

Effects of preservation period on quality of chilled semen of Boer-cross bucks

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Abstract

Characteristics of fresh semen and the effect of chilling on the quality of semen of Boer-cross bucks were studied. Fresh semen from two Boer-cross bucks was evaluated for volume, mass motility, progressive motility, viability, concentration and proportion of spermatozoa with normal acrosome, mid-piece and tail. The semen was diluted at 1:4 ratios with Tris-fructose-citrate-egg yolk extender, preserved at 4°C for 72 hours and evaluated for progressive motility and proportion of spermatozoa with normal acrosome, mid-piece and tail at 24-hour intervals for three days. The mean volume, mass motility, progressive motility, sperm viability, sperm concentration and proportion of spermatozoa with normal acrosome, mid-piece and tail of fresh semen of Buck 1 and Buck 2 were 370.0 ± 0.1 and 410.0 ± 0.1 μl , 3.3 ± 0.4 and 3.4 ± 0.1 , 74.0 ± 13.9 and $76.0 \pm 6.5\%$, 82.3 ± 7.5 and $84.3 \pm 4.2\%$, $2730.0 \pm 740.9 \times 10^6/\text{ml}$ and $2584.0 \pm 470.1 \times 10^6/\text{ml}$, and 93.3 ± 2.9 and $94.9 \pm 0.4\%$, respectively. The progressive motility in chilled semen of Buck 1 at days 1, 2 and 3 was 61.2 ± 7.3 , 51.0 ± 7.3 and $39.0 \pm 5.5\%$, respectively; in Buck 2, it was 60.6 ± 6.8 , 50.2 ± 6.2 and $39.2 \pm 5.4\%$, respectively. The difference in semen parameters among days of preservation was significant ($P < 0.05$) in both bucks. The proportion of spermatozoa with normal acrosome, mid-piece and tail in chilled semen of Buck 1 at days 1, 2 and 3 was 88.0 ± 7.0 , 88.8 ± 5.9 and $87.1 \pm 7.7\%$, respectively; in Buck 2, it was 86.6 ± 4.8 , 84.1 ± 4.6 and $81.8 \pm 4.9\%$, respectively. It is suggested that the characteristics of fresh semen of these two Boer-cross bucks were within normal range. The quality of semen of Boer-cross bucks deteriorated with chilling up to 72 hours. Improvement of the preservation protocol is required to obtain satisfactory quality of chilled semen of Boer-cross bucks. (*Bangl. vet.* 2022. Vol. 39, No. 1 - 2, 34 - 42)

Introduction

After cattle, goats are the second most economically important ruminant in Bangladesh (Shamsuddin *et al.*, 2000). According to FAOSTAT (2022), Bangladesh produced 229580 tonnes of goat meat in 2021 as the 5th largest goat-producing country in the world. Among goats, 90% are of the Black Bengal (BB) breed and 10% are Jamunapari, Boer and crosses (Ahmed, 2017). A mature BB buck weighs around 25 - 30 Kg (Ahmed, 2017). The average birth weight (1.6 ± 0.5 Kg) and the body weight (20.3 ± 2.5 Kg) after 365 days of BB goats are the lowest among the available crossbred goats in Bangladesh (Hassan *et al.*, 2007).

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The Boer is one of the most popular goat breeds in the world for meat production as they have excellent body conformation, fast growth, high carcass quality, and good resistance to diseases and drought (Talukder *et al.*, 2015). The birth weight of Boer kids is 3 to 4 Kg, and mature Boer bucks and does weighing 90 to 130 Kg and 80 to 100 Kg, respectively (Talukder and Choudhury, 2018). Considering the superior traits of the Boer breed and the increasing demand for meat in Bangladesh, Boer goats were introduced from Malaysia in 2012 (Ahmed, 2017). Yousuf *et al.* (2020) reported the adaptability of Boer goats in Bangladesh and found enhanced performance of native breeds through cross-breeding (Lu, 2001; Parajuli, 2020). The offspring of BB × Boer goats are superior to the pure BB breed (Gond *et al.*, 2019).

For cross-breeding of native goats with Boer bucks, natural service (NS) and artificial insemination (AI) are commonly practised (Ciptadi *et al.*, 2014; Nurani *et al.*, 2021). AI in BB goats showed satisfactory performance (Ali *et al.*, 2016). A higher fertility rate is achieved in goats by using chilled semen rather than frozen semen (Mocé *et al.*, 2020). The preservation period has a great effect on chilled semen quality and Pradhan *et al.* (2013) showed that the quality of BB buck chilled semen deteriorated with longer storage. There is a paucity of information on the preservation of chilled semen of Boer-cross bucks in Bangladesh, but AI in BB goats has been practised with frozen semen of Boer bucks (Saha *et al.*, 2020). The present study was carried out to evaluate the characteristics of fresh semen and the effect of the period of chilling on motility and morphology of spermatozoa in the semen of Boer-cross bucks.

Materials and Methods

Animals used and their management

Two cross-bred Boer bucks (Fig. 1 and Fig. 2) were used: both possessed sound health and fair libido. Buck # 1 was 2.0 years and Buck # 2 was 1.5 years old. The body weight of Buck # 1 was 34.5 Kg and Buck # 2 was 29.0 Kg. The scrotal circumference of Buck # 1 was 25.8 cm and Buck # 2 was 24.4 cm. The bucks were housed in the Research Animal Farm (RAF) at Bangladesh Agricultural University (BAU) in a semi-open shed with an iron slat floor. Bucks were allowed to graze on the lush grass around the RAF. Each buck was provided with 500 gm of bran and chickpea every day and had free access to drinking water. The bucks were vaccinated against *Peste des petits ruminants* (PPR) and were dewormed routinely.

Preparation of semen extender

The TRIS-based extender was used for semen dilution. A stock solution of 100 ml diluent was prepared by mixing 3.634 gm TRIS, 1.99 gm citric acid, 0.50 gm fructose and 0.50 ml gentamycin (Genta-10[®], ACME, Dhaka, Bangladesh) with distilled water. On the day of semen collection, 10% (v/v) fresh egg yolk was mixed with the stock solution for preparation of the final extender.



Fig. 1: Buck 1



Fig. 2: Buck 2

Semen collection and evaluation

Semen was collected using an artificial vagina (AV) following standard procedure from each buck once a week with a restrained teaser doe. Typically, the donor bucks were permitted at least one false mount. The graduated tube containing fresh semen was placed in a water bath at 37°C. The volume and colour were evaluated. Mass motility of spermatozoa was evaluated on a 5-point scale by placing a drop of fresh semen on a pre-warmed (37°C) slide without coverslip and examining it under a phase contrast microscope (Olympus CX 43) using 10X objective. The progressive motility of spermatozoa was evaluated in a 10 µl drop of diluted semen under a coverslip using a phase contrast microscope (40X objective) by observing the proportion of spermatozoa moving actively forward.

Sperm concentration was determined using haemocytometer technique following standard procedure and expressed as millions/ml. Sperm viability or the percentage of live spermatozoa was estimated by Eosin-nigrosin stain following standard procedure. As live spermatozoa remained unstained, sperm viability was assessed by counting the number of live spermatozoa under a microscope (40X objective). At least 200 spermatozoa were counted. Semen fixed with buffered formal-saline was used to evaluate the spermatozoa with normal acrosome, mid-piece and tail following standard procedure under a phase contrast microscope using 100X objective. At least 200 spermatozoa were counted.

Semen extension and chilling

After fresh semen evaluation, semen was diluted with TRIS-fructose-egg-yolk extender; 100 µl semen was mixed with 400 µl of extender. Extended semen samples were kept in a refrigerator for chilling for two hours, then preserved separately at 4°C for 24 hours (Day 1), 48 hours (Day 2)++ or 72 hours (Day 3) for further evaluation.

Evaluation of chilled semen quality

To determine the effects of preservation on the quality of chilled semen, progressive motility and proportion of spermatozoa with normal acrosome, mid-piece and tail were evaluated on Days 1, 2 and 3 as described above.

Data analysis

Data were entered into the MS Excel datasheet. The mean, standard deviation of the mean and percentages were calculated. The comparison of fresh semen characteristics between bucks was analysed using Student's t-test. The two-way ANOVA using the SPSS package was used to compare chilled semen qualities. The difference in parameters was regarded as significant when P was <0.05.

Results and Discussion

Fresh semen

In Buck 1 and 2, the mean fresh semen volume was 370 ± 0.1 and 410 ± 0.1 μl , mass motility 3.3 ± 0.4 and 3.4 ± 0.1 , progressive motility 74.0 ± 13.9 and $76.0 \pm 6.5\%$, sperm viability 82.3 ± 7.5 and $84.3 \pm 4.2\%$, sperm concentration $2730.0 \pm 740.9 \times 10^6/\text{ml}$ and $2584.0 \pm 470.1 \times 10^6/\text{ml}$ and proportion of spermatozoa with normal acrosome, mid-piece and tail 93.3 ± 2.9 and $94.9 \pm 0.4\%$, respectively. No significant difference ($p>0.05$) was found with respect to different semen parameters between two bucks.

Table 1: Characteristics of fresh semen of Boer-cross bucks

Buck ID	Volume (μl)	Mass Motility (+)	Progressive Motility (%)	Viability (%)	Concentration ($10^6/\text{ml}$)	Spermatozoa with normal acrosome, mid-piece and tail (%)
1	370.0 ± 0.1	3.3 ± 0.4	74.0 ± 13.9	82.3 ± 7.5	2730.0 ± 740.9	93.3 ± 2.9
2	410.0 ± 0.1	3.4 ± 0.1	76.0 ± 6.5	84.3 ± 4.2	2584.0 ± 470.1	94.9 ± 0.4

All the results were expressed as mean \pm SD. SD = Standard deviation.

The semen volume of both bucks was within standard range. The mean ejaculate volume is in agreement with Kabiraj *et al.* (2011) who reported that the mean volume of semen in BB bucks ranged from 0.32 to 0.68 ml. Higher ejaculate volumes in various breeds of bucks, from 0.54 to 1.97 ml, were found elsewhere (Suyadi, 2012; Kharche *et al.*, 2013; Bastola *et al.*, 2018; Islam *et al.*, 2019). This variation in semen volume can be attributed to age, body weight, body condition score, scrotal circumference of buck and frequency of semen collection as well as the skill of the technician.

Ferdinand *et al.* (2012) recorded higher mass motility (4.6 ± 0.5) in different ages of bucks. The present results agree with those in BB bucks (3.6) by Das *et al.* (2021) and in Indian Surti bucks (3.4) by Kumar *et al.* (2022). Mean progressive motility was similar to the result of Suyadi (2012) and Zaenuri *et al.* (2014) who recorded motility from 70 to 77% in different ages of Boer and Boer-cross bucks. On the other hand, higher motility (81.1 and 84.1%) in BB bucks was reported by Gojen-Singh *et al.* (2016) and Sinha *et al.* (2019), respectively. The variation in motility can be caused by factors such

as age, frequency of collection, environmental conditions and semen handling methods (Apu *et al.*, 2008).

Mean sperm viability conforms to the result obtained by Kabiraj *et al.* (2011) who recorded 76.5 to 85.6%. Higher sperm viability (89.6 to 91.1%) was recorded in various breeds of bucks (Eswaramohan *et al.*, 2014; Siddiqua *et al.*, 2016). Variation in sperm viability could originate from delay between collection and evaluation, and method of transport (Hahn *et al.*, 2019). Concentrations of spermatozoa agree with results from $1522 \times 10^6/\text{ml}$ to $2797 \times 10^6/\text{ml}$ (Apu *et al.*, 2008; Karunakaran *et al.*, 2015). Higher sperm concentrations (2827×10^6 to 3573×10^6 sperm/ml) were reported by others (Kharche *et al.*, 2013; Sultana *et al.*, 2013; Zaenuri *et al.*, 2014). This variation might be due to age, breed, collection frequency, feeding regime and environmental condition. The proportion of spermatozoa with normal acrosome, mid-piece and tail was similar to the results (91 – 94%) in BB goats by Shamsuddin *et al.* (2000) in Bangladesh.

Chilled semen

Progressive motility in the semen of Boer-cross bucks after different periods of chilling is shown in Table 2. The mean progressive motility in chilled semen of Buck 1 at days 1, 2 and 3 was 61.2 ± 7.3 , 51.0 ± 7.4 and $39.0 \pm 5.5\%$, respectively. The value for Buck 2 was 60.6 ± 6.8 , 50.2 ± 6.2 and $39.2 \pm 5.4\%$, respectively. The difference on same day was not significant ($P > 0.05$) between two bucks. However, the difference between days of preservation was significant ($P < 0.05$) in each buck. When the data were pooled, the progressive motility was 60.9 ± 6.7 , 50.6 ± 6.5 and $39.1 \pm 5.1\%$ on day 1, day 2 and day 3, respectively. The difference between days of preservation was significant ($P < 0.05$).

Table 2: Effects of preservation period on motility of chilled semen of Boer-cross bucks

Buck ID	Progressive motility (%)		
	Day 1	Day 2	Day 3
1	61.2 ± 7.3^a	51.0 ± 7.4^b	39.0 ± 5.5^c
2	60.6 ± 6.8^a	50.2 ± 6.2^b	39.2 ± 5.4^c
Overall	60.9 ± 6.7^a	50.6 ± 6.5^b	39.1 ± 5.1^c

Values are mean \pm SD. SD = Standard deviation.

^{abc}Values with different superscripts within same rows differ significantly from each other ($P < 0.05$).

The current study demonstrated a significant reduction in progressive motility of spermatozoa after the buck semen was chilled at 4°C. This finding was similar to Mohamed *et al.* (2012) who found a significant reduction in sperm motility following preservation at 4°C. The mean sperm motility at different days of preservation was very close to that recorded (61.5% on Day 1, 52.1% on Day 2 and 43.7% on Day 3) by Mishra *et al.* (2010). Sarangi *et al.* (2017) and Sinha *et al.* (2019) reported higher sperm motility on different days following chilling. Shamsuddin *et al.* (2000) observed that sperm motility remained 50% or more up to four days during chilling. The variation

might arise due to reduction in sperm metabolism, exhaustion of stored energy in spermatozoa, temperature fluctuations in refrigerators, seasonal variations, or metabolic by-products such as spermicidal endotoxins (Karagiannidis *et al.*, 2000).

The proportion of spermatozoa with normal acrosome, mid-piece and tail in the chilled semen is shown in Table 3. The mean proportion of normal spermatozoa in the semen of Buck 1 on days 1, 2 and 3 was 88.0 ± 7.0 , 88.8 ± 5.9 and $87.1 \pm 7.7\%$, respectively; in the semen of Buck 2, it was 86.6 ± 4.8 , 84.1 ± 4.6 and $81.8 \pm 4.9\%$, respectively. The difference between bucks was not significant ($P>0.05$). When the data were pooled, the mean overall proportion of normal spermatozoa was 87.3 ± 5.7 , 86.5 ± 5.6 and $84.4 \pm 6.7\%$ on days 1, 2 and 3, respectively. The difference between days was not significant ($P>0.05$).

Table 3: Effects of duration of chilling on normal sperm percentage in semen of Boer-cross bucks

Buck ID	Spermatozoa with normal acrosome, mid-piece and tail (%)		
	Day 1	Day 2	Day 3
1	88.0 ± 7.0	88.8 ± 5.9	87.1 ± 7.7
2	86.6 ± 4.8	84.1 ± 4.6	81.8 ± 4.9
Overall	87.3 ± 5.7	86.5 ± 5.6	84.4 ± 6.7

All the results were expressed as mean \pm SD. SD = Standard deviation.

The proportion of spermatozoa with normal acrosome, mid-piece and tail did not differ significantly ($P>0.05$).

The mean proportion of normal spermatozoa in chilled semen coincides with the results obtained by Sarangi *et al.* (2017) who reported sperm abnormality of 12.2% on Day 0 and 12.7% on Day 3. Following chilling, higher normal sperm percentages (95.1% on Day 1, 92.8% on Day 2 and 90.8% on Day 3) were recorded by Pradhan *et al.* (2013). The variations in the results could be due to the physical and chemical environments (Pradhan *et al.*, 2013) and because of the water exchange of spermatozoa during the early stages of the preservation (Ozkavukcu *et al.*, 2008).

In conclusion, the characteristics of fresh semen of two Boer-cross bucks were within normal range. The quality of semen deteriorated with increasing period of chilling up to 72 hours. Further study is required in order to improve the preservation protocol for obtaining satisfactory quality chilled semen of Boer-cross bucks in Bangladesh.

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