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Crossbred Bull Selection for Bigger Scrotum and Shorter Age at Puberty with Potentials for Better Quality Semen

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Contents

Shortening age at puberty of crossbred breeding bull is an important issue in the tropic. This study aimed at selecting crossbred bulls at earliest possible age with bigger scrotum and potential for donating quality semen. One hundred and 31 pre-joining crossbred bulls of Central Artificial Insemination Laboratory, Saver, Dhaka were examined. The bulls being trained by seeing semen collection from mature bulls were allowed ejaculation into the artificial vagina at homosexual mount during a 20 min time at three occasions, every three month. Eighty one of 131 bulls produced at least one ejaculate during the study and their mean ± SD age and scrotal circumference (SC) were 20.3 ± 4.7 months and 28.2 ± 2.7 cm, respectively. Bulls' body weight, body condition score (BCS) and SC influenced the attainment of their puberty ($p < 0.05$). Bull's body weight had positive effects on scrotal circumference and ejaculate volume ($p < 0.05$). Scrotal circumference positively influenced the percentages of normal spermatozoa ($p < 0.05$). Scrotal skin-fold thickness negatively influenced the proportion of spermatozoa with normal head ($p < 0.05$). Based on age at first ejaculate and SC, 29.6% bulls ($n = 24$) were selected by cluster analysis. Selected bulls had mean ± SD age 17.9 ± 2.2 months, body weight 287.3 ± 48.6 kg, SC 30.5 ± 1.5 cm, ejaculate volume 3.4 ± 1.3 ml, sperm motility 50.8 ± 17.2%, total spermatozoa per ejaculate 2541.9 ± 1699.2 million and normal spermatozoa 81.5 ± 14.5%. The selected pubertal bull group was different from the unselected pubertal bulls at MANOVA ($p < 0.0001$). About 30% of pubertal crossbred bulls can be selected with shorter age and larger scrotum at puberty under conditions prevailed in Bangladesh.

Introduction

Obtaining semen at the earliest possible age from bulls being proven tested is desirable to hasten the identification of superior sires. The genetic impacts of superior sires are limited by the number of spermatozoa produced, which is a direct function of a testicular size. Young dairy bulls should be sampled as soon as possible and after breeding soundness evaluation (BSE) be culled rigidly if not selected for AI. Such practice will also benefit producers that use only pedigree bulls by recruiting new sires with better pedigrees.

Early puberty in bulls is positively related to early puberty and subsequent pregnancy rates in their offspring (Werre and Brinks 1986). This means, bulls selection with early puberty, along with other traits, would reduce age at puberty in heifers and improve cow's reproductive performances (Smith et al. 1989; Morris et al. 1999). Therefore, early onset of puberty following sexual maturity and subsequent early recruitment of young bulls at AI is very important. This helps early returning money to the producers and provides economic advantages by not only decreasing feeding and management costs but also adding positive impact on increased reproduction (Hafez and Hafez 2000). Bulls with adequate scrotal development in a definite age have higher probability of becoming satisfactory sires than bulls with small scrotal circumference (Polupan 1994). Therefore, selection of bulls with larger scrotum at an early age will contribute to increasing semen production and subsequently hasten cattle development programs.

In many tropical countries, bull selection protocol is still under development (Anon 2005a). A protocol based on objective data for selection of young bulls has not yet been developed in Bangladesh. Few researchers erratically reported the age at puberty in some breeds but not in crossbred bulls (Shahjalal and Islam 2001). Most of the bulls used for AI in Bangladesh are crossbred. Therefore, the present study was aimed at determining the earliest possible age at which crossbred bulls with larger scrotal circumference would attain puberty with potentials for the production of quality semen for artificial breeding.

Materials and Methods

Animals

One hundred and thirty one pre-joining bulls of Central AI Laboratory, Savar, Dhaka were examined. The bulls were of crossbred where the dam was either indigenous nondescript zebu or Sahiwal or crosses of nondescript zebu with Sahiwal and the sire was Holstein-Friesian. The bulls were free from any venereal or communicable disease.

General information of the bulls

The percentage of Holstein-Friesian genetics and date of birth of the bulls were collected from the register maintained in the AI laboratory. The bulls were weighed to determine their body weight. The body condition of the bulls was scored by two investigators into 1–5 scales (Nicholson and Butterworth 1986).

Measurement of scrotum and scrotal skin-fold thickness

The scrotal circumference was measured following the method described by the Society of Theriogenology (Ball et al. 1983). The testes were first pushed to the lower part of the scrotum, the thumb and the fingers

were placed on either side to put two testes together. A flexible metal tap (scrotal tap; Lane Manufacturing Co., Denver, CO, USA) was looped, placed around the greatest diameter of the scrotum, pulled snugly to establish firm contact of the tap with the entire circumference and reading was taken up to the nearest 0.5 cm. Multiple measurements were done on the same scrotum until the repeatability was ensured. The thickness of scrotal skin was measured with a slide calliper.

Semen collection

Semen was collected in graduated tubes at homosexual mount using artificial vagina (AV). The bulls were allowed usually two false mounts before the collection attempt if any. Each bull was allowed maximum 20 min to make an ejaculate.

Semen evaluation

The volume of semen was recorded by reading the graduation marks of the receptacle. To evaluate sperm motility, a small drop (10 μl) of semen was placed on a pre-warmed (37°C) slide, covered by a coverslip and examined under a microscope equipped with phase contrast optics (400×) and warm stage. Two investigators scored the sperm motility and the average scores were recorded.

A portion of semen was diluted (1 : 200) with water to kill and immobilize the spermatozoa and the concentration of spermatozoa was determined by using a haemocytometer (Bane 1952). The data on the sperm concentration were expressed as million per ml.

To examine the sperm morphology, semen samples were fixed in formol-saline (1 : 100). To prevent any temperature variation-related damage to the spermatozoa, the semen and formol-saline were always mixed at the same temperature. At least 200 spermatozoa were examined from each sample under a microscope equipped with differential interference contrast (DIC) optics (1000×). The proportion of normal spermatozoa was described as that with no abnormalities in the head, acrosome, mid piece or tail.

Experimental design and statistical analysis

The bull calves aging 12 months or more and were not yet selected for routine semen collection were brought to the semen collection premises and allowed them seeing the semen collection from the mature bulls. Individual bulls were allowed to mount the teaser in three occasions at three months intervals. If a bull produced an ejaculate, the semen was evaluated and it was selected for studying the age at puberty.

The puberty of the bull was defined according to Lunstra et al. (1978). Briefly, a bull was considered pubertal if his semen contained 50 million or more spermatozoa per ml with at least 10% motility. The definition of Lunstra et al. (1978) was also used to determine whether or not a bull's semen was freezable, which indicates a semen with 500 million or more spermatozoa per ml with 50% or more sperm motility.

A logistic regression model was fitted estimating the effect of age, body weight, BCS, scrotal skin fold thickness, scrotal circumference and percentage of the Friesian genetics of the bulls on attainment of puberty. A regression analysis was performed to define the best subsets of the variable (age, bodyweight, BCS, scrotal circumference, scrotal skin-fold thickness and percentage of Friesian genetics) influencing on spermatozoa per ejaculate, proportion of normal spermatozoa and proportion of spermatozoa with normal heads (Anon 2000).

Attempts were made to group the bulls according to the age and corresponding scrotal circumference defined by the Society for Theriogenology (SFT), which says that a pubertal bull should have a scrotal circumference of 30, 31, 32, 33 and 34 cm at less than or equal to 15 months, 16–18 months, 19–21 months, 22–24 months and greater than 24 months, respectively. On the basis of age and total spermatozoa per ejaculate, bulls were clustered into two groups by using K-mean cluster analysis (Anon 1996). A second cluster analysis was performed to group the bulls into another two groups on the basis of scrotal circumference and total spermatozoa per ejaculate. Finally, a group was defined by selecting the bulls with the shorter age group as defined in first cluster analysis and the bigger scrotal circumference defined in the second cluster analysis by using data of the pubertal bulls. A discriminant analysis was performed to determine if the selected pubertal bulls' group was different from the rest of the unselected pubertal bulls. Discriminant analysis yielded a canonical correlation (r*) that explains the relatedness of the variables to the groups defined. The eigen values are the vectors that yielded at discriminant analysis showed the apartness between the pubertal bulls' groups defined. The group difference was estimated at multivariate analysis of variance (MANOVA). Univariate ANOVA was also performed to find the difference of the individual variables between the groups.

Discrete and proportion data were transformed to near normality by using log and arcsine square root transformation, respectively.

Results

Eighty-one of the 131 bulls produced at least one ejaculate in any of the three consecutive semen collection attempts. The mean ($\pm$SD) age, body weight, scrotal circumference, scrotal skin-fold thickness and percentage of Friesian genetics of bulls that did or did not produce an ejaculate are shown in the Table 1. Effects of body weight, body condition score and scrotal circumference on the attainment of bull calves' puberty were significant ($p < 0.05$). The mean $\pm$ SD age and scrotal circumference of the 81 bulls that produced at least one ejaculate was 20.5 ± 5.23 months and 28.2 ± 2.7 cm, respectively. Using the 81 pubertal bulls, regression analysis demonstrated significant effect of bulls' body weight, scrotal circumference and scrotal skin-fold thickness on the semen volume and sperm morphology ($p < 0.05$). The ejaculate volume increased when the bull was heavier (Fig. 1; $r = 0.34$, $p < 0.01$). Increasing scrotal circumference was positively related to spermatozoa with normal head (Fig. 2; $r = 0.30$,

Table 1. Mean ± SD measures of age, physical, scrotal and genetics of the crossbred bulls on the attainment of puberty

Parameters studied	Bulls that produced at least an ejaculate (n = 81)	Bulls that did not produce ejaculate (n = 50)	Odds ratios	p-value
Age (month)	20.5 ± 5.23	19.5 ± 6.0	1.05	0.186
Body weight (kg)	284.7 ± 49.6	255.9 ± 61.0	1.01	0.002
Body condition score (1–5 scale)	3.38 ± 0.28	3.12 ± 0.29	42.23	0.000
Scrotal circumference (cm)	28.2 ± 2.7	27.0 ± 3.3	1.16	0.025
Scrotal skin-fold thickness (mm)	5.6 ± 1.4	5.5 ± 1.9	1.04	0.747
Percentage of Friesian genetics	59.1 ± 17.0	60.3 ± 19.4	1.00	0.819

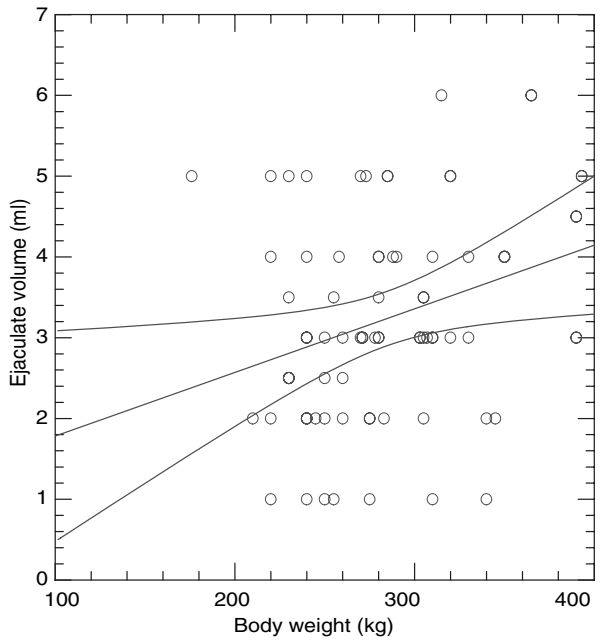

Fig. 1. Relationship between body weight and ejaculate volume

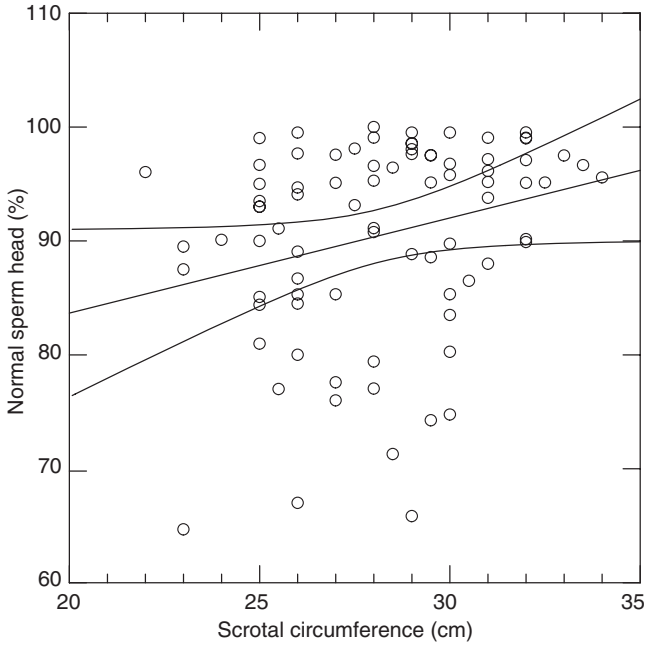

Fig. 2. Relationship between scrotal circumference and percentage of head normal spermatozoa

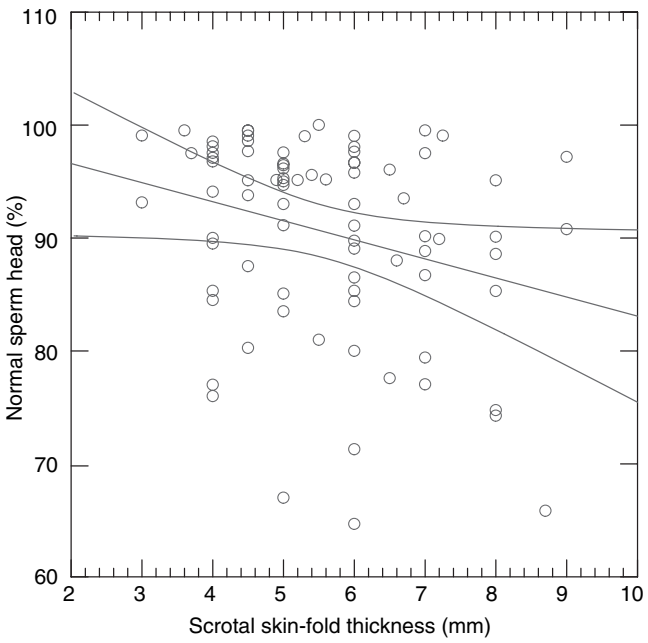

Fig. 3. Relationship between scrotal skin-fold thickness and percentage of head normal spermatozoa

$p < 0.02$). Increasing scrotal skin-fold thickness had a negative effect on the proportion of spermatozoa with normal head (Fig. 3; $r = -0.27$, $p < 0.05$). A positive effect of increasing body weight was also found on the scrotal circumference (Fig. 4; $r = 0.41$, $p < 0.001$).

The cluster analysis selected a population of pubertal bulls (n = 24; 29.6%) with significantly shorter age and larger scrotal circumference than the unselected pubertal bulls (n = 57) ($p < 0.001$). Additionally, the selected bulls' were of uniform age and scrotal circumference, indicated by a smaller SD. (Table 2). Fifteen of the 24 bulls produced ejaculates of freezable quality.

Depending on the groups selected by different methods, the mean ± SD age, body weight, BCS, SC, scrotal skin fold thickness, percentage of Friesian genetics, the ejaculate volume, sperm motility, sperm concentration, spermatozoa/ejaculate, percentage of normal spermatozoa and percentage of spermatozoa with normal head are shown in Table 2.

Discriminant model defined by using age, body weight, body condition score, scrotal skin fold thickness, scrotal circumference, semen volume, spermatozoa per ejaculate and proportion of normal spermatozoa per ejaculate showed that the selected pubertal bulls were different from the unselected pubertal bulls (MANOVA, $p < 0.001$; Table 2). The most discrimination between

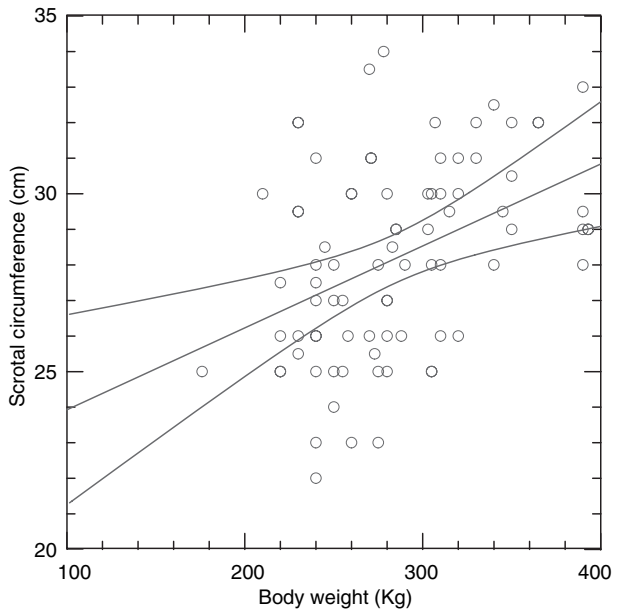

Fig. 4. Relationship between body weight and scrotal circumference

the bulls' groups was due to scrotal circumference ($F = 79.25$) followed by age ($F = 52.23$), scrotal skin-fold thickness ($F = 4.56$) and body condition scores ($F = 4.11$). The canonical correlation of 0.80 demonstrated a strong association of the discriminating variables with the pubertal bulls' groups. The eigen values of the groups mean (−2.034, 0.856) evidenced the groups' separation to the opposite direction on the canonical variable. However, at ANOVA the bulls were differed with regards to age and SC ($p < 0.001$).

Discussion

This study determined a guideline for selecting crossbred bulls with larger scrotal circumference at an earliest possible age at puberty in Bangladesh. It also documented a negative effect of scrotal skin-fold thickness on the proportion of spermatozoa with normal heads. The guideline we determined by using cluster analyses will select about 30% bulls.

Scrotal circumference is moderate to highly heritable and serves as a useful predictor of puberty in bulls (Lunstra et al. 1978; Bourdon and Brinks 1986; Smith et al. 1989). Smith et al. (1989) reported, for each 1 cm increase in sire's scrotal circumference, a 0.31 cm increase in the son's scrotal circumference. It has a favourable association with seminal traits (Smith et al. 1989; Palaz et al. 1994; Kealey et al. 2006). Accordingly, our data confirmed that the larger scrotum has positive effect on proportion of spermatozoa with normal morphology. Therefore, if selection is made based on the guideline of this study, the crossbred bulls in tropics will produce better quality semen at an earlier age. These will definitely have a positive impact on the AI industry of Bangladesh and other countries where crossbred bulls between *Bos indicus* and *Bos taurus* are used as stud.

Bulls with larger scrotum at an earlier age result in reduced age at puberty and increased pregnancy rates in their heifer offspring (Werre and Brinks 1986; Moser et al. 1996). This means, bulls selection with early puberty, along with others traits, would allow breeding heifers earlier and improving cow's reproductive performances (Smith et al. 1989; Morris et al. 1999). This is very important for cattle breeding in tropical countries where delayed puberty and poor reproductive efficiency are blamed to be the major constraints limiting the cattle industry (Syrstad and Ruane 1998). Shamsuddin et al. (2006) documented that the farmers' average income would increase up to US$ 265–561 per year if the age at first calving of heifers could be reduced from 35–44 months to 33–40 months in zebu and crossbred cows depending on the dairy production system in Bangladesh. The average herd size in the study varied between 1.5 and 3.4 lactating cows. Smith et al. (1989) reported a 0.796 day change in the age at puberty and 0.826 day change in the age at first calving of female offspring per cm of SC of the sire. Selection of stud bulls at earliest possible age will not only improve reproduction but also provide economic advantages by decreasing feeding and management costs and early return of money to the producers. The guideline we described selected only 18% bulls that would produce freezable quality semen when compared with the recommendation made by Lunstra et al. (1978). These bulls will be in average 17.4 months old with a scrotal circumference of about

Table 2. Mean ± SD values of the variables separating the bulls' group at discrimination analysis together with values of the bulls that fulfilled the SFT guideline

Variables	Selected pubertal bulls' group (n = 24)	Unselected pubertal bulls' group (n = 57)	Bulls that fulfilled the SFT guideline (n = 7)
Age (month)	[a]17.9 ± 2.2	[b]21.2 ± 5.2	16.7 ± 2.7
Body weight (kg)	287.3 ± 48.6	282.7 ± 50.3	287.3 ± 56.7
Body condition score (1–5 scale)	3.5 ± 0.1	3.45 ± 0.3	3.43 ± 0.2
Scrotal circumference (cm)	[a]30.5 ± 1.5	[b]27.1 ± 2.5	31.8 ± 1.1
Scrotal skin-fold thickness (mm)	6.00 ± 1.5	5.5 ± 1.4	6.1 ± 1.00
Percentage of Friesian genetics	56.0 ± 10.7	60.6 ± 19.0	53.6 ± 9.5
Ejaculate volume (ml)	3.4 ± 1.3	3.2 ± 1.3	4.3 ± 1.4
Sperm motility (%)	50.8 ± 17.2	44.2 ± 17.9	67.1 ± 4.9
Spermatozoa per ejaculate (million)	2541.9 ± 1699.2	2536.3 ± 2117.5	4101.4 ± 2051.4
Spermatozoa with normal head (%)	91.6 ± 9.3	90.0 ± 8.5	89 ± 6
Percentage of normal spermatozoa (%)	81.5 ± 14.5	80.1 ± 13.2	96 ± 4

SFT, Society for Theriogenology

[a,b]Mean ± SD with different superscript letters in the same raw differ between selected and unselected pubertal bulls' groups ($p < 0.001$)

31 cm and will have a narrow variability as demonstrated by smaller standard deviations compared with the unselected population. Hoflack et al. (2006) also reported that 93.7% of the young Belgian Blue bulls and 59.3% Holstein Friesian bulls failed the breeding soundness examination. One may criticize such a high percentage of culling. We have examined the first ejaculate ever collected from the bull. It is likely that the semen quality will improve in the subsequent collections and many of the bulls that produced at least an ejaculate will qualify. The attainment of puberty does not signify full reproductive capacity. Ejaculate volume, output of motile spermatozoa and concentration of spermatozoa increase significantly in bulls for 6–9 months after the onset of puberty (Christian and Wolf 1963; Almquist and Cunningham 1967).

The Society for Theriogenology has recognized the extensive variation of scrotal circumference between breeds of cattle and recommended lower limits for scrotal circumference based on age groups but independent of breed. When we used the Society-set guideline, only 8.5% bulls could be selected as that producing freezable quality semen. However, the Society recommendations were suggested to be considered as a guideline only and emphasis was given to the fact that individual producers will adjust the criteria for the breeds they are concerned (Chenoweth et al. 1993; Hopkins and Spitzer 1997). This further justifies the originality of the present study that has successfully determined a guideline for selecting crossbred bulls at tropical cattle production system.

An interesting finding of the present study is that the bull with a thicker scrotal skin produced a higher proportion of head abnormal spermatozoa. Previous study showed that elevation of testicular temperature, either by exposure to high ambient temperature or thermal insulation of the scrotum, disrupts spermatogenesis with a consequent decrease in both sperm production and semen quality (Austin et al. 1961). Elevation of the testicular temperature results in increased metabolism and oxygen demand which can not be fulfilled because of limited testicular blood flow, resulting in hypoxia, generation of reactive oxygen species and deterioration of semen quality (Setchell 1978, 1998). Although we did not find any report relating sperm abnormalities with scrotal skin thickness, a seasonality study indicated that semen quality decreases to a greater extent in crossbred than in *B. indicus* bulls during the hot summer months in Brazil (Silva et al. 1991). Barth and Oko (1989) indicated a genetic predisposition for the development of specific sperm abnormalities in response to adverse conditions like elevated testicular temperature. The testicular temperature is kept within optimal limits by complex physiological mechanisms involving the scrotum, the testicular vascular cone and testes themselves (Waites and Moule 1961; Kastelic et al. 1996, 1997). Therefore, scrotal skin thickness could be involved in the thermoregulation of the testis and its variation can affect the semen quality. Previous studies reported an increased proportion of abnormal sperm head after scrotal insulation (Vogler et al. 1993; Barth and Bowman 1994; Fonseca and Chow 1995).

Moser et al. (1996) reported a positive response to selection for increased scrotal circumference. The trend in breeding value for SC from 1968 to 2004 of North American Hereford cattle is approximately 0.10 cm per year, based on the data from the national cattle evaluation conducted for the American Hereford Association (Anon 2005b). The greatest positive impact of selection for the increased SC was sperm concentration score and the percentage of spermatozoal abnormalities. Percentages of primary and secondary abnormalities would be expected to decrease by 1.51% and 1.42%, respectively, for each 1.0 cm increase in scrotal circumference (Kealey et al. 2006). The authors also suggested that selection for more sperm-producing tissue, as indicated by increased SC, may lead to more male progeny passing breeding soundness evaluations due to improved sperm morphology. Crossbred bulls selection made on the basis of scrotal circumference will improve the reproductive and growth traits of the progeny at tropical cattle management.

Early recruitment of bulls at AI stud hastens the process of progeny testing. In the cases where progeny testing is not in practice like in Bangladesh, early recruitment of pedigree bulls will hasten the dairy cattle development through quick and efficient dissemination of new genetics (Coulter and Foote 1979). Radostits et al. (1994) reported an average of 20% to 40% low fertility bulls in a non-selected population. Earlier, Fonseca (1989) did andrological examinations on bulls in service and reported as many as 40% sub-fertile bulls. Therefore, the high proportion of culling non-selected bulls according to the guideline we described will not only reduce age at puberty of bulls but also increase fertilizing capacity in the subsequent generations.

In conclusion, cluster analysis of data on age at first ejaculate collection, scrotal circumference, sperm motility, total spermatozoa per ejaculate and proportion of normal spermatozoa resulted in selection of 29.6% bulls with uniform and reduced age at puberty and increased scrotal circumference.

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