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Article

Evaluation of reproductive performance of indigenous cows using Brahman cross-bred bull in a participatory breeding program

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Abstract: The present study was conducted at the Artificial Insemination Center under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University and 3 selected regions of Mymensingh district (Bhabakhali, Boera and Douhakhula) to find out the reproductive performance of indigenous cows using Brahman cross-bred bull in a participatory breeding program. Semen characteristics of Brahman cross-bred breeding bulls were ejaculate volume, mass motility, sperm concentration and live sperm percentage were evaluated. A total of 1087 indigenous cows and four Brahman cross-bred breeding bulls (Bull ID ABG011, ABG012, ABG013 and ABG014) were used. Data on ejaculate volume, mass motility, sperm concentration and live sperm percentage of Brahman cross-bred breeding bulls were analyzed by SAS computer program. The pooled values of ejaculate volume, mass motility, sperm concentration and live sperm percentage were 4.00±0.06 ml, 69.19±0.29%, 1144.59±5.73 million/ml and 83.55±0.97%, respectively. The ejaculate volume, mass motility, sperm concentration and live sperm percentage were differed significantly ($p<0.05$) among the bulls in different seasons. Individual bull had significant ($p<0.01$) effect on the ejaculate volume, mass motility and sperm concentration. Season also had significant ($p<0.01$) effects on the ejaculate volume, mass motility, sperm concentration and live sperm percentage. Highest non-return rate (87.31%) was found on bull ABG012 in Bhabakhali where maximum conception rate **(**71.60%) was observed in Bhabakhali. The highest calving rate (96.29%) at second parity was obtained in Boera. Variations on ejaculate volume, mass motility, sperm concentration and live sperm percentage of Brahman cross-bred breeding bulls indicated that there is further scope to select breeding bull on the basis of semen production potential which would be use to upgrade indigenous cows for improving their beef production.

Keywords: Brahman cross-bred bulls; indigenous cows; reproductive performance

1. Introduction

Poverty alleviation is one of the most important challenges of the twenty first century in Bangladesh. The agricultural sector comprising crops, livestock, fisheries and forestry have been playing important roles to mitigate this challenge (Islam, 1998). Livestock is the most important agricultural component which alone contributes about 1.78% of total GDP in 2013-14 (BER, 2015). Although the cattle population in Bangladesh is considerably high but the productivity is not satisfactory in terms of productive and reproductive performance as well as calf crop production, probably due to lack of technical knowledge and appropriate breeding program. AI is being used as an economical tool for the rapid exploitation of superior germplasm. The basic aim of cattle breeding program in Bangladesh is to improve the genetic potentiality of local cattle through the infusion of exotic blood. But the success of AI program partly depends on semen quality, skill of AI technician, awareness of farmers. Non-return rate is defined as the proportion of inseminated cows which have not been subsequently re-bred within a specified period of time after an insemination (Willett and Salisbury, 1942). The non-return rate of bulls depends on holistic semen characteristics of bull, breeding soundness of cows and appropriateness of time and site for semen deposition. The factors influencing the reproductive performance of cattle such as age, inter-calving period, heat expression, interval between calving to first service, first service conception rate, service per conception and efficiency of estrus detection, are often used as the measures of reproductive performance in dairy herd health program (Rice, 1980). There is no recognized beef breed in Bangladesh. Crossbreeding of native cattle with Brahman breed for beef production has been advocated as a breeding policy across the country (GOB, 2007). Considering weather, agro-climatic condition, heat tolerance, disease and insect resistance, longevity, grazing ability, calving ease, mothering ability and management, Brahman breed is considered to be the most suitable and compatible beef breed in tropical and sub-tropical regions (Antonio *et al*., 2006) that is why evaluation of reproductive performance of Brahman cross-bred bull is necessary to disseminate Brahman breed in Bangladesh for the improvement of beef cattle by up-grading breeding program.

2. Materials and Methods

2.1. Location and duration

The present study was conducted at the Artificial Insemination (AI) Center under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh and 3 selected regions of Mymensingh District (Bhabakhali, Boera and Douhakhula) from May, 2015 to November, 2015.

2.2. Management of experimental animals

A total of 4 (ABG011, ABG012, ABG013 and ABG014) Brahman cross-bred (50%) breeding bulls and 1087 indigenous cows were included in this study. All these bulls are being maintained under uniform conditions of feeding and management at the Artificial Insemination Centre of Bangladesh Agricultural University. Collected semen of those bulls had been evaluated to estimate the non-return rate of the bulls and used to inseminate the indigenous cows in the selected areas.

2.3. Semen collection

Semen was collected by Artificial Vagina (AV) method. The Artificial Vagina was prepared appropriately having proper temperature (110-115ºF). Semen was collected twice a week in the early morning (8.00 am). After collection, semen was kept in water bath at 37ºC until going for further handling.

2.4. Semen characteristics of Brahman cross-bred breeding bull

2.4.1. Semen evaluation

The evaluation of fresh semen was done immediately after collection using the methods described by Herman and Madden (1963). The ejaculate volume (ml), mass motility (%), sperm concentration (%), and live sperm (%) in fresh ejaculate of individual bull were recorded.

2.4.2. Ejaculate volume

The ejaculate volume of semen was measured directly with the help of graduated collection vial in ml.

2.4.3. Mass motility

A clean glass slide was warmed at approximately 37ºC and semen was mixed properly by inverting the vial for 2 to 3 times. One drop of semen (as small as possible) was placed on the pre-warmed (37°C) slide and spreaded. The slide was examined immediately using by lower objective (10X) on the microscope. The number of motile sperm was counted in a field carefully.

Mass motility was expressed as percentage. Mass motility was scored into 5 scales according to Herman and Madden (1963).

5 = Excellent motility (80% or more of the spermatozoa are in very vigorous motion);

4 = Very good motility (approximate 70-80% of the spermatozoa are in vigorous motion. Waves and eddies form and drop rapidly, but not so rapid as in excellent motility);

3 = Good motility (about 50 to 70% of the spermatozoa are in motion. Motion is vigorous but waves and eddies formed moved slowly a Cross-bred the field, in the main);

2 = Fair motility (from 20 to 50% of the sperm are in motion. The movements are largely vigorous but no waves or eddies are formed);

1 = Poor motility (less than 30% sperm are in motion. The motion is mostly weak and oscillatory, not progressive); and

0 = No motility discernable.

2.4.4. Sperm concentration

The concentration of spermatozoa was determined by hemacytometer method according to Herman and Madden (1963). Semen sample was drawn into a standard red cell dilution pipette up to 0.5 marks. Dilution fluid was drawn into the pipette up to 101 marks. Pipette was agitated for 3-5 minutes to ensure proper mixing by eight-knot motion. The first 4-5 drops were discarded to get properly diluted semen. A cover slip was placed over the ruled field of the counting chamber of the hemacytometer and a drop was allowed to run the cover slip. The count was made under low magnification (25X). Five large double ruled squares were counted over the field. This would give a total of small quarters than the number of spermatozoa per ml of semen to be calculated. The concentration of spermatozoa per ml of semen was expressed as million/ml. The following formula was used for calculating total number of spermatozoa per ml of fresh semen as described by Herman and Madden (1963).

The number of spermatozoa per ml of semen = $\frac{\text{C}\times 4000}{\text{S}} \times \text{D}$

Here,
C= Number of sperm counted in given number of small squares;
S= No. of small squares counted; and
D= Dilution ratio.

2.4.5. Live sperm percentage

A one-two drop of previously prepared eosin-nigrosin solution was taken in a glass slide and a very small amount of semen was placed on the solution of the slide. It was spreaded by pulling gently a second glass slide on it. Then the slide was placed on the hot plate (150-200ºF) for drying. The live sperm was counted under 40X objectives. The live spermatozoa were appeared unstained. Live sperm percentage was calculated by the following formula:

$$\%\text{ of live sperm} = \frac{\text{number of live spermatozoa}\times 3}{10}$$

2.5. Non-return rate of bull

Non-return rate of cows are useful to monitor the fertility of the bulls. Pregnancy was confirmed by 60 days non-return rate of cows after service. (Salisbury *et al*., 1978) reported that the cow once inseminated if sold or dead will appear on the record as conceived animal. On the other hand, cows that come in heat (5 to 7%) while pregnant would appear on the records as return**.**

$$\text{Non} - \text{return rate } (\%) = \frac{\text{Cows not return to estrus within 60 days}}{\text{Total number of cows served}} \times 100$$

2.6. Conception rate of cows

The pregnancy was diagnosed between 60 and 90 days after insemination by rectal palpation (Ball, 1980) at the farmer's house. The number of the cows diagnosed pregnant was recorded as 1 for conceived cows and 0 for non-conceived cows and the data were analyzed. First service conception rate for particular group was determined by the number of heifers or cows conceived to the first service divided by the number of heifers or cows given first service multiplied by 100.

$$\text{Conception rate} = \frac{\text{number of pregnant cows}}{\text{total number of cows inseminated}} \times 100$$

2.7. Calving rate of cows

The calving rate of cows was estimated using the following formula:

$$\text{Calving rate } = \frac{\text{number of calves born}}{\text{total number of cows conceived}} \times 100$$

2.8. Season

To evaluate the seasonal variation on semen quality the experimental period was divided into three seasons:
a) Summer season (March to June);
b) Rainy season (July to October);
c) Winter season (November to February).

2.9. Statistical analysis

The data generated from this experiment were entered in Microsoft Office Excel worksheet, checked, organized and processed for further analyses. The processed data was analyzed to obtain the difference in volume per ejaculate, mass motility, sperm concentration, normal sperm percentage and live sperm percentage of ANOVA by using Statistical Analysis System (SAS, 1998) computer package. DUNCAN test was performed to separate mean values in case of significant factors.

3. Results and Discussion

Ejaculate volume was significantly ($p<0.05$) affected by season. Maximum ejaculate volume (4.86±0.18 ml) was observed for Bull ABG013 in winter season and minimum ejaculate volume (2.51±0.19 ml) was observed for Bull ABG014 in summer season (Table 1). This findings is contradict to the studied of Tania (2012) who observed that maximum volume was found in summer season (3.72±0.24 ml) and minimum volume was in rainy season (3.52±16 ml) considering only two seasons.

Table 1. Effect of season on ejaculate volume, mass motility and sperm concentration of Brahman cross-bred (50%) breeding bulls.

Bull ID	Seasons (Mean±SE)		
	Summer	**Rainy**	**Winter**
Ejaculate volume (ml)			
ABG011	3.78^{b}±0.20 (30)	4.33^{a}±0.14 (47)	4.58^{a}±0.20 (37)
ABG012	3.61^{b}±0.60 (30)	3.84^{a}±0.16 (48)	4.22^{a}±0.13 (37)
ABG013	4.75±0.20 (28)	4.44±0.08 (48)	4.86±0.18 (38)
ABG014	2.51±0.19 (27)	3.19±0.14 (47)	3.59±0.15 (38)
Mass motility (%)			
ABG011	70.80^{ab}±1.30 (30)	72.45^{a}±0.66 (47)	69.73^{b}±0.89 (37)
ABG012	66.83±1.41 (30)	70.42±0.83 (48)	65.95±0.64 (37)
ABG013	72.50^{a}±1.19 (28)	70.10^{ab}±0.86 (48)	67.37^{b}±0.88 (38)
ABG011	70.80^{ab}±1.30 (30)	72.45^{a}±0.66 (47)	69.73^{b}±0.89 (37)
ABG014	67.83±1.53 (27)	69.26±0.87 (47)	66.05±0.71 (38)
Sperm concentration (million/ml)			
ABG011	1165.87^{b}±24.55(30)	1217.77^{a}±11.93 (47)	1119.05^{b}±14.44(37)
ABG012	1095.13^{b}±28.22(30)	1181.58^{a}±15.22 (48)	1087.22^{b}±9.96 (37)
ABG013	1202.32^{a}±24.48 (28)	1188.00±14.19 (48)	1132.53^{b}±14.92(38)
ABG014	1044.85^{b}±43.11(27)	1150.57^{a}±15.58 (47)	1088.53^{b}±10.73(38)

Figures in the parenthesis indicate the number of observation; means with different superscript(s) in the same row differed significantly ($p<0.05$).

Mass motility differed significantly ($p<0.05$) among the bulls in different seasons. Maximum mass motility (72.50±1.19%) was observed for Bull ABG013 in summer season and minimum mass motility (65.95±0.64%) was observed for Bull ABG012 in winter season (Table 1). Ahmed *et al.* (2014) conducted an experiment and stated that the highest motility (60.5±1.5%) and lowest (56.6±2.3%) of semen was obtained from local and Friesian bulls, which lower than the present study. This variation was probably due to the genetic potentiality of bulls and season of the year.

Sperm concentration differed significantly ($p<0.05$) among the bulls in different seasons. Highest Sperm concentration (1217.77±11.93 million/ml) was observed for Bull ABG011 in rainy season and lowest sperm concentration (1044.85±43.11 million/ml) was observed for Bull ABG014 in summer season (Table 1). From the Table 1 it was showed that the bull ABG013 produce good quality semen compared to other bulls. This variation was probably due to their genetic potentiality. From the Table 1 it was showed that the bull ABG013 produce good quality semen compared to other bulls. This variation was probably due to their genetic potentiality. Godfrey *et al.* (1990) studied sperm concentrations for the Brahman bulls were lower than for the Hereford bulls in six of the seven sampling periods.

Analysis of variance revealed that live sperm percentage significantly ($p<0.05$) affected by the season in different bulls. Maximum live sperm (86.23±0.22%) was observed for Bull ABG011 in rainy season and minimum live sperm (80.43±3.00%) was observed for Bull ABG014 in summer season (Table 2). From the Table 2 it was showed that the bull ABG013 and bull ABG011 produce good quality semen compared to other bulls. The differences might be due to individuality and variation in body conformation of the different bulls and seasons.

Table 2. Effect of season on live sperm (%) of Brahman cross-bred (50%) breeding bulls.

Bull ID	Seasons (Mean±SE)		
	Summer	Rainy	Winter
Live sperm (%)			
ABG011	82.47^b±1.89 (30)	86.23^a±0.22 (47)	82.37^b±0.33 (37)
ABG012	81.26^b±1.86 (30)	84.47^a±0.32 (48)	82.09^b±0.34 (37)
ABG013	82.98^b±1.13 (28)	85.49^a±0.30 (48)	82.77^b±0.36 (38)
ABG014	80.43^b±3.00 (27)	84.55^a±0.27 (47)	83.76^a±0.36 (38)
ABG011	82.47^b±1.89 (30)	86.23^a±0.22 (47)	82.37^b±0.33 (37)

Figures in the parenthesis indicate the number of observation; means with different superscript(s) in the same row differed significantly (p<0.05).

Table 3. Pooled average and mean±SE of semen characteristics of different Brahman cross-bred bulls.

Semen Characteristics	Pooled values	Graded Brahman bulls			
		ABG011	ABG012	ABG013	ABG014
EV (ml)	4.00±0.06 (455)	4.26^b±0.12 (114)	3.90^c±0.09 (115)	4.65^a±0.08 (114)	3.17^b±0.10 (112)
MM (%)	69.19±0.29 (451)	71.05^a±0.31 (114)	68.04^b±0.26 (115)	69.78^a±0.29 (114)	67.82^b±0.30 (108)
SC (mil/ml)	1144.59±5.73 (455)	1172.07^a±8.12 (114)	1128.67^b±7.25 (115)	1173.03^a±8.21 (114)	1104.04^b±9.55 (112)
LS (%)	83.55±0.97 (451)	83.99^a±1.21 (114)	82.87^a±1.55 (115)	83.97^a±0.98 (114)	83.36^a±1.23 (108)

EV, ejaculate volume; MM, mass motility; SC, sperm concentration; LS, live sperm; means with different superscripts in the same row differed significantly (p<0.01); Figures in the parenthesis indicate the number of observation.

From the Table 3, it was observed that the mean value of ejaculate volume was ranged between 3.17 to 4.65 ml. In fresh semen, there was a significant (p<0.01) bull variation for ejaculate volume. The highest ejaculate volume (4.65±0.08 ml) was found in Bull ABG013 compared to other bulls and the lowest ejaculate volume (3.17±0.1 ml) was found in Bull ABG014. Akhter *et al.* (2013) observed that the overall mean volume of semen was 6.92±0.2 ml in crossbredbred, which was higher than the present study. Mostari (2002) obtained the significant (p<0.01) individual variation in semen volume for Sahiwal bulls, which was almost similar to the present findings.

It was observed from the Table 3, the average value of mass motility (%) was ranged between 67.82 to 71.05. The mass motility was significantly (p<0.01) affected by the bulls. Maximum mass motility (71.05±0.31%) was found in Bull ABG011 compared to other bulls and minimum mass motility (67.82±0.3%) was found in Bull ABG014. Akhter *et al*. (2013) observed that the overall mean (%) motility was 67.90 in crossbred, which was almost similar to the present study.

The mean value of sperm concentration (million/ml) was ranged between 1104.04 to 1173.03. Sperm concentration differed significantly (p<0.01) among the bulls. Sperm concentration was highest (1173.03±8.21 million/ml) in Bull ABG013 compared to other bulls and lowest one (1104.04±9.55 million/ml) was observed for Bull ABG014 (Table 3). The present findings agree with the previous study of the Tania (2012), who studied semen characteristics of Brahman crossbred breeding bulls.

The mean value of live sperm percentage was found to be ranged between 82.87 to 83.99. The variations of live sperm percentage between bulls were not significant (p>0.05). Maximum live sperm (83.99±1.21%) was observed for Bull ABG011 compared to other bulls and minimum live sperm (82.87±1.55%) was observed for Bull ABG012 (Table 3). Akhter *et al.* (2013) observed the average live sperm of semen was 83.56%, which was almost similar to the average live sperm percentage of present study. Talukder *et al.* (2012) observed that the pooled average live sperm percentage 76.6±8.8, which was lower than the present study. The lower percentage of live sperm could be due to younger age of bulls, and breed difference and lower adaptability to the environmental conditions.

3.1. Effects of season on semen characteristics

3.1.1. Ejaculate volume

The variation of ejaculate volume between seasons were significant (p<0.01). From the Figure 1, it was observed that the highest ejaculate volume (4.31 ml) was found in winter season than other two seasons (3.67 and 3.95 ml for summer and rainy, respectively). Snoj *et al.* (2013) conducted an experiment; it was revealed that the ejaculate volume and total sperm output was influenced by season in all breeds. The highest ejaculate volume and total number of sperm in ejaculates were observed during the summer, followed by spring, autumn, and winter, that was lower than the present study.

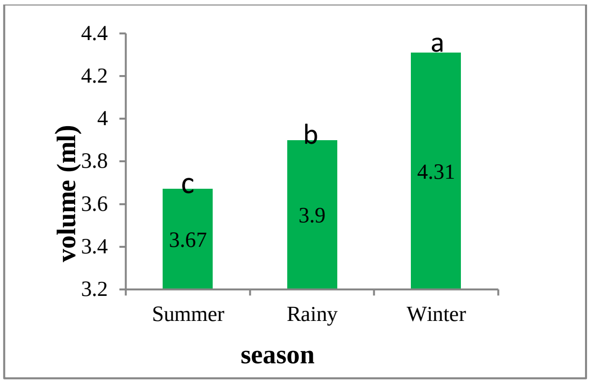

Figure 1. Effects of season on ejaculate volume (letter at the top of the bar indicates significant different p<0.01) between bars).

3.1.2. Mass motility

From the Figure 2, it was observed that the average mass motility (%) in fresh ejaculate was ranged between 67.27 to 70.55. Analysis of variance revealed that season to season variation was significant ($p<0.01$) in motility of fresh semen. Maximum motility (70.55%) was found in rainy season followed by other two seasons (69.46 and 67.27% for summer and winter, respectively). An experiment conducted by Shaha *et al.* (2008), which contradict with the present study. They studied on four bulls of mixed breeds (Sahiwal × Zebu, Sindhi × Zebu, Jersey × Zebu and Holstein-Friesian × 25 Zebu) and examined physically and their semen was evaluated in three seasons: I (rainy): May-November, II (winter): December-February, III (summer): March-April. The semen volume, sperm motility ranged from 56.6 to 76%. They also concluded that the mass motility varied significantly ($p<0.05$) between breeds and seasons.

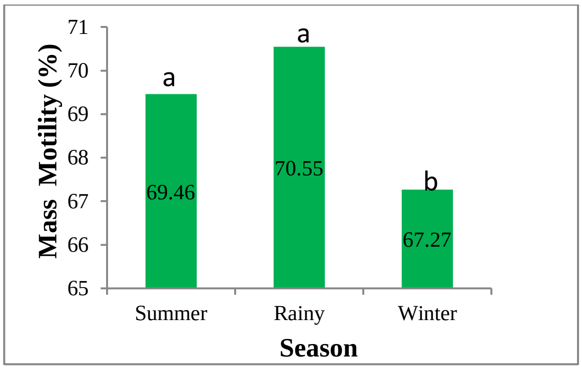

Figure 2. Effects of season on mass motility (letter at the top of the bar indicates significant different p<0.01) between bars).

3.1.3. Sperm concentration

Sperm concentration differed significantly ($p<0.01$) between seasons. From the Figure 3, it was observed that the average sperm concentration in fresh ejaculate was ranged between 1106.88 to 1184 million/ml. Highest sperm concentration (1184.48 million/ml) was found in rainy season followed by other two seasons (1127.88 and 1106.88 million/ml for summer and winter, respectively). This finding is similar with the result of Tania (2012) who found sperm concentration was highest (1246.25±185.53mm^3/ml) in rainy season and lowest one in summer season (1158.33±83.04mm^3/ml) for Brahman crossbred breeding bulls.

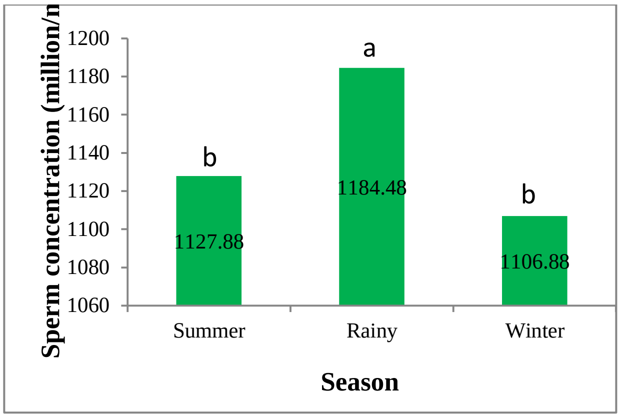

Figure 3. Effects of season on sperm concentration (letter at the top of the bar indicates significant different p<0.01) between bars).

3.1.4. Live Sperm Percentage

Analysis of variance revealed that season to season variation was significant ($p<0.01$) in normal sperm percentage of fresh semen. From the Figure 4, it was observed that the mean live sperm percentage in fresh ejaculate was ranged between 81.83 to 85.19. Live sperm was highest (85.19%) in rainy season followed by other two seasons (81.83 and 82.76% for summer and winter, respectively). Ahmad *et al.* (2011) conducted an experiment on semen evaluation and stated that the percentage of live sperm (67.7±0.7), which lower than the present study. These differences might be due to difference in management or semen collection times.

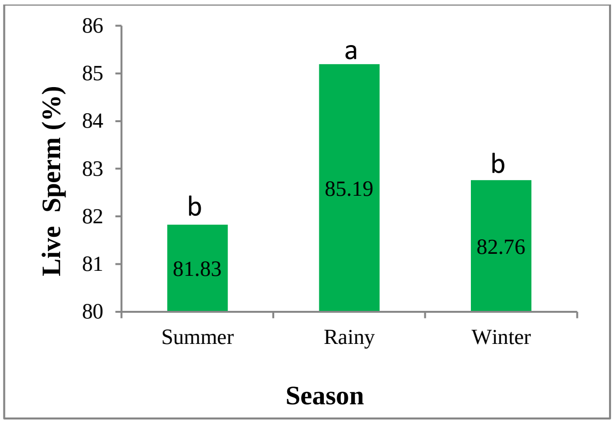

Figure 4. Effects of season on live sperm (letter at the top of the bar indicates significant different p<0.01) between bars).

The average 60 days non-return rate to insemination of 4 bulls is presented in Table 4. It was observed that non-return rate was highest (87.31%) in Bhabakhali of bull ABG012 and the lowest one (67.49%) was found on bull ABG013 in Douhakhula. Talukder *et al.* (2012) observed that the overall non-return rate is 82.7% (72.9～87.5%) which was almost similar to the present study. They also reported that Bulls with semen of higher sperm motility in fresh ejaculates had the higher non-return rate. Bulls having large number of viable spermatozoa in fresh semen achieved the higher non-return rate.

Table 4. Non-return rate of Brahman Cross-bred breeding bull (N=1087).

Bull No.	Bhabakhali	Boera	Douhakhula
ABG011	73.56	75.32	68.77
ABG012	87.31	79.46	69.66
ABG013	68.66	76.36	67.49
ABG014	74.33	69.34	70.66

3.2. Conception rate of indigenous cows

It was observed that the highest conception rate was found in Bhabakhali followed by other two areas, Douhakhula and Boera (Table 5). Kabir (2000) conducted an experiment involving 360 cows and 4 heifers of different genotypes and 3 bulls at the Bangladesh Agricultural University AI Center. He reported that location had significant ($p<0.05$) effect on conception rate on cows. The mean conception rates were 43.81±5.28%, 51.75±4.70% and 33.76±3.78% for location 1, 2 and 3, respectively. The highest (51.75%) and lowest (33.76%) conception rate were found in location 2 location 3. The results indicated that the variation of conception rate in different locations mainly regarded as the differences of overall management of animals by the farmers, the availability of feed resources.

Table 5. Conception rate (%) of indigenous cows breeding with Brahman semen.

Area	Number of cows inseminated	Number of cows conceived	Conception Rate (%)
Boera	236	144	61.02
Bhabakhali	412	295	71.60
Douhakhula	439	247	56.24
Total	1087	686	63.11

3.3. Calving rate of indigenous cows

The calving rate of cows in different areas and parity are presented in Table 6. The overall calving rate of cows was 90.96%. In Boera, the highest calving rate 96.29% was obtained in second parity. On the other hand, maximum calving rate 91.84% at first parity was observed in Douhakhula. Irshad-ul-Haq *et al.* (1996) studied on 200 Sahiwal cows and stated that the overall calving rate was 72 %, which is lower than the present study. The results indicated that the variation of calving rate in different parity and locations mainly regarded as the differences of genotype of the cows, overall management of animals by the farmers and the availability of feed resources.

Table 6. Calving rate (%) of indigenous cows breeding with Brahman semen.

Parity	Boera (135)			Bhabakhali (263)			Douhakhula (226)			Overall calving rate (%)
	CC	CB	Calving rate (%)	CC	CB	Calving rate (%)	CC	CB	Calving rate (%)	
First	39	36	92.31	61	56	91.80	49	45	91.84	
Second	54	52	96.29	110	98	89.09	91	82	90.11	90.96
Third	51	47	92.16	124	109	87.90	107	99	92.52	

Figures in the parenthesis indicate the number of observation; CC, number of cows conceived; CB, number of calves born

4. Conclusions

It can be concluded that the reproductive performance of indigenous cows was satisfactory by using frozen semen of Brahman Cross-bred bull at the community level and it will be possible to improve the performance of indigenous cows. However, further study on this regard is suggested for authentic conclusion.

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Conflict of interest

None to declare.

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