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Quality of ram semen in relation to scrotal size

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Abstract

Aim of this study was to select superior rams by assessing their scrotal size and quality of semen. In ten indigenous Bangladeshi rams, the body weight, scrotal circumference, and scrotal volume of rams increased with age ($P<0.05$). Body weight at 381-410 days (13.6 ± 1.5 kg) was higher than at 169-200 days (10.2 ± 0.1 kg) and 201-230 days (10.4 ± 1.2 kg) ($p<0.05$). Scrotal circumference at 381 - 410 days (19.1 ± 0.3 cm) was higher ($p<0.05$) than at 169 - 200 days (17.2 ± 0.1 cm), 201 - 230 days (17.2 ± 0.6 cm), and 231 - 260 days (17.2 ± 0.5 cm). At 381 - 410 days, scrotal volume (87.4 ± 5.5 cm^3) was higher than in the five youngest groups ($p<0.05$). Both scrotal circumference and volume were positively correlated ($R^2=0.519$) with the increased body weight. Semen volume increased with age, which varied from 0.4 ± 0.0 - 1.2 ± 0.3 ml. The semen volume at 381 - 410 days (1.2 ± 0.3 ml) was higher than other age groups ($P<0.05$) except for 351 - 380 days. The mass activity of sperms of different age groups varied from 2.8 ± 0.4 to 4.3 ± 0.3. Sperm concentration, progressive motility (%), and normal spermatozoa (%) improved progressively with age, but significant changes in these parameters were seen when the animals reached 291 days or more ($P<0.05$). However, normal spermatozoa (%) improved from the age of 261 days. The mass activity was positively correlated with sperm concentration ($R^2 = 0.568$) and with progressive motility (%) ($R^2 = 0.616$). Measurements of scrotum could provide a reliable guide in estimating sperm production capacity, which are important for breeding soundness evaluation, and genetic improvement of indigenous rams. (*Bangl. vet*. 2021. Vol. 38, No. 1 - 2, 1 – 9)

Introduction

The disadvantages of Bangladeshi sheep are that they are small. There is a scarcity of good quality breeding rams. Therefore, for establishing a profitable sheep farm, it is urgent to select high quality rams.

Scrotal circumference (SC), length (SL), and volume (SV) are important factors for selecting quality rams. SC is easy to measure and highly correlated with body weight and high reproductive capacity (Chenoweth, 1981). The scrotal circumference has been correlated with testicular weight and males with longer testes tend to sire daughters that reach puberty at an earlier age (Lino, 1972). In Bangladesh, reports in this area of reproduction are scant (Siddiqui *et al.,* 2008). The study was designed to observe in indigenous rams in Bangladesh the effects of age and body weight on the

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scrotal circumference and volume, and the relationship between scrotal circumference and volume on semen parameters.

Materials and Methods

Animal selection and management

Ten indigenous rams of 5-9 months old were selected and allowed grazing with balanced nutrition. Animals received routine inspection and dipping (ectoparasite), as well as anthelminthic drenching and vaccination against PPR and FMD. Drinking water was provided *ad libitum.*

Animal preparation

The inguinal region of rams was cleaned and dried, for scrotal measurement, and semen was collected by AV methods (Azizunessa *et al*., 2014).

Measurement of body weight

The body weights of the rams were obtained (kg) using a weighing scale at the beginning of the study and weekly.

Measurement of Scrotal circumference (SC)

The scrotal circumference was measured weekly following the method described by the Society of Theriogenology by a flexible metal tape (Ball *et al*., 1983). The rams were restrained in a sitting position.

Measurement of Scrotal length (SL)

The scrotal length was measured from the top of the testis to the bottom of the epididymis with a pair of Vernier callipers.

Calculation of scrotal volume (SV)

The volume of the scrotum was calculated by the equation;

Scrotal volume (cm^3) = 0.0396 $\times$ (average scrotal length) $\times$ (scrotal circumference)^2

Semen collection

Semen was collected by AV once a week and eight ejaculates were collected from each ram. The inner liner temperature of AV was maintained at 42 - 43°C by loading two-thirds of the jacket with 52 - 54°C hot water. The rest of the water jacket was filled with air. The sterile non-spermicidal water-soluble gel was smeared into the inner side of AV by a glass rod. Rams were allowed at least one false mount before collection. The AV was held in a downwards direction at an angle of 45° along the ram’s flank. During mounting, the erected penis was directed to the open end of the AV to permit vigorous thrusts, which signifies ejaculation. The graduated collecting tube was closed with a plastic cap and kept at 37°C in a water bath until evaluation.

Semen evaluation

The density of semen was scored by observing the semen in a slanting tube: 1 = watery, 2 = milky, 3 = creamy, 4 = creamy to grainy. A drop (0.5 μl) of semen was placed on a pre-warmed slide (37°C) without a cover slip and examined under a phase-contrast microscope (100x). The mass activity was scored: 1 = no motion, 2 = free spermatozoa moving without forming waves, 3 = small, slow-moving waves, 4 = vigorous movement with moderately rapid waves and eddies, and 5 = dense, very rapidly moving waves and distinct eddies. For assessing motility, a drop (0.5 μl) of semen diluted 1:4 with tris was placed on a clean pre-warmed slide (37°C) with a cover slip, and viewed at higher magnification (400x). The motility was estimated as the proportion of spermatozoa moving progressively straight forward. The concentration of spermatozoa (million/ml) was calculated by using a haemocytometer and the results were expressed as ($\times 10^6$) spermatozoa/ml.

Statistical analysis

Results were analysed using SPSS version 20. The SC, SCV, SV, and sperm concentration were compared between groups using the independent sample t-test after Levene's test was used to determine the equality of variances. The correlation between scrotal circumference and sperm concentration was measured by the Pearson correlation test (Petrie and Watson, 1999). Data were reported as mean ± SEM. Values were considered statistically significant at $P<0.05$. Data were entered in a Microsoft Excel worksheet for further analysis. Descriptive statistics were performed to calculate the correlation coefficient.

Results and Discussion

The results (Mean ± SEM) of body weight (kg), scrotal circumference (cm), and scrotal volume (cm^3) are presented in Table 1. The estimated body weights were 10.2 ± 0.5, 10.4 ± 1.2, 11.2 ± 0.9, 11.7 ± 0.4, 12.0 ± 0.7, 12.5 ± 0.5, 12.9 ± 0.6 and 13.6 ± 1.8 kg in different age groups started from 169-200 days of age, followed by 201 - 230 days, 231 - 260 days, 261 - 290 days, 291 - 320 days, 321 - 350 days, 351 - 380 days and 381 - 410 days, respectively. The average estimated scrotal circumference (cm) and scrotal volume (cm^3) were 17.2 ± 0.1 cm and 61.3 ± 0.7cm^3, 17.2 ± 0.6 cm and 63.7 ± 4.5 cm^3, 17.2 ± 0.5 cm and 64.9 ± 4.2 cm^3, 17.7 ± 0.4 cm and 67.1 ± 4.2 cm^3, 18.4 ± 0.4 cm and 69.5 ± 4.1 cm^3, 18.5 ± 0.4 cm and 77.2 ± 3.8 cm^3, 18.5 ± 0.4cm and 78.7 ± 4.9 cm^3, 19.1 ± 0.3cm and 87.4 ± 5.5cm^3, respectively in different age groups.

Scrotal circumference increased with age and was highly correlated with the scrotal volume. A similar result was found by Salhab (2001). Scrotal circumference (SC) was highest (19.10 ± 0.30 cm) at 381 - 410 days age with a body weight of 13.6 ± 1.5 kg ($P < 0.05$). Azizunnesa *et al.* (2014) found a scrotal circumference of 22.8 ± 0.2 cm in mature indigenous rams (body weight 20.8 + 0.6 kg) which was more than in the present study. Similar observations were found by others (Adedeji and Gbadamosi, 1999; Akpa *et al.,* 2012). At 381 - 410 days, scrotal volume (87.4 ± 5.5 cm^3) was significantly higher than in the five youngest groups. A decrease in the scrotal volume is correlated with low sperm output (Toe *et al.,* 1994). This effect was observed in the current study as rams with lower scrotal volume within the age group had a lower ejaculate volume. Several researchers (Shannon and Vishwanath, 1995; Garner *et al.,* 1996; Mathevon *et al.,* 1998) reported that the ejaculate volume, sperm concentration, and semen motility improve with the age of the bull. There was a highly significant correlation between body weight and scrotal measurements, which agrees with studies in buffalo (Pant *et al.,* 2003), Sahiwal bulls (Ahmad *et al.,* 2011), and Mithun bulls (Perumal and Rajkhowa, 2013).

Both scrotal circumference and scrotal volume were positively correlated (R2 = 0.519) with body weight (Fig. 1 and 2).

Table 1: Mean (± SEM) body weight and scrotal parameters of rams between 169 and 410 days of age

Age (days)	Body weight (Kg)	Scrotal circumference (cm)	Scrotal volume (cm^3)
169-200	10.2 ± 0.5^{b}	17.2 ± 0.1^{b}	61.3 ± 0.7^{b}
201-230	10.4 ± 1.2^{b}	17.2 ± 0.6^{b}	63.7 ± 4.5^{b}
231-260	11.2 ± 0.9^{ab}	17.2 ± 0.5^{b}	64.9 ± 4.2^{b}
261-290	11.7 ± 0.39^{ab}	17.7 ± 0.4^{b}	67.1 ± 4.2^{b}
291-320	12.0 ± 0.7^{ab}	18.4 ± 0.4^{ab}	69.5 ± 4.1^{b}
321-350	12.5 ± 0.5^{ab}	18.5 ± 0.4^{ab}	77.2 ± 3.8^{ab}
351-380	12.9 ± 0.6^{ab}	18.6 ± 0.4^{ab}	78.7 ± 4.9^{ab}
381-410	13.6 ± 1.5^{a}	19.1 ± 0.3^{a}	87.4 ± 5.5^{a}

Different superscripts within a column differ significantly at the 0.05 level.

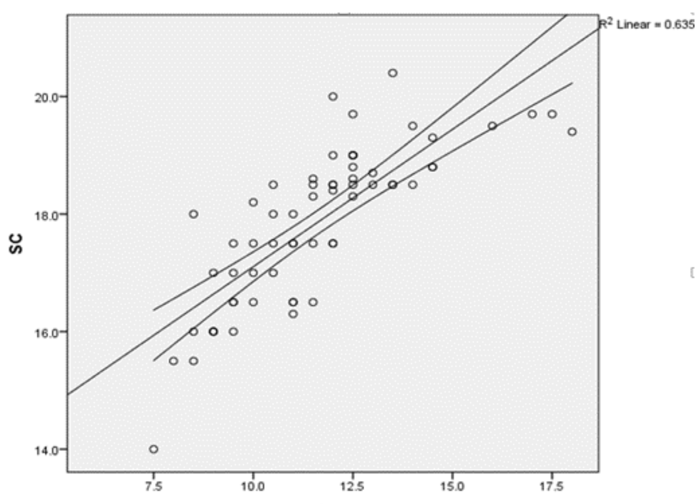

Fig. 1: Relationship between body weight and scrotal circumference (SC) in indigenous rams

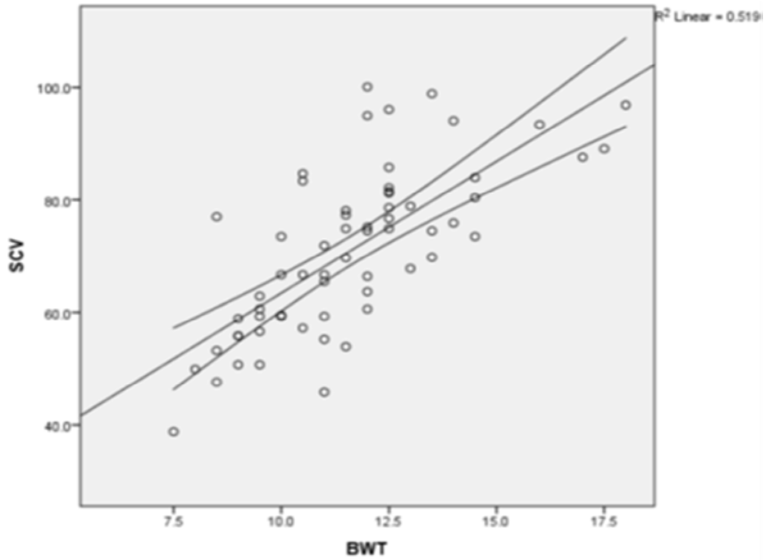

Fig. 2. Relationship between body weight and scrotal volume (SCV) in indigenous rams

Semen volume, mass activity, sperm concentration (X10^6), progressive motility (%), and normal sperm (%) of different age groups are presented in Table 2. Semen volume increased with age, from 0.4 ± 0.0 - 1.2 + 0.3 ml. The semen volume of age group 381-410 days (1.2 ± 0.3 ml) was significantly higher than other age groups (P<0.05) except for age group 351 - 380 days. The mass activity of different age groups varied from 2.8 (± 0.4) to 4.3 (± 0.3) ml on a scale of 1 - 5. Sperm concentration, progressive motility (%), and normal spermatozoa (%) improved with increasing age, but significant changes in these parameters were seen at age 291 days or more (P<0.05). However, normal spermatozoa (%) improved significantly from the age of 261 days.

Table 2: Mean (± SEM) semen characteristics of ram lambs from 169 days to 440 days of age

Age (Days)	Semen volume (ml)	Mass activity (1-5)	Sperm concentration (X10^6) / ml	Motility (%)	Normal spermatozoa (%)
169 - 200	0.4 ± 0.0^{e}	2.8 ± 0.4^{e}	1614.1 ± 230.6^{d}	75.5 ± 2.5^{d}	77.5 ± 2.5^{b}
201 - 230	0.5 ± 0.1^{de}	3.1 ± 0.2^{de}	2205.5 ± 226.5^{cd}	79.6 ± 2.6^{c}	79.8 ± 2.3^{b}
231 - 260	0.6 ± 0.1^{d}	3.1 ± 0.4^{de}	2499.6 ± 352.4^{cd}	79.3 ± 2.3^{c}	82.4 ± 2.3^{b}
261 - 290	0.7 ± 0.1^{cd}	3.3 ± 0.7^{cde}	3249.8 ± 312.5^{bc}	82.8 ± 2.0^{bc}	87.52 ± 1.1^{a}
291 - 320	0.8 ± 0.1^{bc}	3.5 ± 0.4^{bcd}	4014.3 ± 252.4^{ab}	88.0 ± 1.2^{ab}	87.6 ± 0.8^{a}
321 - 350	0.9 ± 0.2^{b}	3.8 ± 0.6^{abc}	4134.3 ± 292.5^{ab}	90.0 ± 1.1^{a}	88.6 ± 1.1^{a}
351 - 380	1.0 ± 0.2^{ab}	4.0 ± 0.4^{ab}	4426.6 ± 167.5^{a}	90.4 ± 1.1^{a}	89.5 ± 1.2^{a}
381 - 410	1.2 ± 0.3^{a}	4.3 ± 0.3^{a}	4686.7 ± 139.7^{a}	92.75 ± 1.0^{a}	90.21 ± 2.2^{a}

Different superscripts within a column differ significantly at the 0.05 level.

The mass activity was positively correlated with sperm concentration (R2 = 0.568) (Fig. 3) and with progressive motility (%) (R2 = 0.616) (Fig. 04).

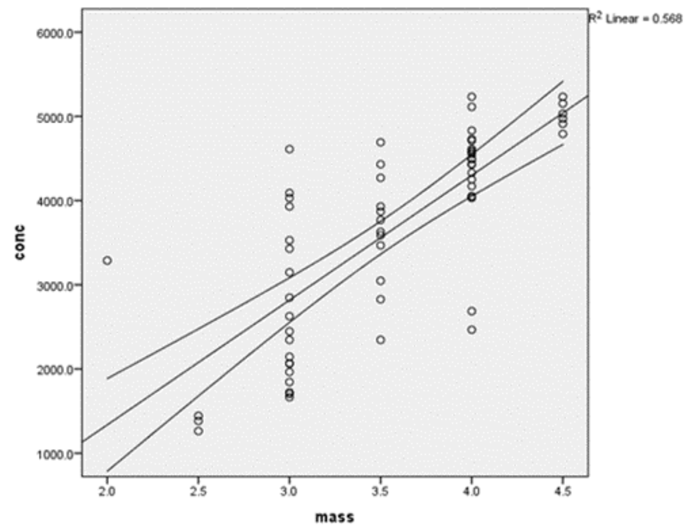

Fig. 3: Relationship between mass activity and sperm concentration

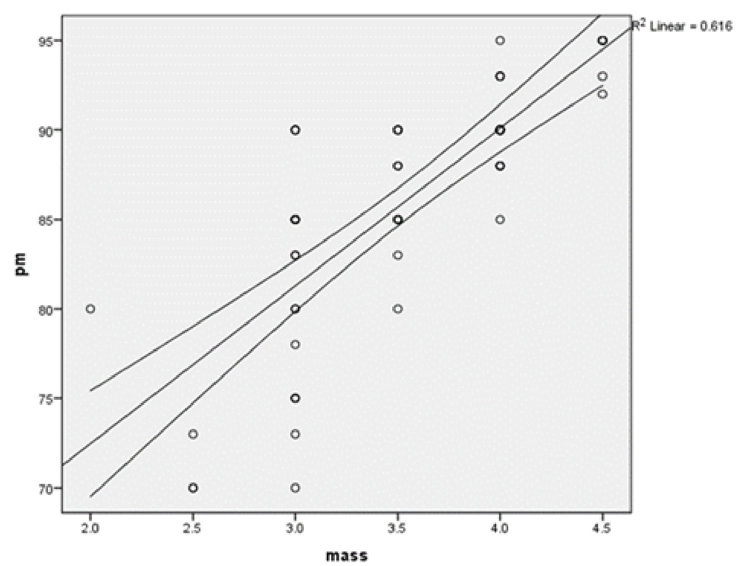

Fig. 4: Relationship between mass activity and progressively motile spermatozoa

This study confirms that testicular weight and sperm concentration were positively correlated, as reported by Vidament *et al*. (2007). The age of rams had a significant effect on ejaculate volume, sperm survival, and the percentage of abnormal spermatozoa (Kanakaraj *et al.,* 1997). There was a significant positive effect of age on sperm production.

Correlation coefficients among age, body weight, scrotal measurements (circumference and volume), and semen characteristics (volume, density, mass activity, concentration, progressive motility, and per cent of normal spermatozoa) are shown in Table 3.

With the increasing age, the measurements of the scrotum and semen parameters were highly correlated (P<0.01) with each other.

The scrotal size was correlated with capacity for sperm production, the number of sperms ejaculated, and sperm reservoirs (Palaz *et al.,* 1994). Scrotal size is strongly correlated with sperm count (Shinobu *et al.,* 2006). The present findings were consistent with those reports.

The correlations among scrotal measurements and semen characteristics with the increasing age were highly correlated. The findings are in the agreement with Akpa (2012).

Table 3: Correlation coefficients among age, body weight, scrotal measurements and semen characteristics in indigenous rams

Measurement	SC	SCV	BW	SV	Density	Conc.	MA	%PM	%NS
Age	.408**	.502**	.368**	.813**	.784**	.739**	.672**	.729**	.631**
SC	-	.940**	.797**	.265*	.441**	.266*	.306*	.457**	.275*
SCV	-	-	.720**	.339**	.518**	.392**	.361**	.534**	.336**
BW	-	-	-	.193	.307*	.052	.148	.262*	.138
SV	-	-	-	-	.695**	.666**	.616**	.687**	.580**
Density	-	-	-	-	-	.831**	.842**	.858**	.678**
Conc.	-	-	-	-	-	-	.754**	.852**	.744**
MA	-	-	-	-	-	-	-	.785**	.616**
%PM	-	-	-	-	-	-	-	-	.714**

SC = Scrotal Circumference, SCV = Scrotal Volume, BW= Body weight, SV = Semen volume, Density = Semen Density, Conc. = Sperm concentration/ml, MA = Mass activity, %PM = Per cent of Progressively motile sperm, % NS = Per cent of Normal spermatozoa.
**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

Measurements of scrotum could provide a reliable guide in estimating sperm production capacity, which are important for breeding soundness evaluation, and genetic improvement of indigenous rams.

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