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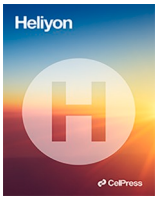

Morphometric features, seminal profile and diluters effect on post-thaw semen quality of Munshiganj cattle in Bangladesh

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ABSTRACT

The study investigates morphometric features, seminal profile and post-thaw semen quality of Munshiganj cattle. Morphometric features were measured using measuring tape from 20 Munshiganj cattle while coat color was measured by observing in naked eye. Fresh and post thaw semen quality parameters were analyzed using Computer Assisted semen analyzer (CASA). Coat color of Munshiganj male cattle were creamy white to dull pinkish and female were white to creamy. The mean body weight, body length, hearth girth, height at wither, head length, head width, ear length, ear width, fore leg length, hind leg length, tail length, tail doc circumference, horn length, horn diameter and mouth circumference were 362.80 kg, 137.31 cm, 160.66 cm, 135.21 cm, 50.97 cm, 20.58 cm, 19.75 cm, 9.88 cm, 73.02 cm, 74.84 cm, 106.10 cm, 20.75 cm, 13.60 cm, 16.12 cm and 43.00 cm, respectively. There was significant difference ($p < 0.05$) between male and female in terms of body weight (418.00 *vs* 307.60 kg), heart girth (173.74 *vs* 147.57 cm), head width (22.50 *vs* 18.67 cm), horn diameter (18.58 *vs* 13.66 cm) and mouth circumference (46.60 *vs* 39.40 cm). Average scrotal length was 16.76 cm while scrotal circumference was 32.70 cm. Age had significant effect ($p < 0.05$) on morphometric characteristics of Munshiganj male and female cattle. On the other hand, season had no significant effect on semen quality. Mean semen volume, sperm concentration, total motile, progressive, static, slow and live spermatozoa were 5.83 ± 0.88 ml, 1510.27 ± 844.07 million/ml, 91.9 ± 2.17 %, 63.80 ± 12.53 %, 8.10 ± 2.17 %, 0.10 ± 0.10 %, 91.38 ± 0.25 %, respectively. On the other hand, sperm head length and width, sperm tail length of Munshiganj cattle were 10.39 ± 0.16 μm, 4.26 ± 0.07 μm, 21.5 ± 0.52 μm, respectively. Individual breeding bull had a significant ($p < 0.05$) effect on post-thawed motile sperm percentage. Four different diluters (Triladyl, Steridyl, Tris-egg yolk-Citrate and Andromed) were used to compare the effects of diluter on post-thaw semen quality of Munshiganj cattle and found that diluter had no significant effect ($p > 0.05$) on post thaw semen quality except slow motility and proximal droplet percentages. Munshiganj cattle had a distinctive phenotypic feature with standard quality semen and had no effect of egg yolk free and egg yolk based diluters on post thaw semen quality.

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1. Introduction

Bangladesh has a rich heritage of native germplasm of livestock. The most commonly found native cattle breeds and varieties in Bangladesh are Red Chittagong cattle (RCC), Pabna variety, North Bengal Gray (NBG), Munshiganj (Mirkadim) variety, Madaripur type, Dinajpur Dwarf cattle (DDC), and most of the Non-descriptive native or local type [1]. The Munshiganj cattle may be one of the potential varieties of domestic cattle genetic resources exclusively found in Munshiganj district of Bangladesh and its surrounding areas and internationally recognized not as a pure breed but as a variety [2]. With a creamy to pale pinkish coat, this breed of cow has a highly attractive appearance and easily distinguishable from others due to its distinctive phenotypic features. Due to unclear history of this variety formation, it is presumed that this variety has evolved in the localities by natural selection and no authentic history of crossbreeding could be traced [3]. It is alarming that now-a days, this variety is under threat due to haphazard breeding. Therefore, it is necessary to take proper steps for its appropriate characterization and conservation both *in situ* and *ex situ*.

Morphometric characterization contributes to the improvement of animal genetic resources in the context of country-level implementation [4]. The morphometric measurement is conducted for the assessment of carcass quantity as well as to characterize breeds of animals. Live weight is an important trait in cattle farming. Weighing is not always feasible and therefore live weight is often estimated from easily accessible morphometric data [5]. However, the information on morphometric characterization on Munshiganj cattle is very scanty. Therefore, it is high time to characterize properly of this valuable genetic resource of Bangladesh and take initiative to conserve properly.

For conservation of the genetic resources, biotechnological tools like sperm cryopreservation and artificial insemination are important. Artificial Insemination (AI) system efficiency in cattle always depends on selection of the best breeding bull [6]. Sub-standard semen use in artificial insemination is one of the important factors of sub fertility in cattle [7]. High quality fresh semen is prerequisite for good quality diluted and frozen semen.

Protocols of semen dilution using different diluters and additives as well as the use of different cooling, freezing and thawing rates have been reviewed over the last decades [8–12]. Several efforts have been made to define the best semen diluter in different species of animals [13]. The most common ingredients added to the diluters when used for freezing semen of different species are Tris buffer and egg yolk [14–17]. Previous studies showed that Triladyl is the best commercial available diluter for camel semen cryopreservation in comparison to steridyl and OPTIXcell [18]. Moreover post-thaw survival and longevity of frozen bull spermatozoa were tested with an egg Yolk-based or egg Yolk-free diluters and found that the use of egg yolk based Biladyl extender results in significantly higher sperm survival and longevity than egg yolk free Andromed or Biociphos Plus extender [19]. However, the use of egg yolk in diluters has been proven problematic for semen cryoprotection, leading to search for the potential animal-free substitutes [14]. Promising results have been published regarding the use of animal-free egg substitutes for freezing semen [14,20–22]. AndroMed and OPTIXcell are the most common bull diluters that are supplied with egg yolk replacer. However, there is no data on the comparison of post thaw semen quality of Munshiganj cattle using egg yolk based and egg yolk free based diluters.

To prevent the loss of genetic diversity, cryopreservation is the process of freezing cells and tissues using liquid nitrogen to achieve extreme low temperatures with the intent of using the preserved sample. The primary benefit of semen cryopreservation is the ability to save germplasms for extended periods of time, therefore maintaining the genetic diversity of a species or breed. Considering the above circumstances, the current study was conducted to characterize Munshiganj cattle morphometrically, know the seminal profile and evaluate the effect of different diluters on post-thaw semen quality of Munshiganj cattle in Bangladesh.

Table 1
Morphological parameters of Munshiganj Cattle.

Trait	Male (10)	Female (10)	Pooled (20)	SEM	Probability value
Body weight (kg)	418.00[a]	307.60[b]	362.80	25.96	0.0217
Body length (cm)	135.43	139.19	137.31	6.88	0.8025
Heart girth (cm)	173.74[a]	147.57[b]	160.66	4.51	0.0019
Height at wither (cm)	141.77	128.66	135.21	5.43	0.2490
Head length (cm)	52.46	49.48	50.97	2.70	0.6113
Head width (cm)	22.50[a]	18.67[b]	20.58	2.88	0.0006
Ear length (cm)	20.80[a]	18.70[b]	19.75	0.534	0.039
Ear width (cm)	10.40	9.35	9.88	0.321	0.226
Foreleg length (cm)	74.54	71.50	73.02	1.74	0.4157
Hind leg length (cm)	71.68	78.00	74.84	2.33	0.1913
Tail length (cm)	112.80	99.40	106.10	3.57	0.0534
Tail doc circumference (cm)	20.70	20.80	20.75	1.69	0.9785
Horn length (cm)	14.40	12.80	13.60	1.51	0.6262
Horn diameter (cm)	18.58[a]	13.66[b]	16.12	0.96	0.0015
Mouth circumference (cm)	46.60[b]	39.40[a]	43.00	1.49	0.0049
Scrotal length (cm)	16.76	–	–	0.34	–
Scrotal circumference (cm)	32.7	–	–	0.69	–

Figures in the parentheses indicate the number of observation; Means with different superscripts within same row differ significantly ($p < 0.05$).

2. Results

2.1. Qualitative traits

Coat color of male Munshiganj cattle were creamy white to dull pinkish whereas female were white to creamy. Neck color was pinkish (50 %), brown (30 %) and white (20 %). While most of Munshiganj cattle (70 %) had pinkish muzzle and only 30 % had pinkish white black dotted muzzle. The hoof color was dominantly pinkish (80 %) with light black (20 %).

2.2. Morphometric characteristics

In male, most of the morphometric traits increased with the advancement of age. Body weight, body length, heart girth, height at wither, head width, hind leg length, tail doc circumference, horn length, scrotal length and scrotum circumference significantly ($p < 0.05$) higher in 60–68 months of age followed by 50–58 and 32–40 months of age (Supplementary Table 1). Like male, all considered morphometric characteristics significantly ($p < 0.05$) increased with the progress of the ages in female Munshiganj cattle except ear length, ear width and horn diameter (Supplementary Table 2).

Table 1 shows the morphometric characteristics (Body weight, body length, heart girth, height at wither, head length, head width, ear length, ear width, fore leg length, hind leg length, tail length, tail doc circumference, horn length, horn diameter, mouth circumference, etc.) of male and female Munshiganj cattle. In the present study, the mean body weight of male (418.00 kg) was significantly ($p < 0.05$) higher than female Munshiganj cattle (307.60 kg) with overall mean of 362.80 kg.

2.3. Seminal profile of Munshiganj cattle

Good quality semen is important for artificial insemination (AI) programs and has positive effect on the fertility of cows and heifers. Seminal profile of Munshiganj cattle is presented in Table 2. The average semen production of Munshiganj cattle was 5.83 ± 0.88 ml/ejaculation, sperm concentration 1510.27 ± 844.07 million/ml, motile 91.90 ± 2.17 %, progressive 63.80 ± 12.53 %, Live spermatozoa 91.38 ± 0.25 %, bent tail 5.10 ± 2.46 % and coiled tail 0.10 ± 0.10 in fresh semen. The average sperm head length, width and tail length were 10.39 ± 0.16 μm, 4.26 ± 0.07 μm and 21.5 ± 0.52 μm, respectively.

Season did not significantly ($p > 0.05$) affect the seminal traits of Munshiganj cattle. The semen production of Munshiganj bulls were 5.69 ± 0.34, 5.66 ± 0.39, 6.5 ± 0.29 ml while the concentration were 1431 ± 134.1, 1613 ± 70.37, 1541 ± 125.0 million/ml, in summer, winter and rainy season respectively (Supplementary Table 3).

2.4. Effect of breeding bull on post-thaw semen quality

Selection of breeding bull is a prerequisite for harvesting high-quality semen for cryopreservation. In order to check the effect of breeding bulls, three selected breeding bulls were used for semen collection. Table 3 shows the effect of breeding bulls on different parameters of semen quality such as motility of sperm namely motile, progressive, static, slow, and morphology of sperm such as bent tail, coiled tail, DMR, distal droplet and proximal droplet. But there was no significant effect of breeding bull in most cases of semen quality parameters except sperm motility.

2.5. Effects of diluters on post-thaw semen quality

Four different diluters were used to evaluate the effects of diluters on post-thaw semen quality of Munshiganj cattle. Table 4 shows

Table 2
Fresh semen quality of Munshiganj cattle.

Parameters	Mean ± SE
Volume (ml)	5.83 ± 0.88 (24)
Concentration (million/ml)	1510.27 ± 844.07 (24)
Motile (%)	91.90 ± 2.17 (24)
Progressive (%)	63.80 ± 12.53 (24)
Static (%)	8.10 ± 2.17 (24)
Slow (%)	0.10 ± 0.10 (24)
Live (%)	91.38 ± 0.25 (24)
Bent tail (%)	5.10 ± 2.46 (24)
Coiled tail (%)	0.10 ± 0.10 (24)
Distal Midpiece Reflex (DMR) (%)	1.60 ± 0.33 (24)
Distal droplet (%)	1.30 ± 0.30 (24)
Proximal droplet (%)	57.90 ± 15.21 (24)
Sperm head length (μm)	10.39 ± 0.16 (24)
Sperm head width (μm)	4.26 ± 0.07 (24)
Sperm tail length (μm)	21.5 ± 0.52 (24)

Parenthesis indicates the number of observations.

Table 3
Effect of breeding bulls on post-thaw semen quality.

Parameters	Bull 31 (8)	Bull 53 (8)	Bull 54 (8)	SEM	Probability value
Motile (%)	91.93[a]	77.83[a]	85.38 [ab]	2.245	0.0165
Progressive (%)	43.88	46.45	62.73	4.258	0.1447
Static (%)	8.08	19.68	14.63	2.387	0.1345
Slow (%)	1.23	1.45	1.63	0.303	0.8857
Bent tail (%)	3.25	4.25	2.68	0.805	0.7593
Coiled Tail (%)	0.23	0.18	0.45	0.121	0.6556
DMR (%)	1.55	2.15	1.25	0.245	0.3415
Distal droplet (%)	2.48	1.60	2.50	0.296	0.4050
Proximal droplet (%)	44.98	41.58	21.58	6.123	0.2605

Parenthesis indicates the number of observations. Means with different superscripts within same row differ significantly ($p < 0.05$).

Table 4
Effects of different diluters on post-thaw semen quality.

Parameters	Triladyl (24)	Steridyl (24)	Tris-egg yolk (24)	Andromed (24)	SEM	Probability value
Motile (%)	86.63	89.86	78.73	84.93	4.156	0.1992
Progressive (%)	51.07	62.63	44.27	46.10	2.751	0.4736
Static (%)	13.36	6.80	21.26	15.06	4.156	0.3966
Slow (%)	1.63[ab]	0.90[b]	2.67[a]	0.53[b]	0.773	0.0283
Bent tail (%)	1.76	1.83	6.33	3.63	0.958	0.1345
Coiled Tail (%)	0.56	0.13	0.40	0.03	0.088	0.4268
Distal Mid-piece Reflex (DMR) (%)	2.10	0.90	1.87	1.73	0.266	0.3696
Distal droplet (%)	2.47	2.67	2.33	1.30	0.483	0.4076
Proximal droplet (%)	34.23[ab]	27.93[ab]	22.60[b]	59.40[a]	2.831	0.1352

Parenthesis indicates the number of observation. Means with different superscripts within same row differ significantly ($p < 0.05$).

the effect of different diluters on different parameters of semen quality. It was revealed that no significant effect was observed among the diluters on post thaw semen quality parameters.

3. Discussion

3.1. Coat colour

Coat color observation is important in the conservation and management of native cattle breeds. Coat color is a heritable trait and can be used as an indicator of breed purity. Native cattle breeds are often at risk of genetic erosion and loss of unique characteristics due to crossbreeding with exotic breeds or genetic drift. Observing coat color and other physical characteristics can help identify purebred animals and prevent genetic dilution or loss. Moreover, coat color can be an important adaptive trait in native cattle breeds. Cattle breeds that have evolved in specific environments over time have developed adaptations that help them cope with local environmental conditions, including coat color. Some breeds may have developed a coat color that provides protection from the sun or camouflage in their natural habitat. Observing coat color can help in identifying animals with desirable adaptive traits and conserving them for future generations. Coat color observation can also aid in the study of genetic diversity and evolution of native cattle breeds.

Previous literature [3] reported creamy to dull pinkish coat color, pinkish muzzle and hoof which support the present study results. In case of North Bengal Gray, another type of indigenous cattle in Bangladesh, researchers [23] found mostly deep gray to white with different shades. Adult bull neck region have ashy shade with ages it increases and become prominent. Muzzle and hooves color were black in North Bengal Gray cattle. Different coat colors and patterns are associated with different genetic markers and can provide insights into the genetic diversity and evolutionary history of a breed. This information can be used in breeding programs to conserve and improve the breed.

3.2. Morphometric characteristics

Phenotypic measurements and observations are important tool for the characterization and conservation of native cattle breeds. They provide valuable information about the physical and production traits of animals with the breed, their adaptation to specific environmental conditions as well as unique features that distinguish the breeds from other breeds. In addition to the physical measurements, observation of coat color, hair length, and other physical characteristics also aid in breed characterization. This information can further be used in breeding programs and conservation efforts aimed at preserving and improving the breed.

In our study, it was observed that morphometric traits were significantly increased with the advancement of the ages in Munshiganj male and female cattle. Some investigators also observed that all the morphometric measurements significantly increased ($p < 0.01$) with the advancement of ages in Red Chittagong cattle in Bangladesh [24]. In a study with indigenous Assam cattle, age had significant effect on morphometric characteristics [25]. Morphometric traits significantly increased up to 4–5 year of age and after that the

increase was non-significant. Similar result was reported by Ref. [26] in Deoni cattle and [27] in Khillar cattle.

Previous results indicated that the results of body weight obtained in this study was higher than the report [3] (223.74 ± 10.20 kg) for Munshiganj cattle, who reported 241.00 kg for North Bengal Gray cattle [23] and 162.77 ± 2.72 kg for indigenous cattle [28] in Sylhet district. The variation might be due to difference in breed, age and body condition score of the animal. The average body length of Munshiganj cattle obtained in this study (137.31 cm) was similar with the findings that reported 144.11 cm for Ongole cattle at 60 month of age [29] and in line with the findings that found 118.11 cm in Munshiganj heifers [3]. The results of this study were also consistent in accordance with the results found for RCC (114.38 ± 1.56 cm) [30] and for Pabna cows (164.39 ± 2.36 cm) [31]. Slightly lower result was found by other investigator [32] for North Bengal Gray cows (105.16 ± 1.21 cm), for RCC (107.13 ± 2.17 cm) [33] and (106.89 ± 3.58 cm) [34]. This variation might be due to difference in age and breed and sex of the animal taken into consideration in the respective studies.

Mean heart girth was 160.66 cm which was comparatively lower than the report [23] that found 127 cm for cow, 101 cm for heifer and 122 cm for bull in North Bengal Gray cattle.

Average height at wither was found 135.21 cm (bull: 141.77 cm and cow: 128.66 cm) which was in line with the findings that found 112.21 ± 0.56 cm in case 66 months old Red Chittagong cattle [24]. Other investigators [31,35] also reported that the average withers height of adult Pabna and Indian Ponwar cows were 118.21 ± 3.25 and 109.0 ± 0.4 cm, respectively.

Overall mean head length of Munshiganj cattle was found 52.46 cm in male and 49.48 cm in female with overall mean 50.97 cm. These results support with the findings of [3] who found 34.29–49.53 cm head length in Munshiganj cattle. Significant effect was observed between male and female Munshiganj cattle in terms of head width with overall mean of 20.58 cm.

Mean ear length and ear width was 19.75 cm and 9.88 cm, respectively. These results were in line with [3,34].

There was no significant ($p > 0.05$) difference between male and female Munshiganj cattle in case of foreleg length and hind leg length. Average foreleg length was observed 73.02 cm and hind leg length 74.84 cm which corroborates with the findings [3] in Munshiganj cattle at *in situ* condition.

The average tail length in male and female was 112.80 cm, 99.40 cm, respectively with an average tail length of 106.10 cm. Several investigator [3] also found similar tail length with an average of 108.50 cm. Overall mean tail doc circumference was 20.75 cm and there was no significant difference between male and female in terms of this trait. Other investigators found tail doc circumference 8.67 ± 0.33 cm in <20 kg and 9.33 ± 0.67 cm in ≥20 kg baby calf [36]. The present study value was higher than the results obtained in the previous studies that might be due to age and body weight variation of the studied cattle.

Average horn length in Munshiganj cattle was 13.60 cm (male: 14.40 cm and female: 12.80 cm). This result agreed with the findings that found 10.38 cm in Munshiganj cattle [3] and 10.33 ± 0.49 cm horn length for Pabna cattle at Baghabarighat BLRI regional station [37] and 9.25 ± 1.26 at rural farms of Sirajganj. The overall horn diameter was 16.12 cm where a strong significant ($p < 0.001$) difference was found between male and female horn diameter. In case of Red Chittagong cattle's horn diameter, researchers [34] reported 11.61 ± 1.28 cm and 9.18 ± 2.07 cm in male and female, respectively. Mean mouth circumference was 43.00 cm while in male it was 46.60 cm and in female 39.40 cm.

The overall mean scrotal circumference of Munshiganj cattle was found 32.7 cm which was similar with the findings [38] that reported 34.0 ± 1.2 cm in local cattle of Bangladesh. In contrary, this result was higher than previous reported 17.68 + 3.51 cm scrotal circumference [39] in adult Pasundan male cattle in Indonesia.

3.3. Seminal profile and fresh semen quality

Fresh semen quality is an important aspect of cattle breeding as it affects the fertility of bulls and the success of artificial insemination (AI) programs [40]. There are several factors that can affect fresh semen quality in cattle breeds, including age, breed, season, and health status [41].

Some of the key parameters that are evaluated for seminal profile andfresh semen quality in cattle include volume, sperm concentration, motility, morphology, and viability. Sperm concentration refers to the number of sperm cells present in a given volume of semen [42], and is an important factor in determining the number of insemination doses that can be obtained from a bull. Motility refers to the ability of the sperm cells to move and swim, which is necessary for fertilization [43]. Morphology refers to the shape and structure of the sperm cells, which can affect their ability to fertilize an egg. Viability refers to the proportion of live sperm cells in the semen sample [44]. Breed can also play a role in fresh semen quality, as different breeds may have different sperm characteristics [41].

Semen quality can be affected by a wide range of genetic and environmental factors including bull age, collection interval, collection frequency, and season [45–47]. Regarding semen volume, present findings are in consistent with the findings 4.93 ± 0.15 ml in Munshiganj bull [48] and 4.88 ± 1.55 to 6.25 ± 1.43 ml/ejaculate in Bali bull in Indonesia [49]. On the other hand, 5.08–6.00 ml/ejaculation were reported for BLRI cattle breed-1 [50]. Present study observation was comparatively lower than 9.3 ml [51], 6.3 ± 0.5 ml [52] in local bull. In contrary, this observation was comparatively higher than the report of 2.58–4.01 ml in RCC [53] and 3.22 ± 0.09 ml in RCC [54] that might be due to breed differences. Accessory gland secretions also play an important role in influencing ejaculate volume [55].

In the current study, average sperm concentration was found 1510.27 ± 844.07 million/ml which was similar with the findings in Munshiganj bull (1669.60 ± 192.07 million/ml) [48]and (1796 ± 122.29 million/ml) [56]. The present study findings was much higher than the report of [54] (907.39 ± 23.01 million/ml in RCC bull), and (1035.24 ± 279.79 to 1088.12 ± 297.14 million/ml) in Bali bull in Indonesia [49]. Breed may be the contributing factor for this difference. Sperm concentration also varied due to the number of spermatogenic cells and their activity in the testes to develop sperm through the process of spermatogenesis. Size of the testes, hormones and environment temperature are responsible for the production of sperm [57].

It was found that the progressive sperm motility in fresh semen of Munshiganj bull was 63.80 ± 12.53 % which was similar with the findings 72.53 ± 2.91 % [56] and 55.80 ± 19.44 % [48] for the same variety bull. In case of RCC bull progressive sperm motility was recorded 63.96 ± 0.76 % [58] and 62.12 ± 0.97 % [59]. This progressive motility was also in agreement with [49] who found 60.282 ± 12.026 % to 63.259 ± 9.409 % in Bali bull in Indonesia. Present study reports 8.10 ± 2.17 % static motility whereas other researchers [56] observed 15.31 ± 4.2 % static motility. Overall mean slow motility was 0.10 ± 0.10 % whereas little higher slow motility (1.23 ± 0.60 %) was found in other report [56]. Moreover, 1.42 ± 0.67 % static motility in Brahman crossbred bull, 1.67 ± 0.61 % in Simmental crossbred bull, 0.00 % in Charolais crossbred bull, 5.48 ± 9.31 % in Limousin crossbred bull and 1.98 ± 2.80 % in pure bred BCB bull were also reported [50]. Mitochondrial generating an energy substrate in the form of adenosine triphosphate (ATP) is responsible for sperm motility. The membrane mitochondria potential, mitochondrial enzyme activity, mitochondrial volume, oxygen consumption, and mitochondrial respiration influenced sperm mitochondrial activity [60].

Live sperm percentage was found 91.38 ± 0.25 in this study while several investigators observed 78.09–81.73 % in Pabna cattle [50], 74.70–78.10 % [53] and 71.64 ± 0.68 % live spermatozoa in RCC cattle [53].

Fertility is strongly related to the proportion of morphologically normal spermatozoa in the sample [61]. Accurate morphological screening of the ejaculates allows elimination of bulls with a potential low fertility, prior to the entrance of bulls a progeny testing program and the preservation of semen, thus contributing to a major savings for AI enterprises [62]. Sperm abnormalities might occur as the result of disturbances in the stage of spermatogenesis or the maturation process in the epididymis [63]. Other researchers states that abnormal spermiogenesis causes abnormalities in the sperm head resulting from a defect in chromatin condensation [64]. Disturbances in the maturation of sperm in the epididymis may result in immature sperm that is identified by the presence of a proximal cytoplasmic droplet.

This study found that the percentages of bent tail, coiled tail, DMR, distal droplet and proximal droplet of sperm were 5.10 ± 2.46, 0.10 ± 0.10, 1.6 ± 0.33, 1.3 ± 0.30 and 57.9 ± 15.21, respectively. Present study output agreed with the report that found 5.26 ± 0.87, 0.56 ± 0.11, 1.75 ± 0.25, 1.32 ± 0.23 and 54.16 ± 4.15% for bent tail, coiled tail, DMR, distal droplet and proximal droplet, respectively in Munshiganj cattle [48].

In another experiment, 19.97 ± 7.32, 0.45 ± 0.64, 1.175 ± 0.11, 1.675 ± 2.37 and 25.45 ± 5.87% of bent tail, coiled tail, DMR, distal droplet and proximal droplet of sperm were found in Brahman crossbred bull while 26.33 ± 5.14, 3.73 ± 3.02, 4.30 ± 3.58, 3.92 ± 1.77 and 42.75 ± 13.03% found in Simmental crossbred bull [50]. On the other hand, in case of Charolais crossbred bull, percentages of bent tail, coiled tail, DMR, distal droplet and proximal droplet of sperm were 6.42 ± 7.17, 4.63 ± 4.07, 4.73 ± 3.80, 0.00 and 7.96 ± 11.20, respectively [50].

Seminal traits of Munshiganj cattle were not influenced by the season of the year. Previous investigators also showed that season had no significant effect on seminal attributes of bull [65]. Some researchers showed no seasonal effect on sperm motilty [66–68] others had shown an increase during summer [69] or (in complete contrast) winter [70,71]. Besides these, season had no effect on total sperm production and sperm morphology [67,72] whilst others showed an increase during summer [69,73], or winter [68,74]. As such, opinions on whether changes of seasons are indeed a source of variation in bull semen qualities are still divided [75].

3.4. Individual bull effect on post-thaw semen quality

The quality of post-thaw semen can be affected by various factors, including the individual bull's genetics [63], age [76], health, and semen collection and processing techniques [77]. Factors such as the type of extender used, the cooling rate, and the method of thawing can all influence the quality of the semen after thawing [78–80].

Individual bull effect was found insignificant on all semen quality parameters except motile sperm percentage. Bull 31 had highest motile sperm percentage (91.93 ± 2.72) whereas lowest value was in percentages of slow motility (1.23 ± 0.57), progressive motility (43.88 ± 6.58) and static motility (8.08 ± 3.68). Among three Munshiganj breeding bulls, Bull 53 had higher value for static sperm (19.68 ± 3.68 %), DMR (2.15 ± 0.42 %) and lower value for motile sperm (77.83 ± 2.72 %), coiled tail (0.18 ± 0.22 %), distal droplet (1.60 ± 0.51 %). On the other hand, Bull 54 had better result in percentages of progressive motility (62.73 ± 6.58), slow motility (1.63 ± 0.57), coiled tail (0.45 ± 0.22) and distal droplet (2.50 ± 0.51) while lower value was found for DMR (1.25 ± 0.42) and bent tail (2.68 ± 1.50). Previous investigators [48] reported that the average total motility was 67.98 ± 7.08 %, progressive motility 43.28 ± 4.94 %, static motility 32.02 ± 7.08 %, bent tail 5.26 ± 0.87 %, coiled tail 0.56 ± 0.11 %, DMR 1.75 ± 0.25 %, distal droplet 1.32 ± 0.23 %, proximal droplet 54.16 ± 4.15 % in post thawed semen which corroborates with the findings of the present study. In Achhami bull, post thaw progressive motility and slow motility were 75 % and 7.4 %, respectively [81] whereas in Gir bull the post thaw progressive motility was 53.81 ± 0.61 % [82]. The post thaw coiled tail were 2.3 ± 0.4 % in local and 2.2 ± 0.3 % in crossbred bull found in Bangladesh [83].

3.5. Effect of different diluters on post-thaw semen quality

Dilution is a common practice in the preservation of semen for artificial insemination (AI) in cattle. Dilution is necessary to provide sufficient sperm numbers per insemination dose and to extend the lifespan of the sperm during storage. However, dilution can have an effect on post-thaw semen quality in cattle. The extent of the effect depends on factors such as the type and concentration of diluent used, the cooling rate, and the freezing rate [84].

Dilution with an appropriate diluent can improve the post-thaw quality of semen by providing a protective environment for the sperm during freezing and thawing. Diluents typically contain ingredients such as egg yolk, glycerol, and antibiotics, which help to protect the sperm against damage from ice crystal formation and oxidative stress [85]. On the other hand, improper dilution or the use

of low-quality diluents can have a negative effect on post-thaw semen quality. Diluents that are not properly balanced in terms of osmolality, pH, and nutrient content can lead to sperm damage during cooling, freezing, and thawing. Over-dilution can also decrease sperm concentration, leading to reduced fertility [86]. Therefore, it's important to use appropriate diluents and to follow recommended dilution protocols to minimize the negative effects of dilution on post-thaw semen quality in cattle.

The cryopreservation process involves departure of the cells from and return to body temperature, both cold shock and warm shock are included as potential stresses to be considered, as well as the stages involving cooling below the freezing point of the medium [11]. Freezing process reduces the activity of sperm so the initial semen quality is critical for final quality after thawing [87]. Extender is generally added to semen to preserve viability and fertility of spermatozoa during storage.

Diluter effect was found non-significant on motile sperm percentage in this study. Motile sperm percentage was higher in Steridyl (89.86 ± 4.41) followed by Triladyl (86.63 ± 4.41), Andromed (84.93 ± 4.41) and Tris egg yolk (78.73 ± 4.41) diluter. Higher percentages of progressive motility were found for Steridyl (62.63 ± 8.61) while lower was found for Tris egg yolk (44.27 ± 8.61). Other investigator [48] stated 43.28 ± 4.94 % progressive motility in post thaw semen of Munshiganj cattle when diluted with commercial Andromed diluter which is in line with the present findings (46.10 ± 8.61 %) diluted with Andromed diluter. Progressive motility of frozen thawed semen diluted with Tris egg yolk was 68.19 ± 0.46 % in Holstein Friesian bull and 56.54 ± 0.25 % in Sahiwal bull [88]. On the other hand, in a study in Achhami bull in Nepal, it was found that post-thaw progressive motility 75.00 % in egg yolk-tris citrate based extender [81].

The mean static motility of frozen-thawed semen was found lower in steridyl (6.80 ± 4.25 %) and higher in Tris egg yolk (21.26 ± 4.25 %) whereas 13.36 ± 4.25 % and 15.06 ± 4.25 % was found in Triladyl and Andromed diluter, respectively. In a study, researchers [48] observed 32.02 ± 7.08 % static motility diluted with Andromed diluter which is much higher compared to current study.

Slow motility was significantly ($p < 0.05$) higher in Steridyl diluter compared to other three diluters. Slow motility was highest in Steridyl (2.67 ± 0.41 %) and lowest in Andromed (0.53 ± 0.41 %). Previous researchers [88] studied Tris egg yolk effect on frozen semen and found that slow motility was 9.21 ± 0.46 % in Holstein Friesian bull and 11.11 ± 0.55 % in Sahiwal bull. In a study with Achhami bull semen in Nepal, it was found that slow motility was 7.4 % in post-thaw semen diluted with egg yolk tris-citrate based extender [81].

Diluter's effect were also non-significant on bent tail percentage of frozen semen and found higher in Steridyl (6.33 ± 1.35 %) and lower in Triladyl (1.76 ± 1.35 %) diluter. Similarly the present study showed a non-significant effect of diluter on coiled tail of spermatozoa after freezing. Coiled tail percentage was higher in Triladyl (0.56 ± 0.24 %) and lower in Andromed (0.03 ± 0.24 %) along with intermediary in Tris egg yolk (0.40 ± 0.24 %) and Steridyl (0.13 ± 0.24 %).

There was no significant effect of diluter in frozen semen in case of DMR and distal droplet though a significant effect was observed in case of proximal droplet. DMR was higher in Triladyl (2.10 ± 0.48 %) and lower in Steridyl (0.90 ± 0.48 %), distal droplet was higher in Steridyl (2.67 ± 0.58 %) and lower in Andromed group (1.30 ± 0.58 %) while in case of proximal droplet highest value was found in Andromed (59.40 ± 10.33 %) and lowest in Tris egg yolk (22.60 ± 10.33 %). Limited literature is available to compare the results with the findings of other studies.

The presence of proximal cytoplasmic droplets (PCD) indicates insufficient sperm maturation during epididymal transit. In contrast, sufficient epididymal maturation of spermatozoa is considered to be a prerequisite for gaining complete functional competence for fertilization [89]. Therefore, when proximal droplets present, results in impairment of fertility [90,91]. On the other hand, distal cytoplasmic droplets (DCD) are not considered serious abnormalities, even at high percentages [92]. Moreover, the presence of sperm-bearing cytoplasmic droplets (CD) is positively correlated with the production of reactive oxygen species (ROS) that affect the acrosome integrity of sperm, ultimately affecting fertilization [93].

Munshiganj cattle are under threat of extinction. So far very few research works have been done to explore the Munshiganj cattle. Therefore, literature is limited. Moreover, only three males and 3 female animals in each age group were used in this study as replication and could not set more number of animals in each age group due to unavailability of the animals in our *ex situ* condition. Besides these, only three mature bulls were used for semen collection and evaluation study. So, number of animals is the limitation of the present study.

4. Materials and methods

4.1. Description of the study area

The data on the considered qualitative and quantitative traits were taken from Munshiganj cattle of different sex located at Pachutia Research farm at Bangladesh Livestock Research Institute (BLRI) which is located at Savar Upazila, 30 km northwest of the capital Dhaka during the period of July 2021 to June 30, 2022. Geographically this study area is located in subtropical region that lies between 23.8583° North latitude to 90.2667° East longitude. It is situated at an elevation of 10.62 m (34.84 feet) above sea level, Savar has a Tropical wet and dry or savanna climate (Classification: Aw). The district's yearly temperature is 30.11 °C (86.2 °F) and it is 2.37 % higher than Bangladesh's averages. The average humidity is 70 %. Savar typically receives about 74.11 mm (2.92 inches) of precipitation and has 120.12 rainy days (32.91 % of the time) annually.

4.2. Experimental design

Three experiments were conducted to fulfill the objectives of the study. In experiment 1, 10 male and 10 female Munshiganj cattle were divided into three age groups: Group A (32–40 months), Group B (50–58 months), Group C (60–68 months). Three animals were

considered in group A and group B whereas 4 animals were considered in group C category for morphometric characterization. In experiment 2, semen was collected from three breeding Munshiganj bulls of aged between 50 and 58 months. Three animals (three breeding bulls bearing ID 31, 53 and 54) were considered for the study to find out the effect of breeding bulls and effect of season on semen quality. Eight ejaculates were taken from each breeding bull and evaluate the seminal profile and semen characterization. In experiment three, a total of 24 ejaculates from three breeding bulls were diluted with four different diluters and used for frozen semen production, thereafter evaluated the post thaw semen quality of Munshiganj cattle.

4.3. Animal management

Total 20 mature Munshiganj cattle (10 male and 10 female) were selected based on the physical appearance, age and their pedigree record from BLRI Farm herd book. Male Munshiganj cattle (Photo 1) age varied from 32 to 68 months with 338–504 kg body weight. While in female (photo 2) age ranged from 29 to 70 months and body weight was 223–384 kg. Physical examination was performed to look for any abnormalities and any clinical signs of infection or infectious diseases. Three mature Munshiganj bulls (ID 31, 53, and 54) of similar age group (50–58 months) were selected for semen collection. The basic criteria for the bull selection were pedigree record, strong body conformation, stout, no sign of physical injury, disease and deformities free in penis and prepuce. Bulls were reared in intensive system and the cow and heifers were reared in semi intensive system in individual pen. Green grass (Napier, German, Maize, Para) was supplied as basal diet twice a day (morning & afternoon) along with a concentrate mixture of 2 kg/head (wheat bran, kheshari bran, soybean oil cake, til oil cake, maize crushed, broken wheat and salt) (see Fig. 2).

Evaluation procedure of qualitative traits of Munshiganj cattle: Qualitative traits like coat color, neck, muzzle and hoof color were observed by naked eye and entered in the record sheet.

4.4. Evaluation procedure of quantitative traits of Munshiganj cattle

Body length (cm): point of shoulder to the point-of-rump or pin bone [94]; *Hearth girth (cm):* around the animal at the point of smallest circumference just behind the forelegs [95]; *Body weight (kg):* measured using Shaeffer's formula, Live weight (kg)=(body length $\times$ (hearth girth)^2)/(300 $\times$ 2.2); *Height at wither (cm)*: distance from the surface of the platform to the dorsal point (Os vertebrae thoracalis III) of the withers [96]; *Head length (cm):* the poll of the head to the rostral end of muzzle [95,97]; *Head width (cm):* broader portion of the head [97] (Photo 3).

Ear length (cm): the root/base of the ear to the tip of the ear; *Ear width (cm):* from the distance of the wider part of the ear [95]; *Horn length (cm):* base of the horn to the tip of the horn; *Horn diameter (cm):* circumference of the base of the horn; *Tail length (cm):* tail head to tail switch (distance from proximal end of the first coccygeal bone to the distal end of the last coccygeal bone); *Foreleg length (cm):* point of shoulder to fetlock joint; *Hind leg length (cm):* stifle to pastern joint; *Tail doc circumference (cm):* around the tail head; *Mouth circumference (cm):* one inch below the eye base, with mouth closed and wrap; *Scrotal length (cm):* base of the scrotum to the end point of scrotum vertically by measuring tape and *Scrotal circumference (cm):* at the area of the largest diameter of the scrotum with a cloth tape as described [98].

4.5. Semen collection and evaluation

Semen was collected from three Munshiganj breeding bulls twice a week by artificial vagina in the early morning between 6:30–8.00 a.m. After semen collection, ejaculate volume was recorded directly from the graduated semen collection tube and stored in water bath at 37 °C [99]. Seminal profile such as volume of semen production, color, concentration, percentages of total motile spermatozoa, progressive, static, slow and live spermatozoa, morphology (vent tail, coiled tail), distal midpiece reflex, distal droplet, proximal droplet, sperm head length, head width, tail length) were evaluated using Computer Assisted semen Analyzer (CASA).

Photo 1. Munshiganj breeding bull.

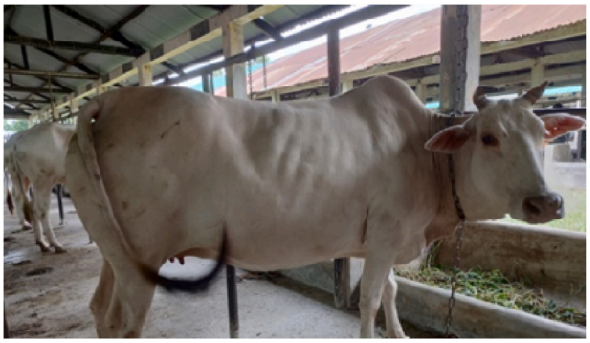

Photo 2. Mature Munshiganj cow.

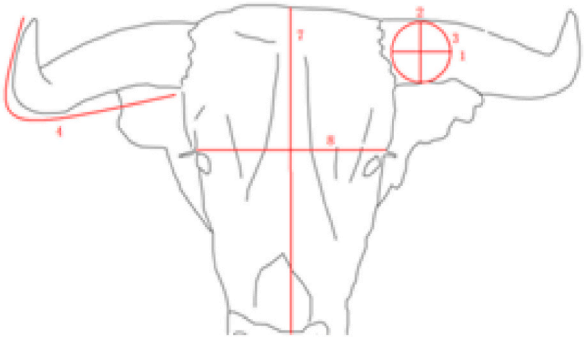

Photo 3. Head width measurement (Lomillos et al., 2020).

4.6. Analysis of sperm motility

Sperm motility analysis was conducted objectively using the Computer Assisted Semen Analyzer (CASA), latest version 6.0.1 (IMV Hamilton, minitube, Germany). One small drop of diluted semen was placed on a clean pre-warmed (37 °C) slide and examined under Computer Assisted Microscope with cover slip at 10× magnification. At least 200 spermatozoa were counted from different fields under microscope [100].

4.7. Analysis of sperm viability

The viability of spermatozoa was assessed by the Eosin (minitube, Germany) staining technique. This method was based on the degree of membrane permeability of dead spermatozoa; due to the damaged membrane, dead sperm cells absorb the stain while living cells remain uncolored. Briefly, a small amount of two drops of Eosin were placed on the edge of a clean, preheated slide and one drop of semen is placed beside the Eosin on slide. Then the smear was prepared by mixing carefully. Then the smear was dried on warm tray (approx. 20 min). Then microscopic analysis of 200 spermatozoa (at 40× magnification) was performed. After that, the percentages of uncolored, living cells were calculated [100].

4.8. Analysis of sperm morphology

Sperm morphology and staining features were assessed using Farelly stain (minitube, Germany) containing a fixative solution, Aniline Blue, and crystal violet. The blue-violet color contrast allows a differential visualization of the head, the acrosome, the equatorial segment, the centerpiece and the tail of sperm cells. A clear distinction between morphologically intact and abnormal sperm cells is possible as well as the identification of the type of abnormality. For analysis of abnormality a thin smear was prepared on a clean slide. Then the air-dried smear was stained and washed by dipping into tap water. The preparation was dried for 12 h. Thereafter microscopic analysis was done using oil immersion (100X, phase contrast) and at least 200 spermatozoa were observed from different fields under microscope [58].

4.9. Dilution and cryopreservation of semen

Four different diluters namely Tris-egg yolk-Citrate diluter, commercial Andromed (plant based), Triladyl and Steridyl were used for comparative analysis of the effect of diluent in semen cryopreservation. Tris-egg yolk-citrate contains Tris 2.24 g, Citric acid

(Monohydrate) 1.48 g, glucose 1.0 g, Water 100 ml, glycerol 12.8 ml, Egg yolk, 20 ml, Penicillin/streptomycin 50000 IU. Diluents were prepared by mixing required quantities of Tris, citric acid, fructose, glycerol, egg yolk and antibiotics [101]. Triladyl (Minitube, Germany) included Tris, citric acid, glucose, buffer, glycerol, pure water and antibiotics (tylosin, gentamicin, spectinomycin, and lincomycin) in 250 g bottles. Triladyl® extender was made up by addition of 60 ml double distilled water and 20 ml egg yolk to 20 ml concentrate [102]. On the other hand, Steridyl (Minitube) contains the same compound as Triladyl as well as egg yolks, in 500 mL bottles. The final diluent, 750 mL of pure double-distilled water was simply added to a complete package of Steridyl diluent in a 1500 mL Erlenmeyer flask. AndroMed (Minitube) consists of phospholipids, Tris, citric acid, sugar, anti-oxidants, buffers, glycerol, antibiotics and the purest water in 200 mL bottles. Each pack of 200 mL of this diluent contained 11.40 mg tylosin, 57.20 mg gentamycin, 68.60 mg spectinomycin and 34.40 mg lincomycin. To prepare the final diluent, 800 mL of double-distilled water was simply added to a complete package of 200 mL AndroMed diluent in a 1000 mL Erlenmeyer flask [103].

Semen was diluted with the extender to give a sperm concentration of 20 million/dose.

The pH was maintained at 6.8. For equilibrium, diluted semen was placed in a cold handling chamber at 4 °C for 4 h. Standard printed ministraws (0.25 ml) was filled and sealed with extended semen samples with automated filling, sealing and printing machine. After equilibration, semen straws were placed in TurboFreezer M (minitube, Germany). One step freezing technique was designed for freezing semen from 5 °C to −140 °C at a rate of (−15.26 °C/minute) within 10 min. This freezing technique requires five steps where the temperature decreases from 5.0 °C to −5.0 °C within 60 s. This temperature remains for 120 s. After that, the temperature again drops from −5.0 °C to −100.0 °C within 143 s. Then again the temperature decreases from −100.0 °C to −130.0 °C within 60 s. This temperature remains for 180 s (Minitube manual). The straws were then frozen in Liquid Nitrogen vapor according to the methods of freezing technique and stored in liquid nitrogen can at −196 °C for long term cryopreservation.

The frozen semen straws (0.25 ml) were thawed in warm water at 37.5–38.5 °C for 10–12 s for post thaw semen analysis [104]. For post-thaw semen analysis, straws were cut after 24 h of cryopreservation to evaluate the quality of cryopreserved semen. Post-thaw semen quality (motility, viability and morphology) was judged by using Computer Assisted Semen analyzer (CASA) [48].

4.10. Statistical analysis

The data generated from this experiment were entered in Microsoft Excel worksheet 2010, organized and processed for further analysis. Descriptive statistics along with SEM were performed in each morphometric and semen quality parameters. One way ANOVA was performed a) to find out the difference of morphometric characteristics among different ages of Munshiganj male and female cattle; b) to observe the difference in seminal quality among different seasons and; c) to demonstrate the difference of post-thaw quality of frozen semen using four different diluters. Moreover, “t” test was performed to find out the difference between male and female morphometric characteristics. Duncan Multiple Range Test (DMRT) was performed to separate mean values for significant independent variables using Statistical Analysis Software (SAS 9.04.01M5P091317) computer package. The difference between values was considered significant when the p value was less than 0.05.

5. Conclusions

In conclusion, the phenotypic characteristics and coat colour of Munshiganj cattle are attractive and easily distinguishable from others due to its distinctive phenotypic features. Seminal profile also showed standard quality in comparison to other native breeds/varieties. Diluters had no significant effect on post-thaw semen quality of Munshiganj cattle. More-in-depth studies are needed to characterize this potential variety genetically and take proper initiative to conserve Munshiganj cattle, a valuable genetic resource of Bangladesh.

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Ethical statement

This study was conducted under the ethical guidelines approved by the Bangladesh Livestock Research Institute (BLRI) with permission number 231.

Data availability statement

Data included in article/supp. Material/referenced in article:

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21967.

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