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Comparative assessment of quality of fresh and frozen semen production in Bangladesh

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Abstract

Background: *cattle is one of most crucial genetic resources among the farm animal genetic resources (FAnGR). Genetic improvement of cattle can be done by planned AI (Artificial insemination), multiple ovulation and embryo transfer (MOET) and in-vitro production (IVP) of embryos in Bangladesh. Among reproductive technologies most popular in Bangladesh is AI. Due to AI, semen quality evaluation is important to selection. To efficient calf good quality semen has positive effect on successful fertility of cows and heifer as well as cattle breeding programme. We conducted this study to evaluate quality of both frozen and fresh production of bull in Bangladesh.*

Materials and methods: *For the purpose of this study, both fresh and frozen semen were collected from CCBDF (Central Cattle Breeding and Dairy Farm) Savar, Dhaka. Fresh semen collected by artificial vaginal method (AV) at early morning. Then sample transported through thermal box to laboratory. This study was conducted at Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207. After collection of semen by AV method from bull, frozen semen straw were prepared and stored at -196^{o}c in liquid nitrogen cane for AI purpose at CCBDF. The frozen sample straw were collected and transported through N_2 cane to laboratory.*

Results: *To assessment semen qualities, the parameters were evaluated such as sperm concentration ×10^{6}/ml, normal sperm, abnormal sperm, dead sperm, live sperm, total motility and progressive motility. The fresh and frozen semen were comparable and statistically significant ($p<0.05$). The abnormal sperm of fresh and frozen semen were 9.98% and 18.2% respectively. The live sperm of fresh and frozen semen were 88.43% and 68.04% respectively. The dead sperm of fresh and frozen semen were 11.8% and 46.3% respectively. The normal sperm of fresh and frozen semen were 87.3% and 65.09% respectively. The sperm concentration ×10^{6}//ml of crossbred is 1370.00×10^{6}/ml and 1223.00×10^{6}/ml were fresh and frozen respectively. The progressive motility of fresh and frozen semen were 75.26% and 44.32% respectively. The motility of fresh and frozen semen were 85.93% and 62.16% respectively.*

Conclusion: *To discard poor fertile bulls in AI programme, semen quality evaluation is a crucial elements. The fresh semen quality better than frozen semen. But to avoid venereal diseases frozen disease straw better than fresh semen and effective to get desirable calf per year. So, frozen semen should be undertaken with special management to maintain maximum quality which can be used in the field level for genetic improvement.*

Key words: *Semen quality; sperm morphology; fresh semen; frozen semen.*

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I. Introduction

For rapid dispersal of desirable genes, artificial insemination (AI) still remained as the most important and main vehicle and has been the method of choice for the farmers to improve the genetic potentiality of their livestock around the world (Vishwanath, 2003). Rapid dissemination of genetic merit of a population can be possible widespread use of artificial insemination with accurate genetic evaluation. Artificial insemination is the most powerful tool for livestock improvement (Robert and Foote, 1989). For increasing milk, meat and skin production could be achieved by the development of artificial insemination (Robert and Foote, 1989). By using AI, semen from a single male can impregnate many thousands of female yearly (Chemineau *et al.*, 1991). A bull is called half of the herd productivity and genetic improvement than a single female. Selection of a good quality breeding bull on the basis of sexual behavior and semen quality (Anzar *et al*., 1993) which is important to breed improvement program and conservation. Also improving the reproductive efficiency of dairy cattle through utilization of highly fertile bull which bear good quality semen. The success of the AI program also depends on

semen quality (Herbowo *et al*., 2019). Artificial insemination can increase pregnancy rate only if the insemination dose contains sufficient numbers of viable spermatozoa. If they are unable to obtain a complete quality semen evaluation, failure must be occurred after AI. So, for successful breeding performance results, evaluation of semen quality and freezing ability of semen is the important aspect (Mandal *et al*., 2009). Quality of semen varies for the different genetic and non-genetic factors such as breed, species, age, temperature, collection frequency, collection timing and season etc has been found. But through better management practices have positive impact on semen quality and quantity and also successful breeding performances. The inner picture of semen which is related to fertility is determined by semen quality which encompasses a package of parameters these are sperm concentration/ml, total motility %, progressive motility%, live sperm%, normal sperm%, dead sperm% and abnormal sperm%. Poor reproductive performance is a major problem which is associated with semen quality of stud bull (Annual report of DAPH, 2011). To meet up the huge demands of milk, milk products, meats, exotic breeding bull have been imported in Bangladesh by different private organizations and entrepreneurs and disseminated the genetic merit of these pure breeding bulls through frozen semen and genetic improvement of our cattle. Fresh and frozen semen straw are using to disseminating the merit of exotic breed in the field level at AI program. But there is no available research works on semen evaluation in Bangladesh. The continuous evaluation of semen quality and quantity is helped to achieve higher breeding performances. Therefore, we conducted this research to evaluate both fresh and frozen semen quality of crossbred in which parameters such as sperm concentration, sperm motility, semen normality and semen viability were considered and these breeding bulls were maintained in the farming condition at CCBDF farm, Savar, Dhaka in Bangladesh. The objectives of this project is to determine the morphological characteristics of fresh and frozen bovine semen and find out the efficacy of fresh and frozen semen.

II. Materials and Methods

This comparative study was done by Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, from July 2021 to December 2021.

Study Design: In lab, observational and experimental study was done.

Study Location: This comparative study was done by Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207.

Study Duration: July 2021 to December 2021.

Experimental Materials

Chemicals and media

All the chemicals, reagents and media constituents were purchased from market before starting experiments.

Preparation of the laboratory

All necessary electrical power and equipment were properly checked and if needed installed before starting the experiment. Before reuse, all reusable equipment were washed properly**,** cleaned and sterilized with 70% alcohol, dried, covered with aluminum foil and kept in cleaned and sterilized chamber until use and every time before reuse follow same procedure was applied.

Collection of semen

Fresh semen

Semen collection: Fresh semen was collected from breeding bull at CCBDF, saver, Dhaka by Artificial vagina method (AV). This sample transported from CCBDF to lab through thermo box.

Semen Preparation: Semen were collected twice/week from bull by artificial vagina method at early morning under sterile conditions. With a gap 20-30 mins generally two ejaculates were collected from most of the bulls in a day. After evaluation of sperm concentration and initial motility, semen samples should be diluted with dilutor by maintaining 34°C. The semen should be extended further after 7 minutes of cooling at 20°C with dilutor maintained at the lab temperature after initial dilution at 1:1 ratio. The semen samples should not kept for long time in water bath, which effect the sperm viability. Volume of semen was noted directly from the graduated collecting test tubes just after collection. Sperm concentration was determined by haemocytometer.

Frozen semen

Semen collection: After semen collection by artificial vagina method from bull, frozen semen straw prepared and these straw was collected from CCBDF, Saver, Dhaka in liquid nitrogen cane (-196^{o}c) within short period to laboratory.

Semen preparation: After evaluate sperm motility and concentration, semen was diluted with TRIS-fructose-egg yolk (TFEY) extender. The basic extender contained TRIS (297.6 mmol/L), fructose (82.6 mmol/L), citric acid (105.3 mmol/L) and to prevent contamination add penicillin G sodium (1000 IU/ml) and streptomycin sulphate (1 mg/ml) in semen sample. Then gradually reduced temperature and preserve at -196^{0}c temperature in

straw. Semen straw was retrieved from liquid nitrogen cane by using forceps and thawed before using in water bath at 37^{o}c for 12 second.

Semen evaluation
Sperm concentration:
Procedure
Apparatus and reagents:

- A Compound microscope
- Hemo-cytometer set
- Standard & red cell dilution pipette or semen dilution pipette.
- Counting chamber
- Cover slip
- Dilution fluid
- Semen sample
- Cotton

Composition of dilution fluid:

- Distilled water: 50 ml
- 2% eosin (for color): 1 ml
- 3% Nacl solution: 1 ml (used for killing the cell)

Sperm concentration of raw semen was enumerated by haemocytometer method. Semen sample was mixed well by slowly inverting vial several times. About 0.5 mm of semen was drawn in to standard red dilution cell pipette. Dilution fluid was drawn in to standard red cell pipette up to 101 mm mark. The pipette was an agitated by grasping it between the thumb and fore finger and it was rotated in one plane by eight knot motion for 3 minutes to ensure thorough mixing. The first 4 or 5 drops were discarded (to get properly diluted semen from the bulb). A cover slip was placed over the ruled field of a cytometer slide and a drop was allowed to run under the cover slip. The count was made under low magnification approximately (10×25). Five large double ruled squares were counted over the field. This will give a total 80 small squares, then the number of spermatozoa per ml of semen to be calculated by using the following formula: The following formula was used for calculating the total number of spermatozoa/mL of fresh semen:

$$\text{Total no. of sperm per ml semen} = \frac{C \times 400 \times d \times 1000}{S}$$

Here: C= No of sperm counted in a given no. of small quarter
S= No. of small square counted
d= Dilution ratio: 200: 1

Motility: Sperm motility was observed just after semen collection and it expressed in percentage.
Procedure: A clean dry glass slide warmed approximately to 100^{o}F (38.7^{o}c). The semen sample was mixed properly by slowly inverting the vial 2 or 3 times (do not agitate vigorously). One drop of semen sample was placed on the slide and was spread on the warm slide. Examined under microscope at low magnification (4X). Percentage of motility was calculated the following formula:

$$\% \text{ of motile sperm} = \frac{\text{No. of motile sperm}}{\text{No. of total sperm}} \times 100$$

There are several systems of motility of rating. The following system using '0-5' is usually recommended.

Scale	Grade	Character
Five	Excellent (+++++)	80% or more then spermatozoa are in very vigorous motion. Swirls and eddies are form caused by the movements of the sperm. Movements are so vigorous that it is impossible to observe individual sperm fresh semen.
Four	Very good (++++)	About 70-80% of the sperm are in vigorous motion. Waves and eddies are form a dropped rapidly.
Three	Good (+++)	About 50-70% of the spermatozoa are in motion waves and eddies are form but dropped rapidly.
Two	Fair (++)	About 30-50% of the sperm are in motion waves and eddies are very slowly across the field.
One	Poor (+)	About less than 30% of the sperm are in motion. No waves and eddies are form the movements are weak and oscillatory not progressive.
Zero	non motile	No motile sperm are observe

Progressive motility

Procedure: Just after collection the semen was diluted with buffer solution and inclined slowly. Then steps were taken from the procedure of mass estimation. When examined under microscope progressive movement of the sperm was observed with care. Percentage of progressive motility was calculated the following formula:

$$\% \text{ of progressive motile sperm} = \frac{\text{No. of progressive motile sperm}}{\text{No. of total sperm}} \times 100$$

Sperm viability: The technique was used by using the nigrosin-eosin stain to evaluate live and dead sperm. It is so called "live-dead" stain and it also allows assessing membrane integrity at the same time as morphology. A high percentage of live, progressively motile, vigorous spermatozoa are necessary for good quality semen. The dead and alive sperm is based upon the difference between dead cells and live cells in absorbing certain dyes. In this test the sperm that are dead at the time of the slide is made will absorb the stain and appear blue. Those that are alive will not absorb the stain and will remain while.

Apparatus and Reagents:

- Microscope
- Two glass slides
- Stain (3 stain available)
- Hot plate (150-200^{o} F)
- Glass rod
- Semen sample
- Cotton

Composition of Stain: Eosin Nigrosin stain:

- Eosin blue – 5 gm
- Nigrosin – 1 gm
- Sodium citrate buffer – 100 ml

Procedure: A clean, dry slide was taken and 1 or 2 drops of stain was placed on the middle of the slide with the help of a glass rod. A small amount of semen was mixed with the stain**.** A second slide was drawn over the semen on the first slide and thus the smear was made. Excess stain was removed by wiping the edges of slides with cotton. The smear was dried rapidly by placing them on a hot plate at 150-200^{o} F temperature (65-93^{o} C). The slide was placed under the microscope, live and dead sperms were counted from randomly selected field. The sperms were counted under 25 x objective.

The dead sperms were those which absorbed dye and appeared blue color under microscope and those alive did not absorb stain; that was showed white in color. A spermatozoa which was partially stained such as nucleus only was counted as dead. Percentage of dead and live sperms were counted by the following formula:

$$\% \text{ of dead sperm} = \frac{\text{Number of dead sperm}}{\text{Total number of sperm}} \times 100$$

$$\% \text{ of live sperm} = \frac{\text{Number of live sperm}}{\text{Total number of sperm}} \times 100$$

Grading of Semen in Respect of Dead and Live Sperm:

i) Excellent quality semen: Contains 5-10 % dead sperm
ii) Good quality semen: Contains 11-20 % dead sperm
iii) Poor quality semen: Contains 21-30 % dead sperm
iv) Very Poor quality (reject): More than 30 % dead sperm.

Normal and abnormal sperm

Equipment and reagents:

- Compound microscope
- Two glass slides
- Rose Bengal stain
- Staining rack
- Cotton
- Semen sample
- Buffer (Any physiological buffer such as citrate buffer)

- Beaker full of distilled water

Composition of Rose Bengal stain:

- Rose Bengal powder – 3gm
- Distilled water – 99ml
- Formalin (40% formaldehyde) – 1ml

Procedure: Two drops of buffer was placed on a clean dry glass slide. One drop of mixed semen was added in buffer. The buffer with semen was spread by covering with another slide. The smear was dried in the air**.** The smear was stained with Rose Bengal stain for 5 minutes. Then remove/rinse of excess stain by dipping slide in distilled water. The smear was dried in the air**.** The slide was placed on the stage of microscope and counted in high magnification. Generally a total of 333 sperms were counted used random fields on different parts of the slide and is recorded in the table.

Grades of semen according to percentage of abnormal sperm:

i. Abnormality < 10 percent: High quality semen
ii. 11-20 percent abnormality: Good quality semen
iii. Greater than 20 percent abnormality: Poor quality semen
iv. Greater than 30 percent abnormality: Rejected grade.

Statistical Analysis: All the data obtained from the locations were organized, structured and analysed using Statistical Analysis System (SAS, 1998), computer programmed to computer analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was performed. Student's *t*-test was used to ascertain the significance of differences between mean values of two variables and confirmed by nonparametric test that compared two paired groups. The level $p< 0.05$ was considered as the cutoff value or significance.

III. Result

Evaluation of freshly drawn and frozen semen is very essential elements before using for successful reproductive performance and successful AI. Therefore, the morphometric characteristics of semen particularly sperm concentration, normal sperm, abnormal sperm, dead sperm, live sperm, total motility and progressive motility were evaluated in this study.

Table 1 shows that the result of semen normality and sperm viabilities. To evaluate semen qualities sperm normality and sperm viabilities are important elements. We can see that in this research the normal sperm of fresh and frozen semen were 87.3% and 65.09% respectively and the abnormal sperm of fresh and frozen semen were 9.98% and 18.2% respectively. We can also see that the results of sperm viabilities of crossbred in table 1. The live sperm of fresh and frozen semen were 88.43% and 68.04% respectively. The dead sperm of fresh and frozen semen were 11.8% and 46.3% respectively.

Table 1: Effect of semen type on sperm morphology and viability

Type of semen	Normality (%)		Sperm viability (%)	
	Normal sperm (%) (Mean±SE)	Abnormal sperm (%) (Mean±SE)	Live sperm (%) (Mean±SE)	Dead sperm (%) (Mean±SE)
Fresh semen	87.3^{a}±0.55	9.98^{b}±0.35	88.43^{a}±0.53	11.8^{b}±0.50
Frozen semen	65.09^{b}±0.72	18.2^{a}±0.56	68.04^{b}±0.69	46.3^{a}±0.64
Level of significant	***	***	***	***

Means with different superscripts within each column differ significantly ***, ($p<0.05$), SE: Standard error.

In the table 2, we can see the sperm concentration and sperm motility. Sperm motility is the crucial elements for successful fertilization. Sperm concentration, semen motility, semen normality and also all parameters which considered for this study were affected by age, collection frequency, storage temperature etc. All effect were considered for this study. The sperm concentration/ml of crossbred is 1370/ml and 1223/ml were fresh and frozen respectively. The progressive motility of fresh and frozen semen were 75.26% and 44.32% respectively. The motility of fresh and frozen semen were 85.93% and 62.16% respectively.

Table 2: Effect of semen type on sperm concentration and motility

Type of semen	Sperm concentration/ml (Mean±SE)	Total motility (%) (Mean±SE)	Progressive motility (%) (Mean±SE)
Fresh semen	1370.00^{a}±4.5	85.93^{a}±0.60	75.26^{a}±0.42
Frozen semen	1223.00^{b}±8.6	62.16^{b}±0.63	44.32^{b}±0.60
Level of significant	***	***	***

Means with different superscripts within each column differ significantly ***, ($p<0.05$), SE: Standard error.

IV. Discussion

Semen normality: The highest normal sperm was observed in fresh semen (87.30%) and lowest in frozen semen (65.09%) in this study (Table 1). The present study reported abnormal sperm percentage of both fresh and frozen semen respectively were 9.98% and 18.20% (Table 1). There is significant ($p<0.05$) effect between fresh and frozen semen. The findings of Mandal *et al*. (2009) and Zodinsanya *et al*. (2015) higher than the present results of abnormal sperm percentage. Habib *et al.* (2003) observed that the normal sperm 82.24% which is lower than the results of this study. Islam *et al.* (2018) observed normal sperm percentage 83.14 which is also lower than the findings of fresh semen but higher than the results of frozen semen. Arefin *et al*. (2022) agreed with the results of normal sperm percentage of fresh semen but smaller than the normal sperm percentage of frozen semen. The abnormal sperm percentage of this study agreed with the findings of the study of Santoso *et al*. (2021). Sankhi *et al.* (2022) observed the abnormal sperm percentage at 8-9 years aged crossbred bulls 19.59% which is higher than the results of abnormal sperm percentages of both fresh and frozen semen. He also reported that the percentage of abnormal sperm 14.3% which is higher than the abnormal sperm percentage of fresh semen and lower than frozen semen percentage. This variation occurred due to the aged and frequency of collection which were considered in the present study. The variation between fresh and frozen semen may be occurred temperature effect, cooling and thawing management. The variation between normal and abnormal sperm may be occurred due to age, season, breed and management practices etc.

Semen viability: The average live sperm in this study of both fresh and frozen semen were 88.4% and 68.04% respectively. There is significant ($p<0.05$) variation between fresh and frozen semen of live sperm percentage (table 1). Kumar *et al*. (2015) found live sperm percentage at pre-freezing condition 71.72% which is lower than the results of the present research. He also found post-freezing live sperm 58.67% which is also lower than frozen live sperm percentage which found in this study. The number of viable bovine sperm inseminated in the reproductive tract of female cattle influences the fertilizing ability of cow upto an upper threshold level (Pace *et al*., 1981; Schenk *et al*., 1987 and Gerard & Humblot, 1991). Rahman *et al*. (2014) reported 77.62% live sperm which is lower than the findings of this study. He also found dead sperm 22.38% which is higher than the percentage of dead sperm of fresh semen but lower than the percentage of frozen semen founded in this project. This variation may be occurred due to low temperature. In this study, the average dead sperm percentage of both fresh and frozen semen were respectively 11.8% and 46.3% (Table 1). There is also significant different between sperm concentration per ml of both fresh and frozen semen. The findings of the percentage of live sperm in the present study higher than the results of the study (Morrell *et al*., 2018). The percentage of dead sperm in this study agreed with the findings of Morrell *et al*. (2018). Sperm viability is a raw material for quality analysis and a prerequisite for success in the fertilization such AI used low number of sperm which is reported by Hossain *et al*. (2011). Felip *et al*. (2008) found the live sperm percentage of both fresh and frozen semen respectively 74.84% and 42.84% which are lower than the results of the live sperm percentage of both fresh and frozen semen found in the present study. Santoso *et al*. (2021) reported the sperm viability of fresh semen which is agreed with the findings of this research. He also found the sperm viability of frozen semen which is lower than the results of this study. Baharun *et al*. (2017) also observed lower sperm viability than the present study. Matahine *et al*. (2014) and Ratnawati *et al*. (2018) reported higher sperm viability than the findings of sperm viability of this present project. Moreover the viability of sperm is influenced by the breed and age of cattle. Samik *et al*. (2014) reported also showed the sperm viability of crossbred 85%. Islam *et al*. (2018) found live sperm percentage about 84.18% which is lower than the results of live sperm percentage of fresh semen in this present study. Hahn *et al*. (1969) and Fatematuzzohora *et al*. (2016) found lower results of live sperm percentage than the present study. Sankhi *et al*. (2022) reported the live sperm of both 8-9 years aged bull and third time collection respectively 45.62% and 54.34% which are lower than the results of live sperm percentage of the present study.

Sperm concentration: The sperm concentration 10^6/ml of both fresh and frozen semen were respectively 1370.00 and 1223.00 (Table 2). There is significant variation between fresh and frozen semen sperm concentration. The results of this present study higher than the Sankhi *et al.* (2022). The results of sperm concentration per ml in the study almost similar with the results of the study of Waltl *et al*. (2004). When compared to the results of the study of Ahmad *et al.* (2003), the findings of this project higher may be due to proper handling, proper stimulation and cooling procedure during preparing semen straw. Arefin *et al*. (2022) reported comparatively lower result than the findings of sperm concentration in this present study. The results of this study significantly higher than the findings of sperm concentration which are reported by Devenath (1999); Habib *et al*. (2003) and Nasrin (2008). This variation may be occurred due to both genetic and non-genetic

factors. Evaluation of semen concentration is used to determine the amount of dilution for preparation of frozen semen straw. Argiris *et al*. (2018) reported almost similar results with the findings of the present study. The sperm concentration decrease with the increasing age according to the study of Fuerst *et al*. (2006). Islam *et al.* (2018) found the sperm concentration 1087×10^6/ml which is lower than this study. The results of the present study higher than the findings of Fatematuzzohora *et al*. (2016). Habib *et al*. (2003) reported almost similar results with the sperm concentration of frozen semen. The slight different value might be due to the age, breed, collection timing and frequency and feeding management (Al-Hakim *et al*., 1984). The results of sperm concentration of this study is higher than the reported by Santoso *et al.* (2021). The sperm concentration means the number of sperm cells per ml of semen. The sperm concentration of bull influenced by testicle size and scrotal circumstance beside collection frequency and timing (Saputra *et al*. 2017). The results of this study agreed with the findings of Mandal *et al.* (2014). Sperm concentration is the initial indicators of semen quality which is used for cryopreservation. Mehedi *et al*. (2020) reported the sperm concentration per ml semen of Holstein bull 1664.28 which is higher than the results of this study. The results of sperm concentration in the present study higher than the results of Ahmed *et al*. (2014). Hossain *et al*. (2012) observed that the semen concentration of fresh semen produced by 75% Holstein bull 1406.40 million/ml which higher than present study. He also reported higher value of sperm concentration than this study of frozen semen. The findings of the present study of sperm concentration is higher than the study of Kumar *et al*. (2015). Rahman *et al*. (2014) reported lower value of sperm concentration than this present experiment.

Semen Motility

Total motility: Total motility of both fresh and frozen semen were respectively 85.93% and 62.16% in this study (Table 2). In this study, between fresh and frozen semen the motility percentage significantly ($p<0.05$) different. Sankhi *et al*. (2022) reported lower value of fresh semen motility than the present study. He also reported lower value of frozen semen motility than the results of this study. The results of this present study of fresh semen motility higher than the findings of the Ahmed *et al*. (2014). The findings of frozen semen motility of this study is higher than the findings of Singh *et al*. 2000; Rao & Rao (1979) and Blom (1950). The present study reported higher results than the study of Arefin *et al*. (2022). Argiris *et al*. (2018) reported lower value of semen motility than the present study. Santoso *et al*. (2018) observed higher value of fresh semen motility than present study. The results of the present study of frozen semen motility lower than the findings of Santoso *et al*. (2018). To evaluation of sperm fertility, sperm motility is one of the parameters. The percentage of sperm motility varies due to cooling temperature. Semen storage at low temperature results in structural damage due to cold shocks (Fattah *et al*. 2017). Mandal *et al*. (2014) reported higher value of fresh semen motility than the motility percentage of this study. The results of the frozen semen motility higher than the findings of Mandal *et al*. (2014). Mehedi *et al*. (2020) reported lower value than the results of fresh semen motility of this study. Hossain *et al*. (2012) found the fresh semen motility are lower than the present study. In this study, the results of frozen semen motility almost similar with the study of Hossain *et al*. (2012). The results of fresh semen motility in this present experiment agreed with the findings of Donham *et al*. (1926). Sperm motility is one of the best single evidence of viability reported by Davis (1939). Lasley (1943) found that there is no significant difference in the fertility of semen with 55-95% live sperm. The sperm motility of frozen semen lower than the fresh semen in this study may be effect of low temperature. This differences occurred due to cryo-injury caused by cryopreservation resulted impaired motility and poor survival in the female reproductive tract (Salmon and Maxwell, 1995). Primary site of sperm plasma membrane damaged by cryopreservation (Hammerstedt *et al*. 1990; Parks & Graham 1992; Watson 1995). Consequently sperm motility reduced by both freezing and thawing implication (Hammerstedt *et al*. 1990). Kumar *et al.* (2015) reported the both fresh and frozen semen motility respectively 62.77% and 46.11% which is lower than the findings of this study. Rahman *et al*. (2014) and Morrell *et al*. (2018) found lower value of sperm motility than the findings of this study.

Progressive motility: This study reported that the progressive motility of both fresh and frozen semen are respectively 75.26% and 44.32%. Progressive motile sperm are responsible for the fertilization. In normal sperm must be present progressive sperm, otherwise fertilization is not possible. Morrell *et al*. (2018) reported higher value than the result of progressive sperm of frozen semen but lower than value of fresh semen progressive motility. The progressive motility ranged from 88-89% which decreased significantly after freezing evaluated 60-71% in crossbred bulls which is reported by Mehedi *et al*. (2020). He reported average fresh semen progressive motility sperm 84.98% which is higher than the present study. He also reported average frozen semen progressive motility 56.54% which was higher than the value of this study. The progressive motility of fresh semen of this study is similar with the findings of Islam *et al*. (2018). The present results are found slightly higher than Rahman *et al*. (2014), Hossain *et al*. (2012) and Sundararaman *et al*. (2012) and Islam *et al*. (2015). The progressive motility of frozen semen reported in this study slightly smaller than the findings of Lecewicz *et al*. (2015), Dolezalova *et al*. (2016), Baloch *et al.* (2019), Mostari *et al.* (2004), Murphy *et al.* (2018) and Dias *et al*. (2018). This difference might be due to raw semen quality, variation in breed, age and freezing procedure. Post freezing motility also can be affected by dilutors (Belorkar *et al*. 1993; Pramanik and Raina 1998) methods

of glycerol addition (Gilbert & almquist 1978; Arancibia *et al*. 1987) and equilibration time (Belorkar *et al*. 1993). Santoso *et al*. (2020) reported fresh semen progressive motility 75.87% which is similar with the present study. He also reported frozen semen progressive motility 46.52% which slightly higher than the present study. Islam *et al*. (2018) reported progressive motility of fresh semen 74.73 which slightly lower than the present findings.

V. Conclusion

This study can be concluded that fresh semen better than frozen semen. There is significant effect ($p<0.05$) to sperm concentration, semen viability, semen normality, total motility and progressive motility. The significant decreasing of frozen semen occurred due cooling-freezing and thawing procedure. But frozen semen used in field level for the genetic improvement of cattle though fresh semen are better. In our country for increasing production AI is most popular at field level. This result have given an insight to the farmers, researchers, policy makers and entrepreneurs about the quality of fresh and frozen semen to use at field condition in BD. We can recommended that the quality of frozen semen can be improved through adapt special management in AI centre.

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