FSHR Gene Polymorphisms & Protein Structure Changes Of Cattle Bred In Iraq

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Abstract: The present study was undertaken to characterize genetic diversity of the follicle-stimulating hormone receptor (FSHR) gene in 35 cows (15 local 15 Holstein and 5 Crosses) in Iraq. The aim of this study was to identify the polymorphism in the exon 10 region of the FSHR gene. Polymorphism of the FSHR gene was detected by DNA sequencing methods. The results showed the presence of 4, 3 and 3 polymorphic sites leading to the construction of 5, 5 and 3 different haplotypes for Holstein, local and crosses respectively. Haplotype diversity were 0.743, 0.695 and 0.800 respectively. While nucleotide diversity was 0.0056, 0.0051 and 0.0056 respectively. Five single-nucleotide polymorphism (SNP) loci of the FSHR gene were detected, namely C2037G (C/G), T2071C (T/C), A2119C (A/C), G2128C (G/C) and T2143C (T/C). Four different structures in protein were identified. Those protein structures were the result of the changes of threonine to serine, tryptophan to arginine, threonine to proline and alanine to proline. FSHR gene in cattle bred in Iraq showed five mutations which have changed the three-dimensional protein structure. Local breeds differ from Holstein by two haplotypes and shared three others.

Index Terms: FSHR gene, Iraqi cattle, Protein 3D, single nucleotide polymorphism.

1. INTRODUCTION

The follicle-stimulating hormone (FSH) is secreted from the anterior lobe of the pituitary gland and has an important reproductive function [1]. In the absence of adequate FSH, oocytes are unable to grow and mature and therefore no ovulation occurs [2]. FSH effect on target cell must be mediated by FSH receptor [3]. This function is governed by hypothalamus-gonad-pituitary and the main role of FSH is the proliferation and differentiation of granulose cells [4]. The FSHR gene is located on the short arm of chromosome 11 of cows and buffaloes, it consists of 10 exons and 11 introns. The first nine exons enclose the extracellular domain, whereas exon ten encloses the transmembrane domain and intracellular domains [5]. The length of the FSHR gene is about 54 kb of genomic DNA. The tenth exons with an extensions range of 69-1234 bp and the 11th introns ranging from 108 bp to 15 Kb. The amino acids encoded in these pieces are often rich in either leucine or isoleucine [6]. The FSHR protein is composed of 695 amino acids, including a signal peptide of 17 amino acids, leading to a mature protein of 678 amino acids with a molecular weight of 75kDa [7]. As many hormones and their receptors mainly affect the reproductive performance of farm animals, researchers have recently focused on genes that regulate hormones that play a role in reproduction, such as FSHR [8]. The present study aims to the discovery of the polymorphism and protein structure of the FSHR gene in cattle bred in Iraq.

2. Materials and methods

2.1. Animals and genomic DNA isolation

This study included the use of 35 cows (15 local 15 Holstein and 5 Crosses). Blood samples (10ml/cow) collected from jugular vein. Genomic DNA was extracted from whole blood using the gSYNC™ DNA Extraction Kit manufactured by the Taiwanese Geneaid Company. A fragment (306 bp, exon 10) of the FSHR gene in cattle was amplified by using the primer F: 5`-CTGCCTCCCTCAAGGTGCCCCTC-3` and R: 5`-AGTTCTTGGCTAAATGTCTTAGGGGG-3` [6]. The PCR amplifications were conducted in a 50 µl volume containing 6 µl genomic DNA, 25 µl of Master Mix, 4 µl each primer, 15 µl free water. The amplification conditions were as follows: initial denaturation at 95˚C for 2 min followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 58.5˚C for 0.5 min, and extension at 72˚C for 0.5 min, and then the final extension at 72 C for 5 min. The PCR results were using electrophoresis at 2% agarose-gel with the visualized by contact with ultraviolet light. For sequencing the PCR product was sent to Yang ling Tianrun Aoka Biotechnology Company, China.

2.2 Bioinformatics analysis

To design the three-dimensional structure of the follicle-stimulating hormone receptor, the RasMol 2.7.5.2 software and Phyre^2 v. 2.0 on the website were used [9]. The sequencing results of the FSHR gene were compared with accession No. XM_010855898 at the NCBI by BioEdit 7.0 software [10]. Haplotype diversity (HD) and nucleotide diversity (π) were analyzed using DnaSP v5. 10 software [11]. The haplotypes network was drawn using Network 5.0.0.0 software [12]. The phylogenetic tree was drawn by using the MEGA X software [13]. All tables and figures will be processed as images. You need to embed the images in the paper itself. Please don't send the images as separate files.

3. Results

The nucleotide sequences concerned in this study in exon 10 of the FSHR gene were registered for local, Holstein and cross cattle in the National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ) and the European Nucleotide Archive (ENA) under the following accession numbers (LC492963, LC492964, LC492965, LC492966, LC492967, LC492968, LC492969, LC492970, LC492971, LC492972).

3.1 GENETIC DIVERSITY

The results of the genetic diversity of the FSHR gene showed that the total number of sequences (N) was 35 and the total number of Haplotypes number (H) was 7 resulted in 5 polymorphisms number (NH) distributed to 3 local, 3 crosses

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and 4 Holstein. Crosses revealed the highest value of haplotype diversity (HD) (0.800), followed by the Holstein cattle (0.743) and the local breed (0.695). The results of the nucleotide diversity (π) of Holstein and crosses were identical (0.0056), followed by local cattle (0.0051) Table 1.

3.2 HAPLOTYPE NETWORK

A total number of haplotypes of the FSHR gene showed by different breeds were seven Fig.1. The first three (H1, H2, and H3) found in all breeds while the remaining four are divided into two for local breeds (H6 and H7) and two for Holstein cattle (H4 and H5). Each pair of haplotypes (H1, H3 or H2. H7 or H5. H6) differs from each other with a nitrogen base (232bp). The branches represented H4 and H5 of Holstein cattle differed from H1 by 251 and 184 bases respectively. Whereas, the haplotypes

TABLE 1

Genetic Diversity of FSHR Gene among Different Cattle Breeds

Breeds	Number of Sequences (N)	Haplotype Number (H)	Number of Polymorphisms (NH)	Haplotype Diversity (HD)	Nucleotide Diversity (π)
Local	15	5	3	0.695	0.0051
Crosses	5	3	3	0.800	0.0056
Holstein	15	5	4	0.743	0.0056
Whole population	35	7	**5**	0.699	0.0053

H6 and H7 represented the local cattle and differed from H2 by 150 and 232 bases respectively.

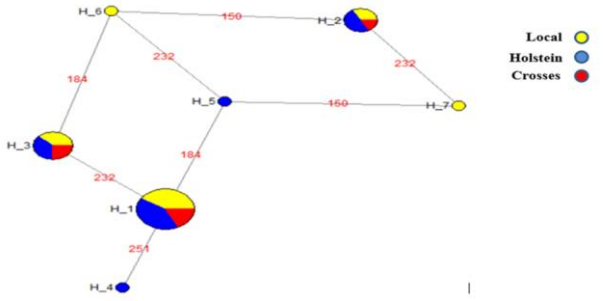

Fig. 1. *Haplotype network of FSHR gene among studied cattle*

SNPs; cytosine (C) to guanine (G), thymine (T) to cytosine (C), adenine (A) to cytosine (C), guanine (G) to cytosine (C) and thymine (T) to cytosine (C) in positions 2037, 2071, 2119, 2128 and 2143 respectively. The SNPs C2037G, T2071C, A2119C and G2128C substitution led to an amino acid mutation (T>S, W>R, T>P, and A>P respectively). While nucleotide change T2143C gave the same amino acid (N>N).

TABLE 2

Nucleotide and Amino acid changing for FSHR Gene at Different Cattle Breeds

SR. NO.	POSITION	NUCLEOTIDE CHANGE	CODON CHANGE	AMINO ACID CHANGE	CHANGE TYPE
1	2037	C > G	ACC > AGC	T > S	Nonsynonymous
2	2071	T > C	TGG > CGG	W > R	Nonsynonymous
3	2119	A > C	ACT > CCT	T > P	Nonsynonymous
4	2128	G > C	GCT > CCT	A > P	Nonsynonymous
5	2143	T > C	AAT > AAC	N > N	Synonymous

T: Threonine; S: Serine; W: Tryptophan; R: Arginine; P: Proline; A: Alanine; N: Asparagine

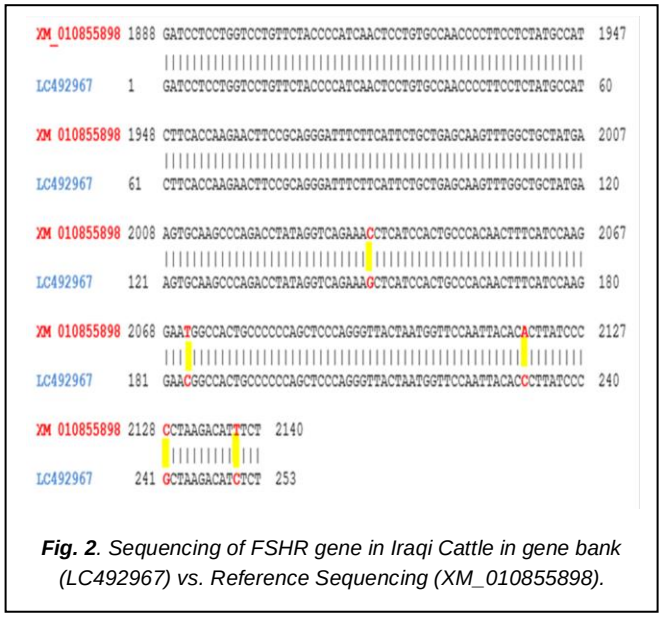

Fig. 2*. Sequencing of FSHR gene in Iraqi Cattle in gene bank (LC492967) vs. Reference Sequencing (XM_010855898).*

3.3 THREE-DIMENSIONAL PROTEIN STRUCTURE

A three-dimensional protein structure was drawn to locate the amino acid change for FSHR protein. The FSHR protein carries various functionally significant regions. Five single-nucleotide polymorphism (SNP) was found in the intracellular domain. The changes of threonine to serine, tryptophan to arginine, threonine to proline and alanine to proline gave rise to some changes in three-dimensional protein structure Fig. 2.

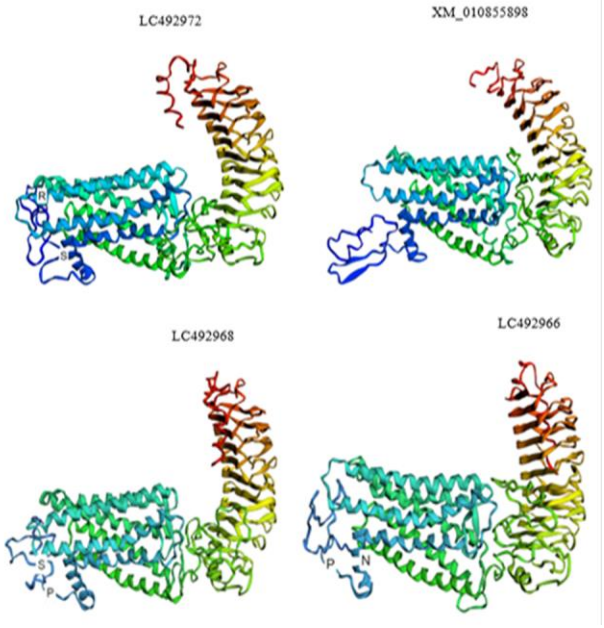

Fig. 3. *The changes of amino acids and resulting changes in FSHR protein structure in studying cattle.*

4. Discussion

The FSHR gene has previously been studied for its relationship with litter size [14]. superovulation traits [15]. and reproductive traits [16]. Furthermore, the identification of different polymorphic sites. Sequencing exon 10 of the FSHR gene showed the same nucleotide sequence of the corresponding region deposited in GenBank (NM_174061) and the polymorphism detected in this study is in agreement with [17]. Although there is little data on the relationship of FSHR with reproductive traits, it showed a positive relationship with fertility and ovulation in numerous breeds of cattle [18], [19]. This confirms that this gene can be considered as a marker for these traits [20]. Research has reported that there is a correlation between the genetic polymorphisms of this gene with the mechanism of ovulation in humans as it plays an important role in the development of ovaries and their response to the FSH hormone [20]. It was also suggested that this gene and its SNPs could be considered as markers for the development of semen quality in bulls used in artificial insemination [21]. Moreover, in the leading dairy cows, including Holstein, it was found that the total number of mature eggs in CC genotypes was higher than that produced by other genotypes [19]. This result leads to a significant increase in the number of embryos in the case of embryo transfer studies. In cattle, it became certain that allele C of the FSHR gene is positively correlated with the number of eggs obtained, thereby improving the quality of embryo transfer processes. Bioinformatics or in Silico technology was used to study the relationship between protein function and molecular structure, it can also be used to analyze the interaction between the hormone FSH and receptor FSHR [22], [23]. The study of [24] showed that differences in nucleotide sequences may lead to changes in the function and shape of the protein.

5. Conclusion

FSHR gene of cattle bred in Iraq showed five mutations at the sites 2037, 2071, 2119, 2128 and 2143. The first fourth mutations were nonsynonymous and new amino acids were recorded (T>S, W>R, T>P, and A>P respectively). These changes have risen some differences in three-dimensional protein structure. Local cattle differ than very well-known dairy breed (Holstein) by two haplotypes (H4 and H5 for Holstein and H6 and H7 for local). However, both share three haplotypes (H1, H2, and H3).

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