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Research Article

Semen quality variations in successively graded up sahiwal crossbred breeding bulls

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The study was conducted to find out the variation of semen quality among three successive graded up (F_1, F_2 and F_3) Sahiwal × local breeding bulls for Artificial Insemination (AI) purposes. Total of 468 ejaculates were collected from 9 breeding bulls throughout the experimental year 2019. The recorded data were summarized using Microsoft Excel 2010 and statistically analyzed using GraphPad Prism 5 software. 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 significant ($P<0.05$) effect on ejaculate volume, consistency, mass activity, sperm concentration and initial and post motility. The highest (5.356 ± 0.10 ml) and the lowest (4.726 ± 0.09 ml) volume of semen were found in third (F_3) and first (F_1) crossing, respectively ($p<0.05$). The mass activity ranged from 3.216 ± 0.04 to 4.389 ± 0.05. Semen pH varied insignificantly but sperm concentration, initial motility and post freezing motility had significant differences ($p<0.05$). Initial and post 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 level of up-gradation and freezing. Semen characteristics were better in F_3 followed by F_2 group.

Key words: Bull semen, ejaculate volume, semen quality

INTRODUCTION

Artificial Insemination (AI) is nowadays widely used as a breeding tool for genetic improvement in farm animals. In this world wide 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 Artificial insemination has been considered as the single most important technology for the genetic improvement of cattle. Quality bull deficiency has 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. Therefore, it is important in selection of breeding bulls to determine the quality of semen. The success and efficiency of AI depends on several factors. Among these semen quality is top of them. Good quality semen is must for successful conception in cattle and therefore, a determinant of reproductive efficiency. A previous study reported that the qualities of semen i.e. ejaculate volume, sperm motility; viability and concentration et cetera were affected by breeds. Therefore, the present study was planned to assess the variation of semen quality among successive upgraded Sahiwal × local breeding bulls [1].

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MATERIALS AND METHODS

Location and time of study

The experiment was carried out in research and development unit of renowned research based animal breeding organization of Bangladesh located at Mymensingh district, around 90 kilometers away from Dhaka. The study was carried out throughout the year 2019.

Animals and their ration

9 breeding bulls (38 to 52 months of age and body weight of 410.50 to 512.00 kg) were selected for this study. Out of 9 bulls, 3 were Sahiwal × Local (SL × L), 3were Sahiwal×Sahiwal × Local (SL1 × L), 3 were Sahiwal×Sahiwal× Sahiwal × Local (SL2 × L) bulls. The bulls were maintained under optimal feeding and management 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% TND and 17.00% CP [2].

Table 1. Ration for breeding bulls at lal teer livestock development limited.

Ingredients	Amount (kg)	DM (kg)	TDN (kg)	DCP (kg)	Ca (kg)	P (kg)
Corn	32	28.8	25.4	3.12	0.0063	0.003
Rice polish	20	17.7	16.02	2.9	0.0011	0.028
Wheat bran	20	17.7	12.34	2.65	0.0027	0.02
Mustard oil cake	12	10.46	7.58	4.01	0.008	0.002
Soybean meal	10	8.9	7.62	4.32	0.0031	0.01
Lime stone powder	2	1.98	0.73		0.074	
D.C.P	2	1.98	0.81		0.046	0.04

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. A male dummy was used for jumping the bulls and after 2 to 3 false jumps semen was collected by a skilled semen collector. After collection each ejaculate was placed in a tube in warm water bath at 37°C and various standard laboratory tests for semen evaluation were performed.

Ejaculate volume of semen was measured directly in milliliter (ml) from the graduated centrifuge collection tube. Color and consistency of semen was observed with the naked eye. By indicator paper strips semen pH was determined. 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 of a digital microscope and graded from 0 to 5 grades. Concentration of sperm per ml of semen was estimated through bovine sperm photometer. Initial motility of fresh semen was assessed by placing a small drop of semen on the wormed glass slide and covering by cover slip under high magnification (40x) using phase contrast microscope [3].

Semen with motility of more than or equal 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 less than 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 saved in liquid nitrogen until using for insemination. After 24 hours post motility of semen was assessed as initial motility was assessed.

STATISTICAL ANALYSIS

Recorded data was compiled by Microsoft Excel 2010. Compiled data was then statistically analyzed using GraphPad Prism 5 software. One way ANOVA and column statistics were performed for mean and standard error. For the multiple comparison and level of significance Tukey test was performed.

RESULTS AND DISCUSSION

In this experiment, total 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 reported that out of 181 seminal ejaculetes 82.3% were creamy 8.8% were yellowish and 2.2% were watery, which are close to the present study. There were significant differences in semen consistancy of crosssbred Sahiwal breeding bulls. 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 F2 of the studied breeding bulls. Probably it is due to the higher exotic blood percentage and environment of experiment time.

Ejaculate volume and Mass activity

Ejaculate volume and mass activity were varried significantly among the three groups of upgraded Sahiwal breeding bulls. The highest volume of semen (5.356 ± 0.10 ml) was found $F_3(SL_2 \times L)$ followed by $F_2(SL_1 \times L)$ and the lowest amount (4.726 ± 0.09 ml) was measured in F_1 (SL × L) bulls. A previous study showed the volume of semen in sahiwal cross local breeding bulls as 3.7 ± 1.8 ml which is slightly lower than the present study results whereas another experiment reported the volume of semen of local cross sahiwal bulls as 5.0 ± 0.5 ml which is almost close to the present study. The highest mass activity was found to be 4.389 ± 0.05 out of 5.00 in F_3 ($SL_2 \times L$) bulls and the lowest mass activity was found in F_1 (SL × L) bulls (Table 2).

Table 2. Variation of semen quality parameters of three successive upgraded Sahiwal × local breeding bulls (F1, F2 and F3)

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	5.099 ± 0.08	5.356 ± 0.10
Mass activity (0-5)	3.216 ± 0.04	4.313 ± 0.04	4.389 ± 0.05
Sperm concentration (millions/ml)	1214 ± 28.67	2021 ± 39.05	1537 ± 44.10
P^H	6.437 ± 0.01	6.539 ± 0.01	6.397 ± 0.01
Initial motility (%)	71.49 ± 0.24	78.89 ± 0.43	76.68 ± 0.46
Post freezing motility (%)	50.29 ± 0.39	53.46 ± 0.36	51.54 ± 0.34

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. In the present study, the results of sperm concentration summarized in Table 2 indicated that sperm concentration varied significantly with level of up-gradation. The highest sperm concentration (2021 ± 39.05 million/ml) was found in F2 (SL1×L) bulls and the lowest sperm concentration (1214 ± 28.67 million/ml) was observed in F1 (SL×L) bulls. It was stated that sperm concentration varies from 500-2500 million/ml whereas this concentration range of 1000-2000 and 800-1500 million/ml, respectively for dairy and beef bulls which is almost close to the present study [4].

In the present study, there was not found significant differences in semen pH ($P<0.05$). Semen pH of the studied bulls ranged 6.397 ± 0.01 to 6.539 ± 0.01. Previous study also reported the 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) which relies on over estimation of motility in more concentrated samples (Everett et al., 1978). Nevertheless, the present time literature regarding whether sperm concentration at the time of semen collection is an indicator of fertilization among normal fertility sire is quite scarce.

Initial motility and Post freezing motility

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 F2 (SL1 × L) bulls and lowest (71.49 ± 0.24%) in F1 (SL × L) bulls. Motility is one of the most important requirements of fertile semen. It was reported that the mean initial sperm motility in fresh ejaculates were between 63.00 ± 0.32 and 64.00 ± 0.35% which is lower than the present study but in another study showed 76.6 ± 2.7% sperm motility that is close to this study. It was also reported that the motility of spermatozoa is one of the best single evidence of viability. Duration of motility in stored semen was reported as another reliable index of fertility. In this study, significant differences were observed in initial motility percentage of semen of graded up Sahiwal bulls which is in agreement with the findings [5].

On the other hand, in Frieswal bulls and in Exotic and crossbred bulls were not found any significant variation in initital motility percentage. In this study, post freezing motility percent ranges from 50.29±0.39 to 53.46±0.36 and there was significant variation within studied breed. It was reported that the motility of sperm after freezing varied from 62.6 to 63.6% in crossbred Sahiwal bulls which is slightly higher than the results of the present study. Lower post freezing motility than initial motility indicated that freezing of semen reduced sperm motility. It might be assumed that the consequences of sperm cryo-injury caused by cryopreservation. The plasma membrane of sperm is the primary site of damage induced by cryopreservation. Both of

freezing and thawing implicate tremendous alteration in volume of cell water, which result considerable mechanical stress on the sperm membrane and consequently reduce sperm motility.

CONCLUSION

It was concluded that ejaculate volume, sperm concentration, and sperm motility were influenced by level of up-gradation and semen quality was better in F2 (SL1 × L) group among the studied three upgraded Sahiwal × Local breeding bulls.

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CONFLICT OF INTEREST

Authors certify that there is no conflict of interest.

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