



## Characteristics of fresh and frozen semen of Red Chittagong Cattle

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### ABSTRACT

Semen production from breeding bulls used in Artificial Insemination (AI) program is of great importance in cattle breeding. Good quality semen has positive effect on the fertility of cows and heifers. The aim of this study was to evaluate the quality and assessed as the freezing ability of Red Chittagong Cattle (RCC) bull with fresh-diluted semen and frozen semen. The parameters of semen characteristics included were volume per ejaculate (ml), percentage of mass motility, normal and abnormal sperm, and sperm concentration (million/ml). The highest mass motility was found in bull ID No. 4 and the lowest in bull ID No. 1 but the highest sperm concentration was found in bull ID No. 3 with lowest in bull ID No. 2 in fresh semen. Highly significant ( $p < 0.001$ ) positive correlation was found between mass motility and sperm concentration in bulls of ID No. 3 and 4, a negative significant ( $p < 0.05$ ) correlation was found between mass motility and normal sperm percentage. Two freezing techniques were followed in frozen semen production: Cork sheet freezing and Cryobath freezing with two freezing times 15 and 30 minutes were also applied. Variations in the motility of frozen semen by Cork sheet freezing with two different times were found significant ( $p < 0.05$ ) between bulls. Furthermore, highly significant ( $p < 0.01$ ) difference of motility by Cryobath freezing was found between the bulls. The highest motility after both 15 and 30 minutes Cork sheet freezing and Cryobath freezing was found in bull ID No. 4 and lowest in bull ID No. 3. In the present study, variability in the potentiality of bulls (semen volume and sperm concentration) was observed and found lower than crossbred bulls due to small size of Red Chittagong, an indigenous zebu cattle of Bangladesh.

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### Introduction

Cattle is an integral part of farming system and its density in Bangladesh is quite high compared to many countries of the world. Most of the indigenous cattle in Bangladesh are of *indicus* type and kept by the 80% of the rural people of the country. Depending upon the climate, soil type and availability of fodder, different types or variety of

cattle genetic resources are available in different parts of the country like Deshi, Red Chittagong, Pabna, North Bengal Grey, Madaripur, Hilly and Munshigonj type. Red Chittagong Cattle (RCC) is one of the most promising varieties of cattle genetic resource of Bangladesh possessing some exceptional qualities like good adaptability to

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traditional husbandry practices, poor quality straw and fodder, regular breeder and better resistance capabilities to withstand environmental stress and tropical diseases (Bhuiyan *et al.*, 2005). Semen production from breeding bulls used in Artificial Insemination program is of great importance in cattle breeding. Cryopreservation of semen allows virtually storage of biological material without deterioration over several decades even probably much longer. This means that we can preserve the present genetic material for long time in a biologically safe deposit vault. The chilled semen is usually preserved at 4 to 5 °C for a short period of time and the frozen semen is usually preserved at -196 °C in liquid nitrogen for long period of time. Freezing of semen at -196 °C in liquid nitrogen is a means to conserve the present genetic diversity for future use. Several reviews have recently appeared on the progress made on the various aspects of freezing and fertility of semen (Dorado *et al.*, 2007). The development and widespread use of frozen semen material paved the way for greater use of superior bulls. The selection of superior quality breeding bulls is essential to support conservation and breed development programs. While selecting breeding bulls, evaluation of semen is a must job. The term "quality of semen" means a package of parameters that represent the inner picture of semen related to fertility. It encompasses motility (%) of spermatozoa, the concentration of spermatozoa, proportion of live and morphologically normal spermatozoa, seminal pH and optimum metabolic features of individual sperm. So, the present study was aimed to evaluate the quality of fresh and frozen RCC semen and compare the effectiveness of different preservation techniques.

## **Materials and Methods**

### **Bull Management**

Four breeding bulls with 2-3 years of age from the Red Chittagong Cattle Project (USDA) being implemented under the Department of Animal Breeding & Genetics, Bangladesh Agricultural University were used for evaluation of liquid semen characteristics and two of them were used for frozen semen production. At the period of semen

collection, the body weight of the individual bulls were 280, 240, 215, and 150 kg, respectively for bulls ID Nos. 1, 2, 3 and 4 and aged 2-3 years. The coat color of the Bull No. 1, 3, 4 were deep red and 2 was light red. The bulls were maintained under standard stall-feeding and management conditions.

### **Semen Collection and Evaluation**

Semen was collected early in the morning from the RCC bulls twice in a week at Bangladesh Agricultural University Artificial Insemination Center by Artificial Vaginal (AV) method. Before collection all the parts of the Artificial Vagina were cleaned, sterilized and assembled properly. The graduated collection vials with the freshly collected semen were immediately transferred to the laboratory and emerged in a water bath at 37 °C. Then evaluated and split into two equal parts, one for the preparation of fresh diluted semen and another for frozen semen. The evaluation of fresh semen was done immediately after collection using the method by Herman and Madden (1963). The data on volume (ml), color, density, mass motility (%), sperm concentration (million/ml), normal and abnormal sperm count (%) were recorded.

### **Semen Processing**

To make egg yolk citrate, a solution of 2.94% sodium-citrate in 100 ml of distilled water was made in which 100000 IU of penicillin and 100000 µg of streptomycin were added per 100 ml. The egg yolk was added with citrate solution at the rate of 1:3 and mixed thoroughly. Semen was diluted with egg yolk-citrate solution and was extended at specific rates so that the volume of semen inseminated contained sufficient sperm to give high fertility without wasting many cells. The semen and diluter were mixed at room temperature. Triladyl based diluter composed of several components such as tris, fructose, glycerol, acidum, citricum, gentamycin, tylosin, spectinomycin and lincomycin, which further supports the freezing and post-thawing survival of spermatozoa was used. The glassware and other autoclavable materials were autoclaved at 121 °C and 15 lb pressure and kept in the dryer before use.

## **Red Chittagong Cattle Semen**

One part of the split semen samples were diluted with egg yolk-citrate diluter. The dilution ratio of semen was determined in such a way so that the concentration of spermatozoa/ml diluted semen was kept 30 million as a minimum. Then the diluted semen was evaluated for motility (%), normal and abnormal sperm count (%) by using the same procedure as used fresh semen and then the semen was preserved in test tubes and stored in a refrigerator at a temperature of 4 °C to 5 °C.

To produce frozen semen, another part of the split semen samples was diluted with Triladyl based diluter. The main reason for using Triladyl based diluter for the frozen semen production is that Triladyl based diluter is a complete diluter for cryopreservation of bull semen along with other livestock species. Different experiments in this regard were performed and obtained better sperm viability with diluter (Afroz et al, 2008). The semen was diluted based on sperm concentration per ejaculation with the Triladyl based cryodiluent (diluter + cry protectant) to obtain a final concentration of 25 million spermatozoa per insemination dose. The motility (%) of the diluted semen was observed and recorded and semen was then packed manually into 0.5 ml straws and was sealed with polyvinyl chloride (PVC) powder. The straws were placed in the refrigerator at 4-5 °C for 3 hours. The motility of equilibrated sperm was checked and only samples with more than 60-70% motility was used for freezing. After equilibration, the straws were placed horizontally on a rack and transferred to the freezer to be frozen in vapor above 15-20 cm of the liquid nitrogen (LN). Two freezing times 15 and 30 minutes were used to become frozen semen. The frozen straws were transferred into the canister within the liquid nitrogen container at -196 °C until used for insemination. After 24 hours, straws were retrieved from the LN container using forceps and thawed in water bath at 37 °C for 12 seconds. The straws were wiped and cut near the cotton plug and PVC sealed end. A drop of thawed semen was placed on a previously warmed (37 °C) slide and post-thaw motility was estimated.

### **Statistical Analysis**

The data generated from this experiment were evaluated in Microsoft Excel program and statistical analyses were performed by using SPSS (Statistical Packages for Social Science) program.

### **Results and Discussion**

The volume of ejaculated semen differed significantly ( $p < 0.001$ ) among the bulls (Table 1) which ranged from 2 - 4 ml with an average of 3.22 ml of semen produced by bulls. Bull ID No. 2 gave the highest amount (4.46 ml) of semen and the lowest amount of semen was given by Bull ID No. 1. Nasrin (2006) studied the semen production of Red Chittagong bulls and did not find a significant difference ( $p > 0.05$ ) in semen volume between bulls which is in contrast with the present study and the variation might be due to sample size, time and duration of data taken. Devnath (1999) and Habib *et al* (2003) found the semen volume of RCC bulls to be 3.00 and 3.25 ml, respectively which agreed with the present findings.

The average mass motility of sperm found in RCC bulls ranged between 55.85-64.31 % and differed significantly ( $p < 0.001$ ) among the bulls (Table 1). The highest mass motility of sperm was found in Bull ID No. 4 than others. The present results agreed with the study of Nasrin (2006) who found a range of mass motility of 52.00-54.00 % in RCC bulls. Nasrin (2006) also found non-significant ( $p > 0.05$ ) difference of mass motility of sperm between RCC bulls which is not in agreement with the present study and might be due to small sample size, time and duration of data taken from bulls.

The concentration of spermatozoa per ml of fresh semen differed significantly ( $p < 0.001$ ) among 4 RCC bulls (Table 1). The bull ID No. 3 produced significantly ( $p < 0.001$ ) higher concentration of spermatozoa than the other bulls. The findings of the present study to some extent agree with the findings of Devnath (1999), Habib *et al* (2003) and Nasrin (2006).

Normal spermatozoa did not differ significantly ( $p > 0.05$ ) among the RCC bulls and are consistent with the results of Devnath (1999), Habib *et al* (2003) and Nasrin (2006).

**Table 1:** Characteristics of fresh semen in RCC bulls (Mean ± SE, #N=20)

Bull ID No.	Volume (ml)	Mass motility (%)	Sperm concentration (%)	Normal sperm (%)
1	2.12±0.14 <sup>b</sup>	55.85± 1.17 <sup>b</sup>	610.39±60.37 <sup>b</sup>	86.25± 1.04
2	4.46±0.25 <sup>a</sup>	64.31± 1.32 <sup>a</sup>	608.42± 47.56 <sup>b</sup>	87.78± 1.46
3	3.6±0.13 <sup>a</sup>	62.97± 0.78 <sup>a</sup>	1031.27± 28.12 <sup>a</sup>	87.63 ±0.38
4	2.94±0.15 <sup>b</sup>	65.18 ±1.02 <sup>a</sup>	1020.36 ±25.31 <sup>a</sup>	87.41± 0.34
Average	3.22±0.09	62.11±0.56	907.39±23.01	87.27±0.31
Level of significance	***	***	***	NS

#N = Replication or number of repeated collections, NS = Non-significant (p>0.05), \*\*\* Significant (p<0.001), Means with uncommon superscripts within the same column differ significantly (p<0.05)

The study was done with Red Chittagong bulls maintained at BAU AI Centre and the numbers of bulls were limited. The management system was moderate and the animal size was lower than other exotics and their crossbreds, so it was very difficult to compare semen quality with others.

**Table 2:** Correlation between fresh semen parameters of Red Chittagong bulls (#N=20)

Bull No	Mass Motility (%) - Volume (ml)	Mass Motility (%) - Sperm Concentration (×10 <sup>6</sup> ml)	Mass Motility (%) - Normal sperm (%)
1	0.22 NS	0.36*	0.52**
2	0.31 NS	0.23 NS	0.30 NS
3	0.29*	0.50**	-0.23*
4	0.23*	0.51**	-0.22*

#N = Replication or number of repeated collections, NS=Non-significant (p>0.05), \* Significance at p<0.05., \*\* Significant at p< 0.01.

The correlation between volume of semen and mass motility are presented in Table 2. Positive and low but significant (p<0.05) correlation was found between Bull No. 3 and 4 and non-significant (p>0.05) correlation was found in Bull No. 1 and 2.

The correlation between sperm concentration and mass motility is presented in Table 2. Positive and highly significant correlation was found between Bull No. 3 and 4. A comparatively low significant correlation was found between Bull No. 1 and 2.

The correlation between normal sperm percentage and mass motility is presented in Table 2 Positive and highly significant correlation was found in only Bull No. 1 and which was shown to have a little bit negative significant effect in Bull No. 3 and 4 and non-significant correlation between mass motility and normal sperm percentage was found in Bull No. 2.

**Table 3:** Characteristics of frozen semen as affected by individual RCC bull (Mean ±SE, # N=20)

Bull No.	Motility % after dilution	Motility % after equilibration	Motility% after freezing		
			Cork sheet freezing		Cryobath freezing
			15 min freezing	30 min freezing	
3	57.76±12.50	55.00± 2.04	9.87± 1.37	12.50± 2.50	44.50± 0.50
4	60.18± 1.02	58.75± 2.39	19.50± 1.50	35.5± 0.50	53.50 ±0.50
Level of significance	NS	NS	*	*	**

#N = Replication or number of repeated collections, \*Significant (p<0.05), \*\* Significant (p<0.01), NS = Non Significant (p>0.05).

Table 3 shows that there is no significant ( $p>0.05$ ) variation for diluted semen motility between two RCC bulls although bull ID no. 4 seemed to be slightly higher motility than other bull (ID. No. 3). The present observation agrees with the previous studies of Foote *et al.* (2002) and Shamsuddin *et al.* (2000).

After three hours of equilibration, the average sperm motility varied from 55.00 to 58.78 %. The analysis of variance revealed that equilibration did not have any significant ( $p>0.05$ ) effect on the sperm motility of different bulls (Table 3). This observation agrees with the previous studies (Correa *et al.*, 1996; Yilmaz and Yurdaydin, 1994). In the present study, an equilibration period of 3 hours was found to be suitable for sperm motility which agrees with Chauhan and Anand (1990) and Das and Rojkonwar (1996). Therefore, an equilibration period of 3 hours might be suitable for bull semen in current experimental condition.

The significantly ( $p<0.01$ ) highest sperm motility at Cryobath freezing was found in Bull ID No. 4. Freezing for 15 and 30 minutes in cork-sheet box had higher significant ( $p<0.05$ ) sperm motility as found in Bull ID No. 4 as Bull ID No. 3 (Table 3). In Cryobath freezing, motility was significantly ( $p<0.001$ ) higher than Cork sheet freezing, because Cryobath freezing totally maintained by fixed protocol management by computerizing rather than manual system. The present findings agree with the findings of other investigations (Nur *et al.*, 2003; Nur *et al.*, 2005). The variations observed from different sources could be due to the different types of diluter, percentage of egg yolk, glycerol, equilibration time and thawing.

Frozen semen production plays a very vital role in distributing semen throughout the country which will provide an alternative approach against the serious shortfall of quality breeding bulls in the country. Production of breeding bulls with quality semen and delivery of that semen in the form of frozen straw to the field could be the most important technical input to support development of indigenous zebu, Red Chittagong Cattle (RCC) of Bangladesh.

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