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Relationship between body morphometry in Bapedi rams and sperm characteristics measured using Computer-aided Sperm Analysis

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Abstract

Ram fertility is not defined by a single trait. Quantifiable physical parameters that are correlated to fertilization capacity of sperm are required to advise farmers on ram selection activities. The objective was to investigate the relationship between body measurements and sperm traits of Bapedi rams conserved *in situ* and *ex situ*. Before semen collection and body measurements, body temperature was taken. Semen was collected from 33 rams (two ejaculates/ram weekly, with 2 d resting period, age = 2–6 y). Body weight (BW, kg), body measurements [body length, head length, head width, rump height, rump width, rump length, tail length, heart girth, and scrotal circumference (SC)]; and semen volume (ml), sperm concentration (billion/ml), and sperm motility parameters were measured using Computer-aided Semen Analysis System (CASA®). The semen pH was measured using a microprocessor pH/mV/°C meter fitted with a glass probe. Body condition scores (BCS) of the rams were recorded on a scale of 1 to 5. Data were analysed using the PROC univariate procedure of SAS. BW of Bapedi rams was 38–57 kg in all groups. There was uniformity in all body measurements of Bapedi sheep regardless of method of conservation. Body temperature during semen collection; scrotal circumference; semen volume, pH, and concentration; sperm total motility; and kinematics in Bapedi rams using both methods of conservation were similar. Strong correlations between BW, BCS, and SC with semen volume were found. Rump length positively influenced sperm normality. BW, BCS, and SC can be included in the selection criteria for improving the reproductive performance of Bapedi breeding rams. Farmers can use SC and rump length to predict semen volume and sperm normality.

Keywords: morphometric traits, semen parameters, body weight, body condition score

Introduction

The influence of climate change on animal production (Nardone *et al.*, 2010) and expansion of animal diseases (Haile, 2020) cannot be overstated. This happens at a time when the human population is increasing drastically in a global space, demanding higher meat production (FAO, 2022). Sheep play a vital role in global food production (FAO, 2022) and maintaining the household (Kunene *et al.*, 2009). South Africa is a home to several indigenous sheep, including the Zulu, BaPedi, Damara, and Namaqua Afrikaner (Ngcobo *et al.*, 2022). These sheep breeds remain vital for crossbreeding in future for adaptability to mitigate the influence of climate change on mutton production.

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Reproductive efficiency, particularly prolificacy and age at first lambing, is vital in sheep farming and these vary with breed. Productivity is the major profit determinant in a sheep farming enterprise and improvement may be attained through good reproductive performance (Akpa *et al.*, 2013; Ramukhithi *et al.*, 2017). The ram contributes up to 50% to the flock in animal husbandry, because it sires most of the lambs in the flock and has more genetic influence on the lamb crop (Perumal, 2014). 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, sex, 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 the rams with high sperm output potential at an early age are important (Suleiman *et al*., 2019). Traditionally, rams were selected based on growth rates, rather than on reproductive traits (Perumal, 2014). However, reproduction is one of the most important factors for an economically-viable livestock production practice. The males with larger testes and scrotal circumference produce more sperm than males with smaller testes. Regression equations have revealed that testicular size is positively correlated to body weight and age (Perumal, 2014).

Sexual behaviour and semen quality are the main factors that limit male reproductive efficiency (Suleiman & Alphonsus, 2012). These are influenced by the breed, photoperiod, geographical location, season, testicular size (Ngcobo *et al*., 2023), and age of the ram (Chella *et al*., 2017). Season of the year, however, has been 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, the evaluation of semen characteristics is the most effective parameter for selecting breeding rams. The volume of ejaculate, sperm concentration, sperm motility, sperm viability, and the morphological features of spermatozoa determine the quality of semen in relation to fertility. Ejaculates containing a high percentage of morphologically-abnormal spermatozoa commonly do not result in fertilization of the egg (Shamsuddin & Rodriguez, 1994). Morphological abnormalities of sperm can have a detrimental impact upon fertilization and embryonic development (Saacke, 2008).

The influence of indigenous sheep breed on spermatozoa characteristics, particularly in South Africa, has received little attention (van der Horst & Maree, 2022). Limited studies have examined the association between body measurements, such as testicular or scrotal size and body weight, with components of semen and spermatozoa quality or sperm output in sheep. Linear body measurements are important in the prediction of carcass weight and the determination of certain body conformation traits that can be taken into consideration in selecting animals for genetic improvement. Any quantifiable physical parameters that are directly correlated with the fertilization capacity of semen can be used as a measure of semen quality. Sperm production and quality can be affected by both animal size and physiological status, leading to the objective of the current study, which was to correlate phenotypic and body measurements of Bapedi rams with semen characteristics.

Materials and Methods

Bapedi sheep are an indigenous South African breed of sheep, believed to have arrived with Pedi people between 200 and 400AD. 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. Ngcobo *et al*. (2022) reported that Bapedi sheep adapt well to hot climatic conditions up to 45 ˚C and are robust, 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, or black to black and white head. The first *in situ* conservation effort with Bapedi sheep was done in the mid-1980s at the Stellenbosch Breeding Station in Sekhukhunland in Limpopo (Ngcobo *et al*., 2022).

The Agricultural Research Council Animal Ethics Committees approved the project (Project no: APIEC 17/13). The study was conducted in four *in situ* conservation stations (Towoomba, Mara research stations; Tompi Seleka and Madzivhandila Agricultural Colleges) in Limpopo and one *ex situ, in vivo* farm conservation (Agricultural Research Council–Animal Production) in Gauteng province of South Africa. Towoomba research station is 308.2 kilometres away from Mara research station, 152 kilometres away from Tompi Seleka, 359.2 kilometres away from Madzivhandila Agricultural College

and 119.7 kilometres away from the Agricultural Research Council, Irene. In the current study, 33 mature Bapedi rams of average body weight 48–68 kg, aged 2–6 y old, were used. The study was conducted during the breeding season (June–August 2017). The rams were grazing on natural pastures with free access to water and shade.

A digital weighing scale was used to determine live weights of the rams and the phenotypic traits were visually observed and recorded. The linear body measurements were measured using a flexible tape when the animals were in standing position with head raised and weight on all four feet without body movement. Physical restraint was sometimes applied to limit movement, as described by FAO (2022) and Akpa *et al.* (2013). Seventeen metric traits were measured (in cm) on each ram: rump width (RW), rump length (RL), tail length (TL), wither height (WH), heart girth (HG), heart girth (HG), rump height (RH), ear length (EL), foreleg length (FLL), rear-leg length (RLL), body length (BL), and shoulder width (SW), using a flexible measuring tape (Figure 1). Scrotal circumference (SC) was measured with a flexible tape each time when collecting semen.

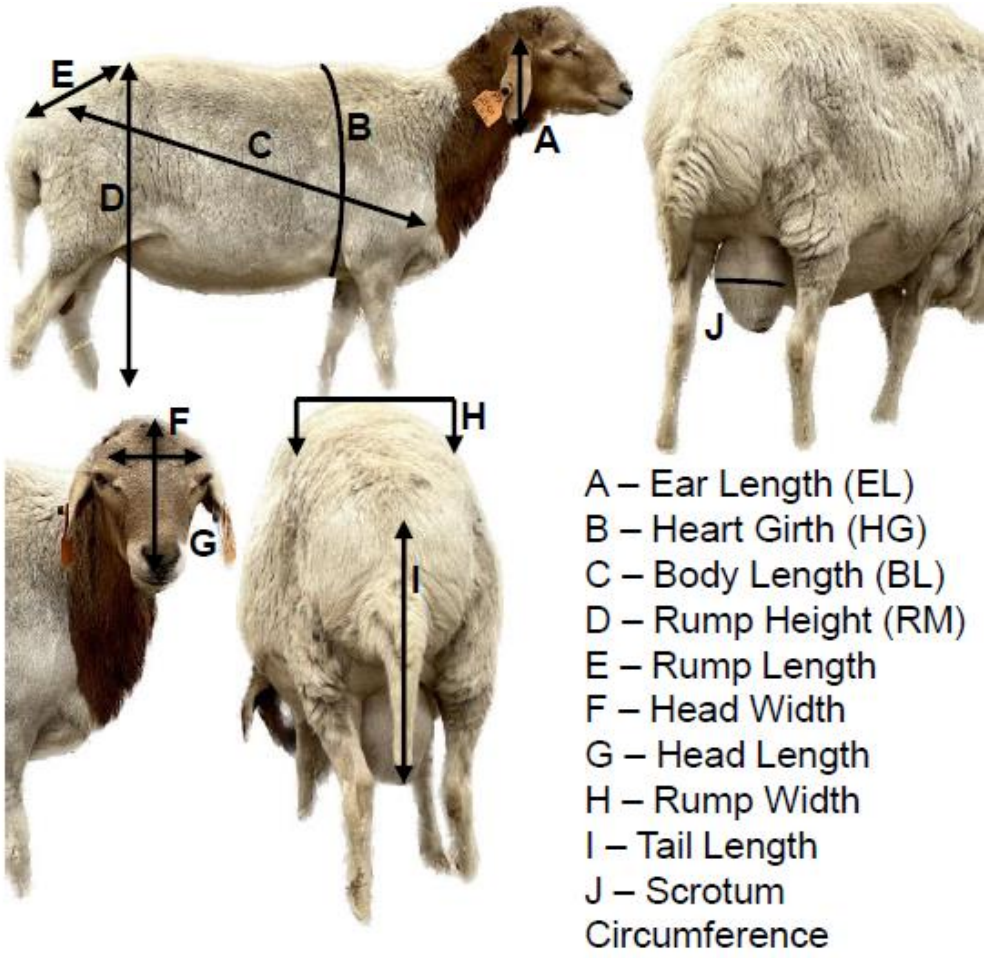

Figure 1 Illustration of body measurements taken on Bapedi rams during the study (South Africa)

Body condition scores of the rams were recorded on a scale of 1 to 5, as described by Abdulkareem & Aljummah (2023). In brief, BCS 1 denoted rams with no perceivable fat between skin and bones; BCS 2 had a light layer of fat on the bones; BCS 3 had a smooth appearance of a layer of fat; BCS 4 had a plump appearance of fat; and BCS 5 was obese, with excessive body fat.

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 rectal probe was lubricated before insertion into the rectum. The probe was inserted and placed in the rectum above the accessory sex glands for stimulation (Dombo, 2002). Semen samples were collected into pre-warmed (37 °C), 15-mL graduated tubes and immediately placed in a thermo flask at 37 °C. Semen was collected twice a week using the electro-ejaculator (RAMSEM, South Africa) with a 2-d resting

period between collections. The electric current was set in four levels of 5 V. The collected semen was then transported to the laboratory within 30 min of collection.

The measurements for semen volume were taken by reading the calibrated 15-mL tube. The semen pH was measured using a microprocessor pH/mV/°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. The absorbance was used to determine the final sperm concentration in millions per millilitre, as described by Ramukhithi *et al.* (2017). The CASA system was used for analysis of sperm motility. The sperm swim-up technique was used, where 10 µl of the semen sample was added into 500 µl of pre-warmed (37 °C) Tris medium. Only 10 µl of the extended semen was placed onto a pre-warmed microscope glass slide and evaluated under (×10) magnification, phase-contrast microscope with the sperm class analyser (SCA®) projecting an image onto a monitor. The 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 included the static (%), slow (%), medium (%), rapid (%); curvilinear (VCL, ms^{-1}), straight-line (VSL, ms^{-1}), average path (VAP, ms^{-1}) velocities; linearity (LIN, %), straightness (STR,%) and wobble (WOB,%).

Eosin Nigrosin stain was used to determine the acrosome integrity, sperm cell viability, morphology, and abnormalities and were evaluated under a fluorescence microscope (Olympus, Japan). The sperm smears were prepared on a clean, warmed glass slide and dried at room temperature (~25 °C). A total of 200 sperm cells per slide were evaluated and counted for each ram per collection using a DBC.6 Model laboratory counter (Han Lien International Corp., Taiwan). The gross structure and sperm morphology were then recorded. The spermatozoa were recorded using two different sets of criteria, i.e., normal and abnormal. Abnormalities were categorised as primary, secondary, and tertiary abnormalities. Primary sperm abnormalities were taken as those abnormalities that occur during the sperm production process in the testicles (head) whereas secondary abnormalities were those that occurred during transition from testicles to the epididymis (mid-piece) and finally, tertiary abnormalities were those that occurred during *in vitro* handling of the semen sample (tail) (Ngcobo *et al*., 2023).

Body measurements were taken in the morning before feeding using a flexible measuring tape. Body weight was recorded using a weighing scale. Body temperature was measured using a digital thermometer before semen collection and body measurements.

Data were subjected to analysis of variance (ANOVA) using Statistics Analysis Software (SAS, 1999). ANOVA was used to test for significant differences in semen concentration, semen volume, semen pH, sperm motility, and sperm morphology. Age of the rams was then added as a covariate in the analysis to block its effect. Treatment means were separated using Fisher's *t*-test and were declared significantly different at $P < 0.05$. Pearson's correlation coefficient was used to assess the relationship between the measured variables.

Results

Table 1 reports the comparison of morphometric traits between Bapedi rams conserved *ex situ* and *in situ*. Body measurements of Bapedi sheep, such as BL, HL, HW, and RH were similar ($P > 0.05$), regardless of the conservation method. The RW and HG measurements were similar between three stations (ARC, Madz, and TMPS) and were substantially higher than the RW and HG observed at the Mara Research Station. Conversely, Mara Research Station produced lower RL measurements, compared to the values observed for Madz and TMPS and did not differ from the ARC. Furthermore, the ear length was longer for rams in Madz and TMPS; Mara and ARC farms produced similar measurements to all farms.

Table 1 Means (± standard error, SE) for morphometric traits (cm) between *ex situ*- and *in situ*-conserved Bapedi sheep

	***Ex situ* conservation**	***In situ* conservation**		
Parameters (cm)	**ARC (n: 9)**	**Madz (n: 8)**	**TMPS (n: 9)**	**Mara (n: 5)**
Body weight (BW)	40.2 ± 4.6	46.9 ± 10.3	47.2 ± 7.6	46.2 ± 6.3
Body length (BL)	67.6 ± 6.5	63.0 ± 3.6	64.3 ± 6.4	62.6 ± 2.5
Head length (HL)	17.8 ± 1.1	17.8 ± 0.8	18.4 ± 1.9	17.3 ± 1.5
Head width (HW)	10.9 ± 0.7	10.8 ± 0.9	10.8 ± 1.2	11.0 ± 1.3
Rump height (RH)	67.2 ± 8.2	66.1 ± 3.5	67.6 ± 5.5	64.6 ± 1.7
Rump width (RW)	17.8 ± 1.4^{a}	18.1 ± 1.1^{a}	15.7 ± 1.7^{a}	24.2 ± 8.5^{b}
Rump length (RL)	19.1 ± 2.4^{ab}	19.8 ± 0.8^{a}	18.9 ± 2.5^{ab}	17.4 ± 1.1^{b}
Tail length (TL)	37.1 ± 4.62^{b}	36.4 ± 5.56^{ab}	34.4 ± 4.8^{a}	32.2 ± 3.2^{a}
Heart girth (HG)	80.4 ± 7.4^{a}	80.3 ± 6.7^{a}	74.2 ± 3.4^{a}	88.0 ± 3.3^{b}
Ear length (EL)	11.3 ± 0.9^{ab}	12.1 ± 1.1^{a}	11.5 ± 1.2^{a}	10.2 ± 1.4^{b}

^{a,b} Values with different superscripts 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 2 shows the comparison between rectal body temperature, body weight, and macroscopic semen parameters. Similar measurements for body weight were obtained for all the rams from different stations (P >0.05). The body temperatures of rams at Mara Farm were substantially higher than the Madz and TMPS farms; ARC farm rams were similar to all other farms. No differences were observed in scrotal circumference measurement in Bapedi rams on all the farms; semen volume, pH, and concentration obtained from these rams were similar (P >0.05).

Table 2 Means (± standard error, SE) for the influence of rectal body temperature on macroscopic semen traits in Bapedi rams

Parameters	***Ex situ* conservation**	***In situ* conservation**		
	ARC (n: 9)	**Madz (n: 8)**	**TMPS (n: 9)**	**Mara (n: 5)**
Body temperature (BT) (°C)	39.0 ± 0.5^{ab}	38.7 ± 0.4^{b}	38.4 ± 0.6^{b}	39.5 ± 0.5^{a}
Scrotum circumference (cm)	28.1 ± 1.5	27.0 ± 2.4	29.4 ± 3.4	28.8 ± 2.0
Semen volume (mL)	1.1 ± 0.4	1.0 ± 0.4	1.1 ± 0.5	0.9 ± 0.3
Semen pH	7.0 ± 0.5	6.9 ± 0.1	6.9 ± 0.2	6.9 ± 0.0
Sperm Concentration (×10^{9})	2.1 ± 0.2	2.2 ± 0.2	2.3 ± 0.3	2.2 ± 0.3

^{a,b} Values with different superscripts 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 shows microscopic sperm traits amongst Bapedi sheep kept *ex situ* and *in situ*. The total sperm motility for TMPS was higher compared to Madz and Mara rams (P <0.05) and the ARC rams were similar to all three farms. The method of conservation did not affect the ram's total sperm motility. Progressive motility was lower for the ARC rams compared to Madz and did not differ from Mara and TMPS rams (P >0.05). The percentages of non-progressive motility and rapidly moving sperm were higher for ARC and TMPS than Madz and Mara. TMPS had lower percentages of MED, SLW, and STC sperm than the other farms. The VCL was higher for TMPS compared to the other three farms (P <0.05).

Table 3 Means (± standard error, SE) for comparison of microscopic semen parameters between *ex situ*- and *in situ*-conserved Bapedi sheep

Parameters	*Ex situ* conservation	*In situ* conservation		
	ARC (n:9)	MADZ (n:8)	MARA (n:5)	TMPS (n:9)
Sperm progression %				
Total motility (TM)	94.2 ± 13.2^{ab}	86.3 ± 11.8^{b}	85.1 ± 14.3^{b}	98.3 ± 1.1^{a}
Progressive motility (PM)	34.5 ± 13.6^{b}	49.9 ± 12.8^{a}	46.6 ± 16.6^{ab}	45.8 ± 9.9^{ab}
Non-progressive motility (NPM)	59.6 ± 11.9^{a}	36.4 ± 8.3^{b}	38.5 ± 12.2^{b}	52.4 ± 10.5^{a}
Static (STC)	4.9 ± 9.8^{ab}	13.7 ± 11.8^{a}	14.8 ± 14.2^{a}	1.6 ± 1.1^{b}
Sperm speed (%)				
Rapid motility (RAP)	46.5 ± 16.1^{b}	36.6 ± 12.8^{b}	36.8 ± 19.2^{b}	70.6 ± 8.5^{a}
Medium motility (MED)	20.6 ± 8.0^{ab}	24.3 ± 14.3^{a}	21.4 ± 8.7^{a}	11.7 ± 4.2^{b}
Slow motility (SLW)	30.5 ± 13.4^{a}	25.3 ± 9.2^{ab}	26.8 ± 10.8^{ab}	16.0 ± 8.4^{b}
Velocity parameters (ms^{-1})				
Curvilinear velocity (VCL)	116.3 ± 22.6^{b}	116.9 ± 13.3^{b}	115.1 ± 17.5^{b}	149.1 ± 9.6^{a}
Straight-line velocity (VSL)	64.1 ± 18.9^{ab}	68.7 ± 14.7^{ab}	61.8 ± 19.2^{b}	82.1 ± 15.4^{a}
Average path velocity (VAP)	82.8 ± 20.7^{b}	89.9 ± 11.8^{ab}	84.8 ± 18.1^{b}	104.5 ± 13.3^{a}
Velocity ratios				
Linearity (LIN)	54.8 ± 10.9^{a}	54.7 ± 9.4^{a}	49.4 ± 9.9^{a}	55.1 ± 10.1^{a}
Straightness (STR)	76.9 ± 6.9^{ab}	70.1 ± 8.2^{bc}	66.4 ± 8.7^{c}	78.2 ± 6.6^{a}
Wobble (WOB)	70.8 ± 9.0^{a}	72.8 ± 5.2^{a}	69.3 ± 6.7^{a}	70.0 ± 7.9^{a}

^{a,b,c} Values with different superscripts 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 4 shows the morphology of sperm in Bapedi sheep kept under different conservation methods. There were no statistical differences between Bapedi ram sperm in terms of viability and percentage of live sperm abnormalities.

Table 5 shows the correlation between the body measurements and the macroscopic semen traits of Bapedi sheep. A strong, positive relationship was found between scrotal circumference and body weight with semen volume. Furthermore, BCS showed a strong, positive correlation with semen volume (P <0.05) and was positively correlated with total motility (P <0.05). RL had a strong, positive relationship with normal sperm (P <0.05). There was a negative relationship between heart girth and linearly-moving sperm. All the other morphometric traits (negative or positive) did not markedly influence the semen volume, pH, and concentration and the sperm total motility.

Table 4 Means (± standard error, SE) for comparison of sperm viability and morphology between *ex situ*- and *in situ*-conserved Bapedi sheep

Parameters		*Ex situ* conservation	*In situ* conservation		
		ARC (n:9)	MADZ (n:8)	MARA (n:5)	TMPS (n:9)
Viability (%)	Live	80.4 ± 5.8	80.8 ± 4.6	74.9 ± 14.4	84.2 ± 7.9
	Dead	20.5 ± 4.9	18.6 ± 4.2	25.1 ± 14.1	15.8 ± 7.8
Abnormalities (%)	Head	0.6 ± 0.8	0.3 ± 0.2	0.7 ± 0.4	0.7 ± 0.2
	Mid-piece	1.8 ± 1.4	1.1 ± 1.2	1.8 ± 1.5	1.3 ± 1.0
	Tail	4.5 ± 1.9a	7.4 ± 7.2a	5.6 ± 2.1ab	6.2 ± 2.7ab

[a,b] Values with different superscripts 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

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Table 5 Pearson's correlations between body measurements and semen parameters in Bapedi rams

	Vol (mL)	pH	Conc ($\times 10^9$)	TM (%)	VCL (ms^{-1})	LIN	Vib (%)	Normal (%)	Abno (%)
BL (cm)	**0.308**	0.037	0.092	0.05	-0.041	0.046	0.282	-0.018	0.106
CC (cm)	-0.155	0.014	0.112	-0.021	0.182	0.035	-0.491	0.06	0.092
HL (cm)	-0.056	0.089	0.075	0.36	0.369	0.087	-0.234	0.078	-0.171
HW (cm)	-0.149	-0.190	-0.169	0.122	-0.232	-0.397*	0.208	-0.052	0.068
RH (cm)	-0.234	-0.193	-0.115	0.108	0.188	-0.254	0.154	0.166	0.057
RW (cm)	-0.241	0.068	0.112	0.060	-0.012	0.354	-0.381	-0.318	0.104
RL (cm)	0.097	0.046	0.33	-0.083	-0.005	-0.001	-0.108	0.566*	-0.157
SW (cm)	0.032	-0.168	-0.017	0.124	0.274	0.067	0.173	0.004	-0.103
TL (cm)	0.235	0.226	0.069	0.146	-0.157	0.364	-0.344	0.197	-0.299
HG (cm)	-0.107	0.040	-0.055	-0.009	-0.187	0.311	-0.106	-0.156	-0.099
EL (cm)	0.046	-0.058	0.122	0.106	0.074	0.088	-0.159	-0.003	0.092
SC (cm)	0.381*	0.092	0.071	0.245	-0.03	-0.116	0.054	0.077	0.230
BCS	0.638**	-0.263	0.020	0.416*	0.042	0.233	-0.321	0.135	0.072
BW (kg)	0.315*	0.062	-0.040	0.311	0.066	-0.104	-0.055	0.102	0.139
BT (°C)	0.105	0.119	-0.031	-0.120	-0.054	0.170	-0.320	-0.227	

* = P <0.05; ** = P <0.01; 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

Discussion

The results shown in Table 1 presented no influence of conservation method/ technique for Bapedi rams when body measurements were compared. Similar body weights and lengths; head length and width; and rump height were obtained in Bapedi rams on all four farms, indicating the homogeneity of the breed. These results are similar to the findings on the Zulu sheep by Mavule (2012), even though Bapedi rams have a higher body weight, rump height, tail length, and ear length than South African indigenous Zulu sheep. However, Bapedi sheep have a smaller head length and width than Zulu sheep. Results from this study concur with findings of Kunene (2009) and Gwala *et al.* (2015) in that Bapedi sheep are heavier than other South African indigenous breeds (Swati and Zulu) and Mozambique Landim sheep. Furthermore, the morphometric measurements of Bapedi sheep were lower than Nigerian indigenous sheep (Yankasa, Uda, and Balami sheep) (Yakubu & Ibrahim, 2011), and higher than 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. Testicles regulate their own temperature, but testicular temperature is dependent on body temperature, which is often measured using rectal temperature (Teodoro *et al.*, 2013). Body growth, 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 year; high temperatures affect the reproductive efficiency of rams. The results obtained from this study indicate that rams from Mara farm had higher rectal temperatures than the rest of the farms. However, this did not affect the scrotal circumference or semen volume, pH, and concentration, as it was similar in all Bapedi rams on different farms, with different 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 the 31.3 ± 0.8 cm recorded by Munyai (2012) on *ex situ*-conserved Bapedi rams.

Higher ejaculate volume and sperm concentration were obtained on all farms in the current study compared to the 0.5 ± 0.1 mL and 0.9 ± 84.2 (10^9/ mL), respectively, of Munyai (2012). For semen pH, the results from this study agree with Munyai (2012). Bapedi sheep in the current study had higher ejaculate volume, semen concentration, and more acidic semen than other South African indigenous breeds (Namaqua Afrikaner, Damara, and Zulu sheep). Semen in this study was collected during the Bapedi sheep-breeding season (June–August) for Bapedi sheep farmers in Limpopo. The ejaculate volume and semen concentration obtained from this study were similar to results found in the Indian Malpura rams and lower than crossbred Barat Merino (Kumar *et al.*, 2009). Awassi and Bangladesh native rams showed similar ejaculate volume with greater semen concentration than Bapedi rams (Salhab *et al.*, 2003; Pervage *et al.*, 2009).

Semen evaluation is a very important element for the 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 method but does not provide estimates as accurate as those obtained using the Computer-aided Semen Analysis system (CASA) (Kumar *et al.*, 2009). The CASA system gives precise, validated, and rapid objective sperm movement measurements.

The type of conservation did not affect the microscopic sperm quality characteristics in this study. Munyai (2012) recorded lower total sperm motility percentages for Bapedi sheep than the current study. However, they recorded a higher number of rapidly-moving sperm than the ARC, MADZ, and MARA farms but lower than TMPS farm, where the first flock of Bapedi sheep was established and maintained at the Stellenbosch breeding station in Sekhukhune district (Ramsay, 2001). Progressive motility percentages 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 the current study. For all velocity parameters, Munyai (2012) obtained better results than the current study. More wobbling sperms were obtained in the current study. All the sperm quality traits obtained from this study were within the acceptable standards for natural mating and AI. The microscopic sperm characteristics of Bapedi rams obtained in the current study were higher than Zulu, Namaqua, and Damara ram sperm traits (Munyai, 2012). For tracked measurements, the results obtained in the current study were lower than the VCL (228.6 ± 6.48; 253.3 ± 6.48 ms^{-1} obtained for Bharat Merino and Malpura rams respectively), however, total motility was higher in Bapedi rams. The total sperm motility obtained in the current study was higher than Bharat Merino and Malpura rams; however, they had higher rapid- and medium-moving sperm than Bapedi rams from the 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.*, 2013; Ahmmed *et al.*, 2016). The results obtained from the current study were better than 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 the fertilising ability of a sperm (Ahmmed *et al.*, 2016). There were no marked influences of conservation method on the percentage of live and dead sperm in Bapedi sheep on all the farms. Higher percentages of live sperm were obtained in this study than in that of Munyai (2012); however, results were similar to those obtained from indigenous Bangladesh ram sperm (Hassan *et al.*, 2009; Mahmuda *et al.*, 2015; Ahmmed *et al.*, 2016; Rekha *et al.*, 2016). The sperm cell abnormalities obtained in the current study were within the range of acceptable standards for breeding rams (Munyai, 2012). The proportions of abnormal sperm obtained in this study were 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 for 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 several 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 would vary with season and body conformation but should be at its maximum during the full breeding season. Body weight, BCS, and scrotal circumference showed a positive 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 positive correlation (P <0.05) between SC and volume of semen, and between SC and semen concentration; however, but semen concentration wasn't the same in the current study. BCS was positively correlated with the total motility of the sperm. This contradicts the findings from a study on Murrah buffalo, where BCS was negatively correlated with sperm total motility. Most body measurements showed no marked influence on the semen characteristics and the results found in the current study were similar to those observed previously (Okere *et al.,* 2014; Ramukhithi *et al.*, 2017).

Conclusion

It was concluded that Bapedi sheep are a uniform breed, regardless of their decreasing numbers. In the conservation programs, the BW, BCS, and SC can be included in the selection criteria for improving the reproductive performance of Bapedi breeding rams. It is recommended that farmers use scrotal circumference and rump length to predict semen volume and sperm normality.

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