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Effect of Skim Milk and Tris-citrate Extenders to Preserve the Semen of Indigenous Ram of Bangladesh

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Authors' contributions

This work was carried out in collaboration among the authors. Authors MSR and NSJ designed the study. Author NSJ wrote the protocol. Authors MSR and MMR collected the semen samples. Author MSR carried out the experiments. Authors NSJ and FYB supervised the experiments. Author MRG performed the statistical analysis. Authors MSR and MRG wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In recent years, there has been an increased interest in the sperm preservation and maintenance of genetic resources of small ruminants. The study aimed to determine and compare the preservation effects of skim milk and Tris-citrate on the quality of chilled and frozen-thawed indigenous ram semen (n=13) during rainy monsoon season (June to October) in Bangladesh. Semen was collected from four indigenous rams by artificial vagina method to observe motility, viability and membrane integrity at 0, 24, 48, 72 hrs of storage. The hypo-osmotic resistance test (HOST) was used to evaluate the sperm membrane integrity. The ejaculates were divided into two groups according to dilution with skim milk or Tris-citric acid egg yolk media for chilling and freezing. Motility, viability and membrane integrity were significantly ($p<0.01$) higher in chilled semen samples diluted with skim milk in comparison to those of semen diluted with Tris-based

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extender at all time-based observations. However, around 50% motility was preserved up to 48 hours by both extenders. Results also showed that motility, viability and HOST (+ve %) decreased drastically after cryopreservation irrespective of diluents. Similar to chilled semen, studied parameters were significantly higher ($p<0.05$) in frozen-thawed semen diluted with skim milk based extender, though quality parameter of frozen-thawed semen was not satisfactory. In conclusion, skim milk based extender appears to be a better option compared to Tris based extenders for chilling semen of indigenous ram up to 48 hours with acceptable quality. Whether the differences found between the extenders will be reflected in the fertility results after artificial insemination (AI) is yet unknown and needs to be further studied.

Keywords: Indigenous ram semen; skim milk; Tris-citrate; motility; viability; HOST.

1. INTRODUCTION

There are about 3.34 million sheep in Bangladesh [1] which plays an important role in the rural economy of Bangladesh supplying meat, wool and skin. Nowadays, due to the high demand of meat and low price of sheep meat, a good number of people, not only rural people but also urban people, prefers sheep meat [2]. Other special attributes of sheep over the other livestock resources include that they are highly adaptable to broad ranges of environment, have short generation cycles, and have high reproductive rates which lead to high production efficiency and poor people can afford few ewes since cost of them is less than a cow [3]. Despite these excellence characteristics, sheep is the lowest in production potential compared to other domestic animals. This is largely due to low improvement in its reproductive potentials. Since Bangladesh has no specific sheep breed, superior ram selection and selective breeding by the use of modern reproductive biotechnology such as Artificial Insemination (AI) seem to be a very important and alternative approach to develop and boost up the production potential of this animal [4]. AI may be regarded as the first generation assisted reproductive technology that is the most widely used and the one that has made the most significant contribution to genetic improvement worldwide [5]. The advantage of AI over natural mating is in its increased number of offspring per sire, allowance of spatial and temporal dissociation between a collection of spermatozoa and fertilization, limitation of disease spread and decrease offspring generation interval [5]. The success of AI in sheep is based on the ability to efficiently collect, evaluate and preserve semen from quality rams [6] and the place of semen deposition also an important factor to the success of AI. In order to maximize the economic benefit of AI, there is a need for an extension of the semen and its preservation.

Semen preservation techniques in small ruminants have been expanded in the last decades to meet the large demands for artificial insemination requirements [7]. Artificial insemination in sheep using fresh or cooled diluted semen has had acceptable success in many countries. Cooled, diluted semen is a good alternative to frozen semen when it is used within a short period of time after collection. As freezing and thawing causes injury to sperm, frozen-thawed semen has generally recorded poorer quality and fertility, compared to cooled diluted or fresh semen [8]. However, the quality and viability of the sperm during liquid storage gradually deteriorates, as the days of storage increase [9]. Sperm characteristics and semen parameters, such as sperm morphology, concentration, viability, capacitation status, acrosomal integrity, osmotic resistance and motility used to evaluate the semen quality of cooled diluted semen and frozen-thawed semen [10].

Extenders have been used to protect and maintain spermatozoa during cryopreservation of the semen as well as to increase ejaculate volume. Good extender must have equal osmotic pressure to the seminal plasma; buffering capacity; protect sperm from cold shock; nutrient for sperm metabolism; controlling microbial contamination; protect sperm against freezing-thawing damages and preserve sperm viability without more decline in fertility [11]. At present, Tris egg yolk based and milk-based extenders are generally used for liquid ram semen storage. While soy lecithin-based extenders have also been tested in different animal species to replace the animal protein component in the situation where there may be a high risk of exotic animal diseases. So, for example, soybean extract based extenders have been shown to have an acceptable cryo-protective effect on ram sperm [12]. However, these extenders were not superior to egg-based extenders. It may thus be a good

animal protein free alternative if there is a concern regarding microbial contamination. Milk is a commonly used component of semen extenders in most species, having good performance both *in vitro* and *in vivo* [9,13]. Skim milk proteins buffer semen pH and may also chelate any heavy metal ions [13]. The addition of egg yolk to skim milk extender improves the viability of spermatozoa during chilled storage [14]. It has also been speculated that milk and egg yolk have a synergistic protective effect on sperm because of their analogous action of the sequestration of seminal plasma proteins. In this context, skim milk with or without egg yolk could be an inexpensive, practical diluent for ram semen.

Preservation of ram semen in Bangladesh had not been established very well although indigenous sheep were reared from many years ago. Preservation of semen of indigenous rams by chilled semen technique is poorly used due to lack of information and shortage in the field of application programs of AI and genetic conservation programs of indigenous sheep. Therefore, the objectives of this study were to observe and compare the efficacy of skim milk and Tris-citrate extenders to preserve the semen of indigenous ram of Bangladesh, particularly the viability, progressive motility and membrane integrity of sperm in terms of refrigeration and cryopreservation.

2. MATERIALS AND METHODS

2.1 Animals and Semen Collection

The work was conducted at the Reproduction Laboratory of the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh. Four adult rams, 24 to 48 months of age and 14 to 16 kg body weight, were used for the study. The general health of the selected animals for this experiment was clinically examined and all rams were apparently healthy. Prior to semen collection, the prepuce of the ram was cleaned to prevent semen contamination. Semen was collected by Artificial Vagina (AV) method. Donor rams were allowed at least 1-2 false mounts before collection of each ejaculation. Semen was collected once in a week and a total 13 ejaculates were collected from used rams during during rainy monsoon season (June to October). Collection was always performed in the morning (9:00 – 10:00 AM). After collection, semen was kept at 37°C in water-bath until the media were added with it.

2.2 Experimental Design

A total of 13 semen samples collected from 4 rams were evaluated. Pooled semen samples were used for the experiment due to low volume of the semen. For refrigeration, each pooled sample was divided into two groups according to extender used. Later each group was divided into three subgroups according duration of preservation *ie*, 24 hrs, 48 hrs and 72hrs. For freezing, semen sample was divided into two groups named as Tris and Skim groups depending on extender used. Extenders used for refrigeration and cryopreservation were prepared in same way.

2.3 Extender Preparation

Extender Tris-based egg yolk (T) was made of Tris (2.42% w/v), citric acid (1.36% w/v), fructose (1% w/v), egg yolk (20% v/v), penicillin (100,000 IU) and streptomycin (100mg), and divided into Part-T1 (without glycerol) and Part-T2 (with 7% glycerol). Extender skim milk based extender (S) was prepared from non-fatty milk powder (11%, w/v) and distilled water, heated to 95°C for 10 min, and after cooling to room temperature egg yolk (5%, v/v), penicillin (100,000 IU) and streptomycin (100mg) were added. Extender S was divided into Part-S1 (without glycerol) and Part-S2 (with 7% glycerol). Extenders Part-T2 and Part-S2 were kept at 4°C until use, whereas, Part-T1 and Part S1 were kept at 37°C in water bath until use.

2.4 Preservation Methods

Two-step dilution method was used to freeze the semen. After collection and proper evaluation, the pooled sample was divided into two parts and diluted with extenders Part T1 and S1 at a concentration rate of 400×10^6 spz/ml accordingly. Viability, motility and plasma membrane integrity tests were performed immediately after dilution of semen and parameters were considered for preservation for 0 hrs. For refrigeration, some straws from both groups were kept at 4°C for observation after 24, 48 and 72 hrs. Two straws were used for each observation. For freezing, diluted semen and extender Part T2 were transferred to a refrigerator for two hours (Tris base medium). In case of skim milk, diluted sample and Part S2 were kept half an hour at 4ºC. After the designated time, the calculated

amount of extenders Part T2 and S2 were poured into the previously diluted semen in three divided parts and filled into the straws. using micropipette. The sealed straws were placed in the refrigerator at 4°C for further equilibration at 2 hours. The freezing was done in liquid nitrogen vapour (temperature -80°C) in a special box for 5-6 minutes. After that straws were transferred into the cryocan at -96°C.

2.5 Thawing of Semen

Semen was thawed by plunging the frozen straws in a water bath at 38-40 C for 10-12 seconds [14].

2.6 Evaluation of Semen

To study the preservation effect of extenders, the following semen parameters were evaluated:

Volume: The volume of semen was measured by reading the graduated tube [15].

Colour: Colour was observed by the naked eye in the collecting tube immediately after collection.

Density: Density was measured observing the viscosity of sperm against the slant position of the tube. Density was scored in 4 scales: 1=watery, 2=milky, 3=creamy, 4=creamy to grainy.

Mass activity: To evaluate the mass activity, a small drop of semen was placed on a pre-warmed slide (37°C) without any cover slip and examined under microscope equipped with phase-contrast optics (100×). The mass activity was scored according to Avdi et al. [16] into 5 scales: 1=no motion, 2=free spermatozoa moving without forming any waves, 3=small, slow moving waves, 4=vigorous movement with moderately rapid waves and eddies and 5=dense, very rapidly moving waves and distinct eddies.

Concentration: The concentration of spermatozoa was determined by means of a hemocytometer [17].

Motility: Progressive motility was determined according to Soltanpour and Moghaddam [11]. Briefly, a drop of 0.5µl semen diluted with Tris at 1:4 ratio was placed on a clean pre-warmed slide (37°C) and covered with a cover slip. The motility was determined by eye-estimation of the proportion of spermatozoa moving progressively straight forward at higher magnification (400×) and expressed as percentage.

Plasma membrane integrity: Hypo-osmotic swelling test (HOST) was used to evaluate the functional integrity of the sperm plasma membrane according to Mansour [18]. Briefly, semen samples were diluted 1:10 (v/v) *i.e.* 20µl semen diluted with 200µl of a 100mOsm hypo-osmotic solution (made of 9g fructose and 4.9g sodium citrate in 100 ml distilled water) and incubated at 37°C for 60 min before examination with a phase contrast microscope (400×). A total of 200 spermatozoa were assessed for their swelling ability in hypo-osmotic solution. The swollen spermatozoa determined by coiling of the tail were considered to have an intact plasma membrane (HOST+ve).

Viability: Viability of sperm was assessed by means of the eosin-nigrosin staining according to Salamon and Maxwell [19].

2.7 Statistical Analysis

The data were entered in Microsoft Office Excel 2007 program, sorted and descriptive statistical was done to get the mean ± SD value. One way ANOVA was done by using SPSS 17.0 package to find out the significant difference between the parameters.

3. RESULTS

3.1 Evaluation of Indigenous Ram Fresh Semen

A total of thirteen samples were collected from the indigenous rams. Values of different sperm quality parameters are shown in Table 1. The color was creamy white to white. Mean values of volume, density, mass activity, concentration, motility, viability and plasma membrane integrity were 0.42 ml, 2.8, 3.27, 1.60×10^9/ml, 78%, 82% and 80%, respectively.

3.2 Effect of Extenders on Motility of Chilled Semen

The sperm motility in refrigerated semen at different durations is presented in Table 2. Motility was 78% in fresh semen. Whereas, after administration of skim milk and Tris, motility reduced to 75.2 ± 0.7% and 72.5 ± 0.9%, respectively. It was found that motility decreased gradually with the increment of duration of preservation in both groups irrespective of extenders, though around 50% motility was

preserved up to 48 hours by both extenders. Motility of sperm between S and T groups varied significantly ($p<0.05$) at different period of time studied (Table 2).

3.3 Effect of Extenders on Plasma Membrane Integrity of Chilled Semen

Plasma membrane integrity was evaluated by determining HOST (+ve %) of sperm in chilled semen at different duration and values are presented in Table 3. We observed 80% HOST (+ve) spermatozoa in fresh semen, which decreased to 76.6 ± 0.6% and 71.0 ± 0.6% when fresh semen was diluted with skim milk and Tris, respectively. It was found that percentage of HOST (+ve) spermatozoa decreased significantly ($p<0.01$) and gradually with the increase of time of preservation in both groups. Results showed that percentage of HOST (+ve) spermatozoa were significantly ($p<0.01$) higher in semen samples diluted with skim milk at different observations in comparison to that diluted with Tris (Table 3).

3.4 Effect of Extenders on Viability of Chilled Semen

The viability of sperm in refrigerated semen at different duration is presented in Table 4. Viability of fresh semen was 82% and it decreased gradually with the increment of duration of preservation in both groups irrespective of diluents. At 0 hour, immediately after administration of skim milk and Tris, viability was 77.8 ± 0.8% and 75.0± 0.5%, respectively and variation was not significant ($p>0.05$). At 24 hour, also insignificant ($p>0.05$) variation was observed whereas, viability varied significantly ($p<0.01$) between skim milk and Tris groups when observed at 48 and 72 hours.

3.5 Effect of Extenders on Different Quality Parameters of Frozen-thawed Semen

The motility, HOST (+ve%) and viability of sperm in freezing semen are presented in Table 5. Motility of sperm in frozen-thawed spermatozoa

Table 1. Characteristics of fresh semen of studied indigenous rams

Parameters	Volume (ml)	Density (1-4)	Mass activity (1-5)	Concentration ($\times10^9$ spz/ml)	Motility (%)	Viability (%)	HOST (+ve %)
Ejaculate-1	0.40	2.50	3.00	1.60	80	84	83
Ejaculate-2	0.45	3.00	3.00	1.50	77	81	78
Ejaculate-3	0.35	3.20	3.50	1.45	75	80	79
Ejaculate-4	0.44	2.60	3.00	1.70	82	86	78
Ejaculate-5	0.45	2.70	3.50	1.55	78	82	81
Ejaculate-6	0.40	2.90	3.30	1.60	78	85	83
Ejaculate-7	0.41	2.60	3.50	1.60	76	82	79
Ejaculate-8	0.42	2.50	3.00	1.55	77	78	76
Ejaculate-9	0.44	3.30	3.20	1.65	80	83	79
Ejaculate-10	0.45	3.00	3.30	1.40	79	82	83
Ejaculate-11	0.50	2.70	3.70	1.80	81	82	81
Ejaculate-12	0.38	2.50	3.00	1.60	75	79	78
Ejaculate-13	0.35	2.90	3.50	1.80	82	84	82
Mean	0.42	2.78	3.27	1.60	78.46	82.15	80

Table 2. Motility of sperm in chilled semen observed at different preservation times. Motility of sperm varied significantly between the extenders studied (n=13)

Extenders	Sperm motility (%)				
	Fresh	0 hour	24 hours	48 hours	72 hours
Skim milk	78.5	75.2 ± 0.7*	61.8 ± 0.6*	52.4 ± 0.6*	45.2 ± 0.8*
Tris	78.5	72.5 ± 0.9	58.8 ± 0.9	49.8 ± 0.5	42.4 ± 0.7

** $p<0.05$*

were 20.00 ± 2.1% and 14.83 ± 1.01% in skim milk and Tris groups, respectively and variation was statistically significant ($p<0.05$). Viability (%) and HOST (+ve %) of sperm in frozen-thawed spermatozoa also varied significantly ($p<0.05$) between skim milk and Tris groups. Moreover, all quality parameters decreased drastically after cryopreservation irrespective of diluents (Figs. 1, 2, 3). Overall, results of present study summarize that skim milk extender gave better protection during preservation process in terms of chilling and freezing of indigenous ram semen.

Table 3. Plasma membrane integrity (HOST +ve %) of sperm in chilled semen observed at different preservation times. Plasma membrane integrity varied significantly both between the extenders and also preservation times studied (n=13)

Extenders	HOST (+ve %)				
	Fresh	0 hour	24 hours	48 hours	72 hours
Skim milk	80	76.6 ± 0.6**	63.8 ± 0.5**	54.3 ± 0.6**	47.5 ± 1.1**
Tris	80	71.0 ± 0.6	58.4 ± 0.6	48.0 ± 0.6	40.4 ± 0.8

***p<0.01*

Table 4. Viability of sperm in chilled semen observed at different preservation times. Viability varied significantly at 48 and 72 hours between the studied extenders (n=13)

Extenders	Viability (%)				
	Fresh	0 hour	24 hours	48 hours	72 hours
Skim milk	82.2	77.8 ± 0.8	64.4 ± 0.6	54.5 ± 0.6**	48.2 ± 0.8**
Tris	82.2	75.0 ± 0.5	62.8 ± 0.5	51.8 ± 0.5	44.5 ± 0.6

***p<0.01*

Table 5. Motility, HOST (+ve %) and viability of sperm in post thawed semen. The studied parameters varied significantly between the extenders (n=13)

Extenders	Frozen-thawed semen		
	Motility (%)	HOST (+ve %)	Viability (%)
Skim milk	20.00 ± 2.1*	17.8 ± 2.1*	23.00 ± 1.9*
Tris	14.83 ± 1.01	12.3 ± 0.9	18.2 ± 1.1

**p<0.05*

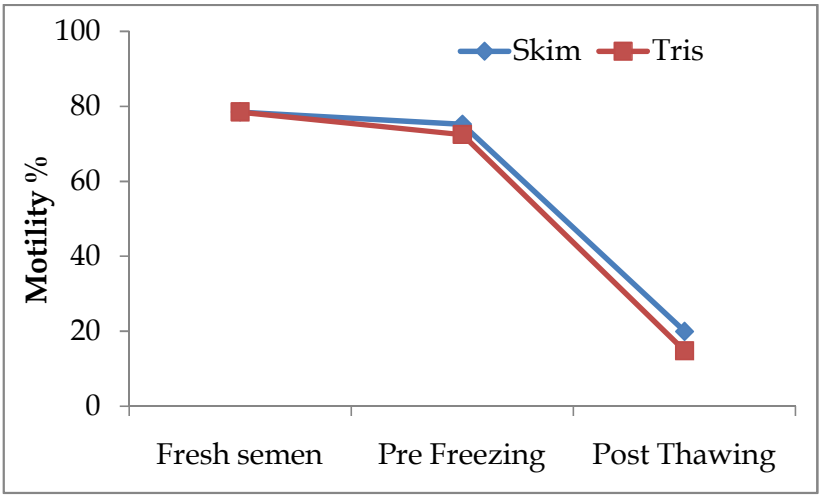

Fig. 1. Motility (%) of sperm in fresh, pre-freezing and post thawing indigenous ram semen preserved by different extenders (n=13)

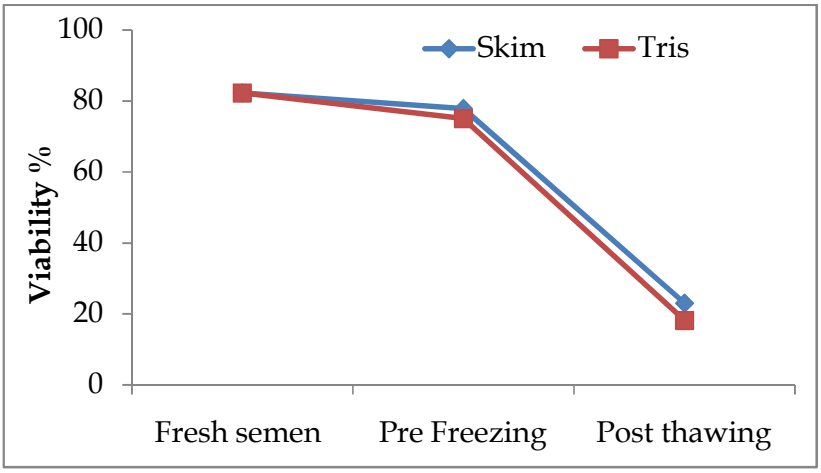

Fig. 2. Viability (%) of sperm in fresh, pre-freezing and post thawing indigenous ram semen preserved by different extenders (n=13)

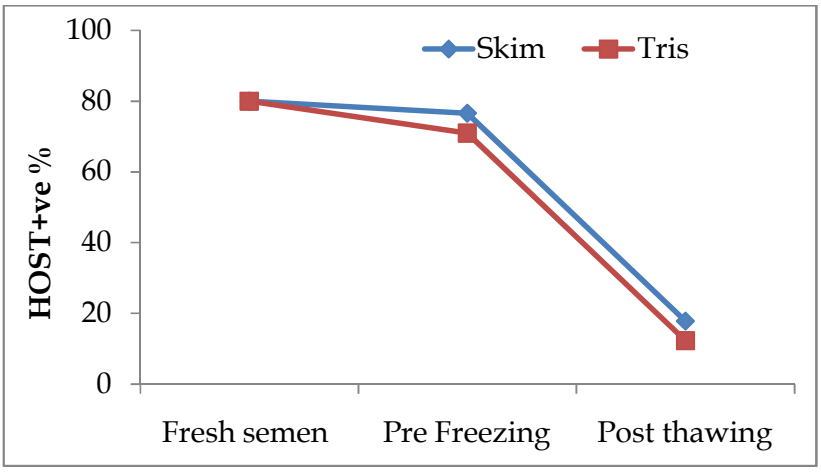

Fig. 3. HOST (+ve %) of sperm in fresh, pre-freezing and post thawing indigenous ram semen preserved by different extenders (n=13)

4. DISCUSSION

Quality semen is the prerequisite for obtaining a good fertility rate in animals. The ram semen varies from milky white to pale creamy in color [20]. The color of indigenous ram fresh semen was creamy white to white in the present study. Volume per ejaculates, motility, sperm concentration and the percentage of normal sperm are the common criteria for evaluating semen quality [21]. The ejaculate volume generally ranges between 0.5 and 2ml in mature rams collected using AV [22]. However, Hafez and Hafez [23] has reported that age of the ram and body condition, season of the year, skill of the technician and the frequency of collection affected the ejaculate volume. We got 0.42 ml ejaculate (in average) from the rams used in this study. Mass activity is the sum of concentration and cumulative motility of the spermatozoa. It is most subjective test and can vary from individual to individual [24]. In the present study, the mean mass activity was 3.3 in 1-5 scale. The sperm plasma membrane functional status is of particular importance since transportation of compounds across plasma membrane is an important biochemical process for sperm viability and maintenance of fertilizing capability [25]. Hypo-osmotic swelling test (HOST) was performed to evaluate the membrane biochemical activity along with evaluation of physical intactness of plasma membrane with vital stains. HOST provides a simple, effective and convenient mean of evaluating viability of spermatozoa [26]. Mean values of concentration, motility, viability and plasma membrane integrity (HOST +ve %) of fresh semen were 1.60×10^{9}/ml, 78%, 82% and 80%, respectively in this study.

Considering the fresh semen characteristics of indigenous ram of Bangladesh, we found the lower volume, mass activity and concentration and the similar motility and viability to Ghezel ram observed by Rasteh and Divandi [27] and lower volume, similar mass activity and higher motility than Awassi rams observed by Albiaty et al. [28] in Iraq. The differences among studies may be due to age of the ram, body condition, season of the year, climate and breed differences.

Extensive research has been conducted in the last few decades on ram semen diluents, semen processing, freezing and thawing methods for improving the post-thaw viability and membrane integrity of motile sperm cells [14,29]. Diluents extend the period of semen storage, improve the motility, reduce the degree of cellular damage, improve the acrosomal integrity, and increase the viability and fertilization capacity of sperm in vitro [30]. Many extenders have been developed as a result of the discovery that sperm in whole semen lived for only short periods of time and that cooling whole semen very slowly to 5°C caused the death of many spermatozoa. Therefore, apart from increasing the ejaculate volume the extender protects the sperm during cooling as well as extends the life of the spermatozoa [31]. Extenders differ in composition, depending on species, method, temperature of diluted semen storage, and the desired duration of storage [7].

Diluted chilled semen provides an efficient and successful means of short-term storage though it has some adverse effects on the spermatozoa [7]. In the present study, the milk-based extenders preserved sperm motility over longer time than tris-based extender in refrigeration. We observed that motility of semen were 61.8± 0.6% vs 58.8 ± 0.9% at 24 hours and 52.4 ± 0.6% vs 49.8 ± 0.5% at 48 hrs in S and T groups, respectively. These findings were in agreement with the results of Badi et al. [32], Acharya [33] in ram and Koray and Ali [34] in goat. Contrary to our results, Quan et al. [35] and Gündoğan [36] reported better spermatozoa motility and membrane integrity rates in a Tris-based extender than in skimmed milk extenders. Lopez et al. [37] observed no differences between sodium citrate-, Tris-, and milk-based extenders when subjected to liquid storage at 4°C. The plasma membrane integrity and the percentage of live spermatozoa (viability) were also significantly higher in skim milk extender compared to Tris–citrate extender. This result made an agreement with finding of Quan et al. [35] and Lopez-Saez et al. [38] in liquid storage of ram spermatozoa. This study demonstrated that the extenders and number of days in storage were significantly associated with the deterioration in the sperm motility, viability and osmotic resistance parameters. This finding supports the findings of Benmoula et al. [39] and Kasimanickam et al. [40] who have also revealed that there is a gradual decrease in motility and morphological integrity, and a rapid decrease in fertility. We found around 50% motility was preserved up to 48 hours by both extenders. The motility obtained in this study is in consistent with some previous studies which showed that optimal motility rate (≥50%) can be achieved using cold liquid semen stored for 2-4 days [14, 40]. Meyers [41] explain the reason for motility decline that the osmotic regulation in lower temperatures affects motility and intact membrane of the spermatozoa, so, the significant differences in motility and acrosome defect percentages between the extenders may due to the differences in osmotic regulation capacity of milk and Tris-citrate.

In order to obtain faster diffusion of individuals of higher genetic merits, AI with cryopreserved semen has been used commercially in dairy cattle for decades. Nowadays, fair to good fertility rates are obtained with AI in comparison to natural mating. Unfortunately, in sheep cryopreservation for AI is commercially limited due to low fertility rates obtained as a result of structural and functional damage caused to the sperm during the freeze-thaw process [14] as well as faulty deposition of semen. Our study demonstrated that all quality parameters decreased drastically after cryopreservation irrespective of diluents. It was found that motility of frozen-thawed spermatozoa was 20.00 ± 2.1% and 14.83 ± 1.01% in skim milk and Tris groups, respectively and the variation was statistically significant ($p<0.05$). The viability and membrane integrity also differed significantly ($p<0.05$) in cryopreserved semen between two groups. The post-thawing sperm motility determined for the extenders used in our study was close to the findings of Soylu et al. [42] but lower to Kulaksiz et al. [43]. Cryopreservation is a non-physiological method that involves a high level of adaptation of biological cells to a series of functions like dilution, incubation, cooling, freezing or thawing, which cause ultrastructural, biochemical and functional alterations of spermatozoa [44]. The generation of ROS resulting from the process of cryopreservation

and reduction in antioxidant enzyme activities in semen after a cycle of freezing [45] may contribute to the biochemical and functional damage caused to cryopreserved sperm. The biggest obstacle in the exploitation of frozen ram semen is that freezing and thawing reduces motility and membrane integrity, which leads to poor fertility following cervical AI [14]. For achieving an acceptable lambing rate following AI with frozen ram semen, it is desirable to achieve good post-thaw survival of spermatozoa.

Overall, skim milk based extender gave better protection during preservation process in terms of chilling and freezing of ram semen in the present study and this is in agreement with the some previous studies. Kulaksiz et al. [46] reported that milk extender is better than Tris-sodium citrate extender and Rota et al. [47] reported that skim milk with egg yolk showed better post-thaw semen parameters compared to Tris based buffer, may be due to caseins, the major proteins of milk and appear to be responsible for the protective effect of milk, buffer semen pH and may also chelate any heavy metal ions [13]. However, Rather et al. [48] found Tris citric acid fructose egg yolk was the best in maintaining the quality of ejaculated ram spermatozoa during preservation for 72 h at 4°C in their study. Gundogan et al. [49] claimed that major differences in the sperm motility were raises due to qualified component of the extender especially the lipoproteins, glucose and egg yolk which had positive impact in the spermatozoa.

5. CONCLUSION

Skim milk based extender was superior to Tris based extender in terms of semen preservation, and the quality of refrigerator preserved indigenous ram semen is acceptable up to 48 hours. More studies needs to conduct in this field to establish best extender and procedures for diluting and preservation of semen of indigenous ram of Bangladesh. However, this study had some limitations. We could not perform AI with these preserved semen and individual ram effect on freezability of semen was also not studied. Future study should be directed to study the effect of these diluents on pregnancy rate after AI.

ETHICAL APPROVAL

The experiments performed in this study were according to the rules of Bangladesh Agricultural University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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