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Molecular characterization of partial FSHβ gene in Indian crossbred (Holstein Friesian and Sahiwal) bull

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Abstract
Follicle-stimulating hormone (FSH) plays a pivotal role in the reproduction of mammals. In this study, partial *FSHB-3* of HFxS 5041 crossbred bull has been characterized and analysed for the presence of single nucleotide polymorphisms (SNPs). Analysis revealed that HFxS 5041 FSHB-3 has 99.1% homology at the nucleotide level and 98.6% homology at the amino acid level with cattle FSHB-3 sequences. Two SNPs were detected in the *FSHB-3* sequence of HFxS 5041 (4453A>G and 4489A>C) compared to cattle M83753 sequence. Amongst the two, 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 the role of this substitution on fertility of crossbred bulls.

Keywords: Crossbred, FSHB, SNP, cattle

1. Introduction
The male reproductive system is regulated by intricate feedback mechanisms involving the hypothalamus, anterior pituitary, and the testes. The gonadotropin-releasing hormone (GnRH) is synthesized and secreted by hypothalamus. The hypothalamic GnRH, a decapeptide, is the master hormone regulating reproduction. The primary target of GnRH is anterior pituitary gonadotropes, which upon stimulation increase the synthesis and secretion of the gonadotropins 1) luteinizing hormone (LH) and 2) follicle stimulating hormone (FSH). The released FSH and LH act on specific receptors present in the testes [1-3].
Follicle-stimulating hormone (FSH) is a pituitary glycoprotein hormone that plays a pivotal role in the regulation of gonadal function, pubertal maturation and reproductive processes in both sexes 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 [4, 5]. Like other members of the pituitary glycoprotein hormones viz. thyroid stimulating hormone, luteinizing hormone, and chorionic gonadotropin, FSH is a heterodimeric glycoprotein composed of an α -glycoprotein subunit (α GSU) and a β -subunit (FSHβ [6, 7] that ensures the binding specificity to FSHR
Although both FSH subunits participate in the binding to FSH receptor, the beta-subunit dictates its binding specificity [8]. 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, 9]. In case of bovines, the published sequence for FSHB (GenBank No.: M83753) [10] 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 [11]. 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 a potentially useful gene for marker assistant selection (MAS) of bull semen quality and fertility traits. However, to the author's knowledge there scanty information 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 the characterization of FSHB-3 exon from the Indian crossbred bull.

2. Materials and methods

Blood sample was collected from Holstein Friesian x Sahiwal crossbred bull (HFxS 5041 Bull number 5041) maintained at Sabarmati Ashram Gaushala, Bidaj Farm, and Gujarat, India. 5 ml of blood was collected from jugular vein of cross bred cattle in polypropylene tube containing 0.5 M Ethylenediaminetetraacetic acid (EDTA) (Thomas Baker, India) as an 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 [12].

A pair of primers for amplification of partial FSHB-3 exon (232bp) were designed based on published FSHB sequence (GenBank No.: M83753) [10]. The forward primer (IDT, India) was of 17 bp (5'- GACCCAGCAAGGCCCAA-3') and reverse primer (IDT, India) was of 19 bp (5'-TAAAGAGCAGCGGATGCTT -3'). Polymerase chain reactions (PCR) was carried out in a final volume of 50 µl reaction mixture containing 100ng of template DNA, 1X PCR assay buffer, 2.0 mM of Mg^{2+}, 200 µM of dNTPs, 1 µM of each primer and 1U of Taq DNA polymerase (HiMedia, India). Amplification was carried out in Thermal cycler (Eppendorf, USA). PCR condition were: initial denaturation at 94 ºC for 5 minutes; followed by 94 ºC for 40sec, 56 ºC for 30sec, 72 ºC for 25 sec, and a final extension of 72 ºC for 5min. The electrophoresis of the amplified products was done at constant voltage of 80 volt for 50 minutes at 37 ˚C using 0.5X TBE buffer (HiMedia, India). The DNA ladder (100 bp ladder) (NEB, UK) as a molecular size marker was used for sizing of the DNA bands.

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 5041 FSHB-3 amplicon sequence to FSHB published sequences available at National Center for Biotechnology Information (NCBI, USA) using DNAstar software (USA).

3. Results and discussion

The PCR amplification of HFxS 5041 partial FSHB-3 is shown in Fig. 1a. PCR amplification of 232 bp was checked using 1.5% agarose gel electrophoresis. PCR product was purified using PCR purification kit and sent for sequencing. The partial HFxS 5041 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 HFxS 5041 partial FSHB-3 was aligned using DNAstar software. Alignment was done using ClustalW method Fig. 2a and 2b. FSHB-3 sequences used for alignment were obtained from the NCBI. HFxS 5041 FSHB-3 nucleotide sequence showed 99.1% homology with cattle FSHB-3 sequences (M83753 & NM_174060). Homology of nucleotide sequence between *FSHB-3* sequence of HFxS 5041 and bison, yak, goat, buffalo, pig and camel was 99.6%, 98.7%, 94.0%, 94.9%, 91.8% and 90.5%, respectively. HFxS 5041 FSHB-3 at amino acid level showed 98.6% identity cattle FSHB-3 sequences (M83753 & NM_174060) whereas it had 100% identity with bison and yak sequence. HFxS 5041 FSHB-3 showed 97.2%, 97.2%, 93.0% and 90.1% identity with camel, pig, goat and buffalo sequence, respectively. Phylogenetic tree at nucleotide level (Fig. 3a) revealed that HFxS 5041 FSHB-3 falls in the cattle group. Next highest identity was seen with bison & yak sequences. Buffalo, pig, camel and goat sequences are distantly related with HFxS 5041 FSHB-3. Phylogenetic tree at amino acid level revealed (Fig. 3b) that HFxS 5041 FSHB-3 falls in the cattle group. Next highest identity was seen with bison & yak sequences. Buffalo, goat, camel and pig sequences are distantly related with HFxS 5041 FSHB-3. This observed close relatedness between HFxS 5041 FSHB-3 sequences and cattle sequences is in agreement with previous study on FSHB-3 sequence analysis from bovines [12].

Two nucleotide substitutions or SNPs were detected in the *FSHB-3* sequence of HFxS 5041 (4453A>G and 4489A>C) Fig. 4 compared to cattle M83753 sequence. The 4489A>C substitution was synonymous. The SNP 4489A>C was also reported previously by Dai *et al* 2009 [11]. However, the novel 4453A>G led to amino acid change Ser103Gly. This finding is different from the earlier report on pure breed bulls of Canada [11]. In that study, they detected different substitution 4453A>C 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 numbers of bulls having this mutation is necessary to confirm role of Ser103Gly on fertility of bulls. Other FSHB-3 gene sequence analysis studies in cattle's are mostly on 5-upstream regulation region (5-URR) or on parts of FSHB-3 gene other than exon 3 [14-17]. Thus findings of the current study could not be compared with these studies.

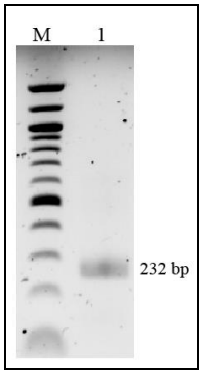

Fig 1a

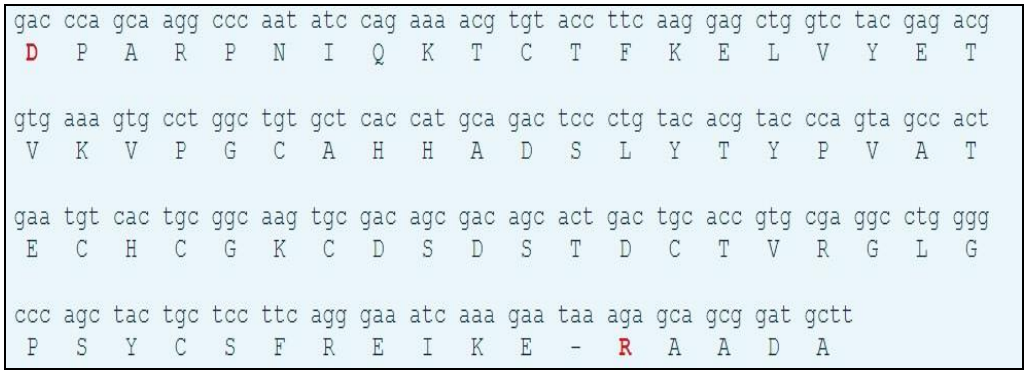

Fig 1b

Fig 1a-b: 1.5% agarose gel electrophoresis of HFxG5041 partial FSHB-3.

Percent Identity

	1	2	3	4	5	6	7	8	9		
1		99.6	99.1	99.1	98.7	94.0	94.9	91.8	90.5	**1**	HFxS 5041 FSHB3.seq
2	0.4		98.7	98.7	99.1	94.4	94.4	92.2	90.9	**2**	bison bison XM_010831795_41.seq
3	0.9	1.3		100.0	97.8	93.1	94.0	91.8	89.7	**3**	Bos taurus NM_174060_41.seq
4	0.9	1.3	0.0		97.8	93.1	94.0	91.8	89.7	**4**	M83753_41.seq
5	1.3	0.9	2.2	2.2		95.3	94.9	92.2	90.9	**5**	bos mutus yak XM_005895599_41.seq
6	6.4	5.9	7.3	7.3	4.9		93.1	89.7	88.4	**6**	capra hircus XM_013969782_41.seq
7	5.3	5.8	6.3	6.3	5.3	7.3		91.2	90.7	**7**	bubalus bubalus KT343910_41.seq
8	8.7	8.3	8.8	8.8	8.3	11.4	9.4		94.4	**8**	sus scrofa NM_213875_41.seq
9	10.3	9.8	11.3	11.3	9.8	13.0	10.0	5.9		**9**	Camelus dromedarius XM_011000359_41.seq
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		

Divergence

Fig 2a: Sequence distance of HFxG5041 FSHB-3 at nucleotide level

Percent Identity

	1	2	3	4	5	6	7	8	9		
1		100.0	100.0	98.6	98.6	97.2	97.2	93.0	90.1	**1**	HFxS 5041 FSHB3.pro
2	0.0		100.0	98.6	98.6	97.2	97.2	93.0	90.1	**2**	bison bison XP_010830097_41.pro
3	0.0	0.0		98.6	98.6	97.2	97.2	93.0	90.1	**3**	bos mutus XP_005895661_41.pro
4	1.4	1.4	1.4		100.0	95.8	95.8	91.5	88.7	**4**	Bos taurus NP_776485_41.pro
5	1.4	1.4	1.4	0.0		95.8	95.8	91.5	88.7	**5**	M83753_41.pro
6	2.9	2.9	2.9	4.4	4.4		100.0	93.0	91.5	**6**	Camelus dromedarius XP_010998661_41.pro
7	2.9	2.9	2.9	4.4	4.4	0.0		93.0	91.5	**7**	sus scrofa NP_999040_41.pro
8	7.4	7.4	7.4	9.0	9.0	7.4	7.4		90.1	**8**	capra hircus XP_013825236_41.pro
9	10.6	10.6	10.6	12.2	12.2	9.0	9.0	10.6		**9**	bubalus bubalus ALQ43817_41.pro
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		

Divergence

Fig 2b: Sequence distance of HFxG5041 FSHB-3 at amino acid level

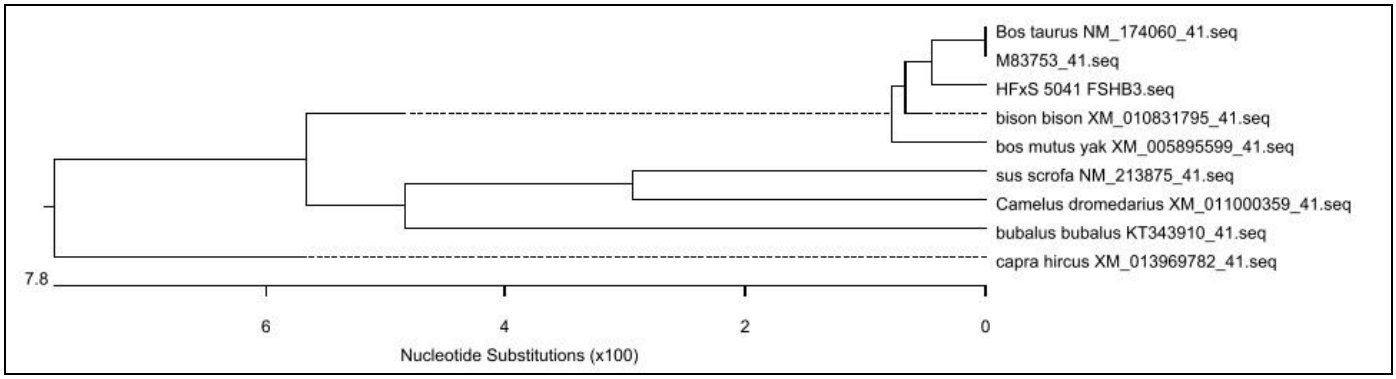

Fig 3a: Phylogenetic tree of HFxG5041 FSHB-3 at nucleotide level

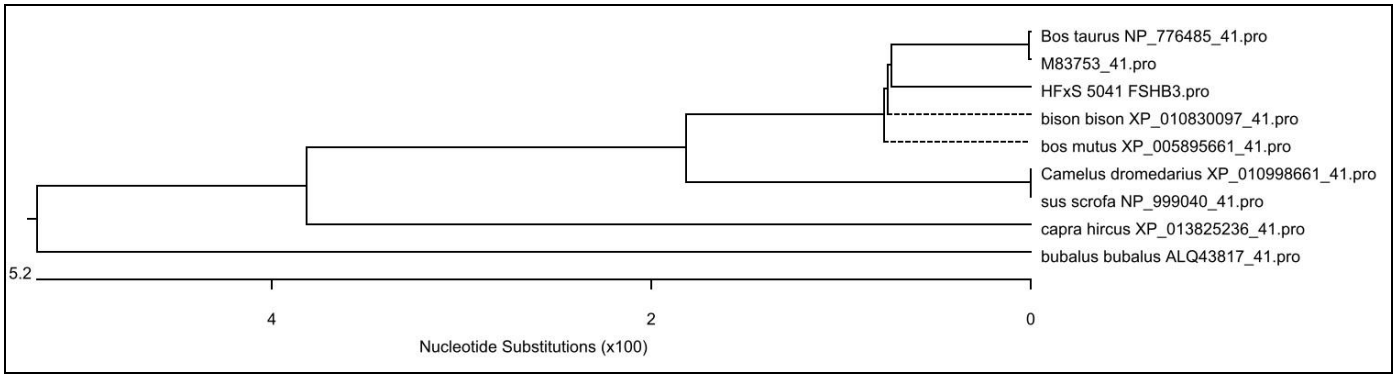

Fig 3b: Phylogenetic tree of HFxG5041 FSHB-3 at amino acid level

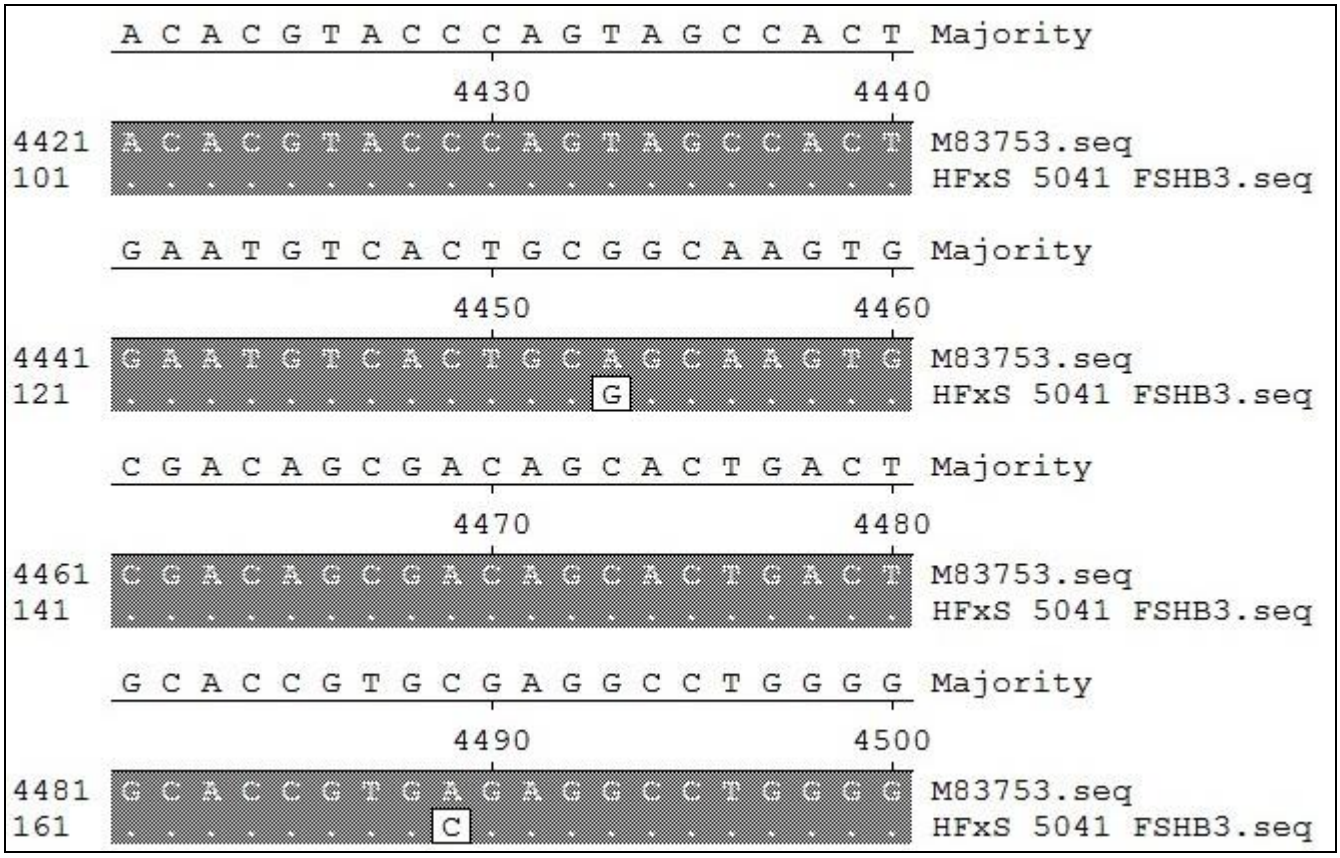

Fig 4: SNP analysis of HFxG5041 FSHB-3

4. Conclusion

In the current investigation, the partial sequence *FSHB-3* of HFxS 5041 crossbred bull was characterized and analysed for the presence of single nucleotide polymorphisms (SNPs). Analysis of partial *FSHB-3* of HFxS 5041 crossbred bull revealed that HFxS 5041 FSHB-3 has 99.1% homology at the nucleotide level and 98.6% homology at the amino acid level with cattle FSHB-3 sequences. Phylogenetic tree showed close relationship between partial sequence *FSHB-3* of HFxS 5041 and cattle congeners. Two SNPs were detected in the HFxS 5041 *FSHB-3* (4453A>G and 4489A>C) compared to cattle M83753 sequence. Amongst the two SNPs, the novel SNP 4453A>G led to amino acid change Ser103Gly. To confirm the role of this novel substitution Ser103Gly on fertility prospective studies on large numbers of crossbred bulls having this novel mutation are necessary.

5. Acknowledgement

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