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Assessment of Frozen Semen Quality and Conception Rate in Cow

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ABSTRACT

This study was conducted in Sadar upazila at Dinajpur district and Ejab Alliance Limited at Thakurgaon district of Bangladesh from March 2017 to February 2018 to investigate the frozen semen quality and conception rate in cow. Total 60 frozen semen straw were collected from six different companies (CCBDF, Ejab, AD, DAIC, Lal-Teer and BRAC) at first week of every month and subsequently up to next ten months. Straw was collected from the dealer of different companies at Upazilla Veterinary Hospital, VTH-HSTU, Dinajpur and Ejab Alliance Limited Breeding Station, Thakurgaon. Then the straw was kept in liquid nitrogen cane and transfer to the laboratory of Ejab Alliance Limited for evaluation**.** The frozen semen straws were thawed at 35-37˚C for 60 seconds and pooled. The post-thaw motility and morphology of spermatozoa were compared and recorded among different season, preservation time, companies and area. Post-thaw motility and number of normal spermatozoa slightly varied on preservation time. Higher motility and number of normal spermatozoa were 73±0.00 and 83±0.75 found in winter season but lowest in summer season and that was 73±0.40 and 82.2±0.47, respectively. The motility and number of normal spermatozoa of BRAC semen were 75±2.27 and 84±1.77 and the values was higher among other companies with lowest in Lal-Teer which were 72±1.61 and 79±3.52, respectively. The conception rate was statistically higher ($p<0.05$) in winter (65%) season, 6 months' storage duration (66%), using BRAC semen (76.5%) and at the area of sadar, Dinajpur (62%). The conception rate was somewhat lower in summer (60%) season, 10 months of duration (62%) using Lal-Teer (53.2%) semen and at the area of Ejab Alliance Ltd. (57%) Thakurgaon.

Keywords: Frozen semen, Spermatozoa, Sperm motility, Assessment, and Conception rate.

INTRODUCTION:

Livestock sector is an integral part of the agricultural economy of Bangladesh. Nearly 85% of total population in Bangladesh is engaged directly or indirectly on agriculture with the livestock sector (Sarma, P. K., and Raha, S. K., 2015). Agricultural development is the main key to alleviate poverty from the country (Gaffar *et al.,* 2004). Nearly 85% of total Statistics showed that about 2 % of national GDP is covered by the livestock sector (Khaleduzzaman *et al.,* 2022), and its annual rate of the productivity is 3.32% (Economic-Review, 2017). The primary function of a bull in a beneficial breeding business is to get a large proportion of cows into calf early, within a mating period of nine weeks or less. To enhance the genetic potential of our native breed. The Artificial insemination (AI) is a worldwide

accepted method of breeding cattle and other species. It is believed that 150 million cows are the artificially inseminated worldwide, but the number of females of other species is unknown (Bonadonna & Succi, 1980). Artificial insemination (AI) contributes to improving dairy productivity and net incomes of livelihood of farmers because a few numbers of highly selected bulls produce large volume of spermatozoa to inseminate a huge number of cows per year. The ultimate goal of AI field services is to maximize the number of viable offspring produced per breeding animal in a given amount of time. This can be accomplished by inseminating cows with a sufficient number of progressively motile spermatozoa from a particular ejaculate without diminishing their fertility. To ensure acceptable pregnancy rates, the limited number of the frozen-thawed spermatozoa in each insemination dose must be of very high quality. Freezing of semen for successful preservation of spermatozoa, for longer period of time, is of great importance in livestock breeding and farm management. Researchers found that the use of frozen semen is one of the spectacular developments in modern-day AI programs and it's could potentially remain viable for 10,000 years. When superior index bulls are the used for a limited amount of time, the frozen semen is useful & economical. This is also a very cost-effective method of the importing exotic germ plasm in the form of deep-frozen sperm straw rather than animal, which is very expensive (Sardar, 2006). When liquid semen is used within the first two days after processing, there are no significant differences in the conception rates between cows inseminated with liquid semen and those inseminated with deep-frozen semen (Satter *et al.,* 1980). The quality of frozen semen can be preserved for years if stored in liquid nitrogen at -196°C (Das *et al.*, 1999; Hassen *et al*., 2022).

Higher viability & motility of spermatozoa are important factors for successful artificial insemination as significant correlation has been reported between the post-thaw sperm viability and the subsequent conception rate (Wongtawan *et al.,* 2006). Duration of the storage period of frozen semen may have great influence on quality and conception of frozen-thawed bull semen (Larsson and Rodriquez-Martinez, 2000). On the contrary, there are many gene-tic and non-genetic factor have direct influence on the increasing conception rate (Gaffar *et al.,* 2004). Freezing and subsequent thawing procedures render reduction of motility and the integrity (Salisbury *et al.,* 1978). They become very sensitive to any stresses by their environment as a result conception failure occur. Taking these factors into account, the current study was designed with goal of evaluating frozen semen quality & cow conception rate and recommending an optimal technique for obtaining maximum conception rate in cattle.

Objectives of the Study

Recently AI is becoming popular in our country, therefore the use of frozen semen for improving the local cattle compromised with several factors. Therefore, the proposed study is designed:

1) To evaluate the quality of frozen semen in the different duration of preservation.
2) To evaluate the conception rate in cow using the frozen semen.

MATERIALS AND METHODS:

Study area

The study was conducted in Sadar Upazila, Dinajpur, Veterinary Teaching Hospital (VTH) of the HSTU and Breeding Bull Station of Ejab Alliance Limited at the district of Thakurgao.

Study period

The duration of the study was one year and conducted from March 2017 to Feb 2018. The total number of in semination were 8511 among which conception were 5190 registered in this respective place of Dinajpur Sadar and Ejab Alliance Limited, Thakurgaon during the course of the study period.

Survey design and sampling

A cross sectional observational study was conducted in different places of Dinajpur town, VTH-HSTU and Ejab Alliance Limited, Thakurgaon. A total of the 60 straws from 6 different companies available in study area were routinely selected for semen sampling and 8155 AI data were collected from AI technicians.

Instruments and Appliance

i. Straw from different companies. ii. Glass slide. iii. Scissors. iv. Syringe. V. Dropper. vi. Hand gloves. vii. Thermometer. viii. Water bath. ix. Microscope (10X, 40X and 100X Lens). x. Bottle. Xi. Slide box. xii. Tissue paper. xiii. Slide. xiv. Marker pen. xv. AI gun.

xvi. Liquid nitrogen cane (-196 degree centigrade).
xvii. Refrigerator.

Fig. 1: Map of study area in Dinajpur sadar.

Table 1: Collection of semen sample.

Areas	Total no. of companies	Total no. of Insemination	No of straw (semen sample) collected
Dinajpur	CCBDF	2799	5
	A.D	655	10
	DAIC	383	10
	Lal-Teer	630	10
	BRAC	1886	10
VTH-HSTU	CCBDF	678	5
Ejab Alliance Limited	Ejab	1480	10

Reagents

1) Eosin stain.
2) Nigrosine stain.
3) Immersion oil.

Laboratory preparation and sterilization of the instrument

All the instruments were properly washed with detergent solution and running tap water and rinsed with 70% alcohol. All items of the glassware soaked in a household dishwashing detergent solution for overnight, contaminated glassware were disinfected in 2% sodium hypochlorite solution prior to the cleaning. The glassware was then cleaned by the brushing, washed thoroughly and finally sterilized either by dry heat at 160 degrees centigrade for 2 hours or by autoclaving at 15 mints. at 121 degrees centigrade under 15 ibs pressure per square inch. Then the instrument was properly dried with drier machine. All the glassware was kept in oven at 50 degrees centigrade for future use.

Methods

Collection of frozen semen

The semen samples were collected from three areas namely Sadar, Dinajpur, Veterinary Teaching Hospital, HSTU, Dinajpur and Ejab Alliance Ltd. Thakurgaon. Semen straw from six different companies were the collected. They were Central Cattle Breeding and Dairy Farm, Ejab Alliance Limited, BRAC, American Dairy, District Artificial Insemination Centre, Dinajpur, Lal-Teer. Samples were collected from the AI technician of these respective companies at first week of every month up to ten months. The AI technician of six companies kept the straw in liquid nitrogen cane., Which were collected directly from them and stored in liquid nitrogen cane. A uniform semen collection, the

evaluation, extension and freezing protocol is followed at the all-bull station of respective companies.

Duration of collection and Shipment of straw

Total 60 straws of 6 different companies were collected at first week of each month up to ten months for this study. Then the collected straw was kept in liquid nitrogen cane. And after the next day of each collection these straws were monthly transferred to the well-organized laboratory of Ejab Alliance Limited for evaluation by own vehicles**.**

Thawing of straw

After shipment thawing was performed to resolute the frozen semen. For thawing water bath was prepared. For thawing water bath was prepared. The water bath was kept in a clean place and connected to the electric switch the temperature was fixed at 35-37 ºc and accurate temperature was determined by observing the reading of thermometer. The frozen straw was taken out from the liquid nitrogen cane. And then immediately thawed at water bath at 37°C for 60 sec.

Evaluation of semen

Individual sample were evaluated for -

1) Concentration
2) Sperm motility,
3) Percentage of live spermatozoa,
4) Proportions of spermatozoa with normal acrosome, mid piece and tail, and with normal head, tail and mid piece morphology

The evaluations of samples were done at every month interval up to 10 months.

Evaluation of Concentration

The post thaw concentration of per straw was average 20 million. For evaluated the concentration at first taken NCN 369 then added 1 drop of semen which diluted with 1 drop of NaCl and sperm were counted.

Evaluation of Motility

A little drop of sperm was placed on a pre-warmed (37°C) slide and inspected under a microscope (40X) without a cover slip to assess sperm motility. The motility was calculated as a percentage by eye-estimating the proportion of spermatozoa moving progressively straight forward at the medium magnification (40X).

Morphological Examination

For the determination of viability, a drop of semen was placed on a glass slide then 2 drop of Eosin and 3 drops of Nigrosine stain was added. The sample was mixed with a clean stick, and a homogenous thin smear was prepared. The smear was observed at a magnification (40X and 100X). Live spermatozoa appeared unstained and dead spermatozoa-stained pink against a brownish purple background. Eosin-Nigrosine stain was used to determine the percentage of spermatozoa morphology. After thawing one drop of semen was taken in a glass slide and two drop of eosin stain were placed closely on this slide, and the semen and the stain were mixed with a clean stick and kept for 30 seconds. Then 3 drop of nigrosine was added and kept for 30 seconds, a thin smear was made, dried in air and examined under microscope (40X and 100X). The abnormalities of the acrosome, mid piece and tail of the spermatozoa were evaluated.

Artificial Insemination

After evaluation of semen AI was done by the quality semen.

Pregnancy diagnosis

Finally, pregnancy was diagnosed by observing the cow non return to the estrous after insemination and waited for next estrous cycle (22-24 days) for confirmation. Rectal palpation was done after 60 -90 days of AI, and the observed closed cervix, tonic uterus, the asymmetry of horn, thinning uterine wall, presence of pregnancy CL then animal was considered pregnant.

Data collection and Processing

Data of conception in cow were collected from the AI technician of respective companies and owner of the cow by using direct interview method.

Statistical analysis

All data were compiled on to SPSS program (Version,16) then analyzed. One-way analysis of variance (ANOVA) was used for semen quality and result were expressed as Mean ± standard deviation. And χ^2 test was used for conception rate. The result was expressed as Mean ± standard error. Difference with values $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION:

The study was conducted in Dinajpur sadar upazila and Ejab Alliance Limited, Thakurgaon of the Bangladesh

from March 2017-February 2018 to investigate the frozen semen quality and conception rate in cow.

Effects of season on semen quality and Conception Rate

The effects of three season summer, rainy and winter on semen quality had not any significant level. The average motility among three seasons presented in the **Table 2**, were in summer 73 ± 0.40, in rainy 73 ± 0.00 and in winter 73 ± 0.00 (Mean ± SD) respectively. There was no any seasonal significant ($p>0.05$) effect in the morphology of the spermatozoa. The value of normal spermatozoa among three seasons were in summer 82.2 ± 0.47, in rainy 82 ± 0.64, in winter 83 ± 0.75. And the value of abnormal spermatozoa in summer 17.8 ± 0.47, in rainy 19 ± 0.64 and in winter 17.3 ± 0.75 respectively. Therefore, it revealed that there were no significant seasonal effects on frozen semen quality.

Table 2: Effects of season on semen quality.

Season	Semen Quality			
	Motility %	Average concentration (Million\Dose)	Morphology (Per 100)	
			Normal	Abnormal
Summer	73±0.40[a]	20	82.2±0.47[b]	17.8±0.47[b]
Rainy	73±0.00[a]	20	82±0.64[b]	19±0.64[a]
Winter	73±0.00[a]	20	83±0.75[a]	17.3±0.75[b]
Significance level	NS			

[a,b,c] *Superscript letters in same column differ significantly ($p<0.05$), NS= Not significant*

In this study, the conception rate was observed in three different seasons that were summer, rainy and winter. In summer, rainy and winter season the total number of inseminations were 2017, 2309 and 2904, following pregnancy were the confirming 1210, 1316 and 1888, respectively. The conception rate was the significantly ($p<0.05$) higher (65%) in winter than summer (60%) and rainy (57%), (**Table 3**).

Table 3: Effects of season on Conception Rate.

Season	Total No. of AI	No. of Pregnant cow	Conception rate%
Summer	2017	1210	60[b]
Rainy	2309	1316	57[c]
Winter	2904	1888	65[a]
Significance level	*		

[a,b,c] *Superscript letters in same column differ significantly ($p<0.05$), * = Significant*

This environmental influence on conception rate was probably exerted through both sexes. The result was concise with Alam and Ghosh, (1988) with their findings as there were the conception rate of the cows significantly differed with seasons of the year due to changes in the nutrition, environmental temperature, and climate and photo period. When temperatures were greater around insemination (Gwazdauskas *et al.,* 1975), cows' CR was significantly lowered. Sonmez *et al.* (2005) & Nabenishi *et al.* (2011), also reported that during summer there was a reduction in the reproductive performance of cows by heat stress. Heat stress caused a reduced the number of conception rate. The follicle developed a low luteinizing hormone environment. Goshu *et al.* (2007) reported that number of services per conception increased significantly with parity number, which was the resemble to this study. Shehab-El-Deen *et al.* (2010) the conception rate was decreased during summer ranged between 18% and 33% compared to the other seasons. Cavestany *et al.* (1985) found that the decrease in conception rate could range between 20% and 30% during the hot season, depending on the severity of heat stress, compared to the cold season. The present research work had some dis-similarities with other studies. Zakari *et al.* (1981) reported that relative humidity, high temperature and poor management affect the conception rate of cow following conception rate (53.07%) in spring significantly higher ($p<0.05$) than the cows inseminated in winter (39.42%). Bilkis *et al.* (2016) reported that the percentage of conception rate was ranges from 50.84 to 76.42% in the studied farms with the highest result

(76.42%) winter and summer season and the lowest (50.84%) in rainy season. According to Al-Katanani *et al.* (1999), the population of small follicles is particularly sensitive to physiological changes produced by heat stress, and developing small follicles injured by heat stress over the summer may ovulate an infertile oocyte, resulting in a reduced conception rate. This observation was similar to the findings of Roth *et al.* (2001) who suggested that heat stress during the hot months had a long-term effect on the antral follicles, which developed into large dominant follicles 40-50 days later. This study found that heat stress reduced the reproductive performance of the dairy and beef cows during the summer.

Effects of preservation duration (Every 1-month interval up to 10 months) on semen quality and Conception Rate

The effect of preservation time on semen quality was significantly ($p<0.05$) varied with the duration. The motility of post-thawed bull spermatozoa in different duration of times sequentially from 1 month to 10 were 73±1.64, 73±1.37, 73±1.21, 73±1.89, 74±2.06, 74± 1.96, 73±1.16, 73±1.04, 73±0.89 and 73±0, respectively, **Table 4**. The numbers of normal and abnor-mal spermatozoa were also significantly associated with duration. The number of normal spermatozoa was 83±2.52, 84±3.14, 84±2.25, 83±5.85, 83±2.16, 85± 2.16, 84±2.09, 83±1.16, 83±1.09 and 83±1.26 respectively in 1-10 months of preservation. The numbers of abnormal spermatozoa were 17±2.52, 16±3.14, 16±2.25, 17±5.85, 17±2.16, 15±2.16, 16±2.09, 17± 1.16, 17±1.09 and 17±1.26 in 1-10 months of preservation duration respectively. The semen quality was slightly varied with the different preservation time.

N.B: Sperm abnormalities: Double head, Swollen and Folded tail, Proximal and distal droplet in neck and tail, Head less tail, Tail less head.

Table 4: Effects of preservation duration (Every 1-month interval up to 10 months) on semen quality.

Months	Semen Quality			
	Motility %	Average concentration (Million\Dose)	Morphology (Per 100)	
			Normal	Abnormal
1	73±1.64^{b}	20	83±2.52^{c}	17±2.52^{a}
2	73±1.37^{b}	20	84±3.14^{b}	16±3.14^{b}
3	73±1.21^{b}	20	84±2.25^{b}	16±2.25^{b}
4	73±1.89^{b}	20	83±5.85^{c}	17±5.85^{a}
5	74±2.06^{a}	20	83±2.16^{c}	17±2.16^{a}
6	74±1.96^{a}	20	85±2.16^{a}	15±2.16^{c}
7	73±1.16^{b}	20	84±2.09^{b}	16±2.09^{b}
8	73±1.04^{b}	20	83±1.16^{c}	17±1.16^{a}
9	73±0.89^{b}	20	83±1.09^{c}	17±1.09^{a}
10	73±0.09^{b}	20	83±1.26^{c}	17±1.26^{a}
Significance level	*		*	*

^{a,b,c} *Superscript letters in same column differ significantly ($p<0.05$), * = Significant*

Table 5: Effects of preservation duration (1-10 months) on Conception Rate.

Months	Total No. of AI	Pregnant cow	Conception Rate%
1	545	349	64^{b}
2	533	336	63^{bc}
3	589	365	62^{c}
4	433	268	62^{c}
5	478	306	62^{c}
6	317	209	66^{a}
7	261	167	64^{b}
8	640	403	63^{bc}
9	423	262	62^{c}
10	430	267	62^{c}
Significance level	NS		

^{a,b,c} *Superscript letters in same column differ significantly ($p<0.05$), NS= Not significant*

The study revealed that the conception rate was the slightly higher (66%) in 6 months of duration than the others (**Table 5**). Though there was slightly variation in conception rate on the basis of the duration but statistically it was not significant (p>0.05). This result was agreeing with Malik *et al.* (2015) who reported that the sperm abnormalities were the significantly (p>0.05) reduce for long time preservation though it is not considerable in earlier period of preservation. Chatterjee and Gagnon *et al.* (2001) reported that the possible damage to spermatozoa after a long time storage in liquid nitrogen that had been generated genetic damage of spermatozoa. Cryopreservation was also inducing damaged to the spermatozoa after thawing, which was similar to this study. Fraser *et al.,* (2007) demonstrated that the prolonged storage had effects to sperm motility, mitochondrial function and morphology. As a result, sperm motility was reduced; Wongtawan *et al.* (2006) reported Length of the storage period of frozen semen had influence on quality and fertility of frozen-thawed bull semen.

According to the research of Larsson and Rodriquez-Martinez, (2000) freezing and the subsequent thawing methods cause the remaining surviving spermatozoa to be physio-logically different from spermatozoa prior to cryopreservation. There was also some dissimilarity that was (Friedmann *et al.,* 1979) stated that the upper limit for preservation in the liquid nitrogen had been estimated to be at least 200 years. Gao *et al.* (1997) declared that no chemical reaction was occur below-120°C in human relevant times and thermally driven reactions were impossible at-196°C. Whittingham *et al.* (1977) suggested that the risk of basal background radiation during storage in liquid nitrogen for several years was insignificant for genetic stocks, which was also inconsistent with this result. Cassou and Roettger *et al.* (1977) reported no difference in fertility when were used frozen semen stored at −196°C for up to 4.5 and 5 years, respectively. As a direct result of "hits" by background ionizing radiation or cosmic rays".

The background ionizing radiation was for a factor that was eventually damaged the DNA. Chatterjee and Lessard *et al.* (2000) reported that loss of the sperm surface proteins, which were necessary for the fertilization, and the result of long storage periods.

Effects of the companies on semen quality and preservation of semen on conception rate

The main data set was used to estimate the significant effect of companies on frozen semen quality before AI. The present study revealed in **Table 6** that higher motility was found in the BRAC semen, which was 75±2.27, and lower motility was 72±1.61 in Lal-Teer. The higher number of normal spermatozoa found in BRAC semen that was 84±3.77 and lower numbers of normal spermatozoa 79±3.52 found in Laal-Teer. The numbers of the normal spermatozoa were 81±1.03, 83±1.63, 84±2.87, and 84±2.27 respectively in CCBDF, EAL, DAIC and AD.

Table 6: Effects of companies on semen quality.

Companies	Semen Quality			
	Motility%	Average concentration (Million\Dose)	Morphology (Per 100)	
			Normal	Abnormal
CCBDF	73±1.31^{bcd}	20	81±1.03^{bc}	19±1.03^{b}
Ejab	73±1.19^{bcd}	20	83±1.63^{b}	17±1.63^{bc}
A.D	73±1.59^{bcd}	20	84±2.27^{a}	16±2.27^{c}
DAIC	74±2.26^{abc}	20	84±2.87^{a}	16±2.87^{c}
Lal-Teer	72±1.61^{cd}	20	79±3.52^{bc}	21±3.52^{a}
BRAC	75±2.27^{ab}	20	84±3.77^{a}	16±3.77^{c}
Significance level	*		*	*

^{a,b,c} *Superscript letters in same column differ significantly (p<0.05),* * *= Significant*

The semen quality was varied among different companies. The overall quality of semen was found higher in companies than government production. The motility and morphology of semen of spermatozoa was also higher in companies.

Table 7: Effects of preservation companies on conception rate.

Name of companies	Total No. of AI	No. of pregnant cow	Conception Rate %
Ejab	1256	772	61.5[b]
A.D	346	219	63.4[b]
DAIC	253	161	63.8[b]
Lal-Teer	306	163	53.2[c]
BRAC	1357	1038	76.5[a]
Significance level	*		

[a,b,c] *Superscript letters in same column differ significantly (p<0.05),* * *= Significant*

As shown in **Table 7**, the average conception rate in private companies that stored sperm was higher than in government production. The conception rate was the significantly higher (p<0.05) in BRAC semen (76.5%) among the different companies with lower value in Laal-Teer semen (53.2%). This result was in agreement with Hossain *et al.* (2015) who reported that the pregnancy rate was higher (58.5%) when the BRAC-derived semen was used compared to semen from DLS (46.7%) these differences were not significant which was dissimilar with this study. The present finding was consistent with other investigators where cows inseminated with frozen semen derived from BRAC showed higher (61.6%) pregnancy rate than that of semen derived from DLS (55.0%) Shikder, (2011). The reason for lower pregnancy rate in cow inseminated with DLS derived semen may be that the quality of semen of DLS might be inferior. Sarder, (2006) conducted a study that found that the conception rate was significantly higher after insemination with frozen sperm from CCBSDF, Savar, and Dhaka than after insemination with locally produced frozen sperm from the RDCIF, Rajabarihat, and Rajshahi, and that the locally produced frozen sperm had more damaged spermatozoa than the frozen sperm from CCBSDF, Savar, and Dhaka, which was not the case in this study. This indicated a lack of precision in the freezing procedure.

Effects of different placement on Conception Rate

Table 8 shows that a total of 8511 cows were inseminated in three areas: 6343 in Dinajpur town, 688 in VTH-HSTU, and 1480 in Ejab Alliance Limited. The number of pregnant cows among 3 areas were 3933, 413 and 844, respectively. The average conception rate of cows was 62%, 60% and 57% in Dinajpur sadar, VTH-HSTU and Ejab Alliance Limited, respectively which was statistically significant (p <0.05).

Table 8: Effects of different placement on conception rate in cow.

Placement	Total No. of AI	Pregnant cow	Conception Rate %
Dinajpur Sadar	6343	3933	62[a]
VTH-HSTU	688	413	60[a]
Ejab Alliance Limited	1480	844	57[b]
Significance level	*		

**= Significant (p<0.05), VTH= Veterinary Teaching Hospital, HSTU=Hajee Mohammad Danesh Science and Technology University*

Some of the scientist agreed and disagreed with this study. Nordin *et al.* (2004) declared that, the better conception rate obtained in the Jasin region, compared with the other regions, it might be associated with the influence of other factors such as feeding system, AI technicians and proportion of time spent on dairying activities. The less skillful AI technician, which were due to the shorter AI training duration had caused improper insemination such as depositing semen in the cervix, which thus contributed to the reduce in the conception rate and his study had resemblance with this study. Magaña and Segura, (2001); Osorio and Segura, (2002) Ayres *et al.* (2014) agreed with this study that they reported that the overall conception rate (44.6%), confirms the low reproductive level in the tropical regions, which was managed under extensive conditions. This result was poor when it was compared to the values of 60 and 79. Campbell *et al.,* (1996)

observed a conception rate of 36.9% in Zebu cattle which was also similar to this study. Siddiqui *et al.* (2003) declared that, Conception rate (%) was differed depending on the different place of the study area, Conception rate was lower in Region-3 (Khulna and Satkhira) than the other regions which had resemblance with this study. Nagamine *et al.* (2013) evaluated the effect of environmental factors in Japan, particularly meteorological conditions, on the fertility of the Holstein-Friesian cattle breed by examining conception rates in different locations.

Sperm abnormalities

There are many abnormalities in sperm. This study found such abnormalities in sperm below. (Double head, Swollen and Folded tail, Proximal and distal droplet in neck and tail, Head less tail, Tail less head).

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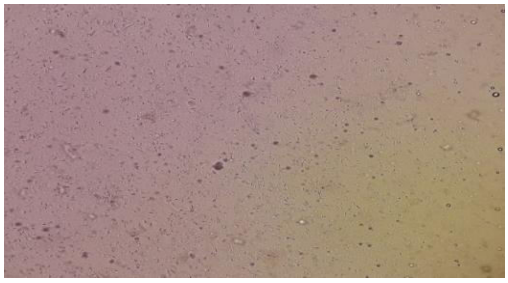

Fig. 2: Motility of spermatozoa.

Fig. 3: Morphology of spermatozoa.

Fig. 4: Folded tail.

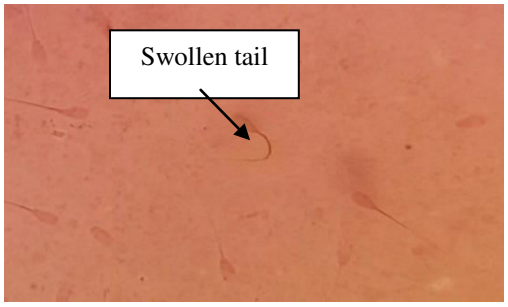

Fig. 5: Swollen tail.

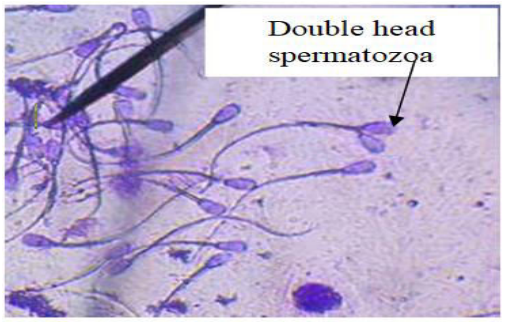

Fig. 6: Double head spermatozoa.

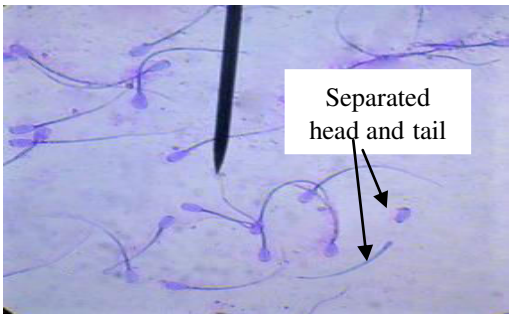

Fig. 7: Separated head and tail.

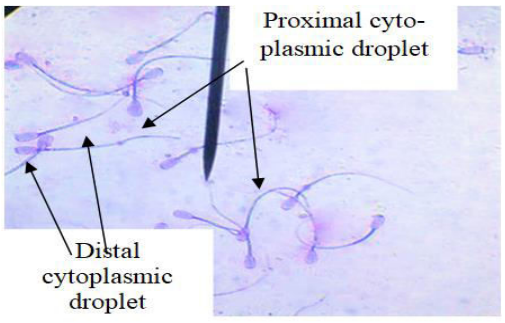

Fig. 8: Proximal and Distal cytoplasmic droplet.

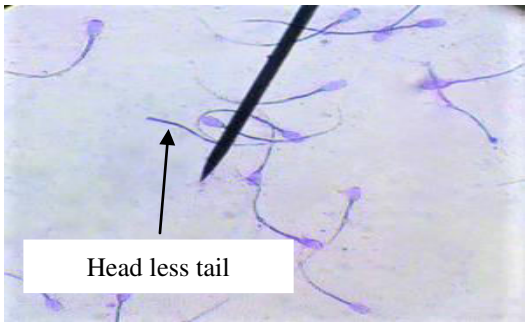

Fig. 9: Head less tail.

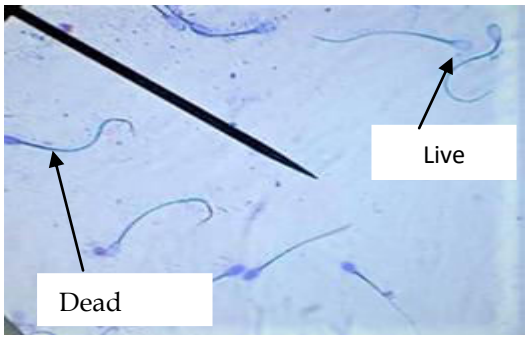

Fig. 10: Dead and Live Spermatozoa.

CONCLUSION AND RECOMMENDATIONS:

Livestock is an important part for supporting the livelihoods of poor farmers, consumers, traders and laborers in developing countries like the Bangladesh. Fertility Improvement is a common goal for many dairy herds or livestock owners. Conception rates are influenced by a wide variety of factors in which semen presservation duration, areas and seasons are very important. The present study was assisted to conclude regarding frozen semen quality and conception rate in cows as follows:

1) The semen quality mainly the sperm motility and percent of normal sperm were slightly varied with preservation duration.
2) The average semen quality and conception rate were higher in cow which inseminated through BRAC semen.
3) The conception rate was higher (65%) in winter than summer and Rainy season.
4) The overall conception rate of frozen semen was higher (62%) at Dinajpur sadar than VTH, HSTU (60%) and Ejab Alliance Ltd. (57%).

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CONFLICTS OF INTERESTS:

The authors declare that there is no conflict of interest.

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