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VARIATION OF SEMEN QUALITY AMONG THREE GENERATIONS (F₁, F₂ and F₃) OF HOLSTEIN FRIESIAN UPGRADED BREEDING BULLS OF BANGLADESH

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ABSTRACT

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The experiment was conducted in a well-known private livestock research and development farm in Bangladesh to find out the variation of semen quality parameter among three generations (F₁, F₂ and F₃) of Holstein Friesian upgraded breeding bulls. A total of 312 ejaculates were collected from 6 upgraded breeding bulls through the experimental year. The recorded data were summarized using Microsoft Excel 2010 and analyzed using GraphPad Prism 5 software. Out of the 312 ejaculates, 273 (87.50%) were found to be creamy in color followed by 26 (8.33%) and 13 (4.17%) as yellowish and watery, respectively. Generation had significant (P<0.05) effect on ejaculate volume, consistency, mass activity, sperm concentration, initial and post freezing motility. The highest (7.389±0.19ml) and the lowest (5.156±0.13ml) volume of semen were found in third (F₃) and first generation (F₁), respectively. The mass activity ranges from 3.74±0.04 to 4.30±0.05. Sperm concentration and pH varied insignificantly (p>0.05) but initial motility and post freezing motility had the significant differences among the three generations. Initial motility ranges from 75.87±0.32 to 78.40±0.38 percent and the post freezing motility ranges from 50.38±0.41 to 52.16±0.43 percent. It could be concluded that most of the semen quality parameters were influenced by generation and freezing. Semen characteristics were better in F₂ followed by F₃ and F₁ generation in upgraded Holstein Friesian bulls.

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INTRODUCTION

Artificial Insemination (AI) is now widely used all over the world to improve the genetic potentiality of livestock species. National program for upgrading local cattle with improved high yielding dairy breeds like Holstein Friesian by artificial insemination (AI) has been in practice in Bangladesh since 1950 as a means for increasing milk production (Ahmed and Islam, 1987). AI has been considered as the single most important technology for the genetic improvement of cattle (Hafez, 1993). Deficiency in bulls has larger impact on herd productivity than fertility problems in a single female: a common thought is that the bull is half the herd. When AI is used, each ejaculate can produce more than 300 inseminations, representing at least 60,000 doses per bull per year (Rodriguez, 2008). Therefore, it is extremely important in selection of breeding bulls to determine the quality of semen. The success and efficiency of AI program depends on several factors. Semen quality is top of them. Good quality semen is obligatory for successful conception in cattle and therefore, a determinant of reproductive efficiency. Upgrading of local cows with Holstein Friesian is widely practiced in Bangladesh for dairy purpose. A previous experiment reported that the qualities of semen i.e. ejaculate volume, sperm motility; viability and concentration et cetera were affected by breeds (Al-Hakim et al., 1986). Therefore, the present study was planned to assess the variation of semen quality among generations within the genotype (Holstein Friesian).

MATERIALS AND METHOD

Place and time of study

The study was undertaken at research and development unit of renowned research based animal breeding organization of Bangladesh, known as "Lal Teer Livestock Development Bangladesh Limited", located at Mymensingh district, around 90 kilometers away from Dhaka City. The experiment was carried out throughout the year 2019.

Animals and their ration

Total 6 (six) breeding bulls from three generations (F_1 , F_2 and F_3) of upgraded Holstein Friesian (nearly 28 to 52 months of age and body weight of 426.00 to 683.50 kg) were selected for this study. Out of 6 bulls, 2 were Holstein Friesian \times Local (HF \times L), 2 were Holstein Friesian \times Holstein Friesian \times Local (HF $_1$ \times L) and 2 were Holstein Friesian \times Holstein Friesian \times Holstein Friesian \times Local (HF $_2$ \times L) bulls. The breeding bulls were maintained under optimal feeding and management during the whole period of the experiment. The bulls were physically fit, free from diseases, clinically normal and sound in breeding. All the bulls were vaccinated against Anthrax, FMD, BQ, and HS according to the schedule. The bulls were allowed ad libitum green grass supplemented with good quality concentrate mixture prepared with maize grain, rice polish, wheat bran, soybean meal, mastered oil cake, DCP, vitamin mineral premix and common salt (Table-1).

Semen collection, evaluation and preservation

Semen was collected early in the morning twice a week from the bulls using sterilized bovine artificial vagina (IMV model-005417) maintaining proper temperature (42°-45°C), pressure and softness (Arthur et al., 1982). A male dummy was used for jumping the bulls and after 2 to 3 false jumps semen was collected from each bull by a skilled semen collector. Just after collection each ejaculate was placed in to hot water bath at 37°C. 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. Semen pH was determined by indicator paper strips (Salisbury et al., 1978). Mass activity of semen was recorded by placing a small drop of fresh semen on the glass slide without cover slip under low magnification (10x) of a digital microscope and graded from 0 to 5 grades. Concentration of sperm per ml of semen was estimated through bovine sperm photometer (IMV technologies, France).

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

Statistical Analysis

The recorded data was compiled by Microsoft Excel 2010. Compiled data was then analyzed using GraphPad Prism 5 software. Column statistics were done for mean and standard error. Tukey test was performed for multiple comparison test and level of significance.

RESULTS AND DISCUSSION

In this study, total 6 bulls from three generations (2 bulls from each generation) were selected and 52 ejaculates from each bull throughout the experimental year were studied, hence, a total of 312 (52x6) ejaculates were evaluated.

Color and consistency

Out of the 312 ejaculates 273 (87.50%) were found to be creamy in color followed by 26 (8.33%) and 13 (4.17%) as yellowish and watery, respectively. A previous study reported out of 181 seminal ejaculates 82.3% were creamy 8.8% were yellowish and 2.2% were watery, which are more or less similar to the present study (Harandra et al., 2017). There were significant differences in semen consistency of upgraded HF breeding bulls. In case of F₁(HF×L) bulls, the highest percentage was thick followed by moderate thick and the lowest number was thin category of semen. Like F₁(HF×L) bulls the similar patterns were found in F₂(HF₁×L) bulls and F₃(HF₂×L) bulls but the highest percentage of thick category semen was found in third generation (F₃) of the studied breeding bulls (Figure1). Probably it is due to the higher exotic blood percentage.

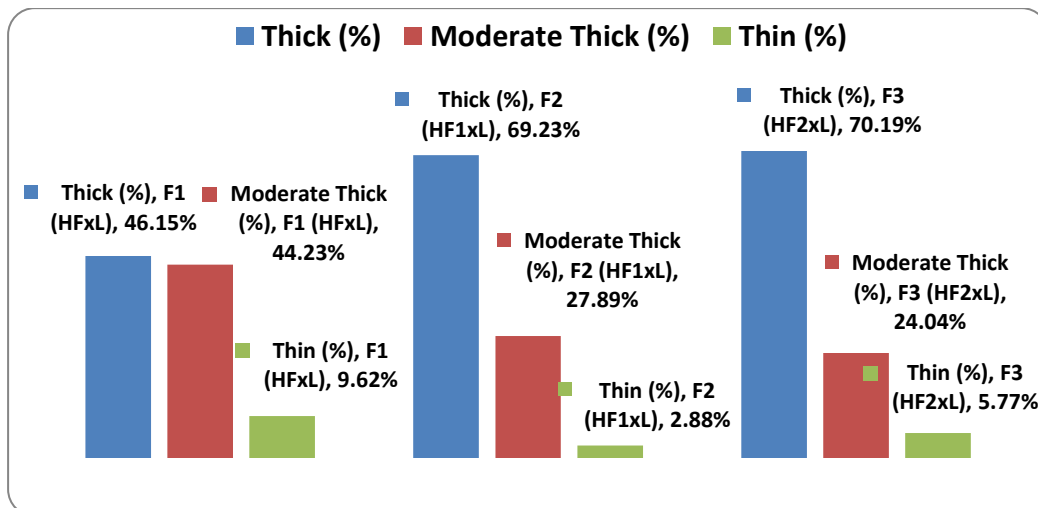
Table1. Ration for breeding bulls at Lal Teer Livestock Development (BD) Limited

Ingredients	Amount (kg)	DM (kg)	TDN (kg)	DCP (kg)	Ca (kg)	P (kg)
Maize	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
Mastered 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

Table 2. Semen quality parameters of three generations (F₁, F₂ and F₃) of upgraded Holstein Friesian bulls

Generation	F ₁ (HF×L)	F ₂ (HF ₁ ×L)	F ₃ (HF ₂ ×L)
Sample size (N)	N=104	N=104	N=104
Volume (ml)	5.156±0.13 ^c	6.255±0.14 ^b	7.389±0.19 ^a
Mass Activity (0-5)	3.745±0.04 ^c	4.077±0.03 ^b	4.308±0.05 ^a
Sperm Concentration (millions/ml)	1239±35.91 ^a	1354±35.80 ^a	1364±34.76 ^a
p ^H	6.428±0.01 ^c	6.494±0.01 ^b	6.764±0.01 ^a
Initial Motility (%)	75.87±0.32 ^b	78.40±0.38 ^a	76.11±0.27 ^b
Post Freezing Motility (%)	50.38±0.41 ^b	52.16±0.43 ^a	51.83±0.45 ^{ab}

*Means with different superscripts within a row differ significantly at 5% level

**Figure 1.** Semen consistency of three generations of upgraded Holstein Friesian bulls

Ejaculate volume and mass activity

Ejaculate volume and mass activity were varied significantly ($p < 0.05$) among the three generations of HF breeding bulls. The highest amount of semen (7.389±0.19 ml) was found in generation number three F₃(HF₂×L) followed by F₂(HF₁×L) and the lowest amount (5.156±0.13ml) was measured in F₁ (HF×L) bulls. A previous study showed the volume of semen in Friesian cross local breeding bulls as 5.8±0.3 ml (Ahmed et al., 2014). Another experiment also reported the volume of semen of Local cross Holstein Friesian bulls as 4.5±1.1 ml (Latif et al., 2009) which is slightly lower than the present study. In a recent experiment it was noticed the ejaculate volume as 7.86±0.19 ml which is very close to present study (Islam et al., 2020). The highest mass activity was found to be 4.308±0.05 out of 5.00 in F₃ (HF₂×L) bulls and the lowest mass activity was found in F₁ (HF×L) bulls (Table 2). It may be due to the higher exotic blood percentage in the third generation. Previous study also observed slightly lower mass activity as 2.81±0.02 in 50% Friesian upgraded breeding bulls than the present study (Islam et al., 2020).

Sperm concentration and pH

Sperm concentration is considered 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 Dhama, 2001). In the present study results for sperm concentration summarized in Table 2 indicated that sperm concentration varied non-significantly with generation of the studied genotype (HF). The highest sperm concentration (1364 ± 34.76 million/ml) was found in F_3 ($HF_2 \times L$) bulls and the lowest sperm concentration (1239 ± 35.91 million/ml) was observed in F_1 ($HF \times L$) bulls. An experiment reported the maximum sperm concentration as 1261.7 ± 193.6 and 1105.03 ± 22.54 million/ml in Friesian cross local bulls (Ahmed et al., 2014 and Islam et al., 2020). In the present study there was not found significant differences in semen pH. The highest and the lowest value of semen pH were 6.764 ± 0.01 in F_3 ($HF_2 \times L$) bulls and 6.428 ± 0.01 in F_1 ($HF \times L$) bulls. Previous study (Hossain et al., 2012) also reported the insignificant differences in semen pH in upgraded Holstein Friesian bulls. Sperm concentration could be considered as an initial indicator of semen quality in semen used for cryopreservation (Shelke and Dhama, 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 overestimation of motility in more concentrated samples (Everett et al., 1978). Nevertheless, still this 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 $75.87 \pm 0.32\%$ to $78.40 \pm 0.38\%$ (Table 2). The highest motility ($78.40 \pm 0.38\%$) was observed in F_2 ($HF_1 \times L$) bulls and lowest ($75.87 \pm 0.32\%$) in F_1 ($HF \times L$) bulls. Motility is one of the most important requirements of fertile semen. It was found (Donham et al., 1926) that semen below normal motility ($\geq 90\%$) was less than half as effective in producing optimum conception rate. It was also reported motility of spermatozoa as one of the best single evidence of viability (Davis, 1939). Duration of motility in stored semen was reported (Comstock, 1939) as another reliable index of fertility. In this study, significant differences was observed in initial motility percent of semen of upgraded Holstein Friesian bulls which is in agreement with the findings of (Hossain et al., 2012) On the other hand, in Friesian bulls and in Exotic and crossbred bulls were not found any significant variation in initial motility percent (Mathur et al., 2002; Rekwot et al., 1987). In the present study the highest value of initial motility percent was recorded as $78.40 \pm 0.38\%$ in F_2 ($HF_1 \times L$) bulls and the lowest value was found as $75.87 \pm 0.32\%$ in F_1 ($HF \times L$) bulls (Table 2). In our study, post freezing motility percent ranges from 50.38 ± 0.41 to 52.16 ± 0.43 and there was significant variation within studied genotype. It was reported that the motility of sperm after freezing varied from 62.2 to 63.6% in crossbred bulls (Hossain et al., 2012) 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 (Salmon et al., 1995). The plasma membrane of sperm is the primary site of damage induced by cryopreservation (Hammerstedt et al., 1990). 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 (Hammerstedt et al., 1990).

CONCLUSION

In conclusion, it was evident that ejaculate volume, concentration, pH and motility of sperm were influenced by freezing and generation of the genotype. Semen characteristics were better in F_2 followed by F_3 and F_1 generation of upgraded Holstein Friesian breeding bulls.

CONFLICT OF INTEREST

Authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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