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Effect of age and body weight on scrotal circumference and testicular growth in Nagaland swamp buffalo bulls

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ABSTRACT

A study was carried out to assess the effect of age and body weight on the scrotal circumference (SC) and testicular growth rate of swamp buffalo bulls in Nagaland. These buffaloes utilized for the present study were maintained around the villages of Medziphema block, Dimapur district, Nagaland, India. In the present study, a total of 24 swamp buffalo bulls were identified, selected and utilized. These buffaloes were grouped into four different categories as per their age and each category comprised 8 buffalo bulls. The groups were Group I: 18 – 24 months (6), Group II: 25–36 months (6), Group III: 37 – 48 months (6) and Group IV: 49 months and above (6). SC and testicular growth parameters were estimated with vernier caliper and simple measurement tape and by using dental formula, the age of animals was estimated.The Shaeffer's formula was used to estimate the body weight of buffalo bulls. Statistical analysis revealed that the SC and testicular growth parameters had significant positive correlation with body weight than age of the bulls. As compared to cattle, testicular growth parameters and SC of the swamp buffaloes were lower. The study result indicated that testicular growth parameters and SC of swamp buffalo are the useful indicators to determine the testicular development to select the buffalo bull for breeding purpose.

Key words: Body weight, Nagaland swamp buffalo bull, Scrotal circumference, Testicular growth parameters

Swamp buffalo in Nagaland is an indigenous unique bubaline species of North Eastern Hilly (NEH) region mainly in Nagaland state of Indian sub-continent.The buffaloes are reared under semi-intensive system and are used for seasonal draft purpose and as the meat and milch animals (Perumal *et al.* 2013, Perumal 2012). Various methods are used to predict semen production potential especially to identify the suitable breeding bulls with high semen production and its quality at an early age is essential, which is directly related with testicular growth parameters and SC of the bulls (Perumal and Rajkhowa 2013). In general, testicular and scrotal measurements at an early age are very useful and helpful to select the breeding bulls for collection of semen for preservation, artificial breeding or insemination purpose in livestock species especially for bubaline species (Perumal *et al.* 2017). The most essential parameters for breeding soundness evaluation are testicular length, testicular diameter, testicular volume, testicular width and SC. The sire having testis with higher SC and testicular growth parameters produce higher sperm with good quality than the sires with smaller SC and testicular weight (Kaymakci *et al.* 1998). Based on regression analysis, it was reported that testicular weight and size were positively correlated with body weight and age of the breeding bulls (Salhab *et al.* 2001). Further, the earlier workers reported that there were positive correlations between subsequent testes size and mating frequency and pre pubertal male hormone levels (Yarney and Sanford 1985, Perumal *et al.* 2017). SC and testicular growth are the potentially important markers of reproductive traits and have been utilized to assemble the selection indexes for swamp buffalo bulls to use in artificial as well as for natural breeding purpose. These traits are easily estimated, calculated and assess the correlation with reproductive performance and body weight for breeding bulls (Morris *et al.* 2000, Boligon *et al.* 2008, Boligon *et al.* 2010).

Estimation of SC and testicular growth parameters is an important aspect of breeding soundness evaluation of breeding sires. Estimation of these reproductive biometric traits mainly SC have a significant effect on onset of puberty and sexual maturity, semen production and its quality, prevalence of various pathological conditions of reproductive system, the fertility status of breeding sires (Ott 1991). Estimation of testicular growth parameters have been used as the markers for assessment of reproductive potentialities in the post-pubertal breeding bulls (Ahmad *et al.* 2010). Body weight and age are highly correlated with scrotal circumference (Devkota *et al.* 2008) and SC is significantly correlated with weight of the testis (Coulter

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and Foote 1977) and testicular consistency is highly associated with fertility (Waldner *et al.* 2010). Body weight and testis size provides to determine the physiological and physical maturity of the breeding bulls, its semen production and birth weight of its offspring (Evans *et al.* 1996).Various authors had reported on the testicular growth parameters and its associated semen quality profiles in matured breeding bulls of cattle (Almquist and Amann 1961, 1962; Andersson and Alanko 1992, Foote *et al.* 1977, Hahn *et al.* 1969), indigenous cattle (Perumal 2014), riverine buffaloes (Bedi 1980, Heur and Bajwa 1986, Pant *et al.* 2003), Malaysian swamp buffaloes (Bongso *et al.* 1984), sheep (Elmaz *et al.* 2008) and mithun (Perumal and Rajkhowa 2013, Perumal *et al.* 2017). Moreover, there are different factors involved in determining the SC and testicular growth parameters such as age, season of the year (Brito *et al.* 2002) and breed of the bulls (Coulter *et al.*1975).

Studies on relationship between body weight, age, testicular growth parameters and SC during the different stages of growth of Nagaland swamp buffaloes are meagre. In this context, it is not only important to determine how these biometric traits respond to selection at different ages, but also to determine the degree of correlation between these biometric traits and age and body weight. Therefore, the present study was designed to measurethe testicular growth parameters and SC at different age groups and correlate with age body weight in Nagaland swamp buffalo bulls to provide a suitable model for the definite selection of buffalo bulls for future breeding and conservation purpose of this precious indigenous bubaline species in NEH regions.

MATERIALS AND METHODS

Experimental animals: Twenty four apparently healthy Nagaland swamp buffalo bulls with different age categories were selected from the buffalo herd of villages around the Medziphema block, Dimapur district, Nagaland, India. The study area is located between 25º54′30″ North Latitude and 93º44′15″ East Longitude and at an altitude range of 250–300 m above mean sea level. Experimental buffaloes were reared under semi intensive system in the forest and they grazed during day time and housed in shed during night time. During cultivation season, these animals are used for ploughing purpose in the paddy field. The experimental animals in shed were fed daily with *ad lib.* quantity of locally available forages without providing concentrate. Fresh water was available throughout the day.

Dental formula was used to determine the age of the buffalo bulls. Body weight of individual bull was estimated with the Shaeffer's formula (Brito *et al.* 2002): $L \times G^2 / 300 \times 2.2$ where L = body length in inch and G = girth in inch.Based on their age, the experimental buffalo bulls were categorised into four different groups and each group consisted of 8 bulls. The groups were Group I: 18 – 24 months (6), Group II: 25–36 months (6), Group III: 37 – 48 months (6) and Group IV: 49 months and above (6).

Testicular measurement: Testicular growth parameters and SC were estimated with a vernier caliper (Mitutoya Digimatic Caliper, Japan) and a simple measuring tape after properly restraining the buffalo bull in the control crate (Elmore *et al.* 1976). Length of testis was estimated by placing the fixed arm of the vernier caliper at the proximal end and the sliding arm at the distal end of the testes. Proper care was undertaken to exclude the epididymides for individual testis. Testicular thickness or depth was estimated by placing the fixed arm of the vernier caliper at the anterior aspect and the sliding arm at the posterior aspect of the each testis, at the point of maximum depth. Testicular width of testis was estimated by sliding the other testes up in the scrotum and placing one arm of the vernier caliper at the medial aspect and the other at the lateral aspect, at the point of maximum width. Measurement of scrotal circumference, the testicle were pushed firmly into the bottom of the scrotum by placing the thumb and fingers laterally on the side of the neck of the scrotum and pushed ventrally. A flexible cloth tape was formed into a loop and slipped over the scrotum and SC was measured in cm by pulling the tape snugly around its greatest diameter (Elmore *et al.* 1976). Volume of the testis was measured by using the following formula for volume of an ellipsoid, i.e. $4/3\pi$ abc, where c = length/2, b = width/2 and a = thickness/2 (Love *et al.* 1991). Testicular weight was estimated by multiplying 1.038 with volume with which is the approximate density of testicular tissue in bovine species (Amann 1990).

Statistical analyses: The results were analysed statistically and expressed as the mean±S.E.M. Means were analyzed by one way analysis of variance between the different age groups of buffalo bulls, followed by the Tukey's post hoc test to determine significant differences between the groups using the SPSS/PC computer program (version 15.0; SPSS, Chicago, IL). Differences with values of $P<0.05$ were considered to be statistically significant. Correlation coefficient was estimated among the testicular parameters, scrotal circumference, age and body weight of the bulls. Differences at $P<0.05$ and $P<0.01$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The statistical analysis on the different testicular growth parameters, SC and body weight at different age of swamp buffalo bulls suggested that these biometric parameters differed significantly ($P<0.05$) between the age groups (Table 1). The average estimated testicular weight was 34.89, 54.86 and 66.47% greater in bulls aged 25–36, 37–48 and >49 months than in 18–24 month old bulls. Likewise the mean estimated volume of the testis was 32.12, 49.74 and 63.32% greater in bulls aged 25–36, 37–48 and >49 months than in 18–24 month old bulls. Whereas the average estimated scrotal circumference was 19.19, 23.25 and 30.38% greater in bulls aged 25–36, 37–48 and >49 months than in 18–24 month old buffalo bulls. Association (correlations) between the different estimation for the 32 bulls are presented in Table 2. As expected, different values of the testicular growth parameters were highly associated

Table 1. Scrotal circumference and testicular growth parameters of Nagaland swamp buffalo bulls at different ages (mean±S.E.)

Scrotal and testicular character	18- 24 months (6)	25- 36 months (6)	37 – 48 months (6)	49 months and above (6)
Age	20.33±0.60^{a}	33.17±0.71^{b}	42.17±0.68^{c}	52.50±0.77^{d}
Body weight (kg)	269.78±1.76^{a}	322.54±1.64^{b}	381.42±2.23^{c}	457.28±2.21^{d}
Scrotal circumference (cm)	19.27±0.38^{a}	22.20±0.48^{b}	25.11±0.46^{bc}	27.68±0.56^{c}
Left testes length (cm)	6.49±0.22^{a}	7.47±0.27^{b}	8.45±0.26^{b}	9.32±0.31^{b}
Left testes width (cm)	4.21±0.18^{a}	4.85±0.22^{a}	5.48±0.21^{b}	6.04±0.26^{b}
Left testes thickness (cm)	4.03±0.17^{a}	4.57±0.21^{b}	5.27±0.23^{bc}	5.89±0.25^{c}
Left testes volume (cm^3)	56.76±1.06^{a}	87.18±1.63^{b}	125.76±1.79^{c}	169.29±2.34^{d}
Left testes estimated weight (gm)	58.91±1.16^{a}	90.49±1.66^{b}	130.54±1.83^{c}	175.73±2.89^{d}
Right testes length (cm)	6.95±0.23^{a}	8.01±0.29^{ab}	9.06±0.28^{b}	9.97±0.34^{ab}
Right testes width (cm)	4.30±0.18^{a}	4.95±0.23^{a}	5.60±0.22^{b}	6.18±0.26^{b}
Right testes thickness (cm)	4.32±0.21^{a}	4.98±0.34^{b}	5.63±0.25^{bc}	6.21±0.48^{c}
Right testes volume (cm^3)	67.79±1.25^{a}	104.13±1.79^{a}	150.22±1.96^{b}	202.21±2.56^{b}
Right testes estimated weight (gm)	70.37±1.27^{a}	108.09±1.82^{a}	155.92±2.12^{b}	209.89±2.62^{b}
Total volume (cm^3)	124.55±1.69^{a}	191.30±2.42^{b}	275.98±2.66^{c}	371.50±3.47^{c}
Total weight (gm)	129.28±1.72^{a}	198.58±2.47^{b}	286.46±2.71^{c}	385.62±3.54^{c}

Figures in parenthesis indicate number of Nagaland swamp buffalo bulls. Figures with same superscript (a, b, c and d) do not differ significantly in rows.

Table 2. Correlation coefficients between scrotal circumference, age, body weight and various testicular measurements in Nagaland swamp buffalo bulls

No	Measurement	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Age	0.83**	0.89**	0.59*	0.92**	0.84**	0.83**	0.85**	0.64**	0.91**	0.89**	0.91**	0.92**	0.89**	0.89**
2	Body weight		0.86**	0.54*	0.84**	0.86**	0.84**	0.82**	0.63**	0.86**	0.86**	0.87**	0.89**	0.84**	0.91**
3	Scrotal circumference			0.71**	0.86**	0.91**	0.91**	0.91**	0.72**	0.83**	0.89**	0.92**	0.91**	0.91**	0.92**
4	Right testes length				0.63**	0.74**	0.83**	0.83**	0.73**	0.59**	0.66**	0.76**	0.70**	0.72**	0.80**
5	Right testes width					0.87**	0.94**	0.94**	0.59**	0.93**	0.83**	0.90**	0.91**	092**	0.92**
6	Right testes thickness						0.96**	0.93**	0.75**	0.85**	0.90**	0.91**	0.90**	0.91**	0.91**
7	Right testes volume							0.95**	0.73**	0.86**	0.85**	0.92**	0.88**	0.94**	0.90**
8	Right testes weight								0.74**	0.88**	0.83**	0.93**	0.92**	0.90**	0.92**
9	Left testes length									0.59**	0.68**	0.73**	0.78**	0.78**	0.81**
10	Left testes width										0.85**	0.91**	0.90**	0.91**	0.87**
11	Left testes thickness											0.92**	0.91**	0.89**	0.89**
12	Left testes volume												0.92**	0.92**	0.92**
13	Left testes weight													0.91**	0.93**
14	Total testes volume														0.91**
15	Total testes weight														1.00

**Correlation coefficient were highly significant (P<0.01). *Correlation coefficient were significant (P<0.05).

/correlated with other parameter (P<0.01) as reported in cattle, buffalo, sheep, goat and mithun (Hahn *et al.* 1969, Pant *et al*. 2003, Perumal and Rajkhowa 2013). In the present study, body weight was significantly correlated with the different testicular growth parameters than its age. Whereas as the age advances, the value of testicular growth parameters increased significantly (P<0.05) in experimental swamp buffalo bulls. Further, age (Fig. 1), testicular weight (Fig. 2) and body weight (Fig. 3) of Nagaland swamp buffalo bulls were significantly associated with SC. Similarly, SC was significantly associated with other testicular growth parameters as well as volume of the testis. In the present study, SC was a simple, repeatable and reliable measurement to obtain and maximum SC is achieved at 5–6 yrs of age and thereafter remains relatively constant. These results suggest that SC is a useful marker and is an essential

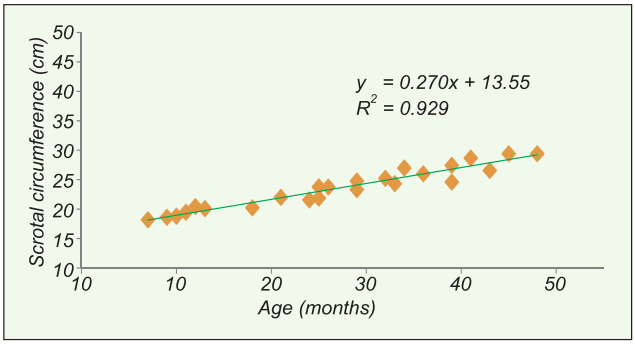

Fig. 1. Relationship between scrotal circumference and age in Nagaland swamp buffalo bulls.

selection parameter to determine the development progress of the testis in young bulls and is highly correlated with tesicular growth parameters.

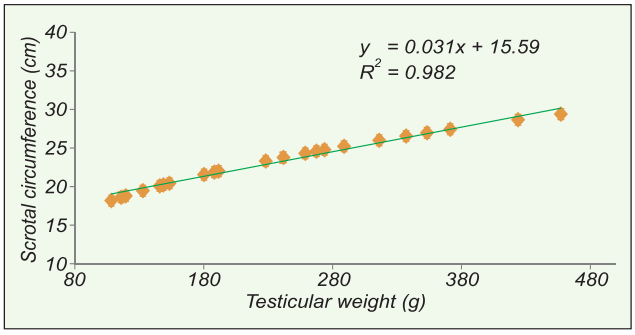

Fig. 2. Relationship between scrotal circumference and testicular weight in Nagaland swamp buffalo bulls.

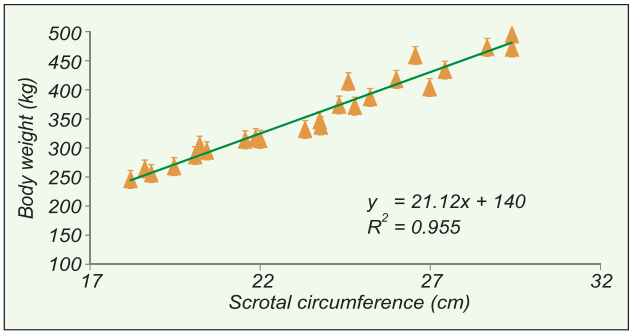

Fig. 3. Relationship between scrotal circumference and body weight in Nagaland swamp buffalo bulls.

In the present study, the statistical results revealed that SC of the swamp buffaloes bulls was significantly correlated with tesicular growth parameters. Therefore, the estimation of SC in buffalo bulls is highly useful to predict the testicular growth parameters and can be utilized in buffalo breeding programme to select elite breeding buffalo male for natural or artificial breeding programme. However, the result in the present in swamp buffaloes showed that the testis size and weight were comparatively smaller than exotic as well as indigenous dairy cattle and the measurements of the swamp buffalo testis were half of the counterpart of the exotic cattle (Bongso *et al.* 1981).

There is no such study on effect of age and body weight on scrotal circumference and testicular growth parameters in Nagaland swamp buffalo bulls and to the best of our knowledge, this is the first report in this bubaline species. Analysis of different testicular growth parameters, viz. testis length, width, thickness, volume and weight are most essential for intensive selection of breeding buffalo bulls for future natural/artificial breeding as well for conservation purpose in the NEH region especially in Nagaland.

Correlation coefficients estimated among the various testicular growth parameters and SC for the 24 buffalo bulls revealed that the body weight was significantly correlated with the different testicular measurements than age in the present study (Table 2). As in other domestic livestock species, the different testicular growth parameters to measure the testis size and weight were significantly correlated with each other in the present study in the bubaline species. All the correlation coefficients were highly significantly ($P<0.01$). Further, circumference of the testis was significantly associated with other testicular growth parameters (Table 2), and it is a simple estimation parameters to obtain & easy to interpret and is highly repeatable (Table 1). The correlations between this trait, SC and testicular growth parameters on a within-age group basis were not given, but remained high. Correlation coefficient of 0.94 between testicular weight and scrotal circumference was similar to the values of 0.90 to 0.96 reported by other researchers (Ahmad *et al.* 2011, Bongso *et al.* 1984) for different comparisons between dimensions and weight of the testis. Since the testicular circumference was easy to estimate, the measurement value had significantly higher repeatability and it was also significantly correlated with weight and volume of testis and this estimation subsequently was undertaken on all the experimental bulls examined in the present study.

In the current study, the correlation relationship among the various testicular growth parameters were in agreement with the research conducted in Nagaland indigenous Tho Tho bulls (Perumal 2014), Nigerian indigenous bulls (Addass *et al.* 2013), Bangladesh indigenous bulls (Sarder 2008), Sahiwal bulls (Love *et al.* 1991), buffalo (Bedi 1980), Malaysian swamp buffaloes (Bongso *et al.* 1984) and mithun bulls (Perumal and Rajkhowa 2013), but are at variance with another report on other bulls in which testicular length and volume were not associated with body weight and age (Heur and Bajwa 1986). In our handling experience in the present study, the swamp buffalo bulls were highly tactile sensitive to handle the scrotum, testes and other reproductive organs and they were invariably pulled them towards making it very difficult to take estimation accurately. In the earlier study in cattle bulls, the volume of testis was measured by multiplying mean values for length, width and thickness of the testes (Bedi 1980). In the present experiment, in this procedure, results were in higher side than those obtained by using the formula for estimating the volume of an ellipsoid, which has a very high accuracy (Willet and Ohms 1957, Perumal and Rajkhowa 2013, Perumal 2014, Perumal *et al.* 2017).

In the present study, interestingly, within each age group, the testicular growth parameters of buffalo bulls were lower than those observed for European breeding cattle bulls (Pant *et al.* 2003, Bongso *et al.* 1984), but highest SC was reached at nearly the similar age (Hahn *et al.* 1969). Nevertheless, there appears to be ample scope to select the buffalo bulls with large SC since about 60 to 70% of bulls, within each age group had a SC higher than the mean SC for that same age group. It was reported that in young dairy as well as beef bulls, the SC and testicular size and weight are significantly correlated (Hahn *et al.* 1969, Almquist *et al.* 1976, Bongso *et al.* 1984) and bulls assumed questionable and/or unsatisfactory breeding potential have smaller SC measurements (Ott 1991). However, the earlier reports in bubaline (Pant *et al.* 2003) and bovine (Andersson and Alanko 1992, Perumal and Rajkhowa 2013) species showed that the spermatogenesis was significantly higher in younger than older age groups. Therefore further research on semen

collection, its analysis and more number of animals need to be involved to pinpoint the correct age at which the mature rate of spermatogenesis is attained.

The mean value of SC in group II (25–36 months) was significantly ($P<0.05$) higher than group I (18–24 months) and group IV had significantly higher value than other three experimental groups. SC increased rapidly in young age groups and gradually in mature groups whereas in old age groups, this parameter decreased due to senile atrophy of the testis (Coulter 1991, Bongso *et al.* 1984, Perumal and Rajkhowa 2013, Perumal 2014, Perumal *et al.* 2017). In the current study, the proportion of increasing the SC and testicular growth parameters were lower in aged bulls than in growing and younger bulls. Based on present experimental results on the SC of 24 buffalo bulls, it was recommended that for higher breeding potential and higher fertility, the buffalo bulls with a average SC of >19.27, 22.20, 25.11 and 27.68 cm in the age categories of 18–24, 25 – 36, 37– 48 and >48 months, respectively may be selected as the breeding bulls.

Scrotal circumference (Table 1) increased with age as well as body weight advances (Fig 1), but testicular size and weight tended to reach mature size and weight more rapidly, as marked by the linear relationship between BW and SC (Fig. 2) and this was consistent with an earlier observation (Coulter *et al*. 1975, Bongso *et al*. 1984, Perumal and Rajkhowa 2013, Perumal 2014, Perumal *et al.* 2017).

High correlation existed between testicular volume and weight and SC, collective use of this information, testicular growth diameter along with SC are excellent markers of spermatogenic function (Coulter 1991, Bongso *et al.* 1984, Perumal and Rajkhowa 2013, Perumal 2014, Perumal *et al.* 2017) and this can be highly beneficial to select the breeding bulls as in the breeding soundness evaluation of swamp buffalo bulls (Bongso *et al.* 1984, Perumal 2014). The study concluded that present results suggest that similar to exotic cattle (Hahn *et al*. 1969), indigenous cattle (Perumal 2014), riverine buffalo (Pant *et al*. 2003), Malaysian swamp buffalo (Bongso *et al.* 1984) and mithun (Perumal and Rajkhowa 2013, Perumal *et al.* 2017) bulls, SC in the swamp buffalo bulls is a useful marker of breeding soundness evaluation and may be used as an important selection criteria to select the young bulls for natural breeding or artificial insemination centre. However, measurements of changes in testicular growth and development can be significantly improved by following the same bulls at different ages to avoid confounding between bulls and ages. Semen collection and analysis of the semen quality parameters will be related with tesicular as well as scrotal parameters are warranted to confirm the present research findings in Nagaland swamp bubaline species.

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