Fundamental and Applied Agriculture
Vol. 8(3), pp. 555–566: 2023
doi: 10.5455/faa.160463

ANIMAL REPRODUCTION | ORIGINAL ARTICLE

Effect of genotypes on reaction time, refractory period, semen index and liquid stored semen quality of breeding bulls in Bangladesh

Sharad Chowdhury [1], Mohammad Mahbubul [2], Md Ahsan Habib [1], Md Younus Ali [1], Auvijit Saha Apu [1*]

[1]Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Haor and Char Development Institute, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ARTICLE INFORMATION

Article History
Submitted: 10 Jul 2023
Accepted: 18 Sep 2023
First online: 30 Sep 2023

Academic Editor
M A M Yahia Khandoker
yahiakhabg@bau.edu.bd

**Corresponding Author*
Auvijit Saha Apu
auvijit_abg@bau.edu.bd

ABSTRACT

The present study was conducted with the aim to reveal the effect of genotypes on reaction time, refractory period, semen index and liquid stored (at 4 °C) semen quality of breeding bulls in Bangladesh. Semen was collected twice a week from Holstein Friesian, Sahiwal and Brahman breeding bulls. Reaction time and refractory period were examined for the breeding bulls. Semen volume, sperm concentration and total spermatozoa/ejaculate were measured immediately after collection. It was revealed that genotype had a significant ($p<0.05$) effect on semen volume, sperm concentration, total sperm output and refractory period but not in reaction time. From the point of view of semen index, semen quality of Holstein Friesian breeding bulls was superior to Sahiwal and Brahman breeding bulls. Progressive motility, live and normal spermatozoa of fresh semen did not differ significantly but after dilution progressive motility differed significantly ($p<0.05$) in different genotypes of breeding bulls. During preservation time (0 to 120 hours), progressive motility, normal and live spermatozoa changed significantly ($P<0.05$) in each genotype with the progress of time. During 0 hour, 72 hours and 120 hours of preservation, progressive motility was found in Holstein Friesian (73.11 ± 1.12%, 53.11 ± 5.14%, 13.40 ± 2.53%), Sahiwal (78.21 ± 1.68%, 64.96 ± 4.60%, 13.22 ± 1.42%), and Brahman (75.21 ± 1.68%, 54.86 ± 4.40%, 16.96 ± 4.42%), respectively. On the other hand, non-return rate was found insignificant ($p>0.05$), where higher fertility was observed in Holstein Friesian bull (67.2%) followed by Sahiwal (63.7%) and Brahman bull (57.38%). In a nutshell, Holstein Friesian bull has better fresh, diluted and preserved semen quality than the other two genotypes and after the 3 days of preservation, semen quality in respect of progressive motility, normal and live spermatozoa drastically deteriorated. Therefore, it is recommended that preserved semen of different genotypes should be used for AI within 3 days of liquid semen preservation at 4 °C.

Keywords: Breeding bulls, genotype, semen quality, storage period

Cite this article: Chowdhury S, Mahbubul M, Habib MA, Ali MY, Apu AS. 2023. Effect of genotypes on reaction time, refractory period, semen index and liquid stored semen quality of breeding bulls in Bangladesh. Fundamental and Applied Agriculture 8(3): 555–566. doi: 10.5455/faa.160463

1 Introduction

Selection of superior breeding bull judged by their breeding soundness is one of the most important decisions in a sound breeding program (Parkinson, 2004). It is noteworthy that breeding soundness is liable, quick and cost-effective method for screening and classifying bulls in terms of fertility as well as minimizes the use of sub-fertile bulls and bulls of questionable fertility (Chenoweth et al., 1994). A breeding bull is considered the half of the herd.

Evaluation of semen is of prime importance to select breeding bulls and one of the most important steps to detect the breeding soundness of bulls. The term quality of semen encompasses motility (%) of spermatozoa, concentration of spermatozoa, and proportion of live and morphologically normal spermatozoa, seminal pH and optimum metabolic feature of individual sperm (Hoque, 1998). On the other hand, the fertilizing capacity of semen can be evaluated by inseminating a reasonable number of cows and calculation the non-return rate after 60 days. The non-return rate of bulls depends on holistic semen characteristics of bull, breeding soundness of cows and appropriateness of time and site for semen deposition (Nasrin et al., 2008). Semen quality can also be affected by collection and subsequent manipulation such as semen dilution, chilling, freezing, storage, transportation and thawing for insemination.

The diluted semen can be preserved either by short term storage (chilling) or by frozen method. The short term chilled preserved semen is usually preserved at 4 °C to 5 °C for a short period of time (2-3 days). Liquid semen is principally used for only 2.5 to 3 days after collection due to reduction in fertility (Vishwanath and Shannon, 2000). Liquid semen has a distinct advantage over frozen-thawed semen as the reduced sperm concentration per straw (approximately 3–5 million vs 15–20 million sperm, respectively (Murphy et al., 2013) allows for approximately 3 times more semen straws to be produced. Hence, compared to frozen-thawed semen the use of liquid semen maximizes the number of insemination straws produced per ejaculate (Murphy et al., 2013).

Several attempts have been made to evaluate the semen but limited information is available on the evaluation of fresh, diluted and short term preservation capability egg yolk-citrate based diluted semen and their fertility of different genotypes of breeding bulls. Moreover, very limited work has yet been done in Bangladesh on libido, reaction time and refractory period of breeding bulls. This study was attempted to reveal a comparative study of above mention attributes of available genotypes of bovine semen in Bangladesh.

2 Materials and Methods

2.1 Location of experiment

The latitude and longitude of Bangladesh Agricultural University Artificial Centre (BAU AI center) is 24.73 North 90.42 East, respectively with average annual high temperature of 32.49 °C and annual low temperature of 23.47 °C. Three genotypes namely Holstein Friesian, Sahiwal and Brahman (aged between 4-5 years) were used in the experiment.

2.2 Measurement of reaction time and refractory period

According to the Hoflack et al. (2006) reaction time was measured as the amount of time between the first contact with the teaser animal and the first false mount with the penis erected. On the other hand, refractory period was measured according to the Prado et al. (2002) as the time taken between first ejaculate till the second false mount for second serving. Reaction time and refractory period of each breeding bull was carefully counted using stop watch by close observation of the two individual operators.

2.3 Semen collection

Semen was collected twice a week by means of artificial vagina (AV) method early in the morning (from 7.00 to 8.00 AM). Before collection, all the parts of Artificial Vagina were sterilized and assembled properly. The AV set used for semen collection was prepared properly with optimum temperature (100-115 °F), pressure and lubricant. The bull was allowed to jump on dummy for two times before collection of semen. The semen was collected during the third jump. After collection, semen was put into water bath at 115 °F until further analysis.

2.4 Ejaculate volume

The volume of ejaculate was measured directly with the help of graduated collection vial (Mortimer, 2000) and expressed in mL.

2.5 Sperm concentration

Sperm concentration was determined by using haemocytometer method according to Herman and Madden (1963). Semen sample was drawn into a standard red cell dilution pipette up to 0.5 marks. Dilution fluid was drawn into the pipette up to 101 marks. Pipette was agitated for 3-5 minutes for ensuring proper mixing by eight-knot motion. First 4 to 5 drops were discarded to get well mixed diluted semen. A cover slip was placed over the ruled field of the counting chamber of the haemocytometer and

a drop was allowed to run the cover slip. Counting was done under low magnification (25×) five large double ruled squares were counted over the field. The concentration of spermatozoa was expressed as million mL^{-1}.

2.6 Morphology and live spermatozoa

Two drops of buffer solution was placed on a clean, dry glass slide. One small drop of thoroughly mixed semen was added in the buffer. It was spread by covering with another slide and it was dried in the air. The smear was stained with rose-bengal stain for 3-5 minutes, then it was rinsed with distilled water for removing additional stain and the smear was dried in the air. The slide was set on the stage of microscope and counted under 40× objectives. Spermatozoa with any of the deformities were considered to be abnormal (Islam et al., 2018). A total of 333 sperms were counted randomly from different parts of the slide.

To measure live sperm count, one drop of Eosin-Nigrosin stain was mixed with a small drop of semen on a pre-warmed slide. After smearing it was placed on microscope and counted under 40× (Islam et al., 2018).

2.7 Semen Index

Semen index is a good indicator for estimating semen quality. Semen index was calculated by using the following formula according to Moghaddam et al. (2012).

$$SI = V \times C \times S_L \times P_M \quad (1)$$

where, SI = Semen index, V = Semen volume, C = Sperm concentration, S_L = Live sperm (%), and P_M = Progressive motility (%).

2.8 Evaluation of semen after diluted with egg yolk–citrate diluter

Egg yolk-citrate diluter was used for the extension of the semen of the breeding bulls for the maximum utilization of the male genetic potentiality. Before use, the Egg yolk-citrate diluter was prepared according to Herman and Madden (1963). A solution of 2.94% sodium citrate in 100 mL distilled water was made in which 100000 IU of diluted pronapen solution was added. After that, one part of egg yolk by volume was mixed with four parts of the citrate solution and mixed thoroughly. The semen and diluter were mixed at room temperature at the proportion of 1:20. After dilution of the semen, it was preserved in refrigerator at 4-5 °C temperature up to 5 days. The preserved diluted semen was tested during the period of 0, 24, 48, 72, 96 and 120 hours respectively for evaluation of progressive motility (%), live spermatozoa and normal spermatozoa by using same method as used for fresh semen, expressed earlier. Thereafter, preserved semen up to three days was used for inseminating the cows in natural estrus arrived at AI center for taking the service.

2.9 Fertility measurement

Fertility of breeding bulls was calculated based on the non-return rate of breeding bulls. Fertility was calculated by the number of cows conceived out of the total number of cows inseminated by the semen of respective breeding bulls and inseminated cows not return to estrous within a period of 60 days.

Non-return rate = (Cows not return to estrous after first service)/(Total number of cows served) × 100

2.10 Statistical analysis

The data generated from this experiment were entered in Microsoft Excel worksheet, organized and processed for further analysis. The data were analyzed to obtain ANOVA by Generalized Linear Model using Statistical Analysis System (SAS 9.0) computer package. DUNCAN was performed to separate mean values for significant independent variables and results were expressed as Mean ± SE.

3 Results

Reaction and refractory period of three genotypes of breeding bulls were evaluated at BAU AI center. Refractory period significantly ($p<0.05$) differed among genotypes while reaction time did not varied (Table 1). Reaction time was shorter in Holstein Friesian bull (28.57 ± 6.66 sec) and refractory period was highest in Sahiwal bull (491.33 ± 63.28 sec).

Evaluation of fresh semen indicated that significant ($p<0.05$) variation in mean values was observed for volume, sperm concentration and total sperm output while interestingly progressive motility percentage did not differed among three genotypes of breeding bulls (Table 2).

There was no significant difference among the three genotypes in terms of normal and live spermatozoa percentage (Table 3). Normal spermatozoa were insignificantly higher in Sahiwal while Holstein Friesian showed better performance in case of live spermatozoa. Calculation of semen index provides an easy comparison of overall semen quality. Table 4 represented the semen index of Holstein Friesian, Sahiwal and Brahman genotype and Holstein Friesian showed best performance and lowest was found for Brahman cattle.

Progressive motility is an important indicator of fresh and preserved semen quality. Preserved semen

quality deteriorate with the passage of time and statistical difference ($p<0.05$) was observed in different preservation period for each genotype. However, progressive motility did not differed within the same preservation hour in different genotypes (Table 5).

With the passage of time, significant ($p<0.01$) decreases in normal spermatozoa percentage in all the three genotype was found (Table 6). However, normal spermatozoa percentage did not vary significantly within the same preservation period in different genotypes of breeding bulls. With the passages of preservation period (0 to 120 hrs) percentage of dead spermatozoa increased i.e. spermatozoa quality decreases (Fig. 1).

Correlation of reaction time with various seminal parameters is presented in Table 7. The correlation was non-significant ($p>0.05$) and varied from moderate negative (-0.43) to strong positive (0.81) correlation. Reaction time was positively correlated with sperm concentration (0.81), abnormal spermatozoa (0.30), oscillatory motility (0.36) and dead spermatozoa (0.35). On the contrary, volume, progressive motility, rotatory motility, and live spermatozoa were negatively correlated with reaction time.

The average 60 days non-return rate of various bulls is presented in Fig. 2. The non-return rate over the breed was affected by some semen evaluation parameters. Non return rate was found non-significant, where higher fertility was observed in Holstein bull (67.2%) followed by Sahiwal (63.7%) and Brahman bull (57.38%).

4 Discussion

The term libido is commonly used to describe sex drive in male animals. It can also be defined as the willingness and eagerness of a male animal to mount and attempt service of a female (Chenoweth, 1981). Reaction time was used as a proxy measure for libido. In breeding bull, good libido is desirable trait for a successful ejaculation and good quality semen collection for an artificial insemination (AI) program. Libido is largely depended on bull genotype (Anzar et al., 1993). Early initiation of sexual desire is important for bulls that is going be used for future successful AI. Genetic, breed differences, hormonal influence, post-weaning management, nutrition, health status and season affects reaction time and refractory period of bull (Chenoweth, 1981). Present study revealed that higher duration of reaction time was observed in Brahman bull (41.00 ± 8.82 sec) followed by Sahiwal (36.00 ± 11.01 sec) and lower in Friesian (28.57 ± 6.66 sec) and breed effect was non-significant. Rehman et al. (2016) stated that Friesian bull reaction time was 17.44 ± 0.95 sec, Sahiwal bull reaction time was 14.78 $\pm$ 4.72 sec where reaction time varied with breed significantly. On the other hand, Abdelrasoul (2017) reported 20.18 ± 2.30 sec and Kumar (1995) reported 24.61 sec reaction time for Sahiwal bull. Nath et al. (1980) studied the reaction time of Holsteins in India and observed an average of 125.1 seconds with the longest (166.5 seconds) reaction time occurring in summer and the shortest (89.2 seconds) in winter. Moreover, hormone like testosterone plays an important role in the initiation of sex drive.

Testosterone is the primary androgen required for spermatogenesis in the testes and is responsible for maintaining secondary sexual characteristics and libido (Seneger, 2012). High levels of testosterone are necessary for normal testicular and epididymal functions. Impairment of the leydig cells may be responsible for alteration in testosterone level as a result decline in libido, increase reaction time, and decrease in sperm outputs accompanied with increased semen abnormalities (Al-Qarawi, 2005). However, Oxytocin also reduced the reaction time in the rabbit (Melin and Erik Kihlstrom, 1963).

Refractory period (sec) for Sahiwal was significantly ($p<0.01$) higher than that of Brahman and Holstein Friesian. Higher duration of refractory period was observed in Sahiwal bull (491.33 ± 63.28 sec) and Friesian bull (318.00 ± 56.54 sec) and lower in Brahman bull (263.00 ± 15.82 sec). Moghaddam et al. (2012) found that mean value of refractory period for ArkharMerino $\times$ Moghani crossbred Sheep is 231.34 sec which is much lower than in case of bull semen. Singh et al. (2015) reported that the Sahiwal bulls are sexually sluggish which can be confirmed by the value of reaction time and refractory period of this study. The temperature of artificial vagina to the penis is very crucial for refractory period. If the temperature is a little bit high then the bull will dismount early and become reluctant to mount again. The refractory period also may prolong due to hyperprolactinemia (Exton et al., 2001). In Gir bull, reaction time was 153.54 ± 4.82 sec in summer and 128.09 ± 4.24 sec in winter (Solanki et al., 2019). Bull's exercise keeps them trimmed and in good physical health and reduces reaction time if given just before collection (Bhosrekar and Nagpual, 1972). These traits are greatly influenced by genetic factors, widely varied among individuals in their expression, season and environmental temperature.

Semen quality is a determinant factor for bull fertility. Good quality ejaculate is made by superior bulls with good generative performance. Breed has highly significant effect on semen volume, sperm concentration and motility, especially in older bulls (Novianti et al., 2020). Low semen quantity and poor semen quality causes subfertility of bull that's results in significant percentage of reproductive failure (DeJarnette et al., 2004). In the present study, volume of ejaculate differed significantly ($p<0.01$) among the bulls. The ejaculate volume of semen is regarded as one of the most important indicators of the genetic po-

Table 1. Reaction time and refractory period of different genotypes of breeding bulls

Genotype	Reaction time (sec)	Refractory period (sec)
Holstein Friesian	28.57 ± 6.66 (n = 18)	318.00^{ab} ± 56.54 (n = 18)
Sahiwal	36.00 ± 11.01 (n = 14)	491.33^{a} ± 63.28 (n = 14)
Brahman	41.00 ± 8.82 (n = 14)	263.00^{b} ± 15.82 (n = 14)
Sig. level	NS	*

Values are mean±SE; a, b values in column with different letters differed significantly; * = Significant at 5% (p<0.05) level of probability; NS = Non significant; Figures in the parentheses indicate the number of observation

Table 2. Quality of fresh semen of different breeding bulls

Genotype	Volume (mL)	Conc. ($\times 10^9$ mL^{-1})	Total sperm output ($\times 10^9$)	Progressive motility
Holstein Friesian	6.70^{a} ± 0.31 (n = 18)	1622.13^{b} ± 62.93 (n = 18)	10.73^{a} ± 2.67 (n = 18)	73.19 ± 1.04 (n = 18)
Sahiwal	4.92^{b} ± 0.76 (n = 14)	1303.75^{c} ± 23.03 (n = 14)	6.37^{b} ± 1.01 (n = 14)	75.76 ± 1.75 (n = 14)
Brahman	2.80^{c} ± 0.29 (n = 14)	1859.25^{a} ± 38.88 (n = 14)	5.04^{c} ± 0.91 (n = 14)	73.46 ± 1.79 (n = 14)
Sig. level	**	**	**	NS

Values are mean±SE; a, b, c values in column with different letters differed significantly; ** = Significant at 1% (p<0.01) level of probability; NS = Non significant; Figures in the parentheses indicate the number of observation

Table 3. Normal and live spermatozoa of fresh semen of different genotypes of breeding bulls

Genotype	Normal spermatozoa (%)	Live spermatozoa (%)
Holstein Friesian	74.08 ± 0.55 (n = 18)	77.03 ± 0.66 (n = 18)
Sahiwal	74.78 ± 2.27 (n = 14)	76.59 ± 2.19 (n = 14)
Brahman	73.43 ± 1.51 (n = 14)	75.48 ± 2.63 (n = 14)
Sig. level	NS	NS

Values are mean±SE; NS = Non-significant; Figures in the parentheses indicate the number of observation

Table 4. Semen index of three genotypes of breeding bulls

Breed	Semen index (Mean ± SE)
Holstein Friesian	60750353.85^{a} ± 2049427.35 (n = 18)
Sahiwal	36533815.27^{b} ± 3981716.93 (n = 14)
Brahman	29269772.83^{b} ± 4274334.10 (n = 14)
Sig. level	**

a, b values in column with different letters differed significantly; ** = Significant at 1% (p<0.01) level of probability; Figures in the parentheses indicate the number of observation

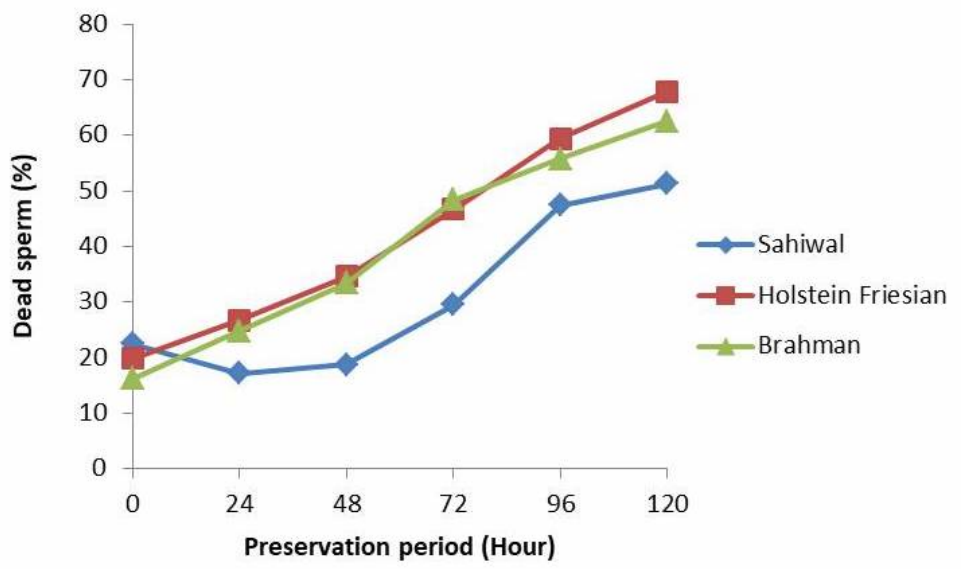

Figure 1. Changes of dead sperm percentage over the preservation time in different genotypes of breeding bulls

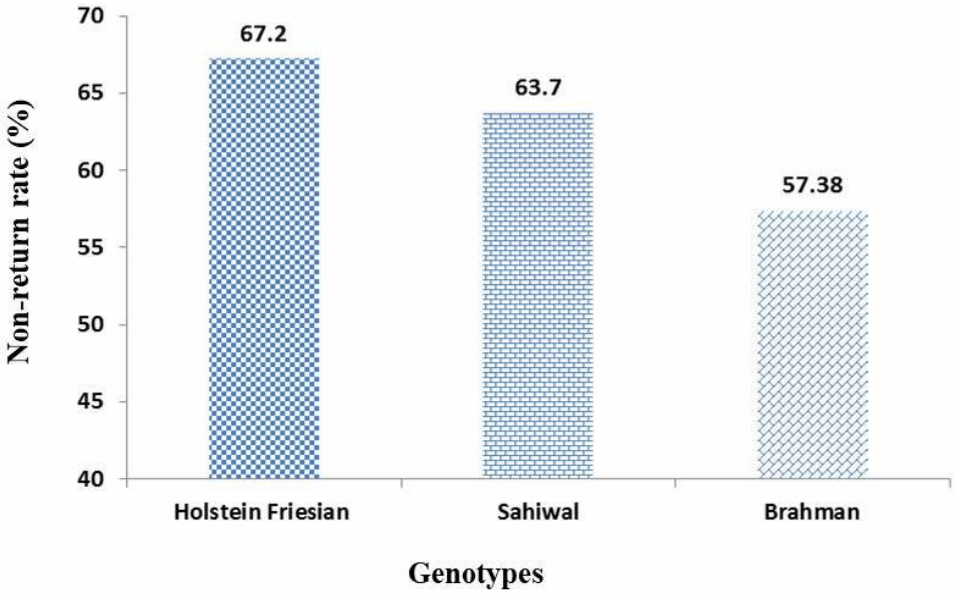

Figure 2. Fertility of three genotypes of breeding bulls

tentiality of bulls. Highest volume per ejaculate (6.71 ± 0.31 mL) was found in Holstein bull followed by Sahiwal bull (4.92 ± 0.76 mL) and the lowest volume (2.80 ± 0.29 mL) was found in Brahman bull. This observation strongly supports with the findings of other published works (Rahman et al., 2014; Akhter et al., 2013; Hossain et al., 2012; Latif et al., 2009; Shaha et al., 2008). Nasrin et al. (2008) found the average semen volume was ranged between 2.58 to 4.01 mL in different breeding bull which coincides with the present study. Islam (2015) reported that the highest (7.86 ± 0.19 mL) volume of semen was obtained in Holstein Friesian bulls which is higher than the value of present study. Semen volume has been reported to increase with age and may decrease at old age due to atrophy or fibrosis of the testicles (Amann et al., 1974; Everett et al., 1978). The size of the seminal vesicles plays a big role in determining the ejaculate volume (Roberts, 1986). The Ejaculate volume also may vary due to scrotal circumference, testicular length, and epididymal length.

Table 5. Effect of preservation time on progressive motility of semen of different breeding bulls

Time (hr)	Holstein Friesian (n = 18)	Sahiwal (n = 14)	Brahman (n = 14)	Sig. level
0	$68.71^{a} \pm 2.00$	$75.21^{a} \pm 1.68$	70.99a ± 2.13	NS
24	$61.95^{ab} \pm 3.51$	$69.27^{ab} \pm 3.24$	$60.94^{ab} \pm 3.34$	NS
48	$53.11^{b} \pm 5.14$	$64.96^{b} \pm 4.60$	$54.86^{b} \pm 4.40$	NS
72	$32.62^{c} \pm 3.38$	$40.52^{c} \pm 3.09$	$39.12^{c} \pm 4.73$	NS
96	$13.40^{d} \pm 2.53$	$13.22^{d} \pm 1.42$	$16.96^{d} \pm 4.42$	NS
120	$6.54^{d} \pm 3.05$	$3.98^{e} \pm 1.17$	$8.09^{d} \pm 4.47$	NS
Sig. level	**	**	**	

a, b, c values in column with different letters differed significantly ; ** = Significant at 1% ($p<0.01$) level of probability, means among genotype differed significantly ($p<0.05$); NS = Non significant; Figures in the parentheses indicate the number of observation.

Table 6. Changes of normal semen percentage over the preservation period in diluted preserved semen sample

Time (hr)	Holstein Friesian (n = 18)	Sahiwal (n = 14)	Brahman (n = 14)	Sig. level
0	$71.49^{a} \pm 0.78$	$73.09^{a} \pm 1.08$	$72.42^{a} \pm 1.05$	NS
24	$64.79^{ab} \pm 2.26$	$70.71^{a} \pm 1.52$	$67.06^{ab} \pm 2.90$	NS
48	$57.53^{b} \pm 3.74$	$67.48^{a} \pm 2.14$	$58.99^{b} \pm 4.83$	NS
72	$45.14^{c} \pm 3.14$	$52.83^{b} \pm 2.05$	$45.48^{c} \pm 3.85$	NS
96	$37.47^{c} \pm 2.55$	$35.01^{c} \pm 2.15$	$37.64d^{c} \pm 2.89$	NS
120	$29.45^{d} \pm 3.14$	$28.25^{d} \pm 2.50$	$29.15^{d} \pm 3.54$	NS
Sig. level	**	**	**	

Means with different superscripts within the same column differed significantly ($p<0.05$), NS = Non-significant; * = Significant at 5% ($p<0.05$) level of probability, ** = Significant at 1% ($p<0.01$) level of probability; Figures in the parentheses indicate the number of observation

Table 7. Correlation between reaction time with different seminal parameters

Parameter	Reaction time	p-value
Volume	−0.43	0.12
Concentration	0.81	0.78
Progressive motility	−0.44	0.11
Oscillatory motility	0.36	0.21
Rotatory motility	−0.40	0.89
Normal sperm	−0.28	0.34
Abnormal sperm	0.30	0.29
Live sperm	−0.35	0.23
Dead sperm	0.35	0.23

From the statistical analysis, it was found that highest value of sperm concentration of semen was found in Brahman bull (1859.25 ± 38.88 million mL^{-1}) followed by Holstein Friesian (1622.13 ± 62.93 million mL^{-1}) and the lowest in Sahiwal bull (1303.75 ± 23.03 million mL^{-1}). Islam et al. (2018) showed that sperm concentration varied significantly among the genetic group of breeding bulls where the highest sperm concentration was found in Brahman crossbred breeding bull (1147.00 ± 28.75 million mL^{-1}) which is nearly similar to the present study. Fatematuzzohora et al. (2016) reported (1144.59 ± 5.73 million mL^{-1}) in Brahman crossbred bull which is lower than the present observation. This variation might be due to body condition, nutritional status, environment and management of individual bull.

The Total sperm output of Holstein Friesian bull was significantly higher ($p<0.01$) than two other breeds. In the present study, the highest value of total sperm output of semen was found in Holstein Friesian bull (10.73 ± 2.67 billion mL^{-1}) followed by Sahiwal bull (6.37 ± 1.01 billion mL^{-1}) and lowest was found in Brahman bull (5.04 ± 0.91 billion mL^{-1}) which is not similar to the observation of Latif et al.

(2009) who found that the mean value of total number of spermatozoa per ejaculate of Holstein Friesian bull is (4.4 billion mL^{-1}) and (3.5 billion mL^{-1}) for Sahiwal bull. Sperm concentration is not related to the size of the testicles, and seminal vesicles but vary according to age, breed, season, volume of semen, and nutrition (Hafez, 1993).

The sperm progressive motility is the ability of sperm to move forward towards an egg for successful pregnancies. It is one of the most important characters related to semen fertilization capacity and recognized as essential for sperm transport and fertilization in female reproductive tract (Januskauskas et al., 1999; Verstegen et al., 2002). Progressive motility (%) was found 73.19 ± 1.04, 75.76 ± 1.75 and 73.46 ± 1.79 for Holstein Friesian, Sahiwal and Brahman bull, respectively and breed effect was non-significant. Almost similar progressive motility (74.73 ± 0.76%) was observed by Islam et al. (2018) for Holstein Friesian × Local crossbred bull. In agreement with our study, Ray and Gosh (2013) also found 76.73% progressive motility for Sahiwal bull at West Bengal, India. Genetic factors influence bull fertility which is well documented by previous researchers (Huang et al., 2011; Corbet et al., 2013). Improved semen quality may results in increased conception rate, which would result in a lower cost per pregnancy for the producer.

Normal spermatozoa percent are a measure of the spermatozoa in an ejaculate that have desirable characteristics. Normal sperm morphology is one of the most important considerations associated with the quantity and quality of semen output that ultimately affect the fertilizing capacity of semen (Akhter et al., 2013). In the hostile environment of female reproductive tract, morphologically abnormal sperm cannot pass and finally may fail to reach the site of fertilization (Hossain et al., 2012). In the present study, morphologically highest normal sperm percentage was observed in Sahiwal cross breeding bull (74.78 ± 2.27%) followed by Brahman cross breeding bull (73.43 ± 1.51%) and for Holstein Friesian (74.08 ± 0.55%). Islam et al. (2018) found that normal sperm percentage was observed in Holstein Friesian cross breeding bull (83.18 ± 1.47%) which is higher than the present study. Structural abnormalities sometimes may occur in the spermatozoa due to faulty spermatogenesis caused by heredity, disease, adverse environmental effects and improper semen handling procedures (Ray and Gosh, 2013).

The quality of semen in relation to fertility is determined by various factors among them live spermatozoa concentration is important one (Akhter et al., 2013). Analysis of variance shows that genetic group of breeding bull had no significant effect on live spermatozoa percentage. The highest live sperm percentage was found in Holstein Friesian cross (77.03 ± 0.66%) and lowest in Brahman cross breeding bull (75.48 ± 2.63%). This finding nearly agrees with the study of Islam et al. (2018) who found that the highest live sperm percentage was found in Holstein Friesian cross (84.18 ± 0.62). Rahman et al. (2014) also reported the live spermatozoa percentage 81.25 ± 0.64 and 79.80 ± 0.89 in Holstein crossbred and Sahiwal crossbred, respectively.

For optimize efficient cattle production, improvement of bull reproductive performance is very important. Semen index is a way to measure semen quality of breeding bull. From the semen index value, it was observed that overall semen quality of Holstein Friesian breeding bulls (60750353.85 ± 2049427.35) were superior than other breeding bulls such as Sahiwal (36533815.27 ± 3981716.93) and Brahman (29269772.83 ± 4274334.10).

Semen preservation is critical for livestock production as it enables and accelerates spread of genetic diversity and facilitates genetically superior animals. In preserved semen, cold shock and presence of free radical can decrease spermatozoa quality. High amount of polyunsaturated fatty acids in plasma membrane makes spermatozoa sensitive to damage caused by cold shock and peroxidation caused by free radicals affects motility (Brouwers et al., 2005; Bansal and Bilaspuri, 2010). Immediately after dilution and during each day of preservation, progressive motility did not differ significantly ($p<0.05$) among the genotypes. Progressive motility was observed in Holstein Friesian (68.71 ± 2.00%, 61.95 ± 3.51%, 53.11 ± 5.14%, 32.62 ± 3.38% and 13.40 ± 2.53%), Sahiwal (75.21 ± 1.68%, 69.27 ± 3.24%, 64.96 ± 4.60%, 40.52 ± 3.09%, and 3.98 ± 1.17%), and Brahman (70.99 ± 2.13%, 60.94 ± 3.34%, 54.86 ± 4.40%, 39.12 ± 4.73%, 8.09 ± 4.47 %) at 0, 24, 48, 72 and 120 hours, respectively. With the passage of preservation time (0 to 120 hours), it was found that progressive motility changed significantly ($p<0.01$) in each genotype. Alam et al. (2005) who worked with the effect of duration of preservation on the quality of chilled bull semen and their observation is almost similar to the above statement of this study. They also added that there was significant decrease ($p<0.01$) in sperm motility found at the day of 3 and 4, compared to day 0, 1 and 2 and it was found 50% in all cases. This finding is nearly similar to the present study. The finding of the present study also coincides with many other research works (Foote et al., 1960; Salisbury et al., 1978; Shamsuddin et al., 1993; Bhuiyan, 1998). Haque et al. (2018) worked with the assessment of sperm viability in extended boar semen during long term storage at 15 °C and concluded that the percentage of sperm motility decreased significantly ($p<0.01$) with increase in hour of preservation. Significant decrease in the sperm motility with increase in hour of preservation could be due to progressive decline in nutrient content in extender with increased periods of preservation and the loss of adenosine triphosphate (ATP) and cyclic AMP, as well as calcium uptake are charac-

teristics of decreased motility (Kadirvel et al., 2016). Progressive motility is affected by some factors like sperm morphology and live dead ratio. The lowest progressive motility means the highest coil tail abnormalities because sperm get energy for movement through glycolysis from the tail. Only sperm with normal chromatin structure is able to fertilize the oocyte (Tsakmakidis et al., 2011). Abnormal chromatin structure may lead to problems in packaging of sperm nuclear material possibly related to morphologically abnormal spermatozoa (Sailer et al., 1995). Normal spermatozoa percentage decreased as the preservation time extended irrespective of genotype. At 0, 24, 48, 72, 96 and 120 hours of storage time normal spermatozoa percentage were 71.49 ± 0.78, 64.79 ± 2.26, 57.53 ± 3.74, 45.14 ± 3.14, 37.47 ± 2.55 and 29.45 ± 3.14% in Holstein Friesian; 73.09 ± 1.08, 70.71 ± 1.52, 67.48 ± 2.14, 52.83 ± 2.05, 35.01 ± 2.15, 28.25 ± 2.50% in Sahiwal; 72.42 ± 1.05, 67.06 ± 2.90, 58.99 ± 4.83, 45.48 ± 3.85, 37.64 ± 2.89 and 29.15 ± 3.54% in Brahman bull. At particular time period genotype effect was non-significant. Alam et al. (2005) worked on preservation quality of bull semen and stated that the proportion of abnormal spermatozoa increased with the advancement of preservation period which is in agreement with present study.

With the advancement of preservation period dead spermatozoa percentage increased i.e. quality of spermatozoa deteriorate. Live spermatozoa percentage at 0, 24, 48, 72, 96 and 120 hours were 73.29 ± 1.29, 65.37 ± 3.21, 63.57 ± 2.71, 53.42 ± 3.01, 40.57 ± 5.38 and 28.89 ± 6.45 for Holstein Friesian; 77.81 ± 1.42, 74.60 ± 2.46, 69.63 ± 5.64, 54.76 ± 5.85, 32.91 ± 3.90 and 27.97 ± 3.58 for Sahiwal and 75.21 ± 2.61, 66.66 ± 4.66, 58.81 ± 2.45, 51.49 ± 3.13, 44.22 ± 1.99 and 35.97 ± 2.24 for Brahamn bull. Strong variation ($p<0.01$) in live spermatozoa percentage in case of Holstein Friesian and Sahiwal bull while in Brahman bull it also differed significantly ($p<0.05$) with different preservation period. At same preservation hour, breed difference was non-significant.

Our study showed that sperm concentration, oscillatory motility, abnormal spermatozoa and dead spermatozoa percentage were positively correlated with reaction time whereas other seminal parameters showed negative correlation with reaction time. Recently, in a study with bull semen, Korkmaz et al. (2023) found very weak (0.014) correlation of reaction time with concentration, weak correlation (0.15) with volume, negative (−1.08) correlation with progressive motility. On the other hand, Moghaddam et al. (2012) reported that reaction time had negative and significant correlation (−0.15; 0.04) with semen volume, negative but non-significant effect with sperm concentration (−0.07; 0.30), progressive motility (−0.01; 0.06), and live spermatozoa percentage (−0.02; 0.05) in ram semen.

Non-return rate is an indirect indicator of fertility and is most frequently used for male fertility measurement under practical farm condition. Better sire fertility estimates would results in more accurate selection. Non-return rate are influenced by various factors like individual bulls, age and parity of the inseminated cows, inaccurate heat detection and recording, season and environmental temperature of AI performed, skill of inseminator and month of insemination, (Rabidas et al., 2012; Khun and Hutchison, 2008; Rycroft and Bean, 1992). The result of the present study was found nearly similar with the observation of Nasrin et al. (2008) who found highest value in Holstein Friesian bull (62.75%) followed by Sahiwal cross (63.7%). Present study findings were comparatively lower than the result of Rabidas et al. (2012) who stated that 60 days non-return rate of 86.0% for Sahiwal × Local and 72.9% for Holstein Friesian × Local crossbred bull. Islam (2015) worked with the semen parameters of Brahman bull and observed that the highest (51.51%) and lowest (48.88%) value of non-return rates were found at two regions which is lower than the result of present study. This variation might be due to semen index, quality, time of insemination and accurate heat detection.

5 Conclusion

The result of the present study revealed that genotype had a significant effect on refractory period, semen volume, sperm concentration, total sperm output of breeding bulls. Semen of Holstein Friesian Bull was superior among the three genotypes used in the present study. The quality of diluted semen remained almost similar in first two days of preservation period but after the 3 days of preservation, semen quality in terms of progressive motility drastically decreased. So, it is recommended to use liquid preserved semen (at 4 °C) of these three genotypes up to 3 days for obtaining satisfactory fertility result.

Acknowledgments

The authors gratefully acknowledge the eminent Professor Dr. Syed Sakhawat Husain to give scholastic suggestions to improve the manuscript. The authors also owe to the contribution of University Grants Commission (UGC) [Project Number 91 (38)] to fund this research and also BAURES for their logistic support to materialize the research project.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Abdelrasoul EKA. 2017. Effect of exercise on sexual behavior and semen quality of Sahiwal bull. International Journal of Biological and Ecological Engineering 11:618–621.

Akhter MS, Azad MAK, Rahman MZ, Ashraf A. 2013. Study on semen of different genetic groups of bull from Khulna region of Bangladesh. International Journal of Pharmaceutical and Medical Research 1:19–23.

Al-Qarawi AA. 2005. Infertility in the dromedary bull: a review of causes, relations and implications. Animal Reproduction Science 87:73–92. doi: 10.1016/j.anireprosci.2004.11.003.

Alam MG, Yeasmin S, Bari FY, Mishra B. 2005. Effect of duration of preservation on the quality of chilled bull semen. The Bangladesh Veterinarian 22:16–22.

Amann RP, Kavanaugh JF, Griel Jr LC, Voglmayr JK. 1974. Sperm production of Holstein bulls determined from testicular spermatid reserves, after cannulation of rete testis or vas deferens, and by daily ejaculation. Journal of Dairy Science 57:93–99. doi: 10.3168/jds.S0022-0302(74)84836-8.

Anzar M, Ahmad M, Nazir M, Ahmad N, Shah IH. 1993. Selection of buffalo bulls: Sexual behavior and its relationship to semen production and fertility. Theriogenology 40:1187–1198. doi: 10.1016/0093-691X(93)90289-H.

Bansal AK, Bilaspuri GS. 2010. Impacts of oxidative stress and antioxidants on semen functions. Veterinary Medicine International :1–7doi: 10.4061/2011/686137.

Bhosrekar M, Nagpual PK. 1972. Studies on effect of exercise on the reaction time and semen production. Indian Journal of Dairy Science 25:194–195.

Bhuiyan MMU. 1998. The quality of bull semen used in artificial insemination programme in Bangladesh. MS Thesis, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University.

Brouwers JF, Silva PFN, Gadella BM. 2005. Assays for detection and localization of endogenous lipid peroxidation products in living boar sperm after BTS dilution or after freeze thawing. Theriogenology 63:458–469. doi: 10.1016/j.theriogenology.2004.09.046.

Chenoweth PJ. 1981. Libido and mating behavior in bulls, boars and rams. a review. Theriogenology 16:155–177. doi: 10.1016/0093-691X(81)90098-4.

Chenoweth PJ, Hopkins FM, Spitzer JC, Larsen RE. 1994. New guidelines for the evaluation of bulls for breeding soundness. The Bovine Proceedings.

Corbet NJ, Burns BM, Johnston DJ, Wolcott ML, Corbet DH, Venus BK, Li Y, McGowan MR, Holroyd RG. 2013. Male traits and herd reproductive capability in tropical beef cattle. 2. genetic parameters of bull traits. Animal Production Science 53:101–113. doi: 10.1071/AN12163.

DeJarnette JM, Marshal CD, Lenz RW, Monke DR, Ayars WH, Sattler CG. 2004. Sustaining the fertility of artificially inseminated dairy cattle: the role of the artificial insemination industry. Journal of Dairy Science 87:93–104. doi: 10.3168/jds.S0022-0302(04)70065-X.

Everett RW, Bean B, Foote RH. 1978. Sources of variation of semen output. Journal of Dairy Science 61:90–95. doi: 10.3168/jds.S0022-0302(78)83555-3.

Exton MS, Krüger TH, Koch M, Paulson E, Knapp W, Hartmann U, Schedlowski M. 2001. Coitus-induced orgasm stimulates prolactin secretion in healthy subjects. Psychoneuroendocrinology 26:287–294. doi: 10.1016/S0306-4530(00)00053-6.

Fatematuzzohora M, Haque MA, Islam MR, Hoque MA, Ali MY. 2016. Evaluation of reproductive performance of indigenous cows using Brahman cross-bred bull in a participatory breeding program. Asian-Australasian Journal of Bioscience and Biotechnology 1:173–181. doi: 10.3329/aajbb.v1i2.61556.

Foote BA, Neff SE, Berg CO. 1960. Biology and immature stages of *Atrichomelinapubera* (Diptera: Sciomyzidae). Annals of the Entomological Society of America 53:192–9. doi: 10.1093/aesa/53.2.192.

Hafez ESE. 1993. Reproduction in Farm Animals. 6th edition. Lea & Febiger, Philadelphia.

Haque A, Ahmed K, Katila D, Talukdar D. 2018. Assessment of sperm viability in extended boar semen during long term storage at 15 °C. Theriogenology Insight 8:15–19.

Herman HA, Madden FW. 1963. The Artificial Insemination of Dairy and Beef Cattle, A Hand Book and Laboratory Manual. Lucas Brothers, Columbia, Missouri, USA.

Hoflack G, Soom A, Maes D, Kruif A, Opsomer G, Duchateau L. 2006. Breeding

soundness and libido examination of Belgian Blue and Holstein Friesian artificial insemination bulls in Belgium and the Netherlands. Theriogenology 66:207–216. doi: 10.1016/j.theriogenology.2005.11.003.

Hoque MD. 1998. Variation and Repeatability of Semen Characteristics of Purebred and Crossbred Bulls. MS Thesis, Department of Animal Breeding and Genetics, Bangladesh Agricultural University.

Hossain H, Khatun MM, Islam MM, Miazi OF. 2012. Semen characteristics of breeding bulls at the central cattle breeding and dairy farm of Bangladesh. Bangladesh Journal of Animal Science 41:1–5. doi: 10.3329/bjas.v41i1.11968.

Huang JZ, Ju Q, Li Q, Hou C, Wang J, Li R, Li L, Wang T, Sun S, Hang S, Gao Y, Hou M, Zhong J. 2011. Solexa sequencing of novel and differentially expressed microRNAs in testicular and ovarian tissues in Holstein cattle. International Journal of Biological Science 7:1016–1026. doi: 10.7150/ijbs.7.1016.

Islam MM, Apu AS, Hoque SAM, Ali MY, Karmaker S. 2018. Comparative study on the libido, semen quality and fertility of Brahman cross, Holstein Friesian cross and Red Chittagong breeding bulls. Bangladesh Journal of Animal Science 47:61–67. doi: 10.3329/bjas.v47i2.40236.

Islam MR. 2015. Evaluation of Semen Parameters of Brahman Cross, Holstein Cross and Local Bulls Using Near-infrared Spectroscopy. MS Thesis, Department of Animal Breeding and Genetics, Bangladesh Agricultural University.

Januskauskas A, Gil J, Soderquist L, Haard MGM, Haard MCH, Johannisson A, Rodriguez-Martinez H. 1999. Effect of cooling rates on post-thaw sperm motility, membrane integrity, capacitation status and fertility of dairy bull semen used for artificial insemination in Sweden. Theriogenology 52:641–658. doi: 10.1016/S0093-691X(99)00159-4.

Kadirvel G, Ponraj P, Thulasiraman S, Andonissamy J, Naskar S, Das A, Hasin D, Baishya S. 2016. Efficacy of different extenders on sperm characteristics and fertility of crossbred pig of north-eastern India. Veterinary Archive 86:515–528.

Khun MT, Hutchison JL. 2008. Prediction of dairy bull fertility from field data: use of multiple services and identification and utilization of factors affecting bulls fertility. Journal of Dairy Science 91:2481–2492. doi: 10.3168/jds.2007-0743.

Korkmaz F, Bastan I, Sahin D, Simsek S, Kaya U, Satilmis M. 2023. Reaction time as a libido indicator and its relation to pre-freeze and post-thaw sperm quality in bulls. Reproduction in Domestic Animals 58:965–971. doi: 10.1111/rda.14374.

Kumar M. 1995. Assessment of sexual behavior in Sahiwal and Murrah bull, NDRI, Karnal-132001, India. Indian Journal of Animal Production and Management 11:217–221.

Latif MA, Ahmed JU, Bhuiyan MMU, Shamsuddin M. 2009. Relationship between scrotal circumference and semen parameters in crossbred bulls. doi: 10.3329/bvet.v26i2.4952.

Melin P, Erik Kihlstrom JAN. 1963. Influence of oxytocin on sexual behavior in male rabbits. Endocrinology 73:433–435. doi: 10.1210/endo-73-4-433.

Moghaddam G, Pourseif MM, Rahimi AA. 2012. Study of correlation between reaction time and refractory period (as indices of libido) with semen parameters in ArkharMerino × Moghani and Baluchi × Moghani rams. Italian Journal of Animal Science 11:363–367. doi: 10.4081/ijas.2012.e66.

Mortimer ST. 2000. CASA-Practical aspects. Journal of Andrology 21:515–524. doi: 10.1002/j.1939-4640.2000.tb02116.x.

Murphy C, Fahey A, Shafat A, Fair S. 2013. Reducing sperm concentration is critical to limiting the oxidative stress challenge in liquid bull semen. Journal of Dairy Science 96:4447–4454. doi: 10.3168/jds.2012-6484.

Nasrin S, Amin MR, Alam MK. 2008. Evaluation of semen and non-return rate of bulls in artificial insemination center. Bangladesh Journal of Animal Science 37:1–7. doi: 10.3329/bjas.v37i2.9875.

Nath S, Mishra HR, Singh CSP. 1980. Note on the breeding behaviour of Holstein-Friesian bulls. Indian Journal of Animal Science 50:1124–1125.

Novianti I, Purwantara B, Herwijanti E, Nugraha CD, Putri RF, Furqon A, Septian WA, Rahayu S, Nurgiartiningsih VMA, Suyadi S. 2020. Effect of breeds on semen characteristics of aged bulls in the Indonesian National Artificial Insemination Center. Indonesian Journal of Animal Science 30:173–179. doi: 10.21776/ub.jiip.2020.030.02.10.

Parkinson TJ. 2004. Evaluation of fertility in natural service bulls. The Veterinary Journal 168:215–229. doi: 10.1016/j.tvjl.2003.10.017.

Prado V, Orihuela A, Lozano S, Perez-Leon I. 2002. Management of the female stimulus during semen collection and its association with libido

reestablishment and semen characteristics of goats. Journal of Animal Science 80:1520–1523. doi: 10.2527/2002.8061520x.

Rabidas SK, Talukder AK, Alam MGS, Bari FY. 2012. Relationship between semen quality parameters and field fertility of bulls. Journal of Embryo Transfer 27:21–28.

Rahman MA, Juyena NS, Ahmed JU, Ferdousy RN, Chakma S, Rine MZ, Tarif AMM. 2014. Evaluation of semen for breeding soundness of four different breeds of bull used for artificial insemination. Bangladesh Journal of Agriculture and Veterinary Science 7:28–34. doi: 10.9790/2380-07922834.

Ray K, Gosh BB. 2013. Semen ejaculate characteristics, in-vitro fertility and their relationships in Sahiwal bull semen. Iranian Journal of Applied Animal Science 3:48–489.

Rehman H, Alhidary IA, Khan RU, Qureshi MS, Sadique U, Khan H, Yaqoob SH. 2016. Relationship of age, breed and libido with semen traits of cattle bulls. Pakistan Journal of Zoology 48:1793–1798.

Roberts SJ. 1986. Infertility in male animals. In: Veterinary Obstetrics and Genital diseases. theriogenology.3rd edition. Woodstock, Vermont Publishers, New York.

Rycroft H, Bean B. 1992. Factor influencing non-return data. In: Proceeding of the 14th Technician Conference in Artificial Insemination and Reproduction. Columbia, MO. Columbia: National Association of Animal Breeders.

Sailer BL, Jost LK, Evenson DP. 1995. Mammalian sperm DNA susceptibility to *in situ* denaturation associated with the presence of DNA strand breaks as measured by the terminal deoxynucleotidyl transferase assay. Journal of Andrology 16:80–87. doi: 10.1002/j.1939-4640.1995.tb01735.x.

Salisbury GW, Vandemark NL, Lodge JR. 1978. Principles and techniques of freezing spermatozoa. San Francisco, CA: Freeman and Co.

Seneger P. 2012. Pathway to pregnancy and parturition. Current Conceptions. Inc, Redmond.

Shaha SP, Alam MGS, Khatun M, Ahmed JU. 2008. Breeding soundness of stud bulls. Bangladesh Veterinarian 25:51–61. doi: 10.3329/bvet.v25i2.4618.

Shamsuddin M, Rodriguez-Martinez H, Larsson B. 1993. Fertilizing capacity of bovine spermatozoa selected after swim-up in hyaluronic acid-containing medium. Reproduction Fertility and Development 5:307–315. doi: 10.1071/rd9930307.

Singh S, Bhakat M, Mohanty TK, Kumar A, Gupta AK, Chakravarty AK, Singh P. 2015. Sexual behavior and its relationship with semen quality parameters in Sahiwal breeding bulls. Veterinary World 8:745–749. doi: 10.14202/vetworld.2015.745-749.

Solanki GB, Kavani FS, Talekar SH, Ahlawat AR, Savsani HH, Gamit PM. 2019. Seasonal influence on sexual behaviour score of Gir bulls. International Journal of Current Microbiology and Applied Sciences 8:3100–3111. doi: 10.20546/ijcmas.2019.806.370.

Tsakmakidis IA, Lymberopoulos AG, Khalifa TAA. 2011. Selectivity of porcine zona pellucida to bind spermatozoa with normal chromatin structure 43:358–360. doi: 10.1111/j.1439-0272.2010.01074.x.

Verstegen J, Iguer-Ouada M, Onclin K. 2002. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology 57:149–179. doi: 10.1016/S0093-691X(01)00664-1.

Vishwanath R, Shannon P. 2000. Storage of bovine semen in liquid and frozen state. Animal Reproduction Science 62:23–53. doi: 10.1016/S0378-4320(00)00153-6.

The Official Journal of the **Farm to Fork Foundation**
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
http://www.f2ffoundation.org/faa