 ± 0.1	0.0 ± 0.1	0.3 ± 0.1	0.0 ± 0.2	0.0 ± 0.2	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Tail	4.2 ± 1.1	1.1 ± 0.9	1.5 ± 0.9	4.0 ± 2.2	4.5 ± 2.2	2.5 ± 2.2	4.1 ± 0.7[1]	1.4 ± 0.8	1.0 ± 0.7[2]

[1, 2] Means with different superscripts within the same cell in the same row differ significantly ($P<0.05$)

4.6. Pearson correlation coefficients for bodyweight, scrotal circumference, and blood serum testosterone concentration

Table 4.6 shows the Pearson correlation coefficients for bodyweight, scrotal circumference, and blood serum testosterone: There existed a positive correlation among all three variables, with the strongest correlation being that between body weight and scrotal circumference (0.60) and the weakest correlation being between body weight and blood serum testosterone (0.28).

Table 4.6: Pearson Correlation Coefficients (*r*) for body weight, scrotal circumference and blood serum testosterone

Parameter	Body weight	Scrotal circumference	Blood serum testosterone
Blood serum testosterone	0.28	0.30	1.00
Body weight	1.00	0.60	0.28
Scrotal circumference	0.60	1.00	0.30

In table 4.7, Pearson correlation coefficients for body weight, blood serum testosterone and scrotal circumference against semen volume, sperm cell concentration, membrane integrity and semen morphology. There existed positive correlations between body weight and semen volume, total motility, NP progression, medium velocity, slow velocity, VAP, VSL, STR, LIN, WOB, intact membrane integrity, live viability, tail abnormalities, and midpiece abnormalities. There also existed positive correlations between blood serum testosterone and semen volume, PR, IM progression, rapid velocity, slow velocity, IM velocity, all average values of speed, non-intact membrane integrity, dead membrane integrity, head abnormalities and midpiece abnormalities. In addition, there existed positive correlations between scrotal circumference and semen volume, sperm cell concentration, NP, IM progression, Slow velocity, IM velocity, intact membrane integrity, live viability, tail abnormalities, and midpiece abnormalities. Moreover, the strongest correlation is between sperm cell concentration and blood serum testosterone (-0.40) and the weakest correlation between tail abnormalities and blood serum testosterone (-0.0011).

Table 4.7: Pearson correlation coefficients (*r*) for body weight, blood serum testosterone and scrotal circumference against semen characteristics

Parameter		Body weight	Scrotal circumference	Blood serum testosterone
Semen volume		0.23	0.33	0.05
Sperm cell concentration		-0.03	0.07	-0.40
Progression	Total motility	0.18	-0.09	-0.0031
	PR	-0.10	-0.15	0.03
	NP	0.30	0.16	-0.04
	IM	-0.18	0.09	0.0031
Velocity	Rapid	-0.09	-0.09	0.04
	Medium	0.20	-0.02	-0.22
	Slow	0.16	0.07	0.09
	IM	-0.18	0.09	0.0031
Average values of speed	VCL	-0.06	-0.0076	0.15
	VAP	0.02	-0.06	0.26
	VSL	0.02	-0.07	0.32
	STR	0.0074	-0.20	0.23
	LIN	0.06	-0.22	0.30
	WOB	0.08	-0.19	0.26
Membrane integrity	Intact	0.23	0.05	-0.06
	Non-intact	-0.28	-0.05	0.03
Viability	Live	0.05	0.13	-0.02
	Dead	-0.05	-0.13	0.02
Abnormalities	Head	-0.22	-0.35	0.35
	Midpiece	0.09	0.05	0.15
	Tail	0.13	0.15	-0.0011

Chapter 5: Discussion

5.1. Sexual behaviour activities of South African indigenous ram lambs

Bapedi sheep began displaying sexual behaviour activities (nosing) at 3.5 months of age, at the same age as Zulu sheep, whereas Namaqua-Afrikaner sheep only began displaying reproductive performance activities a month later. Namaqua-Afrikaner sheep had the lowest cumulative observation percentage compared to Bapedi sheep and Zulu sheep. Thus, this suggests that on a spectrum, Bapedi and Zulu sheep are early maturing compared to Namaqua-Afrikaner sheep. Maqhashu (2019) also reported that Bapedi sheep mature early. It is important to note that Namaqua-Afrikaner sheep are from the Northern Cape province in South Africa (Snyman, 2014); this province has notably extreme weather and it is possible that their 'late maturity' is an adaptability trait: if Namaqua-Afrikaners mature later and reach puberty later, the ewes are better able to protect their young from predators as they will give birth later in life and will be presumably larger in body size. Burger *et al.* (2014) corroborates that Namaqua-Afrikaner sheep are late maturing. Late-maturing breeds are physiologically younger at the same chronological age (a chronological age which would be strongly correlated to body weight (Moulla *et al.*, 2018)) thus they would weigh the same as Bapedi and Zulu sheep but be at a different physiological age with regards to reproduction, as was the case in this study.

Additionally, nosing was the first and most frequently seen sexual behaviour across all three breeds; this supports Edmonson (2012)'s assertion that the olfactory senses are important in the attainment of puberty, and maybe the ram lambs' sensitivity to pheromones is the first indicator of approaching puberty. This is corroborated by Flehmen's response being the second most seen observation across all three breeds as Flehmen's response also makes use of the olfactory system. Banks (1964) stated that nosing is the first sexual behaviour displayed in a sexual interaction, and that it functions to provide the ram with olfactory information. Interestingly, because nosing involves the ram pushing its face into the perineum of the ewe, nosing may also function in providing the ram with information about the ewe's vulva's surface temperature (Banks, 1964). Contrastingly, the sexual behaviour activity that was not observed across any breed was pelvic thrust – This may be because pelvic thrusts happen post-mounting (Banks, 1964), and ram lambs that have not attained puberty or have attained puberty but not sexual maturity, may not have experience with mounting an ewe. The ram lambs used in this study had no interaction with mature rams and were only exposed to ewes for limited periods of time (20 minutes) when studying sexual behaviour activities.

It is also important to also consider whether or not the sexual behaviour that was observed was due to the attainment of puberty, or simply a response to the teaser ewes – The cycling ewe effect (the triggering of puberty in rams periodically exposed to cycling ewes, who will then reach puberty earlier (Edmondson, 2012)) may have played a role in the sexual development of the studied Bapedi, Namaqua-Afrikaner and Zulu ram lambs as they were exposed to teaser ewes that may have been estrus.

In addition, consideration must be given to the results of the Fisher's Exact Test of Independence: In the majority of instances (except for Nosing at 3.5 and 4 months, Mounting at 6 months; and Penile erection at 6.5 months) the P-value given by the Fisher's Exact Test of Independence was above 0.05 and not significant ($P>0.05$) (Kim, 2017). Where the null hypothesis is that breed and sexual behaviour are independent, this means that the null hypothesis is accepted, and it can be assumed that breed is independent of sexual behaviour (McCrum-Gardner, 2008).

Lastly, the seasons during which sexual behaviour activities were monitored are also important to note as sheep are seasonal short-day breeders. This means that they come into season naturally when the daylight length starts to decrease, therefore in autumn. Sheep can also be induced to breed in spring, either with daylight manipulation or hormone supplementation (Department of Agriculture, Forestry and Fisheries, 2008). This study was done from February to June in South Africa i.e., from summer to winter with sexual behaviour activities being monitored from February until May i.e., from summer until Autumn. This may suggest that the increase in sexual behaviour activities may be due to the initiation and progression of the mating season. This finding is particularly interesting because it suggests that increased sexual behaviour, which may be a prerequisite for puberty, may naturally coincide with the natural mating season of sheep. This does not, however, necessarily imply that correlation is equals to causation – It is possible that the observed increase in sexual behaviour seen in this study is due to the natural endocrinological sexual development of the ram lamb, and not due to the mating season. Contrastingly, Bearden *et al*. (2004) suggests that spring born lambs will necessarily achieve puberty during the autumn following their birth and this is corroborated by Ebling (2010). This then means that this study's timing was optimal.

5.2. Body weights of South African indigenous ram lambs

In this study, it was observed that at most ages, the studied breeds had similar bodyweights (4, 5, 5.5, 6.5, 7, 7.5 months) ($P>0.05$). This observation is important because the Bapedi, Namaqua-Afrikaner and Zulu breeds are all indigenous to Southern Africa (Maqhashu, 2019; Snyman *et al.*, 1993). The body weight for Bapedi ram lambs ranged from 21.5 ± 2.4 kg to 27.3 ± 2.4 kg; that of Namaqua-Afrikaner ram

lambs from 17.9 ± 1.7 kg to 24.2 ± 1.7 kg; and that of Zulu ram lambs from 17.3 ± 1.3 kg to 23.1 ± 1.3 kg. Thus, these weight ranges may be indicative of the attainment of puberty even though when compared to many other studies, the results of the studied breeds' bodyweight was low: Compared to Tazegzawt ram lambs used in Moulla *et al.* (2018)'s study which had a body weight at puberty of 38.0 ± 5.0 kg, Saidi ram lambs which had a bodyweight of 50.3 ± 3.0 kg (Anas *et al.,* 2005), Ouled Djellal ram lambs which had a body weight of 40.4 ± 1.2 kg (Boussena *et al*., 2016), and Libyan fat-tailed ram lambs that had a body weight of 39.9 ± 9.3 kg) (Madani *et al.,* 1989), Bapedi, Namaqua-Afrikaner and Zulu ram lambs had lower body weights. Lower body weights at puberty are also reported with Clun Forest sheep (32.5 kg) (Dyrmundsson and Lees, 1972), in Rahmani sheep (33.1 kg) (Regab *et al.,* 1966), and in Merino sheep (24 – 29 kg) (Pretorious and Marincowitz, 1968). Thus, Bapedi, Namaqua-Afrikaner and Zulu ram lambs are closer in weight to, for example, Merino ram lambs at puberty (Pretorious and Marincowitz, 1968). Further, when comparing the body weights at puberty of Bapedi, Namaqua-Afrikaner and Zulu ram lambs to the breeds listed in table 2.1, it is interesting to note that the trend continues and South African indigenous ram lambs may have lower bodyweights at the attainment of puberty: Greek Freisland, Karangouniki, Chios and Serres breeds, along with the Iranian Ghezel breed and the Jordanian Awassi breed, all have bodyweights that are greater than 40 kilograms at the attainment of puberty (Belibasaki and Kouimzis (2000); Nazari-Zenouz et al. (2016); Kridli and Al-Yakoub (2006)).

Accordingly, Bapedi, Namaqua-Afrikaner and Zulu ram lambs' indigenousness to South Africa means that they have specific phenotypic and genotypic traits that are inherited from generation to generation and that are informed by their environment (Mwai *et al.,* 2015). One of these phenotypic traits is bodyweight. Thus, it is expected that members of a breed will have similar phenotypic traits and expected that breeds that share extrinsic factors like environment will have similar phenotypic traits (Mwai *et al*., 2015) as was the case in this study. When comparing Bapedi, Namaqua-Afrikaner and Zulu rams to Tazegzawt, Saidi, Ouled Djellal and Libyan breeds, it is important to remember that these breeds are all from north Africa (Algeria, Libya and Egypt (Moulla *et al.,* 2018)) and are faced with different environmental conditions and genotypes that play a role in their growth rate i.e., different breeds differ on the rate at which they attain puberty (Nishimura *et al*., 2000). Thus, even though the Rahmani sheep are also from Egypt, they also have lower bodyweights at puberty.

In addition, because improved growth rate and body weight advances the attainment of puberty in almost all breeds (Rekik *et al*., 2016), in the absence of data on sexual behaviour activities and solely depending on body weights, one could assume that Bapedi ram lambs may attain puberty at the same time as both Zulu and Namaqua-Afrikaner ram lambs due to the insignificant difference in their weights.

However, it is important to note that in the instances where there was a between-breed significant difference in bodyweight i.e., at 4.5, 6 and 8 months, Bapedi ram lambs had the higher bodyweight compared to Namaqua-Afrikaner and Zulu ram lambs – This is important because it shows that Bapedi sheep may be inherently larger in bodyweight compared to Namaqua-Afrikaner and Zulu sheep. However, Snyman (2014) states that the mature weight of a Bapedi ewe is 45 kg, while that of a Namaqua-Afrikaner ewe is 50 kg.

Lastly, it is also important to note that although bodyweights at say 4 months may be numerically lower than those at 8 months for a specific breed, they are not statistically different ($P>0.05$) and selection strategies that rely on bodyweight should be cautious of this - the change in bodyweight from 4 to 8 months is not a significant one for any of the breeds and suggests the homogeneity of the breeds (Maqhashu, 2019). In addition, the span from of weight observations, from 4 months of age to 8 months of age is from March to June (i.e., summer to winter seasons) which includes the autumn breeding season (April to May) – This means that the weight gain observed in the Bapedi, Namaqua-Afrikaner and Zulu ram lambs may be exacerbated by the approaching breeding season along with being a result of natural metabolic weight gain as the ram lamb ages (Rekik *et al.,* 2016).

5.3. Scrotal circumferences of South African indigenous ram lambs

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had statistically significant between-breed differences in scrotal circumferences ($P<0.05$) for ages 4 to 8 months. Unlike with bodyweight, this heterogeneity between breeds is unexpected because of the correlation between bodyweight and scrotal circumference (Perumal, 2014). However, the cause of this heterogeneity may be individual variation (Eriksson *et al.*, 2012).

Furthermore, testosterone is responsible for sexual maturity and interest, and is produced by the Leydig cells of the testes. Leydig cell parameters (like the number of Leydig cells per gram of testis, total number of Leydig cells per testis and percent cell volume of Leydig cell nuclei) are correlated significantly with testosterone levels (Castro *et al.*, 2002) and testosterone is responsible for secondary sex characteristics like sexual behaviour (Bearden *et al.*, 2004). Thus, because all three studied breeds have different scrotal circumferences from 4 to 8 months ($P<0.05$), they have correspondingly dissimilar Leydig cells and subsequent testosterone production. This suggests, unlike body weight, that these breeds would attain puberty at dissimilar ages. Moreover, Söderquist. and Hultén (2006) found that ram lambs of the Gotlandic breed, a Swedish breed, had a scrotal circumference of 28.9 ± 1.9 cm, which was higher than the scrotal

circumferences found in this study. Despite the difference in scrotal circumference between South African indigenous sheep and Icelandic sheep, scrotal circumference is still a useful selection indicator: Males with larger testes are likelier to sire daughters that reach puberty at an earlier age and ovulate more ova during oestrus (Söderquist. and Hultén, 2006). This would be especially useful for smallholder farmers and livestock keepers as they would be able to get more lambs from one ewe, both because the age at first lambing is lower and the likelihood of multiple births is higher.

However, the significant difference in the scrotal circumferences of Zulu ram lambs at 4 months of age and 7.5 and 8 months of age ($P<0.05$) indicates that there is a substantial change in SC for Zulu ram lambs between these ages and may act as a selection indicator. Also, the significant differences between breeds found from 4 to 8 months have a distinct trend: Bapedi ram lambs generally had the highest SC (except at 7.5 months), and Namaqua-Afrikaner ram lambs had the lowest SC. This difference in SC may be due to the Namaqua-Afrikaner's late sexual maturity.

In addition, SC is affected by the season of the year, breed and body condition score but would usually be at a maximum peak during the breeding season (Perumal, 2014). This study was conducted between February and the following June in South Africa – meaning summer through to autumn and early winter. Because autumn and spring are the breeding seasons of sheep, and an increase (albeit insignificant ($P>0.05$)) in scrotal circumference was observed over this time span, it is possible that there is a correlation between breeding season and scrotal circumference.

Lastly, a bibliographic comparison of the attainment of puberty in ram lambs of different breeds is presented in table 2.1: Of the listed breeds, the lowest scrotal circumference was that of the Moroccan D'Man breed at 21.4 ± 2.0 cm, and the highest scrotal circumference was that of the Greek Friesland breed at 44.5 ± 0.9 cm, with the remaining breeds having scrotal circumferences above 21.4 ± 2.0 cm but below 28.9 ± 2.9 cm. This range in scrotal circumference corresponds to that found in this study: the SC of Bapedi ram lambs ranged from 19.8 ± 1.7 cm to 23.5 ± 1.8 cm; that of Namaqua-Afrikaner ram lambs from 8.5 ± 3.9 cm to 16.2 ± 4.7 cm; and that of Zulu ram lambs from 15.7 ± 1.0 cm to 20.7 ± 1.0 cm. It may be interesting to consider that the scrotal circumferences of the breeds listed in table 2.1 at the attainment of puberty are generally higher than those of Bapedi, Namaqua-Afrikaner and Zulu ram lambs in manner similar to bodyweights – This is to be expected because of the positive correlation between bodyweight and scrotal circumference (Perumal (2014)).

5.4. Blood serum testosterone concentrations of South African indigenous ram lambs

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had similar blood serum testosterone (BST) concentrations to each other ($P>0.05$) except at 6 months of age. This means that although blood serum testosterone levels fluctuated for each breed as they aged from 4 to 8 months of age, this fluctuation was not one that resulted in a significant difference between most samples, both within and between breeds. Thus, the three studied breeds displayed homogeneity in blood serum testosterone levels i.e., there were no significant within-breed differences in BST concentration and minimal between-breed differences ($P<0.05$). In addition, testosterone is needed to initiate spermatogenesis at puberty and to maintain this process in the adult (Castro *et al.*, 2002), thus the presence of blood serum testosterone in all three breeds may indicate the attainment of puberty. In addition, Bapedi, Namaqua-Afrikaner and Zulu ram lambs all had their highest sampled blood serum testosterone levels at 7.5 months: Spermatogenesis and its sustenance takes 2 months (Foster and Hileman, 2015) thus this spike in blood serum testosterone may indicate the attainment of puberty (Bearden *et al*., 2004) at 5.5 months.

Moreover, because the testis of sheep release testosterone that elevates with increasing testicular weight until puberty and maturity (Saaed and Zaid, 2018), it is understandable why the three studied breeds had similar blood serum testosterone levels ($P>0.05$) for most ages: Bapedi, Namaqua-Afrikaner and Zulu ram lambs had similar body weights when compared with each other ($P>0.05$) and since there existed a positive correlation (as per the Pearson correlation coefficients for this study) between body weight, scrotal circumference and blood serum testosterone, this relationship is expected. In addition, blood samples were collected from ram lambs from March (i.e., summer season) and approaching autumn, their natural breeding season. According to Saaed and Zaid (2018), there should have been a significant increase in blood serum testosterone concentration levels as the ram lamb approached puberty and as the ram lamb approached its natural breeding season – This study contradicts that assertion. However, it is interesting to consider that the process of spermatogenesis takes about 6 weeks from start to finish (Foster and Hileman, 2015), thus the expected peak in testosterone and subsequent blood serum testosterone concentration levels may have happened months prior to April (i.e., the start of the autumn breeding season). Also, when comparing the blood serum testosterone concentration of the breeds listed in table 2.1 to Bapedi, Namaqua-Afrikaner and Zulu ram lambs, it is numerically higher in South African indigenous ram lambs (i.e., the highest BST concentration is that of Ouled Djellal ram lambs at 4.0 ± 1.7 ng/ml (Boussena *et al.*, 2016) while the BST ranges of Bapedi sheep were 3.9 ± 1.0 ng/ml to 6.3 ± 1.0 ng/ml; that of Namaqua-Afrikaner sheep were 3.1

± 0.6 ng/ml to 4.6 ± 0.6 ng/ml; and that of Zulu sheep were 3.2 ± 0.9 ng/ml to 6.2 ± 0.9 ng/ml). This may indicate a peak in BST concentration that was caused by the initiation of the breeding season in autumn.

Lastly, as with scrotal circumference, when the difference in BST concentration was significant i.e., at 6 months between Bapedi and Namaqua-Afrikaner ram lambs, Bapedi ram lambs had a higher BST concentration. This is explained by Bapedi ram lambs having higher bodyweights as well as scrotal circumferences, as bodyweight and scrotal circumference are positively correlated to BST concentration.

5.5. Semen characteristics of South African indigenous ram lambs

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had statistically nonsignificant differences in semen volumes to each other from the first semen analysis at 7 months to the last semen analysis at 8 months of age ($P>0.05$) - Homogeneity among the breeds was shown. It is also important to note that the semen volume observed in this study was expected to be low as ram lambs have lower semen volumes compared to rams due to the parabolic nature of semen volume output in rams i.e., semen volume while the ram is very young and very old is low (David *et al.*, 2015). Also, Rege *et al.* (2000) found the semen volumes of Ethiopian highland sheep at 6 months to have a semen volume of 0.30 ± 0.67 mL, which corresponds with the semen volume range found in this study at 7 months of age (0.3 ± 0.1 mL to 0.6 ± 0.1 mL).

Contrastingly, Bapedi sheep had a significant difference in sperm cell concentration at 7 and 8 months of age and Zulu sheep at 7 and 8 months of age, and at 7.5 and 8 months of age ($P<0.05$), with the sperm cell concentration having increased at 8 months. This means that there was still not a significant difference between breeds, but rather within a breed; Bapedi and Zulu ram lambs' sperm cell concentrations thus increased from 7 months to 8 months, as was to be expected if it is considered that semen characteristics improve with age (Rege *et al.*, 2000). In addition, at 8 months of age, both Bapedi and Zulu ram lambs showed a peak in sperm cell concentrations. This peak can be presumably attributed to other factors that occurred 2 months prior to the ram lambs being 8 months old. This is because testicular function is slow in its development, and because spermatogenesis takes 2 months to complete from the creation of spermatogonia to the creation of semen (Foster and Hileman, 2015). It is interesting to note, however, that this change/spike did not occur for Namaqua-Afrikaner ram lambs: As per the results of sexual behaviour activities, it was observed that Namaqua-Afrikaners are late maturing. Namaqua-Afrikaner ram lambs would then not have had the prerequisite trigger that Bapedi and Zulu ram lambs had at 6 months of age that resulted in increased sperm cell concentration at 8 months of age; secondly, only an average of 29% of

Namaqua-Afrikaner ram lambs produced semen during semen collection during the study period, compared to an average of 57% of Bapedi and 71% of Zulu ram lambs per collection day; thirdly, in this study the underdevelopment of many Namaqua-Afrikaner ram lambs' glans penises was observed. This anatomical anomaly meant that many Namaqua-Afrikaner ram lambs could not be collected from; fourthly, various Namaqua-Afrikaner ram lambs in this study had no testes i.e., scrotal circumference could not be measured because the scrotum felt like an empty sack when palpated. This may be because the testes have not yet descended because of the late sexual maturity observed for Namaqua-Afrikaners.

Progression: According to David *et al.* (2015), total motility (TM) is predictive of the overall fertilizing ability of sperm cells (this is because the better able that sperm cells are able to move, the likelier they are to reach the ovum and penetrate the zona pellucida). Thus, overall fertilizing ability can be assumed to be dissimilar at 8 months of age for Bapedi and Namaqua-Afrikaner sheep, and for Zulu and Namaqua-Afrikaner sheep as there existed significant differences in the TM of these breeds. However, overall fertilizing ability between-breeds and within-breeds at the rest of the studied ages can be assumed to be similar. In addition, the semen motility of all three breeds is exceptional (>70%) (Yarney and Sanford, 1993) and this is the case at all three observed ages (7, 7.5 and 8 months); this means that all breeds were producing good quality semen already at 7 months of age and may indicate their attainment of puberty. It is interesting to consider that the TM at 8 months of age was lower than that at 7 and 7.5 months of age for Namaqua-Afrikaner sheep (70.5 ± 15.1 %, 95.4 ± 15.1 % and 95.3 ± 15.1% respecfively) – This may have been a response to breeding season as at 7 months of age, the season was autumn but at 8 months of age, the season was winter, meaning that ram lambs may have produced superior semen for the duration of the breeding season in autumn.

At 7 months of age, a significant difference was found between the PR percentage of Bapedi and Zulu ram lambs, as well as Namaqua-Afrikaner and Zulu ram lambs, where Zulu ram lambs' PR was higher ($P<0.05$). Also, there was a significant difference in the PR of Zulu ram lambs between 7 and 7.5 months of age, with their PR at 7.5 months being lower than that at 7 months ($P<0.05$). This difference contradicts findings by Rege *et al.* (2000) and Hassan *et al.* (1970) that found that the PR should have increased with age as semen progression parameters like PR decline chronologically after 3 years of age but increase from puberty until then. Similarly, for NP, there was a significant difference between Bapedi ram lambs at 7 and 8 months, and between Zulu ram lambs at 7 and 7.5 months of age ($P<0.05$) – Surprisingly, the NP of Bapedi sheep decreased while the NP of Zulu sheep increased when comparing 7 to 8 months of age. Thus, the differences of Zulu ram lambs correspond with Rege *et al*. (2000)'s claim that NP should increase while the differences of Bapedi ram lambs contradict this claim. There was also a significant difference ($P<0.05$)

in the NP of Bapedi and Zulu ram lambs and Namaqua-Afrikaner and Zulu ram lambs at 7 months of age, with Zulu ram lambs having a lower NP. Contradictory to PR, this means that Zulu ram lambs have worse NP than Bapedi and Namaqua-Afrikaner ram lambs at 7 months. At 7.5 months of age, Zulu ram lambs then had a significantly ($P<0.05$) higher NP than Namaqua-Afrikaner ram lambs, and both Bapedi and Zulu ram lambs had a significantly lower NP than Namaqua-Afrikaner ram lambs at 8 months of age. This fluctuation may be an inherent part of the spermatogenetic process of a ram lamb, as suggested by Colas (1983) who states that due to incomplete spermatogenic activity and incomplete epidydimal maturation, the quality of semen produced by a young male may vary. Lastly, the within-breed IM progression for all three breeds was statistically nonsignificant ($P>0.05$), however, there was a significant between-breed difference ($P<0.05$) between Namaqua-Afrikaner and Zulu ram lambs at 8 months of age, where Namaqua-Afrikaner ram lambs had a higher percentage of IM sperm cells. David *et al*. (2015) claims that immotile sperm cells will hinder overall fertilizing ability, meaning that when compared to Zulu ram lambs, Namaqua-Afrikaner ram lambs will have a significantly greater hinderance to their fertilizing ability than would Zulu ram lambs at 8 months of age.

In addition, there was a significant difference in the rapid percentage of Bapedi and Zulu ram lambs and Namaqua-Afrikaner and Zulu ram lambs at 7 months of age ($P<0.05$) with Zulu ram lambs having a higher percentage. It can then be asserted that Zulu ram lambs' semen has a better ability to move through the cervix because rapid velocity of sperm cells is associated with the sperm cell's ability to move through cervical mucus in the cervix (Vasan, 2011). This is also the case for Bapedi and Zulu ram lamb rapid velocity at 8 months compared to Namaqua-Afrikaners. In addition, there was a significant decrease between the rapid percentage of Zulu ram lambs at 7 and 7.5 months of age ($P<0.05$), indicating that this semen characteristic did not improve with age from 7 to 7.5 months of age as Rege *et al.* (2000) would claim it should. Medium velocity did not show any significant differences between breeds ($P>0.05$), however, for slow velocity percentages, there was a significant decrease between Bapedi ram lambs at 7 and 8 months of age, and between Zulu ram lambs at 7.5 and 8 months of age, meaning that this semen characteristic improved with age. This is because slow velocity percentages should be proportionally low compared to medium and fast velocity percentages (Ramu and Jeyendran, 2013) (but, there was a significant increase between Zulu ram lambs at 7 and 7.5 months ($P<0.05$), meaning that this semen characteristic did not improve with age between 7 and 7.5 months for Zulu sheep). However, there was a significant difference between the slow velocity percentage of Bapedi and Zulu ram lambs and Namaqua-Afrikaner and Zulu ram lambs at 7 months ($P<0.05$) with Zulu ram lambs having a lower slow velocity percentage. This again suggests that Zulu ram lambs may have better semen quality at 7 months due to their ability to produce less slow-moving semen.

There is very little literature that explores the correlations associated with and significance of average values of speed. For example, there was a significant difference in the VCL of Bapedi and Zulu ram lambs and Namaqua-Afrikaner and Zulu ram lambs at 7 months of age and between the VCL of Zulu ram lambs at 7 and 7.5 months of age ($P<0.05$). However, it is inconclusive as to whether the average velocity along a sperm cell's average path (VCL definition) affects the fertilizing ability or attainment of puberty of a ram. This is the case for STR and WOB too. Sloter *et al.* (2006) does, however, claim that VSL, LIN and VAP are correlated with fertilization rates *in vivo*, meaning that, because there was a significant decrease between the VAP of Zulu ram lambs at 7 and 7.5 months; and between the VSL of Zulu ram lambs at 7 and 7.5 months, and at 7 and 8 months; and between the LIN of Zulu ram lambs at 7 and 8 months ($P<0.05$), then it can be assumed that the fertilization rates that would have come from Zulu ram lamb semen in the study period would have decreased with age. In addition, a noticeable trend from the average values of speed found in this study is that Bapedi ram lambs had a significantly lower ($P<0.05$) value for VCL, VAP, VSL, STR, LIN and WOB than did Zulu ram lambs at 7 months – This suggests that Bapedi ram lambs fertilization rates *in vivo* may be significantly lower than those of Zulu ram lambs. However, by 8 months of age, this trend had plateaued and there was no significant difference between Bapedi and Zulu ram lambs ($P>0.05$).

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had a statistically nonsignificant difference in intact and non-intact percentages as well as live and dead percentages ($P>0.05$). This further supports the homogeneity of these breeds and suggests that all three breeds will have the same fertilizing ability (Ramu and Jeyendran, 2013). Rege *et al*. (2000) found that Ethiopian highland sheep at 6 months of age produced semen with 29.4 ± 6.0 % dead percentage; this was higher than that of South African indigenous sheep at 7 months (17.1 ± 7.7 % to 23.5 ± 11.4 %). Moreover, because the semen membrane integrity of all three breeds is above 50% but below 90% (Yarney and Sanford, 1993), the semen morphology of all three breeds is satisfactory but not exceptional from the first CASA analysis at 7 months of age, further suggesting that Bapedi, Namaqua-Afrikaner and Zulu ram lambs have attained puberty. Also, according to Yarney and Sanford (1993), having a total motility greater than 70% categorizes semen as exceptional, which is the case for Bapedi, Namaqua-Afrikaner and Zulu ram lambs at 7 to 8 months of age.

With regards to abnormalities, Bapedi ram lambs had a significant decrease in the number of head abnormalities at 7 and 8 months and Zulu ram lambs had a significant decrease in the number of tail abnormalities at 7 and 8 months of age ($P< 0.05$). It is possible that as with sexual behaviour activities, Namaqua-Afrikaner ram lambs are physiologically younger and thus have semen with more abnormalities

(Colas, 1983). Colas (1983) found that ejaculates from younger ram lambs have a greater number of abnormal sperm cells in terms of morphology due to incomplete spermatogenic activity and incomplete epidydimal maturation.

5.6. Pearson correlation coefficients for bodyweight, scrotal circumference, and blood serum testosterone concentration

The strongest positive correlation reported in this study is that between body weight and scrotal circumference, where $r = 0.60$: This means that as body weight increases, so does scrotal circumference. Perumal (2014) concluded that body weight is more highly correlated to scrotal circumference, than scrotal circumference is to age – Although this study did not correlate any parameters to age, it is reasonable to assume that because body weight increases linearly as the Bapedi, Namaqua-Afrikaner and Zulu ram lambs aged, then there would be a similar relationship between indigenous ram body weight, scrotal circumference and age as found in Perumal's (2014) study. Body weight also had a positive correlation to semen volume.

In terms of semen characteristics' Pearson correlations, there existed a positive correlation between semen volume and BW, blood serum testosterone and SC. It can then be assumed that ram lambs with higher semen volume will have higher BW, blood serum testosterone and SC as well (0.23, 0.05 and 0.33 respectively). The positive correlation between scrotal circumference and semen volume (0.33) is corroborated by Dombo (2002) who reported that due to a larger site for semen production and storage, small testes produce a smaller volume of semen than big testes. Latif *et al*. (2009) also found a positive correlation between semen volume and scrotal circumference ($r = 0.72$) in 12 crossbred bulls. In addition, there existed negative correlations and a very weak correlation between sperm cell concentration and BW (-0.03), blood serum testosterone (-0.40) and SC (0.07) respectively. This means that as sperm cell concentration and scrotal circumference increase, BW and blood serum testosterone decrease. In addition, body weight and scrotal circumference are highly heritable phenotypic traits that are easy to measure and can be measured early in life (Rege *et al.,* 2000), thus selection based on these traits may have a favorable effect on other traits like semen volume that are positively correlated.

In terms of the *r* of the HOS test for membrane integrity, there is a positive correlation between coiled tails (intact membranes) and BW (0.23) and SC (0.05), but a very weak negative correlation with blood serum testosterone (-0.06). This may mean that better quality semen (that with intact membranes) comes from ram lambs with higher BW and SC; however, there exists an antagonism between blood serum testosterone and intact membranes. Similarly, there existed a negative correlation between straight tails

(non-intact membranes) and BW (-0.28) and SC (-0.05), and a very weak positive correlation with blood serum testosterone (0.03). This further supports the claim that there may exist an antagonism between blood serum testosterone and membrane integrity.

Interestingly, there existed a negative correlation between live sperm cell percentage and blood serum testosterone (-0.02): This also supports the claim that higher blood serum testosterone may be antagonistic to positive semen characteristics; this is also supported by the very weak positive correlation between dead sperm cell percentage and blood serum testosterone (0.03). However, there does exist very weak positive correlations between live sperm cell percentage and BW (0.05) and SC (0.13), and correspondingly, there existed negative correlations between dead sperm cell percentage and BW (-0.05) and (-0.13). This suggests that ram lambs with higher body weights and scrotal circumferences will present a larger proportion of live sperm cells.

In terms of head abnormalities, there is a weak negative correlation with BW (-0.22) and SC (-0.35) and a weak positive correlation with blood serum testosterone (0.35). This means that the higher the ram lamb's BW and SC, the lower their head abnormalities; and the higher their blood serum testosterone, the higher their head abnormalities. These correlations are corroborated by the *r* of tail abnormalities: There is a very weak positive correlation between tail abnormalities and BW (0.13) and SC (0.15), and a very weak negative correlation with blood serum testosterone (-0.0011), meaning that the higher the BW and SC of a ram lamb, the higher the tail abnormalities; and the higher the blood serum testosterone, the less tail abnormalities. The relationships between tail and head abnormalities are inverse. This is also true for *r* of midpiece abnormalities: There are very weak positive correlations between midpiece abnormalities and BW (0.09), blood serum testosterone (0.15) and SC (0.05). This means that as BW, blood serum testosterone and SC increase, so do midpiece abnormalities.

It is interesting to consider that testosterone level is correlated positively and significantly with sperm total motility and progressive motility (Swelum *et al.*, 2017), thus, study contradicts findings by Swelum *et al.* (2017) as this study found a very weak negative correlation between blood serum testosterone and motile sperm cells (-0.0031). Similarly, there existed a weak negative correlation between motile sperm cells and scrotal circumference (-0.09) which is expected due to the positive correlation between blood serum testosterone and scrotal circumference (0.30). However, there does exist a positive correlation between motile sperm cells and body weight (0.18), suggesting that larger rams will have a higher percentage of motile sperm cells.

PR has a negative correlation with BW and SC (-0.10 and -0.15 respectively) but a positive correlation with blood serum testosterone (0.03). This relationship is confirmed by the inverse correlations between NP and BW (0.30), SC (0.16) and blood serum testosterone (-0.04). The correlations of NP and PR thus suggest that a ram with larger body weight will have less PR sperm cells and more NP sperm cells; a ram with higher SC will have less PR sperm cells and more NP sperm cells; and that a ram with higher blood serum testosterone will have more PR sperm cells and less NP sperm cells. This is important because it suggests that an animal with higher blood serum testosterone will have superior semen in terms of PR and NP. With regards to speed, there existed very weak negative relationships between rapid sperm cell speed and BW (-0.09) and SC (-0.09) but a very weak positive relationship with blood serum testosterone (0.04). Thus, rams with higher body weighs will have lower rapid sperm cell speed and rams with higher SC will have lower rapid sperm cell speed. This relationship is the same for rapid progressive velocity and progressivity. For IM sperm cells, there is a weak negative correlation to body weight (-0.18), a very weak positive correlation to blood serum testosterone (0.0031) and scrotal circumference (0.09). This then means that a ram lamb with higher BW will have less IM sperm cells, but a ram with higher blood serum testosterone and SC will have higher IM sperm cells. In terms of average values of speed, VCL is the average velocity of the sperm head through its actual path and there existed a negative correlation between VCL and BW (-0.06) and SC (-0.0076) and a positive correlation with blood serum testosterone (0.15), as with PR, rapid speed, and rapid progressive velocity and progressivity. Thus, higher BW and SC results in lower VCL, and higher blood serum testosterone results in higher VCL. VAP is the average velocity of the sperm head through its smoothed cell path and there is a positive correlation between it and BW (0.02) and blood serum testosterone (0.26) but a negative correlation with SC (-0.06). This relationship is the same for VSL, STR, LIN and WOB. This is important because it means that ram lambs with higher BW and blood serum testosterone will have higher VAP, VSL, STR, LIN and WOB, but rams with higher SC will have lower VAP, VSL, STR, LIN and WOB.

Chapter 6: General Conclusion

Bapedi sheep began displaying sexual behaviour activities (nosing) at 3.5 months of age, at the same age as Zulu sheep, whereas Namaqua-Afrikaner sheep only began displaying sexual behaviour activities a month later; by the end of the study period, all three breeds had shown sexual behaviour activities. This suggests that on a spectrum, Bapedi and Zulu sheep are early maturing compared to Namaqua-Afrikaner sheep. It was also observed that Bapedi, Namaqua-Afrikaner and Zulu sheep had similar body weights for 66.6% of the study period and blood serum testosterone concentration for 88.9% of the study period, and significantly different scrotal circumferences for the entire study period. This homogeneity in bodyweight and blood serum testosterone is by virtue of all being indigenous Southern African breeds. In addition, all the studied breeds produced what can be considered as satisfactory semen at the first computer aided semen analysis at 7 months of age and although there was a significant difference in most semen characteristics, Bapedi, Namaqua-Afrikaner and Zulu ram lambs showed, in some semen characteristics like semen volume and sperm cell concentration as well as medium velocity and mid-piece abnormalities, no significant differences at different ages and between breeds. Lastly, the strongest Pearson correlation was between body weight and scrotal circumference ($r = 0.60$) and there existed a correlation ($r \neq 0$) between all compared parameters. This study indicated that South African indigenous sheep attained puberty at the age of 7 months while their average body weight was 23.3 kg, their average scrotal circumference was 17.5 cm and their blood serum testosterone was 4.3 ng/ml. The current study also recommended that there should be more research on indigenous sheep breeds for improved reproductive performance and conservation strategies – This study made use of in situ conservation over ex situ conservation and that created various benefits: breeds continued to develop and adapt to changing environmental pressures, enabling research to determine their performance more ingeniously. It was an inexpensive and convenient conservation method, that also allowed for long term study of the ram lambs. This long-term study was advantageous over a short-term study for its allowance of gradual, consistent, progressive observation.

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