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Molecular characterization and polymorphism studies of exon 10 of the Follicle stimulating hormone receptor (FSHR) gene in Indian cattle breeds

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ABSTRACT

FSH receptors are important binding sites of the follicle stimulating hormone (FSH) in ovaries and are coded by the FSHR gene which has 10 exons and 9 introns. Exon 10 is the largest (>1200 bp) of all the exons. In the present study, exon 10 of the FSHR gene partial coding sequence (CDS) was cloned and characterized in Sahiwal and Hariana cattle breeds, and DNA polymorphism was investigated using *AluI*/PCR-RFLP assay. The partial CDS of the Sahiwal and Hariana FSHR exon 10 was 99.3% to 100% similar to exotic cattle breeds at the nucleotide and amino acid level. A missense mutation was found in Sahiwal and Hariana at position 1118 (C→G) that caused an amino acid change at 373 (Thr→Ser) and two nonsense mutations were found at position 729 (G→A), 1180 (C→T). Phylogenetic analysis clearly showed that Sahiwal and Hariana cattle are more closely related to yak and *Bos taurus*. The 306 bp region of exon 10 on digestion with *AluI* restriction enzyme revealed three types of genotypes, namely: CC (243 bp and 63 bp), GG (193 bp, 63 bp and 50 bp) and CG (243 bp, 193 bp, 63 bp and 50 bp), where the CG genotype was more frequent (45.0%) than CC (13.5%) and GG (41.5%) genotypes, and the frequency of the G allele was higher (0.64) than the C allele (0.36) in all the screened animals. Chi square (χ^2) analysis revealed that the screened animal population was in Hardy-Weinberg equilibrium. An association study revealed a significant ($P<0.05$) difference between Total milk yield and Lactation period where the CC genotype showed a higher milk yield than other genotypes.

Key words: Indian cattle; cloning; FSHR; PCR-RFLP; *AluI*; association study

Introduction

The success of a dairy enterprise relies on regular and frequent estrus in animals, that leads to successful insemination and finally the birth of young. However, animal productivity is affected by various factors, including anoestrus, late maturity, inactive ovaries, anovulation etc., most of which occur because of low levels of hormonal secretion or the inability to bind the hormonal receptors to have an effect. The follicle stimulating hormone (FSH) is a major reproductive hormone originating from the anterior pituitary gland, and is necessary for the growth of graafian follicles and the production of estrogen by the ovary (ROBERTS, 2004). In the absence of adequate FSH, follicles are unable to grow and mature beyond the early antral stage, and consequently, ovulation does not occur (HSUEH et al., 1989; HOWLES, 2000). Moreover, FSH is a major hormone used for superovulation in animals,

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multiple ovulation and embryo transfer technology (MOET). FSH maintains follicular development by binding to FSH receptors present in the granulosa cells of ovaries (DIERICH et al., 1988). This binding allows the activation of the FSH receptors encoded by the follicle stimulating hormone receptor (FSHR) gene. The FSHR gene is located on chromosome 11 and has 10 exons and 9 introns. SIMONI et al. (1997) identified the 349 amino acid (aa) sized extracellular domain encoded by exon 1 to 9, whereas the c-terminal part of the extracellular domain, the transmembrane domain (264 aa) and the intracellular domain (65 aa) was encoded by exon 10. HOUDE et al. (1994) reported that the transmembrane domain was 264 aa long, which was a highly conserved region in various species, while the intracellular domain was least conserved. The FSHR gene has been characterized in many species, including sheep (YARNEY et al., 1993; SAIRAM and SUBBARAYAN, 1997), chicken (YOU et al., 1996), cattle (HOUDE et al., 1994), buffalo (PAL, 2006; MINJ et al., 2008), camels (NIARAKI et al., 2014) yaks (XIA et al., 2019), etc. Characterization of the FSHR gene has not been conducted in Indian cattle breeds, including Sahiwal and Hariana. In the dairy industry, milk yield and milk composition are two important economic traits that can be improved to attain maximum profit. Milk yield-related traits can be improved by utilizing molecular markers such as restriction fragment length polymorphism (RFLP) very accurately in a short span of time (ARSLAN et al., 2015). RFLP markers are codominant, showing a high degree of polymorphism, and are widely used in identifying genes that code for important traits (ALMEIDA et al., 2000; MACHADO et al., 2003). They are a useful tool to discover the genotypes associated with a particular gene in a population, and helpful in observing an increased response towards a trait, thus helping in marker assisted selection (MAS). AluI/PCR-RFLP of exon 10 has been conducted in European Zebu (MARSON et al., 2005), Kankrej and Gir cattle (CHANDRAN, 2006), Bos taurus, Bos indicus and Bos taurus×Bos indicus (HERNANDEZ-CRUZ et al., 2009), Egyptian buffalo (OTHMAN and ABDEL-SAMAD, 2013), Turkish cattle (ARSLAN et al., 2015), Sudanese cattle (OMER et al., 2016), and Sahiwal, Rathi and Kankrej cattle (KUMAR, 2018). However, AluI/PCR-RFLP studies of Indian cattle breeds are very limited, or have not yet been conducted in Hariana cattle breed. As this gene is involved in the development of follicles and manifestation of estrous, it can be a suitable candidate for MAS. Sequence analysis and detection of SNP are important to track any change in receptor structure that might affect the binding of receptors with the hormone, thus resulting in infertility. Considering all these points, in the present study cloning and characterization of exon 10 of the FSHR gene partial CDS was conducted in Sahiwal and Hariana cattle. Furthermore, DNA polymorphism using PCR-RFLP, and an association study of genotypes with milk production and reproduction traits were also performed.

Materials and methods

AluI/PCR-RFLP assay. For PCR-RFLP, blood samples were taken from a total of 200 animals (100 Sahiwal and 100 Hariana) and genomic DNA was isolated by using a standard phenol-chloroform DNA isolation protocol (SAMBROOK and RUSSELL, 2001). The primers used for amplification of 306 bp fragment comprising exon 10 (F; 5′-CTGCCTCCCTCAAGGTGCCCCTC-3′ and R; 5′-AGTTCTTGGCTAAATGTCTTAGGGGG-3′) were as per HOUDE et al. (1994). PCR reactions were carried out in a 25 µl reaction mixture containing 1×PCR buffer (NEB, USA), 2 mM $MgCl_2$, 2.5 mM of dNTPs, 5 pmole of each primer and one unit of Taq DNA polymerase (NEB, USA). Cycle conditions were: an initial denaturation of one cycle at 95ºC for 3 min; 35 cycles of exon specific amplification (denaturation at 95ºC for 30 sec; annealing at 58ºC for 30 sec; extension at 72ºC for 30 sec) followed by final extension of 72ºC for 10 min. The restriction digestion was carried out at 37°C for 4 h in a total volume of 7.50 µl, containing 5.0 µl of PCR product, 0.75 µl of 10X RE buffer, 10 units of 0.5 µl AluI restriction enzyme and nuclease free water. Sequence analysis after restriction digestion was conducted for the presence of AluI sites in the PCR product (Fig. 1).

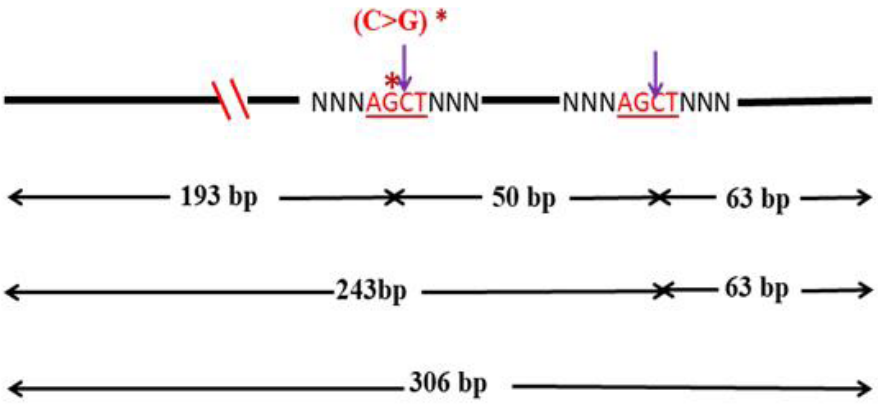

Fig. 1. Schematic representation of AluI/PCR-RFLP assay of the 306 bp region of exon 10 in the FSHR gene. The recognition sequence of AluI is underlined: (↓) indicates the restriction cutting site: (*) (C>G) indicates substitution of G with C from the AluI site

Statistical analysis. The data were generated by estimating the frequency of different FSHR/AluI genotypes. The genotypic and allelic frequencies were estimated by the standard procedure (FALCONER and MACKAY, 1996) using the following formulae:

Genotypic frequency = (Total number of individual of particular genotype) / (Total number of individuals of all genotype)

Allelic/gene frequency = ((2D + H)) / 2N

Where: D is number of homozygotes of a particular gene; H is the number of heterozygotes having that gene, and N is total number of individuals

The chi square (χ^2) test ($P \leq 0.05$) was also performed to test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium (SNEDECOR and COCHRAN, 1989) as follows:

$$\chi^2 = \Sigma \text{ (Observed – Expected)}^2 / \text{Expected}$$

The calculated Chi-square values were compared to the tabulated values at a specific degree of freedom (number of genotypes – number of genes/alleles).

The association study of the different FSHR/AluI genotypes obtained was done with the following reproduction and milk production traits: Age at First Calving (AFC = date of 1st calving – date of birth; DOB), Total Milk Yield (TMY = calculated by totaling the daily milk records of individual cows after completion of lactation), Lactation Period (LP = date of drying – date of calving), Service Period (date of successful artificial insemination (AI) – date of calving) and Calving Interval (CI = the difference between two successive calvings). The association study was analyzed by one way ANOVA with the help of SPSS (version 16.0) statistical software. It was carried out using the General Linear Model (GLM) with following linear model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where: Y_{ij} – observed trait value in animal; μ – mean trait value; G_i – effect of genotype; e_{ij} – random error. Significant differences between the least square means of different genotypes were calculated using Duncan's multiple-range test, and P values of 0.05 were considered statistically significant.

Characterization of FSHR exon 10 partial CDS. Three adult females from both breeds (3 Sahiwal and 3 Hariana) maintained at the livestock farm complex (LFC), DUVASU, Mathura were taken for the characterization study. Blood samples were collected from the two breeds in a sterile vacutainer with EDTA as the anticoagulant. Genomic

DNA was isolated by using a standard phenol-chloroform DNA isolation protocol (SAMBROOK and RUSSELL, 2001). The concentration and purity of the genomic DNA was determined by spectrophotometry at OD_{260} and OD_{280}. The integrity of the DNA was examined by agarose gel (0.7%) electrophoresis, and the gel was visualized under a UV light after staining with ethidium bromide (EtBr). Exon 10 was amplified using specific primers, taking genomic DNA as the template. The primers used for amplification of the FSHR exon 10 (F; 5′-TCTGACCTTCATCCAATTTGC-3′and R; 5′-GTTCTTGGCTAAATGTCTTAGGG-3) were designed using VNTI software, and synthesized commercially (Imperial Life Sciences, Gurugram, Haryana). The PCR reaction mixture components were described earlier. Cycle conditions were: an initial denaturation of one cycle at 95ºC for 3 min; 35 cycles of exon specific amplification (denaturation at 95ºC for 30 sec; annealing at 58ºC for 30 sec; extension at 72ºC for 1 min) followed by final extension of 72ºC for 10 min. The amplified product was run on agarose (1%) gel electrophoresis in 1× TBE buffer including EtBr. The gel was visualized under a UV light and photographed using an automated gel documentation system. The amplified products were cloned into pTZ57R/T cloning vector (PUREGENE, quick clone PCR-cloning kit). The positive recombinant clones were identified from the transformed (E.coli DH5α strain) bacterial colonies using blue and white selection. The positive clones were sequenced commercially (Eurofins genomics India Pvt. Ltd., Bengaluru, Karnataka) by an automated sequencer using standard cycle conditions by Sanger's dideoxy chain termination method. The obtained sequences of exon 10 of the FSHR gene were subjected to BLAST analysis to ascertain whether the sequence obtained was of FSHR. The nucleotide as well as the encoded amino acid sequences of the FSHR gene of Indian cattle breeds were aligned with Bos taurus (EU148061), Holstein cattle (NM_174061), buffalo (DQ845245, DQ785802), sheep (NM_001009289), goats (NM_001285636), yak (MH605506), horse (NM_001164013) donkeys (NM_001323782), Indian camels (XM_010984039), double humped camels (XM_010967756) and pigs (NM_214386) available in the GenBank database, using the ClustalW method of the MegAlign program of Lasergene software (DNASTAR, USA) and BioEdit software.

Results

After performing the PCR-RFLP assay of the exon 10 region of the FSHR gene, a 306 bp amplicon was observed that on digestion with the AluI restriction enzyme revealed three types of genotypes, namely: CC (243 bp and 63 bp), GG (193 bp, 63 bp and 50 bp) and CG (243 bp, 193 bp, 63 bp and 50 bp), as shown in Fig. 2. The CG genotype observed was more frequent (45.0%) than GG (41.5%) and CC (13.5%), and the frequency of the G allele was higher (0.64) than the C allele (0.36) in all the screened animals, hence confirming the polymorphic pattern of this gene in both Sahiwal and Hariana cattle. Sequence analysis also confirmed the genotyping, with the presence of AluI sites. The Chi square (χ^2) test revealed that $\chi^2_{cal\ (0.10)}$

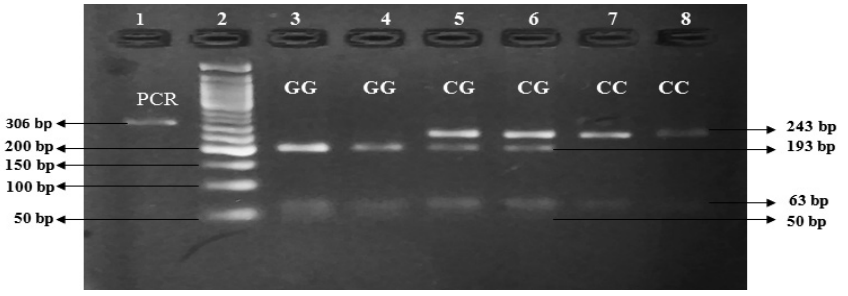

Fig. 2. AluI/PCR-RFLP pattern of exon 10 of the FSHR gene in Sahiwal and Hariana cattle run on 2.5% agarose gel electrophoresis. Lane 1 (PCR product), Lane 2 (50 bp marker), Lane 3-4 (GG genotype), Lanes 5-6 (CG genotype) and Lanes 7-8 (CC genotype).

$< \chi^2_{tab\ (5.99)}$ at a 5% level of significance for degree of freedom 1, indicating that the screened cattle population was in Hardy-Weinberg equilibrium (Table 1). An association study of the observed genotypes with milk production and reproduction traits was also conducted as shown in Table 2. We found a significant (P<0.05) difference in relation to Total Milk Yield and Lactation Period, where the CC genotype showed higher milk yield and had a longer lactation period than the other genotypes.

Table 1. Genotypic and allelic frequencies of AluI/PCR-RFLP of exon 10 of the FSHR gene in Sahiwal and Hariana cattle.

Breed	Genotypic frequency (%)			Allelic Frequency		HWE χ^2 value (df=1)
	CC	CG	GG	C	G	
Sahiwal (n=100)	13 (n=13)	49 (n=49)	38 (n=38)	0.375	0.625	0.187 (P<0.05)
Hariana (n= 100)	14 (n=14)	41 (n=41)	45 (n=45)	0.34	0.66	0.85 (P<0.05)
Total (N= 200)	13.5 (n=27)	45 (n=90)	41.5 (n=83)	0.36	0.64	0.10 (P<0.05)

Where; N= Sample size, n= Number of animals in particular genotype, HWE-Hardy-Weinberg equilibrium

Table 2. Association study of exon 10 of FSHR/AluI genotypes with milk production and reproduction traits (Mean±SEM) in Sahiwal and Hariana cattle

Lact	Breed	Geno	n	AFC (days)	TMY (Ltr)	LP (days)	SP	CI
I	Sahiwal (N=70)	CC	6	2166.7±132.3	2752.3±244.3^a	405.3±40.4	261.2±39.9	556.3±45.9
		CG	37	2209.7±44.9	1795.2±83.49^b	376.8±13.1	236.1±26.6	500.1±22.2
		GG	27	2150.4±46.48	1548.8±127.18^b	329.3±38.1	247.0±24.9	535.9±32.2
	Hariana (N=57)	CC	8	2073.0±108.0	1375.0 ±137.9	286.2±16.3	159.9±54.0	415.0±26.2
		CG	25	2446.0±150.1	1794.3±135.3	354.5±13.1	164.86±24.3	425.6±24.7
		GG	24	2592.0±159.0	1450.0±156.4	273.3±24.3	184.3±33.4	454.1±29.7
	Total (N=127)	CC	14	2113.1±118.4	1965.4±226.0^a	367.3±32.4^a	198.3±47.95	456.8±34.6
		CG	62	2305.0±87.3	1794.8±70.1^{ab}	360.7±9.6^a	219.3±25.6	470.0±23.2
		GG	51	2358.2±99.4	1502.3±99.1^b	303.0±14.6^b	217.5±28.9	497.4±31.0

Table 2. Association study of exon 10 of FSHR/AluI genotypes with milk production and reproduction traits (Mean±SEM) in Sahiwal and Hariana cattle

II	Sahiwal (N=70)	CC	6	---	2535.8±77.4[a]	375.6±22.4	250.0±41.0	520.0.3±57.4
		CG	37	---	1966.4±82.8[b]	359.6±12.3	261.0±26.4	499.0±45.4
		GG	27	---	1840.3±122.9[b]	330.3±27.3	247.0±30.4	530.0±66.3
	Hariana (N=57)	CC	8	---	1485.9±99.1	279.2±38.2	170.5±45.0	413.0±45.3
		CG	25	---	1705.2±104.1	322.0±15.5	175.5±24.0	431.5±28.6
		GG	24	---	1619.5±110.1	295.2±10.1	172.6±65.0	435.0±30.0
	Total (N=127)	CC	14	---	1930.0±138.0	343.4±17.4	204.6±43.3	458.9±50.5
		CG	62	---	1840.2±66.1	334.0±9.9	226.5±25.4	471.8±38.6
		GG	51	---	1750.4±83.8	313.8±20.6	211.9±46.7	485.6±49.2

Lact - lactation, Geno - Genotype, AFC- age at first calving, TMY-total milk yield, LP- lactation period, SP-service period, CI- calving interval; Means with different superscripts (a, b) within the column differ significantly ($P<0.05$);SEM, standard error of mean. Bold values indicate total performances of allanimals.

As expected, agarose gel electrophoresis revealed the amplified product of 1230 bp (Fig 3) encoding exon 10 of the FSHR gene. The partial CDS of Sahiwal and Hariana exon 10 of the FSHR gene were submitted to the GenBank database with accession numbers MT681117 and MT681116, respectively. The multiple sequence alignment of the encoded FSHR amino acid sequence of Sahiwal and Hariana cattle breeds with exotic cattle breeds, buffalo and other species is presented in Fig. 4. The nucleotide, as well as the amino acid sequence of both the breeds was compared with the homologous sequence of other species, as shown in Table 3. Sahiwal and Hariana were 99.3% to 100% homologous to the nucleotide and amino acid sequences of exotic cattle breeds. They were 96.3% to 97.8% homologous to Bubalus bubalis in amino acid sequence. Homologous sequence comparison of small ruminants (sheep and goat) with the Indian

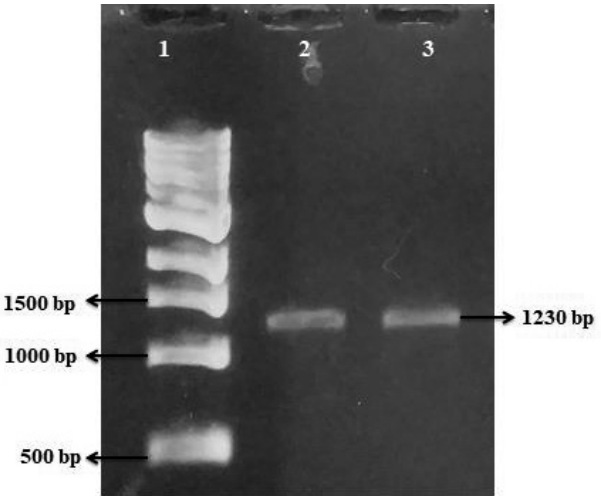

Fig. 3. Amplification of PCR products of FSHR exon 10, partial CDS in Sahiwal and Hariana cattle. Lane 1 (1 Kb Marker), Lanes 2-3 (1230 bp product of FSHR).

cattle breeds studied showed similarity of 97.6% and 98.0% in terms of nucleotide, and 97.3% and 97.6% in terms of amino acid sequences. However, the studied cattle breeds showed less similarity with monogastric animals (Equus asinus; 89.0% and Sus scrofa; 93.2%). Mutations in the partial CDS of the FSHR gene of the Indian cattle breeds studied relative to exotic cattle breeds are presented in Table 4. A missense mutation found at position 1118C→G caused amino acid substitution (Thr→Ser) at position 373 in both the cattle breeds. A nonsense mutation was found at 738T→C in Hariana cattle and at 1200A→C in both the cattle breeds. The phylogenetic tree, shown in Fig. 5, clearly indicated two separate clades of cattle and buffalo. In the cattle subgroup, Hariana and Sahiwal cattle were more closely related with Bos grunniens (MH605506) and Bos taurus (EU148061) than Holstein cattle (NM174061). The Holstein breed was closer to buffalo sequences. In comparison to equidae and camelidae, pigs were closer to the bovidae family.

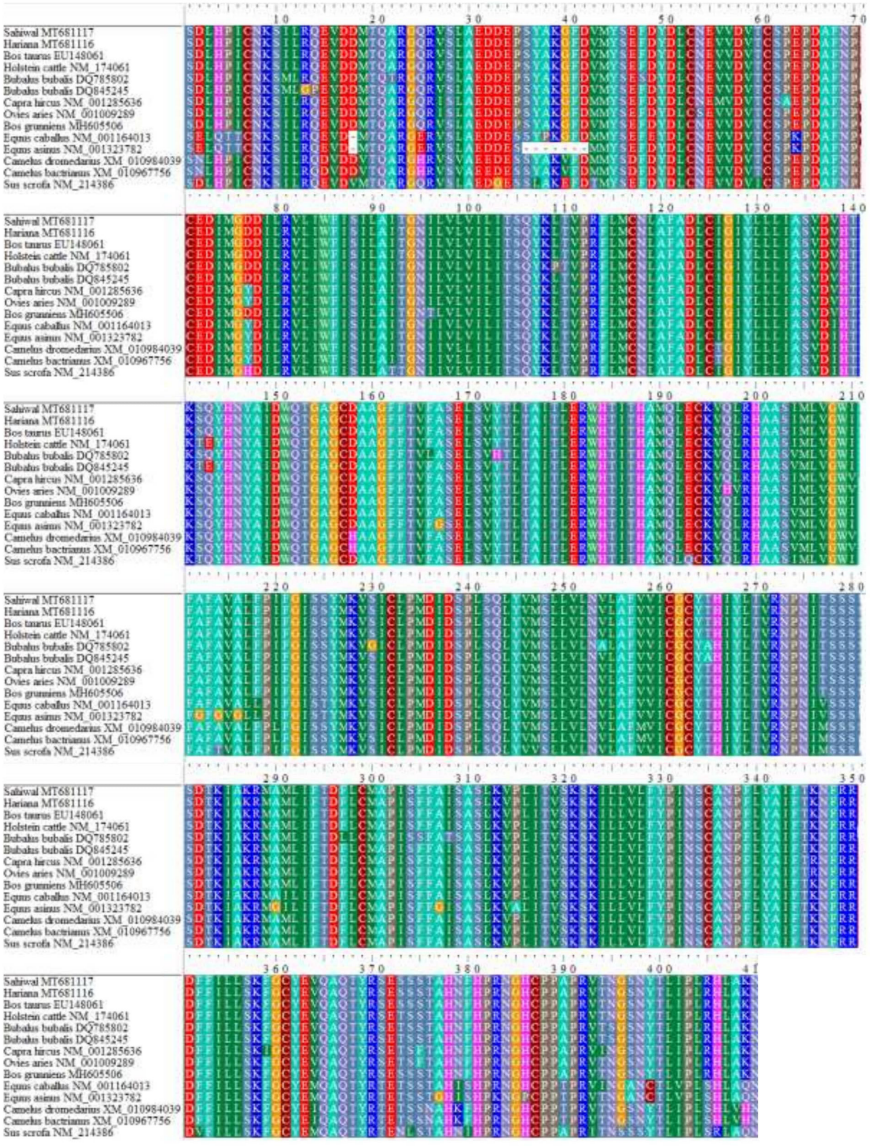

Fig. 4. Multiple sequence alignment of encoded amino acid sequence of FSHR gene exon 10, partial CDS of Sahiwal and Hariana cattle breeds with those of other exotic cattle and other species.

Table 3. Percentage identity of exon 10 of the FSHR gene, nucleotide and amino acid of Indian cattle breeds with other exotic cattle breeds, buffalo and other species. *represents the same accession number of Sahiwal and Hariana in the row and column.

Species and accession no.	Nucleotide %		Amino acid %	
	Sahiwal*	Hariana*	Sahiwal*	Hariana*
Sahiwal_MT681117*	***	99.9	***	100.0
Hariana_MT681116*	99.9	***	100.0	***
Bos taurus_EU148061	99.9	100.0	100.0	100.0
Holstein_NM_174061	99.3	99.4	99.3	99.3
Bubalus bubalis_DQ785802	98.0	97.9	96.3	96.3
Bubalus bubalis_DQ845245	98.8	98.7	97.8	97.8
Capra hircus_NM_001285636	97.6	97.6	97.3	97.3
Ovies aries_NM_001009289	98.0	97.9	97.6	97.6
Bos grunniens_MH605506	99.8	99.7	99.8	99.8
Equus caballus_NM_001164013	92.9	92.8	92.4	92.4
Equus asinus_NM_001323782	90.7	90.6	89.0	89.0
Camelus dromedarius_XM_010984039	93.6	93.6	92.7	92.7
Camelus bactrianus_XM_010967756	93.7	93.7	92.7	92.7
Sus scrofa_NM_214386	93.3	93.2	93.2	93.2

Table 4. Nucleotide and amino acid substitutions identified in exon 10 of the FSHR gene of Indian and exotic cattle breeds.

Nucleotide	425	426	427	651	729	738	1118	1152	1180	1200
Amino acids	142	142	143	217	243	246	373	384	394	400
Majority codon	AGC	AGC	CAG	CTC	CAG	GTT	ACC	AAT	ACC	ACA
Codon Indian cattle breeds	AGC	AGC	CAG	CTC	CAA	GTC	AGC	AAT	ACT	ACC
Codon exotic cattle breeds	ACG	ACG	GAG	CTT	CAA	GTC	AGC	AAC	ACT	ACC
AA Indian cattle breeds	S	S	Q	L	Q	V	T	N	T	T
AA exotic cattle breeds	T	T	E	L	Q	V	S	N	T	T
Breed/ Accession number	Holstein cattle	Holstein cattle	Holstein cattle	Holstein cattle	All cattle	Hariana, Bos taurus, Holstein	Hariana, Sahiwal, Bos taurus	Holstein	All cattle breeds	Hariana, Sahiwal, Bos taurus

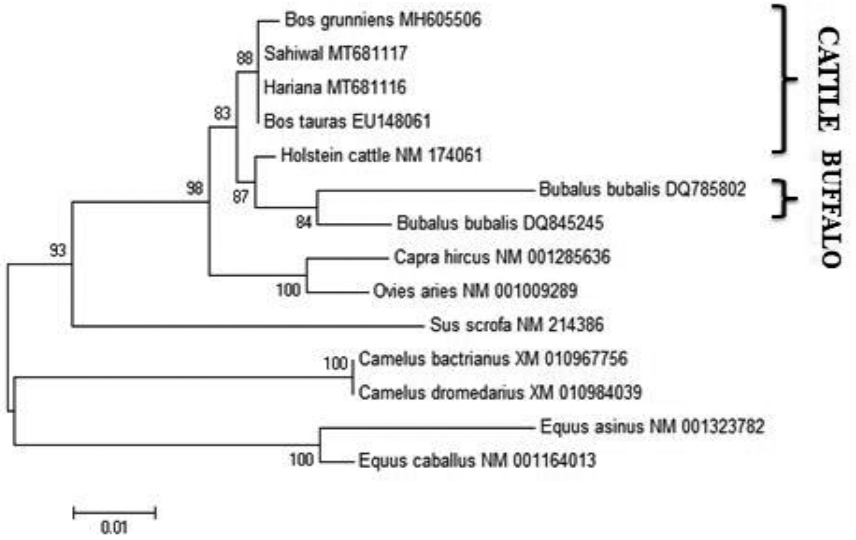

Fig. 5. Phylogenetic tree based on encoded amino acid sequences of exon 10 of the FSHR gene for the Indian cattle breeds, buffalo and other related species.

Discussion

In the present study, we cloned and characterized exon 10 of the FSHR gene partial CDS in Sahiwal and Hariana cattle. The nucleotide sequence was determined by sequencing, and the amino acid sequence was encoded. To the best of our knowledge this is the first study to report the nucleotide and amino acid sequences of any exon of the FSHR gene in Indian cattle breeds. HOUDE et al. (1994) reported that a total of 2034 bp bovine FSHR gene consisted of 10 exons and 9 introns of encoded 684 amino acid proteins. The complete CDS of the FSHR gene was characterized by PAL (2006) and MINJ et al. (2008) in Bubalus bubalis and 97.9% to 98.0% and 96.7% to 98.0% homology was observed with exotic cattle nucleotide and amino acid sequences. XIA et al. (2019) found that yak and cattle showed 99.38% homology in the complete CDS of the FSHR. In the current study, Sahiwal and Hariana cattle breeds showed greater (99.8%) similarity with the nucleotide than amino acid (98.8%) sequences of yak. The studied Indian cattle breeds showed a lesser degree of homology with horses, camels and pigs (91.9% to 93.2%), and least of all, 89.3% with donkeys due to 20 bp nucleotide deletions (107 to 127). Eleven conserved cysteine residues were found in exon 10 at positions 117, 125, 157, 194, 232, 261, 263, 299, 335, 361, and 387, and three potential Asparagine (N) linked oligosaccharide chains at position 8, 275 and 398, important for in vivo activity and function. A similar observation was also made by PAL (2006) in Bubalus bubalis. Conservation of the sequences of both nucleotides and amino acids between related species is due to the fact that exon 10 contains a whole transmembrane domain which is highly conserved between species (HOUDE et al., 1994). In the FSHR exon 10 sequence a total of 3 amino acid substitutions were reported at positions 142Ser→Thr, 143Gln→Glu in Holstein cattle and 373Thr→Ser in Hariana, Sahiwal and Holstein. CORY et al. (2013) reported that 373Thr→Ser (658Thr→Ser in complete CDS) was an important substitution as it has been associated with lower embryonic yield and a high percentage of unfertilized oocytes in some genotypes of animals. This substitution modified the intracellular

carboxyl terminal domain of FSHR, a region involved in signal transduction. In the phylogenetic tree, Sahiwal and Hariana are more closely related to yaks (Bos grunniens) and Bos taurus (EU148061) than Holstein cattle (NM_174061). XIA et al. (2019) also reported yak and cattle in the same clade, indicating that these two species are more closely related to each other. Selection of animals that are carriers of desirable alleles and an association study with milk production and reproduction traits, in order to obtain good performing genotypes using RFLP is an important area of study. In the present study, we performed AluI/PCR-RFLP and found a higher frequency of the CG genotype (49.0%) in Sahiwal cattle. KUMAR (2018) also found a higher frequency of heterozygotes (57.0%) in Sahiwal, but the difference in frequency was perhaps due to the different population size. However, a higher frequency of heterozygotes in all the animals was observed by many researchers, including MARSON et al. (2005), CHANDRAN (2006), YANG et al. (2010) and KUMAR (2018). Contrary to this, ARSLAN et al. (2015) found a higher frequency of CC genotypes in total in Turkish cattle breeds and OMER et al. (2016) did not find any CG and GG genotypes in Sudanese cattle breeds. Differences in genotypic frequencies arose due to different geographical tracts and population sizes. In the present study, allele G was more frequent (0.64) than allele C (0.36), which was in accordance with the observations of CHANDRAN (2006) and KUMAR (2018) in indigenous cattle breeds. However, the C allele was more frequent than G in other cattle breeds reported by ARSLAN et al. (2015), and it was 1.0 in Sudanese cattle observed by OMER et al. (2016). An association study of the observed genotypes revealed significant difference in Total Milk Yield and Lactation Period where the CC genotype showed higher milk yield and a longer lactation period than the other two genotypes. Contrary to these results KUMAR (2018) did not find any significant differences between any of the traits studied.

In the present study, we reported the characterization of exon 10 of the FSHR gene in two Indian cattle breeds. Moreover, the nucleotide and amino acid sequences of Sahiwal and Hariana cattle were compared with homologous sequences of other species. We identified 10 SNPs in Indian and exotic cattle breeds. Furthermore, AluI/PCR-RFLP assay of exon 10 of the FSHR gene was also conducted and we found a polymorphic pattern of this gene in Sahiwal and Hariana cattle breeds. An association study was conducted, and the CC genotype revealed a higher Total Milk Yield and a longer Lactation Period than the other genotypes in Sahiwal and Hariana cattle. As this is a novel study in cattle, especially Indian cattle breeds, it would be interesting to learn more about the important SNPs and their correlation with milk production and reproduction traits in cattle, for use in marker assisted selection.

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SAŽETAK

FSH receptori važna su mjesta vezivanja folikulostimulirajućeg hormona (FSH) u jajnicima, a njihova kontrola je određena genom FSHR koji ima 10 egzona i 9 instrona. Egzon 10 je najveći (>1200 bp) od svih egzona. U ovom istraživanju, egzon 10 kloniran je iz djelomično kodiranog slijeda (CDS) FSHR gena. Kao materijal za istraživanje poslužila su goveda pasmina sahival i hariana u kojih je DNK polimorfizam analiziran pomoću *AluI*/PCR-RFLP testa. Na razini nukleotida i aminokiselina, djelomično kodirani slijed (CDS) egzona 10 FSHR gena u goveda sahival odnosno hariana pasmina bio je 99,3% do 100% sličan onom u egzotičnih pasminama goveda. U navedene dvije pasmine na položaju 1118 (C → G) pronađena je pogrešna mutacija koja je uzrokovala promjenu aminokiseline na 373 (THR → SER), te dvije besmislene mutacije koje su nađene na položajima 729 (G → A) i 1180 (C → T). Filogenetska analiza jasno je pokazala da su goveda pasmina sahival i hariana u većoj mjeri povezana s jakom i domaćim govedom (*Bos taurus*). Područje egzona 10, koje sadrži 306 parova baza pokazalo je nakon cijepanja s *AluI* restrikcijskim enzimom 3 genotipa: CC (243 bp i 63 bp), GG (193 bp, 63 bp i 50 bp) i CG (243 bp, 193 bp, 63 bp i 50 bp). Genotip CG bio je učestaliji (45,0%) od genotipova CC (13,5%) i GG (41,5%). Uzevši u obzir sve pretražene životinje, učestalost G alela bila je veća (0,64) od C alela (0,36). Hi-kvadrat (χ^2) analizom je utvrđeno da je pretražena populacija goveda bila u Hardy-Weinberg ravnoteži. Statistička analiza povezanosti pokazala je znakovite ($P<0,05$) razliku između genotipova za obilježja ukupnog prinosa mlijeka i trajanja laktacije, pri čemu se istaknuo genotip CC s većim prinosom mlijeka u odnosu na druge genotipove.

Ključne riječi: indijske pasmine goveda; kloniranje; FSHR; PCR-RFLP; *AluI*; analiza povezanosti