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Research Article

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

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ABSTRACT

This study investigated the association of *FSH-β* gene polymorphism with semen traits in consecutive ejaculates of Holstein-Friesian crossbred bulls from India. PCR-RFLP was carried out using enzyme *Pst 1*. With regards to sperm concentration, significant differences were observed in the consecutive ejaculates of animals of AB genotype ($p<0.05$). Comparison of consecutive ejaculates between BB and AB genotypes revealed significant differences only in the second ejaculates for two semen traits viz. sperm concentration and initial motility. Sperm concentration was significantly higher in second ejaculates of animals of BB genotype in all four quarters ($p<0.05$). On other hand, initial motility in the second ejaculates was significantly higher in first two quarters for BB genotype ($p<0.05$) while it was significantly higher for AB genotype in last two quarters ($p<0.05$). This is a first report on effect *FSH-β* gene polymorphism on semen traits in consecutive ejaculates of Holstein-Friesian crossbred bulls.

Keywords: Ejaculates, *FSH-β*, Polymorphism, Semen trait

Follicle-stimulating hormone (FSH) plays acrucial role in mammalian spermatogenesis and follicular development. This hormone is one of the principal endocrine factors responsible for the regulation of Sertoli cell function in males. FSH hormone contributes in initiation and maintenance of quality and quantity in spermatogenesis (McLachlan *et al.*, 1996, Shi *et al.*, 2018). Dai *et al.* (2009) conducted a study in pure breed bulls of Canada and reported the associations between a polymorphism in the *FSH β*-subunit gene (FSHB) and sperm deformity, acrosomal integrity and non-return rate. In that study, a total of 13 substitutions and 1 insertion were reported in the *FSHB* gene in pure breed bulls of Canada. Seven substitutions were reported in exon 3 (*FSHB-3*) which significantly infiuenced the quality and fertility traits of fresh and frozen semen. In 2014, effect of *FSHB-3* gene polymorphism (PCR-RFLP using Pst1) on semen traits was reported from 83 Iranian Holstein bulls (Ghasemi and Ghorbani, 2014). After digestion of *FSHB-3* with Pst1, three types of banding pattern have been reported viz. 1) 313 bp for genotype BB, 2) 202 bp and 111 bp for genotype AA and 3) 313 bp, 202 bp and 111 bp for AB, respectively (Ghasemi *et al.*, 2012, Ghasemi and Ghorbani, 2014, Dalvi *et al.*, 2018). Ghasemi and Ghorbani (2014) observed significantly higher post thaw motility (PTM) in semen of bulls of AA genotype (Ghasemi and Ghorbani, 2014). In recent times, 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 of India. They reported non-significant differences between three genotypes (AA, AB, BB), for all traits studied.

With regards to semen traits, consecutive ejaculates differed significantly in ejaculate volumes, sperm concentration and total number of spermatozoa (Everett *et al.*, 1978, Everett and Bean 1982, Fuerst-Waltl *et al.*, 2006). However, studies on the effect of *FSH-β* gene polymorphism on semen traits in consecutive ejaculates of crossbred bulls are absent. Therefore, this study was aimed to study the association of *FSH-β* gene polymorphism on semen traits in consecutive ejaculates of Holstein-Friesian crossbred bulls from India.

MATERIAL AND METHODS

In the present investigation, semen collections were done from 50 HF cross bred bulls maintained at Sabarmati Ashram Gaushala, Bidaj, Gujarat by artificial vagina method. Consecutive semen collections from bulls were carried out with a gap of one hour (GOI, 2014). The following semen attributes were analyzed in triplicates viz. semen volume, sperm concentration, initial motility and post thaw motility. These traits were studied for a year: 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).

Genomic DNA was extracted from venous blood using the phenol–chloroform method (John *et al.*, 1991). PCR was carried out for *FSHB-3* (313 bp) using specific

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primers (Forward primer : 5'CTTCCAGACTACTGT-AACTCATC'3; Reverse Primers : 5'GTAGGCAGTC-AAAGCATCCG'3) as described previously (Dai *et al.*, 2009). For RFLP, the FSHB-3 PCR products were digested using Pst1 enzyme. Statistical analysis was carried out using ANOVA (Analysis of variance) using Web Based Agricultural Statistics Software Package (WASP2) software developed by ICAR (ICAR - Central Coastal Agricultural Research Institute, Ela, Old Goa - 403 402).

RESULTS AND DISCUSSION

In the present study, two banding patterns- (1) 313 bp for genotype BB and (2) 313 bp, 202 bp and 111 bp for AB, were obtained after digestion of *FSHB-3* with *Pst-I* enzyme (Fig. 1). The AA genotype was absent in the present investigation. The genotype frequencies for BB and AB genotypes were 0.40 and 0.60, respectively whereas the allele frequency was 0.7 and 0.3 for the B allele and A allele, respectively. With regards to the consecutive ejaculates, significant differences were observed in sperm concentration in animals of AB genotype ($p<0.05$). This finding is in agreement with previous studies (Everett *et al.*, 1978, Everett and Bean 1982, Fuerst-Waltl *et al.*, 2006). Semen volume revealed non-significant differences in consecutive ejaculates for both BB and AB genotype. Furthermore, significant differences were observed for initial motility (except fourth quarter) in consecutive ejaculates from animals of BB genotype ($p<0.05$). This result could be due to seasonal or environmental variation, type of breed/genotype, number of animals under study or other factors affecting semen quality parameters.

Comparison of consecutive ejaculates between BB and AB genotypes revealed significant differences in values for two semen traits viz. sperm concentration and

Table 1

Effect of *FSH-β* gene polymorphism on semen traits in consecutive ejaculates in first quarter (April to June)

Sr. No.	Particular	Genotypes	
		BB	AB
1	**Semen Volume (ml)**		
	First Ejaculate	5.03±0.32	5.25±0.25
	Second Ejaculate	4.25±0.39	4.83±0.31
	Third Ejaculate	4.70±0.38	4.62±0.45
2	**Sperm Concentration (millions/ml)**		
	First Ejaculate	1325.42±93.72	1224.72±64.39l
	Second Ejaculate	1024.79±113.34^{a}	963.46±65.09^{bm}
	Third Ejaculate	1137.37±135.73	1011.98±96.88^{lm}
3	**Initial Motility (%)**		
	First Ejaculate	63.21±1.822^{m}	62.78±1.86
	Second Ejaculate	67.63±1.08al^{m}	65.46±1.37^{c}
	Third Ejaculate	70.45±0.262^{l}	67.45±2.26
4	**Post Thaw Motility (%)**		
	First Ejaculate	48.23 ± 0.90	47.64 ± 1.07
	Second Ejaculate	49.95 ± 0.38	49.07 ± 0.56
	Third Ejaculate	48.08 ± 1.75	47.36 ± 2.32

Different superscripts indicate significant difference within a row; $p<0.05$ (a, b) and $p<0.01$ (a, c)
Different superscripts indicate significant difference within a column; $p<0.05$ (l, m)

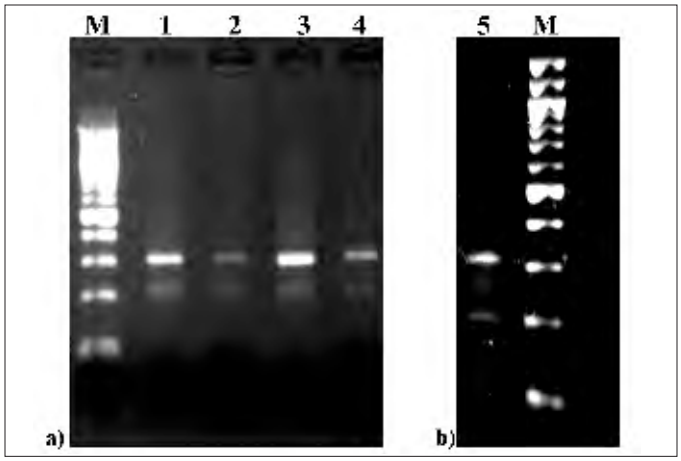

Fig. 1. PCR-RFLP *FSHB-3* gene: Lane M (DNA ladder), Lane 1 (PCR amplification of FSHB-3), Lane 2-4 (BB genotype), Lane 5 (AB genotype)

Table 2

Effect of *FSH-β* gene polymorphism on semen traits in consecutive ejaculates in second quarter (July to September)

Sr. No.	Particular	Genotypes	
		BB	AB
1	**Semen Volume (ml)**		
	First Ejaculate	4.59 ± 0.35	5.28 ± 0.27
	Second Ejaculate	4.30± 0.32	4.534 ± 0.27
	Third Ejaculate	5.03 ± 0.37	5.188 ± 0.22
2	**Sperm Concentration (millions/ml)**		
	First Ejaculate	1326.10±109.70	1215.83±52.63^{l}
	Second Ejaculate	1036.82±127.09^{a}	993.33±63.05^{bm}
	Third Ejaculate	971.54±122.74	1012.21±105.71^{lm}
3	**Initial Motility (%)**		
	First Ejaculate	62.65 ± 3.46^{m}	67.88 ± 0.83
	Second Ejaculate	70.47 ± 0.61^{al}	69.99 ± 0.53^{b}
	Third Ejaculate	70.583 ± 1.38^{l}	69.63 ± 1.19
4	**Post Thaw Motility (%)**		
	First Ejaculate	49.84 ± 0.45	49.22 ± 0.57
	Second Ejaculate	50.65 ± 0.45	49.94 ± 0.63
	Third Ejaculate	49.09 ± 1.38	47.49 ± 3.06

Different superscripts indicate significant difference within a row; $p<0.05$ (a, b) and $p<0.01$ (a, c)
Different superscripts indicate significant difference within a column; $p<0.05$ (l, m)

Table 3

Effect of *FSH-β* gene polymorphism on semen traits in consecutive ejaculates in third quarter (October to December)

Sr. No.	Particular	Genotypes	
		BB	AB
1	**Semen Volume (ml)**		
	First Ejaculate	4.59 ± 0.35	5.28 ± 0.27
	Second Ejaculate	4.30 ± 0.32	4.53 ± 0.27
	Third Ejaculate	5.03 ± 0.37	5.19 ± 0.22
2	**Sperm Concentration (millions/ml)**		
	First Ejaculate	1360.42 ± 101.22	1206.41 ± 62.51^{l}
	Second Ejaculate	1058.18 ± 105.00^{a}	931.33 ± 74.38^{blm}
	Third Ejaculate	1038.35 ± 133.93	835.19 ± 105.51^{m}
3	**Initial Motility (%)**		
	First Ejaculate	65.81± 2.16^{m}	66.88± 1.49
	Second Ejaculate	69.06 ± 1.33^{al}	69.75± 0.82^{b}
	Third Ejaculate	71.47± 0.55^{l}	70.58± 0.26
4	**Post Thaw Motility (%)**		
	First Ejaculate	49.50± 0.86	49.62± 0.62
	Second Ejaculate	50.73 ± 0.39	50.78± 0.36
	Third Ejaculate	51.38± 0.69	51.41± 0.49

Different superscripts indicate significant difference within a row; $p<0.05$ (a, b) and $p<0.01$ (a, c)
Different superscripts indicate significant difference within a column; $p<0.05$ (l, m)

Table 4

Effect of *FSH-β* gene polymorphism on semen traits in consecutive ejaculates in fourth quarter (January to March)

Sr. No.	Particular	Genotypes	
		BB	AB
1	**Semen Volume (ml)**		
	First Ejaculate	5.08 ± 0.35	5.28 ± 0.24
	Second Ejaculate	4.67 ± 0.32	4.62 ± 0.17
	Third Ejaculate	4.86 ± 0.44	5.15 ± 0.23
2	**Sperm Concentration (millions/ml)**		
	First Ejaculate	1414.57±106.19	1334.62±72.19^{l}
	Second Ejaculate	1221.56±114.09^{a}	1175.64±81.06^{clm}
	Third Ejaculate	998.16±145.92	892.98±76.64^{m}
3	**Initial Motility (%)**		
	First Ejaculate	68.48±1.75	70.41±0.57^{m}
	Second Ejaculate	70.52±1.09^{a}	71.42±0..44^{blm}
	Third Ejaculate	69.38±3.33	72.94±0.68^{l}
4	**Post Thaw Motility (%)**		
	First Ejaculate	49.28±0.68	49.67±0.40
	Second Ejaculate	50.17±0.54	50.29±0.43
	Third Ejaculate	49.76±2.03	49.86±0.78

Different superscripts indicate significant difference within a row; $p<0.05$ (a, b) and $p<0.01$ (a, c)
Different superscripts indicate significant difference within a column; $p<0.05$ (l, m)

initial motility. These significant differences were seen between the genotypes only in the second ejaculates for all four quarters i.e. all seasons ($p<0.05$). Sperm concentration was significantly high in second ejaculates of animals of BB genotype in all four quarters ($p<0.05$). On other hand, initial motility in the second ejaculates was significantly higher in first two quarters for BB genotype while it was significantly high in last two quarters for AB genotype ($p<0.05$). To the authors knowledge, this is the first report on effect *FSH-β* gene polymorphism on semen in consecutive ejaculates of Holstein-Friesian crossbred bulls.

Findings of the study indicate that FSH-β gene polymorphism could affect the semen traits viz. sperm concentration and initial motility in consecutive ejaculates of Holstein-Friesian crossbred bulls.

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