International Journal of Pharmaceutical and Medical Research
Volume – 1 Issue – 1 December 2013
Website: www.woarjournals.org/IJPMR

Study on the Quality of Semen of Different Genetic Groups of Bull from Khulna Region of Bangladesh

Md. Shamim Akhter[1]*, Md. Abul Kalam Azad[1], Md. Zahidur Rahman[2] and Ayesha Ashraf[1]

[1]*Biotechnology & Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh.*

[2]*District Artificial Insemination (AI) Center, Daulatpur, Khulna, Bangladesh.*

**Corresponding author's*

***Abstract:** The research work was conducted in District Artificial Insemination (AI) Center, Daulatpur, Khulna and Animal Biotechnology Laboratory, Khulna University, Khulna from July, 2010 to January, 2012. Semen was collected from 3 genetic groups of bulls from the AI center. Five frozen semen ampoules (1/4 Local x3/4 Friesian) was also used in this experiment. After collection, semen was observed to evaluate its quality by volume, pH, sperm concentration, sperm motility (%), live spermatozoa (%) and normal spermatozoa (%). Data were analyzed using 'SPSS' computer program. The results from the study revealed that the effects of genetic groups on volume, live-dead and p^H were non-significant (P>0.05). On the other hand, different genetic groups showed significant (P<0.01) effect on sperm motility, sperm concentration and normal spermatozoa*

Keywords: semen, breed, genetic group, bull, Khulna

1. Introduction

The basic aim of cattle breeding program in Bangladesh is to improve the genetic potentiality of local cattle through the infusion of exotic blood [1]. Globally, more than 100 million Artificial Insemination (AI) was performed in cattle, 40 million in pigs, 3.3 million in sheep and 5 million in goats annually [7]. In order to bring success in AI program, an increase of AI coverage, skilled AI technician, awareness of farmers about the success of AI, satisfactory quality of services, and adaptation rate of AI by livestock owners need to be optimized [6]. Moreover, the success of AI program partly depends on the quality of semen and the semen quality varies according to genotypes and age of bulls. The fertilizing capacity of semen also depends on the quantity and quality of semen. There are many factors associated with the quantity and quality of semen output of which normal sperm morphology is one of the most important considerations. The quality of semen in relation to fertility is determined by the morphological features of spermatozoa, percentage of live spermatozoa concentration and motility of spermatozoa and the ejaculated volume. The length and breadth of sperm cell add to the quality in respect of fertilizing capacity. According to Kumar et al. (1977), conception rate was significantly co-related with sperm head shape[17].

It was reported that total sperm per ejaculate increased with age of the bull up to 7.5 years and then decreased [4]. The normal sperm of farm animals are about 50-60µ long and are similar in appearance and size [12]. The study on measurement of spermatozoa also may help in selecting males for breeding purposes by observing the spermatozoa shape, size, etc. Considering all of the above facts, the present research was undertaken to evaluate semen of different genetic groups of bull and compare semen quality among genetic groups available in Khulna region of Bangladesh.

2. Materials and Methods

2.1 Sample collection:

For this study fresh semen samples were collected from Artificial Insemination (AI) centre laboratory situated at Daulatpur in Khulna, Bangladesh. The average temperature during the study period ranged from 15-20°C and the relative humidity was 80% as recorded by the meteorology division of Khulna. This study was conducted from July, 2010 to January, 2012. After collection of samples semen was examined physically and microscopically both in Artificial Insemination (AI) centre laboratory Daulatpur, Khulna and Animal Cell Culture Lab, Biotechnology and Genetic Engineering Discipline, KU, Khulna. Semen was collected 3 times in a week in the early morning using artificial vagina method considering all aseptic hygienic precautions. Immediately after collection the following tests were done.

2.2 Experimental animals:

Bulls of different genetic groups were namely Raj-08(Local x Friesian x Friesian), CT-28 (Sahiwal x Friesian x Friesian), 985(Sahiwal x Friesian). All these bulls were maintained under uniform conditions of feeding and management. Five frozen semen ampoules of Local x Friesian x Friesian (L×F×F) genetic group was also collected for this experiment. The information of these bulls is presented in table 01.

Table 01: Studied genetic groups of bulls

Bull ID	Genetic group	Date of birth
Raj-08	Local x Friesian x Friesian(L×F×F)	10.09.02
CT-28/A	Sahiwal x Friesian x Friesian(S×F×F)	24.01.04
985	Sahiwal x Friesian(S×F)	31.08.02
1207(Frozen ampoule)	Local x Friesian x Friesian	

2.3 Physical tests:

The volume of semen was recorded by reading the graduated mark of the collection vial just after the collection. The color of semen was observed with naked eye and was recorded and the consistency of semen was observed by inclining and moving the collection vial with care.

pH test

pH of semen was measured by means of nitrizine paper. Immediately after collection a drop of semen was placed on the nitrizine paper of a clean glass rod and allowed to get dried for one minute. The color developed on the paper was compared with the standard color graduation given on the packet cover.

2.4 Microscopic test:

Concentration (millions/ml)

The total number of spermatozoa per ml of raw semen was enumerated by Haemocytometer method [14]. The following formula was used for calculating total number of spermatozoa per ml of fresh semen:

N = C×4000/S × d/ml.,Where N = Number of spermatozoa counted per ml of semen, C = Number of spermatozoa counted in given number of small squares, S = Number of small squares counted, d = Dilution ratio.

2.5 Evaluation of live & dead spermatozoa

Slide preparation

One 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 spirit lamp (with a temperature150-200^{o}F)for few minutes. This helps to dry the slide properly. The live & dead spermatozoa were counted under high power objective microscope.

Observation:

The live spermatozoa were appeared unstained & the dead sperm stained pink against a brownish purple background.

Calculation:

% of live spermatozoa = (number of live sperm/ number of total sperm count)x100

Or

% of live sperm = (number of live spermatozoa x3)/10

Estimation of (%) mass motility

Procedure

A clean glass slide was warmed at approximately 37^{0} C 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. A cover slip was placed over the slide. The slide was examined immediately using by lower objective(10X) on the microscope.The number of motile sperm was counted in a field carefully and several observations were made on the glass slide from different field for determining % motility.

2.6 Morphology of Spermatozoa

Slide preparation

One drop of physiological buffer was taken in a clean glass slide and semen was mixed by inverting the vial 2 to 3 times. A drop (as small as possible) of semen was taken on the buffer of the slide. It was spreaded by pulling gently a second glass slide on it. The smear was dried at room temperature and then stained with RBS for 3-5 minutes. The smear was dried & rinsed into distilled water in a beaker to remove extra stain. The slide was again dried & a total of 333 sperms were counted by using random field on different part of the slide under microscope.

Calculation

% of normal spermatozoa =Total number of normal spermatozoa × 3/10

or

% of abnormal spermatozoa =Total number of abnormal spermatozoa × 3/10

3. Results and Discussion

For this study, 3 genetic groups of bull were selected and quality of semen was evaluated.

Table 02: Evaluation of fresh semen of 3 different genetic groups (Mean±SE)

G. group / Parameter	L×F×F	S×F×F	S×F	L×F×F (frozen)	Overall mean
Volume(ml)	6.67±0.2	6.34±0.2	7.67±0.2	-	6.89±0.2
Color	creamy	light yellowish	creamy	creamy	creamy
Consistency	thick milky	thick milky	thick milky	thin creamy	thick milky
pH	7.2±0.05	6.8±0.05	6.6±0.05	7±0.05	6.9±0.05
Concentration	1225±30.33	1200±30.33	950±30.33	1300±30.33	1168.75±30.33
% Live	84.34±0.35	83±0.35	82±0.35	85±0.35	83.56±0.35
% Motility	66.61±1.22	75±1.22	70±1.22	60±1.22	67.90±1.22
% Normal	80±0.96	82±0.96	78±0.96	86±0.96	81.5±0.96

4. Physical test

4.1.1 Volume of semen

Overall mean volume of semen was 6.92±0.2 ml. Lower volume of semen observed by Dhami et al., (2001). They reported that the ejaculate volume of Jersey, Holstein-Friesian and crossbred was 5.28±0.21, 6.75±0.44 and 5.92±0.45 ml, respectively [8]. This variation may be due to the increased frequency of semen collection or the age and weight of bulls. Similar results were observed by Hafez (1974)[10]. According to his results volume per ejaculate in bull ranged from 3 to 15 ml. Raju and Rao (1982) also reported the significant ($p<0.01$) breed differences in volume of semen [21]. It was observed that the mean volume per ejaculate of Swedish Friesian breed and their crosses were 5.5 ± 0.13 ml [2],[11],[24]. Roy and Rao (1975) observed the volume per

ejaculate for Jersey bulls as 4.02 ± 1.61 ml [22]. The differences in volume per ejaculate among the bulls may be attributed to the variation in the secretary activities of the sex glands, scrotal circumference, body size and body weight [9],[16],[19],[23]. The differences in semen volume among the bulls might be due to individual variation. Highest value was observed in S×F (7.67ml) and lowest was in S×F×F (6.34) (Fig-01). Different genetic groups showed non-significant (P>0.05) effect on volume of sperm in table 03.

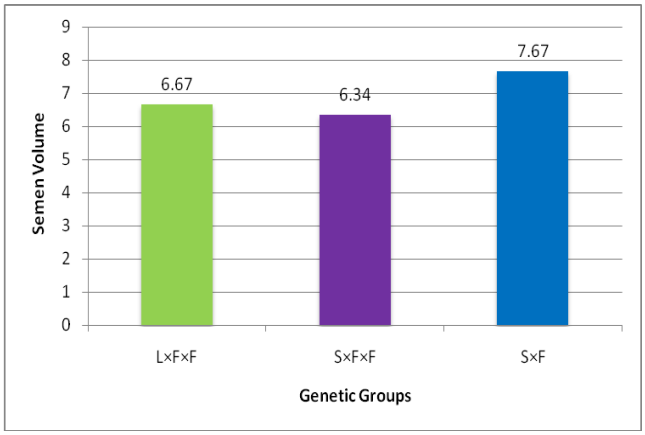

Fig: 01: Histogram showing the average semen volume of different genetic groups of bull.

Table 03: Analysis of variance (ANOVA) for volume

Volume	Sum of squares	df	Mean square	F	Sig
Between Groups	2.407	3	.802	2.102	.153
Within Groups	4.58	12	.382		
Total	6.987	15			

4.1.2 Color of semen

Overall color of semen was creamy. The creamy color was observed in case of the genetic group L×F×F, S×F and L×F×F(frozen). Only the genetic group S×F×F showed the color light yellowish.

4.1.3 Consistency

Overall consistency of semen was thick milky. The highest consistency thin creamy was observed in case of the genetic group L×F×F (frozen). The lowest consistency of thick milky was observed in the genetic group L×F×F, S×F×F and S×F.

4.1.4 P^H test

The overall mean p^H value was 6.9. The semen p^H of the present study was higher than that of Dhami et al., (2001). They described that semen p^H of Jersey, HF, and crossbred was 6.77±0.04, 6.61±0.04, and 6.70±0.04, respectively [8]. Highest value was observed in L×F×F (7.2) and lowest was in S×F (6.6) (Fig: 02). Different genetic groups showed non-significant (P>0.05) effect on sperm (Table-04).

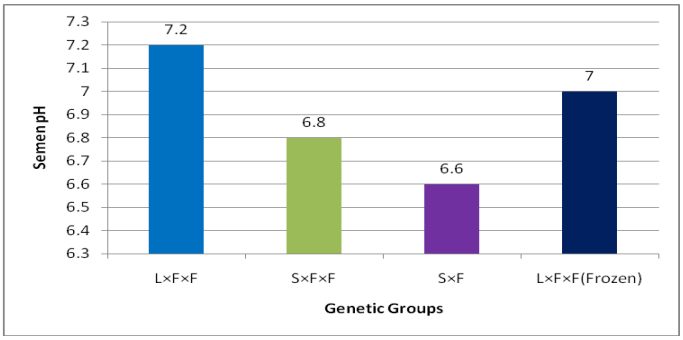

Fig 02 : Histogram showing the average pH values of semen of different genetic groups of bull

Table 04: Analysis of variance (ANOVA) for p^H

p^H	Sum of squares	df	Mean square	F	Sig
Between Groups	.175	4	.044	.454	.768
Within Groups	1.446	15	.096		
Total	1.621	19			

4.2 Microscopic test:

4.2.1 Sperm concentration (million/ml)

The overall mean concentration of semen was 1168.75 million/ml. Similar result revealed that the semen concentration of Jersey, HF, and crossbreed was 1225±95.26, 1155±93.87 and 1165±93.87 million/ml, respectively [8]. Highest value was observed in L×F×F (1225 million/ml) and lowest was in S×F (950 million/ml). (Fig: 03) The effect of genetic groups on concentration of sperm (million/ml) was found significantly (p<0.01) affected among the three genetic groups of bulls. (Table 05)

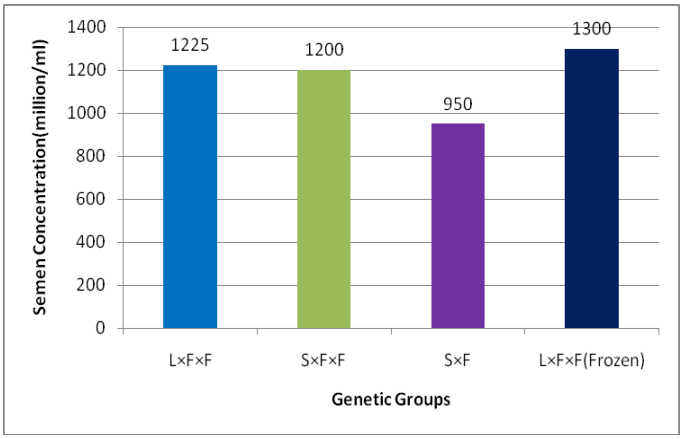

Fig 03 : Histogram showing the average semen concentration of different genetic groups of bull

Table 05: Analysis of variance (ANOVA) for concentration

Concentration	Sum of squares	df	Mean square	F	Sig
Between Groups	167320.0	4	41830.000	32.301	.000
Within Groups	9425.000	15	1295.000		
Total	186745.0	19			

4.2.2 Live sperm percentage

The overall mean live sperm of semen was 83.56%. The findings of the present study collaborate with the results of several findings [2],[13],[20]. Hahn et al., (1969) observed the average live sperm for Holstein-Friesian bulls was 83.5 % and range from 70-90 (13) which were almost similar to the average live sperm percentage of present study. Good quality semen must have 80 percent of live sperm [14]. The lower percentage of live sperm could be due to younger age of bulls, and breed difference and lower adaptability to the environmental conditions. Highest percentage of live sperm 85% was frozen semen in (Local x Friesian x Friesian) and lowest percentage of live sperm (82%) found in genetic group S×F (Fig: 04) The effect of genetic groups on live sperm percentage was found Non-significant (P>0.05) (Table 06)

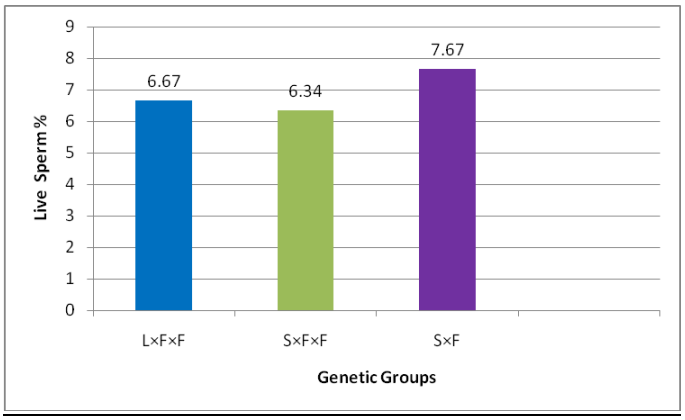

Fig 04: Histogram showing the average live sperm percentage of different genetic groups of bull.

Table 06: Analysis of variance (ANOVA) for live spermatozoa (%)

Live Spermatozoa	Sum of squares	df	Mean square	F	Sig
Between Groups	55.012	4	13.753	2.72	.069
Within Groups	75.849	12	5.057		
Total	130.861	19			

4.2.3 Mass motility

The overall mean (%) motility was 67.90. The motility of sperm (%) in this study was higher with the study of Dhami et al. (2001). They reported that the sperm motility of Jersey, HF, and crossbreed were 65.83±7.96%, 64.50±4.12% and 55.83+8.28%, respectively [8]. Highest value was observed in S×F×F(75%) and lowest was in frozen semen ampoule L×F×F(60%).(Fig: 05). This observation also agrees with the previous studies [15],[18]. However, Average mass motility of bovine semen was reported as 63.3% and range from 50-80 [5] which were almost similar to the average mass motility of the present study. There was a significant difference in mass motility of semen produced by different bulls. This variation might be due to age, breed of bull, inadequate nutrition and poor management. Different effect of genetic groups on motility of sperm was found significantly (p<0.01) among the three genetic groups of bulls (Table 07).

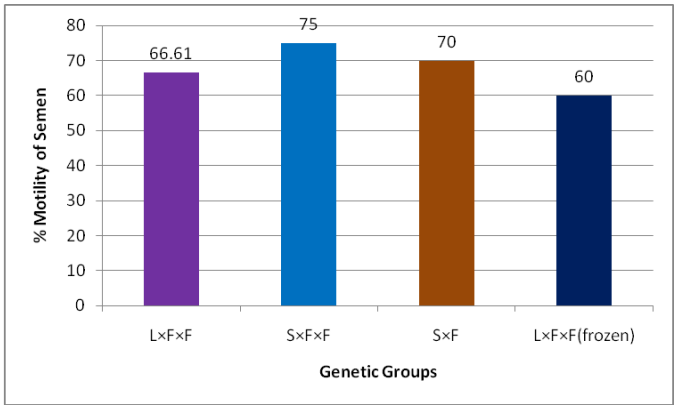

Fig 05 : Histogram showing the average semen motility of different genetic groups of bulls

Table 07: Analysis of variance (ANOVA) for motility

Motility	Sum of squares	df	Mean square	F	Sig
Between Groups	162.688	3	54.229	27.4	.000
Within Groups	23.750	12	1.979		
Total	186.438	15			

4.2.4 Normal sperm percentage

The overall mean (%) normal spermatozoa was 81.5. A slight variation of spermatozoa percentage was also observed [8]. They reported that abnormal sperm (%) of Jersey, Holstein-Friesian and crossbreed was 24.50±4.78, 14.10+2.04 and 17.83±4.74%, respectively. This variation may be due to missing of normal spermatozoa and error during slide preparation. The mean values were differing to the observations where it was obtained average normal spermatozoa percentage 85 and ranged from 75-95 [13]. The findings of the present study also collaborate with other results [20]. Highest value was observed in L×F×F frozen semen ampoule (86%) and lowest was in S×F (78%). (Fig: 06) Different genetic groups showed significant (p<0.01) effect on % normal of spermatozoa. **(**Table 08).

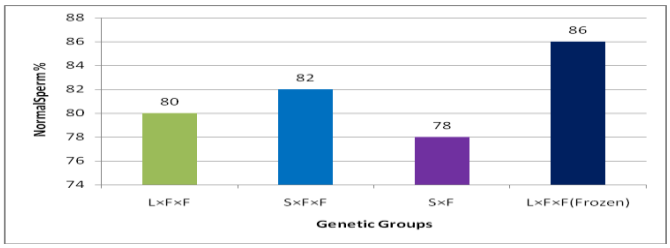

Fig 06 : Histogram showing the average normal sperm percentage of different genetic groups of bull.

Table 08: Analysis of variance (ANOVA) for normal spermatozoa (%).

Normal	Sum of squares	df	Mean square	F	Sig
Between Groups	509.300	4	127.325	43.905	.000
Within Groups	43.500	15	2.900		
Total	552.800	19			

The conception rate of cows is influenced by the quality and quantity of semen which vary according to different breeds of bulls as well as their feeding, management, etc. The results of this work indicate that different genetic groups of bull have significant effect on sperm motility, sperm concentration and normal spermatozoa percentage, whereas non- significant effects were found on the traits like volume, live-dead and P^H of the spermatozoa.

References

[1] Ahmed, Z. and Islam, T.S. (1987). Cattle breeding program through Artificial Insemination in Bangladesh. Artificial Insemination Extension Project, pp.19.

[2] Al-Hakim, M.K., Ali, S.B.A., and Singh, B.P.(1984). Study on semen characteristics of Karadi (Kurdi) bulls. Indian J.Anim. Health, 23(2), 163-169.

[3] Al-Hakim, M.K. (1986). A comparative study on biochemical constituents of semen of Jersey and Jersey Sahiwal bulls. Indian J. Anim. Sci., 57, 1180-1186.

[4] Amann, R.P., Linde-Forsberg, C. (1974). How a bull works. Proc. Eleventh Technical Conference on Artificial Insemination and Reproduction, National Association of Animal Breeders, pp. 6-18.

[5] Bratton, R.W., Foote, R.H. and Henderson ,C.R.(1954). The relationship between fertility and the number of spermatozoa inseminated. J. Dairy Sci. 37, 1353-1356.

[6] Chacko, C.T. and Schneider, F.(2005). Breeding services for small dairy farmers. Oxford &IBH Publishing Co. Pvt. Ltd. New Delhi, pp.84

[7] Donovan, A. and Hanrahan, J.P.(1999). Artificial insemination of sheep- current status and possible developments. Irish Grassland and Animal Production Association. J., 33, 99-106.

[8] Dhami, A. J., Vinaya, B., Dave, R. R. and Kavani, F. S.(2001). Scrotal biometry, sexual behavior and semen production in breeding bulls of different breeds. Indian Journal Dairy Science , 3: 54.

[9] Graves.(1978). Two cases of bovine epididymal dysfunction. Cornell Vet. 62, 393-402.

[10] Hafez, E.S.E.(1974). Reproduction in Farm Animals. 3rd Edition, Lea and Febiger, Philadelphia, pp. 109.

[11] Hafez, E.S.E.(1993). The morphology of boar spermatozoa. Anim. Breed. Abstr., 26: 320.

[12] Hafez, E.S.E. (1970). Study of bull spermatozoa. Quantitative electron microscopy. Anim. Breed. Abstr., 33: 269.

[13] Hahn, J., Foote, R.H. and Seidel, G.E.(1969). Quality and freezability of semen from growing and aged dairy bulls. J. Dairy Sci., 52, 1843-1848.

[14] [Herman, H.A. and Madden, F.W.(1963). The Artificial Insemination of dairy and beef cattle. A hand book of laboratory manual, Freeman and Company, San Francisco, USA., pp. 579-610.

[15] Hoque, M.A. and Krainc, A., (1997). Measurements of bull spermatozoa and their relation to fertility. Anim. Breed. Abstr., 33: 308.

[16] Jainudeen, M.R. and Hafez, E.S.E. (1993). Reproduction failure in males. In: Hafez E.S.E (Ed), Reproduction in Farm Animals. Lea and Febiger, Philadelphia., pp. 287-297.

[17] Kumar, R.P., Bhat, N., and Sezena, M.C. (l977). A note on biometrics of buffalo bull spermatozoa in relation to fertility. Anim. Breed. Abstr., 46: 2101.

[18] Lasely, S.F.(1951). Spermatozoa motility as measure of semen quality. J. Anim.Sci., 10:21.

[19] Leon, H., Porras, A. A. and Galina, C.S.(19910. Effect of the collection method on semen characteristics of Zebu and European type cattle in the tropics. Therioeenolopv., 36: 349-355.

[20] Rao, T.L.N. and Rao, A.R.(1979). Fertility and its relationship with semen characteristics in crossbred bulls. Indian Vet. J., 56: 33-36.

[21] Raju, M.S. and Rao, A.R.(1982). Note on the semen characteristics of crossbred and purebred bulls. Indian J. Anim. Sci., 5(15): 1230-1232.

[22] Roy, R.M .and Rao, A.R.(1975). Studies on semen characteristics of Tharparker and Jersey bulls. Indian Vet. J.,52: 889-900.

[23] Sharma, M.L., Mohan, G and Sahni , K.L.(1991). Characteristics and cryopreservation of semen of Holstein-Friesian bulls under topics. Indian J. Anim. Sci., 61:977-979.

[24] Stalhammar, E.M., Janson, L. and Philipson, J.(1989). Genetic studies on fertility in A I bulls. Anim. Reprod. Sci., 19: 1-17.