The Bangladesh Veterinarian (2018) 35(1 & 2): 32– 39

Factors affecting the semen quality of breeding bulls

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Abstract

This study aimed to investigate the effects of breed, age, scrotal circumference, Body Condition Score (BCS), season and nutrition on bull semen quality at breeding bull station of Ejab Alliance Limited, Thakurgaon, Bangladesh. A total of 777 ejaculates were collected from 25 bulls (Holstein-Friesian-10; Sahiwal-15). All semen parameters were significantly ($P<0.05$) higher in Sahiwal bulls than in Holstein-Friesian. The volume, individual motility, mass activity and sperm morphology were significantly ($P<0.05$) higher in bulls aged >3.5 - 4.5 years than in younger (2.5 - 3.5 years) and older bulls (>4.5 years). Scrotal circumference significantly ($P<0.05$) affected all the parameters of semen quality. The highest values were observed in bulls with scrotal circumference 31.1 - 33.0 cm and the lowest in bulls with scrotal circumference of 33.1 cm and less. The volume, individual motility, mass activity and sperm morphology were higher ($P<0.05$) in bulls with BCS of 4 to 4.5 than in bulls with BCS of >4.5 to 5. Significantly ($P<0.05$) highest values regarding semen quality were in winter and the lowest in summer. Vitamin ADE supplementation significantly ($P<0.05$) improved the semen quality. It is suggested that Sahiwal breeds may be reared at the bull station in the subtropical climate of Bangladesh. Semen should be collected during winter in bulls of >3.5 to 4.5 years of age with moderate scrotal circumference and BCS. (*Bangl. vet*. 2018. Vol. 35, No. 1 & 2, 32 – 39)

Introduction

The production potential of livestock can be increased by genetic improvement using Artificial Insemination (AI). The cattle improvement programme in Bangladesh aims to improve local cattle for milk and meat production by the incorporation of tropical breeds (Red-Sindhi, Sahiwal) and temperate breeds (Holstein-Friesian and Jersey). Quality semen is essential in a successful breeding programme (Dasinaa and Pagthinathan, 2015). Semen volume, sperm concentration, sperm motility, mass activity, sperm morphology and seminal pH are the common criteria for evaluating semen quality (Den Daas, 1992).

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DOI: https://doi.org/10.3329/bvet.v35i1-2.53385

Materials and Methods

Study area and animals

The study was conducted at bull station of Ejab Alliance Limited in Thakurgaon district of Bangladesh from March 2017 to February 2018. A total of 25 bulls were used in this experiment.

Physical examination of the bulls

The age of the bulls was determined by using dental formula according to Johnson (1999). Body condition was measured by the commonly used scale of 1 to 5, with 1 being thin and 5 being optimum conditioned according to Herd and Sprott (1986). Scrotal circumference (cm) was measured by measuring tape around the widest point.

Grouping of the study animals

The bulls were grouped according to breed (Holstein-Friesian=10 and Sahiwal=15), age, scrotal circumference and BCS. Animals were divided into three groups according to age as G I: 2.5 to 3.5 years (n = 6), G II: >3.5 to 4.5 years (n = 10), G III: >4.5 years (n=9). The animals were grouped into four sub-groups based on scrotal circumference, G I: 27-29 cm (n = 2), G II: 29.1-31 cm (n = 6), G III: 31.1-33 cm (n = 5) and G IV: 33.1 cm-above (n = 12). The bulls were grouped into two sub-groups according to BCS, namely-G I: 4 to 4.5 (n = 16), G II: >4.5 to 5 (n = 9).

Nutritional management of bulls

Two types of feed supplement were given to two groups of bulls, Group I (Vit. ADE) and group II without Vitamin supplementation. Each bull of group I was injected with Acivit-ADE (ACI Animal Health Ltd., Bangladesh) 10 ml intramuscularly once a week, and E-Vet plus (ACME Laboratories Ltd., Bangladesh) 10 ml intramuscularly once a week. The bulls of group II were supplemented with same ration as group I, where mustard oil cake was replaced by sesame oil cake (Table 1).

Table 1: Feed supplementation for experimental bulls:

Group I (Amounts/day/Animal)	Group II (Amounts/day/Animal)
Wheat bran (4 kg)	Wheat bran (4 kg)
Rice polish(2 kg)	Rice polish (2 kg)
Molasses (100 gm)	Molasses (100 gm)
Mustard oil cake (200 gm)	Sesame oil cake (300 gm)
Gram (Germinated) (300 gm)	Gram (Germinated) (300 gm)
Pulse (200 gm)	Pulse (200 gm)
Salt (100 gm)	Salt (100 gm)
Straw (1.5 kg)	Straw (1.5 kg)
Green grass (15 kg)	Green grass (15 kg)
Vit. ADE supplementation (10 ml)	No Vit. ADE supplementation

Effects of season on the quality of bull semen

Evaluation was done to assess the effects of seasons (Summer = March - June, Rainy = July - October and Winter = November - February) on semen quality.

Collection of semen

Semen was collected once a week, during summer (March - June), rainy season (July - October) and winter (November - February) using artificial vagina.

Evaluation of semen

Physical examination of semen

The colour observed with naked eye. The volume (ml) was measured, and concentration (million/ml) was evaluated by photometric analysis (imv photometer). pH meter (HI 2211 pH/ORP Meter) was used to measure the pH.

Microscopic examination of semen

Mass activity was observed under microscope at 10x magnification without cover slip. Score (0-4) of mass activity was recorded according to the criteria of Nath (1988); 0 = No mass activity, +1 = Slow moving without forming waves, +2 = Slow waves, +3 = Quick waves, +4 = Waves, churning of whirls and eddies. The motility (%) was examined under microscope with 40x magnification. The morphology of spermatozoa was assessed after staining with Eosin-Nigrosin stain. At least 100 sperm cells were examined under microscope at 100x objective.

Staining procedure

One drop of fresh semen was placed on a slide and mixed with two drops of Eosin stain (0.5%, aqueous) for 30 seconds. Three drops of Nigrosin stain (10%, aqueous) were added and gently swirled to mix. A smear was prepared and the slide was allowed to air-dry. Cover slip was placed on the slide with compatible mounted medium and examined under oil immersion with 100x objective.

Statistical analysis

One Way Analysis of Variance (ANOVA) was used and results were expressed as Mean ± Standard Deviation using SPSS program (version 17.0, SPSS). Difference between means was considered statistically significant with P value <0.05.

Results and Discussion

Effects of breed on the quality of bull semen

Table 2 shows that the mean volume, mass activity, motility and percentage of normal sperm were significantly ($P<0.05$) higher in Sahiwal bulls than in Holstein-Friesian bulls. Sperm concentration was higher in Sahiwal bulls than Holstein-Friesian bulls but the effect was non-significant.

The mean volume of semen was significantly (P<0.05) higher in Sahiwal bulls (13.1 ± 4.1 ml) than in Holstein-Friesian bulls (11.1 ± 3.6 ml). These results were similar to that of Dasinaa and Pagthinathan (2015). The findings differ from that reported by Sane *et al.* (1994). Mass activity of spermatozoa was higher (P<0.05) in Sahiwal bulls (3.4±0.1) than in Holstein-Friesian bulls (2.8±0.2). Rahman *et al.* (2014) observed highest (P<0.01) mass activity in Holstein-Friesian cross and lowest in Red Chittagong bulls. The average sperm motility was significantly (P<0.05) higher (77.4 ± 2.6%) in Sahiwal than in Holstein-Friesian (73.1 ± 2.3%), similar to that of Rahman *et al.* (2014). The average sperm normal morphology was significantly higher (P<0.05) in Sahiwal (85.1 ± 2.2%) than in Holstein-Friesian bulls (82.4 ± 1.8%). The result differs from Buhr *et al.* (1993), where no significant effect of breed was recorded.

Table 2: Characteristics of fresh semen in different breeds of bull (Mean ± SD)

Breed	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
Holstein-Friesian (n = 10)	11.1 ± 3.6	558.4 ± 23.5	73.1 ± 2.3	2.8 ± 0.2	82.4 ± 1.8	17.6 ± 1.8
Sahiwal (n = 15)	13.1 ± 4.1	587.6 ± 33.1	77.4 ± 2.6	3.4 ± 0.1	85.1 ± 2.2	14.9 ± 2.2
Sig. level	*	NS	*	*	*	*

* = Significantly differ (P<0.05); NS = Non-Significant (P>0.05)

Effects of age on the quality of bull semen

The highest mean volume, mass activity, motility and percentage of normal sperm were in G II and the lowest were in G III age group. There was no significant (P>0.05) difference in average sperm concentration of bulls in different age groups, although higher concentrations were in G II (592.2 ± 32.5) and the lower in G III (549.3 ± 27.5) (Table 3).

The mean volume (13.3 ± 3.5 ml) in G II (>3.5-4.5 years) was significantly higher (P<0.05) than others. The findings of Ahmad *et al.* (2003) closely support the result of the present study. The average sperm concentration was higher (592.2 ± 32.5 million/ml) in bulls aged >3.5-4.5 years and lower (549.3 ± 27.5 million/ml) in older bulls (>4.5 years). There was no difference (P>0.05) in sperm concentration among the three age groups. The mean mass activity of sperm was higher (3.3 ± 0.3) in age G II and the lowest value (3.1 ± 0.4) was in GIII, which is similar to that of Ahmad *et al.* (2003). The maximum sperm motility (76.5 ± 3.1%) was in G II (>3.5-4.5 years) and the lowest (75.2 ± 3.3%) in older bulls (>4.5 years). The result is in line with those reported by Galmessa *et al.* (2014) and Ahmad *et al.* (2003). But the present finding is inconsistent with David *et al.* (2007). The percentage of normal sperm was higher (84.4 ± 2.4%) in bulls aged >3.5-4.5 years and lower (84.1 ± 2.3%) in younger and in older bulls (83.7 ± 2.4%). The percentage of sperm morphology differed significantly

(P<0.05) between the age group G II and G III. These results agree with those reported by Vilakazi (2003).

Table 3: Semen characteristics in bulls of different ages (Mean ± SD)

Age groups	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
G I (2.5-3.5 Years)	10.6 ± 3.6[b]	585.5 ± 29.5[a]	75.9 ± 3.1[b]	3.2 ± 0.3[a]	84.1 ± 2.3[a]	15.9 ± 2.3[a]
G II (>3.5-4.5 years)	13.3 ± 3.5[a]	592.2 ± 32.5[a]	76.5 ± 3.1[a]	3.3 ± 0.3[a]	84.4 ± 2.4[a]	15.6 ± 2.4[a]
G III (>4.5 years-above)	10.6 ± 3.9[b]	549.3 ± 27.5[b]	75.2 ± 3.3[b]	3.1 ± 0.4[b]	83.7 ± 2.4[b]	16.3 ± 2.4[b]
Sig. Level	*	NS	*	*	*	*

[a,b] Superscript letters in same column differ significantly (P<0.05), NS = Non Significant

Effects of scrotal circumference on the quality of bull semen

The average volume (12.9 ± 3.6), concentration (696.3 ± 35.2), motility (77.1 ± 2.8), mass activity (3.4 ± 0.1), and percentage of normal sperm (84.7 ± 2.2) were highest in G III and found lowest in G IV (Table 4).

Table 4: Scrotal circumference and semen quality in bulls (Mean ± SD)

Groups	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
G I (27-29 cm)	11.1 ± 4.2[b]	571.4 ± 27.6[c]	76.6 ± 3.2[b]	3.2 ± 0.3[a]	84.7 ± 2.3 [a]	15.4 ± 2.3 [a]
G II (29.1-31 cm)	11.7 ± 3.6[b]	640.0 ± 33.4[b]	77.0 ± 2.6[a]	3.4 ± 0.1[a]	84.7 ± 2.3 [a]	15.3 ± 2.3 [a]
G III (31.1-33 cm)	12.9 ± 3.6[a]	696.3 ± 35.2[a]	77.1 ± 2.8[a]	3.4 ± 0.1[a]	84.7 ± 2.2 [a]	15.3 ± 2.2[a]
G IV (31.1 cm-above)	9.3 ± 3.1[c]	469.9 ± 25.4[d]	74.8 ± 3.2[c]	3.0 ± 0.3 [b]	83.50 ± 2.4 [b]	16.5 ± 2.4[b]
Sig. level	*	*	*	*	*	*

[a,b,c,d] Superscript letters in same column differ significantly (P<0.05), NS = Non Significant

The highest sperm motility (77.1 ± 2.8%) was in bulls with scrotal circumference of 31.1-33.0 cm and the lowest (74.8 ± 3.2%) in bulls with scrotal circumference of 33.1 cm and above. These results are similar to that of Ulfina Galmessa *et al.* (2014). The higher percentage of normal sperm (84.7 ± 2.3%) was recorded in bulls with scrotal circumference of 31.1-33 cm as compared to bulls with 27-29 cm (84.7 ± 2.3%), 29.1-31 cm (84.7 ± 2.2%) and 33.1-above (83.5 ± 2.4%). Menon *et al.* (2011) found no significant effect of scrotal circumference.

Effects of BCS on the quality of bull semen

The mean volume, mass activity, motility and percentage of normal sperm were significantly higher (P<0.05) in G I than in G II bulls. Sperm concentration did not differ significantly (P>0.05) between the two groups.

The mean volume of semen was higher (13.3 ± 3.5 ml) in group G I than group G II. Sitali *et al.* (2017) found that bulls of BCS 3.5 had the highest semen volume while those of BCS 2.0 had the lowest. The average percentage of normal sperm was higher (84.3 ± 2.4%, P<0.05) in bulls with BCS of 4-4.5 than (83.7 ± 2.4%) in bulls with BCS of >4.5-5. The result agrees with that reported by Sarder (2008).

Table 5: Effects of Body Condition Score on fresh semen quality in bulls (Mean ± SD)

Bull groups	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
G I (4-4.5)	13.3 ± 3.5	585.5 ± 29.5	76.1 ± 3.1	3.2 ± 0.3	84.3 ± 2.4	15.7 ± 2.4
G II (>4.5-5)	10.6 ± 3.8	567.6 ± 29.8	75.2 ± 3.3	3.1 ± 0.4	83.71 ± 2.4	16.3 ± 2.4
Sig. level	*	NS	*	*	*	*

* = Significantly differ (P<0.05); NS = Non-Significant

Effects of season on the quality of bull semen

The average sperm concentration, mass activity, motility and percentage of normal sperm were highest in winter and lowest in summer. The highest mean volume was in winter and the lowest in summer but the values did not differ significantly (P>0.05) between seasons.

The results of Salah *et al.* (1992) closely support the findings of present study. Ahmad *et al.* (2003) did not find any significant seasonal variation in sperm concentration. The highest sperm motility was in winter (78.5 ± 2.6%) and the lowest in summer (73.0 ± 2.2%). The result agrees with those reported by Mostari *et al.* (2005) and Li-junjie *et al.* (2001). The season significantly (P<0.05) affected the percentage of normal sperm count, similar to the findings of Coe (1999) and Vilakazi (2003).

Table 6: Characteristics of fresh semen in different seasons of the year (Mean ± SD)

Seasons	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
Summer	11.5 ± 4.1^{b}	538.6 ± 30.8^{c}	73.0 ± 2.2^{c}	3.0 ± 0.3^{b}	81.8 ± 1.4^{c}	18.2 ± 1.4^{c}
Rainy	12.0 ± 3.9^{b}	582.0 ± 27.7^{b}	75.9 ± 2.1^{b}	3.12 ± 0.3^{b}	84.3 ± 1.7^{b}	15.7 ± 1.7^{b}
Winter	12.2 ± 3.6^{a}	613.4 ± 29.7^{a}	78.5 ± 2.6^{a}	3.3 ± 0.3^{a}	86.2 ± 1.7^{a}	13.8 ± 1.7^{a}
Sig. level	NS	*	*	*	*	*

^{a,b,c} Superscript letters in same column differ significantly (P<0.05), NS = Non Significant)

Effects of nutrition on the quality of bull semen

The mean volume, mass activity, motility and percentage of normal sperm were significantly higher (P<0.05) in group I (vitamins) than group II (protein). The sperm

concentration was higher in group I group but did not differ significantly. The mass activity of semen was higher (3.2 ± 0.3) in vitamin supplemented group than the protein supplemented group (3.1±0.3), which was similar to the findings of Susan (2010).

Table 7: Effects of feed supplements on fresh semen quality in bulls (Mean ± SD)

Groups	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
I (n = 12, Vitamin)	12.2 ± 3.8	592.4 ± 28.0	76.8 ± 2.6	3.2 ± 0.3	85.0 ± 1.8	15.1 ± 1.8
II (n = 13, no Vitamin)	11.5 ± 4.0	569.7 ± 31.1	74.6 ± 3.5	3.1 ± 0.3	83.1 ± 2.5	17.0 ± 2.5
Sig. level	*	NS	*	*	*	*

* = Significantly differ (P<0.05); NS = Non- Significant

Conclusions

It is concluded that the semen quality was higher in Sahiwal than Holstein-Friesian bulls. Better quality of semen was recorded in age group II (3.5 - 4.5 years). The semen quality was higher in bulls with BCS ranging from 4.0 to 4.5, and in those with 31.1 to 33.0 cm scrotal circumference. Semen quality was low in summer, while winter was ideal for the best quality of semen. Vitamin ADE supplementation improved the semen quality.

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