Research Journal of Veterinary Sciences

ISSN 1819-1908

Academic Journals Inc.

www.academicjournals.com

Research Journal of Veterinary Sciences 1 (1): 50-55, 2008
ISSN 1819-1908
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Effects of Age and Time of Sampling on Serum Testosternoe and Spermiogram of Bunaji and N'Dama Bulls

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Abstract: Serum testosterone concentrations and spermiogram of Bunaji and N'Dama bulls were investigated at 12, 24, 36 and 48 months of age. Scrotal circumference of the Bunaji bulls were significantly ($p<0.05$) higher than those of the N'Dama bulls. Sperm concentration, total sperm and mean testosterone levels were higher for N'Dama cattle than the Bunaji bulls. However, the sperm motility, total percentage dead sperm and abnormal sperm did not differ with the two breeds ($p>0.05$). Testosterone concentration, scrotal circumference, volume of semen, sperm concentration and sperm output of all the bulls Increased significantly with age ($p<0.05$). The Bunaji and N'Dama bulls had average peak testosterone concentrations of 3.1 and 8.7 ng mL^{-1}, respectively, at 12 months of age and rose to 8.6 and 10.9 ng mL^{-1}, respectively, at 48 months of age. The number of episodic peaks ranged from 3 to 7. Most of these peaks were between 0800 and 1000 h. Duration of peaks ranged from 20 min to a maximum of 2 h. Intervals between peaks ranged from 20 min to 1 h 40 min. The 10 h secretary patterns of testosterone were episodic, pulsatile or temporal in nature. The exact significance of these episodic peaks is not clear but may be related to the sexual and behavioral states of animals or adjustments to photoperiodicity, temperature and postural states of the animals. The testosterone concentrations in this study were closely related to the semen characteristics. Thus, a study of the relationship between follicle stimulating hormone, luteinizing hormone, testosterone, libido and spermiogram in tropical bulls may be required.

Key words: Testosterone profiles, age, time, spermiogram, bulls

INTRODUCTION

There is abundant information regarding the endocrine events in the *Bos taurus* cattle (Rawlings *et al*., 1972; Karg *et al*., 1976; Lunstra *et al*., 1978; McCarthy *et al*., 1979) and ouly limited published information is available for *Bos indicus* (Fields *et al*., 1982; Rekwot *et al*., 1997, 2000). The general study of hormones such as gonadotrophin-releasing hormones, follicle stimulating hormones, luteinizing hormones, estrogens, prostaglandins, testosterone and progesterone has clarified the fundamental mechanisms which regulate puberty, sexual (functions) cycles, gestation and lactation in domestic animals (Edqvist *et al*., 1976; Peters and Ball, 1995; Rekwot *et al*., 2006).

It is well known that adequate nutrition is an important management factor that affects sexual maturity and improved feeding can optimize sperm cell production (Salisbury *et al*., 1978; Rekwot *et al*., 1987, 1988). Testosterone concentration, spermiogram and other parameters increased in levels with increase in age and protein content of the diets (Rekwot *et al*., 1997), thus corroborating the study of Nolan *et al*. (1990), who reported that retarded testicular growth and reduced testosterone concentrations were observed in bulls fed to gain slowly, compared with those fed to gain rapidly.

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Karg *et al*. (1976) reported very low levels of testosterone before puberty, which rose sharply at the onset of puberty, showing numerous pulsatile peaks as a function of time and age. An association between onset of spermatogenic activity and increase testosterone concentrations has also been reported (Karg *et al*., 1976). Testosterone levels may not have a direct relationship to libido and semen quality in bulls (Post and Christensen, 1976). Concentrations of serum testosterone increased linearly with advancing age in bulls (Lunstra *et al*., 1978; Rekwot *et al*., 1997), circadian rhythms and well-defined episodic peaks have been documented in serum testosterone profiles (Sanwal *et al*., 1974; Agarwal *et al*., 1983; Rekwot *et al*., 1997). In evaluating the reproductive function of our zebu bulls, attention is always made to the spermiogram, scrotal size and liveweight. We became aware that vital information on androgen profiles for monitoring male reproductive performance in Bunaji and N'Dama bulls in Nigeria are lacking. The present study was undertaken to investigate the effects of age and time of sampling on serum testosterone and spermiogram of Bunaji and N'Dama bulls in Nigeria.

MATERIALS AND METHODS

Management of Bulls

Thirteen Bunaji and eleven N'Dama yearling bulls, aged approximately 12 months were involved in this experiment. The bulls were grazed on improved pastures and supplemented with a mixture of concentrates consisting of cottonseed cake, maize and wheat offal. Mineral blocks and water were provided *ad libitum*. The bulls were screened for blood and helminthes parasites and appropriate treatments and vaccinations were done. Monthly treatments with anthelmintics and acaricides were implemented throughout the period of study.

Body and Testicular Measurements

Body condition scores indicative of body weight status and muscle cover of the bulls were taken at 12, 24, 36 and 48 months of age using a scale of 0-5 from the most emaciated to the fattest (Pullan, 1978). Scrotal circumference and spermiogram of all the 24 bulls were investigated at the ages of 12, 24, 36 and 48 months.

Semen Collection and Evaluation

Semen was collected by rectal massage specifically or manipulating of the ampullae (Arthur, 1975). Semen volume was measured directly by reading from the graduated centrifuge tube used as the collection vial. The semen was then kept in a water-bath at 37°C until evaluation was completed. Microscopic examination of wave pattern (gross sperm motility) was determined by examining a drop of raw undiluted semen on a pre-warmed slide at 37°C under a light microscope at ×40 magnification (Zemjanis, 1970).

The concentration of the spermatozoa was determined using the red blood cell counting chamber of a haemocytometer that was crossed with microscopic grid or triple lines containing 25 large squares or 400 small squares. Sperm cells were counted diagonally from the left top to the right bottom in 5 large squares or a total of 80 small squares (Coffin, 1953). Semen smears were stained with eosin-nigrosin for the determination of live: dead ratio (Sekoni *et al*., 1981). Also fresh semen samples were fixed in buffered formal saline to determine sperm abnormalities. All slides were examined and at least 400 spermatozoa per slide were investigated or counted using a phase-contrast microscope at ×1000 magnification using oil immersion (Sekoni *et al*., 1981).

Blood Sampling

Blood samples from five of the Bunaji and five of the N'Dama bulls were collected every twenty min from 0800 to 1100 h at 12, 24, 36 and 48 months of age. Blood samples were drawn from the jugular vein by use of an indwelling catheter and serum samples were harvested and stored at -20°C

until analyzed for testosterone concentration. Thus a total of 1600 blood samples were collected from the ten bulls (2 each of Bunaji and N'Dama) and the serum harvested and stored at -20°C and later analyzed for testosterone concentrations. A day before each periodic blood sampling, all the 24 bulls were subjected to semen collection by rectal massage.

Radioimmunoassay (RIA)

Serum testosterone concentrations were determined using a no-extraction, 'Coat-A-Count' solid phase testosterone, RIA kit (Diagnostic Products Corporation, 1987). Human serum based standards with testosterone concentrations ranging from 0, 0.3, 1.0, 3.0, 10.0 and 30.0 ng mL^{-1} were used. The standards provided were validated to cover the physiological testosterone concentration range prevalent in the serum of most domesticated livestock species. The assay procedure was as follows: to antibody-coated tube, 25 µL of standard (0, 0.3, 1.0, 3.0, 10.0 and 30.0 ng mL^{-1}) or serum samples and 1mL buffered ^{125}I-labelled testosterone solutions were added. The mixtures were vortex, incubated for 3 h at 37°C and decanted to remove all visible moisture to separate bound from free testosterone. The tubes were placed in a gamma counter (Beckman 400, Beckman Instruments, Inc) and the potencies of the samples estimated using a linear logit-log dose-response curve.

The antisera were highly specific for testosterone, the cross-reactivity with androstenedione and dihydrotestosterone were 3.0 and 8.1%, respectively, while with other C-19 steroids, the cross-reactivity was <3%. The assays for the bulls were validated for sensitivity and coefficient of variations. The sensitivity of the assay was 0.079 ng mL^{-1}, while intra- and inter-assay coefficients of variation were 8.8 and 9.1%, respectively. Wide variations in protein concentrations of serum samples had no effect on the Coat-A-Count total testosterone assay.

Statistical Analysis

Split-plot analysis of variance (ANOVA) was used to test for the effects of age and time of sampling on testosterone levels and spermiogram using the general linear model procedures (SAS, 1990). Mean basal testosterone levels were defined as the mean of the 5 lowest values observed during the 10 h sampling period, while peak testosterone concentrations were defined as each single or series of values two fold above mean basal concentration (Renaville *et al*., 1983).

RESULTS

Body condition scores, scrotal circumference, spermiogram and testosterone concentrations are shown (Table 1). There were no differences in the body condition scores of the bulls with respect to breed or age of the animals ($p<0.05$). Scrotal circumference of both the Bunaji and the N'Dama bulls increased with age significantly ($p<0.05$). Scrotal circumference of the Bunaji bulls were significantly higher than the N'Dama bulls throughout the sampling period ($p<0.05$). Volume of semen, concentration of semen and sperm output tended to increase with age up to 36 months and stabilized at 48 months of age (Table 1). The Bunaji bulls had higher volume of semen than the N'Dama bulls ($p<0.05$). Sperm concentration and sperm output were significantly higher for the N'Dama bulls than the Bunaji bulls at 24, 36 and 48 months of age ($p<0.05$; Table 1). Total dead sperm and morphologically abnormal spermatozoa were within the normal ranges and were not significantly different for the two breeds or between the varying ages ($p<0.05$). Peak and basal testosterone concentrations were significantly higher for the N'Dama bulls than the Bunaji bulls ($p<0.05$). Peak and basal testosterone concentrations tended to increase with age (Table 1).

The Bunaji and N'Dama bulls had average peak testosterone concentrations of 3.1 and 8.7 ng mL^{-1}, respectively, at 12 months of age and rose to 8.6 and 10.9 ng mL^{-1}, respectively, at 48 months of age. The number of episodic peaks ranged from 1 to 4. Most of these peaks were between 0800 and 1000 h. Duration of peaks ranged from 20 min to a maximum of 2 h. Intervals between peaks ranged from 20 min to 1 h 40 min.

Table 1: Scrotal circumference, spermiogram and testosterone concentrations of Bunaji and N'Dama bulls

Reproductive traits	Breed	No. of bulls	Age (months) 12	2	36	48
Scrotal circumference (cm)	Bunaji	13	*22.3±2.1[a]	*25.9±1.4[b]	*28.4±3.1[c]	*33.4±1.1[d]
	N'Dama	11	20.1±2.5[a]	22.3±1.5[b]	24.9±1.1[c]	28.1±3.1[d]
Body conditions score	Bunaji	13	2.9±0.9	3.6±0.4	3.5±1.1	3.4±0..3
	N'Dama	11	3.1±0.6	3.2±0.5	3.0±1.9	3.4±2.1
Semen volume (mL)	Bunaji	13	*2.1±0.4[a]	*3.5±0.3[b]	*5.8±1.0[c]	*5.6±1.1[c]
	N'Dama	11	1.1+0.2[a]	2.6±0.4[b]	3.7±0.3[c]	3.8±1.2[c]
Gross sperm motility (%)	Bunaji	13	83.3±3.1	86.0±8.1	83.4+5.8	79.5±5.1
	N'Dama	11	80.0±5.0	82.3±4.5	77.1±6.1	81.3±3.9
Sperm concentration	Bunaji	13	0.6±0.2[a]	*1.8±0.3[b]	*2.5±0.6[c]	*2.4±0.5[c]
($\times 10^9$ mL^{-1})	N'Dama	11	0.8±0.1[a]	2.8±2.5[b]	4.4±1.1[c]	4.1±1.0[c]
Sperm output	Bunaji	13	1.3±0.1[a]	*6.3±0.1[b]	*14.5±0.5[c]	*13.4±0.4[c]
($\times 10^9$ ejaculate^{-1})	N'Dama	11	0.9±0.0[a]	7.3±0.2[b]	16.3±0.3[c]	15.9±1.1[c]
Dead sperm (%)	Bunaji	13	14.0±2.4	12.6+4.2	15.6±3.9	16.7±3.1
	N'Dama	11	12.5±1.9	11.2+3.6	13.1±4.5	12.5±3.8
Abnormal sperm (%)	Bunaji	13	8.8±2.2	7.5±3.6	8.9±1.6	9.6±4.1
	N'Dama	11	9.8±5.1	10.1±2.7	11.3±4.9	12.6±3.1
Peak T conc.	Bunaji	5	*3.1+1.3[a]	*4.8+2.1[b]	*6.2+0.6[c]	*8.6+0.7[d]
(ng mL^{-1})	N'Dama	5	8.7+3.1	9.0+2.1	9.2+2.1	10.9+2.2[a]
Basal T conc.	Bunaji	5	*1.3+0.8[ab]	*1.0+0.6[a]	*0.9+0.4[a]	*1.7+0.5[b]
(ng mL^{-1})	N'Dama	5	2.6+0.9[a]	2.6+0.8[a]	3.5+1.9[b]	5.3+1.8[c]
Number of episodic peaks	Bunaji	5	3	5	7	5
	N'Dama	5	3	3	3	4

*Means±SE within breeds in each age group; asterisks indicate significant differences ($p<0.05$). [abcd]Means±SE in rows with different letter superscripts are significantly different ($p<0.05$)

DISCUSSION

The results of this study have shown that scrotal circumference, spermiogram and testosterone concentrations increased with age as has earlier been reported (Lunstra *et al*., 1978; Rekwot *et al*., 1997). Observations in the present study show that testosterone concentrations increased gradually with age, support the hypothalamic desensitization theory of sexual maturation (Odell and Swerdloff, 1974; Lacroix and Pelletier, 1979). The differences observed in the semen characteristics and testosterone concentrations between the two breeds in this study corroborate the works of Hughes and Varley (1980), Rekwot *et al*. (1987) and Cheon *et al*. (2002), who have reported that semen production in male animals is influenced by many factors, such as breed, age, nutrition, season, enviroumental effects, health status, which can result in great variations of semen characteristics. Although the N'Dama bulls had smaller scrotal circumference and lower semen volume than the Bunaji bulls, the former had higher sperm concentration mL^{-1} and hence higher sperm output than the later; possibly due to breed differences.

The peak and basal testosterone concentrations in this study appear to agree with the reports in exotic bulls (Sanwal *et al*., 1974) and zebu bulls (Rekwot *et al*., 19987). The testosterone concentrations of Bunaji and N'Dama bulls showed marked fluctuations with distinct episodic peaks occurring mostly between 0800 to 1000 h. The profiles were episodic, pulsatile or temporal (events existing in time) in nature and this confirms earlier reports in bulls (Thibier, 1976; Agarwal *et al*., 1983). The three to seven testosterone peaks observed in this study, agree with earlier observations of Sanwal *et al*. (1974), Agarwal *et al*. (1983) and Rekwot *et al*. (1997). The exact significance of these episodic peaks is not clear but may be related to the sexual and behavioral states of animals or adjustments to photoperiodicity, temperature and postural states of the animals (Sanwal *et al*., 1974). The testosterone concentrations in this study appear to be closely related to the semen characteristics as has earlier been reported by other workers (Karg *et al*., 1976; Rekwot *et al*., 1997). In the bull, testosterone plays major functions in the onset of puberty such as development of genital tract, sex

drive, initiation and potentiation of spermatogenesis in conjunction with androgen binding proteins and follicle stimulating hormone (Hafez, 1986). With the application of artificial insemination technology in animals, there has been growing interest and need for more knowledge concerning variations of semen characteristics and hormonal profiles. Thus, a study of the relationship between follicle stimulating hormone, luteinizing hormone, libido and spermiogram in tropical bulls may be required.

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