Small Ruminant Research 153 (2017) 57–61

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Research Paper

Attainment of puberty in South African unimproved indigenous bucks

Fhulufhelo Vincent Ramukhithi[a,b], Khathutshelo Agree Nephawe[b], Tlou Caswell Chokoe[c], Matshidiso Bailakaye Matabane[a], Masindi Lotus Mphaphathi[a], Khoboso Christina Lehloenya[d], Tshimangadzo Lucky Nedambale[b,*]

[a] Agricultural Research Council, Germplasm Conservation and Reproductive Biotechnologies, Private Bag X 2, Irene, 0062, South Africa
[b] Tshwane University of Technology, Department of Animal Sciences, Private Bag X 680, Pretoria, 0001, South Africa
[c] Department of Agriculture, Forestry and Fisheries, Directorate: Farm Animal Genetic Resource, Private Bag X 250, Pretoria, 0001, South Africa
[d] University of Pretoria, Department of Animal and Wildlife Sciences, Private Bag X 20, Hatfield, 0028, South Africa

ARTICLE INFO

Keywords:
Puberty attainment
Indigenous bucks
Sexual behaviour activities
Testosterone

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

* Corresponding author.
E-mail address: nedambaletl@tut.ac.za (T.L. Nedambale).

http://dx.doi.org/10.1016/j.smallrumres.2017.05.009
Received 15 November 2016; Received in revised form 17 May 2017; Accepted 20 May 2017
Available online 22 May 2017

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