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Research Article

ASSOCIATION OF SEMEN ATTRIBUTES WITH FSH-β GENE POLYMORPHISM IN HOLSTEIN-FRIESIAN CROSSBRED BULLS FROM INDIA

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ABSTRACT

This investigation was carried out to evaluate the association of FSH-β gene polymorphism with semen attributes and testicular parameters in fifty Holstein-Friesian crossbred bulls. PCR-RFLP was carried out using enzyme Pst1. The genotype frequencies for BB and AB genotypes were 0.40 and 0.60, whereas the frequency of A and B allele was 0.7 and 0.3, respectively. The observations on testicular attributes were non-significant ($p<0.05$) between the two genotypes. Likewise both the yearly and quarter wise observations were non significant between the BB and AB genotypes for some semen attributes viz. semen volume (SV), sperm concentration (SC), initial motility (IM), morphology minor, acrosomal integrity, percent intact acrosome. However, morphology major ($p<0.05$) and hypo osmotic swelling test ($p<0.01$) were significantly different between the two genotypes in yearly analysis. Further, morphology major, hypo osmotic swelling test, post thaw motility & Incubation test showed significant differences in quarter wise analysis. This is a first report on year round association analysis of semen attributes with FSH-β gene polymorphism in bulls.

Keywords: Crossbred bull, FSH- β gene, Polymorphism, Semen

Follicle-stimulating hormone (FSH) is a pituitary glycoprotein hormone that plays an essential role in mammalian spermatogenesis and follicular development. In 2009, associations between a polymorphism in the FSH β-subunit gene (FSHB) and sperm traits in pure breed bulls of Canada were reported (Dai *et al.*, 2009). A total of 13 substitutions and 1 insertion were reported of which seven substitutions were reported in exon 3 (FSHB-3); which signiûcantly inûuenced the quality and fertility traits of fresh and frozen semen (Dai *et al.*, 2009). Further, the effect of FSHB-3 gene polymorphism on semen traits was also reported in 83 Iranian Holstein bulls (Ghasemi & Ghorbani, 2014). Recently, Dalvi *et al.* (2018) studied the association of FSHB-3 gene polymorphism on testicular and semen quality traits in 25 HF crossbred and 6 Jersey crossbred bulls. They reported non-significant differences between three genotypes (AA, AB, BB), for all traits studied. To the authors knowledge, there is no report on effect of FSHB-3 gene polymorphism on year round semen quality traits of bulls. Therefore, the current study was undertaken to study the year round effect of FSHB-3 gene polymorphism on semen quality traits in crossbred HF bulls.

MATERIAL AND METHOD

The current study was carried out on 50 HF cross bred bulls from Sabarmati Ashram Gaushala, Bidaj, Gujarat, India (Commercial semen station managed by NDDB-National Dairy Development Board, Government Project in India, according to "Minimum Standard Protocol (MSP) in semen station", Government of India, Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries from 01/04/2014 to 27/03/2015. The data of other physical parameters like age and body weight of animals were also documented viz. testicular length (TL), testicular width (TW) and testicular thickness (TT) of each testis along with scrotal circumference (Sc). Semen analysis for various semen quality traits was done in triplicates. The semen attributes viz. semen volume (SV), sperm concentration (SC), initial motility (IM), post thaw motility (PTM), sperm abnormalities (major and minor defects; Bloom, 1977), Hypo Osmotic Swelling Test (HOST), Acrosomal integrity, Percent intact acrosome and Incubation test (IT) were studied.

Year round monitoring of semen attributes of the bulls was done in four quarters as follows: first quarter (April to June-Summer); second quarter (July to September-Monsoon); third quarter (October to December-Autumn) and fourth quarter (January to March-Winter).

Isolation of Genomic DNA: Blood samples were periodically collected for disease testing as a routine mandate in accordance to the "Minimum Standard Protocol (MSP)" in semen station. The leftover blood from above testing was utilized for DNA isolation. Genomic

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DNA was isolated from venous blood by the phenol–chloroform extraction method (John *et al.*, 1991)

PCR Amplification and RLFP Analysis: PCR was carried out using FSHB-3 specific primers (Forward primer: 5'CTTCCAGACTACTGTA ACTCATC'3; Reverse Primers: 5'GTAGGCA GTCAAAGCATCCG'3) amplifying part of intron-2 and complete coding of exon-3 (313 bp) (McLachlan *et al.*, 1996). For RFLP analysis, digestion of PCR product was done at 37°C for 4 hours by using Pst1 enzyme.

Statistical analysis: Statistical analysis was carried out using ANOVA using Web Based Agricultural Statistics Software Package (WASP2) software developed by ICAR (ICAR - Central Coastal Agricultural Research Institute, Ela, Old Goa).

RESULTS AND DISCUSSION

PCRRFLP of FSHB-3: The PCR amplicon of 313 bp FSHB-3 was obtained using specific primers(Dai *et al.*, 2009).The PCR products were checked using 1.5 per cent agarose gel electrophoresis (Fig. 1). These findings are in agreement with the previous reports (Dai *et al.*, 2009, Ghasemi *et al.*, 2012, Ghasemi & Ghorbani, 2014).

Genotype Frequency: Three patterns have been reported after FSHB-3 was digested by Pst-I enzymeviz 313 bp for genotype BB, 202 bp and 111 bp for genotype AA and 313 bp, 202 bp and 111 bp for AB, respectively (Ghasemi *et al.*, 2012 and Dalvi *et al.,* 2018). In the current study, the genotype frequencies for BB and AB genotypes were 0.40 and 0.60, respectively (Fig. 1). Our findings concord with previous observation that AB genotype is more frequently observed in Indian crossbred bulls (Dalvi *et al.*, 2018). Similar findings were also reported by Ghasemi and Ghorbani (2014) in Iranian Holstein Bulls.

Allele frequency: The B allele was more frequent than A allele (0.7 vs 0.3) and therefore, most of the bulls (60%) were heterozygous for the B allele and only 40 per cent were homozygous. In contrast, higher frequency of A allele compared to B allele (0.65 vs 0.35) has been reported previously in Indian crossbred bulls (Dalvi *et al.*, 2018). Higher frequency of A allele was also reported in Iranian

Table 1

Effect of FSHB-3 gene polymorphism on testicular attributes and semen quality traits (Yearly analysis)

Sr.No	Particular	Genotype	
		BB	AB
1	Age (days)	3294.359 ±290.231	2842.120 ± 182.625
2	Body weight (kg)	696.883 ± 30.339	702.56 ± 21.153
3	Scrotal circumference (SC) (cm)	40.174 ± 0.887	39.148 ± 0.636
4	Testicular length (TL) (cm)	15.1 ± 2.512	15.44 ± 2.766
5	Testicular width (TW) (cm)	7.7 ± 102	7.76 ± 0.914
6	Testicular thickness (TT) (cm)	8.14 ± 0.941	8.1 ± 1.274
7	Semen volume (SV) (ml)	4.818 ± 0.288	4.996 ± 0.185
8	Sperm concentration (millions/ml)	1119.779 ± 76.864	1041.212 ± 44.472
9	Initial motility (%)	66.825 ± 1.301	67.88 ± 0.655
10	Post thaw motility (PTM) (%)	50.475 ± 0.154	50.4 ± 0.114
11	Morphology major (nos.)	3.278 ± 0.646[a]	3.044 ± 0.158[b]
12	Morphology minor (nos.)	3.026 ± 0.059	3.083 ± 0.074
13	Hypo Osmotic Swelling Test (HOST) (%)	60.2 ± 0.355[a]	61.44 ± 0.261[c]
14	Acrosomal Integrity (%)	84.504 ± 504	84.95 ± 0.321
15	Percent intact acrosome (%)	77.894 ± 405	78.77 ± 0.418
16	Incubation Test (IT)		
	0 mins	51.373 ± 0.502	51.35 ± 0.289
	30 mins	42.305 ± 0.733	42.2 ± 0.486
	60 mins	32.455 ± 0.847	32.2 ± 0.486
	90 mins	22.455 ± 0.847	22.2 ± 0.486

[a,b] Statistically significant difference $p<0.05$
[a,c] Statistically significant difference $p<0.01$

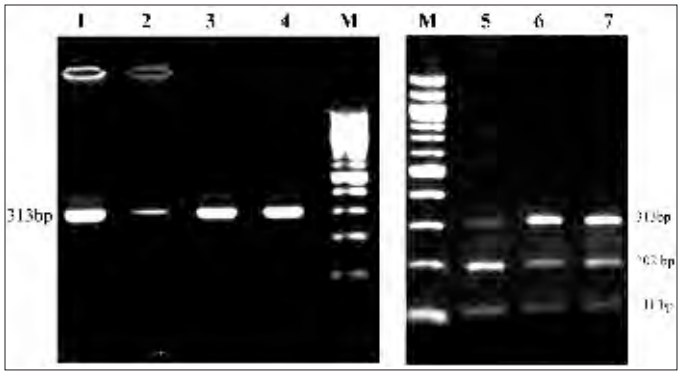

Fig. 1. Lane 1: Amplification of FSHB-3, Lane 2-4: BB genotype Lane 5-7: AB genotype, M: 100 bp ladder

Holstein Bulls (Ghasemi & Ghorbani, 2014), HF, Simental and Limosin bulls (Ishak *et al.*, 2011). Most of the bulls (60%) of the current study were heterozygous for the B allele compared to homozygous which is in agreement with the results of Ghasemi *et al.* (2012) and Ghasemi and Gorbani (2014) who reported higher percentage (65%) of heterozygous B bulls compared to homozygous.

Physical parameters and testicular attributes: In the present study, physical parameters and testicular attributes (TL, TW, TT &Sc) were non-significant ($p<0.05$) between the two genotypes (Table 1). Similar results have been

Table 2
Effect of FSHB-3 gene polymorphism on semen quality traits (Quarter wise analysis)

Sr.No.	Particular	BB	AB
1	**Semen volume (SV)**		
	First Quarter(April to June)	4.681 ± 0.270	5.026 ± 0.232
	Second Quarter(July to September)	4.357 ± 0.290	5.072 ± 0.224
	Third Quarter(October to December)	4.838 ± 0.336	4.944 ± 0.220
	Fourth Quarter (January to March)	4.896 ± 0.323	4.959 ± 0.177
2	**Sperm concentration**		
	First Quarter	1162.172 ± 89.606	1067.231 ± 55.713
	Second Quarter	1117.452 ± 95.554	1067.543 ± 51.033
	Third Quarter	1019.052 ± 97.767	907.855 ± 74.183
	Fourth Quarter	1227.855 ± 93.806	1174.96 ± 62.170
3	**Initial motility**		
	First Quarter	66.144 ± 1.112	64.919 ± 1.273
	Second Quarter	63.7 ± 3.943	69.015 ± 0.614
	Third Quarter	68.461 ± 1.235	68.774 ± 0.833
	Fourth Quarter	69.605 ± 1.514	71.157 ± 0.435
4	**Post thaw motility**		
	First Quarter	47.442 ± 1.547[a]	51.833 ± 0.821[c]
	Second Quarter	47.058 ± 2.981	49.362 ± 0.578
	Third Quarter	50.488 ± 0.508	50.391 ± 0.413
	Fourth Quarter	49.854 ± 0.718	49.884 ± 0.322
5	**Morphology major**		
	First Quarter	3.208 ± 0.148[a]	2.660 ± 0.182[b]
	Second Quarter	3.174 ± 0.105	3.062 ± 0.098
	Third Quarter	3.174 ± 0.152	2.955 ± 0.099
	Fourth Quarter	3.555 ± 0.241	3.5 ± 0.251
6	**Morphology minor**		
	First Quarter	3.359 ± 0.172	3.04 ± 0.172
	Second Quarter	2.941 ± 0.144	2.82 ± 0.106
	Third Quarter	3.325 ± 0.171	3.06 ± 0.173
	Fourth Quarter	4.166 ± 0.183	3.9 ± 0.201
7	**Hypo osmotic swelling test**		
	First Quarter	59.825 ± 0.701	60.6 ± 0.738
	Second Quarter	59.90 ± 1.108	60.88 ± 0.776
	Third Quarter	60.10 ± 0.718	61.1 ± 0.758
	Fourth Quarter	60.975 ± 0.803[a]	63.18 ± 0.572[b]

8	**Incubation tests**		
	First Quarter		
	0 mins	50	50.8 ± 0.371
	30 mins	40	41.6 ± 0.743
	60 mins	30	31.6 ± 0.743
	90 mins	20	21.6 ± 0.743
	Second Quarter		
	0 mins	51.174 ± 0.688	51.4 ± 0.546
	30 mins	42.058 ± 1.131	42.4 ± 0.877
	60 mins	32.359 ± 1.367	32.4 ± 0.877
	90 mins	22.359 ± 1.367	22.4 ± 0.877
	Third Quarter		
	0 mins	50	51 ± 0.402
	30 mins	40	42 ± 0.814
	60 mins	30	32 ± 0.814
	90 mins	20	22.4 ± 0.877
	Fourth Quarter		
	0 mins	52.64 ± 0.969	51.8 ± 0.700
	30 mins	44.417 ± 1.348	42.4 ± 0.877
	60 mins	34.708 ± 1.510	32.4 ± 0.877
	90 mins	24.708 ± 1.510	22.4 ± 0.877
9	**Acrosomal integrity**		
	First Quarter	84.64 ± 0.801	85.4 ± 0.720
	Second Quarter	84.582 ± 0.879	84.76 ± 0.564
	Third Quarter	84.767 ± 0.828	85.24 ± 0.727
	Fourth Quarter	85.24 ± 0.727	85.32 ± 0.589
10	**Percent intact acrosome**		
	First Quarter	77.475 ± 0.846	78.12 ± 0.880
	Second Quarter	79.359 ± 0.846	79.24 ± 0.726
	Third Quarter	77.475 ± 0.846	77.96 ± 0.883
	Fourth Quarter	77.825 ± 1.028	79.2 ± 0.889
11	**Incubation test**		
	0 mins		
	First Quarter	50	50.8 ± 0.371
	Second Quarter	51.174 ± 0.688[a]	51.4 ± 0.546[c]
	Third Quarter	50	51 ± 0.402
	Fourth Quarter	52.64 ± 0.969	51.8 ± 0.700
	30 mins		
	First Quarter	40	41.6 ± 0.743
	Second Quarter	42.058 ± 1.131[a]	42.4 ± 0.877[c]
	Third Quarter	40	42 ± 0.814
	Fourth Quarter	44.417 ± 1.348	42 ± 0.814
	60 mins		
	First Quarter	30	31.6 ± 0.743
	Second Quarter	32.359 ± 1.367[a]	32.4 ± 0.877[c]
	Third Quarter	30	32 ± 0.814
	Fourth Quarter	34.708 ± 1.510	32 ± 0.814
	90 mins		
	First Quarter	20	21.6 ± 0.743
	Second Quarter	22.359 ± 1.367[a]	22.4 ± 0.877[c]
	Third Quarter	20	22.4 ± 0.877
	Fourth Quarter	24.708 ± 1.510	22.4 ± 0.877

[a,b]Statistically significant difference $p<0.05$
[a,c] Statistically significant difference $p<0.01$

reported in HF crossbred's bulls by Dalvi *et al.* (2018).

Semen traits with non-significant findings between genotypes: Semen parameters viz. Semen volume (SV), Sperm concentration (SC), Initial motility (IM), Morphology minor, Acrosomal integrity, Percent intact acrosome were non-significant between the two genotypes in year wise analysis (Table 1) as well as in quarter wise analysis (Table 2).

Semen traits results showing significant differences between genotypes: Yearly mean ± SE values of Morphology major and HOST were significantly different between the two genotypes (Table 1). Major morphological defects were significantly high ($p<0.05$) in BB genotype whereas HOST percentage was significantly high ($p<0.01$) in AB genotype. Similarly, quarter wise analysis revealed significant differences for Morphology major (summer) and HOST (winter) (Table 2).

It is important to note that even though the yearly mean ± SE values of semen parameters PTM and IT did not vary significantly (Table 1), these parameters showed significant differences in quarter wise analysis. PTM was significantly high in AB genotype ($p<0.01$; summer) besides IT was significantly higher in AB genotype ($p<0.01$; Monsoon) (Table 2).

CONCLUSION

In current study, yearly observations (mean ± SE) of two semen parameters viz. morphology major and HOST were significantly different between the two genotypes. Furthermore, morphology major, HOST, PTM and IT showed significant differences in quarter wise analysis.

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