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Attainment of puberty in South African unimproved indigenous bucks

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ABSTRACT

The purpose of the study was to examine attainment of puberty in South African unimproved indigenous bucks (unimproved indigenous bucks). A total of 24 unimproved indigenous bucks aged between 118–123 days (4 months) with a mean body weight of 8.1 ± 0.3 kg were observed for puberty attainment. Their sexual behaviour activities, body weight and scrotal circumference were recorded every two weeks until the onset of puberty. Blood samples were collected every month to evaluate testosterone concentration. Semen samples were collected when the bucks started to show sexual behaviour activities. For continuous and categorical variables, least-square mean and percentage were used to summarise the data, respectively. Bucks started to show sexual behaviour activities at the age of 5.5 months. At the age of 5.5 months, none of the bucks ejaculated semen with the sperm cells. However, at the age of 6 months, 22 bucks ejaculated semen and the semen characteristics observed were good and above the acceptable standards. In conclusion, unimproved indigenous bucks attained puberty at the age of 6 months.

1. Introduction

Goat meat consumption is increasing worldwide due to its high nutritional value, cholesterol composition and its leanness (Supakorn, 2009; Agga et al., 2011; Astro Awani, 2015; Tardiff, 2015). Production of goat meat needs to be increased to keep up with the growing demand (Sodiq, 2004; Sebei, 2005). This may be achieved through good reproductive performance. Moreover, in order to establish the appropriate selection criteria for young breeding goats, it is crucial to understand the reproductive functioning from the onset of puberty (Nishimura et al., 2000; Bezerra et al., 2009). Evaluation of bucks' puberty is a complex process that cannot be defined by single trait. Therefore, evaluation of age, body weight, scrotal circumference and gonadotropins levels are used to determine puberty attainment (Bezerra et al., 2009). Their combined effects cannot be separated when semen-producing capacity is evaluated (Bester, 2006).

Puberty in bucks is attained when they start to produce viable sperm cells that are capable of producing pregnancy (Daramola et al., 2007; Bezerra et al., 2009). During puberty attainment in bucks, the levels of follicle stimulating hormone (FSH) or spermatogenic stimulating hormone (SSH) and luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH) in the testes increase (Bester, 2006). Luteinizing hormone stimulates the leydig cells to produce testosterone hormone (Farshad et al., 2012), which then initiates the onset of puberty and sperm cells development (Bezerra et al., 2009). Then, FSH stimulates spermiogenesis in the presence of testosterone, which is responsible for sexual behaviour activities (nosing, mounting, nudging, bleating, pawing, flehmen, licking, pelvic thrusts and penile erection) which are correlated to sexual maturity (Nishimura et al., 2000; Farshad et al., 2012) and maintenance of ideal conditions for spermiogenesis and semen ejaculation (Bester, 2006).

Puberty attainment varies and it is primarily influenced by body weight, breed type, birth season and management systems (Daramola et al., 2007; Delgadillo et al., 2007; Farshad et al., 2012). In bucks, puberty has been documented to be reached from the age of 5 months (Souza et al., 2011). Bucks that reach puberty earlier have higher reproductive capacity and shorter generation gap (Bezerra et al., 2009; Bitto and Egbunike, 2012). Irrespective of goat being seasonal breeders, different breeds differ on the rate in which they attain puberty (Nishimura et al., 2000; Bester, 2006; Aguirre et al., 2007). Therefore, gathering of information including puberty attainment in different goat breeds, is crucial for good reproductive management of a herd, such as

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selecting of males capable of making females pregnant (Nishimura et al., 2000; Bezerra et al., 2009). However, in unimproved indigenous goats and other indigenous goats in developing countries, there is little information on their reproductive performance including puberty attainment (Webb et al., 1998; Bitto and Egbunike, 2012). Moreover, much attention in unimproved indigenous goats has been paid to the adult goats than in young goats (Matshaba, 2010; Ramukhithi et al., 2011, 2015; Bopape et al., 2015). The purpose of the study was therefore to examine attainment of puberty in unimproved indigenous bucks.

2. Materials and methods

The study was approved by ethic committees of the Tshwane University of Technology (REC2012/10/019-2) and Agricultural Research Council (APIEC15/044). A total of 24 South African unimproved indigenous bucks also known as unimproved indigenous bucks aged around 4 months (range from 118 to 123 days) with a mean body weight of 8.1 ± 0.3 kg were used. Unimproved indigenous goats are mostly small sized animals, tolerate harsh environmental conditions, parasites and diseases, and are able to survive on poor quality grazing when compared to exotic breeds (Ramsay and Donkin, 2000). The bucks were weaned at 4 months of age. The first evaluation was done after weaning (4 months of age) and the last evaluation was done at the age of 6 months. Moreover, the bucks were grazing together on natural pasture (Kikuyu − *Pennisetum clandestinum*) and drinking water was provided *ad libitum* through metal drinking trough.

To determine puberty attainment of the experimental bucks, sexual behaviour activities, body weight (Nishimura et al., 2000) and testosterone concentration (Santiago-Moreno et al., 2005) were evaluated. For sexual behaviour activities evaluation (visual observation), the bucks were given access for 20 min to teaser does which were on heat every second week until they reached puberty (6 bucks versus 1 doe per session). The sexual behaviour activities observed were; nosing, nudging, pawing, bleating, flehmen, licking, mounting, penile erection and pelvic thrusts (Nishimura et al., 2000). For collection of the sexual behaviour activities data, each buck was observed and counted based on number of the times it showed any sexual behavioural activity. Body weight and scrotal circumference were also determined every second week using a weighing scale and flexible tape, respectively.

For determination of testosterone concentration, blood samples were collected once a month until bucks reached puberty. The blood samples were then centrifuged at 1500 x *g* for 10 min (25 °C). Blood serum were harvested and stored in 1 mL tubes at −20 °C until analysed. A liquid chromatography tandem mass spectrometry (LC–MS/MS) method was modified and validated for the detection of seven steroids including testosterone (Guan et al., 2010). In this method, testosterone was extracted from serum with methyl *tert*-butyl ether (MTBE) and analysed using multiple reaction monitoring (MRM) acquisition mass spectrometric detection in positive electron mode using electrospray ionization (ESI). The linear range of the method was from 1 to 50 (parts per billion) ppb. The samples were then quantified using matrix-matched standard addition calibration curves. The limit of quantification for the method was 1 ppb.

Semen samples were collected following the onset of sexual behaviour activities (5.5 months) using an electro-ejaculator (Ramsem, South Africa). 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. Before insertion of the rectal probe, it was washed with 70% ethanol and lubricated with a lubrication jelly. The probe was inserted and placed in the rectum above the accessory sex glands in order to stimulate them (Dombo, 2002). Semen samples were collected in pre-warmed (37 °C) 15 mL graduated tubes. Four levels of 30 voltage were applied when the bucks were lying on their side (Ramukhithi, 2011). From 24 unimproved indigenous bucks that showed sexual behaviour activities, only 22 bucks responded to electro-ejaculation stimulation and ejaculated semen.

Following semen collection, semen samples were then evaluated for volume by reading the measurements on the collection tubes (Yamashiro et al., 2006) and pH using a pH meter (HANNA Instruments®, South Africa). Sperm cell motility was determined using a Sperm Class Analyser® (Microptic S.L, Barcelona), whereby 500 µL of Tris and 10 µL of semen were mixed in a 1 mL graduated tube and incubated for 5 min at 37 °C. After incubation, 10 µL of extended semen was placed on a pre-warmed microscopic slide (37 °C), mounted with a cover slip and examined (x10) under a phase contrast microscope (Ramukhithi, 2011). Sperm cell concentration was determined with a spectrophotometer (Jenway, United Kingdom). A square cuvette was filled with 3 mL of sodium citrate solution and placed in a spectrophotometer for at least 30 s. Raw semen (15 µL) was added in a square cuvette containing the sodium citrate solution, again placed in a spectrophotometer in order to read the absorbance. The absorbance read was used to determine the final sperm cell concentration with the aid of a formula (201 × 25.97 X absorbance − 0.3). The final sperm cell concentration was recorded in millions per millilitre (Seshoka, 2015).

For evaluation of acrosome integrity, sperm cell viability, morphology and abnormalities, samples stained with nigrosin-eosin stain were evaluated under a fluorescent microscope (Olympus, Japan) and 200 sperm cells per slide were counted. Live sperm cells and the sperm cells that have non-reacted acrosomes did not absorb stain (fluorescence), while dead sperm cells and the sperm cells that have reacted acrosomes absorbed stain and become purple in colour (Samper, 2000). Live sperm cells were further evaluated for morphology and abnormalities. Abnormalities were categorised as primary, secondary and tertiary abnormalities (Loskutoff and Crichton, 2001). For evaluation of membrane integrity, a hypo-osmotic swelling (HOS) test was used and the samples were evaluated under a phase contrast microscope (400x) and 200 sperm cells per slide were counted. Sperm cells with swollen and coiled tail were considered intact (Naing et al., 2010).

The data were analysed using Statistical Analysis Software (SAS) (1999), Version 9.2. Sexual behaviour activities data for all the bucks were summarised as percentages. Analysis of variance (ANOVA) and least significant difference (LSD) were applied in order to analyse the influence of bucks' age on the body weight and scrotal circumference. Body weight, scrotal circumference, testosterone concentration ($\alpha = 0.05$) and semen characteristics data were summarised as least square mean ± standard error. The Pearson correlation was done to show the relationship between body weight, scrotal circumference, testosterone concentration and semen characteristics of unimproved indigenous bucks.

3. Results

Sexual behaviour activities of unimproved indigenous bucks are illustrated in Table 1. No sexual behaviour activities were observed at the age of 4–5 months. However, at the age of 5.5 months, unimproved indigenous bucks started to show sexual behaviour activities. The highest proportion of sexual behaviour activity at the age of 5.5 months was mounting (50%) and the lowest proportion was pelvic thrusts (4%). At the age of 6 months, the highest proportion of sexual behaviour activity observed was licking (83%) and the lowest proportion was penile erection (17%). The sexual behaviour activities had increased by 50% from the age of 5.5–6 months.

Body weight, scrotal circumference and testosterone concentration of unimproved indigenous bucks are illustrated in Table 2. The body weights of unimproved indigenous bucks ranges from 8.1 ± 0.3 to 14.6 ± 0.5 kg at the age of 4–6 months. Moreover, body weights of unimproved indigenous bucks observed from the age of 4–6 months were significant different ($p < 0.05$). Scrotal circumferences at ages of 5 (15.5 ± 0.4 cm), 5.5 (16.7 ± 0.4 cm) and 6 months

Table 1
Sexual behaviour activities of unimproved indigenous bucks (%) (n = 24).

Sexual behaviour activities	Age				
	4 months	4.5 months	5 months	5.5 months	6 months
Nosing	0	0	0	17	46
Nudging	0	0	0	21	25
Mounting	0	0	0	50	50
Bleating	0	0	0	29	46
Pawing	0	0	0	21	25
Flehmen	0	0	0	21	75
Licking	0	0	0	8	83
Pelvic thrusts	0	0	0	4	25
Penile erection	0	0	0	25	17
Average	0	0	0	22	44

Table 2
Body weight, scrotal circumference and testosterone concentration of unimproved indigenous bucks (LS-mean ± se) (n = 24).

Age (months)	Body weight (kg)	Scrotal circumference (cm)	Testosterone (ng/mL)
4	8.1 ± 0.3[a]	12.9 ± 0.4[a]	< 1.0
4.5	9.6 ± 0.4[b]	14.1 ± 0.4[a]	
5	11.4 ± 0.4[c]	15.5 ± 0.4[b]	2.1 ± 0.03[a]
5.5	12.8 ± 0.4[d]	16.7 ± 0.4[c]	
6	14.6 ± 0.5[e]	18.1 ± 0.4[d]	4.5 ± 0.2[b]

[a,b]Values with different superscripts within the same column differ significantly ($p < 0.05$).

(18.1 ± 0.4 cm) were significantly different ($p < 0.05$). Testosterone concentration at the age of 4 months was below 1 ng/mL. Testosterone concentration was higher at the age of 6 months (4.5 ± 0.2 ng/mL) when compared to 5 months (2.1 ± 0.03 ng/mL) ($p < 0.05$).

Semen characteristics of unimproved indigenous bucks are illustrated in Table 3. At the age of 5.5 months, none of the unimproved indigenous bucks did ejaculate semen with the sperm cells. However, at the age of 6 months, 22 unimproved indigenous bucks ejaculated, and the semen characteristics observed were good and above the acceptable standards.

Table 3
Semen characteristics of unimproved indigenous bucks collected at the age of 6 months (n = 22).

Characteristics				LS-mean ± se
Semen		Volume (mL)		0.6 ± 0.04
		pH		7.0 ± 0.1
	Concentration (X 10^9/mL)			2.9 ± 0.3
Sperm cell	Motility	Progression (%)	TM	78.7 ± 3.7
			PM	42.8 ± 4.9
			NPM	35.9 ± 3.2
			Static	21.3 ± 3.7
		Velocity (%)	Rapid	28.9 ± 5.3
			Medium	30.5 ± 3.7
			Slow	33.6 ± 9.4
		Average values of velocity parameters	VCL (μm/s)	85.5 ± 7.2
			VSL (μm/s)	62.5 ± 7.6
			VAP (μm/s)	69.4 ± 7.5
			LIN (%)	63.8 ± 3.5
			STR (%)	73.9 ± 3.5
			WOB (%)	80.3 ± 2.9
	Vitality (%)	Acrosome	Intact	96.7 ± 0.6
		Membrane	Intact	82.3 ± 1.8
	Viability (%)	Live		84.9 ± 2.8
	Morphology (%)	Normal		92.1 ± 1.3
	Abnormalities (%)	Primary		1.6 ± 0.2
		Secondary		3.5 ± 0.6
		Tertiary		2.8 ± 0.5

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.

The Pearson correlation between body weight, scrotal circumference, testosterone concentration and semen characteristics of unimproved indigenous bucks are illustrated in Table 4. Body weight did not show any correlation with TM and static sperm cells. On the other hand, body weight had a positive correlation with intact acrosome and membrane ($p < 0.01$). However, body weight had a negative correlation with slow velocity ($p < 0.01$). Scrotal circumference had a positive correlation with intact acrosome ($p < 0.01$). Testosterone concentration did not show any correlation with sperm cell viability and primary abnormalities.

4. Discussion

When the current study was compared with the previous studies: unimproved indigenous bucks had a lower body weight at the age of 4 (8.1 kg) and 6 months (14.6 kg) when compared to other indigenous bucks in the previous studies at the same age. For example, Tokara bucks were weighing 10.6 and 15.8 kg at the age of 4 and 6 months, respectively (Nishimura et al., 2000). Moreover, Boer goat bucks were weighing 16.3 kg at the age of 3 months (Bezerra et al., 2009). The scrotal circumference that was observed at the age of 4 months remained the same at the age of 4.5 months, and it started to increase from 5 to 6 months of age. The results of the current study are in line with the literature, as it has been documented that body weight and scrotal circumference increase with age (Delgadillo et al., 2007; Bezerra et al., 2009). The scrotal circumference of 18.1 cm observed in unimproved indigenous bucks which were 6 months old were lower when compared to Boer goat scrotal circumference of 21 cm which had the same age (Bezerra et al., 2009). The differences between the current study and previous studies might be due to climate, latitude, hormonal concentrations (Bezerra et al., 2009; Farshad et al., 2012), breed and nutritional differences (Gebre, 2007; Akpa et al., 2013).

In the current study, bucks started to show sexual behaviour activities at the age of 5.5 months, with mounting being the highest, followed by licking at the age of 6 months. This is in agreement with the literature as it had showed that mounting of does by bucks is a common and first activity that can be used to evaluate puberty of bucks when introduced to the does (Nishimura et al., 2000; Suyadi, 2012). Generally, sexual behaviour activities had increased exhibition with age of the bucks. In contrast, Suyadi (2012) reported that age of bucks has no influence on the sexual behaviour activities.

At the age of 4 months, unimproved indigenous bucks' testosterone concentration was below 1 ng/mL. This confirmed that at that age those bucks were still immature. Testosterone concentration in the current study increased with the age of the bucks. This also agrees with the previous studies (Bezerra et al., 2009; Souza et al., 2011). Moreover, testosterone concentration of 4.5 ng/mL in unimproved indigenous bucks was high and comparable to Markhoz matured bucks (2–3 years) testosterone concentration (4.8 ng/mL) (Farshad et al., 2012). On the other hand, testosterone concentration at the age of 6 months was even higher when compared to the previous study in Boer goat bucks at the age of 6 months (1.9 ng/mL) (Bezerra et al., 2009). The increase of testosterone concentration with age in the current study is also in agreement with the literature (Farshad et al., 2012). Moreover, testosterone levels in the current study is in agreement with Souza et al. (2011) who indicated that testosterone concentration ranged from 0.4 to 5.4 ng/mL at the age of 5–9.5 months. The reactivation of leydig cells was documented to be associated with the multiplication of germ cells, which is a crucial physiological event on achieving puberty in bucks. The full development of the seminiferous tubules coincides with increase in testosterone concentration (Nishimura et al., 2000).

At the age of 5.5 months, none of the unimproved indigenous bucks

Table 4
The Pearson correlation between body weight, scrotal circumference, testosterone concentration and semen characteristics of unimproved indigenous bucks at the age of 6 months.

Characteristics			Body weight (kg)	Scrotal circumference (cm)	Testosterone (ng/mL)
Semen	Volume (mL)		0.320	0.152	−0.344
	pH		−0.054	0.070	0.054
Sperm cell	Concentration (X 10^9/mL)		0.013	−0.194	−0.181
	Progression	TM	0	−0.162	0.362
	(%)	PM	0.049	−0.114	0.037
		NPM	−0.081	−0.013	0.371
		Static	0	0.161	−0.362
	Velocity	Rapid	−0.027	−0.296	−0.008
	(%)	Medium	−0.106	0.090	0.222
		Slow	−0.658**	−0.389	0.157
	Average values of velocity parameters	VCL (μm/s)	0.004	−0.065	0.069
		VSL (μm/s)	−0.357	−0.215	0.080
		VAP (μm/s)	0.133	0.074	0.029
		LIN (%)	−0.119	0.122	0.039
		STR (%)	−0.091	0.119	−0.098
		WOB (%)	0.011	0.189	0.052
	Vitality (%)	A/intact	0.543**	0.485**	−0.196
		M/intact	0.439**	0.124	−0.028
	Viability (%)	Live	0.089	−0.122	0
	Morphology (%)	Normal	−0.137	−0.008	−0.120
	Abnormalities (%)	Primary	−0.080	0.207	0
		Secondary	0.111	−0.094	0.029
		Tertiary	0.125	−0.214	0.278

**Significant at $p < 0.01$, 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, WOB = wobble, A = acrosome and M = membrane.

did ejaculate semen with the sperm cells. However, at the age of 6 months 22 bucks ejaculated, and the semen characteristics observed were within acceptable limits (Ramukhithi et al., 2011). This shows that the puberty has been reached (Souza et al., 2011). Moreover, this also shows that the semen characteristics of unimproved indigenous bucks were significantly influenced by age like in other goat breeds. Semen production with acceptable characteristics at the age of 6 months was reported to be due to a significant development of seminiferous tubules and sertoli cells (Souza et al., 2011). However, the onset of semen production in the current study (6 months) was late when compared to Tokara bucks (4.5 months) (Nishimura et al., 2000) and West African Dwarf bucks (5 months) (Bitto and Egbunike, 2012). Semen characteristics in the current study were better when compared to Boer goat bucks, which were even older (Suyadi, 2012).

Body weight showed positive relationship with intact acrosome and membrane. This might be an indication that the animals that are well fed and their body condition is improved, as a result some of their semen characteristics are also improved (Akpa et al., 2013). On the other hand, scrotal circumference showed positive relationship with intact acrosome. For the other semen characteristics that were positive or negative with body weight, scrotal circumference and testosterone, their relationships were low and insignificant. However, this contradicts with the literature, whereby most of semen characteristics were reported to have positive and significant relationship with body weight and scrotal circumference (Webb et al., 2004; Pepper-Yowell, 2011).

Unimproved indigenous bucks attained puberty at the age of 6 months with the average body weight of 14.6 kg and scrotal circumference of 18.1 cm. However, these were lower when compared to body weight of 25.7 kg and scrotal circumference of 21.1 cm reported in Anglo-Nubian bucks that reached puberty at the age of 5 months. This show that breed, environment, management systems and genetic type influences attainment of puberty (Daramola et al., 2007; Souza et al., 2011).

5. Conclusion

Unimproved indigenous bucks attained puberty at the age of 6 months with a significant increase in body weight, scrotal circumference and testosterone concentration.

Conflict of interest

There is no conflict of interest to declare.

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