Chapter

The Use of Hormonal Assay, Phenotypic Morphometry, and CASA Semen Analysis to Estimate Attainment of Puberty in Indigenous Ram Lambs

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Abstract

Ruminant landraces that are locally adapted have become crucial for sustainable farming considering climate change. This study sought to evaluate the commencement of reproductive capability of Bapedi, Namaqua-Afrikaner and Zulu ram lambs. Data were collected from a total of 21 ram lambs (7/breed) starting from 3–8 months of age. From four months of age, the scrotal circumference of rams was measured using a flexible tape and it was found that it differed significantly between breeds ($P < 0.05$). Blood was collected from the jugular vein using a 21-gauge needle and red cap vacutainers. Blood serum testosterone levels were obtained using a competitive enzyme immunoassay. It was found that Bapedi, Namaqua-Afrikaner and Zulu ram lambs had similar blood serum testosterone levels ($P > 0.05$). At 7 months, semen was collected using an electro-ejaculator and analysed using a Sperm Class Analyser©. There were significant differences found in semen quality between the studied breeds for various semen parameters ($P < 0.05$). There was a weak positive correlation between blood serum testosterone and scrotal circumference ($r = 0.30$). Conclusively, this study highlights the importance of characterisation for the conversation of landrace breeds.

Keywords: blood serum testosterone, scrotal circumference, semen quality, male reproduction

1. Introduction

Male animals contribute 60% to 70% of a flock's genetics [1]; this is because in many smallholder farming systems, a male animal services more than one female during breeding at a ratio of 1 ram: 45 ewes [2]. This outlines the importance of having 'productive' rams in a flock: 'Productive' refers to the animal's overall health and is

a prerequisite for reproductive efficiency [1]. Having reproductive male is important even when assisted reproductive technologies (ARTs) like Artificial insemination (AI) are used; even in small flocks like those of smallholder farmers.

In the Southern African Development Community (SADC), indigenous sheep play an integral role in the income and sustenance of smallholder farmers and livestock keepers [3]. This is partially due to the arid/semi-arid environment of sub-Saharan Africa - indigenous breeds like the Bapedi, Namaqua-Afrikaner and Zulu are particularly suited to South African environmental conditions due to their continued adaptation. However, despite this adaptation, indigenous breeds are continuously being replaced by exotic breeds like the Merino due to better production [4]. This problem is exacerbated by there being no or slow genetic progress for the indigenous genetic resources used by livestock keepers and smallholder farmers [3].

South Africa houses 46 of the 109 different sheep breeds in sub-Saharan Africa [5]; it also houses 19.9 million of the 39 million sheep in the SADC region [6]. Three of South Africa's oldest sheep breeds are the Bapedi, Namaqua-Afrikaner and Zulu sheep: The Bapedi sheep have small frames, they are polled, and have fat-tails along with long legs and a shallow body; they are hardy and disease tolerant [7]. Similar in general phenotype but multicoloured, the Namaqua-Afrikaner is South Africa's oldest indigenous sheep breed [8, 9], and is used primarily in smallholder farming systems [10] and has a fat tail. They are adapted to the South African Karoo's environmental conditions [4]. Namaqua-Afrikaners are reported to be endangered: the number of breeding females ranges from a hundred to one thousand and the number of breeding males ranges between six and twenty [11]. Thus, urgent intervention to conserve this breed is necessary. Lastly, the Nguni sheep of Zululand, Zulu sheep, are generally dark coloured (sometimes with spots), with fat tails and long legs and shallow bodies. They are classified as insecure [12] because of the limited available information on their characteristics. The Zulu sheep are, however, heat, humidity, and tick tolerant [12].

Indigenous breeds are very important, and their genetic erosion is problematic because of the loss of their adaptability [3]. With climate change an ever-growing concern in sub-Saharan Africa, it is thus important to maintain breeds that are adapted.

Testosterone is considered the most fundamental hormone in male reproduction because although gonadotrophin-releasing hormone (GnRH) and luteinising hormone (LH) play important roles (for example, GnRH acts on the anterior pituitary and LH on the testes), testosterone is important for sexual behaviour [13], secondary sex characteristics and sperm production [14]. Testosterone levels can be ascertained from blood serum or from testicular tissue [13].

Morphologically, scrotal circumference size has been reported to be positively correlated with sperm production as the larger the scrotal circumference, the larger the surface area of testes available for spermatogenesis (testes are made up of 80% seminiferous tubules) [13]. Spermatogenesis is the process by which germ cells in the seminiferous tubules form haploid spermatozoa [15]. It is important to note that testosterone is produced by the Leydig cells of the testes under stimulus from LH, which increase in number the larger the testes are [13]. Similarly, follicle-stimulating hormone (FSH) acts on the testes' spermatogonia to initiate and support spermatogenesis, and on the seminiferous tubules' sertoli cells that assist in the production of sperm [1] - the larger the testes, the larger the available surface area for these processes [13].

This study aimed to use hormonal assays, phenotypic morphometry, and CASA semen analysis to estimate the attainment of puberty in indigenous ram lambs as the age at which a ram attains puberty may be crucial for efficient animal production. This is because the ram lamb can now contribute to the productivity of the herd; A herd which, if a landrace breed, may achieve superior productivity parameters compared to exotic breeds due to their adaptability and hardiness to their respective environments.

2. Materials and methods

Live animal work was conducted at the Agricultural Research Council Animal Production Institute, in Irene, South Africa and laboratory work at the University of Pretoria, in Pretoria, South Africa.

2.1 Statistical analysis

Collected numerical data were analysed using Statistical Analysis Software (SAS University Edition, SAS Institute Inc., 2020). N-way Analysis of Variance (ANOVA) tests was performed with Tukey-Kramer adjustments for multiple comparisons, using PROC GLM with the STDERR statement, to obtain the least square means ± standard error values. Data were graphed using Microsoft Excel 2019. In addition, Pearson correlations were also obtained using Statistical Analysis Software (SAS University Edition, SAS Institute Inc., 2020).

2.2 Ethical approval

Ethical approval was obtained from the University of Pretoria: NAS207/2020, as well as from the Agricultural Research Council: APAEC 2020/07. The study also received Section 20 approval from the South African Department of Agriculture, Forestry and Fisheries.

2.3 Experimental animals

Seven ram lambs of each studied breed (Bapedi, Namaqua-Afrikaner and Zulu) were used in this study, which commenced at 4 months of age (for blood serum testosterone and scrotal circumference) and at 7 months of age (for semen quality) until 8 months of age. The twenty-one ram lambs were kept in a semi-extensive habitat with *ad-libitum* access to water in metal troughs and grazing on natural pasture.

2.3.1 Scrotal circumference

Scrotal circumference was measured using a flexible measuring tape while the ram was in a standing position in a crush. The technician pulled the testicles ventrally into the scrotum and measured the circumference of the scrotum at it its widest; the measuring tape was snug on the scrotum. These measurements were done bi-weekly.

2.3.2 Blood sampling

Blood samples were also collected bi-weekly from the jugular vein: A 21-gauge syringe and red-cap vacutainers were used for all ram lambs, and blood serum was harvested by pipetting the collected blood 24 hours after collection and stored at –20°C until analysis.

2.3.3 Semen collection and analysis

An electro ejaculator was used to collect semen from ram lambs which consisted of a variable source of electric current which varied from 0 to 5 volts and 0.5 to 1.0 amps at 7 months of age. After hair from around the sheath was shaved and the prepuce was cleaned with a sterile paper towel to prevent contamination while the ram was placed in a sitting position, the ram was placed on its side and the electrode was also cleaned with a sterile paper towel. The electrode was then lubricated and inserted transrectally and positioned over the accessory glands.

Semen was collected using 15 mL graduated tubes that were pre-warmed to 37 degrees Celsius and then transported within 30 minutes to the Agricultural Research Council's mobile laboratory for analysis: To determine semen volume, measurements on the graduated tube were read before dilution in mL; to determine semen concentration, a spectrophotometer was used (Jenway, United Kingdom). A square cuvette was filled with 3 mL of sodium citrate solution and placed in the spectrophotometer for at least 30 seconds. Raw semen (20 μL) was added in a square cuvette containing the sodium citrate solution, and again placed in the spectrophotometer to read the absorbance. The absorbance was used to determine the final sperm cell concentration with the aid of a formula: 151 × (25.97 X absorbance – 0.3) where 151 is the dilution factor - the final sperm cell concentration was recorded in millions per millilitre; to determine membrane integrity of sperm cells, hyperosmotic osmotic swelling test (HOS) was performed wherein the samples were evaluated under a phase contrast microscope (400x) and 200 sperm cells per slide were counted. Sperm cells with swollen and coiled tails were considered intact (Naing *et al*., 2010); to determine sperm cell abnormalities and viability, 7 μL of semen was stained with 20 μL of nigrosine-eosin stain in an Eppendorf tube and 5 μL of that mixture was smeared onto a microscopic slide.

3. Results

3.1 Scrotal circumference

The scrotal circumferences of South African indigenous ram lambs are reported in **Figure 1**: There existed a significant difference between the scrotal circumference of Zulu ram lambs at 4 months of age and 7.5 and 8 months of age (15.7 ± 1.0, 20.7 ± 1.0 and 20.7 ± 1.0, respectively) ($P < 0.05$), compared to Bapedi and Namaqua-Afrikaner ram lambs which had no significant within-breed differences ($P > 0.05$). However, there also existed significant differences in between-breed differences ($P < 0.05$): Bapedi and Zulu ram lambs had SC significantly higher than Namaqua-Afrikaner ram lambs at 4 months of age and again at 5 to 8 months of age. In addition, the SC of Bapedi ram lambs ranged from 19.8 ± 1.7 cm to 23.5 ± 1.8 cm; that of

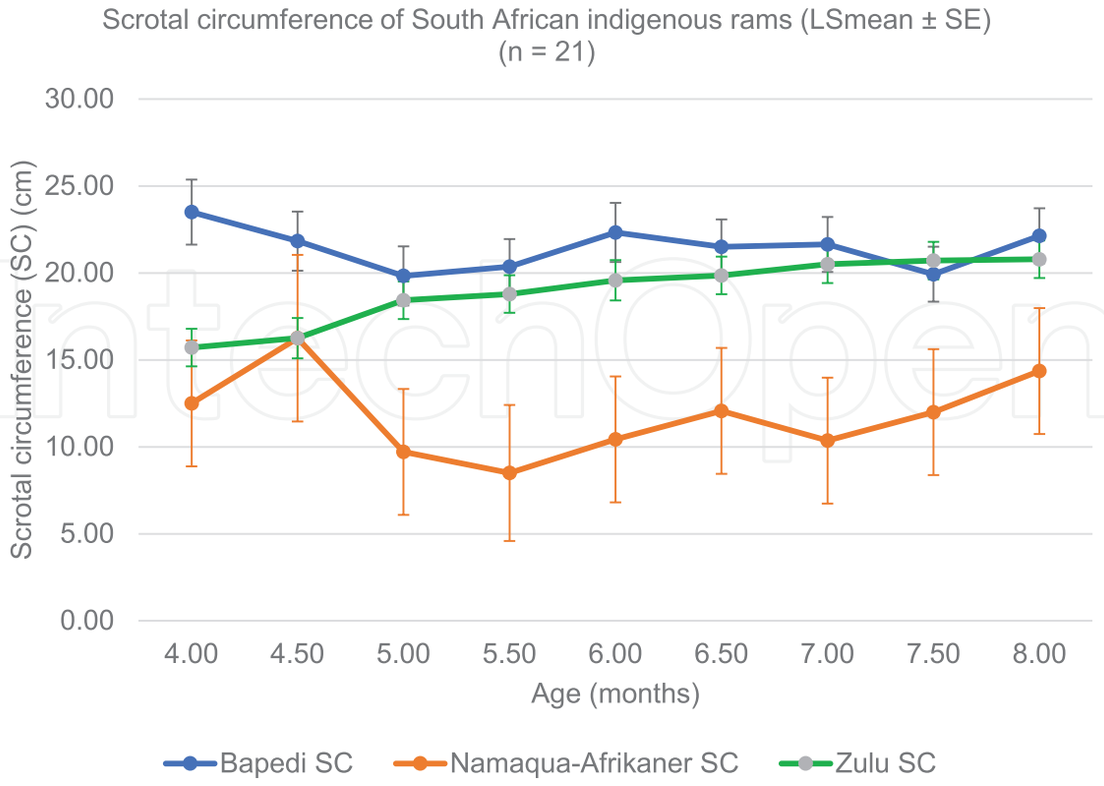

Figure 1.
Scrotal circumference of South African indigenous rams.

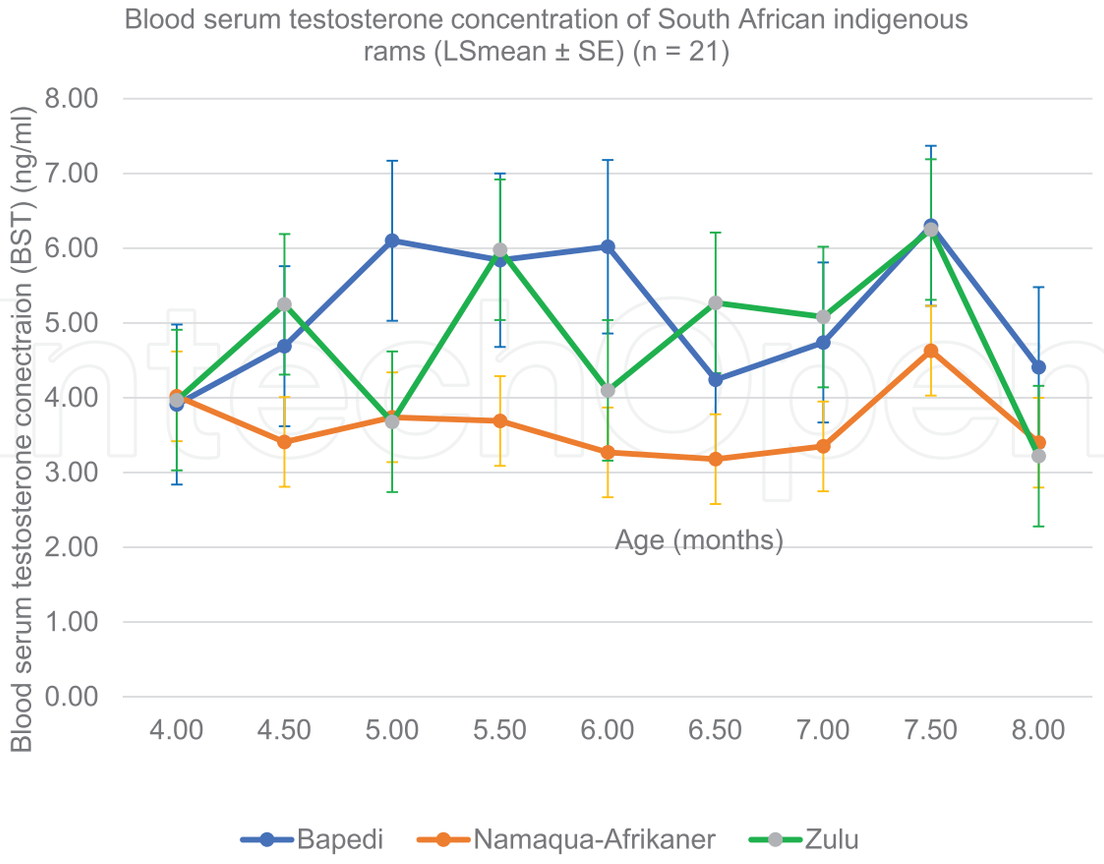

Figure 2.
Blood serum testosterone of South African indigenous rams.

Namaqua-Afrikaner ram lambs from 8.5 ± 3.9 cm to 16.2 ± 4.7 cm; and that of Zulu ram lambs from 15.7 ± 1.0 cm to 20.7 ± 1.0 cm.

3.2 Blood serum testosterone

The blood serum testosterone concentrations of South African indigenous ram lambs are reported in **Figure 2**: There was a significant difference between the blood serum testosterone concentration of Bapedi and Namaqua-Afrikaner ram lambs at 6 months of age (6.0 ± 1.1 and 3.2 ± 0.6 ng/ml, respectively). The blood serum testosterone ranges of Bapedi sheep were 3.9 ± 1.0 ng/ml to 6.3 ± 1.0 ng/ml; that of Namaqua-Afrikaner sheep were 3.1 ± 0.6 ng/ml to 4.6 ± 0.6 ng/ml, and that of Zulu sheep were 3.2 ± 0.9 ng/ml to 6.2 ± 0.9 ng/ml.

3.3 Semen parameters

The semen characteristics of Namaqua-Afrikaner, Bapedi and Zulu ram lambs are compared in **Table 1**: At ages 7, 7.5 and 8 months of age, there was no significant difference between the semen volumes of any of the three breeds ($P > 0.05$). In addition, the semen volumes ranged from 0.3 ± 0.06 mL to 0.6 ± 0.1 mL. Contrastingly, there was a significant difference ($P < 0.05$) between the sperm cell concentrations of Bapedi sheep at 7 and 8 months of age (1.4 ± 0.6 x109/mL and 4.3 ± 0.5 x109/mL, respectively); and of Zulu ram lambs between 7 and 8 months (1.5 ± 0.5 x109/mL and 5.4 ± 0.5 x109/mL, respectively), and between 7.5 and 8 months (2.3 ± 0.5 x109/mL

Breed		**Bapedi**			**Namaqua-Afrikaner**			**Zulu**		
Age (months)		7	7.5	8	7	7.5	8	7	7.5	8
Semen volume (mL)		0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.06	0.4 ± 0.06	0.4 ± 0.06
Sperm cell concentration ($\times 10^9$/mL)		1.4 ± 0.6[a]	2.3 ± 0.5	4.3 ± 0.5[b]	1.1 ± 1.6	2.4 ± 1.6	4.9 ± 1.6	1.5 ± 0.3[a]	2.3 ± 0.3[a]	5.4 ± 0.3[b]
Membrane integrity (%)	Intact	78.8 ± 4.2	81.6 ± 3.5	84.0 ± 3.5	81.0 ± 4.0	80.7 ± 4.0	84.5 ± 4.0	79.5 ± 5.0	85.1 ± 5.4	86.4 ± 5.0
	Non-intact	21.1 ± 4.1	18.3 ± 3.4	15.8 ± 3.4	19.0 ± 1.8	16.7 ± 1.8	15.5 ± 1.8	21.3 ± 4.7	14.9 ± 5.1	13.5 ± 4.7
Viability (%)	Live	79.0 ± 5.3	82.9 ± 4.3	85.3 ± 4.3	76.5 ± 11.4	80.5 ± 11.4	83.7 ± 11.4	82.8 ± 7.7	77.7 ± 8.5	86.0 ± 7.7
	Dead	21.0 ± 5.3	17.0 ± 4.3	14.6 ± 4.3	23.5 ± 11.4	19.5 ± 11.4	16.2 ± 11.4	17.1 ± 7.7	22.3 ± 8.5	14.0 ± 7.7
Abnormalities (n)	Head	1.2 ± 0.2[a]	0.5 ± 0.2	0.3 ± 0.2[b]	2.0 ± 1.3	1.0 ± 1.3	0.5 ± 1.3	1.3 ± 0.5	0.8 ± 0.5	0.1 ± 0.5
	Tail	4.2 ± 1.1	1.1 ± 0.9	1.5 ± 0.9	4.0 ± 2.2	4.5 ± 2.2	2.5 ± 2.2	4.1 ± 0.7[a]	1.4 ± 0.8	1.0 ± 0.7[b]
	Mid-piece	0.0 ± 0.1	0.0 ± 0.1	0.3 ± 0.1	0.0 ± 0.2	0.0 ± 0.2	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a,b] *Values with different superscripts within the same cell in the same row differ significantly ($P < 0.05$).*

Table 1.
Semen parameters of South African indigenous rams.

Parameter		Blood serum testosterone	Scrotal circumference
Blood serum testosterone		1.00	+0.30
Scrotal circumference		+0.30	1.00
Semen volume		+0.05	+0.33
Sperm cell concentration		−0.40	+0.07
Membrane integrity	Intact	−0.06	+0.05
	Non-intact	+0.03	−0.05
Viability	Live	−0.02	+0.13
	Dead	+0.02	−0.13
Abnormalities	Head	+0.35	−0.35
	Tail	−0.0011	+0.15
	Midpiece	+0.15	+0.05

Table 2.
Pearson correlation coefficients for blood serum testosterone and scrotal circumference.

and 5.4 ± 0.5 x109/mL, respectively). In addition, all three breeds had similar percentages of intact and non-intact membranes under membrane integrity ($P > 0.05$). This is the same for live and dead percentages under viability. Lastly, in terms of abnormalities: There was a significant difference ($P < 0.05$) between the number of head abnormalities of Bapedi sheep at 7 and 8 months (1.2 ± 0.5 and 0.3 ± 0.4). Similarly, for Zulu ram lambs, there was a significant difference between the tail abnormalities at 7 and 8 months of age (4.1 ± 0.7 and 1.0 ± 0.7) ($P < 0.05$).

3.4 Pearson correlations for blood serum testosterone and scrotal circumference

Pearson correlation coefficients for blood serum testosterone, scrotal circumference, semen volume, semen concentration, membrane integrity, viability and abnormalities are shown in **Table 2**: There exists a weak positive correlation of $r = 0.30$ between blood serum testosterone and scrotal circumference. There also exists varying positive correlations between blood serum testosterone and semen volume, non-intact membrane integrity, dead viability, head abnormalities and midpiece abnormalities (0.05, 0.03, 0.02, 0.35 and 0.15 respectively); and between scrotal circumference and semen volume, semen concentration, intact membrane integrity, live viability, tail abnormalities, and midpiece abnormalities (0.33, 0.07, 0.05, 0.13, 0.15, and 0.05, respectively).

4. Discussion

4.1 Scrotal circumference

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had statistically significant differences between breeds for scrotal circumferences measurements ($P < 0.05$) for ages 4 to 8 months. This heterogeneity between breeds was unexpected, however, the cause of this heterogeneity may be due to individual animal variation [16]. All three studied breeds had different scrotal circumferences from 4 to

8 months ($P < 0.05$). This may be because testosterone is responsible for sexual maturity and interest and is produced by the Leydig cells of the testes. Leydig cell parameters (like the number of Leydig cells per gram of testis, the total number of Leydig cells per testis and the per cent cell volume of Leydig cell nuclei) are correlated significantly with testosterone levels [17] and testosterone is responsible for secondary sex characteristics like sexual behaviour [18]. Thus, because the Bapedi, Namaqua-Afrikaner and Zulu breeds have dissimilar scrotal circumferences, they will have correspondingly dissimilar Leydig cells and subsequent testosterone production. This suggests that these breeds would attain puberty at different ages.

Söderquist and Hultén [19] found that ram lambs of the Gotlandic breed, a Swedish breed, had a scrotal circumference of 28.9 ± 1.9 cm, which was higher than the scrotal circumferences found in this study. Despite the differences in scrotal circumference between South African indigenous sheep and Icelandic sheep, scrotal circumference is still a useful selection indicator: Males with larger testes are likely to sire daughters that reach puberty at an early age and ovulate more ova during oestrus [19]. This would be especially useful for smallholder farmers and livestock keepers as they would be able to get more lambs from one ewe, both because the age at first lambing is lower and the likelihood of multiple births is higher. However, the significant difference in the scrotal circumferences of Zulu ram lambs at 4 months of age and 7.5 and 8 months of age ($P < 0.05$) indicates that there is a substantial change in SC for Zulu ram lambs between these ages and may act as a selection indicator.

Notably, the significant differences between breeds found from 4 to 8 months have a distinct trend: Bapedi ram lambs generally had the highest SC (except at 7.5 months), and Namaqua-Afrikaner ram lambs had the lowest SC (at all months). This difference in SC may be due to the Namaqua-Afrikaner's late sexual maturity [20]. Late-maturing breeds are physiologically younger at the same chronological age and thus would weigh the same as Bapedi and Zulu sheep but be at a different physiological age with regards to reproduction.

In addition, 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 [21]. This study was conducted between October and the following June in South Africa – meaning Summer through to Autumn and early Winter. Because Autumn and Spring are the breeding seasons of sheep, and an increase (albeit insignificant ($P > 0.05$) in scrotal circumference was observed over this time span, it is possible that there is a correlation between breeding season and scrotal circumference.

4.2 Blood serum testosterone concentration

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had similar blood serum testosterone levels at all ages ($P > 0.05$) except at 6 months of age. This means that although blood serum testosterone levels fluctuated for each breed as they aged from 4 to 8 months of age, this fluctuation was not one that resulted in a significant difference between most samples, both within and between breeds. Thus, the three studied breeds displayed homogeneity in blood serum testosterone levels i.e., there were no significant within-breed differences in BST concentration and minimal between-breed differences ($P < 0.05$). In addition, testosterone is needed to initiate spermatogenesis at puberty and to maintain this process in the adult [17], thus the presence of blood serum testosterone in all three breeds may

indicate the attainment of puberty. Strikingly, Bapedi, Namaqua-Afrikaner and Zulu ram lambs all had the highest sampled blood serum testosterone levels at 7.5 months: Spermatogenesis and its sustenance take 2 months [22], thus this spike in blood serum testosterone may indicate the attainment of puberty [18] already at 5.5 months.

4.3 Semen parameters

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had statistically nonsignificant differences in semen volumes from each other from the first semen analysis at 7 months to the last semen analysis at 8 months of age ($P > 0.05$); Homogeneity among the breeds was shown. It is also important to note that the semen volume observed in this study was expected to be low as ram lambs have lower semen volumes compared to rams due to the parabolic nature of semen volume output in rams i.e., semen volume, while the ram is very young and very old, is low [23]. Also, Rege *et al.* [24] found the semen volumes of Ethiopian highland sheep at 6 months to have a semen volume of 0.30 ± 0.67 mL, which corresponds with the semen volume range found in this study at 7 months of age (0.3 ± 0.06 mL to 0.6 ± 0.1 mL).

Contrastingly, Bapedi sheep had a significant difference in sperm cell concentration at 7 and 8 months of age and Zulu sheep at 7 and 8 months of age, and at 7.5 and 8 months of age ($P < 0.05$), with the sperm cell concentration having increased at 8 months. This means that there was still not a significant difference between breeds, but rather within a breed; Bapedi and Zulu ram lambs' sperm cell concentrations thus increased from 7 months to 8 months, as was to be expected if it is considered that semen characteristics improve with age [24]. In addition, at 8 months of age, both Bapedi and Zulu ram lambs showed a peak in sperm cell concentrations. This peak can be presumably attributed to other factors that occurred 2 months prior to the ram lambs being 8 months old. This is because the testicular function is slow in its development, and because spermatogenesis takes 2 months to complete from the creation of spermatogonia to the creation of spermatozoa [22]. It is interesting to note, however, that this change/spike did not occur for Namaqua-Afrikaner ram lambs: It was observed that Namaqua-Afrikaners are late maturing and would then not have had the prerequisite trigger that Bapedi and Zulu ram lambs had at 6 months of age that resulted in increased semen concentration at 8 months of age; secondly, only an average of 29% of Namaqua-Afrikaner ram lambs produced semen during semen collection during the study period, compared to an average of 57% of Bapedi and 71% of Zulu ram lambs per collection day; thirdly, in this study, the underdevelopment of many Namaqua-Afrikaner ram lambs' glans penises was observed. This anatomical anomaly meant that many Namaqua-Afrikaner ram lambs could not be collected; fourthly, various Namaqua-Afrikaner ram lambs in this study had no testes i.e., scrotal circumference could not be measured because the scrotum felt like an empty sack when palpated. This may be because the testes have not yet descended because of the late sexual maturity observed for Namaqua-Afrikaners.

Bapedi, Namaqua-Afrikaner and Zulu ram lambs had a statistically nonsignificant difference in the percentage of intact and non-intact membranes as well as live and dead percentages ($P > 0.05$). This further supports the homogeneity of these breeds and suggests that all three breeds will have the same fertilizing ability [25]. Rege *et al.* [24] found that Ethiopian highland sheep at 6 months of age produced semen with 29.4 ± 6.0 % Dead percentage; this was higher than that of South African indigenous sheep at 7 months (17.1 ± 7.7 % to 23.5 ± 11.4 %). Moreover, because the

semen morphology of all three breeds is above 50% but below 90% [26], the semen morphology of all three breeds is satisfactory but not exceptional from the first CASA analysis at 7 months of age, further suggesting that Bapedi, Namaqua-Afrikaner and Zulu ram lambs have attained puberty.

With regards to abnormalities, Bapedi ram lambs had a significant decrease in the number of head abnormalities at 7 and 8 months and Zulu ram lambs had a significant decrease in the number of tail abnormalities at 7 and 8 months of age ($P < 0.05$). With Namaqua-Afrikaner ram lambs having a higher number of abnormalities, it is possible that as with sexual behaviour activities, Namaqua-Afrikaner ram lambs are physiologically younger and thus have semen with more abnormalities [27]. Colas [27] found that ejaculates from younger ram lambs have a greater number of abnormal sperm cells in terms of morphology due to incomplete spermatogenic activity and incomplete epidydimal maturation.

4.4 Pearson correlations for blood serum testosterone concentration and scrotal circumference

As shown in **Table 2**, the strongest positive correlation is that between scrotal circumference and blood serum testosterone ($r = 0.30$). This means that as scrotal circumference increases, so does blood serum testosterone concentration. This corroborates the claim that Bapedi and Zulu ram lambs have significantly ($P < 0.05$) higher blood serum testosterone because of their larger scrotal circumferences. Saaed and Zaid [13] corroborate the findings of this study by stating that 'the testis of sheep release testosterone that elevates with increasing testicular weight and age until puberty and maturity age.'

For the Pearson correlations of semen characteristics, there exists a positive correlation between blood serum testosterone and scrotal circumference. It can then be assumed that ram lambs with higher semen volume will have higher blood serum testosterone and scrotal circumference as well ($r = 0.05$ and $r = 0.33$, respectively). The positive correlation between scrotal circumference and semen volume (0.33) is corroborated by Dombo [28] who reported that due to a larger site for semen production and storage, small testes produce a smaller volume of semen than big testes. In addition, there exists correlations between semen concentration and blood serum testosterone ($r = -0.40$) and SC ($r = 0.07$). This means that as semen concentration increases, blood serum testosterone decreases while scrotal circumference increases.

There is also a positive correlation between coiled tails (intact membranes) and scrotal circumference ($r = 0.05$), but a very weak negative correlation with blood serum testosterone ($r = -0.06$). This may mean that better quality semen (that with intact membranes) comes from ram lambs with higher scrotal circumferences; however, there exists an antagonism between blood serum testosterone and intact membranes. Similarly, there exists a negative correlation between straight tails (non-intact membranes) and SC (−0.05), and a very weak positive correlation with blood serum testosterone (0.03). This further supports the claim that there may exist an antagonism between blood serum testosterone and hyperosmotic swelling.

Interestingly, there exists a negative correlation between live viability percentage and blood serum testosterone (−0.02): This also supports the claim that higher blood serum testosterone may be antagonistic to positive semen characteristics; this is also supported by the very weak positive correlation between dead viability percentage and blood serum testosterone (0.03). However, there does exist very weak positive correlations between live spermatozoa percentage and scrotal circumference (0.13),

and correspondingly, there exist negative correlations between dead spermatozoa percentage and scrotal circumference (−0.13). This suggests that ram lambs with higher scrotal circumferences will present a larger proportion of live semen, as in Bapedi and Zulu ram lambs. In terms of head abnormalities, there is a weak negative correlation with scrotal circumference (−0.35) and a weak positive correlation with blood serum testosterone (0.35). This means that the higher the ram lamb's BW and SC, the lower their head abnormalities, and the higher their blood serum testosterone, the higher their head abnormalities. These correlations are corroborated by the *r* of tail abnormalities: There is a very weak positive correlation between tail abnormalities and scrotal circumference (0.15), and a very weak negative correlation with blood serum testosterone (−0.0011), meaning that the higher the scrotal circumference of a ram lamb, the higher the tail abnormalities; and the higher the blood serum testosterone, the fewer tail abnormalities. The relationships between the tail and head abnormalities are inverse. This is also true for *r* of midpiece abnormalities: There are very weak positive correlations between midpiece abnormalities and blood serum testosterone (0.15) and scrotal circumference (0.05). This means that as blood serum testosterone and scrotal circumference increase, so do midpiece abnormalities.

5. Conclusions

Bapedi, Namaqua-Afrikaner and Zulu sheep had different scrotal circumferences but similar blood serum testosterone levels. This lack of significant difference is primarily, a testament to their homogeneity by virtue of age, and secondarily, by virtue of all being indigenous Southern African breeds. In addition, all the studied breeds produced satisfactory semen at 7 months of age. There existed a positive Pearson correlation between blood serum testosterone and scrotal circumference (r = 0.30) and there existed a correlation between all compared parameters. To increase the much-needed conservation and characterisation of these breeds, it is recommended that further studies be conducted on them for robust conservation strategies.

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Declaration of interest

None

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