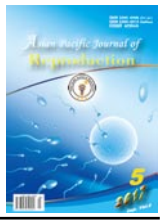
Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net



doi: 10.4103/2305-0500.215928

Genetic characterization of FSH beta-subunit gene and its association with buffalo fertility

Ahmed S.A. Sosa[1], Karima Gh M. Mahmoud[1]✉, Mohamed M.M. Kandiel[2], Hazem A.A. Eldebaky[1], Mahmoud F. Nawito[1], Mahmoud E.A. Abou El-Roos[2]

[1]*Department of Animal Reproduction & A.I, National Research Centre, Dokki, Tahrir Street, 12622 Giza, Egypt*

[2]*Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, Kaliobia, Egypt*

ARTICLE INFO

Article history:
Received 10 June 2017
Revision 30 June 2017
Accepted 10 July 2017
Available online 1 September 2017

Keywords:
Buffalo
FSH beta-subunit gene
Nucleotide sequences
SSCP analysis

ABSTRACT

Objective: To study genetic variation in buffalo follicle stimulating hormone beta-subunit (*FSHB*) gene and its association with fertility. **Methods:** In this experimental study, blood samples were collected by standard methods using EDTA anticoagulant and transrectal ultrasound examination was conducted on fertile (n=74) and infertile buffaloes with a history of anestrum (n=30) or repeat breeding (n=12). The genomic DNA was extracted for PCR followed by single strand conformation polymorphism analysis. DNA sequencing was performed for the determination of single nucleotide polymorphism of *FSHB* gene. **Results:** The study results showed that there was genetic polymorphism with two different single strand conformation polymorphism patterns, AA and AB. The former pattern was associated with fertility in Egyptian buffaloes. Pair wise alignment of the two patterns sequences revealed that *FSHB* pattern II (AB) has C nucleotide insertion as SNP at the site of 208 bp of sequenced fragment. **Conclusions:** *FSHB* is polymorphic in the infertile Egyptian buffaloes, suggesting its practicability as a candidate marker for female fertility.

1. Introduction

Follicle stimulating hormone (FSH) is secreted by anterior pituitary gland under control of the hypothalamus. This hormone is essential for regulation of reproductive processes such as gametogenesis and follicular growth[1,2]. Like other members of the pituitary glycoprotein hormones as luteinizing and thyroid stimulating hormone, FSH is heterodimer containing two subunits, a common alpha and a hormone-specific beta[3]. Although both FSH subunits participate in the binding to FSH receptor, the beta-subunit dictates its binding specificity[4].

Bovine FSH beta-subunit comprises one non-coding and two translated exons that encode 129-amino acid preprotein had important function in reproductive performance. In the bovine dbSNP; nine mutations had been reported including four mutations in the 5-upstream regulation region (5-URR), three in intron two, and two in exon three[5]. Through bioinformatics analysis, Dai *et al*.[6] reported that the mutations in 5'-upstream region possibly altered the gene transcription for protein and consequently lowered the FSH concentrations in bulls with such mutations.

In bovine, Dai *et al*.[6] and Dai *et al*.[7] studied the influence of polymorphism FSH beta-subunit (*FSHB*) gene on reproduction and quality of sperm. They recorded 9 SNPs mutations; four in

✉Corresponding author: Karima Gh M. Mahmoud, Department of Animal Reproduction & A.I, National Research Centre, Dokki, Tahrir Street, 12622 Giza, Egypt.
Tel: 002 01001827716
Fax: 002 02 33370931
E-mail: karimamahmoud@yahoo.com.
Foundation project: This study was funded by project (10060116) at National Research Centre, Egypt.

How to cite this article: Ahmed S.A. Sosa, Karima Gh M. Mahmoud, Mohamed M.M. Kandiel, Hazem A.A. Eldebaky, Mahmoud F. Nawito, Mahmoud E.A. Abou El-Roos. Genetic characterization of FSH beta-subunit gene and its association with buffalo fertility. Asian Pac J Reprod 2017; 6(5): 193-196.

promoter section ('5-URR), three in intron 2, and two in exon 3. The genetic variation in *FSHB* gene in exon 3 significantly affected the quality of frozen and fresh semen. The AA and AB genotypes showed better semen quality as higher sperm concentration and lower sperm deformity than BC genotype[6]. Also, Ishak *et al*.[8] associated the genetic variation of *FSHB* gene with traits of semen quality. Moreover, some authors reported the association of *FSHB* genes with litter size[9] and sperm quality[10] in pigs.

In buffalo, there is no literature exploring the genotyping of *FSHB* gene compared with its receptor which is monomorphic in Egyptian buffalo[11]. Therefore, the present work aimed to study the polymorphism of *FSHB* gene and its association with infertility in female Egyptian buffalo.

2. Materials and methods

2.1. Ethical approval

The permission was obtained from Egyptian Committee of Ethics at National Research Center.

2.2. Animals

In this experimental study, a total number of 116 females Egyptian buffaloes belonged to Meet Kenana village and the farm of Faculty of Agriculture, Menofia University were investigated during the year of 2013 and categorized as in Table 1 with case history of anestrum and repeat breeder. For fertility confirmation, rectal and ultrasonographic examinations were carried out once for three successive weeks to define the animal reproductive status.

Table 1
Area and number of animals investigated.

Animal		Meet Kenana village	Faculty of Agricuture, Menofia University	Total
Fertile		59	15	74
Infertile	Anestrum	27	3	30
	Repeat breeder	10	2	12
Total number		96	20	116

2.3. Blood collection and DNA extraction

Blood samples were collected from all buffaloes by standard methods into vaccum tubes with EDTA anticoagulant. DNA was extracted from blood, according to the instructions of the QIAGEN DNA blood kit.

2.4. PCR and DNA amplification

The primers used for 270-bp amplification of *FSHB* gene were reported by Kim *et al*.[5] with nucleotide sequence (F: CAGCTGATGGCATGTTTATCCT, R: CTCTTTGACTGCCGTGTT).

PCR reactions occurred in 50 μL volume, containing 5.00 μL buffer 10 ×, 1 μL 2.50 mM (dNTPs mixture), 0.25 μL primer, 3.00 μL 25 mM ($MgCl_2$), 0.3 μL Taq polymerase (5 U/μL), 35.20 μL nuclease-free water and 5.00 μL DNA sample. The PCR condition was: one cycle at 95 ℃ for 5 min, and 35 cycles of the sequence: 94 ℃ for 30 s, 56 ℃ for 30 s and 72 ℃ for 30 s. After completion of reaction, PCR products subjected to electrophores was in 2% agarose, TBE 1× buffer with ethidium bromide for 2 h at 60 V. Bands were visualized and photographed under ultraviolet Trans-illumination and in Gel-Doc System (Bio-Rad). The PCR product size was compared with the 100 bp DNA Ladder.

2.5. Single strand conformation polymorphism (SSCP)

The technique was used to identify the mutations in the amplified segment. About 7 μL of PCR products were mixed with 8 μL of denaturing solution (98% formamide, 20 mM EDTA, pH 8.0, 0.05% bromophenol blue, 0.05% xylene cyanol). The samples were denatured by heating for 8 min at 95 ℃, afterwards they were chilled on ice for 8 min and loaded in 1× TBE buffer on to 12% polyacrylamide gel [29:1 acrilamyde:bisacrylamide, 10 mL TBE buffer (Trisbase, Boric acid, Na_2EDTA), 2.5 mL glycerol, 17.5 mL deionized water, 400 μL ammonium per sulfate and 40 μL of TEMED]. Electrophoresis was performed at 4 ℃, 160 V for 14–16 h. Silver staining was used to visualize DNA-fragments on polyacrylamide gels according to Sanguinetti *et al*.[12] with some modification[13].

2.6. Sequence analysis

The PCR product was purified using purification kit (QIAGEN). The PCR products giving unique SSCP band patterns were analyzed by direct sequencing in Macrogen Incorporation (South Korea). Sequence data were analyzed and aligned using NCBI/BLAST/ blastn suite. Sequenced data were further analyzed by BioEdit software for searching single nucleotide polymorphism.

2.7. Statistical analysis

The frequencies of *FSHB* gene patterns distribution among the fertile and infertile animals as well as between various reproductive disorders in infertile animals were analyzed by *Chi*-square test using SPSS program (Ver. 16). The significance level was set at $P<0.05$.

3. Results

In the present work, PCR-SSCP marker was used to determine

the genetic polymorphism of *FSHB* gene in buffaloes. The primers of *FSHB* gene used in our study were flanked 270-bp fragment (Figure 1). Applying SSCP technique in polyacrylamide gel, the gene was polymorphic with two SSCP patterns (Figure 2). Pattern I named (AA) consisted of four bands while pattern II which was named (AB) consisted of two bands. The DNA sequence of 218 bp of buffaloes *FSHB* gene out of the 270 bp was detected and sequence alignment with published sequence of bovine *FSHB* gene, complete cds (accession number: Sequence ID: M83753.1) was performed by BLAST. Data of 218 bp possess similarties at 98% (Figure 3). Nucleotide sequence pair wise alignment of the two patterns sequences (Figure 4) revealed that *FSHB* pattern II (AB) had C nucleotide insertion as SNP at the site of 208 bp of sequenced fragment.

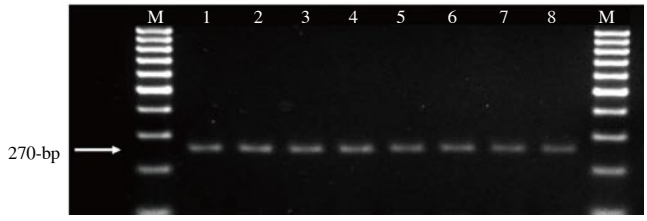


Figure 1. Agarose gel stained with ethidium bromide for *FSHB* gene showing M:100-bp ladder. Lanes 1-8: 270-bp PCR product.

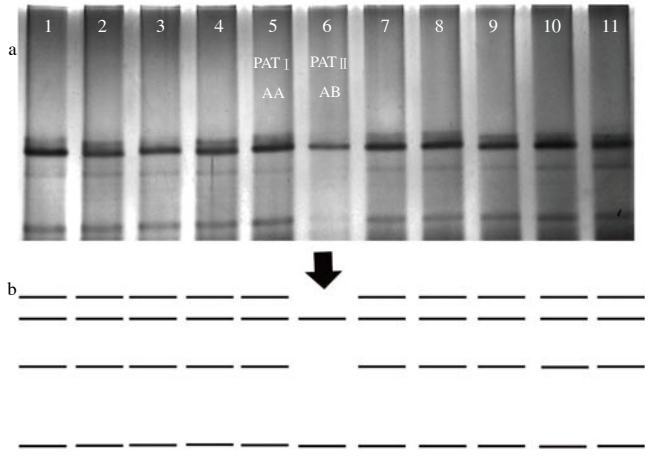


Figure 2. SSCP results.
a, two different SSCP patterns of *FSHB* gene in Egyptian buffalo on 12% silver stained-polyacrylamide gel. Lanes: 1-2-3-4-5-6-7-8-9-10-11: pattern Ⅰ (AA). Lanes: 6: Pattern Ⅱ (AB). b, diagram showing SSCP banding patterns of 270 bp fragment of *FSHB* gene of Egyptian buffalo.

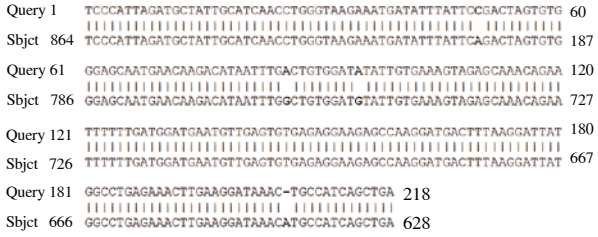


Figure 3. Sequence analysis of 218 segment of Egyptian buffalo *FSHB* compared to bovine follicle stimulating hormone-beta subunit gene, complete cds (accession number: sequence ID: M83753.1 BOVFSHBA).

Figure 4. Pairwise alignment of 2 different patterns of *FSHB* gene of Egyptian buffaloes by BioEdit showed C nucleotide insertion as SNP in the Pattern Ⅱ.

The frequencies of pattern I (AA) and II (AB) were 55.17% and 44.82%, respectively. The phenotype information based on ultrasound investigation showed that the incidence of fertile and infertile animals was 63.79% and 36.20%, respectively (Figure 5).

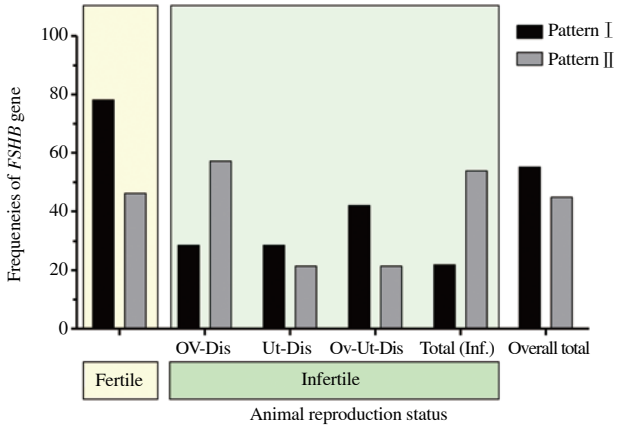


Figure 5. Patterns and frequencies of *FSHB* gene in fertile and infertile female buffaloes.
Ov-Dis: Ovarian disorders, Ut-Dis: Uterine disorders, Ov-Ut-Dis: Ovarian and uterine disorders. Inf.: infertile. Pattern Ⅰ and Ⅱ signified AA and AB patterns of FSHB gene.

The frequency of pattern I among fertile (n=50) and infertile animals (n=14) was 78.10% and 21.87%, respectively. The latter represented animals with ovarian (28.57%), uterine disorders (28.57%) and both ovarian and uterine disorders (42.00%). Nevertheless, the occurrence of pattern II among fertile (n=24) and infertile (n=28) animals was 46.15% and 53.84%, respectively. The later represented animals with abnormal ovarian (57.14%), uterine (21.42%) conditions and both abnormal ovarian and uterine conditions (21.42%). Analysis of the frequency *FSHB* patterns in the studied animals with *chi*-square test verified significant (χ^2=12.70, P<0.005) difference in the pattern distribution between fertile and infertile animals. Nevertheless, the frequency of the patterns did not vary significantly in infertile animals with various reproductive disorders (χ^2=3.30, P=0.19).

The results indicated that *FSHB* might correctly predict the phenotype of fertility with 78.10% accuracy for pattern I (AA) and 46.15% accuracy for pattern II (AB).

4. Discussion

In the current study, follicle stimulating hormone Beta-subunit gene in Egyptian buffalo was recorded to be polymorphic with two SSCP patterns. Pattern I (AA) comprised of four bands, while pattern II (AB) consisted of two bands. For authors' knowledge, this is the first time to study *FSHB* gene in Egyptian buffaloes. Ishak *et al*.[8] stated that the cattle *FSHB* gene was monomorphic in Bali breed, in contrast it was polymorphic with PCR-RFLP in other breeds of Brahman, Simmental and Limmous. It may attributed to the difference in breeds and type of genetic marker.

The phenotype information based on ultrasound investigation showed that the incidence of fertile and infertile animals was 78.10% and 21.87% for pattern I (AA) and 46.15% and 53.84% for pattern II (AB), respectively. This meant that pattern AA was associated with fertile animals and pattern AB was associated with infertile animals (ovarian disorder). Nucleotide sequence revealed that *FSHB* pattern II (AB) showed C nucleotide insertion as SNP at the site of 208 bp of sequenced fragment. In this respect, Dai *et al*.[6] recorded novel SNPs in 5'-URR of bovine *FSHB*. Bulls which had these mutations had SNPs in the coding region of exon 3. These mutations may produce a change in the FSH levels that related to semen quality and fertility traits. In our work, changes in nucleotide sequences of *FSHR* gene in pattern II may alter its expression to protein, which lead to low FSH hormone and consequently the follicles fail to develop as in buffalo with ovarian inactivity. Similarly, Yang *et al*.[14] reported the superovulation response represented by increasing the number of ova was associated with mutation in 5' upstream region of *FSHR* gene. Moreover, in Iranian sheep, mutant alleles of *FSHB* gene can improve considerably mean of litter size than the wild ones[15]. In human, mutation in the beta-subunit of FSH was associated with primary amenorrhoea and infertility[16]. In goats, Nikbin *et al*.[17] found three SNPs of FSH β 3 had significant effect on libido, motility, and viability traits of semen. The genetic variation in *FSHB* revealed the importance of *GnRHR* gene as a candidate marker for fertility in Egyptian buffaloes with its mutation is related to ovarian inactivity[18].

In conclusion, *FSHB* gene in the Egyptian buffaloes is polymorphic and can be used as candidate markers for fertility in buffaloes.

Conflict of interest statement

All authors have no competing interests.

Acknowledgements

KGh M M, MMMK and MFN designed the study, ASAS and MMMKcarried out ultrasonography of genital system for all animals, ASAS and HAAE carried out PCR and genotyping whereas KGhM M, MMM K and MEAA performed data analysis.

References

[1] Ulloa-Aguirre A, Midgley AR, Beitins IZ, Padmanabhan V. Follicle-stimulating isohormones: Characterization and physiological relevance. *Endocr Rev* 1995; **16**: 765–787.

[2] Howles CM. Role of LH and FSH in ovarian function. *Mol Cell Endocrinol* 2000; **161**: 25–30.

[3] Pierce JG, Parsons TF. Glycoprotein hormones: Structure and function. *Annu Rev Biochem* 1981; **50**: 464–495.

[4] Fan QR, Hendrickson WA. Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* 2005; **433**: 269–277.

[5] Kim KE, Gordon DF, Maurer RA. Nucleotide sequence of the bovine gene for follicle-stimulating hormone beta-subunit. *DNA* 1988; **7**: 227–233.

[6] Dai L, Zhao Z, Zhao R, Xiao S, Jiang H, Yue X, et al. Effects of novel single nucleotide polymorphisms of the FSH beta subunit gene on semen quality and fertility in bulls. *Anim Reprod Sci* 2009; **114**: 14–22.

[7] Dai LM, Zao GL, Zang RF, Zhao H, Jiang TH, Ma Y, et al. Molecular cloning and sequence analysis of follicle-stimulating hormone beta polypeptide precursor cDNA from the bovine pituitary gland. *Gen Mol Res* 2011; **3**: 1504–1513.

[8] Ishak, ABL, Sumantri C, Noor RR, Arifiantini I. Identification of polymorphism of FSH beta-subunit gene as sperm quality marker in Bali cattle using PCR-RFLP. *J Indonesian Trop Anim Agric* 2011; **36**: 221–227.

[9] Liu JJ, Ran XQ, Li S, Feng Y, Wang JF. Polymorphism in the first intron of follicle stimulating hormone beta gene in three Chinese pig breeds and two European pig breeds. *Anim Rep Sci* 2009; **111**: 369–375.

[10]Zhang CY, Wu CJ, Zeng WB, Huang KK, Li X, Feng JH, et al. Polymorphism in exon 3 of follicle stimulating hormone beta (*FSHB*) subunit gene and its association with litter traits and superovulation in the goat. *Small Rum Res* 2011; **96**: 53–57.

[11]Sosa ASA, Mahmoud KGhM, Eldebaky HAA, Kandiel MMM, Abou El-Roos MEA, Nawito MF. Genotyping of follicle stimulating hormone receptor gene in fertile and infertile buffalo. *Glob Vet* 2015; **15**: 163–168.

[12]Sanguinetti CJ, Dias Neto E, Simpson AJG. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 1994; **17**: 915–919.

[13]Benbouza H, Jacquemin JM, Baudoin JP, Mergeai G. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. *Biotechnol Agron Soc Environ* 2006; **10**: 77–81.

[14]Yang WC, Li SJ, Tang KQ, Hua GH, Zahang CY, Yu JN, et al. Polymorphisms in the 5' upstream region of the FSH receptpore gene, and their association with superovulation traits in Chinese Holstein cows. *Anim Reprod Sci* 2009; **119**: 172–177.

[15]Nazifi N, Rahimi-Mianji G, Ansari-Pirsaraii Z. Polymorphism in FSH β and *FSHR* genes and their relationship with productive and reproductive performance in Iran black, Arman and Baluchi sheep breeds. Iran. *J Appli Anim Sci* 2015; **5**: 361–368.

[16]Matthews CH, Borgato S, Beck-Peccoz P, Adams M, Tone Y, Gambino G, et al. Primary amenorrhoea and infertility due to a mutation in the beta-subunit of follicle-stimulating hormone. *Nat Genet* 1993; **5**: 83–86.

[17]Nikbin S, Panandam JM, Yaakub H, Murugaiyah M. Association of novel SNPs in gonadotropin genes with sperm quality traits of Boer goats and Boer crosses. *J Appl Anim Res* 2017; DOI: 10.1080/09712119.2017.1336441.

[18]Sosa ASA, Mahmoud KGhM, Eldebaky HAA, Kandiel MMM, Abou El-Roos MEA, Nawito MF. Single nucleotide polymorphisms of GnRHR gene and its relationship with reproductive performance in Egyptian buffaloes. *Egyptian J Vet Sci* 2016; **47**(1): 41–50.