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Allelic and genotypic frequencies in Fshβ- PST-I locus in Iranian Holstein bulls

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ABSTRACT

The follicle-stimulating hormone (FSH) acts on the sertoli cells in the seminiferous tubules of the testis and regulates spermatogenesis up to the secondary spermatocyte stage. Allelic and genotypic frequencies of bovine follicular stimulating hormone (exon III) gene were studied for 83 animals from Iranian Holstein bulls. The blood and semen samples were collected from two artificial insemination and progeny test center (Jahed Co, Karaj, Iran and Shikh Hassan, Tabriz, Iran). We used polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) for animal genotyping. The PCR process conducted with protocol of Dai et al. (2009). PCR products were digested with 4 U of PST-I, using the supplied buffer and maintained at 37°C for1 h. The resulting fragments were separated by vertical electrophoresis (110 W 40 min) in3% agarose gel, stained with ethidium bromide and was visualized under UV light. The A (PST-I) allele had fragment sizes of 111, 202bp, whereas the B (PST-I) allele had fragments of 111, 202 and 313bp. The frequency of the A (PST-I+) and B (PST-I-) alleles are 0.675 and 0.325 respectively. The genotype frequency for AA and AB were 0.35 and 0.65 respectively. The BB genotype was omitted of analysis.

Key word: Follicular stimulating hormone, semen quality, Iranian Holstein bulls.

INTRODUCTION

Follicle-stimulating hormone (FSH) is a glycoprotein hormone secreted from the pituitary, and it is important for regulating reproduction in mammals [12]. FSH is a heterodimer formed by a α-subunit shared with other glycoprotein hormones and a specific β-subunit encoded by the FSHβ gene [9,3]. In males, FSH in combination with testosterone is the most important tropic hormone regulating sertoli cell function, requires for the initiation and maintenance of the quality and quantity in spermatogenesis [6, 8]. The published sequence for bovine FSHβ [5] (GenBank No.: M83753) comprises 1 non-coding exon and 2 translated exons that encode the 129-amino acid pre-protein. Descriptions of cases of naturally occurring mutations of the FSH receptor have highlighted the role of this gonadotrophin in male and female gonadal function [4, 10, 11] providing further evidence that when FSH action is disrupted, spermatogenesis is impaired and follicular maturation is impossible. Findings showed that some bulls with numerous linked mutations in the FSHβ gene, including 13 substitutions and 1 insertion in the upstream regulation region (5'-URR) and the coding region of exon 3 [1]. This study attempts to identify potential single nucleotide polymorphisms bovine FSHβ gene (Exon III).

MATERIALS AND METHODS

Animals

83 bulls of North West AI center (Tabriz, Iran) and Progeny Test center of Jahed Co (Karaj, Iran), were included in the study.

Genotyping

Blood and semen samples were collected from the bulls. An anticoagulant (EDTA) was added to the blood samples and then stored at–20°C. Genomic DNA from whole blood was purified by standard protocol using proteinase K digestion as described by [7] and from semen by DNA extraction kit (DNPTM kit Cinnagen Co. Tehran, Iran). The quality of the DNA was checked on 0.5% agarose gel and the quantity was measured by UV spectrophotometry at A260/A280 nm. Genotyping for FSHβ polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

The PCR reaction conditions were approximately 100 ng of genomic DNA, 10 pmol of each primer, 0.2 mM of each dNTP, 1.5 mM of MgCl2, 1 x PCR buffer [50 mM of KCl and Tris-HCl (pH 8.4)] and 0.4 U of Taq polymerase in a total volume of 25 µl. The PCR was conducted on EppendorfGradientthermalcycler, HotMasterMix (EPPENDORF, Germany) using a preliminary denaturation at 94°C for 1.5 min, 62°C for 1 min and72°C for 1 min, followed by 48 cycles of a specific temperature regime. Each temperature regime consisted of 94°C for 30 s, 62°C for 1 min, 72°C for 30 s and a final extension at 72°C for 5 min. An313 bp fragment of FSHB consisting part of intron 2 and complete coding region of exon 3, was amplified using forward (5`CTTCCAGACTACTGTAACTCATC3`) and reverse (5GTAGGCAGCTCAAAGCATCCG`3`) primers [1].

PCR products were digested with 4 U of PST-I, using the supplied buffer and maintained at 37°C for1 h. The resulting fragments were separated by vertical electrophoresis (110 W 40 min) in3% agarose gel, stained with ethidium bromide and was visualized under UV light. The A (PST-I) allele had fragment sizes of 111, 202bp, whereas the B (PST-I) allele had fragments of 111, 202 and 313bp.

Allele and genotype frequencies

The FSHβ allele frequencies were calculated by simple allele counting [2]. The possible deviations of allele and genotype frequencies from the Hardy–Weinberg equilibrium were examined with PopGene.S2 software by a Pearson's Chi-square test.

RESULTS AND DISCUSSION

Data of 83 bulls were included in the final evaluation. The genotype and allele frequencies at FSHβ exon III loci calculated by PopGene.S2 software are shown in Table 1. Three genotypes for FSHβ gene AB (111, 202 and 313) AA (111 and 202 bp) were observed (Figure 1). The A allele was more frequent than B allele (0.675 vs. 0.325) and therefore most of the bulls (65%) were heterozygous for the B allele and only 35% were homozygous. The BB genotype was not found in animals and their results weren't reported. Pearson's Chi-square test ($P > 0.05$) indicated that the genetic pool were not in Hardy–Weinberg equilibrium.

Table 1.Gene and genotypic frequencies obtained at FSHβ - PSTI loci in Iranian Holsteinbulls

	Genotype			Allele			
	AA	AB	BB	C	D	Chi-square value	Pr>ChiSq
Number	29	54	0				
Frequency	0.35	0.65	0.0	0.675	0.325	19.29**	P<0.05

Dai et al. (2009) has been found 14 mutations in the upstream regulatory and coding regions of FSH gene (exon III) in Chinese Holstein bulls. These researchers have reported that the frequency of allele A, B and C were 0.5, 0.28 and 0.04 and also approximately of 56%, 34% and 9% of bulls had AB, BB and BC genotype. Comparison of observed and expected frequencies showed little difference [AA (29 vs. 37/78), AB (54 vs. 43.36) and BB (zero vs. 8.8)]. Chi-square test for Hardy - Weinberg equilibrium in this locus showed that the population is not in equilibrium ($p<0.05$). These results may be due to a small population size and selection.

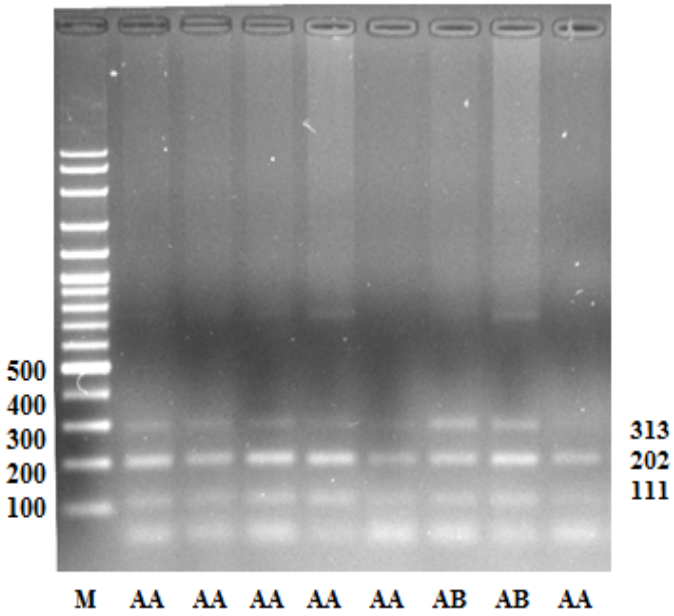

Figure 1. Representative genotyping of FSHβ gene at locus PstI by agarose gel electrophoresis

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