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Association of Bovine Follicular Stimulating Hormone (FSHβ) Gene Polymorphism with Reproduction Traits in Iranian Holstein Bulls

Research Article

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ABSTRACT

The present study was aimed to examine the association of bovine follicular stimulating hormone gene polymorphism with sperm quality traits including sperm volume (SV), sperm concentration (SPCO), total sperm (TS), fresh sperm motility (FSM), total fresh motile sperm (TFMS), post thaw sperm motility (PTSM), total post thaw motile sperm (TPTMS), number of produced payout (NPP), number of fresh motile sperm in each milt ejaculation (NFMSE), motility before and after the freezing (MBATF) and number of post thaw motile sperm in each milt ejaculation (NPTMSE). We used polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) in 83 bulls belonging to two progeny test center. The frequency of the A (PSTI+) and B (PSTI-) alleles were 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 for analysis. Mixed and probity models analyses of sperm quality traits considering genotype and environment as fixed effects and animal as a random effect suggested that sire was a significant source of variation (P<0.001) in all traits. The AB genotype resulted in a significant increase in TS (P<0.0425), NPP (P<0.0302) traits greater than AA genotype. However, AA genotype had significant effect on PTSM (P<0.0001). But not on sperm volume (SV) (P=0.1749), sperm concentration (SPCO) (P=0.1423), fresh sperm motility (FSM) (P=0.5327), number of post thaw motile sperm in each milt ejaculation (NPTMSE) (P=0.5249), total post thaw motile sperm (TPTMS) (P=0.3982), total fresh motile sperm (TFMS) (P=0.2667), total post thaw motile sperm (TPTMS) (P=0.5898) and motility before and after the freezing (MBATF) (P=0.1785). These results indicate that new molecular markers associated with sperm quality traits can be used in marker-assisted selection in bulls.

KEY WORDS follicular stimulating hormone, Iranian Holstein bulls, semen quality.

INTRODUCTION

Artificial insemination (AI) with superior sires is an essential tool for genetic improvement of the traits with economic importance traits in dairy cattle (Simoni *et al*. 1997). The success of AI depends on the quantity and quality of semen affected by environment, management, physiological status (especially hormones, e.g. FSH, LH and GH) and genetics factors (Mathevon *et al*. 1998). Numerous studies have shown that genetic factors influence reproductive and semen quality traits in bulls (Afshar *et al*. 1988; Xinyan *et al*. 2011), goats (Xiaopeng *et al*. 2010), boars (Ren *et al*. 2009; Xing *et al*. 2009), swine (Wimmers *et al*. 2005; Lin *et al*. 2006) and murine Capza3 (Geyer *et al*. 2009). Follicle-stimulating hormone (FSH) is a glycoproteinhormone secreted from the pituitary, and it is important for regulating reproduction in mammals (Ulloa-Aguirre *et al*. 1995). FSH is a heterodimer formed by a α-subunit shared with other glycoprotein hormones and a specific β-subunit encoded by the FSHβ gene (Pierce and Parsons, 1981; Gharib

et al. 1990). In males, FSH in combination with testosterone is the most important tropic hormone regulating sertoli cell function.

It is required for the initiation and maintenance of the quality and quantity in spermatogenesis (Mc Lachlan *et al*. 1996; Ohta *et al*. 2007). The published sequence for bovine FSHβ (Lin *et al*. 2006; genbank No.: M83753) comprises 1 non coding exon and 2 translated exons that encode the 129 amino acid pre- protein.

FSHβ sub unit gene specific unit is located on chromosome 15 that comprises two introns and three exons with a length of 6601 bp (Kim *et al*. 1988; Hediger *et al*. 1991). Variants of this gene were three alleles, namely A, B, and C. Dai *et al*. (2011) found 9 SNPs mutations, i.e. 4 mutations in promoter section (‘5 URR), 3 in intron 2, and 2 in exon 3. Polymorphism of FSHβ sub unit gene in exon 3 on the study significantly influenced the fresh and frozen semen quality.

Dai *et al*. (2009) examined the gene polymorphism FSHβ subunit of production and sperm quality in Simmental, Charolais and Limousin.

Some researchers also associated the diversity of FSHβ subunit genes with litter size in sow (Yaofeng *et al*. 1998; Liu *et al*. 2012), sperm quality in boar (Wimmers *et al*. 2005; Lin *et al*. 2006), litter size in ewes (Yie *et al*. 2006; Xiaopeng *et al*. 2010) and equine sperm quality (Samper, 2010). This study attempts to identify potential single nucleotide polymorphisms bovine FSHβ gene and its relationship with reproductive traits in Iranian Holstein bulls.

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. For each bull the repeated measurements of sperm quality traits of bulls were available from 1991 to 2008 (41890 records).

Phenotypes

Including sperm volume (SV), sperm concentration (SPCO), total sperm (TS), fresh sperm motility (FSM), total fresh motile sperm (TFMS), post thaw sperm motility (PTSM), total post thaw motile sperm (TPTMS), number of produced payout (NPP), number of fresh motile sperm in each milt ejaculation (NFMSE), motility before and after the freezing (MBATF), number of post thaw motile sperm in each milt ejaculation (NPTMSE) were determined from each ejaculation with light microscopy according to the guidelines of the World Health Organization. The semen samples of bulls were collected with date and age of bull records.

Genotyping

Blood and semen samples were collected from the bulls. An anticoagulant ethylene diamine tetra acetic acid (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 some studies 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 m*M* of each dNTP, 1.5 m*M* of MgCl2, 1 × PCR buffer [50 m*M* 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 Eppendorf gradiant thermal cycler, Hot MasterMix (EPPENDORF, Germany) using a preliminary denaturation at 94 ˚C for 1.5 min, 62 ˚C for 1 min and 72 ˚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. A 313 bp fragment of FSHβ consisting part of intron 2 and complete coding region of exon 3 was amplified using forward (5`CTTCCAGACTACTGTAACTCATC3`) and reverse (5GTAGGCAGCTCAAAGCATCCG`3`) primers (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) in 3% agarose gel, stained with ethidium bromide and it was visualized under UV light.

The A (PST-I) allele had fragment sizes of 111 and 202 bp whereas the B (PST-I) allele had fragments of 111, 202 and 313 bp.

Statistical analysis

Allele and genotype frequencies

The FSHβ allele frequencies were calculated by simple allele counting.

The possible deviations of allele and genotype frequencies from the Hardy-Weinberg equilibrium were examined with PopGene.S2 softwear by a Pearson’s Chi-square test. Degree of freedom (df) was according to the method of Allendorf *et al*. (2007)

Where:

df= (number of genotype-i) - (number of allele-j)

Association analysis

Statistical analysis was performed using the MIXED procedure of SAS software (SAS, 1999). The following linear model was used to examine the associations between FSHβ-PSTI, polymorphisms and SV, SPCO, TS, FSM, TFMS, PTSM, TPTMS, NPP, NPTMSE, MBATF and NFMSE traits:

$$y_{ijklm} = \mu + a_i + YS_j + S_k + G_l + \sum_m b_m x_m + \varepsilon_{ijklm}$$

Where:

y_{ijklm}: the above traits, μ is the overall trait mean.

a_i: the random effect of the i^{th} animal.

YS_j: the fixed effect of the j^{th} year-season (j=1-68).

S_k: the fixed effect of the k^{th} station (k=1-2).

G_l: the fixed effect of the l^{th} FSH genotype (l=1-3).

b_m: the regression coefficient of m^{th} covariable (e.g. age).

x_m: the fixed effect of m^{th} covariable.

ε_{ijklm} : is the residual error.

FSM and PTSM traits were categorical variable, hence analyzed with logistic regression and GENMOD procedure by using the following model:

$$\eta_{iklm} = \log[p_i/(1-p_i)] = m + a_i + YS_j + S_k + G_l + \sum_m b_m x_m + \varepsilon_{ijklm}$$

η_{ijklm}: MAF and MBF traits.

m: the overall mean in logarithmic scale.

a_i: the random effect of the i^{th} animal.

YS_j: the fixed effect of the j^{th} year season (j=1-68).

S_k: the fixed effect of the k^{th} station (k=1-2).

G_l: the fixed effect of the l^{th} FSH genotype (l=1-3).

b_m: the regression coefficient of m^{th} covariable (e.g. age).

x_m: the fixed effect of m^{th} covariable.

ε_{ijklm} : the residual error.

RESULTS AND DISCUSSION

Allele frequency

Data of 83 bulls were included in the final evaluation. The genotype and allelic frequencies at FSHβ loci calculated by Pop Gene S2 software are shown in Table 1. Three genotypes for FSHβ gene AB (111, 202 and 313 bp), 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 were not reported. Dai *et al.* (2011) reported that bulls with B allele were more frequent than A and C alleles and most of the bulls were heterozygous for the A allele. Our results were similar to the results of Dai *et al.* (2009). Pearson's Chi-square test (P>0.05) indicated that the genetic pools were not in Hardy-Weinberg equilibrium.

Candidate gene effects

Least square means of sperm quality traits for FSHβ genotypes are presented in Table 2. Analysis of variance indicated significant association of FSHβ genotypes TS (P<0.0425), FSM (P<0.0001) and NPP (P<0.0302) but there were not significant association with SV, SPCO, NFMSE, TFMS, PTSM, NPTMSE, TPTMS and MBATF (P>0.05).

Moreover, year season and age had significant effects on some sperm quality traits (P<0.0001). In this population, least square means comparison with t-test showed that the SPCO, TS, TFMS and NPP traits means in bulls with AB genotype had greater than with AA genotype, but cows with AA genotype were better in FSM, NFMSE, PTSM, MBATF, NPTMSE and TPTMS traits.

The results of the present study showed that the PST-I allele (A) was more frequent than the PST-I (B) (0.675 *vs.* 0.325), so that most of the bulls 65% were heterozygous for the B allele while 35% were homozygous for the A allele. Restriction enzyme pattern in this study was designed for the first time in third exon of FSHβ gene based on the results of Dai *et al.* (2009). So there is no possibility to compare results with previous findings.

First QTL association study in Iranian Holstein semen quality traits was reported by Gorbani *et al.* (2009). They showed three genotypes for bGH gene (CC, CD and DD) and reported the C allele was more frequent than D allele (0.883 *vs.* 0.117) and most of the bulls (78.7%) were homozygous for the C allele and only 19.1% were heterozygous and 2.2% homozygous for the D allele. Also, Grigorova *et al.* (2007) reported two core haplotypes containing 423 major allele homozygotes (GG; 76.4%), 125 heterozygotes (GT; 22.6%) and 6 minor allele homozygotes (TT; 1.1%). The frequencies for G and T-alleles were 87.6% and 12.4%, respectively. The differential effect of the two alleles (G and T) on FSHB gene expression is supported by an independent data set from a large-scale study focusing on the functional analysis of common human promoter polymorphisms across 170 genes (Hoogendoorn *et al.* 2003). Tested by the luciferase assay (Hoogendoorn *et al.* 2003), the relative activity of the FSHβ proximal promoter carrying the rs10835638 T-allele was only half (46%-58%; P=0.0005) compared with the activity of the wild-type promoter variant with the G-allele in cell lines JEG-3 and TE671, known to have progesterone-responsive regulation of transcription (An *et al.* 2005; Yie *et al.* 2006).

Table 1 Gene and genotypic frequencies obtained at FSHB-PSTI loci in Iranian Holstein bulls

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

Table 2 Least square means (±SD) of sperm quality and traits for FSHβ genotypes in Iranian Holstein bulls

Traits	FSHB genotype		P-value
	AA	AB	
Sperm volume	58.3±0.64	4.71±0.146	0.1749
Sperm concentration	1077.06±147.95	1136.34±3381	0.1423
Total sperm	3591.51±913.71	5361.91±203.22	0.0425
Fresh sperm motility	62.1591±4.0944	58.2474±1.6048	0.5327
Number of fresh motile sperm in each milt ejaculation	724.16±97.5355	674.86±22.2077	0.5249
Total fresh motile sperm	2622.15±527.79	3215.22±115.65	0.2667
Post thaw sperm motility	62.1591±4.0944	58.2474±1.6048	0.0001
Number of post thaw motile sperm in each milt ejculation	1670.58±349.80	1432.86±75.2350	0.3982
Total post thaw motile sperm	425.60±76.5987	290.28±172164	0.5898
Motility before and after the freezing	1.6984±0.3818	2.0919±0.1423	0.1785
Number of produced payot	133.61±27.2986	179.47±9.9485	0.0302

Through TFSEARCH predictions, the mutations in the 5'-URR part altered the transcription factor binding sites, which demonstrated that the mutations possibly altered gene transcription and caused the sequence difference in the coding region of exon 3.

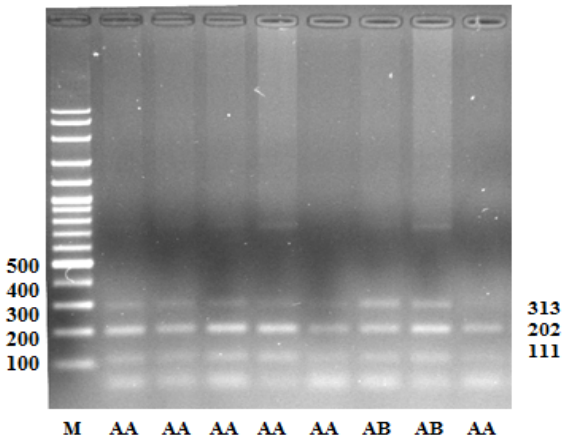

Figure 1 Representative genotyping of FSHβ gene at locus PstI by agarose gel electrophoresis

Radioimmunoassay results revealed that the serum FSH concentrations of bulls with mutations were slightly lower than in bulls of the control group.

Although the difference was not very significative because of the possible presence of compensatory mechanisms in individuals with the heterozygote genotype, we could infer that the mutations of the 5'-URR may influence the level of FSHβ gene expression. However, the exact mechanism is not clear and requires further investigation (Dai *et al*. 2009).

Sequence analysis revealed that the human FSHβ gene is highly conserved and amino acid changing mutations are apparently extremely rare (Layman *et al*. 2002).

The haplotype structure of human FSHβ associated with fertility suggests the possible effect of balancing selection (Grigorova *et al*. 2007). Some homozygous mutations could cause azoospermia in men (Phillip *et al*. 1998; Grigorova *et al*. 2007) which may be due to the crucial role of normal FSH function in the regulation of male fertility. Consistent with the heterozygous FSH receptor, mutant males were viable albeit with reduced fertility (Dai *et al*. 2011) and bulls with the BC genotype in FSHβ-3 were still able to procreate despite the slightly lower semen quality and fertility. These results suggested that breed of cattle is an important source of variation in allelic frequency of FSH-PSTI locus. In the present study AB genotype resulted in a significant increase in TS (P<0.0425), TSD (P<0.0302) traits greater than AA genotype. However, AA genotype had a significant effect on PTSM (P<0.0001), but not on SV (P=0.1749), SPCO (P=0.1423), FSM (P=0.5327), NFMSE (P=0.5249), NPTMSE (P=0.3982), PTSM (P=0.2667), TPTMS (P=0.5898) and MBATF (P=0.1785).

CONCLUSION

In conclusion, the results of the present study showed that including the FSHβ-PSTI polymorphism in breeding program will improve the sperm quality traits in AI bulls. But it is currently unknown how this mutation alters the structure and conformation of FSH. However, further studies are required to test the biochemical effects of FSHβ various isoforms, resulting from this polymorphism on reproduction traits.

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