



The Characterisation of Semen from Zulu Rams raised
under Extensive Management Conditions in
KwaZulu-Natal

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CANDIDATE'S DECLARATION

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

I declare that the work contained in this dissertation is my own, unless otherwise specified. I further certify that this research is original, and that the material submitted for examination has not been submitted, either in whole or in part, for a degree at this or any other university.

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

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

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ABSTRACT

The Zulu sheep is an Nguni breed indigenous to the KwaZulu-Natal (KZN) province in South Africa, and is reported to be under threat of extinction. Studies investigating the factors that may be the cause of the declining numbers are required for the strategic planning of conservation programmes. The study was designed to evaluate some of the factors viz. ram age, season and geographic location, that influence reproductive performance in Zulu sheep populations based on the viability of Zulu ram semen. Spermogramic parameters used to assess quality were: scrotal circumference (cm); semen volume (ml); semen pH; sperm concentration ($\times 10^9$), progressive and mass motility (%), and percentages of live and abnormal spermatozoa. Semen samples were collected via electro-ejaculation and analysed using a microscope while the scrotal circumference was measured using a flexible measuring tape. All data was analysed using the statistical software SPSS version 22. The average semen volume (ml) per ejaculate was 0.66, 1.11, 1.19, 0.82 and the sperm concentration 1.69, 2.79, 3.12, 3.07 ($\times 10^9$) for summer, autumn, winter and spring, respectively. The effects of age on all parameters were significant except for pH. There was a positive correlation between age and semen volume, concentration and semen colour. The values of volume, concentration, motility and live sperm increased linearly up to 3 years of age. Scrotal circumference and live spermatozoa were comparatively higher for rams at 3 years of age than at 4 years of age, while the values were the same at 1 and 2 years of age. The percentage of abnormal spermatozoa decreased down to 3 years of age, thereafter increasing from 4 years of age. The semen quality improved at 3 years of age and thereafter decreased. The effects of season on semen quality positively correlated in some of the geographic locations. For conservation purposes it would therefore be more efficient to select breeding sires from among the 3 year old rams for the autumn and winter breeding programme.

Keywords:

Nguni sheep, Zulu rams, conservation strategies, spermogramic parameters, geographic locations.

TABLE OF CONTENTS

CANDIDATE'S DECLARATION.....	i
SUPERVISOR'S DECLARATION	i
ACKNOWLEDGEMENTS	ii
ABSTRACT.....	iii
TABLE OF CONTENTS	iv
CHAPTER ONE	1
Introduction and Background	1
1.1. Introduction.....	1
1.2 Aim	8
1.3. Hypothesis.....	8
1.4. Objectives.....	8
1.5. Dissertation Outline	8
CHAPTER TWO	9
Literature Review	9
2.1. Introduction.....	9
2.2. Factors affecting semen quality	11
2.2.1. Age of ram	11
2.2.2. Season.....	12
2.2.3. Management.....	14
2.2.4. Testosterone level.....	16
2.3. Conclusion.....	17
CHAPTER THREE.....	18
Methods and Materials.....	18
3.1. Introduction.....	18
3.3. Physical examination (PE), scrotal circumference (SC) measurement and Body Condition Score (BCS)	18
3.2. Location of samples.....	20
3.4. Blood samples.....	21
3.5. Semen samples.....	21
3.6. Gross examination of semen	21
3.7. Quantitative assessment of semen	21
3.8. Statistics analysis	22

3.9. Management review	22
CHAPTER FOUR.....	23
Management Systems	23
4.1 Results	23
4.2. Discussion	25
4.3. Conclusion.....	28
CHAPTER FIVE.....	29
Results.....	29
Effects of age, season and location on body weight, scrotal circumference spermogramic and physiological parameters	29
5.1. The effects of age and season.....	29
5.2. The effect of season in each geographic location	34
The results in Tables 5.7 to 5.15 show the characteristics of Zulu ram semen across all seasons in the areas of data collection.	34
5.3. Spermogramic and physiological parameter quality of Zulu rams among the different geographic locations.....	36
CHAPTER SIX	48
Discussion	48
6.1. The effect of ram age on semen quality of Zulu rams	48
6.2. The effect of season	52
6.2.1. The overall effect of season on Zulu ram semen quality	52
6.2.2. The effect of season on Zulu ram semen quality among the selected geographical locations.....	56
6.3. Conclusion.....	61
CHAPTER SEVEN.....	61
7.1. General conclusion	61
7.2. Recommendations.....	62
7.3. References	63
7.4. Appendices.....	70
7.4.1. Appendix A.....	71
7.4.2. Appendix B.....	73
7.4.3. Appendix C	75
7.4.4. Appendix D	75

CHAPTER ONE

Introduction and Background

1.1. Introduction

The Zulu sheep (also known as *Izimvu* in the local dialect of IsiZulu; species: *Ovis aries*) is an Nguni breed indigenous to the KwaZulu-Natal (KZN) province in South Africa. This rare breed of sheep is adapted to the subtropical climatic conditions of KZN and is tolerant to the parasites prevalent in this region (Kunene *et al.* 2009). This hardiness makes the Zulu sheep a suitable breed for traditional production methods by poorly resourced farmers in the rural areas, where prophylactic care for domestic animals is absent or difficult to obtain. Zulu sheep are therefore important to the livelihood of rural farmers who require hardy, multipurpose breeds that are low-maintenance but have relatively high outputs (Kunene and Fossey, 2006 Mavule, 2013). The Zulu sheep population is reported to be under threat of extinction (Ramsay, 2000), with recent research showing that the population of Zulu has been declining (Kunene and Fossey, 2006)

On a global scale, livestock breeds represent an important resource for economic development and livelihood security. Extensive diversity in these breeds permits their existence in any global environment (Rege and Gibson, 2003). The global demand for animal and animal products is increasing due to an exponentially growing human population. This demand has put a large number of genetically pure livestock breeds at risk of extinction (Roldan and Gomendio, 2008). There are approximately 30 animal species which have been domesticated, of which 30% are at risk of extinction (Hoffman and Scherf, 2005).

According to Rege and Gibson (2003), the four main reasons for the decline in animal diversity are:

i) Genetic dilution or eradication through use of exotic germplasm, a classic example being that of the North American Friesian-Holstein cattle. There have been instances where entire breeds have been replaced by the North American Friesian-Holsteins due to intense marketing, emphasis of a single trait (which in this case is milk production), the use of artificial insemination (AI) and most recently, embryo transfer. As a result, not only is this breed replacing other

breeds of cattle, it is also reducing the diversity within the breed itself. Of the 5000 Holstein bulls born in 18 countries in 1990, 50% bulls were sired by only five 'superior' bulls.

ii) Changes in production systems leading to changes in breed use and crossbreeding.

iii) Changes in response to producer-preference. This is usually influenced by socio-economic reasons.

iv) Droughts, degrading ecosystems, famine, disease epidemics, civil strife/war, political instability and market fluctuations.

Roldan and Gomendio (2008) stated that the cause of endangerment or extinction varies amongst taxonomic groups. However in some cases, such as that of the domesticated terrestrial ungulates (hoofed animals), overexploitation is the most frequent cause of threat.

There are a number of possible ways to conserve the Zulu sheep. This may be done *in-situ*, *ex-situ*, or through a combination of both these methods. *In-situ* is a collective method for keeping animals in their natural state (Ramsay *et al.* 2006). However, this must be done systematically based on the habitat in which they are commonly or typically found and also on the quantitative assessment of the genetic diversity they possess and the implementation of programmes for their effective and sustainable management (Rege and Gibson, 2003). *Ex-situ* conservation is the preservation of semen, oocytes or embryos (Rege and Gibson, 2003) which can be used at a later stage. This may be done to revive a flock by restoring its numbers or to re-distribute genetic material at different locations. Both *in-situ* and *ex-situ* methods are recommended for conservation.

The number of Zulu sheep is rapidly declining. Kunene *et al.* (2007) reported that this decline in numbers is due to replacement by unrelated breeds. This is most probably due to a shift in agricultural paradigm from a traditional system to a more commercialised system to keep up with economic development and other related market trends. However, recent research conducted by Mavule (2013) showed that the major cause of the declining numbers of Zulu sheep in the rural areas of KZN is drought. The drought intensity in some parts of the province was perceived by the farmers to be beyond the adaptive attributes of the Zulu sheep. Disease was ranked as the second major causative factor in the decline of Zulu sheep. Other less common factors included theft and vehicle accidents (Mavule, 2013). In the same study, the author reported that 42% of the 96 Zulu sheep farmers who participated in the survey had crossbred their Zulu sheep with other breeds. Therefore, through cross-breeding or replacement

by other breeds (Kunene *et al.* 2007), the Zulu sheep gene pool is becoming diluted by commercial sheep breeds.

While the diversity of the Zulu sheep species increases by crossbreeding, uncontrolled crossbreeding results in the population of pure breeds diminishing. This means the Zulu sheep genome has been genetically compromised and become less diverse thus leading to an increase in inbreeding within pure Zulu sheep populations. This lack of diversity places Zulu sheep survival at immense risk (Kruger, 2001), caused by inbreeding depression. Mavule (2013) emphasized that lower population variability actually suppresses individual fitness, resistance to diseases and parasites, and flexibility in coping with environmental challenges and long term adaptability. Unless these phenomena are prevented Zulu sheep may well become an extinct breed.

This study was designed to determine factors that influence reproduction in the Zulu sheep population based on the viability of Zulu ram semen. The aim of this pioneer reproductive research on Zulu sheep was to close the gaps in knowledge to enable the development of a more effective conservation programme. The wide geographic spread and extensive collection of data in this research allowed for a more in-depth and accurate representation of Zulu sheep breeding in KZN. Kunene *et al.* (2009) emphasised the need to analyse as many traits as possible to understand the breed and its niche in agriculture and the ecosystem. Mavule (2013) also highlighted that characterisation of this breed is the first essential step in formulating strategies for conservation and sustainable use.

Roldan and Gomendio (2008) stated that the current biodiversity crisis, coupled with the lack of resources, has led to a situation where the establishment of priorities is essential to maximise the effectiveness of conservation measures. This establishment of priorities was further elaborated to include the debate on whether reproductive studies were a priority in conservation due to two main issues:

- 1) Is male reproduction impoverished among endangered species, thus contributing to further declines?
- 2) Is the use of reproductive biotechnologies useful in conservation?

In natural populations males show varying degrees of fertility. In cases where populations are endangered male fertility is a critical factor in the continued survival of the species. Hence it is important to understand the commonality of subfertility or infertility (Roldan and Gomendio,

2008). This enables researchers to determine where the cause of the problem lies and how it can be overcome. Sexual behaviour and semen quality are the main parameters limiting male reproductive efficiency (Zamiri *et al.* 2010).

Conservation programmes (Deen *et al.* 2003) and Performance Testing Centres alike require the quality of semen to be evaluated to establish superior genetics for breeding (Hafez and Hafez, 2000). Apart from breeding for conservation of animal genetic resources (AnGR), semen evaluation may be used in artificial insemination programmes (David *et al.* 2007) for dissemination of superior quality semen (Karagiannidis *et al.* 2000) around the world by improving or emphasising a particular trait (Toe *et al.* 2000) in order to meet demands for meat, wool and other animal-derived products.

Semen quality, which is a phenotypic characteristic, consists of a genetic component, an environmental component, and an interaction between these two components (David *et al.* 2007). Before semen can be utilised or preserved for later use an accurate analysis and evaluation is necessary, because this will determine the breeding potential of a sire and its progeny. This enables researchers to collect semen from only the sires with the greatest breeding potential thus saving irreplaceable resources such as time and genomes.

Semen evaluation of sheep in South Africa has been well documented in the Dorper (Bester *et al.* 2004; Fourie *et al.* 2002) as well as the Dohne Merino sheep breeds (Eastern Dohne Central Nucleus). There has been no study done previously on semen evaluation in Zulu sheep.

While pregnancy is the ultimate test to determine semen quality, it is possible to quantify the properties of the sampled semen using laboratory methods that can be done more quickly than assessing conception rates. Various physical characteristics of semen may be evaluated as no single test accurately predicts the fertility of a sperm sample (Hafez and Hafez, 2000).

The parameters evaluated are semen volume, consistency, pH, sperm concentration and motility, and the percentage of abnormal sperm.

Ram semen should be milky-white or pale cream in colour (Hafez and Hafez, 2000). A pink colour is indicative of the presence of blood, which may be caused by injury during or prior to collection. Contamination or infection of the reproductive tract is suggested by brown or grey semen (Hafez and Hafez, 2000).

Any defects such as abnormal morphology of the spermatozoa or genetic-based disorders must be established. The number of morphologically normal sperm will differ in each ejaculate sample but there should be a normal sperm percentage of approximately 90-95% (Hafez and Hafez, 2000) in order to classify a semen sample as being of good quality.

In most cases, good motility is accompanied by good fertility. Poor motility is almost always an indicator of poor fertility (Oláh *et al.* 2013). Good quality semen should have 80-90% progressive motility (Hafez and Hafez, 2000), based on subjective scoring (David *et al.* 2007; Zamiri *et al.* 2010).

The pH of the semen affects the metabolism of the sperm. This pH may be affected by the diet, extensively (minimum management) raised sheep on open pastures tend to have a higher pH (Moghaddam *et al.* 2012) possibly due to mineral deficiencies in the pastures. A pH of 5.9-7.3 is considered an ideal value for normal sperm (Hafez and Hafez, 2000). Any deviation from this narrow pH range will result in decreased metabolism of the sperm and its eventual death.

The quantity of spermatozoa is also of utmost importance as it is an indication of the quality of the semen (Alm-Packalén, 2009). In rams, at first breeding, the ejaculate is in the region of 0.3-1.0ml with the sperm concentration at around 1.2-2 billion sperm/ml (Hafez and Hafez, 2000). At the peak of sexual maturity, each ejaculate may yield up to 2ml of semen on average with a sperm concentration of 3.6 billion sperm/ml (Oláh *et al.* 2013).

Age is one of the important physiological factors to consider when evaluating semen (Karagiannidis *et al.* 2000), as there is a close relationship between age and the quality and quantity of semen (Hassan *et al.* 2009). As age progresses, semen quality improves. According to Hassan *et al.* (2009) the quantity of semen increases as the animal gets older due to an increase in testicular size. However this testicular growth and semen quantity stabilises around the 3rd to 4th year of age.

Scrotal circumference (SC) is a highly heritable trait and is a superior index for sperm production (Kafi *et al.* 2004; Zamiri *et al.* 2010; Gordon, 1999). Zamiri *et al.* (2010) detailed some of the factors affecting SC including feed quality, average daily weight gain, climatic conditions, and season and day length. A ram which is nutritionally well-sustained in a harsh climate is more likely to have a larger SC than a ram which experiences mild to moderate climatic conditions but is provided inadequate nutrition (Kheramand *et al.* 2006).

The effect of season has been extensively documented and most research has concluded that it is actually photoperiod, which elicits an effect on semen quantity and quality much greater than ambient temperatures. Sheep are short-day breeders (Dorostghoal *et al.* 2009) which means they breed during days with decreasing length. They therefore use photoperiod to synchronize their breeding activity (Moghaddam *et al.* 2012). Hence rams will most likely have the highest quality semen in autumn and summer than in winter and spring (Karagiannidis *et al.* 2000).

The effect of season can also be attributed to the quality of pastures available at certain times of the year (Kheramand *et al.* 2006). It has been widely recognized in many studies that nutrition is perhaps the most crucial factor in terms of its direct impact on reproductive phenomena (Robinson *et al.* 2006) and with the potential to moderate the effects of other factors (Kheramand *et al.* 2006). In essence, adequate nutrition may encourage mediocre biological types to reach their full genetic potential, alleviate the negative effects of a harsh physical environment and minimise the effects of poor management techniques (Kheramand *et al.* 2006). Nutrition can be readily manipulated to ensure positive outcomes. The effect nutrition has on reproductive capacity may be linked with body condition (Bester *et al.* 2004). Rams in good condition are more likely to have better quality sperm than under-conditioned or over-conditioned rams (Bester *et al.* 2004 and Robinson *et al.* 2006).

Kishk (2008) reported that decreased sexual activity in summer may also be due to the lowered levels of testosterone during this period, or an unknown combination of environmental or physiological factors. Since testosterone is the primary male hormone which is responsible for male characteristics and sexual behaviour, there is a high recognisable correlation between testosterone level and semen characteristics (Kishk, 2008). This may be explained in terms of the effect testosterone has on the Sertoli cells in the testes. Testosterone stimulates spermatogenesis since the Sertoli cells are responsible for the nourishment, division and care of the normal spermatozoa. Hence testosterone levels serve as a good marker for semen quality and production due to the fact the testosterone level is correlated to semen quantity and quality (Kishk, 2008).

The many factors that affect the quality and quantity of semen may act either as a single influence, such as temperature and photoperiod (David *et al.* 2007 and Dorostghoal *et al.* 2009), or in conjunction with other factors such as type of management, the individual's age and predisposition to disease, or anatomical defects (Seaman, 2004). These factors tend to become

prominent in endangered species, particularly if the habitat becomes suboptimal (Roldan and Gomendio, 2008).

In most instances, Zulu sheep are raised in an extensive management system (Kunene *et al.* 2007) by subsistence farmers. The effect of this type of management could therefore be a significant factor in the overall quality of the semen, mainly as the availability of veterinary care and supplementary feeding among other factors may be limited or even absent.

A fertility test by means of semen evaluation offers predictive information that may enhance the overall reproductive potential of the flock (Chapwanya *et al.*, 2008).

The importance of semen evaluation is not only significant for conservation, but also for commercial breeding programmes (Giminez and Rodning, 2007). This is because the genetic diversity that the livestock possess is vital to the economic development of the majority of countries in the world as it maintains a sustainable crop-livestock system (Rege and Gibson, 2003). Petrovic *et al.* (2012) also stated that the biological efficiency in terms of meat, milk and wool production is conditioned by fertility.

Male fertility traits are determined by specific sperm traits. Smital *et al.* (2005) also stated that traits like the proportion of normal sperm are thought to have a genetic component, while sperm swimming velocity seems to be influenced to a greater extent by environmental and social factors (Roldan and Gomendio, 2008).

David *et al.* (2007) showed that these factors need to be identified for the improvement of the efficiency of artificial insemination (AI). AI may also be an appropriate tool to be used for increasing flock numbers of Zulu sheep by utilizing genetically superior rams. However due to the difficulties of preserving small ruminant semen, the use of fresh semen is preferred (Cseh *et al.* 2012). To ensure that the reproductive capacity is at its highest, the quantity and quality of the semen should also be substantially high.

Reproductive assessment of semen characteristics has been done in a number of sheep breeds (Karagiannidis *et al.* 2000; Bester *et al.* 2004; Oyeyemi *et al.* 2009; Ceyhan *et al.* 2013) but these evaluations included the control of certain parameters such as nutrition (Dana *et al.* 2000 and Kheradmand *et al.* 2006). This research with Zulu sheep was conducted *in-situ* under the prevailing natural conditions which form the basis of extensive flock management. The results from this study will provide information on the fertility status of Zulu rams.

1.2 Aim

The aim of this work was to assess the quality of Zulu ram semen from the geographically separated and extensively managed Zulu sheep populations in the KwaZulu-Natal (KZN) province. The effect of ram age and season on the viability of Zulu ram semen were also evaluated.

1.3. Hypothesis

There are variations in the quality of Zulu ram semen due to the individual or interactive effects of ram age and season.

The null hypothesis is that there are no variations in the quality of Zulu ram semen due to the individual or interactive effects of ram age and season.

1.4. Objectives

The objectives of this research were to:

1.4.1. Assess the effect of age on the quality of Zulu ram semen.

1.4.2. Evaluate the quality of Zulu ram semen in terms of pH, sperm motility and concentration, numbers of live sperm, and the percentage of sperm abnormality.

1.4.3. Assess the levels of testosterone and white blood cell counts of the rams to establish the correlation with semen quality.

1.4.4. Determine the effect of season within geographically separated Zulu ram semen quality under the extensive management system.

1.4.5. To document the management systems of the selected Zulu sheep farmers.

1.5. Dissertation Outline

Chapter 1 presents the introduction and the objectives of this dissertation. Chapter 2 gives a comprehensive review of pertinent literature. The topics covered were the effects of ram age, season, management and testosterone production on semen quality.

Chapter 3 is devoted to the methods and materials used for the collection of data for this study as well as the locations that were surveyed. Chapter 4 presents the results and discussion of the extensive management system adopted by farmers to rear Zulu sheep.

Chapter 5 presents the results obtained directly from assessing the blood, semen and overall health of the Zulu rams which were influenced by the ram age and season. A discussion follows these results. Chapter 6 covers the results of the rams' semen quality tests in terms of location and particularly the effects of age and season on rams in these locations.

Chapter 7 concludes this study with Zulu rams and also contains references and appendices that accompany this text.

CHAPTER TWO

Literature Review

2.1. Introduction

Manes (2011) described the benefits of rare and indigenous domestic livestock. Some of the valuable characteristics of these species include disease resistance, extreme climate tolerance, high milk production, superior mothering ability and the ability to utilise poor pastures. Indigenous breeds in particular are inextricably linked to the tradition and history of their region and represent a long-term investment of time and intellect (Alderson, 2010).

For thousands of years, man has genetically altered different domestic breeds to produce more products in a particular region, in a much shorter timespan (Manes, 2011). There are breeding programmes that have fuelled the use of exotic breeds to replace indigenous breeds (Gizaw *et al.* 2011; Mavule, 2013) mainly through crossbreeding (Kunene *et al.* 2009). Thus, the existence of indigenous breeds is seriously threatened. There is a need to intervene in their preservation since local breeds are a source of genetic diversity needed by the modern livestock industry for production to ensure stability and continuity (Mavule, 2013) of the existing indigenous and exotic breeds. The same conclusion has also been drawn by Hlophe (2011). Similarly, Notter (1999) stated that farm animal genetic diversity is required to meet production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to ever-changing breeding objectives; while maintaining the core germplasm of unique, rare and endemic species.

Manes (2011) highlighted the main reasons for conservation of indigenous breeds as follows:

- i. Food stability to meet population growth
- ii. Genetic diversity for future livestock industry
- iii. Historical and cultural value
- iv. Resistance to climate stresses and diseases
- v. Cross-breeding potential
- vi. Opportunities for rural development
- vii. Sustainable farming

In South Africa, particularly in KZN (where Zulu sheep are mostly found), Zulu sheep are kept mainly by the subsistence farmers (Kunene *et al.* 2007). Mavule (2013) observed that they are mainly used as a source of meat and trade; however they also provide manure and are used in traditional rituals.

According to a report released by the South African Department of Agriculture (Ramsay *et al.* July 2006), New-World and colonial continents such as the Americas, Australia and New Zealand do not have adapted livestock breeds at their disposal and have to rely entirely on imported material to establish their livestock. In contrast, Africa is richly endowed with a considerable number of breeds that possess an inherent ability to adapt to the prevailing harsh

conditions (Ramsay *et al.* 2006). South Africa possesses a unique and diverse gene pool of indigenous and locally developed livestock breeds which henceforth should be screened and protected (Ramsay *et al.* 2006).

2.2. Factors affecting semen quality

2.2.1. Age of ram

Puberty is the main signal indicating the onset of first sexual activity and has a marked effect on lifetime production (Giminez and Rodning, 2007). According to Hassan *et al.* (2009), small ruminants show various patterns in their reproductive activity that are shaped by age, meaning that selection for fertility may be done by selecting correlated traits such as age.

David *et al.* (2007) reported that in Lacaune and Manech-tête-Rousse rams, there was a decrease in the number of spermatozoa with increasing age. This was attributed to a decrease in ejaculate volume. The number of spermatozoa showed, as expected, a positive phenotypic correlation with volume and concentration, but the correlation was higher with volume than with concentration. Hassan *et al.* (2009) when working with indigenous rams in Bangladesh, found trends similar to David *et al.* (2007).

Hassan *et al.* (2009) also found that with increasing age, the semen quality improved and eventually stabilized at the age of three years. In young rams, the age, testes size and testes length are positively correlated (Belibasaki and Kouimtzis, 2000; Hassan *et al.* 2009).

In general, sexual development of a ram lamb is more correlated with body conformation than with chronological age (Belibasaki and Kouimtzis, 2000; Hassan *et al.* 2009). Akpa *et al.* (2012) suggested that general growth and development are pre-requisites for the initiation of sexual function.

The prevalence of physical abnormalities, particularly arthritis and abnormalities of the testis, epididymis and penis, increases with age (Seaman, 2004). These abnormalities decrease both the quality and quantity of semen.

The prevalence of disease also tends to increase as age progresses (Seaman, 2004). However, there can be bacterial or viral infections that may occur at any stage in a ram's life if it is exposed to such pathogens. Epididymitis is one such reproductive disease in rams which is highly detrimental to semen quality and quantity. This disease results from an injury which

comes into contact with the *Brucella ovis* bacteria. The inflammation which occurs as an immune response to this infection causes a blockage in the epididymis. This prevents the transport of semen from the epididymis to the testicles when the ram is ready to ejaculate. Subsequently, the quantity of semen is reduced. Also, the quality of semen is reduced due to the fact that the small volume of semen contains mainly old semen.

2.2.2. Season

Gordon (1999) stated that natural selection has provided mammals, particularly sheep, with signaling systems which couple certain forms of environmental variation with the appropriate neuroendocrine responses to ensure that reproductive activity occurs at the most favourable time of the year. According to Moghaddam *et al.* (2012), most sheep display a marked seasonality of breeding activity. Although semen quality may decline in non-breeding months, rams do not become azoospermic. Zamiri *et al.* (2010) highlighted the need for understanding the natural breeding season as important for general flock management and for detecting the reproductive potential of rams.

Kafi *et al.* (2004) observed that the reproductive capacity of a ram appears to be influenced by seasons but this is limited to certain breeds and certain regions. Oláh *et al.* (2013) reported that seasonal factors such as temperature, humidity and sunny hours influenced reproduction in sheep. Photoperiod (which is the amount of daylight) has been stated as being the key environmental signal timing the reproductive cycle (Karagiannidis *et al.* 2000). Moghaddam *et al.* (2012) found that photoperiod affected semen quality by marked differences in volume, colour and scrotal circumference.

Kafi *et al.* (2004), Kunene *et al.* (2009) and Zamiri *et al.* (2010) reported a correlation between season and scrotal size. All these studies (Kafi *et al.* 2004; Kunene *et al.* 2009 and Zamiri *et al.* 2010) are in agreement with the fact that the scrotal circumference is larger in summer and autumn than it is in winter and spring. This is most likely attributable to the availability of higher quality grazing during the months of summer and autumn as well as the influence of photoperiod on the hormonal balance in the ram during summer and autumn. The diameter of the seminiferous tubule increases during this period. However interstitial tissue does not increase or decrease significantly across all seasons (Dorostghoal, 2008). An increase in seminiferous tubule diameter is positively correlated with an increase in semen quantity (Kheradmand *et al.* 2006). The quality of semen has not been correlated with the quantity of

semen in other research (Karagiannidis *et al.* 2000; Zamiri *et al.* 2010). However temperature itself does affect the quality of sperm directly. In higher ambient temperatures rams with higher body temperatures have increased metabolic activity in testicular cells, leading to testicular hypoxia which may play a role in heat-induced spermatogenic disturbance (Mohamed *et al.* 2010).

Al-Samarrae (2009) found that the volume of the ejaculate, sperm motility and semen concentration of Iraqi indigenous sheep (Karradi and Arabbi breeds) appeared to be higher in autumn than in other seasons. However, the fact that these Iraqi sheep did not present the same pattern in summer was probably due to differences in genetic make-up. Several authors (Karagiannidis *et al.* 2000; Zamiri *et al.* 2010; Moghaddam *et al.* 2012) have concluded that these differences in semen quality could be as a result of photoperiod, since late summer and autumn are days of decreasing day-length while late winter and spring are days of increasing day-length.

The proportion of normal sperm may have a strong genetic component (Smital *et al.* 2005), while sperm swimming velocity seems to be influenced by the animal's environment and social factors (Roldan and Gomendio, 2008). Oláh *et al.* (2013) found that the highest proportion of normal sperm was found in winter. The possible increase in semen production in autumn may be due to the seasonal fluctuation in seminal plasma protein components.

According to Santolaria *et al.* (2011), in sub-tropical and tropical climates, local sheep show restricted reproductive activity in summer months as a result of high ambient temperatures. This occurs because of a decrease in mating behaviour and redirection of energy to other physiological processes to maintain homeostatic body temperature. The heat stress is further aggravated when the high ambient temperature is coupled with a high humidity. As a result, this heat stress evokes a series of drastic changes in biological functions which include a decrease in feed intake efficiency and use, disturbances in the metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites (Marai *et al.* 2007). The effect of cold stress is mostly pronounced when coupled with a high wind-chill factor. Internal effects include piloerection and vasoconstriction of blood vessels for prolonged periods of time. Other more visible effects include frostbite and excessive shivering to increase endogenous heat production (Cannon and Nedergaard, 2010). The detrimental effects of cold stress can be overcome by a high caloric intake.

2.2.3. Management

The type of management indicates the level of care administered by the livestock owner. Management covers all aspects from nutrition to veterinary care and the provision of housing especially during inclement weather (Santolaria *et al.* 2011). The type of management used by subsistence farmers in particular includes minimal inputs due to the limited resources available (Mavule, 2013) and is referred to as extensive management. Zulu sheep are a hardy breed and therefore do not require intense forms of management. However, there is no documented evidence on how extensive management has an impact on semen characteristics in Zulu sheep populations.

The nutritional status of a flock is a factor that a farmer has the most control over (Giminez and Rodning, 2002). Rams which are genetically low performers, which are fed on a high plane of nutrition, outperform superior rams that are on a lower nutritional plane (Kheradmand *et al.* 2006). One of the manifestations of a particular diet in animals is the effect it has on the scrotal circumference (SC) which, according to Dorostghoal *et al.* (2009) influences the quantity and quality of semen produced. Kheradmand *et al.* (2006) found that the SC diameter was lower in unimproved diets and this low diameter reduced the quantity of semen produced. Kunene *et al.* (2007) also found that in summer and autumn, Zulu rams generally had a larger SC than rams in winter and spring. The authors attributed this finding to the seasonal fluctuation in fodder quality and quantity and the effect it had on the amount of fatty tissue deposited in the scrotal sac.

Bester *et al.* (2004) found that the quantity of fat deposited in the scrotum was directly proportional to the concentration of energy in the diet. An increase in scrotal circumference invariably increases a ram's fertility (Akpa *et al.* 2012). However, over fattening of rams also reduces fertility (Bester *et al.* 2004).

Animals that are in good health generally perform better and are more resistant to disease and infection or may be able to fight off infection faster than those with compromised immune systems. Mavule (2013), most of the rural farmers do not follow routine systems for parasite control and animals are usually treated for such infestations when symptoms are displayed.

Healthy animals are more likely to possess a higher quality of semen as nutrients are not diverted from reproductive processes to fight infections or disease. However physical impairments may jeopardise both quality and quantity. Thus animals which are also fed a well-

balanced diet are equipped with the necessary biological components to strengthen immune function (Santolaria *et al.* 2011). The most reliable method for assessing each individual's health status is to examine the basic white blood cell (WBC) count (Egbe-Nwiyi *et al.* 2000). In general, younger individuals in tropical zones tend to have a higher WBC. This, according to the authors, is due to the fact that tropical environments tend to be havens for parasites and diseases; therefore this high WBC is more of an environmental disposition which becomes an inherent part of the animals' immunity. High WBC is also attained through colostrum consumption in the first few months of life or, in older rams, through physiological stimuli such as excitement or stress during handling (Egbe-Nwiyi *et al.* 2000).

The effect of white blood cell (WBC) count on reproduction has not been intensively studied in sheep reared under natural conditions. Therefore this study was to determine if there are any significant effects to the reproductive potential of Zulu rams. An increase above the normal accepted ranges for WBC counts indicates an immune response to fighting off disease or an infection. This could also mean that during this process, reproductive potential is diminished.

Swellings in joints, foot rot or foot abscesses caused by a number of management related factors (e.g. muddy rest areas) are easy to overlook when assessing the quality of the ram's reproductive capacity. Severe pain may affect a ram's ability to effectively mount and this in turn may affect the semen quality. This reduction in semen quality is brought upon due to a reduced libido. Animals in pain automatically have a suppressed libido. Libido is controlled by testosterone by a feedback system and therefore if testosterone levels diminish, libido decreases. However, it is the decrease in testosterone itself that affects semen production and quality rather than libido.

The effect of nutrition also ties in with the effects of season on scrotal circumference (SC). Kheramand *et al.* (2006) found that the SC value is lower in unimproved diets, which in this case would refer to lower quality grazing forage. However, seasonal effect can be countered by the fact that well-fed rams in spring may have a larger SC than poorly-fed rams in autumn (Kheramand *et al.* 2006). This may be because nutrition mediates its effects through increasing the frequency of pulses of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Dana *et al.* (2000) also found that Ethiopian rams which were not provided with high quality nutrition (in this case, a mixture containing *Leucaena leucocephala*), did not show changes in the overall body weight, but the SC was reduced by about 10%. The authors attributed this loss

of fat from the scrotal tissue possibly to the rams being maintained on a poor quality roughage diet.

The scrotum plays an important role in a male individual as it contains the testes which are the male gonads and production site of spermatozoa. Since optimum sperm formation occurs at 4-6 degrees lower than actual body temperature, the scrotum is important for thermoregulation of the testicles. This mechanism is controlled by the cremaster muscle located in the wall of the scrotum. This muscle is temperature-sensitive and therefore relaxes when ambient temperatures increase and contracts when ambient temperatures decrease. Excessive fat deposits interfere with the sensitivity of the cremaster muscle to detect temperature changes. This interference prevents the scrotum from being moved away or drawn closer to the body in adverse temperatures and may thus result in a decrease in fertility.

The effects of geographic location on reproductive performance of rams are caused mainly by the prevailing climate and availability of grazing pastures and water. These are extrinsic effects as they can be manipulated in order to maximize the genetic (intrinsic) potential of each ram.

Davendra *et al.* (2013) described how extremes in climatic variables alter energy transfer between an animal and its environment which ultimately has negative effects on reproductive performance. Among the variables listed by Davendra *et al.* (2013) as having adverse effects on fertility, access to drinking water is of lesser impact than ambient temperatures, excess humidity and severe cold.

2.2.4. Testosterone level

Gundogan and Serteser (2005) found that using semen characteristics alone does not suffice for a complete semen appraisal. A complete appraisal requires the biochemical evaluation of Zulu ram blood in order to determine a possible relationship between testosterone level and semen quality. Gundogan and Serteser (2005) further stated that with better knowledge of ram reproductive physiology, a more accurate andrological evaluation could be conducted. This would improve reproductive efficiency and enhance breeding schemes and the rate of genetic gain.

Hafez and Hafez (2000) observed that the control of mammalian reproduction is regulated by the central nervous system (CNS) and the endocrine system. The hypothalamus links the two

systems through the hypothalamo-hypophyseal portal system to co-ordinate the functions of the gonads. The gonads play a dual role of production of the germ cells (gametogenesis) and secretion of the gonadal hormones i.e. luteinizing hormone (LH) and follicle stimulating hormone (FSH). These hormones influence the production and secretion of testosterone.

Preston *et al.* (2012) found that testosterone is ubiquitous among male vertebrates and plays a central role in the expression of numerous sexually selected traits, for example, the seasonal strengthening of weaponry such as horns, and seasonal changes in musculature. Although testosterone is a key mediator in the expression of numerous morphological and behavioural traits in mammals, the factors underlying individual variation in circulating testosterone levels are poorly understood.

The duration of the male breeding season is longer than the female and although males can mate all year round, testosterone and gonadotropin levels are minimal during the non-breeding season. Preston *et al.* (2012) tracking testosterone production in Soay sheep in the Scottish Archipelago, also indicate that testosterone production is affected by photoperiod. Photoperiod affects the secretion of melatonin which can either inhibit long-day breeders or stimulate short-day breeders (Ungerfeld and Bielli, 2008). According to Kishk, (2008), testosterone secretion is correlated to external stimulants such as behaviour of ewes, odour of ewes and ewe oestrus manifestation. This means that sexual behaviour and pheromone production are also dependent on the action of androgens such as testosterone. Gordon (1999) found that sheep sexual activity depends on gonadotrophic function. The author found further evidence supporting previous research which noted differences in LH concentrations according to the time of year.

Most seasonal breeding mammals that have been studied have been shown to possess a self-sustaining endogenous rhythm of seasonal reproductive activity which is either synchronized or entrained by photoperiod (Ungerfeld and Bielli, 2008). While the ram does not exhibit a restricted mating season, sexual activity tends to be highest in autumn and thereafter declines in late winter, spring and summer.

2.3. Conclusion

The vast majority of the human population rely on animal products for food and to lose an entire breed either through genetic dilution, over-diversity or total extinction represents a global ecological loss. Indigenous animals hold an enormous amount of clues and information about the cultural and continental history about a region. This information is vital for scientific

advancement as it provides the basis for understanding the mechanisms for survival and proliferation of various animal species. Global research has been conducted on the reproductive physiology of indigenous sheep species however none of such research has been conducted with Zulu rams. If environmental factors (such as season and management) can be maximised Zulu ram reproductive capacity can be improved upon. Such improvement relies heavily on the understanding of how the various environmental, physiological and anatomical factors exert an effect on reproduction in Zulu rams.

CHAPTER THREE

Methods and Materials

3.1. Introduction

Semen and blood samples from Zulu rams were obtained from locations where the Zulu sheep were predominantly found. Locations and co-ordinates of the eight experimental sites are as presented in Table 3.2. The pedigree of rams that were sampled was established through interviews with the farmers. All sampled rams were raised under varying degrees of extensive flock management.

3.3. Physical examination (PE), scrotal circumference (SC) measurement and Body Condition Score (BCS)

The physical examination was conducted to determine if there was any visible impairment to the ram's body such as lesions, lumps, tumors or abrasions. Impairment has the potential to compromise the reproductive capacity of the ram. Rams so identified were not eliminated from the study but used as a reference point for other unwell rams. The scrotal circumference (SC) was measured at the widest part of the scrotum (Appendix C, Figure 8.1.) using a flexible measuring tape as done by Fourie *et al.* (2002). The body condition score (BCS) conducted was

based on a score of 1 to 5 with 1 being emaciated condition and 5 obese (Appendix C, Figure 8.2.). Body temperature was measured (using a rectal thermometer) to ensure that the rams did not suffer from fever or display any immune response to infection.

3.2. Location of samples

Table 3.1: The geographic locations of Zulu rams used for semen collection

Location	Co-ordinates	Grazing pastures	N	Mean annual rain (mm)	Mean summer temp 2013/14 (°C)	Mean autumn temp 2014 (°C)	Mean winter temp 2014 (°C)	Mean spring temp 2014 (°C)
Makhathini Research Station	27°38'S; 32°10'E	<i>Pennisetum clandestinum</i> (kikuyu)	22	≈520	29.4	28.1	22.3	26
Jozini	27°25'S; 32°04'E	Mixed veld	30	498	30.3	28.7	24.3	26
Kwa-Mthetwa	28°37'S; 31°55'E	<i>Cynodon nlemfuensis</i>	24	890	28	26.6	24	25.1
UNIZULU	28°51'S; 31°51'E	Sporobolus sp.; kikuyu	16	948	27.7	27.6	23.4	24.3
Stanger	29°20'S; 31°17'E	Lucerne	16	866	26.7	27	25.7	26.4
Mooi River	29°12'S; 29°59'E	Kikuyu	21	648	25.1	24.9	20.7	23.2
Estcourt	28°44'S; 30°27'E	Mixed veld	20	589	28.2	27.6	24	26
Tugela Ferry	28°44'S; 30°27'E	Mixed veld	29	≈300	31	29.3	22	24.1

3.4. Blood samples

Blood samples from each ram were collected from the jugular vein in EDTA-containing tubes and thrombin-containing tubes for white blood cell count and testosterone levels respectively. The blood samples were sent to a Lancet Laboratory® for the analysis of blood testosterone and leukocyte levels. These blood samples were acquired each time a semen sample was taken from each ram. Due to the influence of photoperiod on the testosterone levels, blood samples were only taken after each animal was exposed to at least 2 hours of daylight to allow a uniform and accurate measurement. The length of daylight hours was calculated from the official sunrise and sunset hours provided by the South African Weather Service to ensure accuracy and consistency of results.

3.5. Semen samples

Semen from each ram was collected at the beginning, middle and end of each season (spring, summer, autumn, and winter) to find the averages for each season. This was done in the mornings between 06:00 and 08:00 as described by Yue *et al.* (2009) and Zamiri *et al.* (2010). The same rams were used for the duration of this study to ensure consistency of the results. Semen was collected in a graduated measuring tube using electro-ejaculation (3-15 volts for 3 seconds, 7 seconds interval). All semen samples were analysed on-site.

3.6. Gross examination of semen

A gross examination was initially conducted based on volume and appearance of any contaminants (such as blood, urine or dirt) and consistency. Assessment of these parameters was conducted on fresh semen using visual assessment. The volume was obtained directly from the measuring tube (Yue *et al.* 2009; Zamiri *et al.* 2010). Consistency (colour) was determined subjectively on a scale of 0-5 with 0 being clear and 5 being thick/creamy (Hafez and Hafez, 2000). The pH was measured using a pH electrode (Hanna equipment) with a digital scale.

3.7. Quantitative assessment of semen

The next step was the subjective estimation of the progressive motility (10µl semen with an added 2µl of 1% saline solution) of the spermatozoa using a microscope (10 x

magnifications) with a warm stage (37°C). A score was assigned (David *et al.* 2007), with 0% being 'no movement' and 100% being 'rapid movement'. The abnormal sperm percentage and proportion of live and dead sperm was determined by microscopic inspection. The proportion of live and dead spermatozoa and abnormalities was determined using a nigrosin eosin staining technique (5µl semen; 2µl nigrosin eosin) at 400x magnification (Akpa *et al.* 2012; Kafi *et al.* 2004). The concentration of the sperm was estimated by diluting 25µl semen into 5.0ml distilled water then pipetting 15µl of this solution into each chamber of a Neubauer Haemocytometer (Karagiannidis *et al.* 2000; Kheramand *et al.* 2006).

3.8. Statistics analysis

An analysis of the quality and quantity of the semen was done using SPSS statistical software. The parameters that were analysed were consistency, pH, volume, semen concentration and sperm motility. The quantity of testosterone in the blood was analysed to define the relationship between the quality of semen and the level of testosterone present. The analysis of variance (ANOVA) test was used to check the significance of the various factors (age and season) affecting semen characteristics (i.e. volume, concentration, pH, etc.). The Pearson Test was carried out to examine the correlation between the seminal and physiological parameters.

3.9. Management review

A questionnaire was handed out to farmers whose rams were involved in this study. The survey (See Appendix A) covers aspects such as the uses of Zulu sheep, nutrition, breeding practices, and veterinary care. The results of this survey were used to determine the effects of the varying degrees of extensive management. The results emanating therefrom were used to assess if there were some factors within the existing type of management that had an effect on the quality and quantity of Zulu sheep semen.

CHAPTER FOUR

Management Systems

4.1 Results

In total, there were 12 farmers and two farm managers (UNIZULU and Makhathini Research Station) that participated in this research. Table 4.1 shows the results of the farmers' survey and these results revealed that the participants used varying degrees of extensive management (Table 4.1).

All locations showed an approximately 1:10 ratio of rams to ewes except for UNIZULU and Stanger which showed an approximately 1:2 and 1:3 ram to ewe ratio respectively. Controlled breeding was not practised in any of the areas chosen for sampling.

Veterinary care was employed at UNIZULU, where a veterinarian was consulted. Routine external and internal parasite control was practised at MRS, UNIZULU and Estcourt. Only one of the two respondents in Jozini routinely controlled external parasites, while both farmers in Stanger practised routine external parasite control. The farmers at Stanger practised parasite control via pharmaceutical suppliers. Farmers in KwaMthethwa, Mooi River and Tugela Ferry only provided external and internal parasite control when it was required. None of the rams was ill during the course of this study.

In all surveyed locations the sheep were grazed on natural pastures. Only UNIZULU provided hay from mid-winter to spring. Supplements in the form of licks were provided only by farmers in Stanger, Mooi River and Estcourt. Water was provided *ad libitum* in all locations except Jozini and Tugela Ferry. None of the animals used for research was reported sick in the last 6 to 12 month period.

Table 4.1: The farmers survey on management practices

	MRS	Jozini	KwaMthethwa	UNIZULU	Stanger	Mooi River	Estcourt	Tugela Ferry
Total No. of farmers	1	2	2	1	2	2	2	2
No. of rams	22	30	24	16	16	21	20	29
No. of ewes	64	255	76	30	49	300	250	130
External Parasite Control (%)								
Routinely	14	14	0	14	28	0	28	0
Only when needed	0	14	28	0	0	28	0	28
Internal Parasite Control (%)								
Routinely	14	14	0	14	14	0	28	0
Only when needed	0	14	28	0	14	28	0	28
Breeding (%)								
Uncontrolled	14	28	28	14	28	28	28	28
Controlled	0	0	0	0	0	0	0	0
Nutrition								
Forage	Natural	Natural	Natural	Natural/Hay	Natural	Natural	Natural	Natural
Supplements	None	None	None	None	Licks	Licks	Licks	None
Water	Ad libitum	Limited	Ad libitum	Ad libitum	Ad libitum	Ad libitum	Ad libitum	Limited

The majority of the farmers (50%) kept Zulu sheep mainly for sale (Figure 4.1) and while 36% kept the sheep mainly for meat. The remaining 14% of farmers kept Zulu sheep for research purposes. Farmers in Stanger kept sheep as a hobby while the Makhathini Research Station (MRS) and UNIZULU kept the Zulu sheep mainly for research and conservation purposes. Farmers in Mooi River and Estcourt were commercial; farmers in Jozini, KwaMthethwa and Tugela Ferry were subsistence.

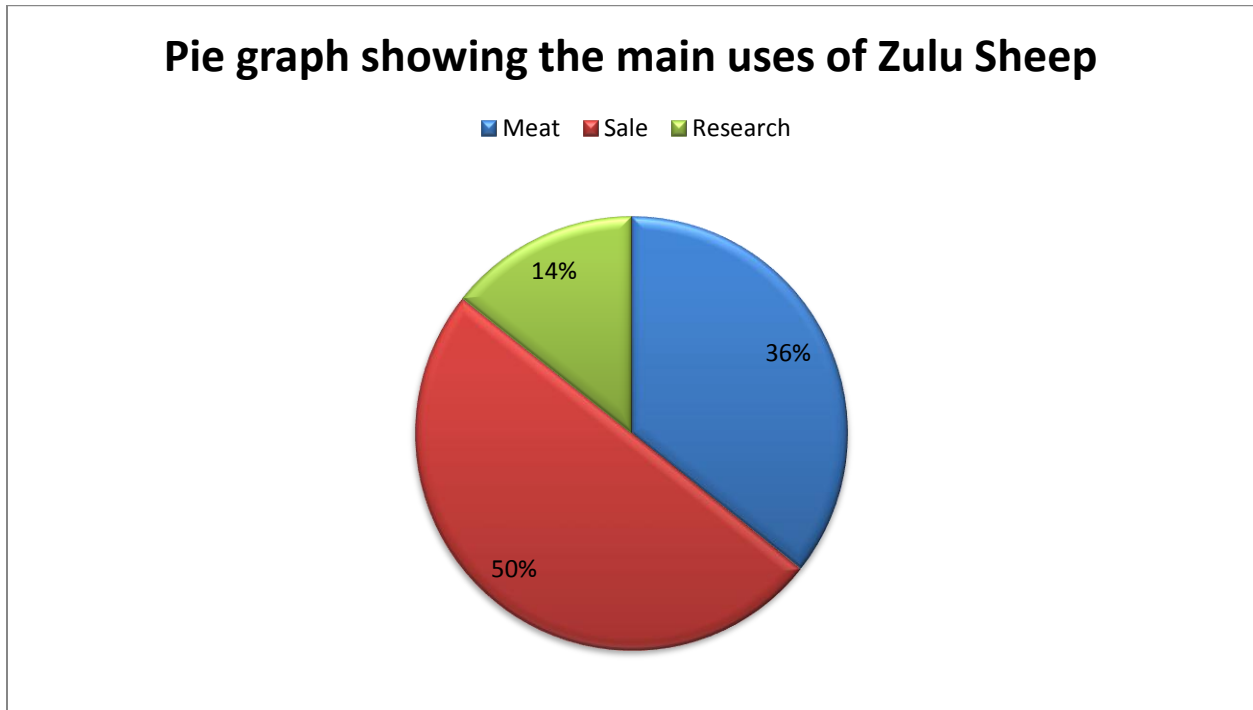


Figure 4.1.: The main uses of Zulu sheep at the surveyed locations

4.2. Discussion

Santolaria *et al.* (2011) suggested that although fertility in rams is a combination of genetic and environmental influences, ejaculate differences are caused by management. It therefore makes sense to study the types of management for Zulu rams that were used for semen evaluation.

While Zulu sheep have become an established part of the KZN ecosystem through years of adaptation (Mavule, 2013), their numbers are not constant. This study has revealed that subsistence farmers integrated Zulu sheep farming into their livelihood as these sheep can survive on crop residues and can utilise land that cannot be ploughed. This was evident in locations such as KwaMthethwa, Jozini and Tugela Ferry. Ahmed and Egwu (2014) also reported that sheep provide practical means of using vast areas of natural grasslands that cannot be

used for planting crops and thus sheep can be successfully incorporated into a subsistence lifestyle.

Out of the total of 12 farmers, there was a grand total of 140 Zulu rams. This is an exceptionally large population of Zulu rams in KZN and the potential to conserve the breed via the development of a sustainable breed improvement programme appears more feasible. The rams at UNIZULU and MRS were used as control groups as these are both recognized Zulu sheep research centres. These two centres also demonstrated an extensive management system.

Mavule (2013) reported that the degree of management and flock productivity for small ruminants such as sheep depend on the flock composition, which is the ratio of rams to ewes. As shown in Table 4.1., there exists an approximately 1:10 ratio of rams to ewes in Estcourt, Jozini, Mooi River and Tugela Ferry while UNIZULU and Stanger show a 1:2 and 1:3 ram to ewe ratio respectively. The ratio of ewes to rams observed in all these flocks is small compared to the suggestions of Geenty (2013). The author recommended a ram to ewe ratio of 1:350. The need for such a high ewe to ram ratio is to ensure that the maximum amount of genetic gain is transferred from the ram to the progeny (Geenty, 2013). While it may seem sufficient to have fewer ewes being served per ram, this decreases the number of services per ewe over time. The author noted that should ratios reach 1:210, the proportion of services to the ewes in a cycle does not change despite the number of ewes decreasing. However, these small ratios are expected as Zulu sheep are mainly kept by small-scale farmers who have been reported to have an average of between 10 and 30 sheep with only approximately 7.4% of all subsistence Zulu sheep farmers owning more than 50 head of sheep (Kunene and Fossey, 2006; Mavule *et al.* 2013). The knowledge that the breed is declining in numbers may also be a factor in the number of Zulu sheep owned by subsistence farmers.

All the farmers that participated in this study practised extensive management, although there were variations in the degree of extensive management administered.

Fourie *et al.* (2004) showed that factors such as nutritional management and genetics affect the reproductive potential of sheep. The same could apply for the Zulu rams. The rams in this study were grazed predominantly on natural pastures. Bester *et al.* (2004) noted that there have been very few studies conducted to estimate the effect of nutrition on reproductive performance in rams. The authors suggested that it is necessary to evaluate the various dietary concentration effects on ram fertility before evaluating the routine feeding practices used by South African farmers. Therefore the effects of nutritional management of Zulu sheep cannot be accurately ascertained, as the effects of different dietary energy concentrations on Zulu ram fertility have

not been determined. The effect of feeding regimen on Zulu ram semen quality has to be further studied.

The extended drought in KZN during 2014 resulted in farmers supplementing the sheep feeding regimen with mineral licks (Stanger, Mooi River and Estcourt). Other farmers simply could not afford the costs of supplementing nutrition, particularly in Jozini and Tugela Ferry. Robinson *et al.* (2006) supported the fact that nutrition influences ruminant fertility directly by the supply of specific nutrients for spermatogenesis. The authors further asserted that nutrition indirectly influences fertility through its impact on the circulating concentrations of hormones and other nutrient-sensitive metabolites that are required for sustaining an optimum level of fertility.

The provision of water is important to aid in digestion by absorption of nutrients, excretion of wastes, and to maintain homeostasis in the animals. It is therefore likely that at Jozini and Tugela Ferry, since water is limited, could have a negative effect on the fertility of the Zulu rams by reducing the quantity and quality of semen produced. However, this research did not focus on evaluating the impact of water on semen characteristics.

At UNIZULU, Estcourt, Stanger and MRS, shelter was provided for the sheep during the night and during inclement weather such as storms or heat waves. Davendra and Kalyan (2013) noted that harsh weather conditions can be deleterious to reproduction as this is the first physiological phenomenon to be abandoned in order to conserve or dissipate energy for survival.

The veterinary care provided by the farmers was consistent with accepted flock management practices. However farmers at KwaMthetwa, Mooi River and Tugela Ferry did not routinely follow parasite control. Reasons given for these measures in these particular locations were: that farmers did not understand the need for regular control; in order to keep animals true to type (sustaining Zulu sheep hardiness and parasite infection resistance); and lack of affordability. Veterinary care is essential in locations that have a higher incidence of common sheep diseases such as bluetongue and heartwater. Farmers in all areas except for Jozini and Tugela Ferry vaccinated against these diseases, including tetanus. Alawa *et al.* (2002) highlighted that a factor which limits ruminant productivity is poor animal health. The author further reported that this limitation is more prevalent in developing countries due to a decline in funding of veterinary services.

Disease has the potential to reduce the fertility and reproductive processes of rams, as body temperature increases as an immune response to fight off infection. A prolonged elevated body temperature results in degeneration of germinal epithelial tissue in the testes and partial cellular

atrophy of the seminiferous tubules (Al-Ghetaa, 2012). These events are irreversible and thus affected rams will never reach their maximum level of reproductive potential and fertility. Rams were not ill during the course of this study, except that they did display unusually shabby coats and high levels of tick infestation in all locations.

One of the findings of this survey was the revelation of the uncontrolled breeding occurring at these different locations. Getachew *et al.* (2010) also found that uncontrolled breeding is prevalent in extensive farming systems, particularly in subsistence farming, and it is a major challenge in terms of effecting breeding programmes. Uncontrolled breeding actually increases the incidence of inbreeding and this phenomenon has the tendency to cause an accumulation of lethal recessive alleles. It was recommended by Getachew *et al.* (2010) that mixing of flocks (Zulu sheep in this case) in a commune should be encouraged to allow for an increase in intensity selection and a decrease in inbreeding. This should result in a more diverse Zulu sheep gene pool without the genetic makeup of these sheep being diluted by the genetics of other sheep breeds, thus promoting Zulu sheep survival.

4.3. Conclusion

This research shows that there were limited resources for the care of sheep and all the farmers that participated in this research adopted an extensive system of management which requires minimum inputs. A lack of supplements and access to water in some areas still exist. The climatic phenomenon known as El Niño during the latter part of the year (2014) occurred in KZN and most areas suffered remarkably from the extended drought. There is no evidence that some rural farmers have mitigations on the effects of climate change.

CHAPTER FIVE

Results

Effects of age, season and location on body weight, scrotal circumference spermiogramic and physiological parameters

5.1. The effects of age and season

Tables 5.1 to 5.3 show the characteristics of Zulu ram semen across all seasons and the effect of age on the semen parameters.

5.1.1. Body weight

The body weight of the rams increased continually from 1 to 4 year old rams (40.54 ± 1.16 kg to 50.16 ± 1.65 kg respectively), but the weights of the 1 and 2 year old rams were statistically similar. The body weight was highest in summer (47.17 ± 1.33 kg) but there was no significant difference ($P > 0.05$) between the weight of Zulu rams in autumn, spring and winter.

5.1.2. Scrotal circumference (SC)

The scrotal circumference was similar ($P > 0.05$) for the 1 and 2 year old rams, and for the 3 and 4 year old rams, with the SC of the 3 and 4 year olds being larger than the SC of the younger rams (Table 5.1.). The largest SC seasonally was observed during summer at 29.61 ± 0.51 cm; there was no significant difference of SC in autumn spring and winter. Scrotal circumference varied more significantly ($P < 0.05$) with age than with season.

5.1.3. Semen volume

The highest semen volume was for the 3 year old rams (1.14 ± 0.07 ml). The volume was similar for the 1, 2 and 4 year old rams and was not significantly correlated to SC ($P > 0.05$, Table 5.3). The volume was highest during autumn and winter (1.12 ± 0.09 ml and 1.21 ± 0.10 ml, respectively), and lowest during summer (0.67 ± 0.59 ml). The semen volume was significantly similar in autumn and in winter (Table 5.2)

5.1.4. Semen pH

Semen pH did not vary significantly ($P>0.05$) at different ages and during the different seasons. The pH values ranged from 6.23 ± 0.20 to 6.74 ± 0.19 for rams of the different age groups while the pH values ranged from 6.26 ± 0.17 to 6.75 ± 0.15 between seasons. The Pearson correlation test revealed that there was no significant correlation between age and semen pH level but the pH was positively although poorly correlated to the SC ($r = 0.182$; $P<0.05$).

5.1.5. Sperm motility

The highest amount of mass motility was realized in 3 year old rams ($84.02\pm 2.58\%$). Mass motility ($P<0.05$) amounts for the 1 year old, 2 year old and the 4 year old rams were similar to each other. The values for the mass motility increased up to the 3 years old but decreased for the 4 year old rams. Table 5.3 shows that the mass motility was also significantly and positively correlated to semen volume ($r=0.298$; $P<0.01$). Table 8.1 of Appendix C tabulates the different degrees of movement of spermatozoa.

Progressive motility was highest for the semen of the 3 year old rams ($82.84 \pm 2.58\%$) and was lower and similar ($P>0.05$) for the 1, 2 and 4 year old rams. Progressive motility was significantly and positively correlated to volume ($r=0.290$; $P<0.01$).

Mass and progressive motility were not significantly ($P>0.05$) correlated to SC (Table 5.3). Progressive motility was highly and positively correlated to live sperm percentage as well as mass motility ($r=0.798$ and $r=0.788$ respectively, $P<0.01$). This indicates that as the live percentage increases, so does progressive or mass motility. Mass motility is a gross measurement of progressive motility.

5.1.6. Sperm concentration

Table 5.1 shows that the highest spermatozoa concentration was for the 3 year old rams ($4.04\pm 0.22 \times 10^9$ /ml) while the sperm concentrations for the 1, 2 and 4 year old rams were similar to each other ($P>0.05$). There was a significant ($P<0.01$) and positive correlation between ram age and sperm concentration ($r=0.208$). The spermatozoa concentration was also significantly and positively correlated to volume and sperm motility (Table 5.3). The lowest spermatozoa concentration was in summer ($1.70\pm 0.14 \times 10^9$ /ml) while it was similar ($P>0.05$) for the other three seasons.

5.1.7. Live sperm percentage

The number of viable sperm increased from 2 year old to 3 year old rams (Table 5.1.) with the percentage of live sperm for the 3 year old rams at $81.47\pm 2.72\%$. The live sperm percentage

was similar ($P>0.05$) for the 1, 2 and 4 year old rams. The number of live sperm was positively correlated to age ($r=0.206$; $P<0.01$). Live sperm percentage was also highly and positively correlated to sperm concentration ($r=0.626$; $P<0.01$). Summer had the lowest percentage of live sperm ($48.72\pm 4.08\%$), while the sperm viability was not significantly different ($P>0.05$) between autumn, winter and spring.

5.1.8. Abnormal sperm percentage

The age of ram or season did not have a significant ($P>0.05$) influence on the percentage of abnormal sperm present in the ejaculate sample. The averages for abnormalities were below 10% for all of the sampled rams.

5.1.9. Testosterone level

The highest testosterone levels ($17.10\pm 1.89\text{ng/ml}$) were found in the 3 year old rams (Table 5.5). There were no significant differences ($P<0.05$) in the testosterone levels between 1, 2 and 4 year old rams. Winter displayed the highest levels of testosterone with an average of $16.28\pm 1.94\text{ng/ml}$. Testosterone levels in the summer, autumn and spring were not significantly different ($P>0.05$).

5.1.10. White blood cell (WBC) count

The white blood cell count was highest in 3 year old rams ($17.07\pm 1.41 \times 10^9/\text{l}$), but was similar ($P>0.005$) for the 1, 2 and 4 year old rams. The Zulu rams had the highest levels of circulating WBC ($15.85\pm 0.97 \times 10^9/\text{l}$) in winter. The WBC counts were relatively lower and similar ($P>0.05$) in autumn, spring and summer.

Table 5.1: The least mean squares for Zulu rams from different age groups

PARAMETERS	AGE OF RAM				Significance
	1	2	3	4	
Body weight	40.54 ^a ± 1.16	42.42 ^{ab} ± 1.27	46.10 ^b ± 1.56	50.16 ^c ± 1.65	P <0.05
Scrotal circ.	26.35 ^a ± 1.16	27.76 ^a ± 0.41	28.90 ^b ± 0.52	30.34 ^b ± 0.57	P <0.05
Semen volume	0.83 ^a ± 0.07	0.94 ^a ± 0.06	1.14 ^b ± 0.07	0.98 ^a ± 0.10	P <0.05
Semen pH	6.23 ± 0.20	6.69 ± 0.11	6.37 ± 0.10	6.74 ± 0.19	NS
Mass motility	61.93 ^a ± 4.14	63.13 ^a ± 3.28	84.02 ^b ± 2.58	65.52 ^a ± 4.98	P <0.05
Prog. motility	59.47 ^a ± 4.28	60.80 ^a ± 3.23	82.84 ^b ± 2.58	62.59 ^a ± 5.14	P <0.05
Semen conc.	2.34 ^a ± 0.20	2.34 ^a ± 0.16	4.04 ^b ± 0.21	2.47 ^a ± 0.34	P <0.05
Live sperm (%)	53.89 ^a ± 4.18	54.48 ^a ± 3.66	81.47 ^b ± 2.72	60.00 ^a ± 6.11	P <0.05
Abn. sperm (%)	6.14 ± 0.75	5.49 ± 0.50	3.92 ± 0.43	5.86 ± 0.66	NS

abc: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different.
 Scrotal circ: scrotal circumference, Prog motility: progressive motility, semen conc: semen concentration, Abn sperm: abnormal sperm

Table 5.2: The least mean squares for spermogrammic parameters during different seasons

PARAMETERS	SEASONS				Significance
	SUMMER	AUTUMN	WINTER	SPRING	
Live Mass	47.17 ^b ±1.33	41.87 ^a ±1.38	42.95 ^a ±1.63	43.38 ^a ±1.38	P <0.05
Scrotal Circ.	29.61 ^b ± 0.51	27.60 ^a ±0.44	26.79 ^a ±0.47	28.08 ^a ± 0.50	P <0.05
Semen Volume	0.67 ^a ± 0.59	1.12 ^b ± 0.09	1.21 ^b ± 0.07	1.08 ^b ± 0.06	P <0.05
Semen pH	6.75 ± 0.15	6.26 ± 0.17	6.69 ± 0.12	6.25 ± 0.16	NS
Mass Motility	65.93 ± 3.23	67.31 ± 3.40	69.46 ±4.01	70.00 ± 4.84	NS
Prog. Motility	63.52 ± 3.13	65.38 ± 3.58	67.32 ± 4.13	67.80 ± 4.91	NS
Semen Conc.	1.70 ^a ± 0.14	2.95 ^b ± 0.24	3.32 ^b ± 0.23	3.12 ^b ± 0.22	P <0.05
Live Sperm (%)	48.72 ^a ± 4.08	64.17 ^b ± 4.27	65.00 ^b ± 4.07	68.90 ^b ±4.38	P <0.05
Abn. Sperm (%)	6.48 ± 0.62	5.33 ± 0.55	4.82 ± 0.62	4.70 ± 0.63	NS

abc: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different

Table 5.3: The Pearson correlation co-efficient (r) matrix for spermiogramic and physiological parameters

	Volume	pH	MM	PM	Abnormal %	Live%	Conc.	Age
pH	0.149							
MM	0.296**	0.132						
PM	0.290**	0.101	0.970**					
Abnormal%	0.093	0.122	0.144*	0.145				
Live%	0.312**	0.011	0.788**	0.798**	0.131			
Conc.	0.501**	0.040	0.531**	0.557**	-0.036	0.626**		
Ram age	0.149*	0.100	0.170*	0.170*	-0.094	0.206**	0.208**	
SC	0.006	0.182*	-0.015	-0.017	0.122	-0.052	-0.080	0.358**
Consistency	0.360**	0.189**	0.706**	0.715**	0.127	0.708**	0.591**	0.120
Body weight	0.114	0.193*	0.028	0.020	0.057	0.006	-0.017	0.298

*P<0.05 **P>0.01. MM: mass motility, PM: progressive motility, Conc: concentration SC: scrotal circumference.

Table 5.4: The Pearson correlation co-efficient for anatomical parameters

	SC	Body weight	BCS
Body weight	0.700**		
BCS	0.289**	0.327**	

*P<0.05 **P>0.01. BCS: Body condition score.

Table 5.5: The average testosterone level (ng/ml) and WBC counts (x10⁹) for age groups of Zulu rams

PARAMETERS	AGES				Probability
	1	2	3	4	
Testosterone	12.83 ^a ±1.18	11.43 ^a ±1.86	17.10 ^b ±1.89	9.55 ^a ± 1.68	P <0.05
WBC Count	13.57 ^a ±0.76	14.08 ^a ±0.98	17.07 ^b ±1.41	13.77 ^a ±1.09	P<0.005

WBC: White blood cell

Table 5.6: The averages for testosterone levels (ng/ml) and WBC counts ($\times 10^9$) for Zulu sheep during four seasons

PARAMETERS	SEASONS				Probability
	Summer	Autumn	Winter	Spring	
Testosterone	9.06 ^a ± 1.90	11.61 ^a ± 1.53	16.28 ^b ± 1.94	12.77 ^a ± 1.68	P < 0.05
WBC Count	15.85 ^a ± 0.97	13.20 ^a ± 1.55	17.07 ^b ± 1.55	14.20 ^a ± 1.04	P < 0.005

WBC: White blood cell

5.2. The effect of season in each geographic location

The results in Tables 5.7 to 5.15 show the characteristics of Zulu ram semen across all seasons in the areas of data collection.

5.2.1. Makhathini Research Station (Table 5.8)

The Body weight, scrotal circumference (SC), semen volume, progressive motility (PM), and live sperm percentage were affected by season ($P < 0.05$) at Makhathini Research Station (MRS). However the body condition score (BCS), semen pH, semen concentration, mass motility (MM), and abnormal sperm percentage were not affected by season in this location. Body weight was higher in summer ($46.30 \pm 8.42\text{kg}$) and in spring ($41.00 \pm 7.83\text{kg}$). The SC was smallest in winter ($23.80 \pm 1.64\text{cm}$) but semen volume was greatest in this season ($1.10 \pm 0.55\text{ml}$). Although the effect of season on progressive and mass motility was significant ($P > 0.05$), the values did not differ significantly. This could be attributed to the high standard errors depicting a large variation on the performance of rams per season in this area. A larger variation was realized in spring. The live sperm percentage was the highest in autumn and winter ($90.00 \pm 14.14\%$ and $83.00 \pm 7.58\%$ respectively).

5.2.2. Jozini (Table 5.9)

The traits that were affected by season in this location were Body weight, semen volume, MM, and sperm concentration ($P < 0.05$). Higher Body weights were found in spring and summer ($49.66 \pm 8.11\text{kg}$ and $54.00 \pm 8.01\text{kg}$ respectively) than autumn and winter. Semen volume produced by the Zulu rams was higher in autumn ($1.70 \pm 0.76\text{ml}$) and winter ($1.35 \pm 0.51\text{ml}$) compared to the other seasons. Semen mass motility was higher in autumn ($75.00 \pm 17.11\%$) and spring ($71.25 \pm 34.91\%$). BCS, SC, PM, and abnormal sperm percentage were not affected by season in Jozini.

5.2.3. KwaMthethwa (Table 5.10)

The seasons of the year affected SC, MM, PM, sperm concentration, live sperm percentage, and abnormal sperm percentage in KwaMthethwa. Body weight, semen volume and semen pH were not affected by seasons in this area. The main difference in SC was found in winter (26.33 ± 2.34 cm) which had the lowest SC. Semen mass motility ($71.67 \pm 22.06\%$) was the highest in winter whereas the progressive motility percentage was higher in winter and in autumn ($75.00 \pm 23.02\%$ and $65.00 \pm 33.17\%$ respectively). Live sperm percentage ($73.33 \pm 21.60\%$) was the highest in winter, followed by autumn. The lowest percentage of sperm abnormalities was observed in autumn and spring ($2.86 \pm 3.934\%$).

5.2.4. University of Zululand (Table 5.11)

The values for Body weight and SC of the Zulu rams at the University did not vary according to season ($P > 0.05$). However semen volume, semen pH, MM, PM, sperm concentration, live sperm percentage, and abnormal sperm percentage were affected by the different seasons of the year. The rams performed the best in terms of semen volume during autumn (1.05 ± 0.44 ml) and winter (1.03 ± 0.56 ml). Sperm concentration was found to be the highest during autumn and winter ($3.68 \pm 0.59 \times 10^9$ /ml and $3.55 \pm 1.30 \times 10^9$ /ml respectively). Progressive and mass motility was greatest during spring ($98.75 \pm 2.50\%$ and $96.25 \pm 4.79\%$ respectively). Higher live sperm percentages were realized in autumn and in spring ($91.25 \pm 8.54\%$ and $88.75 \pm 4.79\%$; $P > 0.05$). Summer and autumn were similar ($P > 0.05$) to each other in terms of abnormal sperm percentage and displayed the greatest number of abnormalities ($5.00 \pm 0.00\%$ and $6.25 \pm 4.79\%$ respectively).

5.2.5. Stanger (Table 5.12)

Seasons did not affect Zulu ram Body weight, SC, semen pH, live sperm percentage, and abnormal sperm percentage. However season did affect semen volume, MM, PM, and sperm concentration in Stanger. Spring was the season in which Zulu rams produced the most viable semen in terms of MM, PM and sperm concentration ($88.33 \pm 16.07\%$, $91.67 \pm 16.43\%$ and $71.67 \pm 28.43\%$; $P < 0.05$ respectively). The semen volume was found to be highest in winter (1.23 ± 0.53 ml).

5.2.6. Mooi River (Table 5.13)

Body weight and SC were impacted on by season ($P < 0.05$) and were both greatest in summer (45.86 ± 10.19 kg and 28.57 ± 4.12 cm respectively) and lowest in spring (33.50 ± 8.96 kg and 24.50 ± 3.00 cm respectively). Semen mass motility was highest during summer ($77.86 \pm 10.75\%$)

and winter ($84.29 \pm 18.80\%$). Progressive motility also exhibited the same pattern as mass motility in summer and winter. Autumn and winter were recognized as the seasons in which semen volume was the greatest ($1.33 \pm 0.93\text{ml}$ and $1.13 \pm 0.47\text{ml}$ respectively) while spring and summer displayed the lowest semen volumes. There were no significant variations in the BCS, semen pH and abnormal sperm values between the seasons ($P > 0.05$).

5.2.7. Estcourt (Table 5.14)

There were no significant differences in SC, sperm concentration and abnormal sperm percentage of the Zulu sheep between the seasons ($p > 0.05$). Zulu ram semen was most viable during spring since parameters such as semen volume, MM, PM and live sperm percentage were observed to be the highest in this season ($P < 0.05$). Semen volume was found to be the highest during winter and spring while summer and autumn had the lowest semen volumes. The pH of Zulu ram semen was greatest during summer (7.53 ± 1.24) while pH levels in autumn, winter and spring were not significantly different from each other. Large standard errors were observed for the values of MM, PM and live sperm percentage although the highest MM, PM and live sperm percentage was in spring ($82.00 \pm 32.52\%$; $74.00 \pm 35.95\%$ and $73.00 \pm 38.83\%$ respectively).

5.2.8. Tugela Ferry (Table 5.15)

Zulu rams were not sampled in summer as there were no farmers located in this area for sampling at the time. There were no significant differences in Body weight, SC, semen pH, progressive motility and abnormal sperm percentages between the seasons. Semen volume was found to be the highest during autumn and winter ($1.31 \pm 0.57\text{ml}$ and $1.39 \pm 0.47\text{ml}$ respectively; $P < 0.05$) while spring had the lowest semen volume ($1.01 \pm 0.40\text{ml}$). Mass motility was the greatest in spring ($77.73 \pm 32.59\%$) while the live sperm percentage was the greatest in winter ($87.14 \pm 29.70\%$).

5.3. Spermio graphic and physiological parameter quality of Zulu rams among the different geographic locations

Table 5.7 describes the means and standard errors for each of the spermio graphic parameters as well as scrotal circumference and Body weight of the Zulu sheep populations.

5.3.1. Body weight

The locations with the highest Body weight were Estcourt and Jozini (52.96 ± 1.51 kg and 49.29 ± 1.66 kg respectively; $P < 0.05$). Body weight did not differ significantly between the locations of Makhathini Research Station (MRS), KwaMthetwa, UNIZULU, Stanger, Mooi River and Tugela Ferry.

5.3.2. Scrotal circumference

Scrotal circumference (SC) varied significantly ($P < 0.05$) between each geographic location. The results showed that Zulu rams from Jozini possessed a greater SC (30.09 ± 0.56 cm) compared to the other locations. The SC sizes in the other locations was not significantly different ($P > 0.05$) from each other.

5.3.3. Semen volume

There were no significant differences in semen volume between all the locations.

5.3.4. Semen pH

The pH did not vary significantly between the different geographic locations.

5.3.5. Mass and progressive motility

A difference in mass motility of rams was apparent between UNIZULU ($83.67 \pm 4.82\%$), KwaMthetwa ($58.79 \pm 5.929\%$) and Estcourt ($56.74 \pm 7.31\%$). A similar pattern of results was observed in progressive motility, with significant differences ($P < 0.05$) recorded between UNIZULU and KwaMthetwa and between UNIZULU and Estcourt. Makhathini Research Station, Jozini, KwaMthetwa, Stanger, Mooi River, Estcourt and Tugela Ferry did not display significant differences ($P > 0.05$) in mass and progressive motility.

5.3.6. Sperm concentration

There were no significant differences ($P > 0.05$) in the concentration of spermatozoa in Zulu ram semen between any of the locations.

5.3.7. Live sperm percentage

There were no significant differences in the live sperm percentage ($P < 0.05$) although UNIZULU ($78.33 \pm 4.46\%$) and Tugela Ferry (75.32 ± 5.13) showed a greater live sperm percentage as compared to the rest of the locations. Jozini, KwaMthetwa and Estcourt had Zulu rams which produced the lower percentages of viable spermatozoa ($53.11 \pm 4.81\%$; $51.14 \pm 6.40\%$ and $48.13 \pm 7.53\%$ respectively).

5.3.8. Abnormal sperm percentage

Abnormal sperm percentage did not vary significantly ($P>0.05$) between locations. Moreover, the value of the abnormal sperm was below 10% in all the areas.

Table 5.7: Least mean squares for spermiogramic parameters from different locations

PARAMETERS	LOCATION							
	MRS	Jozini	KwaMthetwa	UNIZULU	Stanger	Mooi River	Estcourt	Tugela Ferry
Body weight	39.95 ^a ± 2.22	49.29 ^c ± 1.66	40.55 ^a ± 1.72	37.67 ^a ± 1.29	41.98 ^{ab} ± 2.78	41.04 ^{ab} ± 2.05	52.96 ^c ± 1.51	41.13 ^{ab} ± 1.04
SC	27.43 ^{ab} ± 0.79	30.09 ^c ± 0.56	28.10 ^b ± 0.55	24.60 ^a ± 0.95	27.00 ^{ab} ± 0.92	27.33 ^{ab} ± 0.67	29.39 ^b ± 0.46	27.03 ^{ab} ± 0.44
Volume	0.73 ± 0.93	0.61 ± 0.89	0.81 ± 0.91	0.89 ± 0.11	0.90 ± 0.88	0.91 ± 0.14	0.80 ± 0.11	1.22 ± 0.91
pH	6.06 ± 0.15	6.93 ± 0.11	6.17 ± 0.29	6.33 ± 0.17	6.77 ± 0.22	6.39 ± 0.16	6.63 ± 0.26	6.30 ± 0.25
MM	69.76 ^{ab} ± 5.80	67.23 ^{ab} ± 4.18	58.79 ^a ± 5.63	83.67 ^b ± 4.81	68.18 ^{ab} ± 5.99	76.67 ^{ab} ± 4.07	56.74 ^a ± 7.31	71.61 ^{ab} ± 4.78
PM	68.33 ^{ab} ± 5.40	63.51 ^{ab} ± 4.28	58.97 ^a ± 5.94	84.00 ^b ± 4.00	66.36 ^{ab} ± 6.10	74.79 ^{ab} ± 3.84	51.30 ^a ± 7.50	69.84 ^{ab} ± 4.93
Sperm Conc.	2.19 ± 0.34	2.50 ± 0.19	2.66 ± 0.37	3.05 ± 0.34	3.12 ± 0.41	2.72 ± 0.33	2.40 ± 0.34	3.60 ± 0.28
Live sperm(%)	64.52 ^{ab} ± 6.09	53.11 ^a ± 4.81	51.14 ^a ± 6.40	78.33 ^b ± 4.46	65.00 ^{ab} ± 6.08	66.04 ^{ab} ± 4.91	48.13 ^a ± 7.53	75.32 ^b ± 5.13
Abnormal (%)	6.67 ^c ± 6.0	5.11 ^b ± 4.81	4.66 ^a ± 6.40	3.67 ^a ± 4.46	5.00 ^b ± 6.08	5.42 ^b ± 4.91	6.30 ^c ± 7.53	5.71 ^c ± 5.13

^{abc} least square means with different superscripts in the same row differ significantly (P<0.05). Semen volume, pH and concentration least mean squares are not significantly different. SC:Scrotal circumference, MM: Mass motility, PM: Progressive motility, Sperm Conc.: Sperm concentration

Table 5.8: The means and standard errors for semen parameters for Makhatini Research Station during the seasons

PARAMETERS	MAKHATINI RESEARCH STATION				
	Summer	Autumn	Winter	Spring	Significance
Body weight (kg)	46.30 ^a ± 8.42	30.00 ^b ± 1.41	30.40 ^b ± 6.73	41.00 ^a ± 7.83	P<0.05
BCS	3.10 ± 0.32	3.00 ± 0.00	3.00 ± 0.00	3.25 ± 0.50	NS
SC (cm)	28.80 ^a ± 3.88	26.50 ^a ± 0.71	23.80 ^b ± 1.64	29.00 ^a ± 2.83	P<0.05
Semen Volume (ml)	0.61 ^a ± 0.34	0.70 ^b ± 0.00	1.10 ^c ± 0.55	0.60 ^a ± 0.41	P<0.05
Semen pH	6.00 ± 0.74	5.35 ± 0.67	6.37 ± 0.79	6.17 ± 0.19	NS
Mass Motility (%)	66.50 ^{ab} ± 23.34	85.00 ^b ± 14.14	91.00 ^b ± 10.25	43.75 ^{ab} ± 31.98	P<0.05
Progressive Motility (%)	66.50 ^{ab} ± 20.42	77.50 ^b ± 24.75	85.00 ^b ± 15.00	47.50 ^{ab} ± 35.00	P<0.05
Sperm Conc. (x10⁹sperm/ml)	1.34 ± 0.68	2.80 ± 3.54	3.48 ± 1.79	2.38 ± 0.77	NS
Live sperm (%)	56.00 ^a ± 29.79	90.00 ^b ± 14.14	83.00 ^b ± 7.58	50.00 ^a ± 29.44	P<0.05
Abnormal (%)	6.00 ± 6.15	7.50 ± 3.54	8.00 ± 8.36	6.25 ± 7.79	NS

abc: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. BCS: Body condition score, SC:Scrotal circumference, Sperm Conc.: Sperm concentration

Table 5.9: The means and standard errors for semen parameters for Jozini during the seasons

PARAMETERS	JOZINI				Significance
	Summer	Autumn	Winter	Spring	
Body weight (kg)	54.00 ^a ± 8.01	46.19 ^b ± 15.57	46.87 ^b ± 12.99	49.66 ^a ± 8.11	P<0.05
BCS	3.17 ± 0.57	3.25 ± 0.38	3.00 ± 0.00	3.00 ± 0.00	NS
SC (cm)	32.42 ± 3.48	29.38 ± 5.32	28.47 ± 3.34	30.25 ± 2.86	NS
Semen Volume (ml)	0.81 ^a ± 0.46	1.70 ^{bc} ± 0.76	1.35 ^b ± 0.51	0.88 ^a ± 0.43	P<0.05
Semen pH	6.93 ± 0.92	6.98 ± 0.63	7.19 ± 0.76	6.57 ± 0.54	NS
Mass Motility (%)	65.83 ^a ± 22.75	75.00 ^b ± 17.11	61.00 ^a ± 33.12	71.25 ^b ± 34.91	P<0.05
Progressive Motility (%)	60.83 ± 19.40	72.50 ± 17.73	56.67 ± 35.28	68.75 ± 35.75	NS
Sperm Conc. (x10⁹sperm/ml)	1.73 ^a ± 0.69	3.33 ^{bc} ± 1.56	2.60 ^b ± 1.36	2.61 ^b ± 1.38	P<0.05
Live sperm (%)	33.00 ^a ± 32.69	68.13 ^b ± 31.38	44.33 ^{ab} ± 24.92	74.17 ^{bc} ± 29.45	P<0.05
Abnormal (%)	6.67 ± 3.89	4.38 ± 3.20	4.33 ± 4.58	5.00 ± 3.69	NS

^{abc}: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. BCS: Body condition score, SC:Scrotal circumference, Sperm Conc.: Sperm concentration

Table 5.10: The means and standard errors for semen parameters for KwaMthetwa during the seasons

PARAMETERS	KWAMTHETWA				Significance
	Summer	Autumn	Winter	Spring	
Body weight (kg)	41.56 ± 6.59	39.14 ± 12.03	41.67 ± 10.97	39.71 ± 9.54	NS
BCS	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.14 ± 0.378	NS
SC (cm)	29.67 ^b ± 2.59	26.71 ^b ± 2.36	26.33 ^a ± 2.34	29.00 ^b ± 3.51	P<0.05
Semen Volume (ml)	0.74 ± 0.548	0.89 ± 0.539	1.02 ± 0.549	0.66 ± 0.310	NS
Semen pH	6.60 ^b ± 1.37	5.64 ^b ± 2.54	6.46 ^b ± 0.72	5.90 ^{ab} ± 0.96	P<0.05
Mass Motility (%)	62.22 ^b ± 25.14	63.57 ^b ± 32.37	71.67 ^c ± 22.06	38.57 ^{ab} ± 36.14	P<0.05
Progressive Motility (%)	60.00 ^b ± 24.62	65.00 ^{bc} ± 33.17	75.00 ^c ± 23.02	37.86 ^a ± 40.09	P<0.05
Sperm Conc. (x10⁹sperm/ml)	1.48 ^a ± 1.12	3.21 ^b ± 2.10	4.05 ^c ± 2.25	2.43 ^{ab} ± 1.96	P<0.05
Live sperm (%)	42.22 ^a ± 32.32	65.71 ^b ± 35.52	73.33 ^c ± 21.60	41.43 ^a ± 39.66	P<0.05
Abnormal (%)	7.22 ^{bc} ± 4.410	2.86 ^a ± 3.934	5.00 ^b ± 4.472	2.86 ^a ± 2.675	P<0.05

abc: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. BCS: Body condition score, SC:Scrotal circumference, Sperm Conc.: Sperm concentration

Table 5.11: The means and standard errors for semen parameters for University of Zululand during the seasons

PARAMETERS	UNIVERSITY OF ZULULAND				Significance
	Summer	Autumn	Winter	Spring	
Body weight (kg)	39.67 ± 6.51	39.75 ± 5.25	38.50 ± 4.43	37.25 ± 5.85	NS
BCS	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	NS
SC (cm)	24.67 ± 2.52	25.00 ± 2.71	23.25 ± 4.50	25.50 ± 5.26	NS
Semen Volume (ml)	0.83 ^a ± 0.29	1.05 ^b ± 0.44	1.03 ^b ± 0.56	0.65 ^a ± 0.40	P<0.05
Semen pH	6.78 ^b ± 0.25	6.13 ^a ± 0.72	6.84 ^b ± 1.31	7.04 ^b ± 0.66	P<0.05
Mass Motility (%)	83.33 ^a ± 20.21	83.75 ^a ± 10.31	68.75 ^b ± 25.29	98.75 ^c ± 2.50	P<0.05
Progressive Motility (%)	85.00 ^a ± 17.32	82.50 ^a ± 5.00	72.50 ^b ± 22.17	96.25 ^c ± 4.79	P<0.05
Sperm Conc. (x10⁹sperm/ml)	2.17 ^a ± 2.27	3.68 ^c ± 0.59	3.55 ^c ± 1.30	2.60 ^b ± 0.68	P<0.05
Live sperm (%)	70.00 ^b ± 17.32	91.25 ^c ± 8.54	61.25 ^a ± 16.52	88.75 ^c ± 4.79	P<0.05
Abnormal (%)	5.00 ^b ± 0.00	6.25 ^b ± 4.79	1.25 ^a ± 2.50	2.50 ^a ± 2.89	P<0.05

^{abc}: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. BCS: Body condition score, SC:Scrotal circumference, Sperm Conc.: Sperm concentration

Table 5.12: The means and standard errors for semen parameters for Stanger during the seasons

PARAMETERS	STANGER				Significance
	Summer	Autumn	Winter	Spring	
Body weight (kg)	41.00 ± 14.61	42.21 ± 12.32	41.29 ± 17.00	44.67 ± 3.51	NS
BCS	3.50 ± 0.54	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	NS
SC (cm)	27.40 ± 4.78	28.00 ± 3.92	24.57 ± 4.72	29.67 ± 1.16	NS
Semen Volume (ml)	0.67 ^a ± 0.28	0.79 ^a ± 0.21	1.23 ^b ± 0.53	0.80 ^a ± 0.27	P<0.05
Semen pH	6.99 ± 1.22	6.43 ± 0.78	6.84 ± 1.31	7.04 ± 0.66	NS
Mass Motility (%)	72.00 ^b ± 11.51	61.43 ^a ± 14.64	63.57 ^a ± 45.71	88.33 ^b ± 16.07	P<0.05
Progressive Motility (%)	66.00 ^a ± 6.52	60.71 ^a ± 13.97	61.43 ^a ± 46.79	91.67 ^b ± 16.43	P<0.05
Sperm Conc. (x10⁹sperm/ml)	1.68 ^a ± 0.82	3.80 ^a ± 1.95	2.63 ^a ± 1.99	5.07 ^b ± 1.15	P<0.05
Live sperm (%)	64.00 ± 19.49	69.29 ± 18.80	58.57 ± 43.37	71.67 ± 28.43	NS
Abnormal (%)	6.00 ^b ± 2.24	4.29 ^a ± 3.45	4.29 ^a ± 3.45	6.67 ^{ab} ± 7.64	NS

^{abc}: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. BCS: Body condition score, SC:Scrotal circumference, Sperm Conc.: Sperm concentration

Table 5. 13: The means and standard errors for semen parameters for MOOI RIVER during the seasons

PARAMETERS	MOOI RIVER				Significance
	Summer	Autumn	Winter	Spring	
Body weight (kg)	45.86 ^c ± 10.19	42.17 ^{bc} ± 7.64	39.57 ^b ± 10.42	33.50 ^a ± 8.96	P<0.05
BCS	3.40 ± 0.55	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	NS
SC (cm)	28.57 ^b ± 4.12	27.83 ^b ± 2.64	27.29 ^b ± 2.49	24.50 ^a ± 3.00	P<0.05
Semen Volume (ml)	0.51 ^a ± 0.492	1.33 ^b ± 0.93	1.13 ^b ± 0.47	0.58 ^a ± 0.340	P<0.05
Semen pH	6.60 ± 1.14	6.24 ± 0.81	6.46 ± 0.63	6.13 ± 0.30	NS
Mass Motility (%)	77.86 ^b ± 10.75	71.67 ^a ± 25.43	84.29 ^b ± 18.80	68.75 ^a ± 27.50	P<0.05
Progressive Motility (%)	77.86 ± 8.59	65.83 ± 24.58	82.14 ± 19.55	70.00 ± 21.21	NS
Sperm Conc. (x10⁹sperm/ml)	2.49 ^b ± 1.35	1.62 ^a ± 0.99	3.93 ^c ± 1.99	2.65 ^b ± 0.55	P<0.05
Live sperm (%)	65.00 ^b ± 15.81	50.83 ^a ± 32.93	77.14 ^c ± 24.81	71.25 ^b ± 10.31	P<0.05
Abnormal (%)	5.71 ± 4.49	6.67 ± 4.08	4.29 ± 5.35	5.00 ± 0.00	NS

abc: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. SC:Scrotal circumference, MM: Mass motility, PM: Progressive motility, Sperm Conc.: Sperm concentration

Table 5.14: The means and standard errors for semen parameters for ESTCOURT during the seasons

PARAMETERS	ESTCOURT				Significance
	Summer	Autumn	Winter	Spring	
Body weight (kg)	52.13 ^a ± 6.36	46.00 ^a ± 4.90	56.00 ^b ± 8.52	58.20 ^b ± 3.56	P<0.05
BCS	3.50 ± 0.93	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	NS
SC (cm)	30.50 ± 0.93	26.40 ± 1.14	30.20 ± 1.92	29.80 ± 2.39	NS
Semen Volume (ml)	0.54 ^a ± 0.41	0.48 ^a ± 0.36	1.10 ^b ± 0.27	1.24 ^b ± 0.68	P<0.05
Semen pH	7.53 ^b ± 1.24	6.17 ^a ± 1.64	6.16 ^a ± 0.41	6.11 ^a ± 0.72	P<0.05
Mass Motility (%)	48.75 ^a ± 32.81	39.00 ^a ± 30.08	62.00 ^b ± 39.47	82.00 ^c ± 32.52	P<0.05
Progressive Motility (%)	45.63 ^{ab} ± 34.48	32.00 ^a ± 35.64	57.00 ^b ± 35.79	74.00 ^c ± 35.95	P<0.05
Sperm Conc. (x10⁹sperm/ml)	1.50 ^a ± 0.85	1.54 ^a ± 1.31	3.04 ^{ab} ± 1.10	4.06 ^b ± 1.87	NS
Live sperm (%)	38.75 ^{ab} ± 31.25	23.40 ^a ± 24.45	63.00 ^b ± 37.01	73.00 ^c ± 38.83	P<0.05
Abnormal (%)	7.50 ± 5.98	6.00 ± 4.18	6.00 ± 4.18	5.00 ± 8.66	NS

^{abc}: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. BCS: Body condition score, SC:Scrotal circumference, Sperm Conc.: Sperm concentration

Table 5. 15: The means and standard errors for semen parameters for TUGELA FERRY during the seasons

PARAMETERS	TUGELA FERRY			
	Autumn	Winter	Spring	Significance
Body weight (kg)	41.85 ± 6.05	43.43 ± 5.99	38.88 ± 5.06	NS
BCS	3.00 ± 0.00	3.14 ± 0.38	2.91 ± 0.30	NS
SC (cm)	28.08 ± 2.25	27.00 ± 1.73	25.82 ± 2.68	NS
Semen Volume (ml)	1.31 ^b ± 0.57	1.39 ^b ± 0.47	1.01 ^a ± 0.40	P<0.05
Semen pH	6.30 ± 0.77	6.54 ± 1.20	6.15 ± 2.08	NS
Mass Motility (%)	68.85 ^a ± 24.25	67.14 ^a ± 22.15	77.73 ^b ± 32.59	P<0.05
Progressive Motility (%)	69.23 ± 24.99	66.43 ± 24.62	72.73 ± 33.57	NS
Sperm Conc. (x10⁹sperm/ml)	3.08 ^a ± 1.63	4.27 ^b ± 1.38	3.79 ^{ab} ± 1.45	NS
Live sperm (%)	67.69 ^a ± 28.11	87.14 ^c ± 29.70	76.82 ^b ± 28.13	P<0.05
Abnormal (%)	6.31 ± 4.44	5.71 ± 3.45	5.00 ± 4.47	NS

abc: means with different superscripts in the same row differ significantly (P<0.05) NS: means Not Significantly different. BCS: Body condition score, SC:Scrotal circumference, Sperm Conc.: Sperm concentration

CHAPTER SIX

Discussion

6.1. The effect of ram age on semen quality of Zulu rams

The age of the ram is important because not only does it influence the number of ewes a ram is capable of serving in its lifetime, but it also influences the quality of semen produced by the ram at each stage in its life. The results obtained in this research have shown that there are age-induced differences in the reproductive capacity of Zulu rams.

An increase in quality of spermiogramic parameters such as semen volume, sperm motility, sperm concentration and live sperm percentage were seen between the ages of 2 and 3 years old compared to rams from 1 to 2 years old. These results also seem to indicate that in Zulu rams, quality of the spermiogramic parameters reach their optimum at 3 years old. The volume of semen, mass motility, progressive motility, live sperm percentage and concentration were positively correlated to age ($P < 0.01$). Mandiki *et al.* (1998) studied the effects of age on reproductive performance of rams in France and also found that rams that had just reached puberty and up to the age of 1 year had a far lower reproductive potential than 2 and 3 year old rams. This lower reproductive potential in the rams between 1 and 2 years old could be the consequence of a high growth potential that suppresses reproductive development until the animal has reached a mature age.

The low correlation of age to semen volume ($r = 0.149$) in Zulu rams, although significant ($P < 0.05$), was surprising, as Hassan *et al.* (2009) and Mandiki *et al.* (1998) reported a high correlation between age and semen volume. This low correlation may be attributable to other factors such as management, season or even possibly stress. However a study conducted by Tabbaa *et al.* (2006) showed that semen volume in Awassi rams had a poor ($r = 0.01$) and not significant correlation to age. Semen volume was also found to be insignificantly correlated to SC ($r = 0.006$), which was surprising since other research has shown a positive correlation between semen volume and SC (Akpa *et al.* 2012). The average SC increased with age (Table 5.1.), but this could be more related to animal maturation than to semen production itself.

Scrotal circumference is a highly heritable trait and is a superior index for sperm production in rams (Zamiri *et al.* 2010). Gimenez and Rodning (2002) suggested that the SC is one of the most useful measurements for estimating breeding ability and it is highly correlated to semen quality, quantity and reproductive success. The authors also noted that rams with larger testicles sire lambs that reach puberty earlier than rams with smaller testicles. Lambs that reach puberty sooner have a longer reproductive lifespan than those that take longer to reach puberty.

The medium correlation of semen volume to sperm concentration ($r=0.501$; $P<0.01$) was expected, as a higher volume of semen does not necessarily indicate that it has a higher concentration of sperm. Sperm concentration was also poorly correlated to ram age ($r=0.208$) and this result was highly significant at the $P<0.01$ level. As the method of electro-ejaculation was used for semen extraction, the rams may have become mildly stressed during the process. A consequence of stress can exhibit as an incomplete extension of the sigmoid flexure triggering ejaculation within the penile sheath. Such an event reduces the volume and purity of the extracted semen. Hence the concentration of the sperm becomes diluted with impurities. The results with Zulu rams has shown that 3 year old rams exhibit the highest level of sperm concentration compared to the 1, 2 and 4 year old rams. Similarly, David *et al.* (2007) found that in Manech tete Rousse and Lacaune rams, sperm concentration was higher between the ages of 2 and 3 years old, thereafter decreasing chronologically. Concentration of Zulu ram sperm peaked at age 3, confirming that this is the best age for stud ram selection since the live sperm percentage was also at its highest at this age while abnormal sperm was at its lowest.

Live sperm percentage was poorly correlated to age ($r=0.206$) while being highly significant. Rams aged 1, 2 and 4 years old exhibited a similar live sperm percentage while 3 year old rams had the highest percentage of live sperm. Hassan *et al.* (2009) found that live sperm percentages in 2, 3 and 4 year old rams were not significantly different from each other ($P>0.01$). However the author suggested that there is a chronological increase in live sperm percentage.

The pH for Zulu rams ranged from 5.9-7.3. This range was maintained irrespective of ram age, thus the correlation between pH and age was found to be non-significant ($P>0.05$). But this non-deviation from the ideal pH range indicates that Zulu rams have healthy seminal plasma to sustain the viability of the semen irrespective of age. The alkalinity of semen depends on the electrolyte and protein concentration of the semen plasma which is secreted by the accessory glands at ejaculation. This plasma binds to the spermatozoa as they traverse the male

reproductive tract (Hafez and Hafez, 2000). This is important since in natural mating, seminal plasma serves as a carrier and protector of the spermatozoa. Such a result bodes well for Zulu sheep because there is a positive and significant correlation between pH and mass motility, pH and progressive motility, as well as between pH and abnormal sperm percentage ($r=0.132$; $r=0.101$ and $r=0.122$ respectively). Hafez and Hafez (2000) classified pH as an exogenous factor affecting sperm motility, with imbalances in pH resulting in decreased sperm motility.

Mass and progressive motility are important semen parameters for measuring semen quality as these are indicators of sperm performance in its own accessory fluid (Hafez and Hafez, 2000). Motility is measured as a percentage in terms of mass motility and progressive motility. Mass motility is focused on the gross swirling movement of the semen, and progressive motility in terms of strong, forward directional motility of individual spermatozoa.

Mass and progressive motility were both the greatest for the semen of 3 years old Zulu rams. Although there was a low correlation between age and these parameters ($r=0.170$; $r=0.170$, respectively), this result was still significant. Mandiki *et al.* (1998) found that mass and progressive motility were similar at ages 2 and 3 but this study has shown that Zulu ram sperm motility (mass and progressive) were significantly different between the 2 and 3 years olds. Similar results were obtained by Hassan *et al.* (2009) with indigenous rams in Bangladesh which exhibited significantly different motility percentages at 2 and 3 years old. In a study done with Garole rams in a semi-arid tropical environment, Joshi *et al.* (2003) found that the age of the ram did not have a significant effect on sperm motility. However Kumar *et al.* (2011), using Computer Aided Sperm Analysis (CASA), found that age significantly influenced sperm motility.

Hafez and Hafez (2000) documented age, along with spermatogenesis and availability of energy stores, as an endogenous factor affecting spermatozoa motility. Energy stores refer to the ATP (adenotriphosphate) stores which are required in ion transport, flagella movement and molecular transport in spermatozoa. Low ATP reserves inhibit the abovementioned processes and therefore impede sperm motility. Reduced motility or complete immobility of spermatozoa result in the inability of sperm to swim upwards into the female reproductive tract, thereby preventing conception.

The percentage of abnormal sperm in Zulu ram semen did not vary significantly at any age ($P>0.05$). A negative and insignificant correlation was found between age and abnormal sperm percentage ($r=-0.094$). This result implies that age is not a factor that influences the percentage

of abnormal sperm in Zulu rams. Focșăneanu *et al.* (2014) also reported that there is no linear relationship between abnormal sperm percentage and age.

Scrotal circumference (SC), which is a measure of testicular size, was similar for the 1 and 2 years old as well as for the 3 and 4 years old rams. Focșăneanu *et al.* (2014) also found that there was a chronological increase in testicular size in Turcana Alba (Romanian indigenous sheep) rams, most likely brought on by sexual maturation. There was also a high and positive correlation between SC and Body weight ($r=0.700$, $P<0.05$). Allaoui *et al.* (2014) similarly found a positive and significant correlation between scrotal measurements and body weight. However, this correlation decreased significantly with age. Toe *et al.* (2000) supported the finding that a well-established relationship exists between testicular size and Body weight, while Fourie *et al.* (2002) asserted that SC is an important trait for selection of genetically superior rams for fertility.

Preston *et al.* (2011) affirmed that SC actually indicates the capacity for sperm production, as SC is a direct reflection of the quantity of spermatogenic tissue the testes contain, which in turn indicates the maximum potential of sperm production. This study with Zulu rams has shown that while SC and Body weight increase chronologically, the 3 year old rams are still the better performing rams compared to the 4 year old rams. Further research may need to be conducted to find out the exact reasons why this is the case.

Preston *et al.* (2011) found that there is a strong relationship between an individual ram's testes size and testosterone production. Testosterone requirements for spermatogenesis have a significant influence on the production of this male androgen. Mahmoud (2013) reported that with increasing age plasma testosterone concentration also increased. No difference between 1, 2 and 4 year old rams was observed. However, 3 year old rams had the highest levels of plasma testosterone. The result obtained in 3 year old rams was significantly different ($P<0.05$) to the other age groups.

Preston *et al.* (2011) also highlighted that plasma testosterone concentrations are directly proportional to age. This means that an increase in age would result in an increase in plasma testosterone. However this was not the case with the Zulu rams. Preston *et al.* (2011) went on to further suggest that the concentration of plasma testosterone levels may be influenced by association with ewes and display of natural male aggression and sexual behaviour, but these assumptions are limited to natural circumstances. Studies to determine the effect of sexual behaviour must be conducted in Zulu sheep to determine if testosterone levels are linked to sexual behaviour. The Zulu rams in this study were isolated from the ewes overnight. Zulu rams

are kept in flocks with rams of all ages. Therefore a natural pecking order may be established, with older rams forcing younger rams into submission and therefore diminishing their natural aggression. The sexual behaviour of Zulu sheep has not been studied, so this present study may be used as a guide to their sexual behaviour patterns and how this links to the quality of semen produced in rams. Therefore, age of ram does not act as an isolated factor to influence the reproduction potential of rams but most likely works with a combination of influencers such as management, social and sexual behaviour.

The white blood cell (WBC) count was found to be the highest in 3 year old rams and was significantly different ($P < 0.005$) from 1, 2 and 4 year old rams. There was no apparent pattern in the change in WBC according to age. Egbe-Nyiwi *et al.* (2000) also found that the age has no effect on the WBC in sheep. Njidda *et al.* (2014) noted that a higher WBC count is an indicator of an immune response to infection or toxic substances, while a low count is an indicator of pathogenic infection or the presence of antigens. However, the author did not provide a WBC range for indigenous sheep to use as a benchmark for low and high WBC counts.

6.2. The effect of season

6.2.1. The overall effect of season on Zulu ram semen quality

Environmental temperature averages change seasonally in KwaZulu-Natal (KZN). Summer is characterized by high temperatures with most of the annual rainfall occurring during this season. Winter is usually the coldest and driest season (Table 3.2.1.). Autumn and spring exhibit mild temperatures. This climate pattern therefore classifies KZN as a sub-tropical location and this climate is more apparent in the coastal regions of the province. According to Rosa and Bryant (2003), in tropical climates the annual rainfall cycle, with the consequent cycles in food availability, plays an important role in reproduction. The results of this study have shown that there are some spermiogramic parameters that are affected by change of season.

Ungerfeld and Bielli (2003) highlighted the intrinsic and extrinsic factors that affect seasonal reproduction in mammals. Intrinsic factors incorporate genetics and life expectancy while extrinsic factors broadly incorporate environmental factors such as latitude and season. Because sheep have a life span that can exceed a year, they are not opportunistic breeders but

seasonal breeders, especially in temperate climates (Ungerfeld and Bielli, 2003). Other authors (Karagiannidis *et al.* 2000; Rosa and Bryant 2003 and Oláh *et al.* 2013) have also suggested that seasonal variation is more evident in temperate climates compared to tropical or sub-tropical climates.

The semen volume (in this study with Zulu rams) was similar during autumn (1.12 ± 0.09 ml), winter (1.21 ± 0.07 ml) and spring (1.08 ± 0.06 ml) and was significantly different ($P < 0.05$) to the volume obtained in summer (0.67 ± 0.59 ml). Similar findings in Ile de France rams were also reported by Oláh *et al.* (2013) where semen volume was highest in autumn, winter and spring while summer had the lowest semen volume. As with the Ile de France breed, it appears that there is a seasonal effect on Zulu rams' semen volume, irrespective of KZN not being a temperate climate. Karagiannidis *et al.* (2000) noted that the seasons can be divided into quality periods, as summer signals the onset of quality improvement, autumn the peak of improvement, winter the onset of decline, and spring the lowest quality. Even though this study of Zulu sheep identified summer as the worst performing season in terms of semen volume, the trend discussed by Karagiannidis *et al.* (2000) could still be plausible for the Zulu rams. This is because even though the results were similar for autumn, winter and spring, the volume does appear to improve in autumn and peak in winter.

Volume was significantly correlated to sperm concentration ($r = 0.501$) at a medium level. Sperm concentration was similar during autumn, winter and spring while summer had the lowest concentration ($1.70 \pm 0.14 \times 10^9$ /ml). Even though autumn, winter and spring were not significantly different from each other, sperm concentration appeared to peak in winter ($3.32 \pm 0.23 \times 10^9$ /ml). In terms of the quality period described by Karagiannidis *et al.* (2000), sperm concentration also seems to increase from summer, peak in winter and decrease in spring like the semen volume. These findings coincide with those of Hamidi *et al.* (2011) where sperm concentration was the highest in the breeding season, which was during autumn and winter. Hamidi *et al.* (2011) also revealed that even though quality and quantity of ram sperm parameters may decrease in the non-breeding season, a ram is still capable of copulating and reproducing. Research done by Mohamed and Abdelatif (2010) suggested that the decrease in concentration during the warm seasons such as spring and summer could be related to the reduction in spermatogenic activity and epididymal reserves during the hot seasons.

Sperm concentration in Zulu sheep was positively and highly correlated to the live sperm percentage ($r = 0.626$, $P < 0.01$). This suggests that a higher sperm concentration is most likely to

yield a higher live sperm percentage while a lower sperm concentration would contain a lower live sperm percentage. Similar findings were made by Boussena *et al.* (2014) where live sperm percentage was significantly and highly correlated to concentration ($r=0.64$, $P<0.05$). Just as with semen volume and sperm concentration, the live sperm percentage in summer ($48.72\pm 4.08\%$) was the lowest and significantly different from the other seasons. In a similar study with Dorper rams Malejane *et al.* (2014) found that live sperm percentage (or viability) was similar but high in spring and summer and significantly different to autumn and winter. In Dorper rams, winter resulted in the lowest percentage of live sperm ($39.0\pm 14.6\%$).

The mass and progressive motility of Zulu ram semen across all four seasons were not significantly different ($P>0.05$) to each other. These findings are in agreement with those of Karagiannidis *et al.* (2000) and Malejane *et al.* (2014). The high and positive correlation between mass and progressive motility ($r=0.970$, $P<0.01$) was significant and expected as mass motility represents the gross wave motion of the total number of individual spermatozoa. This suggests that there is a direct proportionality between mass and progressive motility. However, as mass motility is entirely based on progressive motility, if progressive motility decreases or increases, mass motility will behave exactly as progressive motility. The high correlation between live sperm percentage and mass motility ($r=0.788$, $P<0.01$), as well as with progressive motility ($r=0.798$, $P<0.01$) shows that motility is dependent on live sperm percentage. Hence only live sperm are capable of creating motility.

The pH of Zulu ram semen was similar for all seasons ($P>0.05$). In a study with Dorper rams (Malejane *et al.* 2014), the same results for semen pH were obtained. Semen pH is correlated to mass and progressive motility ($r=0.132$ and $r=0.101$ respectively) and although these correlations were not significant, they were still positive. This indicates that pH does affect the quality of the spermatozoa itself, as pH is one of the biochemical properties that are responsible for sustaining the viability of the spermatozoa.

A very alkaline pH (8.0 upwards) is indicative of infection while a very acidic pH (lower than 5.3) suggests that there could be obstructions in the ejaculatory ducts, seminal vesicles could be blocked or there could be urea contamination. Malejane *et al.* (2014) also suggested that a delay in processing fresh semen could result in getting a false acidic result as over time, degradation of fructose by the sperm cells results in semen becoming more acidic. The fact that the average pH for each season hovered in the acceptable pH range shows that all the sampled rams were in good health.

The abnormal sperm percentage in Zulu ram semen was not significantly different ($P>0.05$) from season to season. Malejane *et al.* (2014) obtained the same results with Dorper ram semen.

In terms of the hormonal control of reproduction it appears that winter is the most favourable season as the average plasma testosterone concentration in Zulu ram is the highest during the course of winter ($16.28\pm 1.94\text{ng/ml}$, $P<0.05$) while summer, autumn and spring are not significantly different from each other. Benia *et al.* (2013) found that there are significant differences ($P<0.001$) in the plasma testosterone concentrations seasonally. Testosterone is responsible for the proliferation of Leydig cells, Sertoli cells and germ cells which inevitably lead to increased testicular size.

Dorostghoal *et al.* (2009) have shown that scrotal circumference (SC), which is an indicator of testicle size, changes according to the breeding and non-breeding season in sheep. The author suggested that it was the circannual variations in follicle stimulating hormone (FSH), luteinizing hormone (LH), plasma testosterone levels and inhibin levels which actually brought on these changes in physiological structures rather than the seasons itself. This study with Zulu rams has shown that there is a significant difference ($P<0.05$) in SC during summer compared to autumn, winter and spring. The SC was the largest in summer ($29.61\pm 0.51\text{cm}$). This is in contrast to the findings of Dorostghoal *et al.* (2009) where a seasonal variation was found. However Oberst *et al.* (2011) found that there was no significant difference in SC during any season. Avdi *et al.* (2004) highlighted that sheep originating in tropical regions show little or no variation in SC.

As previously stated, the scrotum plays an important role in a male as it contains the testes which are the male gonads and the production site of spermatozoa. Since optimum sperm production occurs at 4-6 degrees lower than actual body temperature, the scrotum is important for thermoregulation of the testicles. This mechanism of thermoregulation is controlled by the cremaster muscle located in the wall of the scrotum. This muscle is temperature-sensitive and therefore relaxes when ambient temperature increases and contracts when temperatures decrease. An excessive fat deposit interferes with the sensitivity of the cremaster muscle to detect temperature changes and is thus regarded as one of the factors limiting ram reproductive capacity.

Kheradmand *et al.* (2006) found that the diameter of the seminiferous tubules increased at certain times in the year and this was positively correlated to an increase in semen quantity. However this study with Zulu sheep did not find a high or significant correlation between semen

volume and SC ($r=0.006$; $P>0.05$). But there was a high and significant correlation between SC and Body weight ($r=0.700$; $P<0.01$) and this was expected. Ungerfeld and Bielli (2003) suggested that larger animals have larger energy stores and are therefore capable of postponing breeding to a time of the year which is most conducive for offspring survival. The largest Body weight average was found in summer ($47.17\pm 1.33\text{kg}$). Therefore if the duration of gestation lasted for a period of 4-5 months and ewes conceived in winter, then progeny would be lambled in early spring or summer when the climate is favourable.

It therefore makes sense that rams are heavier in spring and summer owing to other factors controlled by season (such as availability of nutritious grazing pastures) but maintain a higher reproductive potential during autumn and winter. However Zulu rams have not shown a distinct breeding season due to the fact that parameters such as motility (mass and progressive), sperm abnormality percentage and semen pH do not significantly differ seasonally ($P>0.05$). The average white blood cell (WBC) was also evidently highest during the course of winter ($17.07\pm 1.55\times 10^9/\text{l}$, $P<0.005$). This could be a natural defence mechanism in Zulu rams to ensure that reproduction occurs without the interruption of disease. Njidda *et al.* (2014) found that indigenous sheep in Nigeria also possessed WBC counts that were higher than the $11\times 10^9/\text{l}$ limit, but this was deemed as normal. The author also found that age, sex and breed significantly affected WBC counts in sheep.

6.2.2. The effect of season on Zulu ram semen quality among the selected geographical locations.

Over a period of time, the Zulu sheep must have adapted to the climate, topography, altitude and nutrition of the area. Adaptability is the key to survival. Gizaw *et al.* (2011) suggested that an initial step in characterising animal breeds would be to identify distinct populations using information on their geographic and ecologic isolation.

In MRS, the Body weight was higher in summer ($46.30\pm 8.42\text{kg}$) and in spring ($41.00\pm 7.83\text{kg}$). During summer and spring, the kikuyu pastures available at the MRS looked fresh, green, highly nutritious and succulent. This was possibly one of the main factors influencing the Body weight. The average summer temperature was 29.4°C and spring averaged 26°C (Table 3.2.1.). The low Body weights in autumn and winter at MRS could possibly be attributed to the fact that rams were already tugging and were not feeding well. Geenty (2013) highlighted that rams lose a significant amount of weight during mating particularly when there are high ewe to ram ratios.

MRS had a very low ewe to ram ratio ($\approx 3:1$) so this may have resulted in dominant behaviour in rams which in turn could have prevented the rams from eating, in order to display their dominance against competing rams.

The SC at MRS (Table 5.8.) was smallest in winter (23.80 ± 1.64 cm) but semen volume was greatest in this season (1.10 ± 0.55 ml). Live sperm percentages were also high in winter. This is in comparison to spring and summer when nutrition was good and temperatures were higher but viability was low. Bester et al. (2004) highlighted that excessive fat reduces the fertility of rams. This reduction in semen quality results from the disruption of thermoregulation in the scrotum due to excessive fat accumulation in the scrotal neck. Therefore the results obtained from this study with Zulu rams at MRS appear to be in agreement with the findings of Bester et al. (2004) on the accumulation of fat in the scrotal neck and its influence on semen quality.

The values for Body weight and SC of the Zulu rams at UNIZULU did not vary according to season ($P > 0.05$). This makes sense as the rams were kept on a constant level of nutritional inputs. There was supplementation during pasture shortages hence the rams did not suffer as a consequence of an inadequate feeding regimen. However, semen volume, semen pH, MM, PM, sperm concentration, live sperm percentage, and abnormal sperm percentage were affected by the different seasons of the year. The rams performed the best in terms of semen volume during autumn (1.05 ± 0.44 ml) and winter (1.03 ± 0.56 ml). These results are in agreement with studies done by Karagiannidis *et al.* (2000) in which the authors found that autumn and winter generally display a higher semen quality although semen quality does tend to show a negligible decrease from autumn to winter.

The sperm concentration in UNIZULU rams was found to be the highest during autumn and winter ($3.68 \pm 0.59 \times 10^9$ /ml and $3.55 \pm 1.30 \times 10^9$ /ml respectively). These results are similar to reports by Hamidi et al. (2011) in which it was found that sperm concentration in Arabic rams was greatest during autumn and winter and diminished after these months. These results indicate that while Zulu sheep may be fertile all year, they may still possess a breeding season. This is surprising as Rosa and Bryant (2003) and Thiery et al. (2002) found that breeds which have been domesticated tend to move away from a distinct breeding season, but the rams at UNIZULU showed a seasonal pattern.

Rams at KwaMthetwa exhibited semen quality that was comparable to that of rams from other locations (Table 5.8.). It was predictable that Body weight, SC, semen volume, and semen pH were not affected by seasons in this area (Table 5.10.) as the quality of the pastures remained

constant throughout the year. In terms of seasonal performance, autumn and winter were the seasons in which the semen parameters were of the highest quality. The average temperature for these two seasons was 26.6°C and 24°C respectively.

Semen mass motility ($71.67 \pm 22.06\%$) in KwaMthetwa Zulu rams was the highest in winter whereas the progressive motility percentage was higher in winter and in autumn ($75.00 \pm 23.02\%$ and $65.00 \pm 33.17\%$ respectively). It has already been established in this research the mass motility and progressive motility are highly and positively correlated to each other ($r=0.970$; $P<0.01$). The live sperm percentage was highest in winter ($73.33 \pm 21.60\%$). It may therefore be concluded that rams at KwaMthetwa exhibit a breeding season at this time of year as the highest live sperm percentage is coupled with the highest motility. It is apparent that the greater the number of viable sperm that are highly mobile, the greater the chances of conception in the ewe. However, winter displayed similar results in terms of abnormal sperm percentage to summer. This may be a natural occurrence for Zulu rams in this area but this abnormal sperm percentage can be overridden by the fact that it is still under 10% and its effects on the overall quality of the semen may be negligible.

In Jozini (Table 5.9), the Zulu ram semen volume produced was higher in autumn ($1.70 \pm 0.76\text{ml}$) and winter ($1.35 \pm 0.51\text{ml}$) compared to the other seasons. The average temperature in autumn was 28.7°C and 24.3°C in winter. This location was one of the hardest hit by the drought in KZN and it was expected that the rams would perform poorly throughout the year. The drought had a direct impact on the nutrition at Jozini because by early winter most of the pastures had withered and were visibly poorer in quality. The mixed veld found at Jozini was a combination of thorny grasses and very few succulent drought-resistant plants.

The Body weight of the rams was also lower in autumn and winter than it was during summer and spring. It was therefore expected that the observed nutritive effects would show a direct proportionality on the quality of the semen. Semen mass motility was higher in autumn and ($75.00 \pm 17.11\%$) and spring ($71.25 \pm 34.91\%$). By spring, the pastures did show a slight visible improvement in quantity rather than quality, evidenced by the increase in Body weight from winter to spring. These results obtained in Jozini are in conflict with the observations of Karagiannidis et al. (2000), as the overall quality of semen in terms of motility, live sperm percentage and sperm concentration improved from winter to spring, whereas the author found a general decline in semen quality after autumn.

Scrotal circumference (SC), PM and abnormal sperm percentage was not affected by seasons of the year in Jozini. The fact that SC correlated to Body weight ($r=0.700$, $P<0.01$) is not surprising. Literature also indicates a positive correlation between SC and Body weight (Fourie et al. 2002 and Allaoui et al. 2013).

The seasons did not affect the Body weight, SC, semen pH, live sperm percentage and abnormal sperm percentage of Zulu rams at Stanger, however but season did affect semen volume, MM, PM and sperm concentration. The fact that seasons did not affect the Body weight of the Zulu rams at Stanger could have been influenced by the management practices at this location. The farmer supplemented the feeding regimen during the pasture shortages and ensured that during the coldest season (winter, Table 3.2.1.) rams were kept sheltered and well fed. This also impacted on the semen volume as it was found to be highest in winter (1.23 ± 0.53 ml). Spring was the season in which Zulu rams produced the most viable semen in terms of MM, PM and sperm concentration ($88.33\pm 16.07\%$, $91.67\pm 16.43\%$ and $71.67\pm 28.43\%$; $P<0.05$ respectively). Malejane et al. (2014) also found Dorper rams to be at their best in terms of semen quality during spring.

Body weight and SC were impacted on by season ($P<0.05$) in Zulu rams at Mooi River (Table 5.13) and were both highest in summer (45.86 ± 10.19 kg and 28.57 ± 4.12 cm respectively) and lowest in spring (33.50 ± 8.96 kg and 24.50 ± 3.00 cm respectively). These results indicate that the feeding regimen at Mooi River was not adequate enough to overcome the effects of environmental pressure caused by season in this location.

Semen mass motility was highest during summer ($77.86\pm 10.75\%$) and winter ($84.29\pm 18.80\%$) at Mooi River. Progressively motile sperm also exhibited the same pattern as mass motility with summer and winter. This makes sense, as mass and progressive motility are highly correlated to each other. Autumn and winter were recognized as the seasons in which semen volume was the greatest (1.33 ± 0.93 ml and 1.13 ± 0.47 ml respectively) while spring and summer displayed the lowest semen volumes. Although summer had one of the lowest semen volumes for the year, the fact that there was greater motility indicates that quality should take precedence over quantity. There were no significant variations in the semen pH and abnormal sperm values between the seasons ($P>0.05$).

There were no significant differences in SC, sperm concentration and abnormal sperm percentage in Zulu rams at Estcourt (Table 5.14) between the seasons ($P>0.05$). Zulu ram semen was most viable during spring since parameters such as semen volume, MM, PM, and

live sperm percentage were observed to be the highest in this season ($P < 0.05$). Similar results were reported by Kafi et al. (2004). The average temperature during this season at this location was 26°C. It was established during data collection in this season that animals were provided with supplements to counteract the effects of the extended drought being experienced in Estcourt at the time. Supplementary feeding boosts the performance of animals and it is now evident that the same booster effects are visible in the reproductive potential of Zulu rams.

Semen volume in Estcourt rams were found to be the highest during winter and spring while summer and autumn had the lowest semen volumes. The highest MM, PM and live sperm percentage was observed in spring ($82.00 \pm 32.52\%$; $74.00 \pm 35.95\%$ and $73.00 \pm 38.83\%$ respectively). This could possibly be due to the fact that the farmer supplemented the rams at this part of the year to make up for the low quality pastures that were available.

The pH of Zulu ram semen in Estcourt was greatest during summer (7.53 ± 1.24), while pH levels in autumn, winter and spring were not significantly different from each other. Extensive research has not been done on the factors influencing semen pH. None of the rams in this location was sick during the time of semen collection, thus eliminating the possibility of an internal infection raising the pH of the seminal fluid. A possibility could be that the composition of the pastures found at Estcourt may influence the pH of Zulu ram semen. There is a combination of sweet veld and sour veld grasses in this location, known collectively as mixed veld. Further studies must be carried out to establish the effects of veld type on the reproductive performance of Zulu sheep.

Zulu rams were not sampled in summer at Tugela Ferry (Table 5.15) as there were no farmers located in this area for sampling at the time. There were no significant differences in Body weight, SC, semen pH, progressive motility, and abnormal sperm percentages between the seasons. However, semen volume was found to be the highest during autumn and winter ($1.31 \pm 0.57\text{ml}$ and $1.39 \pm 0.47\text{ml}$ respectively; $P < 0.05$) while spring had the lowest semen volume ($1.01 \pm 0.40\text{ml}$). This result was expected as Karagiannidis et al. (2000) highlighted that autumn and winter are usually expected to display peak performances in terms of semen quality, and spring becomes a transitional period in such a case. However, mass motility was the greatest in spring ($77.73 \pm 32.59\%$). This result is similar to those obtained at Stanger. The live sperm percentage was the greatest in winter ($87.14 \pm 29.70\%$). Such a result indicates that a high semen volume coupled with a high average percentage of live sperm creates a greater chance for conception.

6.3. Conclusion

This study with Zulu rams has revealed some information about the factors which may affect or influence Zulu sheep viz. age, season and management, making it easier to understand how these factors can be manipulated to improve Zulu rams breeding potential and aid conservation efforts of this breed.

This work has shown that Zulu rams reach their peak reproductive potential at 3 years old. The quality of semen improved as age increased up to 3 years old. These results lead to the conclusion that age may be used as a criteria for breeding ram selection.

Although season had an influence on semen quality, the level of management seems to be more important in determining the quality of semen in a particular season. However in general, Zulu rams performed better in the cooler seasons of autumn and winter. Nevertheless, this degree of these seasonal differences should not prevent the rams from being used for breeding throughout the year.

CHAPTER SEVEN

7.1. General conclusion

Conservation programmes require information on semen quality in order to establish superior genes for nucleus breeding flocks and for artificial insemination. This study was necessary as in cases where populations are at a risk of extinction, male fertility is a critical factor for the continuation of the species. The aim of this study with Zulu rams was to assess the quality of Zulu ram semen of some geographically separated and extensively managed Zulu sheep populations in the KwaZulu-Natal (KZN) province by evaluating the effect of ram age and season on the virility of Zulu rams. This aim was achieved as there now exists a better understanding as to what degree age and season affect the reproductive capacity of Zulu rams in their respective locations. If the characteristics of these sheep are understood in their natural habitats, it allows for sound conservation practices that take into consideration the role of these sheep in the human sphere and environmental sphere. The results of this study unearthed the adaptive attributes that extend to reproductive adaptation in Zulu sheep. This is a significant

finding as it provides a better understanding of the mechanisms and signals timing and affecting reproduction in Zulu rams in particular.

The hypothesis that there are variations in the quality of Zulu rams semen due to the individual or interactive effects of ram age and season was proven to be true and therefore the null hypothesis was rejected. The first objective was to evaluate the quality of Zulu ram semen in relation to the effects of age. This objective was achieved. The objective to evaluate the quality of Zulu rams semen in terms of pH, sperm motility and concentration, numbers of live sperm and percentage of sperm abnormality was acquired. The testosterone level and white blood cell count was assessed and its correlation to semen quality was established. The effect of season on semen quality within geographically separated rams under the extensive management system was determined and the management system of the farmers that participated in this study, were documented.

The fact that semen quality parameters of Zulu rams reared in cooler locations such as Mooi River were still comparable to locations in Northern KZN which were hotter indicates that Zulu sheep can adapt to different climatic areas.

7.2. Recommendations

This knowledge must be used by scientists in partnership with the farmers and other stakeholders to ensure the preservation of Zulu sheep in their natural habitats. While Zulu sheep are important to the livelihood of some, they also represent a significant pool of information, of which the surface has only been slightly scratched. Further studies on spermatogenic activity in Zulu rams must be piloted to provide a deeper understanding on other possible intrinsic and/or extrinsic factors that may influence reproductive potential in Zulu rams.

The results in this dissertation may be used in breeding programmes as no other study of the reproductive patterns in Zulu rams has ever been conducted.

The results from this study may be used by scientists to design preservation protocol for Zulu ram semen as a form of *ex-situ* conservation.

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7.4. Appendices



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MANAGEMENT SURVEY

GENERAL

- a) Farm type: (Commercial or subsistence)
- b) Number of sheep in residence: Rams _____ Ewes _____
- c) Reason for keeping sheep (x): Meat ___ Wool ___ Manure ___ Sale ___

NUTRITION

- a) Type of feed/pasture provided: _____
- b) Most common grass grazed (provide sample): _____
- c) Supplementary feeding? (x): Yes ___ No ___
- d) Provision of licks? (x): Yes ___ (specify) _____ No ___
- e) Provision of water? : _____

BREEDING

- i) Type of mating? (x):

Uncontrolled	Group	AI	Other
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- ii) Source of ram used for breeding

Own (bought)	Own (bred)	Borrowed	Other
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VETERINARY CARE

Have any of the rams been sick in the past 6 to 12 months? (x): No ___ Yes ___

If 'yes', then state the sickness or disease: _____

i) External parasite control (x): Only when need arises ____ Routinely ____

ii) Method of External parasite control:

Method	(x)	Frequency: Winter	Frequency: Summer
Dip			
Spray			
Hand dressing			
Pour on			
Traditional			
None			

iii) Internal parasite control (x): Only when need arises ____ Routinely ____

iv) Method of Internal parasite control:

Method	(x)	Frequency: Winter	Frequency: Summer
Drench			
Traditional			
None			

v) Vaccinations? (x): No ____ Yes ____ (specify) _____

7.4.2. Appendix B



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CONSENT AND INDEMNITY FORM

I, the undersigned _____ (full names) have been fully informed of:

1. The purpose of this study is to assess the fertility of Zulu sheep (rams).
2. That my participation is voluntary and I will not be compensated for participation.
3. That I cannot withdraw at any time without prior indication.
4. That all information provided by me will be as accurate as possible and may be used for research and publication.
5. I will receive feedback in the form of presentation by the Agricultural extension officers regarding the results obtained from the study.
6. The researcher intends publishing the research results. However, confidentiality and anonymity of records will be maintained and my identity will not be revealed to anyone who has not been involved in this research.
7. There may be risks associated with my participation in the project. I am aware that the animals may injure the researcher and vice-versa however, if animals are injured, the researcher will ensure that treatment is immediately administered to the best of his/his capabilities.

8. Any further questions that I might have concerning the research or my participation will be answered by Miss Chella (researcher) or Professor NW Kunene (0359026264).
9. By signing this informed consent declaration, I am not waiving any legal claims, rights or remedies.
10. A copy of this informed consent declaration will be given to me, and the original will be kept on record.

I,have read the above information/confirm that the above information, has been explained to me in a language that I understand and I am aware of this document's contents. I have asked all questions that I wished to ask and these have been answered to my satisfaction. I fully understand what is expected of me during this research.

This consent and indemnity form has been explained to me by (Full name) in (Language) and I confirm that I have understood the conditions of my participation.

I,(full name) agree to voluntarily participate in this research project.

Signature and Date:

Participant

Witness

.....

.....

Researcher

Witness

7.4.3. Appendix C

Table 2.1: Table showing the degrees of movement for spermatozoa (Hafez and Hafez, 2000)

MASS ACTIVITY	RATING	PROGRESSIVE MOTILITY
Rapid swirling	Excellent	71-100%
Slower swirling	Very good	50-70%
Generalized oscillation	Good	31-49%
Sporadic oscillation	Fair	11-30%
No movement	Poor	0-10%

7.4.4. Appendix D

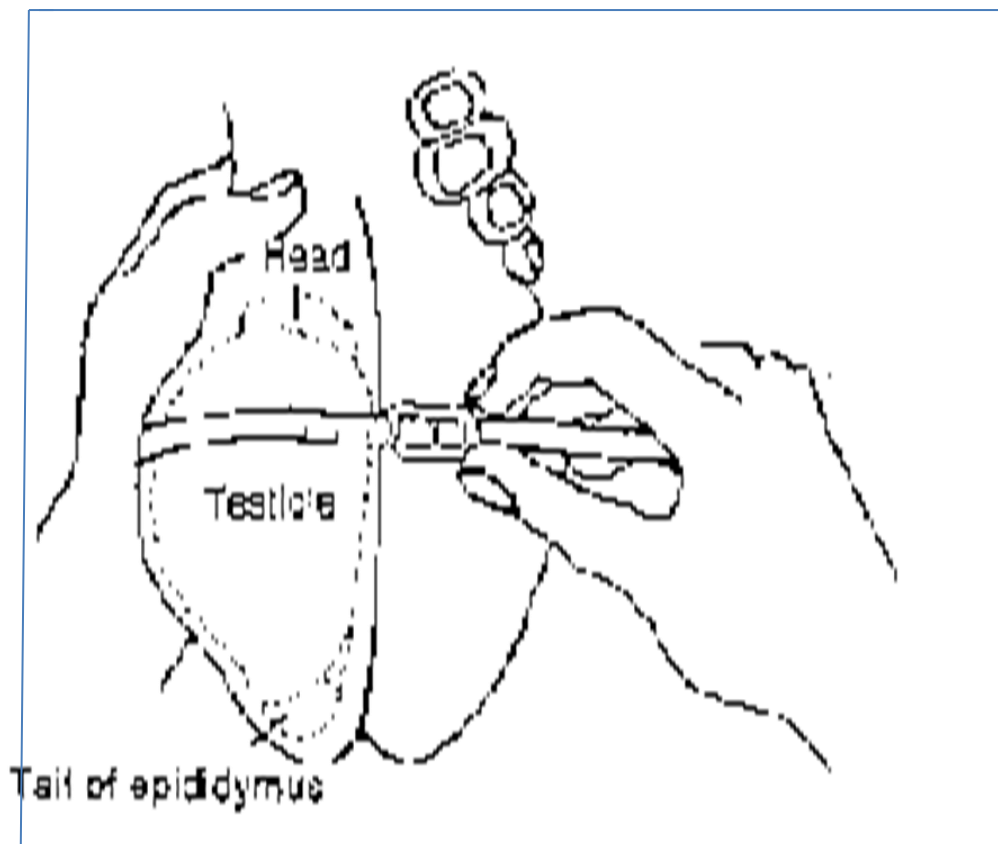


Figure 8.1: Diagram illustrating scrotal measurement

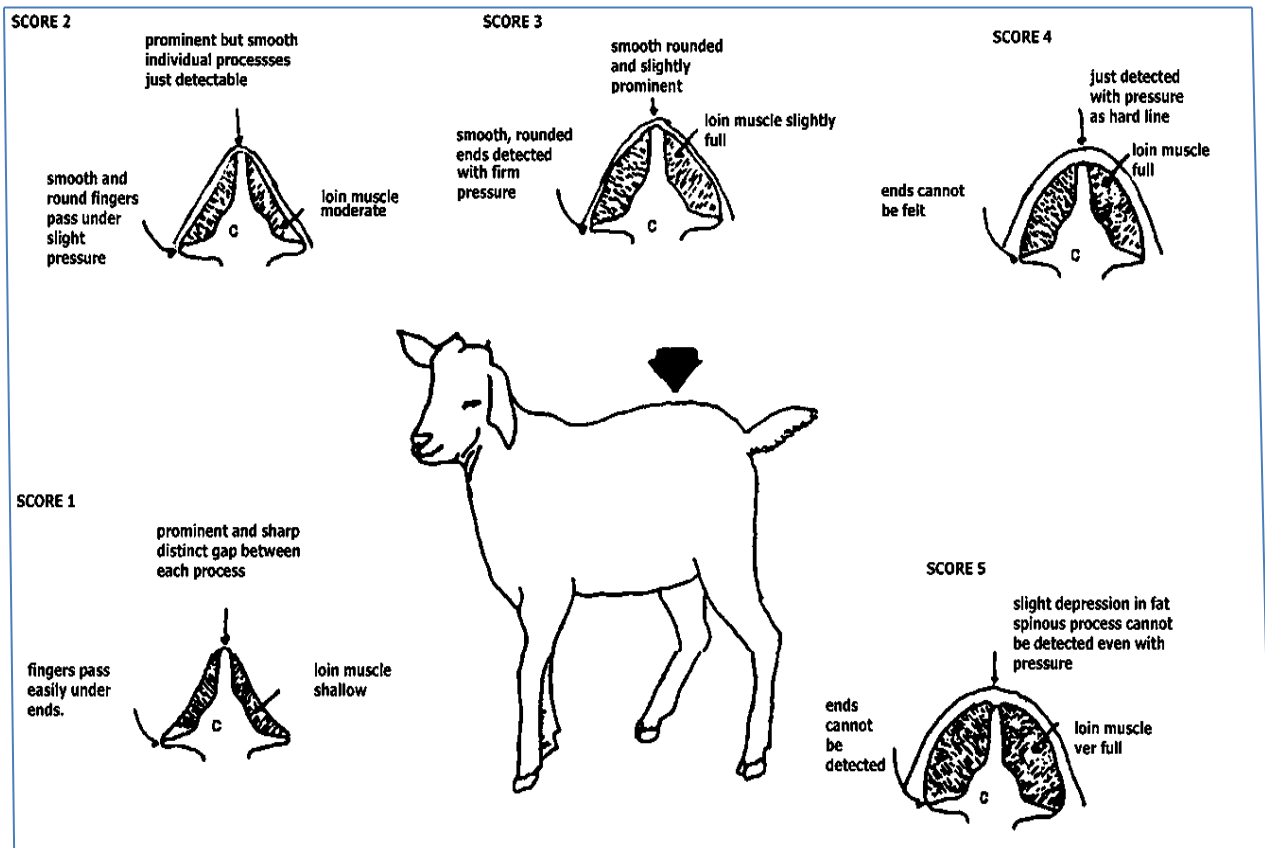


Figure 8.2: Diagram illustrating Body Condition Scoring procedure