Effect of Different Preservation Time of Chilled Semen on the Fertility of Field Indigenous Ewes

A. Saha¹, M. Asaduzzaman², S. Akter¹, F.Y. Bari¹

ABSTRACT

Background: Artificial insemination (AI), among all the fundamental systems of animal breeding, has proved to be the best and efficient method for the rapid improvement of livestock for maximum use of superior genetic merit of males on numerous dams. The frozen AI in sheep is, to date, not fulfilling the farmer’s need. Therefore, the present study aimed to determine the pregnancy rate in indigenous ewes with chilled semen preserved at different hours.

Methods: Semen was collected from three indigenous rams using an artificial vagina and evaluated for its quality both in the fresh and chilled stage at 12 h, 24 h and 48 h before insemination. Trans-cervical insemination was performed in PGF2α synchronized ewes. Pregnancy was confirmed by ultrasonography at 50-60 days of gestation.

Result: The motility, viability and normal sperm values of chilled semen decreased significantly (p < 0.01) with increasing the duration of preservation. However, the quality of chilled semen was acceptable level for insemination. The pregnancy rate was significantly varied (p < 0.01) and the higher pregnancy rate (64.28%) was achieved when AI was performed using semen stored at 4°C for 12 h than 24 h (58.33%) and 48 h (50%), respectively. Therefore, increased the duration of preservation time of different chilling process negatively affect the semen quality and the pregnancy rates.

Key words: Artificial insemination, Chilled semen, Pregnancy rates, Preservation time.

INTRODUCTION

Among the livestock, sheep is one of the important commodities, estimated at 3.4 million sparsely distributed with an annual growth rate of 5% in Bangladesh. Bangladeshi climate is favorable for sheep breeding and sheep has got special importance due to their multifaceted utility among all other livestock species (Prabhu et al., 2009; Prasad et al., 2019). Sheep is more resistant to parasitic and infectious diseases compared with goats. Sheep contribute significantly to meat production, employment and regular income to the farmers (Prasad et al., 2019). The handling of sheep is easy with low input and feeding with minimum supplements. However, the farmer’s dream is not full filling due to low genetic merit, nutrition and lack of management and proper breedings (Alam et al., 2001). Farmers are not aware of the benefits of selection, breeding and assisted reproductive techniques for breed improvement programs and profitable marketing. AI is the technology to increase production, genetic progress of breed in sheep and meet up the protein deficiency in human society (Campbell et al., 1996). The selection of best quality rams could only be exploited, by using artificial insemination. To increase production and profitable income, the introduction of assisted reproductive techniques is necessary for Bangladesh. AI is the technology to reduce inbreeding depression and facilitate the genetic development of sheep. But AI in sheep with liquid or frozen semen is not yet popularized in Bangladesh. Liquid semen could be an appreciated alternative to fresh and frozen semen and stored between 0-5°C. A higher conception rate occurs when extended semen is cool stored instead of cryopreserved. The conception rate was higher with extended semen stored at 4°C for 72 h (70%) or 23°C for 24 h (76%) compared with cryopreservation (-196°C, 50%) (Wusiman et al., 2012). Higher pregnancy rates were found when cooled semen deposited at the cervix compared with frozen semen (Salmon and Maxwell, 2000). A higher percentage of motile sperm, in the AI dose, was maintained in liquid semen compared with frozen-thawed semen at the same concentration and the pregnancy rate is comparatively higher in liquid semen than frozen semen (Roca et al., 2006). Liquid semen is more economical with no hazard to maintain liquid nitrogen and use even if they are bad freezer (Vera-Munoz et al., 2011). Liquid semen can be stored long period compared with fresh semen. Therefore, the present study aimed to observe the effects of preservation time of chilled semen to evaluate semen quality and to determine the conception rate of indigenous field ewes.
MATERIALS AND METHODS

Study area
The ram semen was processed and preserved in the Reproduction Laboratory of the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, located from N 24.73 latitude to E 90.44 longitude from June 2019 to September 2020. Sheep farms located at the different villages of Muktagacha and Sadar Upazila under Mymensingh district, that located between N 24.76 latitude and E 90.27 longitude and N 24.75 latitude and E 90.42 longitude, respectively.

Animal management
A total of three Muzaffarnagar cross-bred rams of 18 to 24 months of age and 35 to 45 kg of body weight, were selected for semen donor and maintained at Sheep Research Animal Farm, under Bangladesh Agricultural University, Mymensingh. The rams were grazed on natural pasture for about 7 to 8 hrs, with a concentrate supplement, at the rate of 250-300 gm per ram once daily, consist of 50% wheat bran, 25% crushed maize, 20% soybean meal, 1% fish meal, 2% di-calcium phosphate (DCP® Plus, Opsonin Pharma Ltd., Bangladesh) powder, 0.5% vitamin-mineral premix (Megavit®, Opsonin Pharma Ltd., Bangladesh) and 1.5% common salt. They were allowed to free access to water three times a day ad-libitum. Field ewes used in artificial insemination were to be under the farmer’s management. The farmer’s ewe underwent routine vaccination and deworming program before study work.

Initial evaluation of seminal traits
The semen samples were collected by artificial vagina (AV) prepared following the guideline of Vishal (2014). The ejaculate volume was measured directly from the graduated collecting tube (Elsharif, 2010; Mafolo, 2018). The colour of ejaculate was assessed directly by visual observation (Elsharif, 2010) and was graded as per Jha et al., (2013). Mass activity, sperm motility and sperm concentration were studied according to procedure of Avdi, (2004), Moghaddam et al., (2012) and Jha et al., (2018), respectively. The viability (live-dead) was studied using eosin-nigrosin stain as per standard procedure (Sitai et al., 2017). Normal sperm percentage were determined using the same slide of eosin-nigrosin stain.

Diluents preparation
The home-made media tris-citrate-fructose-egg yolk was used for semen dilution. Tris-citrate-fructose-egg yolk diluents were prepared by dissolving TRIS 3.634 g, 1.99 g citric acid to 100 ml Milli-Q water, one half gram fructose, 0.5 ml of gentamicin (10 mg/ml), 5% egg yolk was added 95 ml of stock solution (Acharya, 2017). On the day of semen collection, early in the morning, Tris-citrate-fructose-egg yolk diluent was prepared to use in fresh condition.

Semen extension and chilling
All of our selected rams had possessed ≥2800 × 10⁶ of sperm per ml and were for semen extension and chilling. Only the ejaculates with ≥ 0.5 ml of volume and ≥ 80% of sperm motility were used in chilled semen preservation. After a short evaluation, the semen samples were diluted with Tris-fructose -egg -yolk extender at a rate of fixed sperm concentration at 300 × 10⁶/ml. Then the diluted semen samples have transferred to the refrigerator to chilled and preserved at 4°C for 12 h, 24 h and 48 h respectively to use.

Quality evaluation of chilled semen
Chilled semen was evaluated and processed further for motility, viability and normal sperm percentage in 12 h, 24 h and 48 h, respectively, as procedures described earlier in the initial semen evaluation before transporting and shifting for field ewe artificial insemination.

Oestrus induction
The selected field ewes received 175 μg of Cloprostenol (Oxupropro®, Bomac laboratories Ltd, Manukau) i.m. injection and the ewes were not in oestrus, underwent Cloprostenol injection again at the previous dose rate following at 9 days oestrus induction protocol (Jha et al., 2013).

Performance of artificial insemination
On the morning of the day of artificial insemination, the oestrus-induced ewes were identified, selected and separated by an aproned ram from the neighborhood. At 12-14 h of onset of oestrus, trans-cervical artificial insemination was performed following traditional technique. Briefly, an assistant holds the ewes lifting of its two hind limbs in a slanting position with the head downwards. The operator inserts the lubricated (KLY gelly®) vaginal speculum with a light source (pen-torch) into the vaginal lumen up to the os-cervix. Then he inserts a semen straw loaded artificial insemination gun into the cervix and expel out the semen by mild pressuring the plunge.

Pregnancy diagnosis
Trans-abdominal ultrasonography (DRAMIŃSKI ANIMAL profi portable ultrasound scanner, Poland) was performed to confirm the pregnancy after 45-55 days of artificial insemination.

Data analysis
All the data of seminal traits were entered first into the MS-Excel datasheet. Then the mean values and standard errors were determined by descriptive statistics. The one-way ANOVA (single factor) was done to compare the significant mean differences between seminal traits. The completely randomized design of WASP software was used to compare the significant percentages of the pregnancy rates.

RESULTS AND DISCUSSION
The mean ejaculate volume of the current study (Table 1) was in agreement with Menchaca et al. (2005) and Malama et al. (2013) reported 0.75-0.94 and 0.59-0.99 ml per ejaculates, respectively. In contrast, Zohara et al. (2013) reported higher semen volume 1.05-1.6 ml in the...
Bangladeshi rams. These variations of semen volume may be due to the age of rams, management and frequency of semen collection. The color of the ram’s semen was consistent with Zohara et al. (2013). In the present study, the sperm mass activity was comparable with Jha et al. (2015) and, Moghaddam et al. (2014) reported 3.80 ± 0.09 and 3.88 ± 0.91, respectively. However, sperm mass activity was lower in agreement with Cunha et al. (2012). Sperm motility constitutes an integral part of semen quality and male fertility is reflected significantly by sperm motility. The presence of a higher concentration of spermatozoa in semen hampers their transportation and fertilizing capacity is critical. The extension of spermatozoa is, therefore, plays a significant role in sheep breeding insemination. Regardless of storage temperature, sperm motility decreases by increasing the duration of chilled semen preservation. In this study, however, the motility was recorded for quality assessment of chilled semen to be used in artificial insemination. The motility of spermatozoa observed in the present study for different chilling stages is in Table 2. These findings explore significant (p < 0.05) differences in chilled sperm motility (12h, 24h and 48h, respectively) than the fresh semen samples. The mean motility of fresh semen was similar to the findings of many researchers Rahman et al. (2015), Azizunnesa et al. (2014). The motility of chilled semen samples was in agreement with Pervage et al. (2009), who reported 76.45 ± 2.82 and 75.31 ± 3.72% of motility in indigenous ram semen 24 and 48 hours of chilling, respectively. However, the lower sperm motility (68.9%) was in Merino chilled ram semen by Gil et al. (2011) at 5°C in 12 hours of chilling. This result may be due to breed differences and types of extenders used in chilling.

It is urgent to know the semen quality, especially sperm viability, at every stage of semen processing that corroborates successful breeding and fertilization. The present findings of semen viability at different stages of the chilling procedure are in Table 2. The result showed a significant (p < 0.05) difference in sperm viability of semen samples at different chilling stages from the fresh semen sample. However, the current findings of chilled semen were similar to referred value (> 70%), which is essential for successful artificial insemination (Nilani et al., 2012). The viability in our study of fresh semen was in agreement with the findings of Hassan et al. (2009) and, the value for chilled (at 24 and 48 hours) semen was in line with the observation of Rather et al. (2017) to be 85.37 ± 4.19, 82.22±3.26 and 78.36±3.89%, respectively. The sperm viability of chilled semen (10.7h storage) was 60% in small-tail Han sheep (Zhou et al., 2002) that was lower than in the current study. This result might be due to reduced sperm metabolism during the chilling procedure stated by Salamon and Maxwell (2000) and toxic metabolic products such as ROS (Reactive oxygen species) that exert a decrease in sperm viability (Griggers et al., 2001) (Vishwanath and Shannon, 1997). The amount (%) of Normal sperm is of outstanding importance for semen quality. Sperm abnormality assessment considers as a prerequisite in both liquid and frozen semen in United States, Sweden and Netherlands (Arifiantini et al. 2006). The results of the Normal sperm percentage of the current study are in Table 2. Like other traits, normal sperm percent reduces significantly (p< 0.05) at 12h, 24h and 48h of the chilling stage than fresh semen. The present observation of Normal sperm percentage of fresh semen was in line with the findings of 86-98 of Azizunnesa et al. (2014). The Normal sperm percentage at 12h of chilling for ram semen was not available published online. However, Perumal et al. (2013) found similar results in Mithun bull semen. Reka et al. (2016) reported higher Normal sperm percentage at 24h but the same at 48h of chilling semen samples that corroborated the present findings.

Artificial insemination (AI), to date, among all the fundamental systems of animal breeding, has proved to be the best and efficient method for the rapid improvement of livestock for maximum use of superior genetic merit of males on numerous dams. The widespread use of AI facilitates to increase in the productivity and profitability of a particular commercial herd by increasing the number of offspring. The success of AI depends on the dose, the number of motile spermatozoa, the site and methods applied for the ewe insemination. In the present study, diluted chilled ram semen at 0h, 12h, 24h and 48h of preservation having > 70 % motile spermatozoa. The results on pregnancy rates of transcervical artificial insemination are in Table 3. The pregnancy rates in ewes following artificial insemination using

### Table 1: General Characteristics of ram semen.

| Parameters               | Mean±SE | Range |
|--------------------------|---------|-------|
| Volume (ml)              | 0.79±0.02 | 0.7-0.9 |
| Color (1-4 grades)       | 3.6±0.09  | 3-4   |
| Sperm concentration (million/ml) | 3308±54.80 | 2912-3625 |
| Mass activity (1-5 grades) | 3.78±0.06 | 3.5-4 |

SE-Standard error.

### Table 2: Semen qualities at different stages of chilling procedure.

| Semen stage         | Duration of evaluation | Motility (%)   | Viability (%)   | Normal sperm (%) |
|---------------------|------------------------|----------------|----------------|-----------------|
| Fresh semen         | 0h                     | 80.07±0.77<sup>a</sup> | 87.31±0.39<sup>a</sup> | 88.00±1.09<sup>a</sup> |
| Chilled storage     | 12h                    | 76.81±0.56<sup>a</sup> | 84.75±0.52<sup>b</sup> | 83.94±0.93<sup>b</sup> |
|                     | 24h                    | 74.31±0.35<sup>c</sup> | 79.38±0.77<sup>c</sup> | 80.38±0.81<sup>c</sup> |
|                     | 48h                    | 72.31±0.39<sup>d</sup> | 75.44±0.59<sup>d</sup> | 77.31±0.57<sup>d</sup> |

<sup>a,b,c,d</sup> Different superscripts in the same column indicate significant differences (p<0.01).
Effect of Different Preservation Time of Chilled Semen on the Fertility of Field Indigenous Ewes

Table 3: Effects of chilled storage of ram semen on pregnancy rates in ewes.

| Chilled storage of ram semen | Pregnancy rate (%) | (n) |
|-----------------------------|-------------------|-----|
| 12h                         | 64.28             | (9/14)  
| 24h                         | 58.33             | (7/12)  
| 48h                         | 50                | (5/10)   |

Different letters indicate significant differences, (P< 0.01) between treatments.

liquid chilled ram semen stored for 12h was comparatively higher (64.28%) than 24h (58.33%) and 48h (50%), respectively. These findings agreed with the observation of pervage et al. (2009). The theme of the current study- the increase in the duration of chilled semen, the decrease in conception rates is similar to the study by the observation Boyd and Reed (1961). The conception rates for 12h of chilled preserved semen was 42.7 and 35% (Mencha et al., 2005 and Olivera-Muzantea et al., 2011) and 34.5% for 24h chilled storage ram semen (Olivera-Muzantea et al. 2011) that were lower than our current findings. These differences might be due to breed differences.

CONCLUSION
From the observed findings of the current study, it could be concluded that the two days chilling of ram semen shows acceptable semen quality and conception rate in ewes in the farmer’s field.

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Effect of Different Preservation Time of Chilled Semen on the Fertility of Field Indigenous Ewes

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