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Scrotal circumference and its relationship with testicular growth, age and semen production in mithun bulls

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Mithun (*Bos frontalis*), a unique free – range bovine species of North Eastern Hilly (NEH) region of India (Simoons 1984). It is a beef animal and rearing under semi-intensive system. The demand for sperm from outstanding sires has increased with the development of frozen semen and the growth of large artificial breeding organizations. Methods for predicting potential sperm production and particularly for identifying bulls with high sperm output potential at an early age are important. Generally testicular measurements at early ages are very useful in selection of breeding sire to collect semen for artificial insemination. Testicular parameters have a direct relationship with sperm production. There are various reports on the testicular growth and related semen characteristics in different species (Pant *et al.* 2003, Elmaz *et al.* 2008). But studies on mithun species are scarce. Therefore the present study was designed to estimate the testicular parameters at different ages and correlate with body weight and semen production in mithun to provide data for the definite selection of mithun bulls.

A total of thirty two apparently healthy mithun bulls with different age groups were selected from the herd derived from various hilly tracts of the NEH region with good body condition (score 5–6) maintained under uniform feeding, housing and lighting conditions at the NRC on Mithun, Nagaland, India. According to their age, the experimental bulls were equally divided into four groups each of 8 bulls viz., group 1: 24 – 36 months (n=8), group 2: 37–48 months (n=8), group 3: 49 – 72 months (n=8) and group 4: 72 months and above (n=8).

Testicular and scrotal measurements were taken with a caliper (Mitutoya Digimatic Caliper) and a tape after restraining the mithun bull in the chute (Elmore *et al.* 1976). Testes volume was calculated by using the formula for volume of an ellipsoid, ($4/3\pi abc$) where, a, thickness/2, b, width/2 and c, length/2 (Love *et al.* 1991). Weight of the testes was measured by multiplying volume with 1.038 which is the approximate density of testicular tissue in cattle (Amann 1990).

Semen was collected from the animals through rectal massage method twice a week for 10 weeks. Immediately after collection, the samples were kept in a water bath at 37°C and these ejaculates were subjected to routine semen evaluation (Perumal *et al.* 2013). After the preliminary evaluations, samples were subjected to the initial dilution with pre-warmed (37°C) Tris Egg Yolk Citrate extender (TEYC). The partially diluted samples were then brought to the laboratory in an insulated flask containing warm water (37°C) for further processing and make concentration of 50×10^6 sperm per ml of diluent. The diluted semen samples were packed in 0.25 ml French straws. During the study, all the experimental protocols met the Institute Animal Care and Use Committee regulations.

Means were analyzed by analysis of variance between the different age groups of mithun 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).

The results of different testicular and seminal parameters at different age of mithun bulls were significantly ($P< 0.05$) differed between the age groups. Similarly as expected, various measurements of the testis size were highly correlated (Table 2) with each other ($P< 0.01$) as in cattle (Hahn *et al.* 1969). Body weight was more highly correlated with the various testicular measurements than age. But also according to age, the testicular and seminal parameters were increased significantly ($P< 0.05$) in mithun bulls. Moreover age (Fig. 1) and testis weight (Fig. 2) of mithun bulls was highly correlated with scrotal circumference. Scrotal circumference was highly correlated with other testicular measurements as well as testicular volume. The correlation between scrotal circumference and testis volume is similar to reported by various studies (Hahn *et al*. 1969) of several comparisons between testicular dimensions and testicular weight in European breeds of cattle. The values of sperm output were also correlated with the testicular parameters

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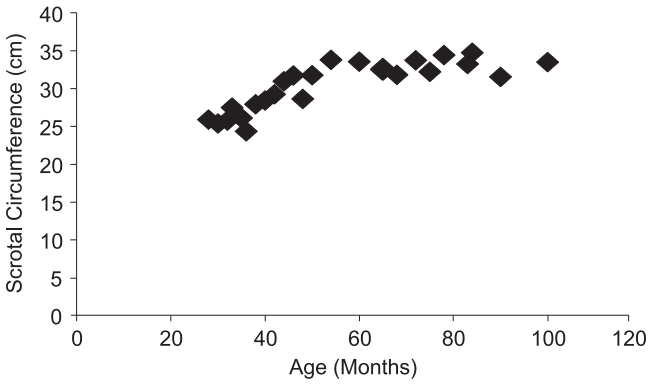

Fig. 1. Relationship between scrotal circumference and age in mithun bulls.

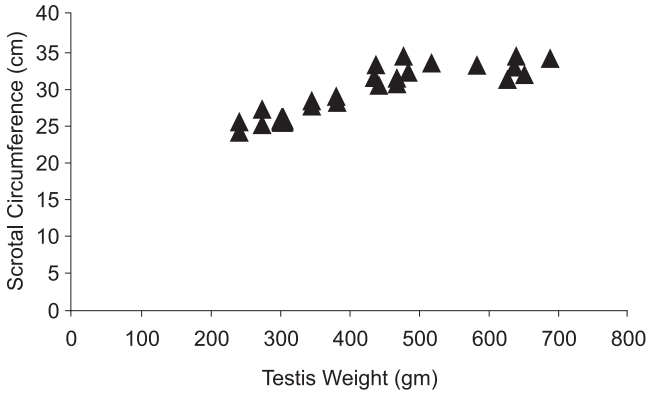

Fig. 2. Relationship between scrotal circumference and testis weight in mithun bulls.

and scrotal circumference (Table 1). Sperm output per bull per unit of time was lower in bulls 25 – 36 months of age than in the bulls of higher age groups.

These results indicate that scrotal circumference is a useful indicator of potential current sperm output and subsequent testicular development in young bulls. However, it has little value in bulls more than 5 to 6 years of age, where other changes in the testis apparently mask the relationship of testis size to total spermatogenic potential. The results revealed that scrotal circumference have highly correlated with tesicular and seminal parameters and is very useful tool to predict the semen production capacity. In the present study, the correlation between various testicular measurements are in agreement with a study in buffalo bulls (Bedi 1980) but are at variance with another report on other bulls in which testes volume and length were not correlated with age and body weight (Heuer and Bajwa 1986). Mithun bulls were very sensitive to handling of the testes and they invariably pulled them towards making it difficult to take measurement accurately. Further more, in the earlier study of other cattle

Table 1. Scrotal circumference, testicular and seminal parameters of mithun bulls at different ages (mean ± S.E.)

Characters	25- 36 months (8)	37- 48 months (8)	49 – 72 months (8)	73 months and above (8)
Age	32.33 ± 1.73^{a}	43.26 ± 1.93^{b}	61.52 ± 2.90^{c}	85.67 ± 2.99^{d}
Body weight (kg)	278.5 ± 19.97 ^{a}	347 ± 42.07 ^{b}	393.25 ± 13.81^{c}	428.43 ± 58.42 ^{d}
Scrotal circumference (cm)	25.22 ± 0.84^{a}	29.12 ± 1.20^{b}	32.39 ± 0.89^{b}	33.14 ± 1.17^{c}
Left testes length (cm)	8.82 ± 0.87^{a}	9.49 ± 0.62^{b}	9.90 ± 0.56^{b}	10.46 ± 0.77^{c}
Left testes width (cm)	5.19 ± 0.38^{a}	5.87 ± 0.55^{b}	6.59 ± 0.43^{c}	7.09 ± 0.47^{d}
Left testes thickness (cm)	5.20 ± 0.28^{a}	6.18 ± 0.50^{b}	6.18 ± 0.27^{b}	7.16 ± 0.34^{c}
Left testes volume (cm^3)	124.94 ± 3.92^{a}	180.82 ± 4.90^{b}	211.05 ± 3.87^{c}	277.99 ± 4.70^{d}
Left testes estimated weight (g)	129.69 ± 3.99^{a}	187.69 ± 4.99^{b}	219. 07 ± 3.94^{c}	288.55 ± 4.79^{d}
Right testes length (cm)	8.78 ± 0.24^{a}	9.73 ± 0.69^{ab}	10.12 ± 0.49^{bc}	10.83 ± 0.50^{d}
Right testes width (cm)	5.50 ± 0.39^{a}	6.19 ± 0.53^{b}	6.96 ± 0.41^{c}	7.77 ± 0.56^{d}
Right testes thickness (cm)	5.53 ± 0.39^{a}	6.47 ± 0.56^{b}	6.50 ± 0.39^{b}	7.68 ± 0.56^{c}
Right testes volume (cm^3)	140.03 ± 3.41^{a}	204.96 ± 5.51^{b}	239.74 ± 4.24^{c}	338.51 ± 5.57^{d}
Right testes estimated weight (kg)	145.35 ± 3.47^{a}	212.75 ± 5.62^{b}	248.85 ± 4.32^{c}	351.57 ± 5.68^{d}
Total volume (cm^3)	264.98 ± 5.17^{a}	385.79 ± 7.36^{b}	450.80 ± 5.64^{c}	616.51 ± 5.74^{d}
Total weight (g)	275.04 ± 5.27^{a}	400.45 ± 7.50^{b}	467.93 ± 5.74^{c}	639.93 ± 5.85^{d}
Semen volume (ml)	0.92 ± 0.46^{a}	1.40 ± 0.35^{b}	1.73 ± 0.34^{b}	2.21 ± 0.65^{c}
Concentration (10^6/ml)	532.83 ± 7.00^{a}	590.83 ± 10.05^{ab}	611.66 ± 6.10^{ab}	680.83 ± 6.28^{b}
Total sperm output (10^6/ml)				
Per ejaculate	485.66 ± 10.36^{a}	833.81 ± 14.20^{b}	1060.41± 10.16^{b}	1512.66 ± 17.74^{c}
Per week	1942.66 ± 20.72^{a}	3335.26 ± 28.40^{b}	4241.66±20.33 ^{b}	6050.66 ± 35.49^{c}
Per day	277.52 ± 7.83^{a}	476.46 ± 10.73^{b}	605.95 ± 7.68^{b}	864.38 ± 13.41^{c}
Sperm ejaculate/g of testes (10^6/ml)				
Per ejaculate	1.78 ± 0.709^{a}	2.07 ± 0.59^{ab}	2.27 ± 0.54^{ab}	2.35 ± 0.65^{b}
Per week	7.14 ± 1.41^{a}	8.29 ± 1.18^{ab}	9.10 ± 1.08^{ab}	9.42 ± 1.30^{b}
Per day	1.02 ± 0.53^{a}	1.18 ± 0.44^{ab}	1.30 ± 0.40^{ab}	1.34 ± 0.49^{b}

Figures in parenthesis indicate number of mithun 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 mithun bulls

No	Measurement	Correlation between the measurement												
		2	3	4	5	6	7	8	9	10	11	12	13	14
1	Age	0.82**	0.80**	0.83**	0.89**	0.84**	0.87**	0.86**	0.67**	0.90**	0.87**	0.88**	0.86**	0.42*
2	Body weight		0.90**	0.88**	0.90**	0.92**	0.89**	0.90**	0.86**	0.88**	0.92**	0.87**	0.89**	0.56*
3	Scrotal circumference			0.94**	0.95**	0.88**	0.94**	0.93**	0.78**	0.93**	0.87**	0.93**	0.90**	0.51*
4	Left testes length				0.94**	0.95**	0.96**	0.94**	0.86**	0.94**	0.92**	0.95**	0.95**	0.43*
5	Left Testes width					0.95**	0.98**	0.96**	0.79**	0.96**	0.93**	0.95**	0.96**	0.50*
6	Left Testes thickness						0.97**	0.95**	0.78**	0.89**	0.97**	0.94**	0.92**	0.47*
7	Left testes volume							0.97**	0.73**	0.92**	0.95**	0.96**	0.95**	0.49*
8	Left testes weight								0.79**	0.92**	0.95**	0.93**	0.96**	0.50*
9	Right testes length									0.86**	0.78**	0.90**	0.91**	0.22
10	Right Testes width										0.91**	0.97**	0.95**	0.41*
11	Right Testes thickness											0.95**	0.93**	0.44*
12	Right testes volume												0.97**	0.38
13	Right testes weight													0.36
14	Sperm con per ejaculate													

** Correlation coefficient were highly significant, P< 0.01. * Correlation coefficient were significant, P< 0.05.

bulls, testicular volume was calculated by multiplying mean values for length, thickness and total width of the testes (Bedi 1980).

Results revealed that within each age group, the testicular measurement of mithun bulls were lower than those reported for Holstein bulls and higher than buffalo bulls (Pant *et al*. 2003), but maximum scrotal circumferences was reached at nearly the same age (Hahn *et al*. 1969). Nevertheless, there are ample scopes for selecting mithun bulls with large scrotal circumference since about 60–70% of bulls, within each age group, had a scrotal circumference greater than the mean scrotal circumference. In young dairy and beef bulls, scrotal circumference, testes size and sperm production are highly correlated (Hahn *et al*. 1969). In the present study, the semen production was increased gradually and higher in aged animal than young animals. But the reports in other species like bubaline (Pant *et al*. 2003) and bovine (Andersson and Alanko 1992) species the spermatogenesis was higher in younger than older age. But further studies with artificial vaginal method of semen collection and more number of animals will help to pinpoint the exact age at which the mature rate of spermatogenesis is attained.

In the present study, daily sperm output ranged from 2.77 X 10^8 to 8.64 X 10^8. We are not aware of any earlier report on sperm output in the mithun bulls. However, compared to European cattle these values are lower. Amman and Almquist (1962) reported daily sperm output of 4.58 X 10^9 for young dairy bulls (average age 29 months) and 5.25 X 10^9 for mature bull, when bulls are ejaculated six times per week. Hahn *et al*. (1969) reported a daily sperm output range from 4.02 X 10^9 (age 17–22 months) to 6.10 X 10^9 (age 59 – 69 months) in Holstein bulls ejaculated four times per week, we believe that the lower daily sperm output in the mithun bulls may be primarily attributed to their lower testicular measurement than in cattle bulls. Correlation between scrotal circumference and sperm output per week was gradually increased with age in mithun bulls. These results emphasize the importance of scrotal circumference in selecting mithun bulls with greater sperm output. Based on our results on the mean testicular circumference of 32 mithun bulls, we recommend that for optimum sperm output and possible higher fertility, mithun bulls with a mean scrotal circumference of > 25.22, 29.12, 32.39 and 33.14 cm in the age groups of 25– 36, 37 – 48, 49 – 72 and > 72 months, respectively should be selected as AI sires.

Because of the high correlation between testis size and scrotal circumference, collective use of this information, testicular diameter and along with scrotal circumference are excellent indicators of spermatogenic function (Almquist *et al*. 1976), this can be beneficial for breeding soundness evaluation of mithun bulls. In conclusion, present results indicate that as in cattle and buffalo bulls scrotal circumference in the mithun bulls is a useful indicator of sperm output and should be used as an important criteria for selecting young bulls. However, estimates of changes in testicular development and semen production can be considerably improved by following the same bulls at different ages to avoid confounding between bulls and ages.

SUMMARY

The present study was undertaken to assess the relationship between the scrotal circumference and testicular parameters with seminal parameters in mithun, maintained at Semen Collection Centre, National Research Centre on Mithun, Jharnapani, Nagaland. Total of 32 mithun bulls were selected and divided into four groups according to their age

and each group consisted of 8 bulls viz., group 1: 24 – 36 months (n=8), group 2: 37–48 months (n=8), group 3: 49 – 72 months (n=8) and group 4: 72 months and above (n=8). The scrotal circumference and testicular parameters were measured with caliper and tape. The semen was collected through rectal massage method and analysed for concentration and volume and then preserved in LN_2 with 0.25 ml French straws. Scrotal circumference was highly correlated with testicular parameters and body weight than age. Compared to exotic cattle (*Bos taurus*), testicular parameters and scrotal circumference of mithun were lower, while higher than buffalo bulls. The result in mithun is useful indicator of sperm output and will be used as an important criterior for selecting mithun bulls for breeding purpose.

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