UNIVERSITY OF THE FREE STATE

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Characterization and evaluation of reproductive performance in Bapedi sheep breed

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DECLARATION

I, Ayanda Maqhashu declare that "Characterization and evaluation of reproductive performance in Bapedi sheep breed" is my own work, has not been submitted before for any degree or examination in any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.

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Maqhashu A (Miss) **Date**

DEDICATION

This thesis is dedicated to my son Oyintanda Athi Maqhashu

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ABSTRACT

Reproduction is an important field of animal production, as it ensures continuation and maintenance of different animal species and their production. Bapedi sheep are indigenous to South Africa and predominantly found in the Limpopo province. They are reared for lean meat production and as a source of income to resource limited farmers. However, there is limited documented information on the morphometric characteristics and reproductive performance of the breed. The objectives of this study were to (i) determine the relationship between Bapedi rams morphometric characteristics with semen parameters, (ii) evaluate the effect of age and body condition on oestrous synchronization response of Bapedi ewes, (iii) assess the conception, and lambing rate following synchronization and natural mating and (iv) validate the genetic structure of Bapedi sheep using microsatellite markers to relate to each farm's reproductive performance.

Body measurements and semen data were collected from 31 rams conserved *in situ* (Mara Research Station, Madzivhandila and Tompi Seleka Agricultural Colleges) and *ex situ in vivo* (Agricultural Research Council). Rams aged 1-5 years, grazing on natural pastures with free access to water and shade were used for semen collection by means of an electro-ejaculator. Parameters measured were body condition scores (BCS), frame size (FS), body weight (BW), scrotal circumference (SC), semen volume, semen pH, spermatozoa concentration ($X10^9$), motility (%), viability and morphology. Live weight was measured with an animal weighing scale, SC was measured with a flexible measuring tape, semen volume was measured using a graduated tubes (mL), semen pH was measured using a microprocessor pH/mV/°C, and spermatozoa concentration was measured with spectrophotomer ($X10^9$). Computer aided sperm analysis (CASA®) was used for analysis of spermatozoa motility rate. Spermatozoa morphology was determined using an eosin-nigrosin stain. Experiment 2; Ninety-one Bapedi ewes (aged <2 and 3-6 years) were synchronized for oestrous and the influence of age and BCS

on the oestrous response of these ewes were measured, water was provided *ad libitum*. Ewes were assigned to (BCS) <3 and BCS≥3 on a scale of 1–5. For oestrous synchronization, controlled intravaginal drug release (CIDR®) dispensers were inserted for 9 days and 300 IU of equine chorionic gonadotrophin was injected intramuscularly after CIDR removal. Oestrus detection was done for a period of 72 h, from CIDRs withdrawal with a vasectomized ram. All ewes observed to be on heat were exposed to fertile rams for mating. Assessment of the genetic variation within and between Bapedi sheep was conducted using 14 microsatellite markers were used. Blood samples were collected from 174 unrelated Bapedi sheep in 6 farms in different districts of Limpopo and one conservation farm in Gauteng. Other South African sheep breeds such as Zulu, Damara, Dorper and Namaqua were included to assess the genetic relationship between these breeds and the Bapedi sheep as reference populations. There were no significant difference when the body weight of Bapedi rams was compared in all the farms ($P>0.05$). Moreover, there was uniformity in all body measurements of Bapedi sheep regardless of conservation method. There were no significant differences in body temperature during semen collection, SC, semen volume, pH, and concentration, sperm total motility and sperm kinematics in Bapedi rams in both methods of conservation ($P>0.05$). Pearson correlations revealed significant positive relationships between BW, BCS and SC ($r = 0.315$; $r = 0.638$; $r = 0.381$ respectively) with semen volume in Bapedi rams. Rump length was also found to positively influence sperm normality. There were no significant differences observed in oestrus response of ewes regardless of age ($P>0.05$) and method of conservation. Oestrus response was higher when ewes with BCS≥3 (91%) compared to lower BCS group (71%) ($P< 0.05$). Old and lower BCS ewes showed estrus signs earlier (23 ± 2.8; 21 ± 4.1); (22 ± 4.1; 20 ± 5.3) and with a shorter duration (23 ± 8.2; 20 ± 6.2); (22 ± 4.0; 23 ± 3.2) compared to young and ewes with higher BCS groups (onset of estrus: 34 ± 2.0; 32 ± 2.4); (36 ± 1.3; 35 ± 2.3) duration (30 ± 1.3; 29 ± 1.5);(33 ± 5.0; 32 ± 6.0) ($P<0.05$). Conception rate was 65, 67, 53, and 70% for ARC,

Towoomba, Tompi Seleka and Mara farms respectively. Toowoomba had a significantly lower litter size recorded compared to all the other farms. There were no significant difference ($P<0.05$) between the two conservation methods on the gestation length of Bapedi sheep. Prolificacy of Bapedi sheep was 1.30±0.6 1.28±1.3; 1.29±0.8 and 1.31±0.5 for ARC, Towoomba, Tompi Seleka and Mara farms respectively. To assess the genetic variation between and within populations the results obtained showed a mean number of alleles (MNA) to be 9, indicating that the panel of used markers were highly informative, however, no private alleles were obtained. Observed heterozygosity (Ho) and expected heterozygosity (He) values were ranged between (0,555±0.03 to 0,827± 0,027) and (0,754±0.02 to 0,883±0.004) respectively. These heterozygosities are indicative of a considerable genetic diversity among the Bapedi sheep populations. Within population inbreeding estimates (FIS =0,173±0,029) did not the influence heterozygosities obtained as it was high supported low rate of inbreeding, most likely as a result of the mating structure of Bapedi sheep. Both the unweighted pair group method with arithmetic mean (UPGMA) tree and Principal component analysis (PCA) results were in agreement and revealed that Bapedi sheep from Mopani commercial farm, Sekhukhune communal farm, ARC and Mara research station clustered together and share common genetic material. Towoomba population of Bapedi sheep did not cluster with the Bapedi sheep or any other reference population. Based on the findings it was concluded that Bapedi sheep are still a uniform breed, regardless of decreasing numbers. Higher oestrus was observed on ewes with BCS≥3. Young ewes with high BCS showed a delayed onset of estrus that lasted longer compared to old ewes with lower BCS. Conservation methods did not affect the reproductive performance of Bapedi sheep. It is recommended that BW, BCS and SC should be included in the selection of criteria to improve reproductive performance of breeding rams. Bapedi ewes can be synchronized successfully with an acceptable conception rate without supplementary feeding.

Based on the currents results it was identified that there is a need for sustainable breeding and conservation programs to control inbreeding and gene flow of Bapedi sheep, in order to stop possible genetic dilution of Bapedi sheep. It is recommended that flush feeding should be considered to improve the fecundity and prolificacy of this breed. More research is required to assess correlation of body measurements, testicular morphometry and semen parameters in this breed.

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ABBREVIATIONS

AMOVA: Analysis of molecular variance

ART: Assisted reproductive biotechnologies

BCS: Body Condition Scoring

CASA: Computer-aided sperm analysis

CIDR: Controlled internal drug release

DNA: Deoxyribonucleic acid

ECG: Equine chorionic gonadotrophin

FAO: Food and Agricultural Organisation

FIS: Inbreeding coefficient of individuals within subpopulation

FIT: Inbreeding coefficient of individuals within total population

FSH: Follicle stimulating hormone

FST: The amount of genetic differentiation within the total population

GnRH: Gonadotrophin realising hormone.

HWE: Hardy- Weinberg equilibrium

i.m: Intramuscular

ISAG: International Society for Animal Genetics

MNA: mean number of allele

NPM: non-progressive motility

OS: Oestrous synchronization

P4: Progesterone

PCA: Principal Component Analysis

PM: progressive motility

SASSCAL: Southern African Science Service Centre for Climate Change and Adapted Land Use

SCA®: Sperm Class Analyser

SD: Standard deviation

SE: standard error

STR: Straightness

TM: Total motility

UPGMA: unweighted pair group method with arithmetic mean

VAP: Average-path velocity

VCL: Curvilinear velocity

VSL: Straight-line velocity

WOB: Wobble

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Chapter 1

1.1 Background to the problem

Small ruminants (sheep and goats) are a vital part of smallholder farming systems. They significantly contribute to the total farm income, the stability of farming systems and human nutrition (Tshabalala, 2000; Meissner *et al*., 2013). Sheep have lower per-herd nutrient requirements compared to cattle and that makes them suitable for the limited resources imposed by drought and global warming (Tshabalala, 2000). During periods of drought, livestock experience rapid reduction in weight and reproductive efficiency thus resulting in considerable economic losses and a subsequent reduction on food supply for humans. Five provinces of South Africa were severely affected by drought that reached a critical stage where commercial and smallholder farmers lost 5-10 % of livestock in 2016 (Janse van Vuuren & Mokhema, 2016). Climate change challenges are predicted to continue into the future. South Africa has an opportunity to better utilize indigenous small ruminants (sheep) that are well adapted to harsh environmental conditions to mitigate these loses. However, most of the indigenous sheep breeds have been neglected as pure breeds and face the risk of extinction (FAO, 2000; 2007; Macaskill, 2016) and there is limited information about their productivity (Ameha *et al*., 2011).

There is a need for development of strategies to prevent and reduce the degradation of indigenous farm animal genetic resources; consequently, establish programs for their conservation and sustainable use to secure food for the future. Small ruminants have shorter production cycles, faster growth rates and greater environmental adaptability as compared to large ruminants (Nigussie, 2015). Cloete *et al.,* (2014), also reported that sheep and goats are reasonably tolerant to higher temperatures compared with other livestock. Higher temperatures would bring about increasing numbers of sheep and goats. South Africa has a wide range of

indigenous sheep breeds including Bapedi, Zulu, Swati and Namaqua Afrikaner that walk long distances in search of pasture and are adapted to harsh environmental conditions (Kunene, 2007). Furthermore, their tolerance to gastrointestinal nematodes increases their importance for sustainable livestock production (Baker *et al*., 2002).

Indigenous sheep breeds are an under-utilized resource that can be improved to uplift the supply of meat locally. This would in turn assist in the conservation of these adapted breeds. Africa as a whole is facing problems with preventing loss of indigenous sheep breeds due to lack of proper documentation of what exists. Sustainable utilization of livestock diversity requires characterization of the available resources and development of sustainable genetic improvement strategies that consider the needs and perceptions of target groups and that minimizes loss of genetic diversity (Solomon, 2008b; FAO, 2011). In sub-Saharan Africa, it has been estimated that 30% of indigenous animal genetic resources are at risk of becoming extinct before they are characterized and documented (Muigai *et al*., 2009). Conversely, in most indigenous breeds of South Africa such as Bapedi and Swati sheep. Characterization has only been done partially on phenotypic traits with limited information on genetic distinctness (Qwabe, 2013) and how it relates to resulting reproductive performance.

Good reproductive performance is a requirement for any successful genetic improvement and it controls production efficiency, which depends on various factors including age at first lambing, litter size, lambing interval and the lifetime productivity of the ewe (Abate, 2016). Kunene (2010), reported that for many decades indigenous genetic resources have been perceived as unproductive and inherently inferior to improved breeds. As a result, information on the reproductive performance of indigenous sheep is limited. Furthermore, information on the endocrine regulation of oestrous cycle, lambing, twinning, and weaning percentages, age

of ewes at first mating, age of rams at first mating, age at first lambing, weaning age, and lambing interval are limited for indigenous sheep breeds in South Africa. This might be among other things due to the limited information on detailed phenotypic, morphometric and genetic traits of indigenous sheep breeds.

1.2 Objectives

The aim of the study is to characterize the Bapedi sheep breed and evaluate their reproductive performance.

The specific objectives were:

a) To determine the relationship between Bapedi rams' morphometric characteristics with semen parameters
b) To evaluate the effect of age and body condition score on oestrous synchronization response of Bapedi ewes
c) To assess the conception, lambing and growth rates of lambs following oestrous synchronization and natural mating
d) To validate the genetic structure of Bapedi sheep using microsatellite markers and relate to each farm's reproductive performance.

Chapter 2: Literature review

2. Literature review and discussion

The review will focus on conservation of animal genetic resources, *in situ* conservation, *ex situ* conservation, sheep distribution in South Africa risk and status classification, Bapedi sheep breed, characterization of sheep genetic resources, reproductive efficiency in sheep, male reproductive performance, breeding strategies, and application of assisted reproduction technologies.

2.1 Conservation of animal genetic resources

Conservation of livestock genetic material involves all human strategies, policies, plans and actions taken to ensure maintenance of genetic diversity of animals. The idea behind conservation is to maintain the contribution of animals towards food production and increase in production at present and for the future (Hanotte *et al.,* 2000). Conservation of animal genetic resources involve two interlinked concepts, which is the conservation of genes and conservation of the breed (FAO, 2005). These are equally important in ensuring food production for the future, increase options to improve the sustainability of livestock production and increases the options to improve the quality of our food. There are two methods that are used for conserving farm animal genetic resources (AnGR), namely *ex situ* and *in situ*.

2.1.1 *In situ* conservation

The *in situ* method is the conservation of live animals with utilization in their production system, or in the area where the breed developed its characteristics. It can also be referred to as “on-farm conservation”. Such conservation consists of entire agro-ecosystems including

immediately useful species of crops, forages, agroforestry species, and other animal species that form part of the system (Rege, 2001; Chokoe and Shole, 2013). Under *in situ* conditions, breeds continue to develop and adapt to changing environmental pressures, enabling research to determine their genetic uniqueness. It is an inexpensive and convenient, conservation method for emerging farmers or resource poor farmers.

2.1.2 *Ex situ* conservation

The *ex situ* method involves conservation of animals outside of their habitat. It involves most of the technologies such as semen, ova, somatic cells, blood and embryo freezing (Collins *et al*., 2001). *Ex situ* conservation can also be achieved through keeping live populations in zoos and on experimental or show farms (Pieters, 2007). This method helps supports *in situ* conservation and serves as an insurance for the future. It enables recreation of extinct breeds/breeding lines. In addition, it can serve as a backup in case genetic problems occurs. It allows development of new lines or breeds for research and genetic characterization purposes (Hiemstra, 2015).

2.1.3 Sheep distribution in South Africa: risk and status classification

It was reported that sheep and goats occupy 50% of agriculture land suitable for extensive livestock farming (National Department of Agriculture, 2011). The largest numbers of sheep are found in the Eastern Cape (30%), Northern Cape (25%) and Free State (20%) with the Western Cape having about 11%, Mpumalanga 7%, North West 3%, and Limpopo 1% (National Department of Agriculture, 2014). An assessment of the risk status of different livestock breeds or populations is an important element in the national planning of AnGR management. It informs stakeholders whether, and how urgently, actions need to be taken to conserve a certain breed. Gandini *et al*. (2004) define “degree of endangerment” as “a measure

of the likelihood that, under current circumstances and expectations, the breed will become extinct". Accurately estimating degrees of risk is a difficult task and incorporates both demographic and genetic factors. Qwabe (2011) reported that 20% of the breeds are currently at risk and 14% of sheep breeds worldwide have disappeared. Seventeen sheep breeds are known to exist in South Africa (DAGRIS, 2007). However, Macaskill (2016) reported about nine sheep breeds [Izimvu (Zulu), Namaqua Afrikaner, Pedi, Persian (Blackhead or Redhead), Ronderrib Afrikaner (gladde- or Blinkhaar), Ronderrib Afrikaner (steekhaar), Speckled Persian (Black or Red), Vandor and Van Rooy] to be at risk of extinction. These indigenous sheep breeds are mainly used for mutton production.

2.2 Bapedi sheep breed

2.2.1 General characteristics of Bapedi sheep

Bapedi sheep is an indigenous breed of South Africa believed to have arrived with Pedi people between 200 and 400 AD. This breed is predominantly found in the Limpopo province of South Africa. The Bapedi sheep is a non-selective mixed feeder with outstanding veld utilization habits. It fully utilizes any type of grazing or roughage. Ferreira (2013) reported that Bapedi sheep adapts well to hot climatic conditions up to 45˚C and is tolerant, with virtually limited disease problems. Bapedi ewes are excellent mothers with limited assistance required at lambing and have no lambing problems. This breed is small framed, polled, with a flat shallow body, long legs and fat-tail. Coat colour varies from uniform brown through white with a red to brown head, black to black and white at time as shown in figure 2.1 (Collins *et al*., 2001). Additionally, Bapedi sheep mature early, have a strong flocking instinct, domesticate well are efficient on veld and water (Ferreira, 2013). Ewes' average from 35-40 kg, rams 50-60 kg whereas rams reach an ideal slaughter weight at 12 months and ewes at 14 months. It is not

costly to keep Bapedi sheep. This breed is easy to manage; survives well on natural pasture and lambs twice a year (Buduram, 2004). Meat from Bapedi sheep is tender and tasty and most of the fat is localized in the tail (Snyman, 2004).

Figure 2.1. Bapedi sheep breed (Nthakheni, 2015)

2.3 Characterization of sheep genetic resources

2.3.1 Phenotypic characterization

Characterization of animal genetic resources involves all the activities that will help in identification, quantitative and qualitative description of breeds (Caballero and Toro, 2002). Physical characteristics of livestock are often correlated with various productive and adaptation characteristics. However, contrary to characterization using adaptive physical characters, neutral markers sometimes do not reflect the diversity in production and adaptive traits. Physical traits can be used to separate genetically distinct populations, classify and categorize each animal according to specific production environments (FAO, 2011).

2.3.2. Breed identification and description

Populations of livestock of the same species, that are sometimes found or originated from the same geographical region and are recognized by ethnic owners, are referred to as a breed or distinct eco-type. Physical identification involves sampling of the animals and targets traditionally recognized and unrecognized features for that certain breed. Qualitative and quantitative physical measurements are also used in the description of the breed (Caballero and Toro, 2002). The qualitative traits observed in most studies are coat colour, face profile, ear form, presence or absence of horns, tail type, fibre type and shape of the tail. Quantitative or morphometric traits are body weight and length, withers and substernal height as well as ear and hair length (FAO, 2011).

2.3.3 Overview of indigenous sheep production and environment description

The focus of surveys on production systems and environments are to describe and identify the constraints to increase productivity. Description of geographical and ecological distribution aids in estimates of population size of animal genetic resources and is very important for characterization (FAO, 2011). These are most important for monitoring risk status of populations and design conservation and improvement programs. The major indicators of production systems are used to give a fair picture of a breed's position. It is important to assess the whole range of production systems in where a breed is raised so that conclusion on the range of adaptability be documented. Moreover, added benefits can be merited to the breed for example if it can be raised in more than one environment (Harkat *et al*., 2015). These also help identify the flock structure of a certain breed compared to other breeds.

2.3.4. Socio economic value

In developing countries small stock breeding programs are highly affected by the socio economic and cultural values. The failure of the development agencies to achieve sound breeding programs is not understanding the needs and desires of farmers. Social and cultural values attached to livestock and livestock products vary from society to society (Kunene, 2010). Breeders and consumers inclinations have been identified as one of the main factors responsible for the declining genetic diversity of livestock (Harkat *et al*., 2015).

2.4 Genetic characterization

Genotypic characterization is the evaluation of genetic potential of breeds under an on station or well-controlled farming conditions (Abera, 2013). Genotypic characterization involves a systematic comparative evaluation of breeds over the same environment using appropriate designs, which include sufficient numbers of representative animals and sires. It also allows comparing breeds with respect to genetic parameters such as genetic variances and covariance (Solomon, 2008). Genotypic characterization studies provide estimates of heritability and breeding values of sires and dams that help to predict the response of the breed to selection and development. The practical importance of genotypic characterization is providing information on genetic differences of breeds that help in decision making for the rational utilization of breeds as sources of genes in any development program (Rege and Okeyo, 2006).

2.4.1 Molecular characterization

Recent developments in molecular biology and statistics have opened the possibility of identifying and using genomic variation and major genes for the genetic improvement of livestock (Yilmaz, 2016). It enables the establishment of the genetic distances between breeds and the genetic structure of breeds (Abera, 2013). Complementary procedures are used to loosen the genetic basis of the phenotypes of AnGR, their patterns of inheritance from one generation to the next and to establish relationships between breeds. Molecular characterization is too costly and requires exceptional expertise. Several molecular genetics techniques have been developed to achieve genetic structure and diversity. Microsatellites, which are highly polymorphic and abundant, often found in non-coding regions of the genes and are widely used for paternity and genetic diversity studies (Yilmaz, 2016).

2.4.2 Microsatellite markers

Microsatellites are the preferred technique for population studies. These reveal genetic variation both on the standard and new variation generated by mutations. They are short tandem repeats (STR's) of genomic sequences. The repeated unit can be single, double or triple, however double is common. These units usually occur in non-coding regions of the genome. Microsatellite markers are reliable because they reveal differences between and within individuals (Abegaz and Duguma, 2000). These markers are used in a wide range of samples such as hair, meat, saliva, blood and skin (Yilmaz, 2016).

2.4.3 Single nucleotide polymorphism

Single nucleotide polymorphism (SNP) technology is a new method of analyzing thousands of parameters simultaneously within a single experiment (Qwabe, 2011). These deoxyribonucleic acid (DNA) chips have been used in studies involving gene expression for identification of single nucleotide polymorphism or sequences among DNA genotypes. The potential of this DNA technique has been identified in livestock but is still unavailable for some species. The disadvantage of this technique is that it is costly.

2.5 Reproductive efficiency in sheep

Reproductive efficiency is a performance factor measured by the timeliness of getting ewes bred and producing healthy lambs within a year. Any measure of reproductive efficiency must take into account allowances for management (Webb *et al.,* 2016). In this regard, the number of lambs reared to weaning is of great practical importance. Other important indicators of reproductive efficiency include puberty, oestrous, size and age at first mating orartificial insemination (AI), litter size, lambing rate, lambing interval, non-return rates and age and size vs. scrotal circumference and semen production (Safdarian *et al*., 2006).

2.5.1 Puberty

Puberty in female sheep is the age at which the growing female displays first estrus. Onset of puberty is earlier with higher weaning weights, but poor nutrition can delay puberty. Improved growth rate and body weight, resulting from better post-weaning nutrition, advances the attainment of puberty in almost all the breeds (Rekik *et al.,* 2016). It usually occurs from 6-9 months in ewes (Hafez and Hafez, 2009).

2.5.2 Ovarian cycle and related endocrine events

Estrus in ewe lambs is observed later than the age at which they reach puberty, meaning that even though puberty is attained at an earlier age, fertility will only improve with age and weight (Mukasa-Mugerwa *et al*., 1993). Measuring the concentration of hormones associated with reproductive functions in female animals provides insight about their reproductive status. In particular, measuring the level of progesterone secreted by the corpus luteum can be very informative. Rekik *et al*. (2016) observed progesterone profiles in Menz sheep during pubertal development, estrus cycle, pregnancy, and post-partum anestrus and discovered that they were similar to those of temperate breeds.

2.5.3 Daylight length (photoperiod) effects on oestrous cycle and seasonality of breeding in sheep

Seasonality of reproduction is a characteristic of sheep breeds from temperate latitudes where variations in the photoperiod trigger changes in ovarian cyclic activity between seasons. In tropical and equatorial latitudes, seasonality of reproduction is less important. While sheep in these latitudes might not be susceptible to changes in the photoperiod, alterations can result from other environmental and social cues, such as feed availability, ambient temperature, and social interactions (such as the presence of rams or ewes that are cycling in the flock) (Rekik *et al*., 2016). The natural sexual season is positioned so that lambs will be born in the spring when the weather is warmer and grass is available (Kennedy, 2012). The length of the breeding season varies from one breed to another. Breeds that originated closer to the equator tend to have longer breeding seasons than those that originated further north (Safdarian *et al*., 2006).

2.5.4 Parturition interval and post-partum anestrus

Lambing of improved sheep breeds were reported to be twice a year (Kennedy, 2012). However, for indigenous sheep in Ethiopia lambing interval of eight months was achieved with three lambings in 24 months (Abate, 2016). Lambing interval is influenced by many factors such as previous litter size, parity and lambing season but it is not influenced by either birth or weaning weights (Rekik *et al*., 2016).

2.5.5 Age at first lambing

The age at which a ewe gives birth for the first time is highly influenced by the management system in that particular farm. Ewes under smallholder farmer's management demonstrated to be around 404 days and it's not different from indigenous sheep under semi-intensive management (Gizaw *et al*., 2013).

2.5.6 Conception rate and fertility

Mukasa-Mugerwa and Lahlou-Kassi (1995) reported that indigenous ewes of Ethiopia have high conception rates (>= 90%) at first lambing. However, lambing rate was 20% lower than conception. This suggests moderate embryonic losses. Conception rate by rank of mating is affected by the age of the ewe. Research findings indicated that lambing rates (lambs born/ewes mated) vary within breeds (Gizaw *et al.,* 2013; Rekik *et al*., 2016).

2.5.7 Gestation Period

Gestation period is usually defined as the period from conception to parturition. It has been reported to be between 145 and 150 days in ewes across all breeds (Abate, 2016).

2.5.8 Litter size and lamb survival

Various studies (Abegaz *et al.,* 2000; Dibissa, 2000; Mukasa-Mugarwa *et al*., 2002) reported litter size between 1.04 and 1.34 in indigenous goats. Agyemang *et al*. (1985) reported a twinning rate of 4.2%. Mamabolo and Webb (2005) reported a litter size of 1.7 with the most frequent litter size being twins in autumn and spring for indigenous goats. Winter twinning was reported to be lower in goats (Webb, 2005). Survival of lambs within the first four days in indigenous sheep was lower compared to improved breeds (Rekik *et al*., 2016), this might be due to poor management, harsh environmental conditions and poor disease control.

2.6 Male reproductive performance

2.6.1 Puberty

Puberty mean age for South Africa indigenous rams was reported to be 288±6 days with weights of 19.3±0.4 kg and condition score of 2.6±0.06 (Abate, 2016). Age of puberty attainment is affected by a number of factors such as season of birth weight, level of nutrition and weaning weight. The age of sexual maturity is estimated to be later than puberty and semen and spermatozoa quality improved with age (Rege *et al*., 2000).

2.6.2 Breeding soundness evaluation

The ram is half of the herd in animal husbandry, which indicates that the sire fathers many lambs in the flock when natural mating is practiced. Since the ram has more genetic influence (80–90%) on the lambs in the flock. fertile ram selection can be the most powerful method to improve the flock (Perumal, 2014). Farmers should be sure of sufficient numbers of rams that are available for the breeding program and that the rams are fertile. Rams should possess characteristics that will upgrade the production potential of the flock in which it is used, and must successfully mate to transmit these traits (Abebe, 2017). Traditionally evaluation of a ram's breeding abilities came from the observation of a ram's breeding behavior after introduction into the ewe flock. The results were only acquired after the end of the breeding season, and failures were not rectified on time. Development of new assisted reproductive technologies allows for the breeding soundness examination to be conducted before breeding season, which includes, health history, physical fitness, particularly of feet and legs and eyesight. Pedigree, confirming the sire is free from known hereditary defects. Evaluating the smoothness of the hair coat for evidence of malnutrition or chronic infection, body condition score (BCS) and noting of the score on a scale of 1-5. The score of 1 being emaciated and 5 obese. Checking for and noting any defects that could interfere with the breeding process such as cryptorchidism. A thorough examination of the scrotum (as it directly influences sperm production), palpation of testicles, and examination of sheath and penis to make sure they are free of abnormalities and diseases (Abebe, 2017).

2.6.3 Scrotal circumference measurements

Scrotal circumference (SC) is measured to give an indication of a ram's breeding endurance. The SC is affected by the season of the year, breed and body condition but would usually be at

a maximum peak during the breeding season (Perumal, 2014). Ram lambs with a SC of less than 30 cm and adult rams with less than 33 cm should usually not be approved as acceptable breeders (Perumal, 2014). Söderquist and Hultén (2006) reported that males with larger testes tend to sire daughters that reach puberty at an earlier age and ovulate more oocytes during each oestrus period. The demand for sperm from outstanding sires has increased with the development of frozen semen technology, for conservation purposes and application of artificial insemination (AI) technologies.

2.6.4 The importance of semen evaluation

Semen quality is reported to be a major contributing factor that affects fertility and is an aspect of major concern in the animal production industry (Munyai, 2012). The average ejaculate volume of ram semen is 1.1 ml (Munyai, 2012). Semen needs to be evaluated using a light microscope to estimate sperm viability and percentage motile (and progressively motile) sperm cells, prior to its use in AI or cryopreservation**.**

2.7 Breeding strategies

Strategies for genetic improvement of livestock involves the decisions about the use of either crossbreeding or pure breeding. The choices that are made are the major reason for reduced animal biodiversity (FAO, 2007). There are very few indigenous sheep pure breeding programs. Cross breeding in developing countries assist in acceleration of AnGR degradation (Rege *et al*., 2006). This practice has been mainly due to farmers desiring higher production performance, however in low-input conditions cross breeds have been indicated not to perform any better than pure breeds (FAO, 2007). Selective pure breeding of indigenous breeds have been neglected and hence optimal production performance of these breeds remains unknown. The design of breeding programs requires definition of the objectives for breeding, estimation

of genetic parameters of the breed of interest, gametes evaluation, selection responses, genetic evaluation and design of an optimal breeding program (Bijma *et al*., 2006). Wollyn (2003) reported that an enabling farm animal genetic resource conservation policy could be successful if high priority is placed on a community-based participatory approach and focusing on food security and poverty alleviation. However, information on sheep breeding objectives targeting the needs and perceptions of farmers in a community- or village is absent.

2.8 Application of assisted reproduction technologies

Reproductive biology as a discipline has valuable contributions towards the broad aims of conservation. It provides insight into the many reproductive specialties and adaptations of different species, and is crucial for understanding different factors that deleteriously affect the survival of populations and also provides information for making strategic management decisions aimed at alleviating these threats to survival (Holt and Pickard, 1999). Emerging farmers in South Africa face various challenges that impede their productivity and ability to effectively contribute to food security relative to the commercial farmers. This can be attributed to lack of information on breeding plans. Without all these factors, it is unlikely that farmers will have the desired productivity. A proper breeding plan includes evaluation of all the rams that are going to participate in breeding for all venereal diseases, mounting ability, libido, sperm quality and maintaining of all the females in good body condition for breeding. There are worldwide improvements in sheep management practices such as assisted reproductive technologies (ART). Assisted reproductive technologies such as oestrous synchronization, semen quality assessment, AI and embryo transfer may be adopted for introducing superior genetic material in areas of reduced genetic biodiversity.

2.8.1 Oestrous synchronization

Oestrous synchronization is the modification of the ovarian cycle of the ewes to release an oocyte at a given time in order for AI/natural mating to be achieved. Ramukhithi *et al*. (2012), reported that high fertility rates depend on balanced endocrine responses. The protocols used for synchronization and ovulation are expected to maintain hormonal balance for satisfactory results after fertilization (Lehloenya *et al*., 2005). This process eases management and increases lambing rates, prolificacy in and out of breeding season and reduces the costs of heat detection and mortality during lambing by avoiding out of season lambing when weather conditions are not favorable for newborn lambs. Synchronization offers an opportunity to increase the efficiency of an animal to produce, as it allows mating or AI at a predetermined time. Oestrous synchronization in ewes is achieved by the control of the luteal phase of oestrous cycle either by providing exogenous progesterone or by inducing premature luteolysis (Shahneh *et al*., 2006). There are different protocols used for synchronization of ewes. These range from short to long differing on the number of days the progestogen device is kept (sponges or pessary) in the ewe.

2.8.1.1 Methods of oestrous synchronization in sheep

The focus on livestock oestrous synchronization (OS) is based on manipulating either the luteal or the follicular phase of the oestrous cycle. Luteal phase is the most preferred for sheep and goats because of the longer duration and responsiveness to manipulation (Wildeus, 2000). Methods that are considered successful for OS are methods that not only establish synchrony but also results in acceptable fertility after artificial insemination, natural mating and embryo transfer. In OS of ewes the luteal phase is either extended by supplying exogenous progesterone

or shortened by CL regression. Increase in fertility following OS is often achieved by co-treatments with gonadotrophins.

2.8.1.2 Progesterone methods

Oestrous synchronization protocols that involve the use of progesterone are common in cycling or seasonally anestrus ewes and have been used with or without other treatments such as prostaglandin or gonadotropin equivalents (Whitley and Jackson, 2003). The main purpose for using various forms of progestogens with different methods of administration is to try and extend the lifespan of the CL. Progestogens are manufactured in the form of vaginal sponges containing flourogestone acetate (FGA) and Methyl acetoxy progesterone (MAP). Similarly, a controlled internal drug-release device in a form of silicone intravaginal insert can be used. These vaginal implants are usually left in the vagina for 9 to 21 days with eCG or PGF2α48 hours before pessaries removal depending on the protocol being used (Wildeus, 1999; Omontese *et al*., 2016). Variation in the oestrous response and fertility have been reported in ewes and goats following intravaginal sponges treatment, as affected by the breed, co-treatment, management and mating system (Wildeus, 1999; Whitley and Jackson, 2003; Muna, 2012). Short-term progestogen treatments are encouraged because they minimize vaginal discharge, onset of oestrous, infection, and increase fertility (Omontese *et al*., 2016). Controlled internal- drug release implants are the preferred vaginal implants because they are easy to use, it is made of medical silicone elastomer prepared over a nylon staple with natural progesterone. Silicon controlled intravaginal drug release devices are preferred because they do not stick to vaginal walls and cause discomfort as much as sponges do. Gonadotrophins are often used together with progestogen for a tighter synchrony and induction of superovulation and improve fertility (Whitley and Jackson, 2003). There are controversial reports on the proper dosage of progesterone to induce optimum oestrous and there are no differences found where

CIDR or MAP were used, however the onset of oestrous was prolonged where MAP was used compared to CIDR (Omontese *et al.,* 2016). According to Omontese *et al*. (2016), the use of eCG increases the cost of this technology and reduces the fertility endurance of does and repeated administration builds up antibodies against eCG which serve as anti-eCG thereby reducing ovarian stimulation. It is also reported to have a long –acting biological activity and results in a large number of unovulated follicles (Armstrong *et al*., 1983; Wildeus, 1999).

2.8.1.3 Prostaglandin and their synthetic equivalents

Prostaglandin administration is another preferred way of inducing oestrous in ewes and does. This method is mostly used because prostaglandins are rapidly metabolized in the lungs and converted to 15- keto-prostaglandin F2α and 13, 14-dihydro-15-keto-prostaglandin F2α (Muna, 2012). Prostaglandin-based OS induce successive follicular phases of the oestrous cycle by terminating the CL. The limitation of this approach is that it can only be applied to cyclic ewes; it is not suitable for inducing oestrous and are most suitable during the breeding season (Whitley and Jackson, 2003). Prostaglandin injections requires assurance that ewes are not pregnant before injection because abortions may occur at any stage of the gestation. Injecting the ewes twice is a requirement for success of this method and 9-11 days apart, because prostaglandins are only effective if there is an active CL. Oestrous response and fertility following prostaglandin treatment, or its analogue, is affected by the dose level of prostaglandin, responsiveness of the CL, stage of OS, season, and inclusion of gonadotrophins as co-treatments (Wildeus, 1999). Omontese *et al*. (2016) reported a compromise in the follicular function of does following prostaglandin administration and a great variability in the timing of ovulation as a consequence. Variability is eliminated by the use of males, pre-treatment with progesterone or concurrent administration of gonadotrophins to increase LH secretion.

2.8.1.4 Natural oestrous synchronization method 'Ram effect'

In ewes oestrous is naturally induced by a planned introduction of rams into a flock of anestrus ewes. This method is advantageous in reduction of exogenous hormones that are used during other methods of OS (Omontese *et al*., 2016). Aggression of the ram used, lower ovulation rates of the first cycle, breeding season and loss of synchrony in subsequent cycles influence the response of ewes to this method of oestrous synchronization. This method is mostly efficient in anovular ewes than already cycling ones (Ungerfeld, 2003).

2.8.1.2 Factors affecting oetrous synchronization in ewes

Seasonality of breeding patterns in sheep is a major factor affecting OS especially in breeds of temperate regions. Seasonality of breeding in sheep is controlled by photoperiod, which adjusts hormonal balances and causes variation in reproduction. This affects fertility even if hormonal treatment has been applied to induce oestrous before AI or natural mating (Santolaria, 2017). Seasonality was discovered to affect sperm transport because of compromised cervical mucus quality, however Anel *et al.* (2005) argued that photoperiod changes progestogens and cervical mucus characteristics reducing its quantity and making it thicker. The induction of successful out of season OS and an increase in the number of pregnancies, litter size and treatment of anestrus have been reported where melatonin was administered, melatonin treatment imitate a short day like response (Mukasa-Mugerwa *et al*., 1994).

Conclusion

Indigenous sheep breeds are an under-utilized resource that can be improved to uplift the supply of meat locally. This would in turn assist in the conservation of these adapted breeds. Africa as a whole is facing problems with preventing loss of indigenous sheep breeds due to lack of proper documentation of what exists. Sustainable utilization of livestock diversity requires characterization of the available resources and development of sustainable genetic improvement strategies that consider the needs and perceptions of target groups and that minimizes loss of genetic diversity

Most indigenous breeds of South Africa such as Bapedi and Swati sheep characterization has only been done partially on phenotypic traits with limited information on genetic distinctness and how it relates to resulting reproductive performance.

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Chapter 3

Relationship among Bapedi rams' phenotypic and morphometric characteristics with semen parameters

Abstract

South African indigenous Bapedi sheep breed is facing genetic degradation due to unselective crossbreeding and irregular mating. Breeding strategies for urgent conservation of Bapedi sheep breed are a prerequisite. The aim of the present study was to investigate the relationship between body measurements, SC and semen traits of Bapedi rams conserved *in situ* and *ex situ in vivo*. In the present study, body measurements and semen traits of Bapedi rams were studied along with the inter-relationship between body measurements and semen parameters. A total of 31 rams were used in this study. Body measurements (cm), BCS (scale 1-5), BW (kg) semen volume (ml), sperm concentration (billions/ml) and sperm motility parameters as measured by computer aided semen analysis system (CASA®), four ejaculates were collected per ram. Data was analysed using Proc univariate procedure of SAS. Body weight (BW) of Bapedi rams ranged from 38-57 kg between the *ex situ in vivo* and *in situ* conservation methods. There was uniformity in all body measurements of Bapedi sheep regardless of conservation method $P<0.05$. There were no significant differences on the rectal body temperature during semen collection, SC, semen volume, semen pH, semen concentration, sperm total motility and sperm kinematics in Bapedi rams between methods of conservation ($P>0.05$). Furthermore, no significant differences were observed between live and dead sperm and sperm abnormalities. Pearson correlation revealed significant positive relationships between BW, BCS and SC ($r = 0.315$; $r = 0.638$; $r = 0.381$ respectively) with semen volume in Bapedi rams. Rump length was also found to positively influence sperm normality ($r=0.566$). It was concluded that Bapedi sheep are still a uniform breed, regardless of their decreasing numbers, and BW, BCS and SC can be included in the selection criteria for improving the reproductive performance of Bapedi

breeding rams. It is recommended that more studies be done in the correlation of body measurements of testicular morphometry and semen parameters in this breed.

Keywords: Bapedi rams, body condition score, body weight, morphometric traits, semen parameters.

3.1 Introduction

Production rate is the major profit determinant in a sheep farming enterprise and improvement may be attained through good reproductive performance (Akpa *et al*., 2012; Ramukhithi *et al.,* 2017). The ram contributes 50% to the flock's genetic material in animal husbandry, because it sires most of the lambs in the flock and has more genetic influence on the lamb crop (Perumal, 2014) compared to the ewe. Selection of fertile rams can be the most powerful method for improvement and conservation of indigenous sheep. However, prediction of ram fertility is an intricate process that is not defined by a single trait. Information on quantifiable physical parameters that directly correlate to fertilization capacity of sperm is required to advise farmers on ram selection activities. Body measurements reflect breed standards and are also very important in giving information about morphological structure and development ability of animals (Shirzeyli *et al*., 2013). Body measurements differ according to breed, gender and age.

The demand for semen from outstanding sires has increased with the development of frozen semen technology and the growth of artificial breeding organizations. Methods to predict sperm production potential and particularly to identify rams with high sperm output potential at an early age are important (Suleiman and Alphonsus, 2012). Traditionally, rams have been selected based on growth rates, rather than on reproductive traits (Perumal, 2014). However, reproduction is one of the most important factors for the economically viable livestock production practice. Males with larger testes and SC produce more sperm than males with

smaller testes. Regression equations revealed that testicular size was positively correlated to body weight and age (Santolari *et al*., 2009). Positive correlations between pre-pubertal male hormone levels and subsequent testes size and mating frequency have also been reported in bulls (Perumal, 2014).

Mating behaviour and semen quality are the main factors that limit male reproductive efficiency (Suleiman and Alphonsus, 2012). These may be influenced by the breed, geographical location, seasons, testicular size and age. Season of the year, however, was reported to be the principal factor affecting semen quality in goats of temperate regions with limited information on tropical regions (Mia *et al*., 2012). Irrespective of these factors, evaluation of semen characteristics is the most effective parameter for selecting breeding rams. Semen volume, sperm concentration, sperm motility, sperm viability and morphological features of spermatozoa determine the quality of semen in relation to fertility. Ejaculations containing a high percentage of morphologically abnormal spermatozoa commonly do not result in fertilization of the egg (Shamsuddin and Rodriguez, 1994). Morphological abnormalities of sperm can have a detrimental impact on fertilization and embryonic development (Saacke, 2008). The effects of indigenous sheep breeds on spermatozoa characteristics, particularly in South Africa, have received little attention (FAO, 2011). There are few studies that examined the association between body measurements such as testicular or scrotal size, body weight with components of semen and spermatozoa quality or sperm output. Linear body measurement is important in prediction of carcass weight and determination of certain body conformation traits that can be taken into consideration in selecting animals for genetic improvement. Any quantifiable physical parameters that directly correlate with the fertilization capacity of semen could be potentially used as a measure of semen quality. Sperm production and quality can be affected by both animal size and

physiological status. Which led to the objective of the current study, to evaluate possible correlation of Bapedi rams' phenotypic and morphometric traits with semen characteristics.

3.2 Materials and methods

3.2.1 Study sites, animals and experimental design

The Agricultural Research Council Animal Ethics Committees approved the project (Project no: APIEC 17/13). The study was conducted in four *in situ* conservation farms (Towoomba, Mara research stations; Tompi Seleka and Madzivhandila Agricultural Colleges) in Limpopo and one *ex situ in vivo* farm (Agricultural Research Council-Animal Production) in Gauteng provinces of South Africa. In this study a total 31 matured Bapedi rams available on each farm were used with average BW 48-68 kg; aged (2-6 years). The study was conducted during the breeding season of June-August 2017. Rams grazed on natural pastures with free access to water and shade. A randomized complete block design was used in this study to account for differences in management of individual farms and potential environmental factors.

3.2.2 Phenotypic characterization and morphometric trait measurements

A digital weighing scale was used to determine live weights of rams, and phenotypic traits were visually observed and recorded. Linear body measurements were measured using a flexible tape when animals were in standing position with head raised and weight on all four feet without body movements. Physical restraint was sometimes applied to limit movement as described by FAO (2012) and Akpa *et al* (2012). Seventeen metric traits were measured on each ram using the identification rump width (RW), rump length (RL), tail length (TL), wither height (WH), heart girth (HG), paunch girth (PG), rump height (RH), ear length (EL), foreleg

length (FLL), rear-leg length (RLL), body length (BL) and shoulder width (SW). Body condition scores (BCS) of the rams were recorded on a scale of 1 to 5. Scrotal circumference (SC) was measured with a flexible tape.

3.2.3 Semen collection

Prior to semen collection, hair around the sheath was shaved and the prepuce was cleaned with a sterile paper towel containing 70% ethanol for the prevention of contamination. A sterile probe of the electro ejaculator was lubricated before insertion into the rectum. The probe was inserted and placed in the rectum above the accessory sex glands for stimulation (Dombo, 2017). Semen samples were collected into pre-warmed (37°C) 15 ml graduated tubes and immediately placed in a thermos flask at 37 ˚C. The semen from all the rams was collected once a week, using an electro-ejaculator (RAMSEM, South Africa). The electric current was set in four levels of 30 volts. The collected semen was then transported to the laboratory within 30 minutes of collection. Fresh semen samples were macroscopically and microscopically evaluated for sperm concentration, sperm cell motility rate, and semen pH. A Computer Assisted Sperm Analysis (CASA®) system was used to evaluate the spermatozoa motility rates. A total of four ejaculates were collected per ram.

3.2.4 Semen evaluation

Measurements for semen volume were taken from reading the calibrated 15 ml tube. The semen pH was measured using a microprocessor pH/mV/^{0}C meter fitted with a glass probe. Semen concentration (sperm/mL) was determined with the aid of spectrophotometer (Jenway 6310, UK). A 15 µL semen sample was added in a cuvette containing 1000 µl sodium citrate solution and the cuvette was inserted into the spectrophotometer to give automated absorbance.

Absorbance was used to determine the final sperm concentration in millions per ml as described by Ramukhithi *et al*. (2017). The CASA system was used for analyses of sperm motility. The sperm swim-up technique was used where 10µl of the semen sample was added into a pre-warmed (37°C) 500µl of the tris medium. Only 10µl of the extended semen was placed into pre-warmed microscope glass slide and evaluated under (x10) magnification phase contrast microscope with the sperm class analyser (SCA®) projecting an image onto a monitor. The spermatozoa motility evaluated was expressed as the percentage progressively motile sperm (sperm with forward movement), percentage non-progressively motile sperm and percentage static (immotile) sperm. Sperm velocity parameters evaluated included static, slow, medium, rapid, curvilinear (VCL), straight-line (VSL), average path (VAP), linearity (LIN), straightness (STR) and wobble (WOB) velocities.

Eosin nigrosin stain was used to determine acrosome integrity, sperm cell viability, morphology and abnormalities, and evaluated under fluorescent microscope (Olympus, Japan). Semen smears were prepared on a clean warmed glass slide and dried at room temperature. A total of 200 sperm cells per slide were evaluated and counted for each ram per collection using DBC.6 Model laboratory counter (Han Lien International Corp). Gross structure sperm morphology was then recorded. The spermatozoa were recorded using two different sets of criteria, which are normal and abnormal. Abnormalities were categorised as primary, secondary and tertiary abnormalities.

3.2.5 Statistical analysis

Data was analysed with statistics analyses software (SAS, 2009). Analysis of variance (ANOVA) was used to test for significant differences in semen concentration, semen volume, semen pH, spermatozoa motility and morphology. Correlation analysis procedure of SAS (2009) was used to assess the relationship between the measured variables. The morphometric

traits data was analysed using the general linear model procedure of SAS (2009). Means between the two conservation methods (*ex situ in vivo* and the *in situ*) were compared using contrasts.

3.3 Results

Table 3.1 shows comparison of morphometric parameters between Bapedi rams conserved *ex-situ in vivo* and *in situ*. There were no significant differences on body measurements of Bapedi sheep such as BL, HL, HW and RH ($P>0.05$) regardless of the conservation method. The BW was higher for the in situ conserved Bapedi rams compared with the ex situ in vivo rams. The RW and HG measurements were similar between three stations (ARC, Madz and TMPS) and were significantly higher than RW (24.2±8.5) and HG (88.0±3.3) observed in Mara Research Station ($P<0.05$). Conversely, Mara Research Station had lower RL (17.4±1.1) measurements compared to the values observed for Madz (19.8±0.8) and did not differ with TMPS (18.9±2.5) and ARC (19.1±2.4). Furthermore, the ear length was longer for rams in Madz (12.1±1.1) and TMPS (11.5±1.2) compared to Mara (10.2±1.4) conservation farms and ARC farm obtained similar measurements to all the farms ($P<0.05$).

Table 3.1: Morphometric traits between *ex- situ in vivo* and *in situ* conserved Bapedi sheep (Mean ± SE)

Variables		N	BW	BL	HL	HW	RH	RW	RL	TL	HG	EL
Farm	ARC	8	40.2±4.6	67.6±6.5	17.8±1.1	10.9±0.7	67.2±8.2	17.8±1.4^{a}	19.1±2.4^{ab}	37.1±4.2^{b}	80.4±7.4^{a}	11.3±0.9^{ab}
	Madz	8	46.9±10.3	63.0±3.6	17.8±0.8	10.8±0.9	66.1±3.5	18.1±1.1^{a}	19.8±0.8^{a}	36.4±5.5^{ab}	80.3±6.7^{a}	12.1±1.1^{a}
	TMPS	8	47.2±7.6	64.3±6.4	18.4±1.9	10.8±1.2	67.6±5.5	15.7±1.7^{a}	18.9±2.5^{ab}	34.4±4.8^{a}	74.2±3.4^{a}	11.5±1.2^{a}
	Mara	7	46.2±6.3	62.6±2.5	17.3±1.5	11.0±1.3	64.6±1.7	24.2±8.5^{b}	17.4±1.1^{b}	32.2±3.2^{a}	88.0±3.3^{b}	10.2±1.4^{b}
Method	*Ex-situ in vivo*	8	40.2±4.6^{a}	67.6±6.5	17.8±1.1	10.9±0.7	67.2±8.2	17.8±1.4	19.1±2.4	37.1±4.2	80.4±7.4	11.3±0.9
	In-situ	23	46.7±8.1^{b}	63.3±4.1	17.8± 1.4	10.8±1.1	66.1±3.5	19.3±3.8	18.7±1.5	34.3±4.5	80.8±4.5	11.2±1.2

BW: Body weight BL: Body length; HL: Head length; HW: Head width; RH: Rump height; RW: Rump width; RL: Rump length; TL: Tail length; HG: heart girth; EL: ear length. ^{a,b} Values with different superscripts for a variable (farm or method) within a column differ significantly ($P< 0.05$). ARC; Agricultural Research Council, Madz: Madzivhandila Agricultural College, TMPS: Tompi Seleka Agricultural College, Mara; Mara Research station.

Table 3.2 shows a comparison between rectal body temperature, body weight and macroscopic semen parameters. Similar measurements for body weight were obtained for all rams from different stations (P>0.05). Body temperature for Mara Farm rams (39.5±0.5) was significantly higher compared to Madz (38.7±0.4) and TMPS (38.4±0.6) farms, and no significant differences were observed for the ARC farm rams (39.0±0.5) compared to the other rams. No significant differences were observed on scrotal circumference measurement in Bapedi rams from all the farms. Correspondingly, semen volume, semen pH and semen concentration obtained from these rams did not differ (P>0.05).

Table 3.2: Influence of rectal body temperature on macroscopic semen traits in Bapedi rams (Mean ±SE)

Variables		N	BT	SC (cm)	SV (mL)	pH	Conc
Farm	ARC	8	39.0±0.5^{ab}	28.1±1.5	1.1±0.4	7.0±0.5	2.1±0.2
	Madz	8	38.7±0.4^{b}	27.0±2.4	1.0±0.4	6.9±0.1	2.2±0.2
	TMPS	8	38.4±0.6^{b}	29.4±3.4	1.1±0.5	6.9±0.2	2.3±0.3
	Mara	7	39.5±0.5^{a}	28.8±2.0	0.9±0.3	6.9±0.0	2.2±0.3
Method	*Ex situ in vivo*	8	39.0±0.5	28.1±1.5	1.1±0.4	7.0±0.5	2.1±0.2
	In situ	23	38.8±0.5	28.4±2.6	1.0±1.4	6.9±0.1	2.2±0.3

BT; body temperature; SC: scrotal circumference SV: semen volume; Conc: sperm concentration. ^{a,b} Values with different superscripts for a variable (farm or method) within a column differ significantly ($P< 0.05$). ARC; Agricultural Research Council, Madz: Madzivhandila Agricultural College, TMPS: Tompi Seleka Agricultural College, Mara; Mara Research station.

Table 3.3 shows microscopic sperm traits amongst Bapedi sheep kept *in ex situ in vivo* and *in situ*. The total sperm motility for Tompi Seleka farm (98.3±1.1) was significantly higher compared to Madz (86.3±11.8) and Mara (85.1±14.3) at $P<0.05$ and the ARC rams did not differ with all the three farms. The method of conservation did not affect the ram's total motility. Progressive motility was lower ($P<0.05$) for the ARC (34.5±13.6) rams compared to Madz (49.9±12.8) and did not differ from Mara (46.6±16.6) and TMPS (45.8±9.9) rams ($P>0.05$). The percentage of non-progressive motility and rapidly moving sperm, were higher for ARC (59.6±11.9; 46.5±16.1) and TMPS (52.4±10.5; 70.6±8.5) compared to Madz (36.4±8.3; 36.6±12.8) and Mara (38.5±12.2; 36.8±19.2), respectively. Tompi Seleka rams had lower percentages of MED, SLW and STC sperm compared to the other farms even though statistically insignificant. The VCL was significantly higher for TMPS compared to other three farms ($P<0.05$).

Table 3.3: Comparison of microscopic semen parameters between *ex-situ in vivo* and *in situ* conserved Bapedi sheep (Mean± SD)

Variables	Farm				Method	
	ARC	MADZ	MARA	TMPS	*Ex-situ in vivo*	*In-situ*
N	8	8	7	8	8	23
TM	94.2±13.2^{ab}	86.3±11.8^{b}	85.1±14.3^{b}	98.3±1.1^{a}	94.2±13.2	89.9±9.1
PM	34.5±13.6^{b}	49.9±12.8^{a}	46.6±16.6^{ab}	45.8±9.9^{ab}	34.5±13.6	47.4±13.1
NPM	59.6±11.9^{a}	36.4±8.3^{b}	38.5±12.2^{b}	52.4±10.5^{a}	59.6±11.9	42.4±10.3
RAP	46.5±16.1^{b}	36.6±12.8^{b}	36.8±19.2^{b}	70.6±8.5^{a}	46.5±16.1	48.0±13.5
MED	20.6±8.0^{ab}	24.3±14.3^{a}	21.4±8.7^{a}	11.7±4.2^{b}	20.6±8.0	19.1±9.0
SLW	30.5±13.4^{a}	25.3±9.2^{ab}	26.8±10.8^{ab}	16.0±8.4^{b}	30.5±13.4	22.7±9.5
STC	4.9±9.8^{ab}	13.7±11.8^{a}	14.8±14.2^{a}	1.6±1.1^{b}	4.9±9.8	10.0±9.0
VCL	116.3±22.6^{b}	116.9±13.3^{b}	115.1±17.5^{b}	149.1±9.6^{a}	116.3±22.6	127.6±13.5
VSL	64.1±18.9^{ab}	68.7±14.7^{ab}	61.8±19.2^{b}	82.1±15.4^{a}	64.1±18.9	70.9±16.4
VAP	82.8±20.7^{b}	89.9±11.8^{ab}	84.8±18.1^{b}	104.5±13.3^{a}	82.8±20.7	93.1±14.4
LIN	54.8±10.9^{a}	54.7±9.4^{a}	49.4±9.9^{a}	55.1±10.1^{a}	54.8±10.9	53.1±9.3
STR	76.9±6.9^{ab}	70.1±8.2^{bc}	66.4±8.7^{c}	78.2±6.6^{a}	76.9±6.9	71.6±7.8
WOB	70.8±9.0^{a}	72.8±5.2^{a}	69.3±6.7^{a}	70.0±7.9^{a}	70.8±9.0	70.7±6.6

TM = total motility, PM = progressive motility, NPM = non-progressive motility, VCL =curvilinear velocity, VSL= straight-line velocity, VAP = average path velocity, LIN = linearity, STR = straightness and WOB = wobble. Values with different superscripts for a variable (farm or method) within a row differ significantly ($P<0.05$). ARC: Agricultural Research Council, Madz: Madzivhandila Agricultural College, TMPS: Tompi Seleka Agricultural College, Mara: Mara Research station.

Table 3.4 shows the morphology of sperm in Bapedi sheep kept under different conservation methods. There were no significant differences observed between Bapedi ram semen on viability, and percentage of live sperm abnormalities**.**

Table 3.4: Comparison of sperm viability and morphology between *ex-situ in vivo* and *in situ* conserved Bapedi sheep (Mean ± SE)

Variables		N	Viability		Abnormal sperm (%)		
			LIVE	DEAD	Head	Mid piece	Tail
Farm	ARC	8	79.4±5.8	20.6±4.9	0.8±0.6	1.8±1.4	4.5±1.9[a]
	Madz	8	80.8±4.6	19.2±4.2	0.3±0.2	1.1±1.2	7.4±3.2[a]
	TMPS	8	84.2±7.9	15.8±7.8	0.7±0.2	1.3±1.0	6.2±2.7[ab]
	Mara	7	74.9±14.4	25.1±14.1	0.7±0.4	1.8±1.5	5.6±2.1[ab]
Method	*Ex situ- in vivo*	8	79.4±5.8	20.6±4.9	0.8±0.6	1.8±1.4	4.5±1.9
	In situ	23	79.9±8.9	20.0±8.7	0.5±0.2	1.4±1.2	6.4±2.7

[a,b] Values with different superscripts for a variable (farm or method) within a column differ significantly (P< 0.05). ARC: Agricultural Research Council, Madz: Madzivhandila Agricultural College, TMPS: Tompi Seleka Agricultural College, Mara: Mara Research station.

Table 3.5 shows the correlation between the body measurements and the macroscopic semen traits of Bapedi sheep. A significant positive relationship was found between SC, BW, and semen volume. Furthermore, BCS showed a strong positive correlation with semen volume $P<0.05$ and was positively correlated with total motility at $P<0.05$. Rump Length (RL) showed a significant positive relationship with normal sperm at $P<0.05$. There was also a significant negative relationship between heart width and linearly moving sperm. All the other morphometric traits (negative or positive) were found not to significantly influence the semen volume, pH, concentration and the sperm total motility.

Table 3. 5: Pearson correlations among body measurements, semen parameters and sperm morphology

Body measurements	Semen parameters								Sperm morphology	
	Vol	pH	Conc	TM	VCL	LIN	Vit	Vib	Normal	Abnormal
BL	0.308	0.037	0.092	0.05	-0.041	0.046	0.063	0.282	-0.018	0.106
CC	-0.155	0.014	0.112	-0.021	0.182	0.035	0.021	-0.491	0.06	0.092
HL	-0.056	0.089	0.075	0.36	0.369	0.087	-0.020	-0.234	0.078	-0.171
HW	-0.149	-0.190	-0.169	0.122	-0.232	-0.397*	-0.11	0.208	-0.052	0.068
RH	-0.234	-0.193	-0.115	0.108	0.188	-0.254	-0.075	0.154	0.166	0.057
RW	-0.241	0.068	0.112	0.060	-0.012	0.354	0.009	-0.381	-0.318	0.104
RL	0.097	0.046	0.33	-0.083	-0.005	-0.001	-0.008	-0.108	0.566*	-0.157
SW	0.032	-0.168	-0.017	0.124	0.274	0.067	0.191	0.173	0.004	-0.103
TL	0.235	0.226	0.069	0.146	-0.157	0.364	0.277	-0.344	0.197	-0.299
HG	-0.107	0.040	-0.055	-0.009	-0.187	0.311	-0.022	-0.106	-0.156	-0.099
EL	0.046	-0.058	0.122	0.106	0.074	0.088	0.070	-0.159	-0.003	0.092
SC	0.381*	0.092	0.071	0.245	-0.03	-0.116	0.148	0.054	0.077	0.230
BCS	0.638**	-0.263	0.020	0.416*	0.042	0.233	-0.025	-0.321	0.135	0.072
BW	0.315*	0.062	-0.040	0.311	0.066	-0.104	-0.009	-0.055	0.102	0.139
BT	0.105	0.119	-0.031	-0.120	-0.054	0.170	0.005	-0.320	-0.227	

BW: Body weight BL: Body length; HL: Head length; HW: Head width; RH: Rump height; RW: Rump width; RL: Rump length; TL: Tail length; HG: heart girth; EL: ear length. Vol: semen volume; TM: total sperm motility; Vit: vitality; Vib: viability; Abno: abnormal sperm*= Significant if P <0.05

3.4 Discussion

The results shown in Table 3.1 presented no influence of conservation method for Bapedi rams when body measurements were compared. Similar body weight, length; head length, width and rump height were obtained amongst Bapedi rams in all four farms, this indicated the homogeneity of the breed. These results are similar to the findings on the Zulu sheep by Mavule, (2012) even though Bapedi rams have higher body weight, rump height, tail length and ear length compared to South African indigenous Zulu sheep. However, Bapedi sheep had smaller, head length (average 18.1± 1.3) and width (10.8±1.0) compared to Zulu sheep. Results from this study concur with findings from (Kunene, 2010; Gwala *et al*., 2015) in that Bapedi sheep is heavy compared to other South African indigenous breeds (Swati and Zulu) and Mozambique (Landim) sheep. Furthermore, Bapedi sheep's morphometric measurements were lower compared to Nigerian indigenous sheep Yankasa, Uda and Balami sheep (Yakubu *et al.*, 2011), and higher compared to Mexican Croele sheep without ears (Israel *et al*., 2013).

Increase in testicular temperature is an important factor in determining the thermal stress and reproductive efficiency of rams. Testes regulate its own temperature but testicular temperature is dependent on body temperature, which is often times measured through rectal temperature (Teodoro *et al*., 2013). Body growth and biological functions and semen quality of rams are affected by environmental temperatures higher than 29°C (Ahmmed *et al*., 2016). The climate in Limpopo where Bapedi sheep originate is characterised by high temperatures throughout the whole year, high temperatures affect reproductive efficiency of rams. The results obtained from this study-depicted that rams from Mara have higher rectal temperature compared to the rest of the farms. However, that did not affect the scrotal circumference measurements, semen volume, semen

pH and concentration as it was similar in all Bapedi rams in different farms and conservation methods. The absence of differences in the parameters analysed between farms could be due to adaptability of animals. Scrotal circumferences (SC) obtained in this study were lower in all farms than 31.3±0.8 cm recorded by Munyai (2012) on *ex situ in vivo* conserved Bapedi rams. Furthermore, high semen volume and spermatozoa concentration were obtained in all farms used in this study compared to 0.5±0.1 mL and 0.9±0.2 (109/ mL) respectively (Munyai, 2012). For semen pH, the results from this study agree with findings by Munyai (2012). Bapedi sheep in this study had higher ejaculate volume, semen concentration, and more acidic semen pH compared to other South African indigenous breeds (Namaqua Afrikaner, Damara and Zulu sheep) (Buduramp, 2004). Semen in this study was collected during the Bapedi sheep-breeding season, which is June to August according to Bapedi sheep farmers in Limpopo. Ejaculate volume and semen concentration obtained from this study were similar to results found in Indian Malpura rams and lower compared to crossbred Barat Merino (Kumar *et al.,* 2009). Awassi and Bangladesh native rams showed similar ejaculate volume with greater semen concentration compared to Bapedi rams (Salhab *et al*., 2003; Pervage *et al.,* 2009).

Semen evaluation is a very important element for selection of rams for natural mating or artificial insemination (AI). Semen can be analysed either subjectively or objectively depending on availability of equipment. Subjective analysis of semen quality is cheaper and easier to perform than objective methods but do not provide accurate estimates compared to computer aided semen analysis system (CASA) (Kumar *et al*., 2009). The CASA system gives precise, validate and rapid objective sperm movement traits. The conservation area did not affect the microscopic sperm quality characteristics in this study. Munyai (2012) recorded lower total sperm motility (74%) percentages for Bapedi sheep compared to this study. However, they recorded high number of

rapidly moving sperm than ARC, MADZ and MARA farms but lower than TMPS farm where the first flock of Bapedi sheep was established and maintained in Stellenbosch breeding station in Sekhukhune district (Ramsay, 2001). Progressive motility percentage of 52.7±13.3 and non-progressive motility of 22.2±19.3 were obtained by Munyai (2012) in Bapedi sheep and were better than results obtained in this study. For all velocity parameters Munyai (2012) obtained better results compared to this study. Furthermore, more wobbling sperms were obtained compared to this study. All the sperm quality traits obtained from this study were within the acceptable standards for natural mating and AI (Anel *et al*., 2005). The Bapedi ram microscopic sperm characteristics obtained in this study were higher than Zulu, Namaqua and Damara rams' sperm traits (Munyai, 2012). For track dimensions the results obtained in this study were lower compared to VCL (228.6±6.48; 253.3±6.48 obtained for Bharat Merino and Malpura rams respectively), however, total motility was high in Bapedi rams. The total sperm motility obtained in this study was higher compared to Bharat Merino and Malpura rams however they had higher rapid and medium moving sperm compared to Bapedi rams from ARC, MADZ and Mara farms and their results were similar to those of TMPS farm (Kumar, 2009). Similar total motility was obtained in indigenous rams of Bangladesh (Azizunnesa *et al*., 2014; Ahmmed *et al*., 2016). The results obtained from this study were better compared to other studies (Pervage *et al*., 2009; Mahmhda *et al.*, 2015).

Sperm morphology is used as an important standard in semen quality evaluation, as it is associated with good prediction of fertilising ability of a sperm (Ahmmed *et al.,* 2016). There were no significant influences of conservation method evident on the percentage of live and dead sperm in Bapedi sheep from all the farms. Higher percentage of live sperm was obtained in this study compared to findings by (Munyai, 2012). However, similar findings were obtained from

Bangladesh indigenous ram sperm (Hassan *et al*., 2009; Mahmuda *et al*., 2015; Ahmmed *et al*., 2016; Rekha *et al*., 2016). The sperm cell abnormalities obtained from this study are within the range of acceptable standard for breeding rams (Munyai, 2012). The proportion of abnormal sperms obtained in this study was lower than the results obtained by Taha *et al*. (2000) and Langeveldt (2016) in Dormer (12.70%) and Merino rams (10.36%), respectively.

There is a general lack of good quality breeding rams in smallholder sheep farming in South Africa, therefore there is a need to find a valid, affordable diagnostic approach for selecting good breeding rams. There are many techniques that are used to test vital aspects of sperm function. However, these can be very complex, expensive and there are no universal standards. There is a need for a model that would enable farmers to predict advanced variables from a number of basic morphometric traits. Scrotal circumference of Bapedi rams in this study was measured during the breeding season for accuracy of the results. Etim (2015) reported that SC will vary with season and body conformation, but should be at its maximum peak during the full breeding season. Body weight (BW), BCS and SC ($r = 0.315$; $r = 0.638$; $r = 0.381$ respectively) showed a positive and significant influence on the semen volume in Bapedi rams, these findings are similar to a study that was conducted by Rajashri *et al*. (2016) who reported a significant ($P<0.05$) positive correlation ($r=0.298$) between SC and semen volume; SC and semen concentration ($r=0.836$). However, semen concentration in this study showed no relationship with SC. In the current study BCS had a positive correlation with the total motility of the sperm, this contradicts finding from a study in Murrah buffalo where BCS was negatively correlated to sperm total motility (Sing *et al*., 2017). However, is in agreement with findings by Addass (2011) who reported that generally sperm production increases in bulls with increasing age and BCS suggesting that breeding bulls (natural/artificial) should attain full maturity age and at a higher body condition scores, however

not more than seven years of age. Most body measurement showed no significant influence on the semen characteristics and the results found in this study is similar to result found by other researchers (Ostermeier *et al*. 2001; Ramukhithi *et al*., 2017).

3.5 Conclusion

It was concluded that Bapedi sheep do not change regardless of the conservation method. The body weight, BCS and SC can be included in the selection criteria for improving the reproductive performance of Bapedi breeding rams. It is recommended that more studies be done in the correlation of body measurements of testicular morphometry and semen parameters in this breed.

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Chapter 4

Influence of age and body condition score on oestrous synchronization response in Bapedi ewes

Abstract

Oestrous synchronization programme in sheep is associated with genetic selection schemes of improved breeds, but it has not been applied to South African indigenous sheep breeds that are on the verge of extinction. The present study evaluated the effects of age and body condition score on the response and duration of oestrous in synchronized Bapedi ewes, conserved *ex situ in vivo* and *in situ*. A total of 91 Bapedi ewes (<2 and 3-6 years of age) were used in the study, ewes used in this study were conserved *ex situ in vivo* (grazing and supplementation) and *in situ* (grazing only) and water was provided *ad libitum*. Ewes were assigned to body condition scores (BCS) <3 and BCS≥3 on scale of 1–5 where 1 is very thin and 5 obese. For OS, controlled intravaginal drug release(CIDR®) dispensers were inserted for 9 days and 300 IU of equine chorionic gonadotrophin was administered intramuscularly immediately after CIDR removal. Oestrous detection was done for a period of 72 h, from CIDRs withdrawal at 12 h interval with a vasectomised ram. Data was analysed using Proc GLIMMIX of SAS version 9.1. There were no significant differences observed in oestrus response of ewes regardless of age (P>0.05) and method of conservation. The oestrous response was higher in ewes with BCS≥ 3 compared to lower BCS group (P< 0.05). Old and lower BCS ewes showed oestrus signs earlier (23±2.8; 21±4.1); (22 ± 4.1; 20 ±5.3) and with a shorter duration in hours (23 ± 5.2; 20 ±6.2); (22 ± 4.0; 23 ± 3.2) compared to young and higher BCS groups (onset of oestrus: 34±2.0; 32±2.4); (36 ± 1.3; 35±2.3) duration (30±1.3; 29±1.5); (33±5.0; 32 ± 6.0) (P<0.05). In conclusion, age did not affect the proportion of ewes that responded oestrous synchronization. However, higher BCS ewes had higher percentage of ewes that

responded to oestrous. Young and high BCS ewes had a delayed onset of oestrus that lasted longer compared to old and lower BCS ewes.

Keywords: Age, body condition, oestrous synchronization.

4.1 Introduction

South Africa has 23, 71 million sheep but remains an importer of mutton (Department of Agriculture Forestry and Fisheries, 2016). To grow and diversify production from livestock in South Africa sheep farming might be the best choice as indigenous sheep are well-known for adaptability, survivability and disease tolerance. Production of mutton locally needs to be increased to meet up with the increasing demand. Ensuring good reproductive performance of locally adapted breeds would be a great strategy, and only then will the aim to secure food for the future be a reality (Kunene, 2010; Ramukhithi *et al*., 2017). Indigenous sheep secure food and provide a source of income to smallholder farmers (Webb *et al*., 2004). Bapedi sheep is a naturally selected, minimum care veld sheep that thrives well in harsh environmental conditions. Bapedi sheep are resilient to diseases and seldom deworming is practiced to a few that need it and never the whole flock. They produce lean healthy meat because the fat is centralized in the tail. In South Africa, Blackhead Persian has a yearlong estrus activity, with Merinos and Dorset having an extended estrus period such that they could have two lamb crops per year (Debaca *et al*., 2017). The importance of studying the approximate time of onset of oestrous in ewes assists not only for mating on time but also for having a uniform lambing time. The reproductive performance of indigenous sheep breeds such as Bapedi, Zulu and Namaqua Afrikaner has not been fully studied and hence there are no strategies in place for conservation and utilization (Nsoso *et al*., 2004). There is limited information on the onset and duration of estrus of Bapedi sheep. Hafez 2007

reported that breeding season is controlled by both genetic and environmental factors and hence breeds differ significantly in this regard. Reproductive efficiency is a key factor in any livestock enterprise (Perry, 2005). Assisted reproductive technologies such as oestrous synchronization may be adopted to improve ovulation rates. Animals of different ages have different physiological developments and respond differently to hormonal treatments, this might reduce the impact of reproductive technologies on livestock improvement programs (Lehloenya and Greyling, 2010).

Estrus synchronization in ewes is an effective reproductive technology that favours productivity, eases management and offers homogeneous groups of lambs at suitable seasons to take advantage of niche markets, feed supplies and rising price trends (Whitley and Jackson, 2004). Synchronization of oestrous is achieved by hormonal treatments such as natural progesterone or synthetic progestogens in combination with gonadotrophin releasing hormones for increased ovulation rates (Iida *et al.,* 2004). The ovarian response of ewes to oestrous synchronization changes according to type of intravaginal device, kind of progestogen, body condition, presence of stress factors, environmental aspects, male effect, age of the ewes and breed (Cavalcanti *et al*., 2012). Body condition score (BCS) is a management tool used to monitor nutrition of flocks, especially when they are on pastures. Body condition scores are scored on a scale of 1 to 5. Animals with scores of 1 to 2 are thin and it is easy to feel the bones in loin region, firm pressure is needed to feel the bones in condition scores of 3 to 4. In animals of BCS of 5 it is difficult to feel the bones (Gootwine, 2016). It is used to subjectively estimate fat deposition level and muscle thickness on the backbone behind the ribs (Jalilian and Moeini, 2013). Body condition is used to predict negative energy balance severity, where the loss of energy is associated with suppressing the luteinizing hormone and ewes with higher body condition were reported to have increased ovulation rates and higher circulation of follicle stimulating hormone and lower estradiol

concentration (Vinoles *et al.,* 2002). Vinoles *et al*. (2005) reported that nutrition plays a key role in encouraging proliferation in ovulation rates, and ewes in acceptable body condition for breeding had higher number of gonadotrophin dependent follicles compared to lower body condition ewes. Ewes with BCS of 3 were reported to have high number of lambs born per ewe while less number of lambs per ewe were reported for BSC of 3.5 (Jalilian and Moeini, 2013). Opposing results from other studies were observed, females with lesser BCS that were found to have a short breeding season, abnormal oestrous cycles and fewer ovulations than ewes with greater body condition (Yilmaz *et al.,* 2011; Wang *et al*., 2016). Low body condition scores are associated with many reproduction disorders such as delayed puberty, reduced ovulation rates, pregnancy rates and postpartum anestrus. Age is another important factor reported to affect ovulation rates in ewes, adult ewes are said to have higher fertility compared to young and maiden ewes (Santolaria *et al*., 2017). Scaramuzzi and Radford (1983) argued that the weight of ewes is a major determinant of whether ovulation will occur or not. The discrepancy reports about the effect of BCS and age on the oestrous response has led to the objective of this study; to evaluate the effects of age and BCS on the response and duration of oestrous in synchronized indigenous Bapedi ewes.

4.2 Materials and Methods

4.2.1 Animals and experimental design

This experiment procedures and management of animals was evaluated and approved by the Agricultural Research Council ethics committee under the Germplasm Conservation and Reproductive Biotechnologies department (APIEC 17/13).

The trial was conducted on ewes at five indigenous animal conservation farms *ex situ in vivo* and *in situ*. Agricultural Research Council (n = 40), Mara Research Station (n=20), Towoomba

Research stations (n = 12) and Tompi Seleka College of Agriculture (n=19). Madzivhandila Agricultural College had 3 ewes and was excluded from the study for reliability and validity of the data. Ewes of average body weight 48-68 kg, aged 2- 6 years were used in this study. The ewes used in this study were grazing on natural pasture, and water was provided *ad libitum*. This study followed a factorial design to evaluate the influence of age, body condition score and method of conservation on ewes' response, onset and duration of oestrus following synchronization with exogenous hormones. Ewes were grouped according to their body condition on a scale of 1-5 and age. Ewes with body condition scores of BCS of <3 for *ex situ in vivo* group (n = 15) and BCS≥3; (n = 25) and; ≥3 for *in situ* BCS <3 (n = 22) BCS≥3 (n = 29) respectively. Ewes were further grouped according to age counting the number of permanent incisors and farm records.

4.2.2 Oetrous synchronization

Oestrous cycles of ewes were synchronized using a short-term progestogen protocol as described by Ramukhithi *et al.* (2014), where a controlled internal drug release device (CIDR) (0.3 g progesterone) [(Pfizer^{TM}. New Zealand) (Ltd)], was inserted in the vagina of the ewe for 9 days. Ewes were injected intramuscularly with 300 IU of equine chorionic gonadotrophin (eCG) (Intervet Schering-Plough Animal Health, South Africa) on the day of progestogen removal. At 0, 12, 24, 36, 48, 60 and 72 hrs following progestogen withdrawal ewes were observed for signs of heat for 30 minutes using a vasectomised ram. The time of onset of oestrus and the duration were recorded in both groups 1 and 2.

4.2.3 Statistical analysis

Comparison between two conservation methods on the percentage of ewes showed oestrus, time of onset of oestrus, duration of oestrus were determined by using student t-test. Comparison among two conservation areas on the percentage of ewes showed oestrus, duration of oestrus and onset of oestrus were determined using one-way ANOVA. Ewes on each conservation method were grouped according to BCS and age. Data regarding the effect of BCS and age on oestrus onset and duration were analysed using the GLIMMIX procedure of SAS version 9.1. The results were measured significant when $P<0.05$.

4.3 Results

The oestrous response of Bapedi ewes was not influenced by the conservation method and age ($P>0.05$). The results were similar when young ewes on the *ex situ in vivo* group (34±2.0; 30 ± 1.3: h) were compared with young Bapedi ewes on the *in situ* group (32 ± 2.4; 29 ±1.5: h) for the onset and duration of oestrus respectively. A similar trend was also observed with older ewes and no significant differences were observed on the onset and duration of oestrus between *ex situ in vivo* (23 ± 2.8; 23 ± 8.2*)* and *in situ* (21 ± 4.1; 20 ±6.2) old ewes ($P>0.05$). The time interval between CIDR® removal and onset of oestrus was significantly earlier with short duration for the old ewes compared to the young ewes in both *ex situ in vivo* and *in situ* conservation methods ($P< 0.05$) as shown in Table 4.1.

Influence of body condition on the oestrous response, onset of oestrus and duration are set out in Table 4.2. Oestrus response was higher for BCS≥3 ex situ in vivo (92%) and in situ (90%) compared to BCS <3 ex situ in vivo (73%) and in situ (73%) ($P<0.05$). Ewes with lower body

condition showed heat signs earlier (P<0.05) (22±4.1h; 20 ±5.3) than ewes with BCS≥3 (36±1.3h; 35± 2.3b). Similarly, duration of the oestrous was longer (P<0.05) in high BCS ewes (33 ± 5.0; 32 ± 6.0) compared to low BCS ewes (22 ± 4.0; 23 ± 3.2) regardless of the conservation method.

Table 4.1: Effect of Age on oestrus synchronization response, onset and duration of oestrus (Mean ± SE) in young and old Bapedi conserved *in situ* and *ex situ in vivo*

Method	Age	N	Oestrus response (%)	Onset of oestrus (h)	Duration of oestrus (h)
Ex situ- in vivo	Young (1-2)	18	83 (15/18)	34 ± 2.0[a]	30 ± 1.3[a]
	Old (3-6)	22	82 (18/22)	23 ± 2.8[b]	23 ± 5.2[b]
In situ	Young (1-2)	20	80 (18/20)	32 ± 2.4[a]	29 ±1.5[a]
	Old (3-6)	31	84 (26/31)	21 ± 4.1[b]	20 ±6.2[b]

[ab] Means within the same column with different superscripts differ significantly (P<0.05).

Table 4.2: Effect of body condition score on the oestrus synchronization response (%), onset of and duration of oestrus (h) (Mean ± SE) conserved *in situ* and *ex situ in vivo*

Method	Age	N	Oestrus response (%)	Onset of oestrus (h)	Duration of oestrus (h)
Ex situ- in vivo	BCS<3	15	73 (11/15)[a]	22 ± 4.1[a]	22 ± 4.0[a]
	BCS≥3	25	92 (23/25)[b]	36 ± 1.3[b]	33 ± 5.0[b]
In situ	BCS<3	22	73 (16/22)[a]	20 ±5.3[a]	23 ± 3.2[a]
	BCS≥3	29	90 (26/29)[b]	35± 2.3[b]	32 ± 6.0[b]

[ab] Means within the same column with different superscripts differ significantly (P <0.05).

4.4 Discussion

In the current study, no significant differences were observed on the estrus response of ewes in all age groups. These results agree with findings by Webb *et al*. (2010) where the age of the ewe did not affect ovulation rates. The onset of oestrus was earlier in old ewes with a shorter duration compared to young ewes. Similar degree of synchrony was obtained in other studies (Roy *et al*. 2014). When evaluating onset of estrus adult ewes recorded a shorter time from CIDR® removal to first signs of heat compared to the young ones (Simonetti *et al*., 1999; Omontese *et al*., 2014). Results obtained from this study are comparable to results obtained from work done in goats by Lehloenya and Greyling 2010, where time to onset of oestrus was shorter for the adult compared to young does. Moreover, Debaca *et al*. (2017) also reported similar findings. Menzies (2018) reported conflicting findings where it was reported that maiden ewes have a shorter and less intense oestrus than old ones. Contentious, to these findings no differences were reported with regard to onset of oestrus when adult and lamb ewes were synchronized using medroxyprogesterone and pregnant mare serum gonadotrophin over 14 days (Simonetti *et al*., 1999). Age influences ovulation rate and lambing performance (Debaca *et al.,* 2017). It was reported that adult ewes produced more multiple births than younger ewes and that the percentage of multiple births increased with age of the ewe (Roy *et al.,* 2014; Debeca *et al*., 2017). Furthermore, in another study it was observed that yearlings and 5-7-year-old ewes had lower ovulation rates compared to 4-6-year-old ewes (Menzies, 2018) and concluded that lower percentages of multiple births in yearlings was as a results of lower ovulation rates.

Body condition affects oestrous, endocrine, follicle development and conception for both livestock and human beings (Yilmaz *et al*., 2011). Ewes that are adequately nourished and maintained in

good body condition respond most rapidly to oestrous synchronization with an increase in ovulation rate (Satolaria *et al*., 2013; Todorov and Nedelkov, (2015). Results obtained from this study indicated that body condition influences the response, onset and the duration of oestrous in Bapedi ewes. Higher BCS ewes responded better to oestrous synchronization (92%) compared to lower BCS (73%) and the duration of oestrous was longer on high BCS compared to lower BCS ewes irrespective of conservation method. For ewes with low BCS's onset of oestrous from CIDR removal was shorter ($P<0.05$) compared to high BCS group. There is limited information on the influence of BCS on synchronization response of ewes. Similar results were obtained when BCS 2 and 3 does were synchronized for oestrous and BCS 2 showed oestrous signs earlier (25-60 h) after CIDR® removal compared to the higher BSC group (30-70h), however synchrony and duration were significantly higher and longer in does on the BCS 3 group compared to lower BCS group (Widayati *et al*., 2011). Todorov and Nedelkov (2015) obtained similar results, where ewes of different body condition scores were synchronized by introduction of a teaser ram and the high BCS ewes responded better compared to ewes with BCS < 2. There is limited information on the influence of BCS on the response, onset and duration of ewes to oestrous synchronization with exogenous hormones. Yilmaz *et al.* (2011) reported that BCS>2.1 positively affected reproductive performance and lower BCS ewes during mating resulted in poor pregnancies, lower litter size and were most likely to abort. Similarly, in Malpura ewes BCS 3-3.5 was reported an optimum score for improving fertility (Sejian *et al*., 2009). According to Widayati *et al*. (2011), BSC affects the circulation of reproductive hormones in goats, and does with BCS 3 had higher concentration of circulating progesterone, estradiol -17ß and luteinizing hormone. Furthermore, oestrous cycle was affected by BCS. Fertility of BCS 3 was better compared to BCS 2 and delays puberty and stops oestrous in cycling heifers (Short and Adams, 1988).

4.5 Conclusion

It was concluded that, older ewes show signs of heat earlier than young ewes and the oestrus is less intense compared to younger ewes. Furthermore, body condition score affected the response, duration and length of estrus cycle in Bapedi ewes regardless of the conservation method. For the maintenance of maximum reproduction rates in a flock, ewes must have an ideal BCS value. Active management efforts to improve indigenous sheep breeds of South Africa should focus mostly on maintaining BCS 3-4 for successful application of assisted reproductive technologies to increase production and profits.

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Chapter 5

Reproduction performance of Bapedi ewes following oestrous synchronization and natural mating.

Abstract

The objective of this study was to measure reproductive performances of Bapedi sheep conserved *in situ* and *ex situ in vivo*. A total of 79 Bapedi ewes were synchronised for oestrous and ovulation using a controlled internal drug release device (CIDR) and were injected with 300 IU of eCG on the day of progestogen withdrawal and heat was observed using a vasectomised ram. All ewes observed to be on heat were exposed to fertile rams for mating. Bapedi ewes were synchronised successfully and the average response rate was 81%. No significant differences were observed for conception rates between *ex situ in vivo* (65%) and *in situ* (63%) conservation methods ($P<0.05$). Toowoomba had a significantly lower litter size recorded compared to all the other farms. There were no significant differences ($P<0.05$) between the two conservation methods on the gestation length of Bapedi sheep. No significant differences were observed for lambing rate between ex situ in vivo (70%) compared to *in situ* (73%) ($P< 0.05$). Prolificacy of Bapedi sheep was 1.30±0.6 1.28±1.3; 1.29±0.8 and 1.31±0.5 for ARC, Towoomba, Tompi Seleka and Mara farms respectively. It was concluded that conservation method did not affect the reproductive performance of Bapedi sheep. Bapedi ewes can be synchronised successfully with an acceptable conception rate without supplementary feeding. It is recommended that flush feeding should be done to improve the litter size.

5.1 Introduction

Bapedi sheep are indigenous to South Africa, classified under the Nguni sheep breeds. Bapedi sheep is a fat-tailed, fine wool and a multi-coloured breed, however the red head with a white body is a dominating colour due to selection that was done at Stellenbosch in the Sekhukhune land. Furthermore, Bapedi sheep are resistant to diseases and parasites, have a good mothering ability, good flocking instinct and ability to walk long distances in search of food. Bapedi sheep have long reproductive lives, tolerant to harsh climatic conditions especially hot and dry weather and survive well in low and fluctuating nutrient availability (Snyman, 2013). These animals have better adaptability, higher reproductive capacity, shorter gestation, fast growth rates and easy to handle, they are a better option for resource limited smallholder farmers including landless youth and women. Livestock farming assists greatly in South African rural communities with job opportunities, alleviate household food insecurity supporting the objectives of the National Development Plan 2030 of the South African Government (NDP, 2012).

In light of all the advantages indigenous sheep of South Africa are under the danger of becoming extinct and once these genetic resources are lost they can never be recovered. For so long these animals were perceived to be inferior to exotic sheep breeds and were crossed indiscriminately, and the sector has not received enough attention from scientists, and legislators hence the numbers are reduced significantly without proper documentation of their unique characteristics and reproductive performance (Macaskill, 2018). These provide important sources of genetic material for research and development, scientific reference, future utilization, as well as rehabilitation of breeding stock for national recovery from natural disasters. Due to these benefits indigenous sheep

need to be conserved as pure breeds or used as the dam line in cross breeding programs to safeguard adaptation traits of crossbred offspring (Lehloenya *et al.,* 2004).

Reproduction in sheep production is a trait of economic importance and can be manipulated among ewes using hormonal treatments to synchronise their oestrous (Kor *et al*., 2011). Oestrous synchronization (OS) forms part of management and was reported as successful in increasing reproductive efficiency in ewes and allows for timed breeding and lambing (Kusina *et al*., 2000). This assisted reproductive technology allows for breeding when the body condition of the females is favourable and lambing when forage is available, taking advantage of seasonal variations, photoperiod and market demands (Safdarian *et al*., 2006). Many methods are used to synchronise oestrous in small ruminants with prostaglandin method mostly used during natural breeding season as it requires an active corpus luteum and other methods include the use of progesterone and ovulation synchronization methods, these either reduce the length of luteal phase of the oestrous cycle with PGF2α or artificially extend it with progesterone injections (Safdarian *et al*., 2006).

Measurement of reproductive efficiency of indigenous sheep such as Bapedi sheep would be first step in an attempt to increase productivity. Reproductive performance (RP) is a requirement for the success of livestock production (FAO, 2011). In sheep reproductive performance is measured by age at first lambing, parturition interval, fertility, prolificacy, fecundity and survival. There is a general lack of information when it comes to reproductive and productive performances of Bapedi sheep which are early signs of adaptability and management sufficiency. Performance recording of these breeds in their native environment, under farmer's conditions is a requirement to identify their contribution to livelihoods and only then can areas that need intervention can be highlighted (Talore, 2009). This is to avoid capturing misleading results from on-station research that are of little relevance to traditional on farm production systems. Hence the objective of this

study was to measure reproductive performances of Bapedi sheep conserved *in situ* and *ex situ in vivo.*

5.2 Materials and methods

5.2.1 Study sites, animals and experimental design

The trial was conducted at five indigenous animal conservation farms in Gauteng and Limpopo provinces to measure reproductive performance of Bapedi ewes under *in situ* where an extensive management system in practised and *ex situ in vivo* conservation methods (semi intensive management method). The animals were selected randomly based on availability in each farm and blocked for influence of age and breed. Agricultural Research Council (n = 40), Mara Research Station (n=20), Towoomba Research Stations (n=12) and Tompi Seleka College of Agriculture (n=19). Madzivhandila Agricultural College was excluded for this trial due to lower number of ewes. Ewes of average body weight 48-68 kg and age 3- 6 years were used in this study. The ewes used in this study were grazing on natural pasture, and water was provided *ad libitum.* The reproductive performance of Bapedi sheep was studied from June 2017- January 2019. A randomized complete block design was used in this study to account for differences in management of individual farms and impending environmental factors

5.2.2 Oestrous synchronization and natural mating

Oestrous cycles of ewes were synchronized using a short-term progestogen protocol as described by Ramukhithi *et al*. (2014), where a controlled internal drug release device (CIDR) (0.3 g progesterone) [(PfizerTM. New Zealand) (Ltd)], was inserted in the vagina of the ewe for 9 days. Ewes were injected intramuscularly with 300 IU of equine chorionic gonadotrophin (eCG) (Intervet Schering-Plough Animal Health, South Africa) on the day of progestogen removal to prompt oestrous and ovulation. Following progestogen withdrawal ewes were observed for signs of heat for 30 minutes using a vasectomised ram twice a day. Ewes that were on heat were separated and were put together with a ram and observed to ensure that mating occurred. All rams were tested for breeding soundness before taking part in breeding. Ewes that were on heat were left with fertile rams until they rejected to be mounted by the rams for a period of three days in both conservation methods. The data was recorded for the response of ewes to hormonal synchronization, conception rate, gestation length, litter size, birth and weaning weights. The effect of dam weight before mating, litter size and sex of the lamb on birth weights and neonatal loss were also studied.

5.2.3 Conception rate

Ewes were observed closely for return to oestrous and non-return to oestrous. All ewes were then subjected to pregnancy diagnosis by an Ibex pro transabdominal ultrasound scanning machine between 30-35 days following mating (EI Medical, USA). Conception rate was then calculated as the number of ewes pregnant in relation to the number of ewes exposed to the fertile rams. Lambing rate was calculated as number of lambs born alive/number of ewes mated x 100.

5.2.4 Statistical analysis

Conception rate was expressed as the ratio of the number of sows that conceived / number of ewes that were inseminated. The lambing rate, expressed as the ratio of number of ewes lambed / number of ewes exposed to the ram. Litter size was expressed as the number of total lambs born (live and dead). The comparison of means between the groups was analysed using analysis of variance (ANOVA), means were separated using Tukey's test and general linear model of (SAS, 2009) for unbalanced groups. Descriptive statistics including the LSM, SEM and ranges of all reproductive performance data were calculated. The response of ewes to oestrus synchronization, lambing rate and number of multiple births were also analysed. Significance level was considered at $P<0.05$. Contrast comparison was used to compare the *ex situ in vivo* and the *in situ* conservation methods Significance level was considered at $P<0.05$.

5.3 Results

The Bapedi ewes were synchronised successfully and an average of 81% of the ewe in all the farms expressed oestrus following the introduction of the vasectomised ram and were exposed to fertile rams for mating. No significant differences were observed for conception rates between *ex situ in vivo* and *in situ* conservation methods ($P<0.05$). Toowoomba had a significantly lower litter size recorded compared to all the other farms. There were no significant differences ($P<0.05$) between the two conservation methods on the gestation length of Bapedi sheep. No significant differences were observed for lambing rate between ex situ in vivo (70%) compared to in situ (73%) ($P< 0.05$). No significant differences were observed between the *in- situ* and the *ex- situ in vivo* conserved

Bapedi sheep with regards to gestation length (P>0.05). Towoomba farm only had single births compared to all the other stations even though the percentage of multiple births was low in all the farms as shown in Table 5.1

Table 5.1 Expression of oestrus (%), conception rate (%), gestation length (Mean ± SE) of indigenous Bapedi sheep following oestrous synchronization and natural mating on *in situ* and *ex situ in vivo* conservation methods

Variables		N	Expression of Oestrus	Conception rate	Gestation length	Litter size
Farm	ARC	40	39/40 (98%)	26/40 (65%)	148.9±1.2	1.3±0.2[b]
	Towoomba	12	10/12 (83)	8/12 (67%)	150.1±0.4	1.0±0.0[a]
	Tompi Seleka	19	19/19 (100%)	10/19 (53%)	149.2±0.6	1.2±0.4[b]
	Mara	20	17/20 (85%)	14/20 (70%)	146.8±2.9	1.2±0.3[b]
Method	*Ex-situ in vivo*	40	39/40 (98%)	26/40 (65%)	148.9±1.2	1.3±0.2
	In-situ	51	46/51(90%)	32/51 (63)	148.7±1.3	1.1±0.4

[ab] Values with different superscripts for a variable (farm or method) within a column differ significantly (P< 0.05).

Table 5.2 shows the reproductive performance among Bapedi sheep. The *in situ* conservation method had a higher number of multiple births compared to the *ex situ in vivo* method. No significant differences were observed for all the other parameters between the two conservation methods. Mara farm had a higher number of lambs born alive compared to all the other stations, even though in general the prolificacy of all the Bapedi sheep farms was above 50%. Bapedi sheep had lower numbers of multiple births in all the farms with Towoomba farm having only single births. It was highlighted that Bapedi sheep had no lamb mortalities or ewe mortalities in both conservation methods. In all the Bapedi sheep on both the conservation methods most of lambs born were male compared to females. The average birth weights of male were higher ($P<0.05$) than those of females. The males were heavier ($P<0.05$) at birth than the females.

Table 5.2: Lambing rates (%), prolificacy (Mean ± SE), multiple birth rate (%), ewe and lamb mortality rates (%), sex of lamb (%) birth and weaning weights of lambs (Mean ± SE)

Variable		N	LR	Prolificacy	MBR	EMR	LMR	ML	FL	FBW	MBW
Farm	ARC	40	70	1.30±0.6	7.5	0	0	83	17	2.9±0.9	3.6±1.2
	Towoomba	12	67	1.28±1.3	0	0	0	98	2	3.2±0.3	3.6±0.6
	Tompi Seleka	19	68	1.29±0.8	15.7	0	0	84	16	3.0±0.6	3.5±0.6
	Mara	20	85	1.31±0.5	15.4	0	0	88	12	3.0±0.4	3.8±0.7
Method	*Ex-situ in vivo*	40	70	1.30±0.6	7.5[a]	0	0	83	17	2.9±0.9	3.6±1.2
	In-situ	51	73	1.29±0.9	10.4[b]	0	0	90	10	3.1±0.4	3.6±0.6

[a,b] Values with different superscripts for a variable (farm or method) within a column differ significantly ($P< 0.05$).
LR; lambing rates, MBR; multiple birth rates, EMR; ewe mortality rates, LMR; lamb mortality rates, ML; Male lamb, FL; Female lamb FBW; female birth weights; MBW; male birth weights.

5.4 Discussion

Hormonal treatment for synchronization of Bapedi sheep was successful and a conception rate of 53, 65, 67 and 70 percent were obtained from Tompi Seleka, ARC, Towoomba and Mara research Station, respectively, following natural mating. Conception rates obtained in this study were lower compared to 83 % obtained from non-supplemented group of Bangladesh indigenous ewes (Zohara *et al*., 2014). However, they were comparable to findings by Lehloenya *et al.* (2005) done in South African Nguni goats. The percentage of oestrous response and the conception rate in this study were comparable to finding from other studies (Kridli *et al*., 2009; Zohara, *et al.,* 2014). The gestation length of Bapedi sheep ranged from 146.8±2.9 to 150.1±0.4 days and there were no significant differences among the farms and within individual conservation methods. The gestation period for multiple and single births was similar in all the Bapedi sheep breed in all the farms. The gestation length of Bapedi sheep was longer compared to gestation length from the Bangladesh indigenous ewes which ranged from 141 to 145 days (Roy *et al*., 2014). The gestation length was also similar to that obtained from South African Boer and Nguni does (Lehloenya *et al*., 2005). Nutrition during pregnancy has been reported to be a major influencer of gestation length, with maternal undernutrition resulting in longer pregnancies. Lower energy diets reduced pregnancy rates (Kusina, *et al*., 2001; El-Hag *et al*., 2007; Zohara *et al.,* 2014). Towoomba farm had the smaller ($P<0.05$) litter size compared to other farms with ewes giving birth to only one lamb per ewe. There were no significant differences on litter size between ARC, Mara and Tompi Seleka farms. These findings might be due to the fact that litter size is influenced by a number of factors such as genotype, parity, season, and ewe body weight at mating (Mukasa-Mugarwa and Lahlou-

Kassi, 1995). Litter size similar to the results of this study were also obtained by Mukasa-Mugarwa *et al*. (2002).

No significant differences were observed for lambing rate between ex situ in vivo (70%) compared to in situ (73%) ($P< 0.05$). The results from this study were lower compared to lambing rates of (85.3%) brown and (89.7%) black faced Awassi ewes kept under the same traditional management conditions (FAO, 2011). These results were similar to indigenous ewes of Bangladesh where 75% lambing rates were obtained (Roy *et al*., 2014). The prolificacy of Bapedi sheep was similar to that obtained from the brown and black head Awassi, the Horro ewes from western Ethiopia and Begayt ewes (Mukasa Mugrewa *et al*. 2002; Kridli *et al*., 2009; Ashebir *et al.,* 2016). Bapedi sheep had the lowest number of multiple births compared to Awassi and Begyat ewes. The twinning or percentage of triplets can be improved by flush feeding and better management, through selection (Mukasa Mugrewa *et al.,* 2002). There were no ewe and lamb mortalities in all the farms. This can be attributed to the adaptation traits of the Bapedi sheep. Interestingly, most of Bapedi ewes gave birth to mostly male lambs than females. No significant differences were observed for birth weights in both the conservation methods. These findings were similar to the results of indigenous Tswana goats (Nsoso *et al*., 2004)

5.5 Conclusion

The results of this study indicated that semi extensive to extensively reared Bapedi sheep can be synchronised successfully even though the conception rate is still low (50% from the standard 85% for improved breeds). Bapedi sheep in all the farms gave birth to mostly single lambs and the sex ratio was mostly male lambs. No mortalities were recorded all the lambs that were born survived until weaning.

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Chapter 6

Assessment of genetic variation within Bapedi sheep using microsatellite markers

Abstract

The study was conducted to assess the genetic variation within Bapedi sheep using 14 microsatellite markers. Blood samples were collected from 174 unrelated Bapedi sheep in six farms in different districts of the Limpopo province and one conservation farm in Gauteng. Other South African indigenous sheep such as Zulu, Damara, Dorper and Namaqua were included as reference populations to assess the genetic relationship between these breeds and the Bapedi sheep. The results obtained in this study showed a higher mean number of alleles (9). The observed heterozygosity (Ho) and expected heterozygosity (He) values ranged between (0,555±0.03 to 0, 8270± 0, 027) and (0,754±0.02 to 0,883±0.004) respectively. These heterozygosities are indicative of a considerable genetic diversity among the Bapedi sheep populations. Within population inbreeding estimates FIS =0,173±0,029 did not affect the heterozygosities. The UPGMA tree and PCA results revealed that Bapedi sheep from Mopani commercial farm, Sekhukhune communal farm, ARC-AP and Mara research station clustered together and share common genetic structure and showed no relatedness to the reference populations. Towoomba population did not cluster Bapedi sheep or any other breed. In conclusion the panel of used markers were highly informative for Bapedi sheep. There is a need for sustainable breeding and conservation programs to control inbreeding and gene flow, in order to stop possible genetic dilution of Bapedi sheep.

6.1 Introduction

Bapedi sheep is an indigenous breed of South Africa originating from the semi-arid regions of Limpopo. They are very important to resource limited farmers as they have overtime adapted to harsh environmental conditions, opposed by challenges of drought, lack of good quality feed, exposed to diseases and resistance to gastrointestinal nematodes (Kunene *et al*.,2014). Indigenous sheep are perceived to be inferior and were crossbred incalculably to improve their productivity traits before they were even characterized and documented. The biasness in the resource distribution by the South African government that supports only cattle also contributes in the degradation of indigenous sheep. Food and Agricultural organization (2007) reported that about 30% of indigenous genetic resources are at risk of becoming extinct before they are characterized and acknowledged. Crossbreeding and inbreeding of indigenous sheep resulted in the decline of locally adapted low inputs breeds (Kunene *et al*., 2014). Johannesburg Zoo and many government institutions are involved in conservation of Bapedi sheep with an objective to increase their numbers. However, the prerequisite for starting any conservation plan is genetic evaluation of the existing population structure and differences among or within the breed (Tariq *et al*., 2011; Qwabe, 2013). Research like that would serve as an initial guide to simplify conservation activities. Bapedi sheep is characterized under the Nguni sheep breeds which are Zulu and Swazi. Surprisingly in a study by Buduram (2004) Bapedi sheep was genetically distant with the Nguni breeds and was clustered with the Dorper sheep breed and these findings were supported by Hlope (2011). These findings left curiosity of whether Bapedi sheep is a subpopulation of Dorper or its sharing of genes between breeds because they have different phenotypic characteristics. There is limited information on the genetic variability of Bapedi sheep.

The evolution in the genomic studies has introduced many tools including genetic markers to study the unique genetic features of a breed (Nguluma *et al.,* 2017). Microsatellite markers are one of important DNA markers for genetic diversity of closely related populations. Microsatellite are determined at a known loci and can accommodate a wide range of population parameters (Hanotte and Jianlin 2005; FAO, 2011; Kunene *et al*., 2014). This study was conducted to assess genetic variation of Bapedi sheep population from *ex situ in vivo* and *in situ* conservation programs in varying agro-ecological climates.

6.2 Materials and methods

The study sites, coordinates, average temperatures and rainfall of seven farms that samples were collected from for genetic variation assessment of the Bapedi sheep breed using microsatellite markers are shown in Table 6.1.

Table 6.1. Coordinates, altitude, temperatures and rainfall for sampled Bapedi sheep farms (http://www.southafrica.info/travel/advise/climate.htm).

Method	Farm	Coordinates	Altitude	Max Temp	Min Temp	Rainfall/year
Ex situ in vivo	ARC- AP	25º 53' 59.6" S 28º 12' 51.6" E	1524m	27.0°C	17.6°C	556 mm
In situ	Towoomba	24°54'0" S 28°19'60" E	1,109 m	28.8°C	19.9°C	481 mm
	Mopani	23.0353° S, 29.6583° E	764 m	29.1°C	21.9°C	881 mm
	Madzivhandila Agricultural College	22.9870° S, 30.5508° E	764 m	30.3°C	22.9°C	752 mm
	Tompi Seleka Agric College	24.7892° S, 29.4548° E	873 m	29.4°C	21.5°C	473 mm
	Capricorn	23.8962° S, 29.4486° E	1329 m	26.6°C	19.2°C	389 mm
	Mara	23°.05S 29°.25E	1020m	30.0°C	20.2°C	495 mm

6.2.1 Blood sampling and DNA extraction

A total of 174 blood samples were collected from unrelated individuals in seven different farms of Bapedi sheep (15- 30 samples/ farm depending on the availability). Blood samples from the Zulu, Namaqua, Damara and Dorper sheep breeds were also collected and used as reference populations from the Limpopo province. Whole blood samples were collected from the jugular vein into using an 18 gauge veterinary needles and a 6ml vacutainer tubes containing ethylenediaminetetra-acetic acid (EDTA) and were stored at 5 °C immediately after collection. Samples were then transported to the laboratory and blood samples were aloquated into 2ml cryo-tubes and stored at -20 degrees Celsius DNA isolation. DNA isolation was done using a Roche High Pure PCR template preparation kit (Roche, IN, USA) following the manufacturer's protocol. The isolated DNA was assessed for concentration and quality using a spectrophotometer (Nano-drop 2000) at 260/280 absorbance ratio (Thermo Fisher Scientific, Waltham, MA, USA) and further visualized at 0.8% agarose gel electrophoresis. Eluted DNA was stored at -20 degrees Celsius until it was required for PCR.

6.2.2 Polymerase Chain Reaction and genotyping

The DNA was subjected to a Polymerase Chain Reaction (PCR). DNA samples were amplified using 14 microsatellite recommended by Food and Agricultural Organization (FAO) and International Society for Animal Genetics (ISAG) for diversity studies. Microsatellite markers were selected based on levels of polymorphism including the number of alleles per locus and genome coverage as suggested by (Buduram, 2004). The following markers were used in this study

HSC, OARFCB20, OARFCB304, CSRD2115, THRA, MCM527, CSRD2111, ILSTS39, OARFCB128, CSRD247, BMS1967, INRA63, MAF65 and TCRBV6.

For PCR amplification a reaction mixture of 7.5 µl consisting of 2.5 µl of the DNA, 3.225µl deionized water, 0.75 µl buffer, 0.15 µl of the dNTPs, 0.375 µl $MgCL_2$, 0.2 µl of Taq and 0.3 µl primers was used. The amplification was performed using a Perkin Elmer Gene Amp PCR machine 9700 thermocycler. The amplification included 5 minutes at 95˚C 35 cycles of 45 seconds, 94˚C 35 cycles for 45 seconds. The samples were further subjected to annealing temperature of 59 ˚C and for one minute at 72˚C and continued at the same temperature for 10 minutes. Ovine DNA sample was concurrently ran with each PCR for accuracy and avoiding errors since it's of known size and labeling.

6.2.3 Statistical analysis

The data analysis included several computer software, Microsatellite Toolkit was used in this study to calculate the allele frequencies, both the expected and observed heterozygosity values, polymorphic information content mean number of alleles per locus and number of private alleles (Park, 2001). MS Toolkit were used to prepare genotypic data to formats suitable for other software used in this study (Glaubitz, 2004). The fixation index F_{ST} values were obtained from Arlequin version 3.1 (Excoffier *et al*., 2005). Deviation from Hardy Weinberg Equilibrium (HWE) was tested at each locus in all the populations using Genepop version 4 (Raymond and Rousset, 1995). FSTAT version 2.9.3.2 was used to compute Wright's statistics (F_{IS}, F_{ST}, F_{IT}) (Goudet, 1995). Pairwise genetic distances between the Bapedi sheep populations were obtained following Nei's method of unbiased genetic distance in POPGENE computer software (Nei, 1978). The UPGMA dendogram was constructed based on Nei‟s (1972). Assignment of animals to genetic groups was

done using Principal Component Analysis to reveal the ancestral history and admixture among the Bapedi sheep populations (Peakall and Smouse, 2006)

6.3 Results

All 14 loci used in this study were found to be polymorphic with a mean number of 9 alleles when pooled across the 11 populations (Table 6.2). The mean number of alleles ranged from 5.7±0, 512 for the Mara Research Station being the lowest to 12.644±0.87 for the ARC-AP Bapedi sheep with the highest number of alleles. The number of effective alleles were lower for Mara Research Station (4,465±0.34) and higher for the Towoomba (8,737±0, 32) Bapedi sheep. The expected heterozygosities were higher than the observed heterozygosities in all of the Bapedi sheep populations studied. The observed heterozygosity ranged from 0,555±0.03 for the Sekhukhune communal Bapedi sheep to 0, 827± 0, 027 for the Towoomba Bapedi sheep. The expected heterozygosity ranged from 0,754±0.02 for Mara Bapedi sheep to 0,883±0.004 for the Towoomba sheep. Shannon index ranged from 1,688±0.08 to 2,266±0.02 indicating that the markers used were very informative. The overall genetic diversity expressed as heterozygosity in the populations was moderate to high, with moderate Mara (75%) the highest value in Towoomba (95%). The ARC-AP, Sekhukhune communal, Mopani commercial and Madzivhandila Agricultural College had higher FST values indicating reduction in heterozygosity. The deviation from HWE was tested for in all 14 microsatellite markers for all the populations and the results showed all the loci were in HWE (P>0.05) as shown in Table 6.2

Table 6.2: Measures of genetic variation in the Bapedi sheep populations

Population	N	NA	Ne	I	Ho	He	uHe	F_{ST}
1	15	9,571±0,22	8,737±0,32	2,266±0,02	0,8270±,027	0,883±0.004	0,952±0.00	0,065±0.02
2	20	11,143±0,79	7,345±0.82	2,261±0.07	0,629±0.04	0,842±0.02	0,864±0.02	0,253±0.04
3	15	9,143±0,60	5,364±0.43	1,866±0.08	0,625±0.04	0,794±0.02	0,827±0.02	0,210±0.05
4	44	9,857±0,57	4,759±0.35	1,864±0.06	0,555±0.03	0,777±0.01	0,786±0.01	0,284±0.04
5	20	7,143±0,55	5,019±0.29	1,741±0.07	0,672±0.04	0,790±0.01	0,834±0.02	0,148±0.05
6	20	5,714±0,51	4,465±0.34	1,688±0.08	0,634±0.04	0,754±0.02	0,784±0.03	0,160±0.04
7	40	12,643±0.87	5,754±0.60	2,124±0.09	0,556±0.03	0,793±0.02	0,803±0.03	0,296±0.03

1: Towoomba, 2: Mopani commercial farm, 3: Madzivhandila Agric College; 4: Sekhukhune communal farm, 5: Polokwane commercial farm, 6: Mara, 7: ARC-AP. N: Sample Size, NA: number of alleles, Ne: No. Effective Alleles, I: information index, Ho: observed heterozygosity, He: expected heterozygosity, uHe: unbiased expected heterozygosity, FST: fixation index

Fixation indices (FIS, FST and FIT) were used for population differentiation for all 14 markers across the 7 Bapedi sheep populations as indicated in Table 6.3. The average F- statistics of the all the markers for the Bapedi sheep populations, FIS = 0,173±0,029; FST 0,251±0,027 and FIT =0,096±0,005. The FIS obtained from this study were moderate to high and positive due to the smaller sample sizes. The mean gene flow (nM) was 2,469±0,163, highest for OarfCB20 = 3,814 and lower for Inra63= 1,734.

Table 6.3: F-Statistics and Estimates of Nm over All Pops for each Locus

Locus	FIS	FIT	FST	Nm
HSC	0,177	0,255	0,095	2,378
OarfCB20	0,191	0,241	0,062	3,814
OarfCB304	0,131	0,196	0,075	3,092
CSRD2115	0,114	0,200	0,097	2,323
THRA	0,090	0,158	0,076	3,061
MCM527	0,180	0,244	0,078	2,960
CSRD2111	0,215	0,309	0,120	1,842
ILSTS39	0,099	0,193	0,105	2,125
OarfCB128	0,222	0,288	0,085	2,692
CSRD247	0,184	0,284	0,122	1,798
BMS1967	0,144	0,217	0,086	2,656
Inra63	0,152	0,259	0,126	1,734
MAF65	0,020	0,118	0,100	2,243
TCRBV6	0,497	0,557	0,119	1,850
Mean	0,173±0,029	0,251± 0,027	0,096±0,005	2,469±0,163

FIS: Inbreeding coefficient of individuals within subpopulation, FIT: Inbreeding coefficient of individuals within total population, FST: The amount of genetic differentiation within the total population

Table 6.4 shows Nei's genetic distances between populations. The distance between the Mopane commercial farm was closer to the Sekhukhune communal farm Bapedi sheep (0.071); ARC-AP Bapedi sheep (0.070). The Mara and ARC-AP Bapedi sheep were also genetically close (0.063). The Sekhukhune communal farm was also closely related to the ARC-AP (0,081). In general, Bapedi sheep were genetically distant from Dorper, Damara, Zulu and Namaqua.

Table 6.4: Pairwise population matrix of Nei's genetic distance for 7 Bapedi sheep population and 4 reference populations

	1	2	3	4	5	6	7	8	9	10
1	0,000									
2	0,122	0,000								
3	0,408	0,203	0,000							
4	0,291	0,071	0,330	0,000						
5	0,198	0,112	0,411	0,162	0,000					
6	0,239	0,100	0,262	0,164	0,264	0,000				
7	0,484	0,123	0,258	0,152	0,356	0,245	0,000			
8	0,202	0,070	0,293	0,081	0,207	0,130	0,063	0,000		
9	0,547	0,344	0,452	0,314	0,398	0,372	0,304	0,308	0,000	
10	0,537	0,372	0,510	0,501	0,605	0,492	0,463	0,464	0,449	0,000
11	0,393	0,254	0,615	0,249	0,395	0,432	0,390	0,392	0,719	0,866

1: Towoomba, 2: Mopani commercial farm, 3: Madzivhandila Agricultural College, 4: Sekhukhune communal farm, 5: Polokwane commercial farm, 6: Zulu sheep, 7: Mara, 8: ARC- AP, 9: Damara, 10: Dorper, 11: Namaqua.

The UPGMA tree in figure 6.1 was used, Bapedi sheep from Mopani commercial farm, Sekhukhune communal farm, ARC-AP and Mara research station clustered together. The Zulu, Damara, Dorper and Namaqua sheep populations were used as outliers to root the tree and they were clustered separately.

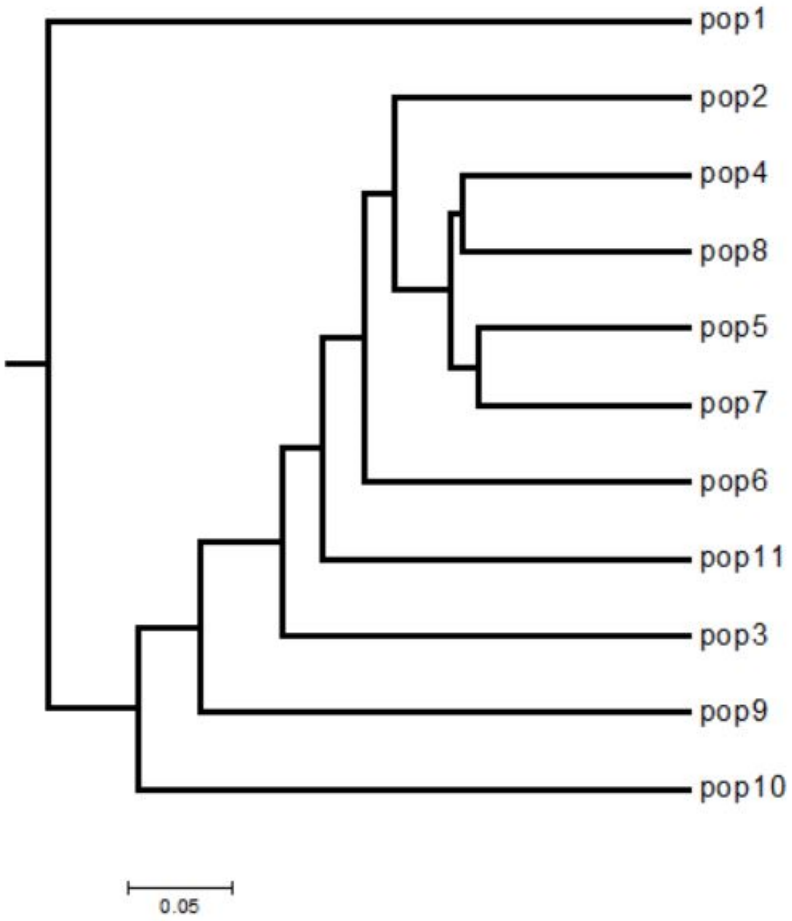

Figure 6.1: Neighbor joining UPGMA tree rooted with Dorper sheep population as an out group

Population 1: Towoomba; population 2: Mopani commercial farm; population 3: Madzivhandila Agricultural College; population 4: Sekhukhune communal farm, population 5: Polokwane commercial farm, population 6: Zulu sheep, population 7: Mara; population 8: ARC- AP, population 9: Damara, population 10: Dorper, population 11: Namaqua.

Principal Coordinates Analysis (PCA) as presented in figure 6.2 was used to further examine possible genetic relationship among Bapedi sheep and four reference populations. The percentage of variation is explained by the first three axis PC1 (40.5) PC2 (28.7) PC3 (10.9) This analysis verified that the Mara and ARC-AP population were closely related together with Sekhukhune communal, Mopane commercial, Madzivhandila Agricultural college, Polokwane commercial. The Towoomba Bapedi sheep clustered far from all the other sheep. This analysis also distinguished Damara and Dorper from other populations.

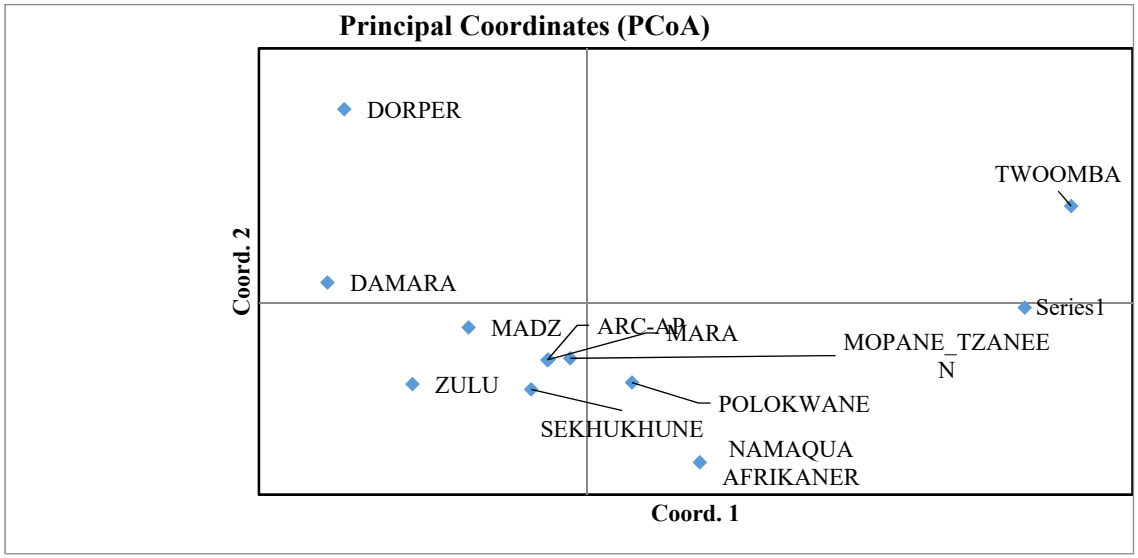

Figure 6.2: Principal coordinates Analysis for 11 sheep populations

Table 6.5 shows the AMOVA analysis for 7 Bapedi sheep populations for further evaluation of genetic variation amongst, within and within individual Bapedi sheep. The results obtained from AMOVA analysis showed that 72% of genetic diversity occurred within individuals, followed by 23% within populations and least among populations (6%).

Table 6.5: AMOVA analyses for Bapedi sheep samples

Source of variation	Sum of Square	Variance components	Percentage variation
Among Populations	98.422	0.369	6.239
Within populations	1300.682	1.371	23.124
Within individuals	844.000	4.274	72.097
Total	2243,104	6,014	

6.4 Discussion

South Africa lacks official data regarding on the census of indigenous sheep numbers and hence strategies and breeding plans for their sustainable conservation. Indigenous sheep such as Bapedi are reported to be endangered (Macaskill, 2018). Attention should be given to strategic breeding to reduce the decline of indigenous sheep populations.

All markers yielded a mean number of 9 alleles and was observed was higher compared to findings reported in other indigenous sheep in South Africa for Swazi (6.5), Nguni (5.4) and Pedi (7) (Buduram, 2004).In Namaqua Afrikaner the mean number of alleles was (5); Zulu sheep (3.73-6.5) (Kunene *et al*., 2014; Selepe *et al*., 2018). Internationally lower MNA were observed in African fat tailed sheep and Red Maasia-Mutara (Muigai *et al*., 2009). These results were similar to those observed for Najdi sheep of Saudi Arabia (9.11) (Musthafa *et al*., 2012), however, lower compared to Egyptian (10.30) EI-Nahas *et al*., 2008), Spanish (13.30) (Calvo *et al*., 2011) and Bhutan sheep breeds (13.38) (Dorji *et al*., 2010).

No private allele were observed in all the populations studied in the current study, this might be due to migration and gene flow from the selection that was done over the years. Private alleles are important for genetic distinctiveness of a population, however there were no remarkable distinctiveness in Bapedi populations. Bapedi sheep are in danger of losing variation due to smaller population sizes. Overall the mean number of alleles observed in this study was high due to highly polymorphic markers used (Buduram, 2004).

In the current study a high number of expected heterozygosities were observed for all the seven Bapedi sheep populations indicating that the populations that were sampled have high genetic diversities within populations. Similar findings were observed in other African indigenous breeds

the Red Maasai-Matura (0.70±0.03), Migori (0.70±0.03) and Transmara (0.70±0.03) for expected heterozygosities. In South Africa the heterozygosities were similar to those found in the Zulu sheep for both observed and expected heterozyogosities (Kunene *et al.,* 2014). In this study all the He were higher than Ho and these findings contradicts observations from a study in Kail sheep (Ahmed *et al*., 2014).

The overall genetic diversity expressed as unbiased heterozygosity in the populations was moderate to high with the highest value in Towoomba (95%) and moderate Mara (75%). These estimates are higher than those obtained by Qwabe (2011) who obtained heterozygosities ranging from 49- 55% for Namaqua Afrikaner sheep breed, also findings by Buduram (2004) Pedi (67%), Nguni (69%), Swazi (69%), and Karakul (67%).The high genetic variation in Bapedi sheep population indicates that Bapedi sheep were not kept as a closed population. Farmers permitted the animals to be bred naturally, no controlled mating was practiced in their flocks The number of effective alleles ranged from (4,465±0.34 - 8,737±0, 32) in Bapedi sheep which was higher when compared to findings by (Sodhi *et al*., 2006; Ahmed 2014).

The FST values for some of Bapedi sheep populations were high indicating reduction in heterozygosity, similar results were obtained for some Namaqua Afrikaner population subdivisions. Additionally, similar FST was observed in Zulu sheep populations and in Nigerian sheep breeds (Agaviezor *et al*., 2013; Kunene *et al*., 2014). These high values indicate separations among subpopulations. The FIS obtained from this study were moderate 0,173±0,029 due to the smaller sample sizes. These findings were lower compared to those obtained from the Kail sheep (0.327-0.655) (Ahmed *et al*., 2014); Hamdani sheep (0.469) (Al-Barzinji *et al*., 2011) this might be due to the fact that indigenous sheep are most found where breeding is not controlled. The results obtained for inbreeding coefficient from the 7 Bapedi sheep populations were higher

compared to those obtained from Namaqua Afrikaner (0.019) and Zulu sheep (0.118) (Kunene *et al*., 2014). The gene flow was 2,469±0,163 lower than the one reported by Buduram (2004). The AMOVA showed a moderate genetic differentiation among and within populations.

Nei's genetic distance and the UPGMA tree revealed close genetic distances between Bapedi sheep populations from Mopani commercial farm, Sekhukhune communal farm, Mara, ARC-AP and Polokwane commercial farm. These findings are interesting as the results from the conservation stations Mara and ARC-AP showed a close relationship even though these stations are far apart and no known exchanges of genetic material has been reported. It was not surprising that the population from Sekhukhune was closely related to most population as seen on the UPGMA tree as Sekhukhune was where the Bapedi sheep were maintained and selected (Snyman, 2014). The genetic distances ranged from.

A moderate to high genetic estimate was observed between Bapedi sheep and Damara, was higher than findings by Mugai (2002), but lower than genetic distance between Damara and Pedi (0.579) (Buduram, 2004). A high genetic estimated was observed between, Bapedi sheep with Dorper, Zulu and Namaqua Afrikaner as shown in table 3, These results contradicts the findings by Hlope, 2011 and Buduram 2004, that reported Bapedi sheep clustered with Dorper. These finding indicate that genetic differences exist between South African indigenous sheep.

6.5 Conclusion

The results from this study indicated that Bapedi sheep have a high genetic diversity and most of them clustered together, which can be incorporated to future breeding programs and conservation strategies for this breed. The Towoomba Bapedi sheep did not cluster with any other breed, this indicates a possible cross breeding or development of new fixed genes. All the reference populations used in this study did not cluster with Bapedi sheep. The inbreeding coefficient were high and positive so intervention to reduce inbreeding in Bapedi sheep is required. Genetic diversity that is reported in this study should be expanded by incorporating more Bapedi sheep farms and exploring advanced analysis of genetic markers such as SNPs.

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Chapter 7

General discussion, conclusion and recommendation

7.1 Discussion

South Africa has 23, 71 million sheep but remains an importer of mutton. To grow and diversify production from livestock in South Africa sheep farming might be the best choice as indigenous sheep are well known for adaptability, survivability and disease tolerance. Production of mutton locally needs to be increased to meet up with the increasing demand. Ensuring good reproductive performance of locally adapted breeds would be a great strategy, and only then will the aim to secure food for the future be a reality (Webb *et al*., 2010; Kunene, 2010; Ramukhithi *et al*., 2017). Bapedi sheep are indigenous to South Africa, classified under the Nguni sheep breeds. Bapedi sheep is a fat-tailed, fine wool and a multi-coloured breed, however the red head with a white body is a dominating colour due to selection that was done at Stellenbosch in the Sekhukhuneland district of Limpopo. They are resistant to diseases and parasites, have good mothering ability, good flocking instinct, ability to walk long distances in search of food, longevity, high tolerance to harsh climatic conditions especially hot and dry weather and survive well in low and fluctuating nutrient availability (Snyman, 2013). These sheep have better adaptability, higher reproductive capacity, shorter gestation length, fast growth rates and easy to handle, they are a better option for resource limited smallholder farmers including landless youth and women. Livestock farming assists greatly in South African rural communities with job opportunities, alleviate household food insecurity supporting the objectives of National Development Plan 2030 of the South African Government (NDP, 2012).

In Chapter 3 of this study morphometric traits obtained amongst Bapedi rams in all four farms, were consistent indicating a level of homogeneity of the breed. These results are similar to the findings on the Zulu sheep by (Mavule, 2012) even though Bapedi rams have higher body weight, rump height, tail length and ear length compared to South African indigenous Zulu sheep. However, Bapedi sheep had smaller, head length and width compared to Zulu sheep. Results from this study concur with findings from Kunene (2010); Gwala *et al*. (2015) in that Bapedi sheep is heavy compared to other South African indigenous breeds (Swati and Zulu) also Mozambique (Landim sheep). Furthermore, Bapedi sheep morphometric measurements are low compared to Namaqua Afrikaner, an indigenous breed of South Africa (Qwabe, 2011), Nigerian Yankasa, Uda and Balami sheep (Yakubu and Ibrahim, 2011), and larger compared to Mexican Croele sheep without ears (Israel *et al*., 2013).

Body growth and biological functions and semen quality of rams are affected by temperatures higher than 29°C (Ahmmed *et al*., 2016). The climate in Limpopo where Bapedi sheep originate is characterised by high temperatures throughout the whole year, whereas high temperatures affect reproductive efficiency of rams. The results obtained from this study-depicted rams from Mara farm to have higher rectal temperature compared to the rest of the farms, however, that did not affect the scrotal circumference measurements, semen volume, semen pH and concentration as it was similar in all Bapedi rams in different farms and conservation methods. The absence of differences in the parameters analysed between the farms could be due to adaptability of the animals. The scrotal circumferences obtained in this study were lower in all farms than 31.3±0.8 cm recorded by Munyai (2012) on *ex situ in vivo* conserved Bapedi rams. Furthermore, high

ejaculate volume and sperm concentration were obtained in all farms used in this study compared to 0.5±0.1 mL and 0.9±84.2 (109/ mL) respectively (Munyai, 2012). For semen pH, the results from this study agree with Munyai (2012).

There is a general lack of good quality breeding rams in smallholder sheep farming in South Africa, therefore there is a need to find a valid, affordable diagnostic approach for selecting good breeding rams. There are many techniques that are used to test vital aspects of sperm function. However, these can be very complex, expensive and there are no universal standards. There is a need for a model that would enable farmers to predict advanced variables from a number of basic morphometric traits. Scrotal circumference of Bapedi rams in this study was measured during the breeding season for accuracy of the results. Etim (2015) reported that SC will vary with season and body conformation, but should be at its maximum peak during the full breeding season. Body weight, body condition score and scrotal circumference ($r= 0.315$; $r = 0.638$; $r = 0.381$ respectively) showed a positive and significant influence on the semen volume in Bapedi rams, these findings are similar to a study that was conducted by Rajashri *et al*. (2016) who reported a significant ($P<0.05$) positive correlation ($r=0.298$) was observed between scrotal circumference (SC) and volume of semen and between scrotal circumference and semen concentration ($r=0.836$). However, it was not the case when about semen concentration in this study. Furthermore, BCS had a positive correlation with the total motility of the sperm, this contradicts finding from a study in Murrah buffalo where BCS was negatively correlated to sperm total motility. However, is in agreement with findings by Addass, (2011) who reported that generally sperm production increases in bulls with increasing age and BCS suggesting that breeding bulls (natural/artificial) should attain full maturity age and at a higher body condition scores, however not more than 7

years of age. Most body measurement showed no significant influence on the semen characteristics and the results found in this study is similar to result found by other researchers (Ramukhithi *et al*., 2017).

Onset of oestrus was earlier on the old (3-6 years) ewes with a shorter duration compared to young (1-2) ewes (Chapter 4). Similar degree of synchrony was obtained in other studies, when evaluating onset of oestrus adult ewes recorded a shorter time from CIDR® removal to first signs of heat compared to the young ones (Simonetti *et al*., 1999; Omontese *et al*., 2014). Results obtained from this study are comparable to results obtained from work done in goats by Lehloenya & Greyling (2010), where time to onset of oestrus was shorter for the adult compared to young does. Moreover, Debaca *et al*. (2017) also reported similar findings.

Body condition score affects the oestrous, endocrine, follicle development and conception for both livestock and human beings. Ewes that are adequately nourished and maintained in good body condition respond most rapidly to oestrous synchronization with an increase in ovulation rate (Satolaria *et al*., 2013; Todorov and Nedelkov, 2015). Results obtained from this study indicated that body condition influences the response, onset and the duration of oestrous in Bapedi ewes. Higher BCS (≥3) ewes responded better to oestrous synchronization (92%) compared to lower BCS (<3) (73%) and the duration of estrus was longer on the high BCS compared to lower BCS ewes. Ewes with low BCS onset of estrus from CIDR removal was shorter compared to high BCS group.

In Chapter 5 reproductive performance of Bapedi sheep was investigated and the conception rates obtained in this study were lower compared to 83 % obtained from non-supplemented group of

Bangladesh indigenous ewes (Zohara *et al*., 2014). But was comparable to findings by Lehloenya *et al*. (2005) done in South African Nguni goats. The percentage of oestrous response and the conception rate in this study were comparable to finding from other studies (Kridli *et al*., 2009; Zohara, *et al*., 2014). The gestation length of Bapedi sheep ranged from 146.8±2.9 to 150.1±0.4 days and there were no significant differences among the farms and within individual conservation methods. The gestation period for multiple and single births was similar in all the Bapedi sheep breed in all the farms. The gestation length of Bapedi sheep was longer compared to gestation length from the Bangladesh indigenous ewes which ranged from 141 to 145 days (Roy *et al*., 2014). The gestation length was also similar to that obtained from South African Boer and Nguni does (Lehloenya *et al*., 2005)

The Mara farm had a higher lambing rate compared to all the other farms ($P<0.05$) with 85% lambing rate, compared to 68%.67 and 70 % for Tompi Seleka, Towoomba and ARC respectively. The results from this study were lower compared to lambing rates of (85.3%) brown and (89.7%) black faced Awassi ewes kept under the same traditional management conditions. These results were similar to indigenous ewes of Bangladesh where 75% lambing rates were obtained. The prolificacy of Bapedi sheep was similar to that obtained from the brown and black head Awassi, the Horro ewes from western Ethiopia and Begayt ewes (Getachew *et al*., 2016).

A validation study of Bapedi sheep genetic structure was done in chapter 6. Indigenous sheep such as Bapedi are reported to be endangered before any molecular and phenotypic characteristics are studied and documented (Macaskill, 2018). Attention should be given to strategies to reduce the decline of indigenous sheep populations. All 14 microsatellite markers yielded a recommended number of alleles per locus. All markers yielded 1 a mean number of 9 alleles and was higher to

ones reported on other indigenous sheep in South Africa for Swazi (6.5), Nguni (5.4) and Pedi (7) (Buduram, 2004). Additionally, for Namaqua Afrikaner the mean number of alleles was (5); Zulu sheep (3.73- 6.5) (Kunene *et al*., 2014; Selepe *et al*., 2018). Internationally lower MNA were observed in African fat tailed sheep and Red Maasia-Mutara (Muigai *et al*., 2009).

In this study high number of expected heterozygosities were observed for all the 7 Bapedi sheep populations. Meaning that the populations that were sampled have high genetic diversities within populations, similar findings were observed in other African indigenous breeds the Red Maasai-Matura (0.70±0.03), Migori (0.70±0.03) and Transmara (0.70±0.03) for expected heterozygosities. In South Africa the heterozygosities were similar to those found in the Zulu sheep for both observed and expected heterozyogosities (Kunene *et al*., 2014). In this study all the He were higher than Ho and these findings contradicts observations from a study in Kail sheep.

Nei's genetic distance and the UPGMA tree revealed close genetic distances between Bapedi sheep populations from Mopani commercial farm, Sekhukhune communal farm, Mara, ARC-AP and Polokwane commercial farm. These findings are interesting as the results from the conservation stations Mara and ARC-AP showed a close relationship even though these stations are far apart and no known exchanges of genetic material has been reported. It was not surprising the population from Sekhukhune was closely related to most population as seen on the UPGMA tree as Sekhukhune was where the Bapedi sheep were maintained and selected (Snyman, 2014).

7.2 Conclusion

It was concluded that Bapedi sheep are still a uniform breed, regardless of their decreasing numbers, and BW, BCS and SC can be included in the selection criteria for improving the reproductive performance of Bapedi breeding rams.

Higher oestrus was observed on ewes with BCS≥3. Young and high BCS ewes had a delayed onset of estrus that lasted longer compared to old and lower BCS ewes. Bapedi sheep can be synchronized successfully and with a reasonable conception rate. Bapedi sheep in all the farms gave birth to mostly single lambs and the sex ratio was mostly male lambs. No mortalities were recorded all the lambs that were born survived until weaning. The results from this study indicated that Bapedi sheep have a high genetic diversity and most of them clustered together, which can be incorporated to future breeding programs and conservation strategies for this breed. The Towoomba Bapedi sheep did not cluster with any other breed, this indicates a possible cross breeding or development of new fixed genes.

7.3 Recommendations

1. Body Weights, BCS and SC can be included in the selection criteria for improving the reproductive performance of Bapedi breeding rams and more studies be done in the correlation of body measurements, testicular morphometry and semen parameters in this breed so that a cheaper tool for selecting breeding rams can be developed.
2. It is recommended that breeding ewes should be of BCS ≥3 and flush feeding should be done to improve the litter size.
3. Oestrous synchronization can be used to shorten breeding season and uniform lamb crop in extensive production systems.
4. Genetic diversity that is reported in this study should be expanded by incorporating more Bapedi sheep farms and exploring advanced analysis of genetic markers such as single nucleotide polymorphism (SNPs).

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