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Research Article

MOLECULAR CHARACTERIZATION AND SNP ANALYSIS OF PARTIAL FSHB GENE IN INDIAN CROSSBRED (HOLSTEIN FRIESIAN AND GIR) BULL

KADAM V.K.[1], BARATE A.K.[2*], SHENDE T.C.[3], BIRADE H.S.[4]

[1]Department of Animal Reproduction Gynaecology & Obstetrics, KNP College of Veterinary Science, Shirwal 412 801, Maharashtra Animal and Fishery Sciences University, Nagpur, 440001, India

[2]Department of Veterinary Biochemistry, Department of Veterinary Biochemistry, KNP College of Veterinary Science, Shirwal 412 801, Maharashtra Animal and Fishery Sciences University, Nagpur, 440001, India

[3]Department of Animal Genetics and Breeding, KNP College of Veterinary Science, Shirwal 412 801, Maharashtra Animal and Fishery Sciences University, Nagpur, 440001, India

[4]Department of Animal Reproduction Gynaecology & Obstetrics, Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra Animal and Fishery Sciences University, Nagpur, 440001, India

*Corresponding Author: Email - abhijit.barate@gmail.com

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Abstract: Follicle-stimulating hormone (FSH) is a key regulator of reproduction in mammals. In this study, partial FSHB-3 of HFxG 5040 crossbred bull has been characterized and analysed for the presence of single nucleotide polymorphisms (SNPs). Analysis revealed that HFxG5040 FSHB-3 has 98.4% homology at nucleotide level and 96.4% homology at amino acid level with cattle FSHB-3 sequences (M83753 & NM_174060). Three SNPs were detected in the FSHB-3 sequence of HFxG5040 (4377G>T, 4453A>G, 4489A>C) compared to cattle M83753 sequence. Amongst the three, the novel SNP 4453A>G led to amino acid change Ser103Gly. Future study with larger number of bulls having Ser103Gly mutation is necessary to confirm role of this substitution on fertility of bulls.

Keywords: Crossbred, FSHB, SNP, cattle

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Introduction

The male reproductive system is regulated by intricate feedback mechanisms involving the hypothalamus, anterior pituitary, and the testes. The hypothalamus synthesizes and secretes the gonadotropin-releasing harmone (GnRH). GnRH acts directly on the gonadotropic cells in the anterior pituitary. Upon stimulation by GnRH the gonadotropes synthesize and secrete the gonadotropins 1) Follicle stimulating harmone (FSH) and leutinizing harmone (LH). The released FSH and LH act on specific receptors present in the testes [1, 2]. Follicle-stimulating hormone (FSH), a glycoprotein hormone derived from adenohypophyseal parenchymal cells in the pituitary, is a key regulator of the reproductive process in mammals. In males, FSH and testosterone are principal endocrine factors responsible for the regulation of Sertoli cell function. FSH is required for the initiation and maintenance of the quality and quantity in spermatogenesis [3, 4]. Like other members of the pituitary glycoprotein hormones *viz*. thyroid stimulating hormone, luteinizing hormone, and chorionic gonadotropin, FSH is heterodimer comprising of two subunits, a common alpha and a hormone-specific beta[5, 6]. Although both FSH subunits participate in the binding to FSH receptor, the beta-subunit dictates its binding specificity [7]. Previous research in mice has shown that spermatogenesis is not completely normal in FSH-deficient mice. It was observed that FSH-deficient mice are fertile; however epididymal sperm numbers and the number of motile sperm in the FSH-deficient mice were lower compared to that in normal mice [1, 8]. In case of bovines, the published sequence for FSHB (GenBank No.: M83753) [9] comprises 1 non-coding exon and 2 translated exons that encode the 129-amino acid preprotein. It is important to note that single nucleotide polymorphisms (SNPs) in the bovine FSHB gene have been shown to affect quality of fresh and frozen semen and fertility in bulls.

A total of 13 substitutions and 1 insertion was reported in the FSHB gene in pure breed bulls of Canada [10]. Seven substitutions were reported in the FSHB-3 which caused significantly influenced some of the observed fresh and frozen semen quality and fertility traits. FSH is potentially useful gene for marker assistant selection (MAS) of bull semen quality and fertility traits [10]. However, to the author's knowledge there are no reports on FSHB gene polymorphism from Indian crossbred bull. Due to the lack of information about the SNPs present in FSHB-3 exon from Indian crossbred bulls, we report here characterization of FSHB-3 exon from Indian crossbred bulls.

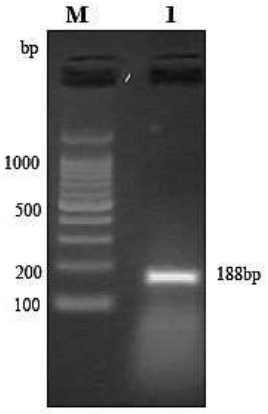

Fig-1 1.5% agarose gel electrophoresis of HFxG5040 partial FSHB-3

ggtc tac gat acg gtg aaa gtg cct ggc tgt gct cac cat gca gac tcc ctg tac acg tac cca gta gcc act gaa tgt cac tgc ggc aag tgc gac agc gac agc act gac tgc acc gtg cga ggc ctg ggg ccc agc tac tgc tcc ttc agg gaa atc aaa
V Y D T V K V P G C A H H A D S L Y T Y P V A T E C H C G K C D S D S T D C T V R G L G P S Y C S F R E I K
gaa taa aga gca gcg gat gct ttga
E - **R** A A D A L

Fig-1b

Percent Identity

Divergence		1	2	3	4	5	6	7	8		
	1		88.3	88.3	93.1	89.9	89.9	89.4	89.9	1	*Sus scrofa* NM_213875_40
	2	12.9		97.9	89.9	94.7	94.1	94.7	94.1	2	*Ovies aries* NM_001009798_40
	3	13.0	2.2		89.9	94.1	94.1	94.7	94.1	3	*Capra hircus* XM_013969782_40
	4	7.3	11.0	11.1		91.1	90.4	91.0	90.4	4	*Camelus dromedaries* XM_011000359_40
	5	10.9	5.5	6.2	9.5		94.7	95.3	94.7	5	*Bubalus bubalus* KT343910_40
	6	11.0	6.1	6.1	10.4	5.5		98.4	100.0	6	*Bos Taurus* NM_174060_40
	7	11.6	5.5	5.5	9.7	4.9	1.6		98.4	7	*HFG5040*
	8	11.0	6.1	6.1	10.4	5.5	0.0	1.6		8	*Bos taurus M83753_40*
		1	2	3	4	5	6	7	8		

Fig-2a Sequence distance of HFxG5040 FSHB-3 at nucleotide level

Percent Identity

Divergence		1	2	3	4	5	6	7	8		
	1		92.9	92.9	100.0	91.1	94.6	94.6	94.6	1	*Sus scrofa* NP_999040_40
	2	7.5		96.4	92.9	91.1	91.1	91.1	91.1	2	*Ovies aries* NP_001009798_40
	3	7.5	3.7		92.9	91.1	91.1	91.1	91.1	3	*Capra hircus* XP_013825236_40
	4	0.0	7.5	7.5		91.1	94.6	94.6	94.6	4	*Camelus dromedaries* XP_010998661_40
	5	9.5	9.5	9.5	9.5		87.5	87.5	87.5	5	*Bubalus bubalus* ALQ43817_40
	6	5.6	9.5	9.5	5.6	13.7		100.0	96.4	6	*Bos Taurus* NP_776485_40
	7	5.6	9.5	9.5	5.6	13.7	0.0		96.4	7	*M83753_40*
	8	5.6	9.5	9.5	5.6	13.7	3.7	3.7		8	*5040 Protein*
		1	2	3	4	5	6	7	8		

Fig-2b Sequence distance of HFxG5040 FSHB-3 at amino acid level

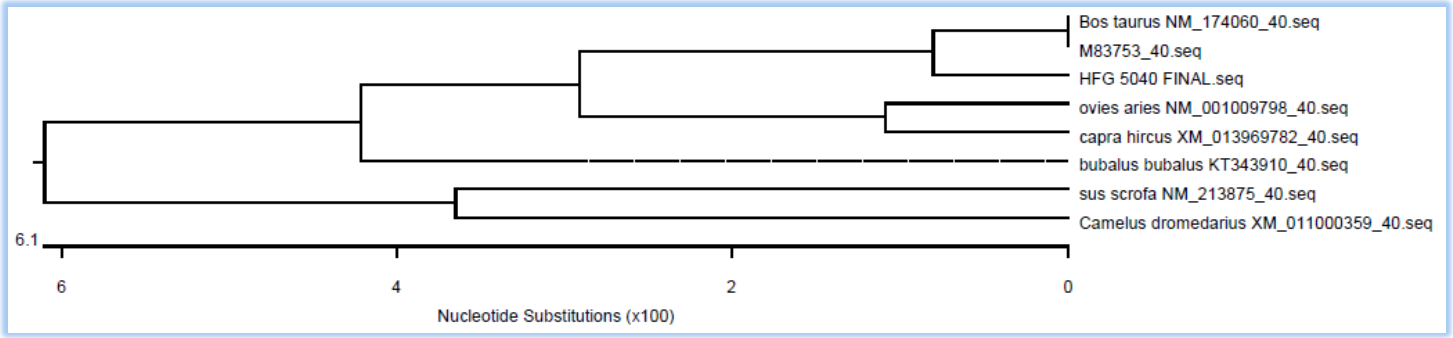

Fig-3a Phylogenetic tree of HFxG5040 FSHB-3 at nucleotide level

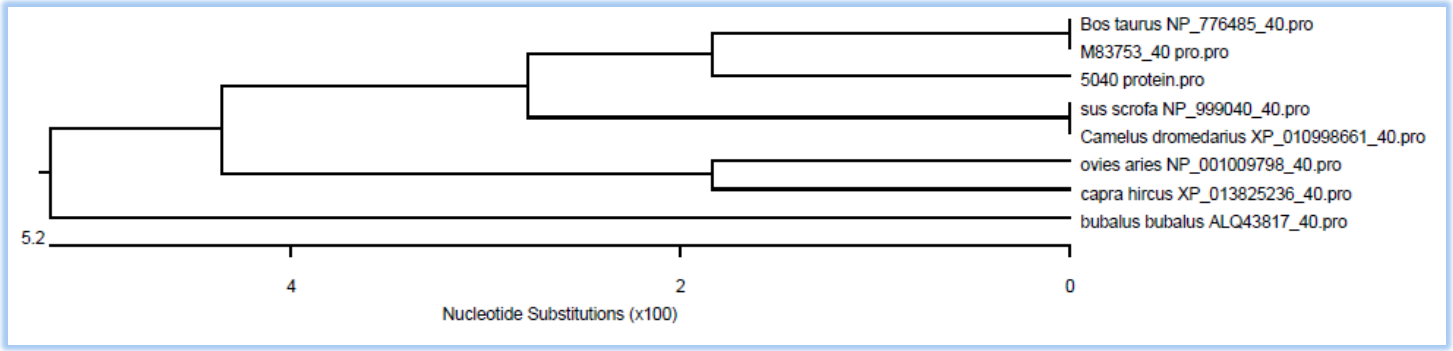

Fig-3b Phylogenetic tree of HFxG5040 FSHB-3 at amino acid level

Materials and Methods

Blood sample was collected from Holstein Friesian x Gir crossbred bull (HFxG5040 Bull number 5040) maintained at Sabarmati Ashram Gaushala, Bidaj Farm, Gujarat, India. 5 ml of blood was collected from jugular vein of cross bred cattle in a 15 ml polypropylene tube containing 250µl of 0.5 M Ethylenediaminetetraacetic acid (EDTA) as anticoagulant. After collection of blood, the vials were shaken gently to facilitate through mixing of blood. The vials were then kept immediately in ice box containing ice and gel cool pack and were transport to the laboratory immediately. Genomic DNA was extracted by phenol-chloroform method as described previously [11]. A pair of primers for amplification of partial FSHB-3 exon (188bp) were designed based on published FSHB sequence (GenBank No.: M83753) [9]. The forward primer was of 18 bp (5'-GGTCTACGATACGGTGAA-3') and reverse primer was of 17 bp (5' TCAAAGCATCCGCTGCT-3'). Polymerase chain reactions (PCR) was carried out in a final volume of 50 µl reaction mixture containing 100ng of template DNA, 1XPCR assay buffer, 2.0 mM of Mg^{2+}, 200 µM of dNTPs, 10 pM of each primer and 1U of Taq DNA polymerase. Amplification was carried out in Thermal cycler (Eppendorf, USA). PCR condition were: initial denaturation at 94ºC for 5 minutes; followed by 94ºC for 40sec, 53ºC for 30sec, 72ºC for 25 sec, and a final extension of 72ºC for 5min.

PCR products were purified and quantified according to manufacturer's instructions (QIAquick PCR Purification Kit; Qiagen Inc). Purified PCR products were submitted to geneOmbio technologies pvt ltd. Sequence analysis was done by comparing HFxG5040 FSHB-3 amplicon sequence to FSHB published sequences available at National Center for Biotechnology Information (NCBI, USA) using DNA star software (USA).

Results and Discussion

The PCR amplification of HFxG5040 partial FSHB-3 is shown in [Fig-1a]. PCR amplification of 188 bp was checked on 1.5% agarose gel electrophoresis. PCR product was purified using PCR purification kit and sent for sequencing. The partial HFxG5040 FSHB-3 sequence and its protein translation obtained by using the ExPASy translate tool (ExPASy software, Swiss Institute of Bioinformatics, Geneva, Switzerland) is shown in [Fig-1b]. The nucleotide sequence and the deduced amino acid sequence of HFxG5040 partial FSHB-3 was aligned using DNAstar software. Alignment was done using ClustalW method [Fig-2a] and [Fig-2b]. FSHB-3 sequences used for alignment were obtained from the NCBI. HFxG5040 FSHB-3 nucleotide sequence 98.4% homology with cattle FSHB-3 sequences (M83753 & NM_174060) [Fig-2a]. Homology of nucleotide sequence between FSHB-3 sequence of HFxG5040 and buffalo, camel, goat, sheep and pig was 94.7%, 90.4%, 94.1%, 94.1% and 89.9%, respectively. HFxG5040 FSHB-3 at amino acid level [Fig-2b] showed identity 96.4%, 87.5%, 94.6%, 91.1%, 91.1% and 94.6% identity with cattle FSHB-3 sequences (M83753 & NM_174060), buffalo, camel, goat, sheep and pig sequence, respectively. Phylogenetic tree at nucleotide level [Fig-3a] revealed that HFxG5040 FSHB-3 falls in the cattle group. Next highest identity was seen with goat & sheep sequences. Buffalos, pig and camel sequences are distantly related with HFxG5040 FSHB-3. Phylogenetic tree at amino acid level [Fig-3b] revealed that HFxG5040 FSHB-3 falls in the cattle group. Next highest identity was seen with pig & camel sequences. Buffalos, sheep and goat sequences are distantly related with HFxG5040 FSHB-3. Three nucleotide substitutions or SNPs were detected in the FSHB-3 sequence of HFxG5040 (4377G>T, 4453A>G, 4489A>C) [Fig-4] compared to cattle M83753 sequence; of which novel 4453A>G led to amino acid change Ser103Gly. The remaining two substitutions (4377G>T and 4489A>C) were synonymous. This finding is different from the previous study on pure breed bulls of Canada [10]. In that study, they detected 4453A>C substitution which resulted in Ser103Arg amino acid change. It is important to note that the sample used in the study was obtained from the breeding bull at Sabarmati Ashram Gaushala, Bidaj Farm, Gujarat. Also, there is no previous report about the effect of FSHB-3 Ser103Gly on fertility of bulls. Thus, we presume that amino acid change Ser103Gly might not affect semen/ fertility. Nevertheless, further studies with larger number of bulls having this mutation is necessary to confirm role of Ser103Gly on fertility of bulls.

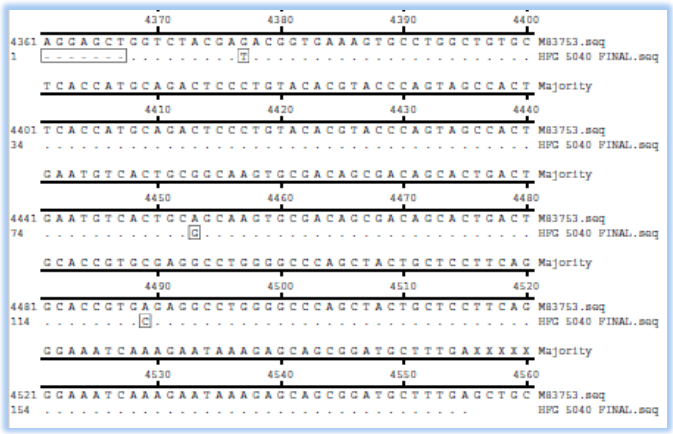

Fig-4 SNP analysis of HFxG5040 FSHB-3

Conclusion

Analysis of partial FSHB-3 of HFxG 5040 crossbred bull revealed that HFxG5040 FSHB-3 has 98.4% homology at nucleotide level and 96.4% homology at amino acid level with cattle FSHB-3 sequences (M83753 & NM_174060). Three SNPs were detected in the HFxG5040 FSHB-3 (4377G>T, 4453A>G, 4489A>C) compared to cattle M83753 sequence. Amongst the three, the novel SNP 4453A>G led to amino acid change Ser103Gly. Prospective study on Ser103Gly mutation is necessary to confirm the role of this substitution on fertility of bulls.

Abbreviations: Follicle-stimulating hormone (FSH); single nucleotide polymorphisms (SNPs); gonadotropin-releasing harmone (GnRH); leutinizing harmone (LH); marker assistant selection (MAS); Ethylenediaminetetraacetic acid (EDTA); Polymerase chain reactions (PCR); National Center for Biotechnology Information (NCBI)

Application of research: Study useful as molecular markers for selection of animals.

Research Category: Veterinary Science

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Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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