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Comparative Study on Semen Quality and Fertility of Red Chittagong Cattle, BCB1 and Munshiganj Bulls of Bangladesh

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Abstract

The present study was conducted to analyze the fresh and post-thaw semen quality and fertility from native bulls of Red Chittagong Cattle (RCC), BLRI Cattle Breed 1 (BCB1), and Munshiganj Cattle of Bangladesh. One hundred and seventy-two ejaculates were collected by artificial vagina set and semen analysis was performed using Computer Assisted Sperm Analyzer (CASA) at Bangladesh Livestock Research Institute. Commercial extender (AndroMed) was used to dilute the fresh semen. After equilibration (4˚C for 4 hr), freezing was done using a programmable bio-freezer. Post-thawed semen was evaluated for sperm motility and kinematics. Cryopreserved semen straws were used for artificial insemination (AI) and determined the bull fertility based on 60 days non-return rate. Motility of the sperm differs significantly ($p < 0.01$) among the genotypes. Total motility was higher in Munshiganj bulls and static motility was higher in BCB1 bulls. However, the semen volume and sperm concentration did not vary significantly ($p > 0.05$) among the bulls but the highest concentration was found in Munshiganj bull (1669.60 ± 192.07 million/ml) followed by RCC (1648.70 ± 91.07 million/ml) and BCB1 bull (1481.60 ± 167.35 million/ml). Moreover, the highest bent tail (5.89 ± 0.75%), coiled tail (1.01% ± 0.22%) and distal mid-piece reflex (2.26% ± 0.28%) were observed in BCB1 followed by Munshiganj and RCC. Amplitude of lateral head displacement (ALH) was recorded higher in post-thaw than in fresh semen. Kinematics parameters of post-thaw semen decreased than fresh semen irrespective of genotypes. More number of doses/ejaculates can be produced from Munshiganj bull (394.34 ± 127.95) followed by RCC (349.01 ± 120.91)

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and BCB 1 bulls (331 ± 98.99). Fertility rate does not differ among the bulls ($p > 0.05$) but the highest value was found for RCC (62.06% ± 1.94%) bulls. Therefore, it can be concise that, the quality of Red Chittagong Cattle semen is better than BCB 1 and Munshigang bull.

Keywords

Native Bulls, CASA, Sperm Kinematics, Morphology and Fertility

1. Introduction

The productive performance of native cattle of Bangladeshi is low compared to other existing exotic breeds and their crosses but they are well adapted to the tropical environment and can maintain their body condition on poor quality feed stuffs and are well resistant to local diseases [1]. Red Chittagong cattle found in the Chattogram district of Bangladesh have distinct identity with attractive red body color, delicious milk and meat compared to other indigenous genotypes, which make them top preferred in that region [2]. Munshiganj type, mostly of creamy to dull pinkish coat color with milk type body conformation and have great demand as milking cow in the surrounding regions of their habitat. Bangladesh Livestock Research Institute (BLRI) developed BLRI Cattle Breed-1 (BCB1) through selection and breeding of local Pabna cattle with higher milk production potentialities. Due to lower productivity than exotic breeds and their crosses native cattle population is going to decline day by day because of indiscriminate crossbreeding with exotic breeds. Although, the lifetime productivity is high compared to crossbred, which is neglected by the farmers to get higher production from crossbreed. Considering the above fact, Bangladesh livestock Research Institute is working with the conservation and subsequent development of native species like cattle, buffalo, sheep and goats for the last few decades. For subsequent improvement of native stock proper breeding plan with good quality semen from elite bull is a prerequisite. Therefore, semen analysis and bull fertility are equally important for breeding planning and selection of suitable breeding bull. Spermatozoa are present in the ejaculates as a heterogeneous group that's why sperms motility is an important and critical parameter that indicates the progressive movement of the sperm when moving in the female reproductive tract to reach the oocyte and initiate fertilization [3] [4]. The evaluation of sperm motility provides important information on the energy status [5] and mitochondrial activity within the sperm mid-piece [6] of mammalian sperms. Before the 1980s, the motility of sperm was measured manually [7] [8] but the variation in the result was found so high. Therefore, to address this problem, Computer Assisted Sperm Analysis (CASA) technology was developed to improve the accuracy and precision of semen analysis [9] [10]. CASA systems provide information based on values of thousands of sperm tracks that allows for individual motion analysis and accurate assessment of important kinetic pa-

rameters and are considered powerful tools for semen analysis across the world [11] [12]. Sperm kinematics includes the measurement of the distance between each head point for a given sperm during the acquisition period [13]. The kinematic parameters (VSL, VAP, VCL, LIN, ALH, STR, and BCF) usually estimate to find out the fertility potential of the bulls [14]. Now a day, the evaluation of semen motility and kinetic parameters is an essential part of sperm quality evaluation in livestock species [15]. Motility and morphology of spermatozoa are accepted as a benchmark for fertility status in bulls that are used for acceptance or rejection of ejaculates for artificial insemination (AI) in farms [16] [17] [18] [19]. Droplets can be identified as a regularly shaped remnant of cytoplasm under the plasma membrane of the spermatozoa which have negative impacts on the spermatozoa quality, both *in vivo* [20] and *in vitro* [21]. However, information on the use and application of CASA in Bangladeshi native bull semen analysis is very scanty, that is why this study was carried out to correlate the semen quality of the RCC, BCB1, and Munshiganj bulls in relation to their fertility as well as to establish a semen bank of native cattle germplasm for their conservation and development.

2. Materials and Methods

2.1. Bull Selection, Management, and Semen Collection

The breeding bull of Red Chittagong Cattle, BLRI Cattle Breed 1, and Munshiganj were selected as genetic material for this experiment. The age of the RCC and Munshiganj bull was 3 - 5 years and BCB1 bull was 4 - 8 years. The bulls were housed individually in intensive housing and fed green fodder (Napier, German, and Maize), concentrates, and mineral mixture supported by ad-libitum drinking water. Deworming and vaccination were practiced on a regular basis. Semen was collected twice a week in the early morning from 6.0 to 6.30 A.M by using an artificial vagina (AV) set [22] at the Cattle research farm of Bangladesh Livestock Research Institute, Savar, Dhaka. Collected ejaculates were immediately transferred into a water bath at 37˚C - 38˚C and measured the volume.

2.2. Motility, Kinematic Parameters, and Morphology Assessment

One hundred and seventy-two (172) ejaculates from three genotypes (BLRI Cattle Breed 1-33 ejaculates, Red Chittagong Cattle-104 ejaculates and Munshiganj cattle-35 ejaculates) were used for semen analysis. Motility, morphology, concentration, and kinematics parameters were measured using a Computer Assisted Sperm Analyzer (CASA) (Hamilton Throne, IVOS II) [23]. The CASA software settings for recording sperm motility were set as; Frame rate 60 Hz, Frames acquired 30, Minimum contrast 35, Minimum cell size, 5 pixels, Cell size, 9 pixels, Cell intensity 110 pixels, Path velocity (VAP) 50 μm/s, Straightness (STR) 70%, VAP cut-off, 30 μm/s, and VSL cut-off 15 μm/s. In a pre-warmed (38˚C) Leja® 4 chamber slide (depth 20 μm), a 1 μl prepared semen sample was loaded and analyzed for sperm motility characteristics. For each sample, at least

five optical fields around the central reticulum of the chamber were used to count spermatozoa. Besides motility (%) and concentration ($\times 10^6$ mL^{-1}) of semen sample the following kinematic parameters were evaluated (Table 1) & (Figure 1). During semen analysis, the standard temperature was maintained carefully 37˚C - 38˚C.

2.3. Dilution, Equilibration, and Freezing

Commercial extender (AndroMed®, Minitube, Germany) was used to dilute the fresh semen where 80% Mili-Q Water was added with 20% AndroMed® powder as per manufacturer's guidelines to make a final concentration of 80×10^6 spermatozoa/ml. The semen was diluted with the extender to give a sperm concentration of 20 million/dose. Diluted semen was placed in a cold handling cabinet (Minitube, Germany) for 4 hr at 4˚C for equilibration. The semen samples were

Table 1. Sperm kinematic parameters measured by the CASA system [15] [24] [25].

Parameters	Parameter description	unit
Motility	Percentage of sperm in different motility group based on velocity and progression	%
Concentration	Number of spermatozoa per milliliter	$\times 10^6$ mL^{-1}
VAP	Average path velocity based on every 11th frame of VCL path	μms^{-1}
VSL	Straight-line velocity along shortest path from start to end point	μms^{-1}
VCL	Curvilinear velocity along actual swimming path	μms^{-1}
ALH	Amplitude of lateral head displacement	μms^{-1}
BCF	Beat cross frequency based on VCL crossing VAP per second	Hz
STR	Straightness, expressed as VSL/VAP	%
LIN	Linearity of a curvilinear path, expressed as VSL/VCL	%

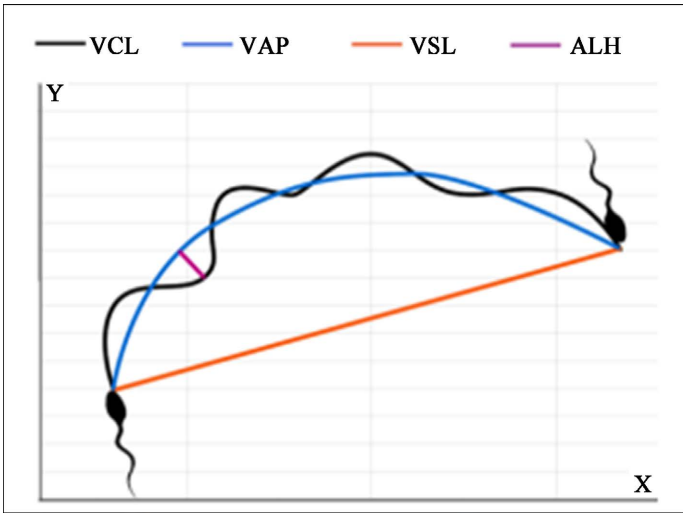


Figure 1. Sperm velocity parameters (VCL, VAP, VSL and ALH) [26].

filled and sealed in standard printed straws (0.25 ml) using an automated filling, sealing, and printing machine (MPP Uno, Minitube, Germany). After equilibration, freezing (15 minutes) was carried out in liquid nitrogen (LN_2) vapor using a programmable bio-freezer (Turbo Freezer M, Minitube, Germany). The straws were then plunged into the LN_2 (−196˚C) for overnight storage.

2.4. Post-Thaw Evaluation and Artificial Insemination (AI)

Thawing of the semen straws was performed at 37˚C for 30 sec after 24 hours of storage. The post-thawed semen was evaluated for sperm motility and kinematics using the same protocol as described previously for fresh semen. For each sample, at least five optical fields around the central reticulum of the chamber were used to count spermatozoa. The cryopreserved semen straws were used for artificial insemination (AI) of naturally estrus cows at on-farm and on-station. Finally, the fertility of the bull was determined based on 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 estrus within a period of 60 days.

2.5. Statistical Analysis

All data were recorded in Excel data sheet and One-way ANOVA followed by DMRT'S was performed by using SPSS (16.0) to assess differences among mean of motion characteristics of fresh and frozen semen of three genotypes and their sperm morphology, kinematics, fertility and dose/ejaculates. P value ($p < 0.05$) was considered as statistically significant.

3. Results

3.1. Volume and Concentration

Freshly ejaculated semen from RCC, Munshiganj and BCB1 bull were evaluated and quantified. There was no significant differences among the mean volume and concentration among the genotypes, although Munshiganj bull (4.93 ± 0.15) produced higher quantity of semen followed by BCB1 (4.45 ± 0.24) and RCC bull (4.10 ± 0.11). Highest sperm concentration was found in Munshiganj bull (1669.60 ± 192.07 million/ml) followed by RCC (1648.70 ± 91.07 million/ml) and BCB1 bull (1481.60 ± 167.35 million/ml) respectively (**Figure 2**).

3.2. Fresh and Frozen Sperm Motility

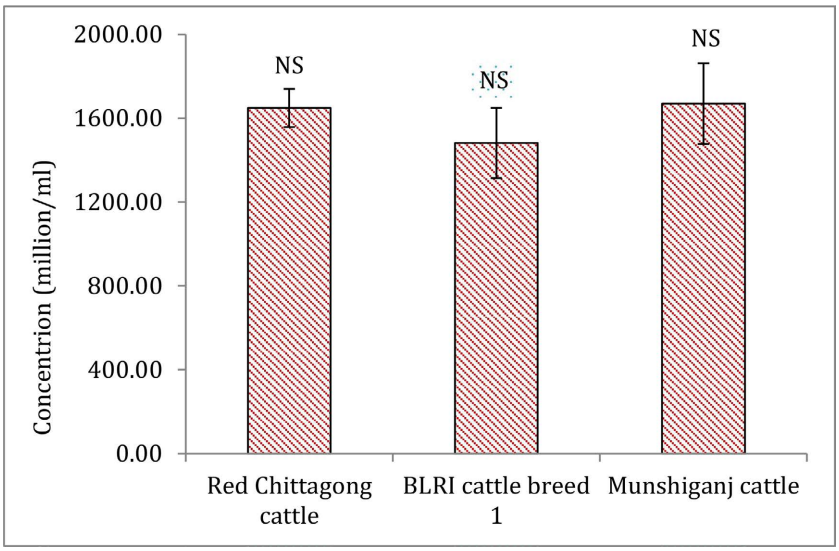
Except progressive motility of fresh semen, the total and static motility differ significantly ($p < 0.01$) among the bulls. The highest total, progressive, and lowest static motility was observed in both fresh and post-thaw semen of RCC bull followed by Munshiganj and BCB1. The mean value of total and progressive motility of post-thaw semen was found lower than in the fresh samples of the bulls but in contrast to static motility a higher value was observed in post-thaw semen than in fresh semen sample (**Table 2**).

3.3. Sperm Morphology

The mean values of the coiled tail, DMR, and distal droplet differ significantly ($p < 0.01$) whereas bent tail and proximal droplet did not differ significantly ($p < 0.05$) among the three native genotypes. The highest value of distal droplet and lowest value of coiled tail was found in RCC followed by Munshiganj and BCB 1 bull semen (Table 3).

3.4. Sperm Kinematics

The kinematics parameters of fresh and post-thaw semen did not differ significantly but the beat cross frequency of post-thaw semen differs among the bulls ($p < 0.05$). The ALH value was recorded higher in post-thaw than in fresh semen. However, the value of other kinematics parameters of post-thaw semen decreases from the value of fresh semen of the bulls (Table 4).



NS = Non significance.

Figure 2. Sperm concentration (mean ± SD) of RCC, BCB1 and Munshiganj bull.

Table 2. Genotype effects (mean ± SD) on the fresh and post thaw semen motility.

Parameters/Bulls		Red Chittagong Cattle	BLRI Cattle Breed 1	Munshiganj Cattle	Sig. level
Total Motility (%)	Fresh	78.31 ± 1.26[a]	70.56 ± 3.21[b]	79.19 ± 2.02[a]	**
	Post-thaw	61.88 ± 1.11[a]	50.48 ± 3.16[b]	67.98 ± 7.08[a]	**
Progressive Motility (%)	Fresh	57.35 ± 15.14	53.02 ± 19.58	55.80 ± 19.44	NS
	Post-thaw	44.94 ± 0.96[a]	25.35 ± 1.55[b]	43.28 ± 4.94[a]	**
Static motility (%)	Fresh	21.74 ± 1.26[b]	29.44 ± 3.21[a]	20.81 ± 2.02[b]	**
	Post-thaw	38.12 ± 1.11[b]	49.52 ± 3.16[a]	32.02 ± 7.08[b]	**

Mean with different superscripts within same row differ significantly ($p < 0.05$), ** ($p < 0.01$); NS= Non significance ($p > 0.05$); Sig. = significant.

Table 3. Genotype effects (mean ± SD) on fresh sperm morphology.

Bulls	Bent tail	Coiled tail	DMR	Distal droplet	Proximal droplet
Red Chittagong Cattle	4.56 ± 0.23	0.49 ± 0.04^{b}	1.35 ± 0.11^{b}	2.79 ± 0.23^{a}	55.93 ± 1.59
BLRI Cattle Breed 1	5.89 ± 0.75	1.01 ± 0.22^{a}	2.26 ± 0.28^{a}	1.60 ± 0.28^{b}	52.42 ± 4.03
Munshiganj Cattle	5.26 ± 0.87	0.56 ± 0.11^{b}	1.75 ± 0.25^{ab}	1.32 ± 0.23^{b}	54.16 ± 4.15
Significance level	NS	**	**	**	NS

Mean with different superscripts within same column differ significantly ($p < 0.05$), **($p < 0.01$); NS = Non significance ($p > 0.05$); Sig. = significant; DMR= distal mid-piece reflex.

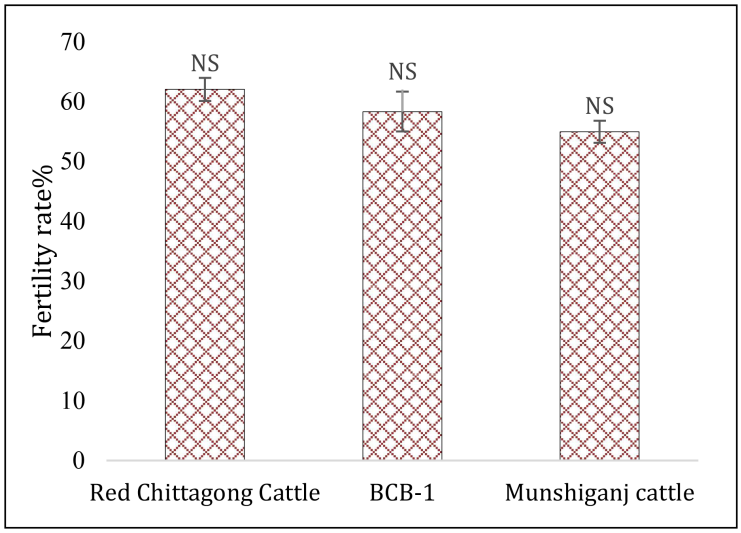
Table 4. Mean (± SD) values of sperm kinematics of fresh and frozen sperm of RCC, BCB1 and Munshiganj bull based on total motile sperm.

Parameters/Bulls		Red Chittagong Cattle	BLRI Cattle Breed 1	Munshiganj Cattle	Sig. level
VAP (μm/s)	Fresh	143.99 ± 4.15	156.68 ± 3.08	145.15 ± 27.10	NS
	Post-thaw	107.25 ± 8.85	96.04 ± 0.78	121.82 ± 20.09	NS
VSL (μm/s)	Fresh	128.50 ± 5.53	139.18 ± 3.83	137.13 ± 28.12	NS
	Post-thaw	90.46 ± 7.12	69.00 ± 1.48	107.42 ± 22.32	NS
VCL (μm/s)	Fresh	232.21 ± 23.59	256.06 ± 3.41	208.28 ± 54.84	NS
	Post-thaw	188.62 ± 12.97	179.8 ± 1.41	186.92 ± 27.91	NS
STR (%)	Fresh	88.35 ± 3.45	89.07 ± 0.38	93.73 ± 1.33	NS
	Post-thaw	84.19 ± 2.33	73.21 ± 1.91	84.66 ± 5.63	NS
LIN (%)	Fresh	58.57 ± 6.63	56.20 ± 2.13	69.46 ± 6.73	NS
	Post-thaw	50.37 ± 1.59	37.79 ± 1.00	54.70 ± 5.75	NS
ALH (μm)	Fresh	8.33 ± 1.62	9.53 ± 0.78	6.83 ± 1.97	NS
	Post-thaw	8.84 ± 0.63	10.05 ± 0.80	8.10 ± 0.52	NS
BCF (Hz)	Fresh	34.66 ± 4.97	32.96 ± 2.52	35.89 ± 3.36	NS
	Post-thaw	25.77 ± 1.73^{ab}	20.19 ± 0.82^{b}	32.92 ± 3.42^{a}	**

VAP: average path velocity, VSL: straight line velocity, VCL: curvilinear velocity, STR: straightness, LIN: linearity, ALH: Amplitude of lateral head displacement and BCF: beat cross frequency. Mean with different superscripts within same row differ significantly ($p < 0.05$), **($p < 0.01$); NS= Non significance ($p > 0.05$); Sig. = significant.

3.5. Frozen Semen Production

Average number of frozen semen dose can be produced per ejaculates from RCC, BCB 1 and Munshiganj bull was calculated. There was no significant differences was found among the genotypes, although more number of doses/ejaculates can be produced from Munshiganj bull (394.34 ± 127.95) followed by RCC (349.01 ± 120.91) and BCB 1 bulls (331 ± 98.99).



NS = Non significance.

Figure 3. Fertility rate (%) of RCC, BCB1 and Munshiganj bull.

3.6. Fertility Evaluation

Comparative fertility rate following AI of three genotypes of semen is presented in **Figure 3**. The fertility rate did not vary among the genotypes ($p > 0.05$) but the highest value was found for RCC (62.06 ± 1.94) bulls (**Figure 3**).

4. Discussion

Bovine spermatozoa goes through different challenges in the female reproductive tract to reach the site of fertilization. Morphologically normal sperm with acceptable motility and viability only can participate the race and out of them only one sperm finally wins the race. So, the quality of fresh and frozen semen is important to achieve the acceptable conception rate.

In this experiment, the volume and concentration of fresh semen of RCC, BCB1, and Munshiganj bull ranged from 4.10 to 4.93 ml and 1481.60 - 1669.60 million/ml respectively. Mean value of semen volume and concentration of Holstein-Friesian × Zebu, Sahiwal × Zebu, Sindhi × Zebu and Red Chittagong Bull were found 5.81 ± 0.16ml and 1115.97 ± 16.08 million/mm^3 [27]. Mean semen volume and concentration of pure breed BCB1, Limousin cross, Charolaise cross, Simmental cross and Brahman cross were 4.79 ± 0.65, 6.62 ± 0.09, 6.80 ± 0.05, 6.85 ± 0.07, 6.71 ± 0.30 and 800 to 1775 million/mm3 respectively [28]. Semen volume and concentration of Munshiganj bull was found 4.273 ± 0.54 ml and 1796 ± 122.29 million/ml respectively [29]. There are variations of semen volume and concentration of different breeds and such variability might be attributed to difference in age, breed, nutritional status, geographic location, season of the year, method of the semen collection procedure and collection frequency [30] [31]. Concentration of bull sperm ranges from 800 - 2000 million/mm^3 [30]. Sperm concentration of buffalo bull increased significantly with the increasing age of the animals [32]. However, the range of semen volume and

concentration found in this experiment agrees with the available literature [30] [32] and indigenous cattle genotypes produces semen with almost similar quantities and concentrations.

Motility of mammalian spermatozoa is an important trait as sperm has to swim through the female reproductive tract to unite with the oocytes in the oviducts to fertilize and subsequently produce viable zygote. In this experiment, significantly higher total and static motility of fresh and frozen semen was found for Munshiganj bull sperm followed by RCC and BCB1. However, in fresh semen although progressive motility is almost similar ($p > 0.05$) among the genotypes but in frozen semen progressive motility differs significantly among the genotypes ($p < 0.05$). After freezing significantly lower progressive motility was observed in BCB1 semen, this might be due to the higher age (4 - 8 years) of BCB1 bull. Comparatively lower total initial mass motility (61.25% - 64.38%) was found [34] from BCB1 bull although no published record was found for frozen semen quality of BCB1 genotype so far. Total, progressive, static and slow motility of the fresh vs. post thawed Munshiganj semen were (84.69 ± 4.28 vs. 52.97 ± 3.13), (72.53 ± 2.91 vs. 43.71 ± 1.57), (15.31 ± 4.28 vs. 47.03 ± 3.13) and (1.23 ± 0.60 vs. 0.58 ± 0.18) respectively [29]. The average mass motility of fresh and frozen sperm in RCC bulls ranged between 55.85% - 64.31% and 44.50% - 53.50% respectively [35]. Fresh sperm motility of Red Chittagong bulls was 62.12 ± 0.97% [27]. Average motility of bovine fresh semen was 63.3% and the range was 50% - 80% [36]. In this experiment, Munshiganj bull has higher percentage of total and progressively motile sperm in fresh semen in comparison with RCC and Munshiganj and freezability of BCB 1 semen is poor than RCC and Munshiganj semen. This variation in semen quality and freezability could be caused by the genotypes, age of animals, climate and management [36].

Morphologically abnormal sperm cannot pass through the hostile environment of female reproductive tract and finally may fail to reach the site of fertilization. Especially if the sperm subpopulation has higher tail deformities then they can't swim in linear motion through the higher viscous environment into the uterus. In this experiment, bulls from three genotypes showed different morphological deformities whereas coiled tail, distal sidepiece reflexes (DMR) and distal droplet varied significantly ($p < 0.01$). Morphological abnormalities were lower in RCC bull followed by Munshiganj and BCB1 bulls. The spermatozoa of normal fertile bull has been recommended not to contain more than 20% total abnormality, and individual head, midpiece and tail abnormality of 10% or more [30]. Tail and midpiece abnormalities of this experiment were within the normal range. Stressful condition is associated with abnormal sperm quality due to higher ROS production which may result in cellular damage through structural and functional changes [37]. Climatic stress has significant effects on bent tail, coiled tail, distal droplet and distal mid-piece reflex percentage of Murrah buffalo bull [38]. In this experiment, bulls from all three genotypes were kept in uniform feeding and management practices hence the variations may be due to genotypes. Although there were significant differences in the

morphology of sperm irrespective of genotypes but still their values are within the normal range and suitable for breeding purposes.

Sperm motility is associated with sperm kinematics, in general highly motile sperm shows higher VAP, VSL, VCL, STR and LIN in CASA. Sperm kinematics are not significantly different among the genotypes however higher kinematics value were observed for Munshiganj bull. In general, there was a reduction of motion characteristics for all three genotypes of semen during collection to post-thaw stages. This reduction may be due to holding time before semen dilution and equilibration time before freezing and stress during freezing [39]. In Mithun bull, average VCL, VSL and VAP were found 188.83 ± 4.65, 89.77 ± 3.49 and 118.58 ± 3.72 μm/s [40]. Kinematic value of Holstein-Friesian bull was found as VCL, VSL and VAP of 193.93 ± 71.50, 90.69 ± 40.60 and 111.37 ± 40.97 μm/s respectively [41]. In this study, the observed kinematic values of all three genotypes were higher than Mithun and Holstein-Friesian bulls and this variation may be due to genotype differences or differences in CASA set up. More number of frozen straw can be produced from Munshiganj bull followed by RCC and BCB 1. This information will be useful for set up and planning in AI center for estimation of yearly frozen semen production from each genotypes. However, this production efficiency will largely vary on collection interval, seasonal variation, freezing efficiency and bull management.

There were no significant differences among the genotypes for fertility rate following AI. Although the post thaw motility of Munshiganj bull semen was better than RCC semen but fertility rate was better for RCC semen. This may be due to renowned higher reproductive status of RCC cows over Munshiganj cows because RCC semen was used to inseminate RCC cows and Munshiganj bull semen was inseminated in Munshiganj cows. On the contrary, the progressive motility of BCB1 was significantly lower than the RCC and Munshiganj semen but their fertility did not vary significantly, this may be due to double dose of straw was used for AI for BCB 1. Non-return rate of Sahiwal bull ranged from 57.16% - 70.90% when AI was conducted in Pabna cow [42]. Conception is a complex science largely depends on semen handling, number of sperm deposited, site of insemination, efficiency of AI worker, semen quality, fertilization status, bull effect and time of AI [43]. Sperm motility is related to its energy status [5] and sperm with high progressive motility have more chances of fertilization [44]. However, it was reported that sperm motility and the kinetic parameters cannot be a reliable fertility marker [45]. So the variations of the present study may be due to semen handling, genotype, reproductive health of the female and other factors. However, in this experiment acceptable non-return rate following AI was achieved irrespective of genotypes.

5. Conclusion

Fresh and frozen semen of RCC, BCB1 and Munshiganj bulls were evaluated in terms of semen quality and fertility. Irrespective of differences in fresh and fro-

zen semen quality of the bulls they are suitable for AI with considerable fertility rate. In this present experimental condition semen quality of RCC bull was better than BCB1 and Munshiganj bull.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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