Research Article

Effect of Semen Collection Frequency and Scrotal Circumference on Semen Quality Parameters in Brahman x Local Crossbred Bulls

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Abstract | Five Brahman crossbred bulls were ejaculated twice weekly (2x) and 5 bulls four times weekly (4x) for 16 weeks to determine the effect of ejaculation frequency on semen characteristics and sperm output. Each of 10 bulls was ejaculated 4 times weekly for 4 weeks to determine sperm output and the relationship between scrotal circumference and semen parameters. For 4x bulls as compared to 2x bulls, ejaculation volume was smaller (P<0.01) but sperm concentration per ejaculate and total progressive motile sperm (TPMS) output per week were greater (P<0.01). Weekly semen output was greater (P<0.01) in 4x bulls (11.4 mL) compared to 2x bulls (7.99 mL). Average weekly TPMS output for 4x bulls was 1.61 times higher compared to 2x bulls. Occurrence of total abnormalities, proximal droplets and tail abnormalities was higher (P<0.05) in 4x bulls as compared with 2x bulls. When 2x bulls were placed at 4x for 4 weeks, they produced numerically higher TPMS ($14.2x10^9$) per week than that ($12.8x10^9$) of continued 4x bulls. Scrotal circumference was highly correlated (P<0.01) with semen volume and TPMS per ejaculate while the correlations between scrotal circumference and sperm motility and scrotal circumference and sperm concentration were found at medium and weak levels, respectively. Therefore, it seems appropriate to recommend the collection of semen from Brahman crossbred breeding bulls at the rate of 4 ejaculates per week and recording of scrotal circumference as a criterion to select young bulls as sires.

Keywords | Ejaculation frequency, Sperm abnormality, Sperm output, Morphology, Motility

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INTRODUCTION

The cattle population of Bangladesh is 23.30 million, which consists of Local zebu, Friesian- Local crossbred, Friesian-Sahiwal crossbred and Sahiwal-Local crossbred (DLS, 2013). Out of 6.9 million breedable cows and heifers, Friesian-Local crossbred contributes about 24% (Huque et al., 2011). More than 80% of the breeding bulls used in national artificial insemination program are Friesian-Local crossbred. Therefore, the proportion of this dairy genotype increased in the cattle population rapidly which contributed to long term growth in the dairy sector (Jabbar, 2010). Due to absence of any beef type breed, local zebu cattle and Friesian-Local crossbred cattle are being used for beef production to meet huge demand of meat. In the year of 2009, National beef cattle development program was undertaken for crossbreeding between local zebu cows and Brahman sires through artificial insemination. Thereafter, more than 10,000 Brahman crossbred cattle were born throughout the country. Greater utilization of genetically superior Brahman-Local crossbred bulls for flourishing beef industry can be achieved by frequent semen collection from them. Weekly collection of two ejaculates from breeding bulls is a common practice at Central Artificial Insemination Laboratory in Bangladesh. According to DAHDF (2014), semen stations must follow the norm of minimum two ejaculates per collection and minimum two collections per bull per week for taking at least 90 collections and 180 ejaculates annually from each adult bull. In previous studies, the effect of age and ejacula-

tion frequency on semen characteristics and sperm output of young Angus, Hereford, Charolais and Friesian x Sahiwal crossbred bulls has been reported (Almquist and Cunningham, 1967; Almquist et al., 1976; Sattar and Mirza, 2002). However, a maximum number of collection per bull would depend on the individual capacity of the bull.

A positive correlation between testicular development and semen quality has been documented through various studies (Spitzer et al., 1988; Arteaga et al., 2001). Testicular size, weight, semen volume and motile sperm output of young or adult bulls have been reported to be highly correlated with in situ measurements of scrotal circumference (Latif et al., 2009; Ha et al., 2012). Scrotal circumference is intimately correlated to capacity of sperm production, number of sperms ejaculated and sperm reserves (Palasz et al., 1994). However, information related to optimum ejaculation frequency, semen characteristics, sperm output and scrotal circumference of Brahman F_1 crossbred beef type bulls in hot and humid tropical regions including Bangladesh are scanty. Thus, this study was conducted to determine: (a) the effect of ejaculation frequency on the sperm characteristics and sperm output of Brahman x Local F_1 crossbred bulls and to evaluate (b) the relationship between scrotal measurements and semen parameters.

MATERIAL AND METHODS

Animal Management and Experiment Design

Ten Brahman x Local F_1 crossbred bulls, having average age of 28.0±4.0 months, body weight of 460±20 kg and scrotal circumference of 33.4±1.8 cm were selected from the Central AI Laboratory of Central Cattle Breeding Station, Savar, Dhaka. The bulls were reared in a separate clean, dry and regularly washed-floor shed and maintained under similar feeding and management. Total required feed included 1/3 concentrates and 2/3 roughages. Bulls had free access to fresh water.

The bulls were then divided into 2 groups having 5 bulls in each group and assigned to a high (4x) and low (2x) ejaculation frequency. The average age, body weight and scrotal circumference of the bulls between high and low ejaculation frequency group were similar. Five bulls were ejaculated twice weekly (2x) on Sunday and 5 bulls four times weekly (4x) consisting of two successive ejaculations on Sunday and Thursday for a period of 16 weeks from April to July 2013 (Phase-1). After completion of 16 weeks collection period, each of the 10 bulls was ejaculated at 4x for 5 weeks and data for the last 4 weeks (Phase-2) were used to determine mean weekly semen and sperm output.

Testicular Measurements

Scrotal circumference was measured using a flexible metallic tape graduated in centimetre (cm) around the greatest diameter of both testes and the scrotum maintaining the testicles toward the bottom of the scrotum by moderate digital pressure as described by Foote (1969).

Semen Collection and Laboratory Evaluation

To maximize sperm output per ejaculate, the bulls were allowed at least two false mount before collection of each ejaculate. The semen was collected in graduated tubes at homosexual mount using artificial vagina (AV). Each ejaculum was evaluated for volume, sperm concentration, total motility of sperm, progressive motility of sperm, total sperm, total motile sperm and total progressive motile sperm. First and 2nd ejaculate of each bull on Sunday of 1st, 5th, 9th and 13th week of 16 week experiment were evaluated to determine the percentage of spermatozoa with normal and abnormal morphology. When all bulls ejaculated at 4x for 5 weeks, 1st ejaculate of each bull on Sunday from each of last 4 weeks were also evaluated to determine the normal and abnormal spermatozoa.

The volume of fresh semen was recorded from the graduated mark of the semen collecting tube. The concentration of spermatozoa was determined by using Density Spectrophotometer (SDM-5, Minitüb, GmbH, Germany). Semen was diluted in cuvettes with 0.9% sodium chloride solution at the ratio of 1:100. The reading was recorded from the Density Spectrophotometer in million/mL. Total sperm per ejaculate was determined by multiplication of sperm concentration with volume of ejaculate and expressed as million per ejaculate. Total motile sperm and total progressive motile sperm per ejaculate were determined.

Spermatozoa Motility Analysis

Each fresh ejaculate was evaluated for percentage of motile spermatozoa (motility>5µm/s) and percentage of progressive motile spermatozoa (motility>20µm/s) using the Computer Assisted Semen Analysis (CASA-Plus) system-AndroVision (Minitüb, Tiefenbach, GmbH, Germany) was equipped with Zeiss Scope A1 phase contrast microscope (Zeiss, Germany), a camera, a mini-therm stage warmer, an image digitizer and a computer to store data. To estimate total motility and progressive motility, a small drop of raw semen (20 µL) was diluted with physiological solution (0.9% NaCl, Merck, Germany) using a dilution ratio of 1:25. One small drop of this diluted semen was placed on a clean pre-warmed glass slide maintained on a heating plate (37°C), covered with a cover slip and examined under phase-contrast microscope Zeiss Scope A1 phase contrast microscope at 20x. Three to five randomly selected microscopic fields were scanned for each sample and 500-1000 sperms were assessed in each analysis (Massányi et al., 2008).

Spermatozoa Morphology

The proportion of normal spermatozoa with respect to acrosome, midpiece and tail was evaluated in buffered formal saline-fixed semen. One or 2 drops of raw semen was diluted in 0.5 mL of buffered formol saline containing 0.2% glutaraldehyde. The buffered formol saline was prepared according to the procedure described by Perera (2005). A drop (10µL) of formol saline fixed semen was placed on a clean glass slide with a cover slip and the edged were soaked with tissue paper to remove excess fluid. The slide was then held for 5 minutes to allow spermatozoa to settle down and examined under a phase contrast microscope (400x) that was equipped with differential interference contrast (DIC) optics (Olympus, BH-2, Japan).

A total of 200 spermatozoa per sample were evaluated to determine the percentage of spermatozoa with normal and abnormal morphology and the abnormalities with respect to acrosome, midpiece and tail. The abnormalities were classified as head abnormalities (detached head, giant head, pyriform), midpiece abnormalities (presence of proximal cytoplasmic droplet and distal cytoplasmic droplet) and tail abnormalities (coiled tail, bent tail, DAG and folded tail). The abnormalities were recorded individually. The spermatozoa having no abnormalities with respect to acrosome, midpiece and tail were considered as normal spermatozoa. Percentages of proximal droplets, distal droplets, head abnormalities and tail abnormalities were determined according to Barth and Oko (1989).

Statistical Analysis

The data on semen parameters and morphology were subjected to analysis of variance using Proc. GLM to determine treatment effects. Semen parameters of 1st and 2nd ejaculate within the frequency of ejaculation were also compared. Pearson product-moment correlation coefficient analysis for pair-wise correlation was used to assess correlations between scrotal circumference and semen parameters. Regression analyses were used to evaluate the importance of scrotal circumference measurement on prediction of different semen parameters. All analyses were conducted using Statistical Analysis System (SAS, 2003). P-values at P<0.05 were considered as statistically significant.

RESULTS

Out of an expected number of 160 and 320 ejaculates from bulls of 2x and 4x group, 159 and 304 were collected, respectively during the first 16 weeks semen collection period, which was 96.5% of total specified number of 480. Ejaculation during false mount and or before the penis could be covered with the artificial vagina accounted for 1 failure in 2x group and 2 failures in 4x group, while 14 failures happened because of not giving second ejaculate by 4x bulls.

Table 1: Semen parameters (Mean±SE) per ejaculate of Brahman crossbred bulls at different ejaculation frequency

Ejaculation frequency	Volume (mL)	Concentration (10^6/mL)	TM (%)	PM (%)	TS (10^6)	TMS (10^6)	TPMS (10^6)
2x	4.02[a]± 0.13	1379[b]±32.2	81.5±0.61	67.1±0.85	5578[a]±232	4584[a]±199	3804[a]±176
4x	3.00[b]± 0.08	1543[a]±24.0	80.7±0.53	67.1±0.68	4658[b]±151	3818[b]±131	3202[b]±117
SEM	0.07	19.6	0.40	0.53	129	111	98.2
P-value	<.0001	<.0001	0.37	0.95	0.0007	0.001	0.0035

TM=Total motility; **PM**=Progressive motility; **TS**=Total sperm; **TMS**=Total motile sperm; **TPMS**=Total progressive motile sperm; **SEM**= Standard error of mean; [a,b] Values with different superscript in a column differ significantly (P<0.05)

Table 2: Semen parameters of 1st and 2nd ejaculate within frequency of ejaculation

Ejaculation frequency	2x ejaculation				4x ejaculation			
Parameters	1st ejaculate (n=80)	2nd ejaculate (n=79)	SEM	P-value	1st ejaculate (n=157)	2nd ejaculate (n=147)	SEM	P-value
Volume (mL)	4.23	3.81	0.13	0.11	3.18[a]	2.8[b]	0.08	0.025
Concentration (10^6/mL)	1445[a]	1313[b]	32.2	0.04	1571	1512	24.0	0.22
TM (%)	81.4	81.5	0.61	0.93	80.8	80.6	0.53	0.85
PM (%)	66.9	67.3	0.85	0.81	67.0	67.1	0.68	0.94
TS (10^6)	6174[a]	4975[b]	232	0.009	5040[a]	4250[b]	151	0.008
TMS (10^6)	5088[a]	4074[b]	199	0.011	4129[a]	3487[b]	131	0.014
TPMS (10^6)	4232[a]	3371[b]	176	0.014	3459[a]	2928[b]	117	0.022

TM=Total motility; **PM**=Progressive motility; **TS**=Total sperm; **TMS**=Total motile sperm; **TPMS**=Total progressive motile sperm; **SEM**= Standard error of mean; [a,b] Values with different superscript in a column differ significantly (P<0.05)

Volume of Semen

Mean semen volume per ejaculate for 2x and 4x bulls are shown in Table 1. Ejaculate volume varied between ejaculation frequencies and among bulls within frequency (P<0.01). Mean ejaculate volume of 4.02 mL for the 2x bulls was larger (P<0.01) than that of 3.00 for 4x bulls.

For 4x bulls, the average of 3.18 mL for first ejaculate was greater (P<0.05) than that of 2.81 mL for second ejaculate, while 2x bulls did not show any variation between 1st and 2nd ejaculate volume (Table 2). Table 3 demonstrated that the weekly semen output for 4x bulls was larger (P<0.01) than that of 2x bulls. For bulls previously ejaculated at 2x, weekly semen output at 4x averaged 14.2 mL in Phase-2. This value is higher than the average of 12.4 mL for the 4x bulls kept on 4x during the same period, but the difference was not significant.

Sperm Concentration

Sperm concentration varied (P<0.01) between ejaculation frequencies (Table 1) and among bulls within frequency (not presented). Mean concentration of 1543 x 10^6 sperm/mL for the 4x bulls was superior (P<0.01) than that of 1379 x 10^6 sperm/mL for 2x bulls. In 2x bulls, the sperm concentration of 1st ejaculate was greater (P<0.05) than that of 2nd ejaculation while the corresponding values of sperm concentration for 4x bulls did not show any variation (Table 2). Table 8 indicated that when all bulls ejaculated at 4x, the sperm concentration per ejaculate did not vary between previous 2x group and 4x group.

Table 3: Weekly sperm output from 2x and 4x bulls (n=80)

Ejaculation frequency	Volume (mL)	TS (10^6)	TMS (10^6)	TPMS (10^6)
2x	7.99[b]	11086[b]	9111[b]	7561[b]
4x	11.4[a]	17701[a]	14510[a]	12167[a]
SEM	0.38	705	601	536
P-value	<.0001	<.0001	<.0001	<.0001

TM=Total motility; **PM**=Progressive motility; **TS**=Total sperm; **TMS**=Total motile sperm; **TPMS**=Total progressive motile sperm; **SEM**= Standard error of mean; [a,b] Values with different superscript in a column differ significantly (P<0.05)

Sperm Motility

Total motility and progressive motility of sperm were neither influenced by ejaculation frequency nor by the number of ejaculation within frequency (Table 1 and 2). On the other hand, total motility and progressive motility varied among bulls (P<0.01) of both groups except for progressive motility in 2x bulls (not presented). Progressive motility of 1st ejaculation averaged 66.9 and 67.0% and 2nd ejaculation averaged 67.3 and 67.1% in 2x and 4x bulls, respectively. In Phase-2, the total motility and progressive motility of sperm did not vary significantly between two groups.

Sperm Output

The mean of 5578 x10^6 total perm (TS), 4584 x10^6 total motile sperm (TMS) and 3804 x10^6 total progressive motile sperm (TPMS) output per ejaculate for 2x bulls was greater (P<0.01) than that of 4658 x10^6 TS, 3818 x10^6 MS and 3202 x10^6 PMS output per ejaculate for 4x bulls (Table 1). Difference associated with ejaculation number within frequency (Table 2) and bulls within frequency (not presented) were significant (P<0.01). Weekly output of TS, TMS and TPMS differed significantly between ejaculation frequencies (P<0.01) and those of 4x bulls

Table 4: Morphological sperm abnormalities per ejaculate semen at 2x and 4x bulls (n=40)

Ejaculation frequency	Head abnormalities (%)	Proximal droplet (%)	Distal droplet (%)	Tail abnormalities (%)	Total abnormalities (%)
2x	1.26	0.18[b]	0.94	1.64[b]	4.01[b]
4x	1.69	0.51[a]	1.06	2.51[a]	5.78[a]
SEM	0.15	0.07	0.21	0.20	0.40
P-value	0.15	0.014	0.76	0.026	0.026

SEM= Standard error of mean; **n**= Number of observation of each treatment; [a,b] Values with different superscript in a column differ significantly (P<0.05)

Table 5: Semen parameters per ejaculate of Brahman crossbred 2x and 4x bulls ejaculated at 4x (n=80)

Ejaculation frequency	Volume (mL)	Concentration (10^6/mL)	TM (%)	PM (%)	TS (10^6)	TMS (10^6)	TPMS (10^6)
2x bulls ejaculated at 4x	3.56[a]	1420[b]	82.0	69.6	5001	4155	3540
4x bulls remained at 4x	3.10[b]	1472[a]	81.8	69.7	4531	3750	3211
SEM	0.10	32.4	0.49	0.63	179	159	140
P-value	0.025	0.42	0.85	0.95	0.19	0.20	0.24

TM=Total motility; **PM**=Progressive motility; **TS**=Total sperm; **TMS**=Total motile sperm; **TPMS**=Total progressive motile sperm; **SEM**= Standard error of mean; [a,b] Values with different superscript in a column differ significantly (P<0.05)

Table 6: Semen parameters and output of 1st and 2nd ejaculate of all bulls ejaculated at 4x (n=80)

Ejaculation number	Volume (mL)	Concentration (10^6/mL)	TM (%)	PM (%)	TS (10^6)	TMS (10^6)	TPMS (10^6)
1st ejaculate	3.44	1431	81.5	69.3	4911	4058	3475
2nd ejaculate	3.22	1461	82.2	70.0	4621	3847	3276
SEM	0.10	32.4	0.49	0.63	179	159	140
P-value	0.28	0.63	0.47	0.56	0.42	0.51	0.47

TM=Total motility; **PM**=Progressive motility; **TS**=Total sperm; **TMS**=Total motile sperm; **TPMS**=Total progressive motile sperm; **SEM**= Standard error of mean; [a,b] Values with different superscript in a column differ significantly (P<0.05)

Table 7: Comparative weekly sperm output of 2x and 4x bulls ejaculated at 4x (n=20)

Ejaculation frequency	Volume (mL)	TS (10^6)	TMS (10^6)	TPMS (10^6)
2x bull ejaculated at 4x	14.2	20005	16620	14160
4x bull remained at 4x	12.4	18125	14998	12844
SEM	0.53	908	848	801
p	0.08	0.33	0.37	0.45

TM=Total motility; **PM**=Progressive motility; **TS**=Total sperm; **TMS**=Total motile sperm; **TPMS**=Total progressive motile sperm; **SEM**= Standard error of mean; [a,b] Values with different superscript in a column differ significantly (P<0.05)

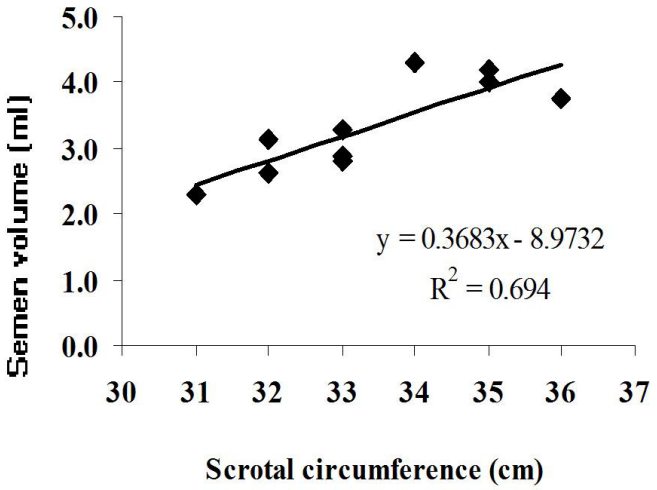

Figure 1: Relationship between scrotal circumference and volume of semen per ejaculate

increased 1.60, 1.59 and 1.61 folds, respectively compared to those of 2x bulls (Table 3). When 2x bulls were placed on 4x, weekly mean TS, TMS and TPMS were 20005 x10^6 sperm, 16620 x10^6 and 14160 x10^6, respectively (Table 7). These values compared favorably to the corresponding values of 4x bulls maintained at 4x during the same period, but the differences were not significant (Table 7).

Sperm Morphology

Occurrence of total abnormalities, proximal droplet and tail abnormalities was higher (P<0.05) in 4x bulls as compared with 2x bulls (Table 4). Difference associated with number of ejaculation was not significant (not shown). When all bulls ejaculated at 4x, only tail abnormalities showed discrepancy between two groups of bulls (Table 8). Overall sperm abnormalities in both groups were increased by double in Phase-2 than that in Phase-1, except for proximal droplet.

Relationship between Scrotal Circumference and Semen Parameters

Pair wise correlation between scrotal circumference and semen parameters of all bulls ejaculated at 4x in Phase-2 period are shown in Figure 1, 2, 3 and 4. The semen volume, TMS and TPMS per ejaculate seemed to be better in bulls with bigger scrotal circumference while sperm concentration and progressive motility of sperm did not show

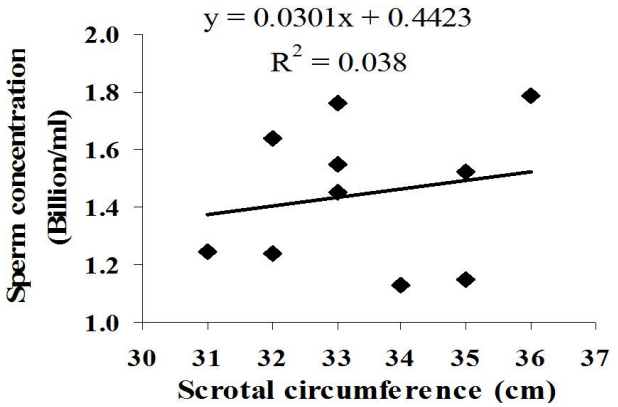

Figure 2: Relationship between scrotal circumference and sperm concentration

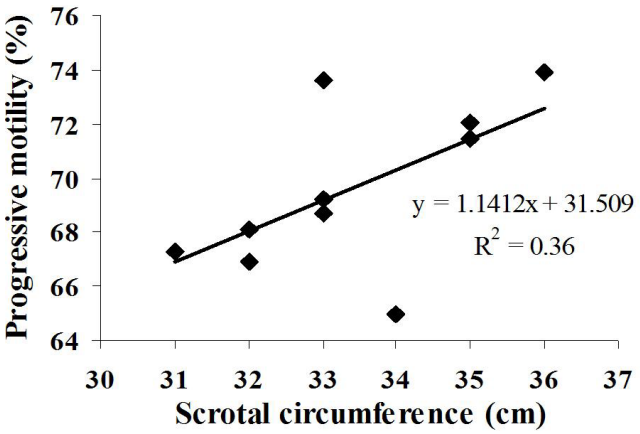

Figure 3: Relationship between scrotal circumference and progressive motility of semen

Table 8: Morphological sperm abnormalities of 2x and 4x bulls ejaculated at 4x (n=15)

Ejaculation frequency	Head abnormalities (%)	Proximal droplet (%)	Distal droplet (%)	Tail abnormalities (%)	Total abnormalities (%)
2x bull ejaculated at 4x	2.71	0.25	3.39	2.21[b]	8.57
4x bull remained at 4x	3.07	0.40	2.67	5.77[a]	11.9
SEM	0.43	0.09	0.63	0.65	0.95
P-value	0.68	0.39	0.57	0.004	0.08

SEM= Standard error of mean; **n**= Number of observation of each treatment; [a,b] Values with different superscript in a column differ significantly (P<0.05)

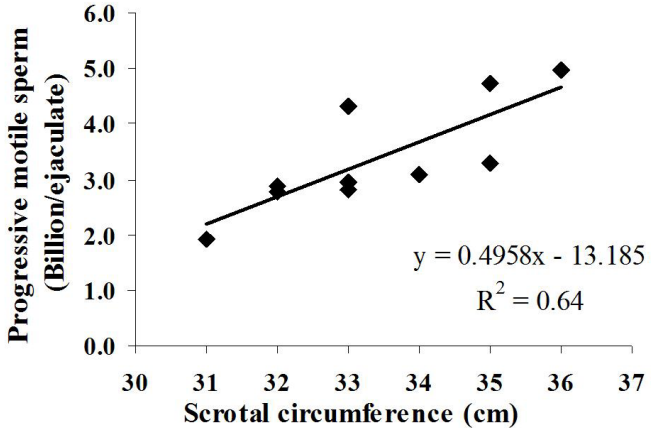

Figure 4: Relationship between scrotal circumference and progressive motile sperm per ejaculate

clear trend. The scrotal circumference had positive correlations with semen volume (P<0.01) and progressive motile sperm output and the determination coefficients were 0.69 and 0.64, respectively (Figure 1 and 4). Scrotal circumference also had positive influence to the progressive motility of sperm and sperm concentration, although correlations (P>0.05, R^2=0.36 and 0.04, respectively) were not as high as that on semen volume (Figure 3 and 2).

DISCUSSION

In this study, ejaculate volume and weekly semen output were significantly affected by bulls (although not shown) and ejaculation frequency. Volume of semen has been reported to be affected by the bulls and ejaculate number (Andrabi et al., 2002), and weekly semen output were influenced by frequency of semen collection (Sattar and Mirza, 2002). In 4x zebu and crossbred bulls, Shaha (2008) found that semen volume ranged from 4.1 to 7.6 mL. Greater semen volume in 1st ejaculate than 2nd ejaculate of this study was consistent with the results of Andrabi et al. (2002) while Sattar and Mirza (2002) found no variation between 1st and 2nd ejaculates. On the contrary, Almquist et al. (1976) reported greater ejaculate volume (3.3 mL) in second ejaculations than that (3.1 mL) for first ejaculations in 6x Charolais bulls from 1 to 2 years of age (P<0.05). The present findings showed that weekly semen output of 4x bulls increased 1.43 times compared to 2x bulls. In comparison, Sattar and Mirza (2002) demonstrated increased semen output in 2 folds at 4x bulls than 2x bulls and Almquist et al. (1976) found 3.5 times more semen production in 6x bulls compared to 1x bulls (P<0.01). Greater sperm output in 4x bulls (1.6 times) compared to 2x bulls in the present study was fairly comparable to the results of Almquist et al. (1976) who reported that Charolais bulls ejaculated at 6x yielded 3.3 times more motile sperm per week than ejaculation at 1x weekly.

In this study, sperm concentration was higher in 4x bulls than in 2x bulls, which was disagreed by Almquist et al. (1976) who found no influence of ejaculation frequency (1x vs 6x). Andrabi et al. (2002) obtained higher sperm concentration in 1st ejaculate compared to 2nd ejaculate in crossbred bulls ejaculated at 2x weekly, which supports our results. In contrast, greater (P<0.01) sperm concentration was found in 1st ejaculate (1.4 x10^9 sperm/mL) than in 2nd ejaculate (1.2 x10^9 sperm/mL) at 6x bulls (Almquist et al., 1976). The present study showed that motility of sperm was not affected by ejaculation frequency, which is in line with the results of Almquist et al. (1976) who evaluated 1x and 6x bulls. Sattar and Mirza (2002) reported significantly higher sperm motility in 4x Friesian-Sahiwal crossbred bulls than that in 2x bulls. Better sperm motility was observed by Andrabi et al. (2002) in 1st ejaculate than that in 2nd ejaculate at 2x crossbred bulls whereas Almquist et al. (1976) reported greater motility in 2nd ejaculate than that of 1st ejaculate in 6x Charolais bulls (P<0.01). The differences between those studies and present work might be due to varied age or genotype and number of animals.

Proximal droplet arises in the epididymis and high percentage indicates maturation problem which might be common findings in bulls ejaculated at high frequency. Although 4x bulls showed higher abnormalities and proximal droplet, these are within acceptable range described by McGowan et al. (1995). Comparing with the present findings, Sattar and Mirza (2002) observed greater number of abnormal spermatozoa and tail abnormalities in 2x bulls than in 4x bulls while frequency of proximal and distal droplet did not vary between two. Sperm abnormalities were observed to be low in our study at Phase-1 compared to the findings of Farooq et al. (2013) in Cholistani bulls and Ahsan et al. (2003) in pure *Bos indicus* and crossbred

bulls. Overall sperm abnormalities in both groups ejaculated at 4x increased by double at Phase-2 (August) compared to Phase-1 period, which could be attributed to mainly nutrition factor (Chacon et al., 2002) or more humidity in that month or some others factors like- scrotal length and testicular consistency (Chacon, 2001).

The present results of positive correlations between scrotal circumference and semen parameters were supported by Ha et al. (2012) who demonstrated strong correlation of scrotal circumference with semen volume, sperm motility and total number of motile sperm in Holstein Friesian and Brahman bulls. Furthermore, significant positive correlations between scrotal circumference and weekly sperm output ($P<0.01$, $r=0.78$) and between scrotal circumference and semen volume ($P<0.05$; $r=0.72$) were reported by Almquist et al. (1976) and Latif et al. (2009), respectively. However, Siddiqui et al. (2008) concluded that crossbred bulls having bigger scrotum could produce better semen.

CONCLUSION

Ejaculation of Brahman x Local F_1 crossbred breeding bulls at 4x weekly was not deleterious to semen traits and yielded 1.6 times more motile sperm output per week than ejaculation at 2x weekly. The scrotal circumference was highly correlated with semen volume and total progressive motile sperm per ejaculate. Therefore, it seems appropriate to recommend the collection of semen from Brahman x Local crossbred bulls at 4x weekly and the recording of scrotal circumference as a criterion to select young bulls as sires.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors contributed equally to this research paper.

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