

*Original Research***Association of FSH beta Gene Polymorphism on Testicular Parameters and Semen Quality in HF and Jersey Crossbred Bulls**

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Abstract

The present research was conducted to study the association of FSH beta gene polymorphism on testicular parameters and semen quality in twenty five HF (75%) as well as Jersey (50%) crossbred bulls. Three different genotypes BB, AB and AA having amplification size 313 bp; 313 bp, 202 bp, 99 bp and 202 bp, 99 bp, respectively were identified. Genotypic frequencies were 0.38, 0.54 and 0.08 for AA, AB and BB genotype while gene frequency of A allele was 0.65 and B allele was 0.35. The testicular parameters like scrotal circumference (SC), testicular length (TL), testicular width (TW), testicular thickness (TT) as well as seminal attributes like semen volume (Vol) concentration (Conc.) initial motility (IM), post thaw motility (PTM), hypo osmotic swelling test (HOST), abnormal sperm rate (ASR) and acrosome integrity test (AIT) were non significant ($P < 0.05$) between the three genotypes.

Key words: Crossbred Bulls, FSH Beta Gene, Polymorphism, Semen Quality, Testicular Parameters

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Introduction

Bull fertility is essential since bull DNA is the primary mechanism through which genetic improvements can be efficiently accomplished. Although 20 to 40 per cent of bulls may have reduced fertility, few are completely sterile (Kastelic and Coulter, 1999). Sub fertile bulls delay conception, prolong the calving season, reduce calf weaning weights and increase the number of females culled, thereby resulting in

economic losses and threatening sustainability of a livestock population. Bull semen quality is a quantitative trait controlled by multiple genes. By identifying various genes that have effects on fertility, the genetic cause behind sub fertility can be explored.

The process of spermatogenesis is regulated by reproductive hormones in gonadotropin axis and controlled by large number of genes. Therefore, hormone and their receptors are presumed to be good candidate genes for reproductive traits (Vincent *et al.*, 1998). Follicle Stimulating Hormone (FSH) is a glycoprotein hormone secreted by the anterior pituitary gland primarily in response to gonadotropin releasing hormone (GnRH) and plays an important role in the growth, development and function of the ovaries and testes and it is also important for regulating reproduction in mammals (Ulloa-Aguirre *et al.*, 1995). The biological protein consists of an alpha (α) and beta (β) subunit; however the β subunit is responsible for the specific biological actions of FSH and its interactions with the FSH-receptor (Bernard *et al.*, 2010). In males, FSH promotes spermatogenesis, the process of sperm development, in the seminiferous tubules primarily via actions on sertoli cells (Sairam and Krishnamurthy, 2001). Follicle stimulating hormone plays a critical role in the hormonal control of spermatogenesis by binding to FSH-receptors on sertoli cells and stimulating primary spermatocytes to undergo the first meiotic division to form secondary spermatocytes. Since each sertoli cell can only support a limited number of developing spermatozoa at a time, it is important to understand how polymorphisms of the FSH β subunit gene can affect male fertility (Sairam and Krishnamurthy, 2001).

In males, FSH in combination with testosterone is the most important tropic hormone regulating sertoli cell function initiate and maintain the quality and quantity in spermatogenesis (Ohta *et al.*, 2007). The published sequence for bovine FSH β gene (Lin *et al.*, 2006), (genbank No: M83753) comprises 1 non coding exon and 2 translated exons that encode the 129 amino acid pre- protein. Bovine FSH β gene is located on chromosome 15 that comprises two introns and three exons with a length of 6601 bp (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 FSH β subunit gene in exon 3 on the study significantly influenced the fresh and frozen semen quality. Polymorphism of FSH β subunit affects the production and sperm quality in Simmental, Charolais and Limousin (Dai *et al.*, 2009). The diversity of FSH β subunit genes with litter size in sow (Yaofeng *et al.*, 1998) and (Liu *et al.*, 2012), sperm quality in boar (Lin *et al.*, 2006), litter size in ewes (Xiaopeng *et al.*, 2010) and equine sperm quality (Samper and Plough, 2010) was co-related. Considering the paucity of research in this area the present research work was planned to study the polymorphism of FSH beta subunit on testicular and seminal attributes in Holstein Friesian and Jersey crossbred bulls.

Materials and Methods

The research work was carried on twenty five HF (75%) crossbred as well as six Jersey (50%) crossbred bulls from Frozen Semen Laboratory Harsul, Aurangabad and Nagpur. The testicular length (TL), testicular width (TW) and testicular thickness (TT) of each testis along with scrotal circumference (SC) were measured with a metric tape in the widest region of the scrotum after adequate ventrocaudal traction of the gonads. The semen was collected by artificial vagina method twice weekly for analyzing various semen quality traits and six replicates of samples were studied. The semen volume, concentration, initial motility, post thaw motility, acrosome integrity, hypo osmotic swelling test and abnormal spermatozoa rate were studied. Genomic DNA was extracted from the collected blood samples using DNA extraction Mini kit and the quality and the purity of the DNA were checked and quantification was done by agarose gel electrophoresis.

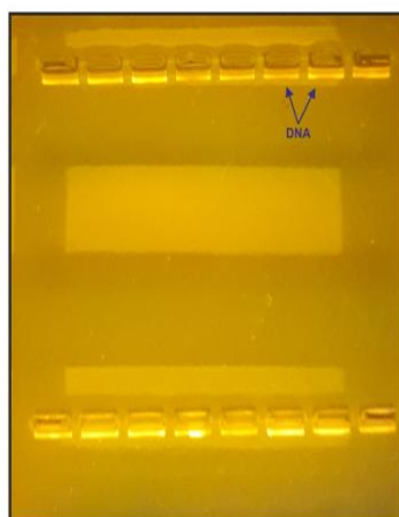


Fig. 1: DNA in agarose gel electrophoresis

The DNA samples were diluted at different dilutions 1:40, 1:50, 1:80 and 1:100 (20 µl of DNA in 980 µl nuclease free water) and subjected to agarose gel electrophoresis for quality check. FSH beta subunit gene specific primers were custom synthesized and utilized in the study is given below in Table 1.

Table 1: Primer sequence for FSH beta subunit gene used to evaluate the presence of SNP

Primer	Primer sequence (5'-3')	Product size	Reference
Forward	5'-CTTCCAGACTACTGTAATCATC -3'	313 bp	Dai <i>et al.</i> (2009)
Reverse	5'-GTAGGCAGTCAAAGCATCCG -3'		

Primers supplied in freeze dried powder form were reconstituted in nuclease free water to the volume 291 (in µl) and 347 (in µl) as in the case of Forward and Reverse primers, respectively. PCR was carried out in a final reaction volume of 27 µl Table 2.

Table 2: Components used for each PCR reaction for amplification of FSH beta subunit gene

PCR Components	Final Concentration	1X
Dream taq Green PCR master mix (2X)	12.5 µl	12.5
Forward Primer (20pmole/ µl)	1µl	1
Reverse Primer (20pmole/ µl)	1µl	1
DNA	4 µl	4
Nuclease- free water	8.5 µl	8.5
	Total	27

All reaction was carried out in 0.2 ml thin wall PCR tubes. PCR tubes containing mixture were taped gently and quickly spinned @ 10,000 rpm for few seconds. PCR amplification was confirmed by running 9 µl of PCR product from each tube plus 1 µl of DNA loading dye on 2% agarose in 1X TAE buffer (depending on the expected size of amplified product) at a voltage of 120 V and 100 V for 10 min and 30 minutes, respectively. Ethidium bromide was incorporated @ 2 µl of 1% solution/40 gel solution in the gel itself. The amplified product was visualized as a single compact fluorescent band of expected size under UV light. The PCR product for FSH beta subunit gene was digested by restriction enzyme Pst 1 using above protocol and kept in water bath for 1 hour at 37°C. Enzyme digestion was analyzed by running 9 µl of digested PCR product from each tube plus 1 µl of DNA loading dye on 2% agarose in 1X TAE buffer at a voltage of 120 V and 100 V for 10 min and 30 minutes, respectively. Statistical analysis was carried out by using ANOVA (Analysis of variance) using statistically Web Based Agricultural Statistics Software Package (WASP).

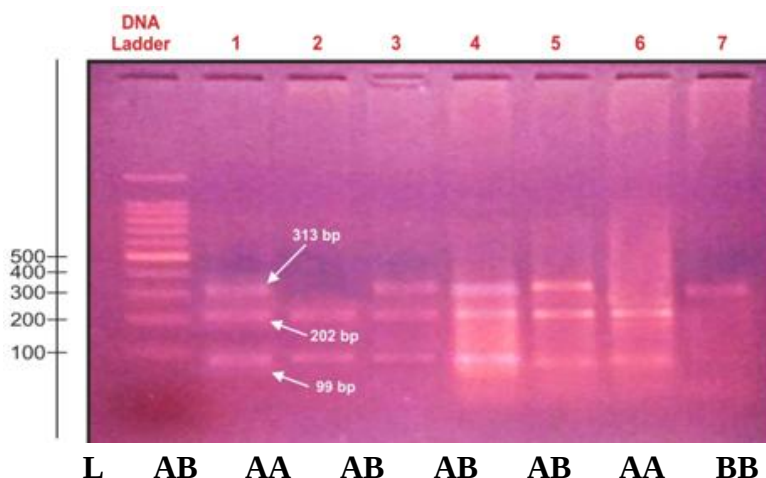


Fig. 2: Genotypes observed after RE digested product in agarose gel electrophoresis

Results and Discussion

In the present study, three different genotypes BB, AB and AA having amplification size 313 bp; 313 bp, 202 bp, 99 bp and 202 bp, 99 bp, respectively were identified. The results of present study for the three genotypes are in concurrence with Dai *et al.* (2009), Ghasemi and Ghorbani (2014) and Kadam *et al.*

(2015). Genotypic frequencies were 0.38, 0.54 and 0.08 for AA, AB and BB genotype while gene frequency of A allele was 0.65 and B allele was 0.35. Kadam (2015) found the gene frequency for A and B allele to be 0.702 and 0.307, respectively while the genotype frequency for BB and AB genotypes were observed to be 0.404 and 0.595, respectively. Ghasemi and Ghorbani (2014) observed the A allele was more frequent than B allele (0.675 vs 0.325) which is in relation with result of the present study. However, Dai *et al.* (2011) reported that bulls with B allele were more frequent than A and C alleles.

Co-relation of FSH beta Subunit Gene with Testicular and Seminal Attributes

Testicular Parameters

The scrotal circumference, testicular length, testicular width and testicular thickness (mean $\pm$ S.E) in BB, AB and AA genotypes are depicted in Table 3.

Table 3: Testicular parameters (mean $\pm$ S.E) of BB, AB and AA genotypes in H.F and Jersey crossbred bulls

S. No.	Breed	Genotype	SC	TL	TW	TT
1	HF	BB	33.5 $\pm$ 1.89	15.3 $\pm$ 0.59	6.45 $\pm$ 0.35	6.53 $\pm$ 0.22
		AB	33.84 $\pm$ 0.38	15.36 $\pm$ 0.16	6.46 $\pm$ 0.07	6.81 $\pm$ 0.07
		AA	34.88 $\pm$ 0.52	15.41 $\pm$ 0.31	6.29 $\pm$ 0.05	6.74 $\pm$ 0.09
2	JY	BB	31 $\pm$ 2	15.63 $\pm$ 0.12	6.45 $\pm$ 0.09	6.37 $\pm$ 0.12
		AB	32.46 $\pm$ 0.55	16.58 $\pm$ 0.3	7 $\pm$ 0.14	6.80 $\pm$ 0.09
		AA	32.45 $\pm$ 0.67	16.53 $\pm$ 0.35	6.77 $\pm$ 0.13	6.58 $\pm$ 0.13

In the present study, all the testicular parameters are statistically non significant ($P < 0.05$) between the three genotypes. The results of the present study are in correlation with Kadam *et al.* (2015) observed 40.174 $\pm$ 0.88 and 39.148 $\pm$ 0.636 cm average scrotal circumference in BB and AB genotypes, respectively. There was no statistically difference ($P < 0.01$) among the scrotal circumference among BB and AB genotypes. The mean age, mean body weight, scrotal volume and scrotal weight were statistically non significant among the genotypes.

Seminal Attributes

The semen volume, sperm concentration, initial motility, post thaw motility, HOST, ASR and AIT(mean $\pm$ S.E) in BB, AB and AA genotypes are depicted in Table 4. In the present study, all the seminal attributes differed non significantly ($P < 0.05$) between the BB, AB and AA genotypes. The results of present study for semen volume (4.181 $\pm$ 0.288 and 4.996 $\pm$ 0.185 ml), sperm concentration (1119.779 $\pm$ 76.86 and 1041.212 $\pm$ 44.47 millions/ml), initial motility (66.82 $\pm$ 1.301 and 67.88 $\pm$ 0.655 %), post thaw motility(50.47 $\pm$ 0.15 and 50.40 $\pm$ 0.11), acrosome integrity (84.504 $\pm$ 0.504 and 84.95 $\pm$ 0.321 %) and per cent intact acrosome (77.89 $\pm$ 0.405 and 78.77 $\pm$ 0.148) are in accordance with Kadam *et al.* (2015) who reported non significant difference for these seminal attributes between BB and AB genotypes, respectively.

Table 4: Seminal attributes (mean $\pm$ S.E) of BB, AB and AA genotypes in H.F and Jersey crossbred bulls

S. No.	Breed	Genotype	Vol.	Conc.	IM	PTM	HOST	ASR	AIT
1	HF	BB	8.96 $\pm$ 0.97	876.54 $\pm$ 77.96	70 $\pm$ 0	57.5 $\pm$ 0.83	42.84 $\pm$ 0.83	12.09 $\pm$ 2.08	71.165 $\pm$ 1.33
		AB	7.10 $\pm$ 0.6	899.76 $\pm$ 34.5	70.00 $\pm$ 0	58.89 $\pm$ 0.18	43.08 $\pm$ 0.26	10.69 $\pm$ 0.28	71.37 $\pm$ 0.15
		AA	7.22 $\pm$ 0.91	968.10 $\pm$ 43.67	70.00 $\pm$ 0	58.85 $\pm$ 0.41	42.90 $\pm$ 0.22	11.25 $\pm$ 0.39	71.42 $\pm$ 0.22
2	JY	BB	5.34 $\pm$ 1.11	992.80 $\pm$ 25.92	70.42 $\pm$ 0.41	50.42 $\pm$ 0.41	42.59 $\pm$ 0.41	9.58 $\pm$ 1.25	70.09 $\pm$ 0.41
		AB	6.05 $\pm$ 0.46	1186.41 $\pm$ 99.54	71.60 $\pm$ 0.74	51.46 $\pm$ 0.43	42.50 $\pm$ 0.21	11.29 $\pm$ 0.29	71.36 $\pm$ 0.21
		AA	5.51 $\pm$ 0.32	1035.65 $\pm$ 94.1	71.06 $\pm$ 0.66	52.20 $\pm$ 0.69	42.91 $\pm$ 0.39	10.65 $\pm$ 0.55	71.29 $\pm$ 0.16

However, hypo osmotic swelling test (60.02 ± 0.355 and $61.44 \pm 0.261\%$) and abnormal sperm major defect (3.27 ± 0.646 and 3.044 ± 0.158) were significant at ($p < 0.01$) in BB and AB genotypes in HF bulls. The results of present study are not in agreement with Dai *et al.*, (2009) reported bulls with mutation in exon 3 gene of FSH β exhibited a significantly lower sperm concentration in fresh semen and a lower percentage of acrosome integrity in both fresh and frozen semen ($P < 0.05$), higher percentage of sperm deformity in fresh semen ($P < 0.05$), which was more pronounced in frozen semen ($P < 0.01$), and a significantly lower sperm motility in frozen semen ($P < 0.05$). For fertility evaluation, the non return rates obtained from 14,416 inseminations revealed that bulls with this genotype showed significantly lower non return rates ($P < 0.05$). Ghasemi and Ghorbani (2014) reported significant association of AA and AB genotype on total sperm, fresh semen motility and number of produced pay out but there was non significant association with semen volume, concentration, post thaw motility and sperm in each milt ejaculation in 83 bulls.

Davis *et al.* (2012) reported percent motile, progressive sperm and rapid sperm were statistically significant ($P < 0.01$) while per cent major, minor and total abnormalities in sperm were non significant in GG and GA genotypes of SNP 485 A for FSH beta subunit in Angus and Balancer bulls. Ishak *et al.* (2009) reported that Bali cattle was monomorphic and the other cattle's in study i.e. Simmental, Limousin, Brahman were polymorphic for FSH beta subunit gene polymorphism with sperm quality traits in 470 samples and the higher incidence of percentage of sperm abnormalities in Simmental, Limousin, Brahman compared to Bali were reported.

Conclusion

In the present study, three different genotypes BB, AB and AA having amplification size 313 bp; 313 bp, 202 bp, 99 bp and 202 bp, 99 bp, respectively were identified and which has non significant ($P < 0.05$) effect on testicular parameters as well as seminal attributes in H.F and Jersey crossbred bulls.

References

1. Bernard DJ, Fortin J, Wang Y and Lamba P. 2010. Mechanisms of FSH synthesis: what we know, what we don't, and why you should care. Science Direct. 93:2465-2485.

2. Dai L, Zao YM, Zang GL, Zhao RF, Jiang H and Ma TH. 2011. Molecular cloning and sequence analysis of follicle-stimulating hormone beta polypeptide precursor cDNA from the bovine pituitary gland. *Gen. Mol. Res.* 3 : 1504-1513.
3. Dai L, Zhihui Z, Ruifeng Z, Shuqi X, Hao J, Xupeng Y, XichunLi, Yan G, Jifeng L and Jiabao Z. 2009. Effects of novel single nucleotide polymorphisms of the FSH beta-subunit gene on semen quality and fertility in bulls. *Animal Reproduction Science.* 114(1-3):14-22.
4. Davis AJ, Kreider DL, Rosenkrans CF, Powell JG, Rorie RW, Looper ML, Rowe MP, Williams CL, Page RJ, Lester TD and Woolley JB. 2012. Single nucleotide polymorphisms of the follicle-stimulating hormone- β gene and effects on semen quality. *Arkansas Animal Science Department Report.* 39-42.
5. Ghasemi MV and Ghorbani A. 2014. Association of Bovine Follicular Stimulating Hormone (FSH β) Gene Polymorphism with Reproduction Traits in Iranian Holstein Bulls. *Iranian Journal of Applied Animal Science.* 4(2):275-280.
6. Hediger R, Johson SE, and Hetzel DJS. 1991. Localization of the P-subunit of follicle stimulating hormone in cattle and sheep by in situ hybridization. *Anim. Gen.* 22:237-244.
7. Ishak ABL, Sumantri C, Noor RR, and Arifiantini I. 2009. Identification of polymorphism of FSH beta-subunit gene as sperm quality marker in Bali cattle using PCR-RFLP. *J. Indonesian Trop. Anim. Agric.* 36(4):222-227.
8. Kadam VK. 2015. Correlation of FSH beta subunit gene polymorphism with semen quality traits in HF crossbred bulls, M.V.Sc. thesis submitted to MAFSU, Nagpur.
9. Kastelic JP and Coulter GH. 1999. Scrotal / Testicular Thermoregulation in the bull. *Ann. Rev. Biomed. Sci.* 1:87-101.
10. Lin CL, Ponsuksili S., Tholen E, Jennen DG, Schellander K and Wimmers K. 2006. Candidate gene markers for sperm quality and fertility of boar. *Anim. Reprod. Sci.* 92:349-363.
11. Liu X Z, Wang JL, Zhang Y, Huang J, Qi C, Li J, Zhong J, Yang G and Wang C. 2012. Effects of DraI, StyI, and MspI polymorphisms and haplotypic combinations of the transferrin (Tf) gene on the sperm quality of Chinese Holstein bulls. *Afric. J. Micro. Res.* 6 : 594-602.
12. Ohta T, Miyake H, Miura C and Miura T. 2007. Follicle-Stimulating Hormone Induces Spermatogenesis Mediated by Androgen Production in Japanese Eel, *Anguilla japonica*. *Biol. Reprod.* 77(6):970-977.
13. Sairam MR and Hanumanthappa K. 2001. The Role of Follicle-Stimulating Hormone in Spermatogenesis. *32(6):601-608.*
14. Samper, JC and Plough T. 2010. Techniques for the Insemination of Low Doses of Stallion Sperm. *Reprod. Dom. Anim.* 45 (2) : 35-39.
15. Ulloa-Aguirre, Timossi C and Mendez JP. 1995 Is there any physiological role for Gonadotrophin oligosaccharide heterogeneity in humans. *Human Reproduction.* 16(4):599-604.
16. Vincent L, Evans G, Short TH, South wood OI., Plastow G S, Tuggle C K and Rothschild MF. 1998. The prolactin receptor gene is associated with increased litter size in pigs. In: *Proceedings of the 6th World Congress on Genet. Appl. Live. Prod.* 27:15-18.
17. Xiaopeng AN, Dan H, Jin-Xin H, Guang L, Ya-Na Wand Ling L. 2010. Polymorphism of exon 2 of FSH β gene and its relationship with reproduction performance in two goat breeds. *Agri. Sci.* 6: 889-886.
18. Yaofeng Z, Ning L, Xiao L, Gengsheng C, Yizhen C, Zhang S, Chen Y, Changxin W, Zhang J and Shiquan S. 1998. FSHB subunit gene is associated with major gene controlling litter size in commercial pig breeds. *Sci.* 41(6): 664-668.