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Research Article

Semen Quality Variations in Successively Graded up Sahiwal Breeding Bulls

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ABSTRACT

The study was conducted to find out the variation in semen quality among three successive graded up (F_1, F2 and F_3) Sahiwal×local breeding bulls for artificial insemination (AI) purposes. There were three (3) groups (F_1, F2 and F_3) and three bulls were selected from each group for this experiment. F_1 group means 100% Sahiwal×Local (SL×L) bulls, F_2 and F_3 are 100% Sahiwal×Sahiwal×Local (SL_1×L) bulls, and Sahiwal×Sahiwal× Sahiwal×Local (SL_2×L) bulls, respectively. 52 ejaculates from each of 9 breeding bulls were collected, evaluated and processed following standard procedure throughout the experimental year 2019. A total of 468 (52×3×3) samples (52×3=156 from each group) were examined in this study. Out of 468 ejaculates,385 (82.26%) were found to be creamy in color followed by 64 (13.68%) and 19 (4.06%) as yellowish and watery, respectively. Level of up-gradation had a significant (P<0.05) effect on ejaculate volume, consistency, mass activity, sperm concentration and initial and post-thaw motility. The highest (5.356±0.10 ml) and the lowest (4.726±0.09ml) volume of semen were found in the third (F_3) and the first (F_1) crossing, respectively (p<0.05). The mass activity ranged from 3.216±0.04to 4.389±0.05. Semen pH varied insignificantly (p<0.05) but sperm concentration, initial motility and post-thaw motility had significant differences (p<0.05). Initial and motility ranged from 71.49±0.24% to 78.89±0.43% and from 50.29±0.39% to 53.46±0.36%, respectively. It was concluded that most of the semen quality parameters were influenced by the level of up-gradation and freezing. Semen parameters were observed better in F_2 followed by F_3 group.

Introduction

Artificial Insemination (AI) is nowadays widely used as a breeding tool for genetic improvement in farm animals. In this worldwide technique, semen from genetically superior males is collected, diluted, processed and inseminated to a large number of female populations that otherwise is quite impossible to cover through natural mating. AI has been considered as the single most important technology for the genetic improvement of cattle (Hafez, 1993). Quality bull deficiency has a greater impact on herd productivity than fertility problems in a single female: a common belief is that the bull is half the herd. When AI is used, each ejaculate can produce more than 250 inseminations, representing at least 50,000 doses per bull per year (Rodriguez, 2008). Therefore, it is important in the selection of breeding bulls to determine the quality of semen. The success and efficiency of AI depend on several factors. Among these semen quality is top of them. The semen quality varies breed to breed as well as grade to grade in same breed in different up-gradation level. Good quality semen is a must for a successful conception in cattle and therefore, a determinant of reproductive efficiency (Latif et al., 2009). A previous study reported that the qualities of semen i.e. ejaculate volume, sperm motility; viability and concentration etc. were affected by breeds (Al-Hakim et al., 1986). Therefore, the present study was planned to assess the variation of semen quality among successive upgraded Sahiwal×local breeding bulls.

Materials and Methods

Location and time of study

The experiment was carried out in the research and development unit of research-based animal breeding organization of Bangladesh (Lal Teer Livestock Development Bangladesh Limited) located at Mymensingh district. The study was carried out throughout the year 2019.

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Animals and their ration

Nine breeding bulls (30 to 52 months of age and body weight of 390.50 to 512.00 kg) were selected for this study. Out of 9 bulls, 3 were Sahiwal×Local (SL×L), 3were Sahiwal×Sahiwal×Local (SL_1×L), 3 were Sahiwal×Sahiwal× Sahiwal×Local (SL_2×L) bulls. The animals were maintained in a subtropical environment and reared in an intensive farming system following the ethical approval rules of the institute as well as the Government of Bangladesh. The bulls were farmed under optimal feeding with regular exercise during the whole period of the experiment. All the bulls were physically fit, free from diseases, clinically normal and sound in breeding. The bulls were vaccinated against Anthrax, Foot and Mouth Disease (FMD), Black Quarter (BQ), and Hemorrhagic Septicemia (HS) according to the schedule. They were fed *ad libitum* green grass supplemented with good quality concentrate mixture prepared with wheat bran, corn, rice polish, soybean meal, mustard oil cake, DCP, vitamin-mineral premix and common salt (Table 1). The concentrate mixture was adjusted with 70.00% TDN and 17.00% CP.

Table 1. Feed ingredients in supplemented ration for breeding bulls at Lal Teer Livestock Development (BD) Limited

Ingredients	Amount (kg)	DM (kg)	TDN (kg)	DCP (kg)	Ca (kg)	P (kg)
Corn	32.00	28.80	25.40	3.12	0.0063	0.003
Rice Polish	20.00	17.70	16.02	2.90	0.0011	0.028
Wheat Bran	20.00	17.70	12.34	2.65	0.0027	0.020
Mustard Oil Cake	12.00	10.46	7.58	4.01	0.0080	0.002
Soybean Meal	10.00	8.90	7.62	4.32	0.0031	0.01
Lime stone powder	2.00	1.98	0.73	-	0.0740	-
D.C.P	2.00	1.98	0.81	-	0.0460	0.04
Common salt	1.00	0.99	-	-	-	-
Vita. Min premix	1.00	0.99	-	-	-	-
Total	100.00	89.50	70.50	17.00	0.14	0.10

Semen collection, evaluation and preservation

From the experimental bulls **s**emen was collected early in the morning twice a week using sterilized bovine artificial vagina (IMV model-005417) maintaining optimum temperature (42°-45°C), pressure and softness (Arthur et al., 1982). Semen was collected by a skilled semen collector. After collection, each ejaculate was placed in a tube in the warm water bath at 37°C and various standard laboratory tests for semen evaluation were performed. Ejaculate volume of semen was measured directly from the graduated centrifuge collection tube. Color and consistency of semen were observed with the naked eye. By indicator paper strips semen pH was determined (Salisbury et al., 1978). The mass activity of semen was recorded by placing a small drop of fresh semen on a wormed glass slide without cover slip under low magnification (10x) of a digital microscope and graded from 0 to 5 grades. The concentration of sperm per ml of semen was estimated through a bovine sperm photometer (IMV technologies, France).

Initial motility of fresh semen was assessed by placing a small drop of semen on the wormed glass slide and covering it by cover slip under high magnification (40x) using phase contrast microscope. Semen with motility of more than or equal to 70% was diluted with egg yolk-citrate-glycerol semen extender (laboratory prepared extender). The diluted semen was subsequently loaded in 0.25 ml/straw (IMV technologies, France), cooled at 4°C for 3.5 to 5 hours. Semen straws were then frozen using IMV bio freezer following the standard procedure of IMV technologies. After that, frozen straws were stored in liquid nitrogen until used for insemination. After 24 hours post-thaw motility of semen was assessed as initial motility was assessed.

Statistical Analysis

Necessary information and the recorded data collected from the study was coded and recorded in Microsoft Excel. Descriptive statistics such as number, percentage distribution, mean, maximum, minimum, standard error, etc. were used in describing the variables. One way ANOVA, column statistics, Tukey test, all this analysis was performed using GraphPad Prism 5 software.

Results and Discussion

In this study, a total of 9 bulls (3 bulls from each graded up population) were selected and 52 ejaculates from each bull throughout the experimental year were studied, hence, a total of 468 (52×9) ejaculates were examined and evaluated.

Color and Consistency

Out of the 468 ejaculates,385 (82.26%) were creamy in color followed by 64 (13.68%) and 19 (4.06%) as yellowish and watery, respectively. A study (Harandra et al., 2017) reported that out of 181 seminal ejaculates 82.3% were creamy 8.8% were yellowish and 2.2% were watery in Frieswal bulls, which are close to the present

study. There were significant differences in semen consistency of upgraded Sahiwal breeding bulls.

The thick category of semen was found to be the highest followed by moderate thick and thin category semen in all studied bulls' semen. Among the three groups of upgraded bulls, the highest percentage of thick category semen was found in F_2 upgraded breeding bulls (Fig. 1).

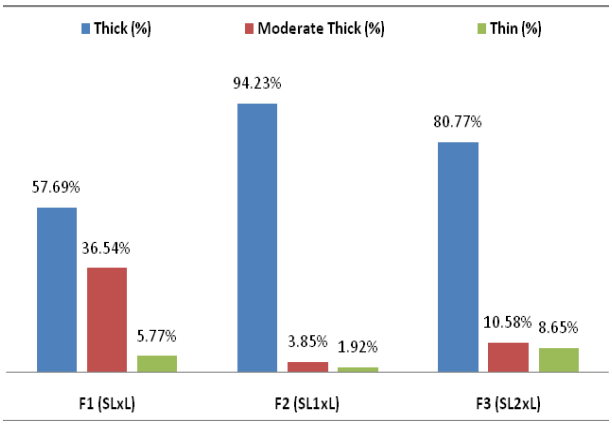

Figure 1. Semen consistency of three successive upgraded Sahiwal×local breeding bulls

Ejaculate volume and Mass activity

Ejaculate volume and mass activity were varied significantly ($p<0.05$). Among the three groups of upgraded Sahiwal breeding bulls. The highest volume of semen (5.356±0.10ml) was found in F_3 (SL_2xL) followed by F_2 (SL_1xL) and the lowest amount (4.726±0.09 ml) was measured in F_1 (SLxL) bulls. A previous study (Latif et al., 2009) showed the volume of semen in Sahiwal crossbred breeding bulls as 3.7±1.8 ml,which is slightly lower than the present study results.Whereas another experiment (Ahmed et el., 2014) reported the volume of semen of local cross sahiwal bulls as 5.0±0.5ml,which is almost close to the present study. The highest mass activity was found to be 4.389±0.05out of 5.00 in F_3 (SL_2×L) bulls and the lowest mass activity was found in F_1 (SL×L) bulls (Table 2).

Table 2. Variation in Semen quality parameters of three successive upgraded Sahiwal ×local breeding bulls (F_1, F_2 and F_3)

Group	F_1 (SL×L)	F_2 (SL_1×L)	F_3 (SL_2×L)
Sample size (N)	N=104	N=104	N=104
Ejaculate volume (ml)	4.726±0.09^c	5.099±0.08^b	5.356±0.10^a
Mass activity (0-5)	3.216±0.04^b	4.313±0.04^a	4.389±0.05^a
Sperm concentration (millions/ml)	1214±28.67^c	2021±39.05^a	1537±44.10^b
P^H	6.437±0.01	6.539±0.01	6.397±0.01
Initial motility (%)	71.49±0.24^c	78.89±0.43^a	76.68±0.46^b
Post-thaw motility (%)	50.29±0.39^b	53.46±0.36^a	51.54±0.34^{ab}

Mean± SE with different superscripts within a row differ significantly ($P<0.05$)

Sperm concentration and semen pH

Sperm concentration is regarded to be one of the most important semen attributes and significant differences in the concentration of sperm have been shown in semen from different bulls (Graffer et al., 1988; Shelke and Dhami, 2001). In the present study, the results of sperm concentration summarized in Table 2 indicated that sperm concentration varied significantly with the level of up-gradation. The highest sperm concentration (2021±39.05million/ml) was found in F_2 (SL_1×L) bulls and the lowest sperm concentration (1214±28.67 million/ml) was observed in F_1 (SL×L) bulls. It was stated that sperm concentration varies from 500-2500 million/ml (Lating et al., 1988). Another study reported that the sperm concentration of dairy and beef bulls were from 1000 to 2000 and 800-1500 million/ml, respectively (Hafez, 1993) which is almost close to the present study.

In the present study, there were no significant differences ($p>0.05$) in semen pH. Semen pH of the studied bulls ranged from 6.397±0.01 to 6.539±0.01. A previous study (Hossain et al., 2012) also reported insignificant differences in semen pH in crossbred Sahiwal bulls.

Sperm concentration could be considered as an initial indicator of semen quality in semen used for cryopreservation (Shelke and Dhami, 2001). A positive correlation between motility and sperm concentration at semen collection has been reported (Everett et al., 1978; Mathevon et al., 1998). Nevertheless, the present time literature regarding whether sperm concentration at the time of semen collection is an indicator of fertilization among normal fertility is quite scarce.

Initial motility and Post-thaw motility

The average initial motility was varied ($p<0.05$) from 71.49±0.24%to 78.89±0.43% (Table 2). The highest motility (78.89±0.43%) was observed in F_2 (SL_1×L) bulls and lowest (71.49±0.24%) in F_1 (SL×L) bulls. It was reported that the mean initial sperm motility in fresh ejaculates was between 63.00±0.32 and 64.00±0.35% (Sarder, 2003), which is lower than the present study but in another study (Latif et al., 2009) showed 76.6±2.7% sperm motility that is close to this study. In this study, significant differences ($p<0.05$) were observed in the initial motility percentage of semen of graded up Sahiwal bulls,which is in agreement with the findings of Hossain et al.,(2012). In contrast, other studies (Rekwot et al., 1987; Mathur et al., 2002) did not find any significant variation in initial motility percentage in Frieswal bulls and in Exotic and crossbred bulls.

In this study, post-thaw motility percent ranged from 50.29±0.39to 53.46±0.36and there was significant variation ($p<0.05$) within the studied breed. It was reported that the motility of sperm after thawing varied from 62.6 to 63.6% in crossbred Sahiwal bulls (Hossain et al., 2012),which is slightly higher than the results of the present study. Lower post-thaw motility than initial motility indicated that freezing of semen reduced sperm motility. It might be assumed that the consequences of sperm cryo-injury are caused by cryopreservation (Salmon et al., 1995). The plasma membrane of sperm is the primary site of damage induced by cryopreservation (Hammerstedt et al., 1990). Both freezing and thawing implicate tremendous alteration in the volume of cell water, which results in considerable mechanical stress on the sperm membrane and consequently reduce sperm motility (Hammerstedt et al., 1990).

Conclusion

It was found significant variation in semen quality among the studied groups of breeding bulls and It was concluded that ejaculate volume, sperm concentration, and sperm motility were influenced by the level of up-gradation, processing and freezing. Semen parameters were better in F_2 (SL_1×L) group among the studied three groups of upgraded Sahiwal × Local breeding bulls.

Competing interests

The authors have declared that no competing interests exist.

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