ISSN 1819-1878

Asian Journal of Animal Sciences

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OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2017.132.139

Research Article
Molecular Detection of Polymorphism of Heat Shock Protein 70 (*Hsp70*) in the Semen of Iraqi Holstein Bulls

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Abstract

Background and Objective: *Hsp70* is a molecular chaperone and cell preservation against heat shock, considered as the ideal biological marker for measuring heat stress in animals, its polymorphism may affect stress tolerance bulls' fertility. The study aimed to identify polymorphism of *hsp70* gene in Holstein bulls born in Iraq. **Materials and Methods:** The study was carried out during 1st November, 2015 to 31th January, 2016, 29 Holstein bulls born in Iraq, 2.5-3.5 years old, back to Artificial Insemination Center of Abou-Ghareeb Baghdad, Iraq, were used. Semen was collected by using the artificial vagina. DNA extracted then PCR amplified, sequencing, BLAST analysis and multiple sequence alignment were carried out. **Results:** The results showed three haplotypes, haplotype G1, haplotype G2 and haplotype G3 as a compare with the same gene in GenBank, they all showed silent mutations in different positions except haplotype G2 showed a missense mutation in position 1451 as well as silent mutations. **Conclusion:** It is concluded that the Holstein bulls born in Iraq have new polymorphism of *Hsp70* gene as a compare with same gene in GenBank, because of mutations.

Key words: Molecular detection, heat shock protein 70 polymorphism, Holstein bulls, heat stress

Received: March 15, 2017 Accepted: April 06, 2017 Published: April 15, 2017

Citation: Hassan Nima Habib, Amad Falah Hassan and Bassam yasein Khudaier, 2017. Molecular detection of polymorphism of heat shock protein 70 (*Hsp70*) in the semen of Iraqi Holstein bulls. Asian J. Anim. Sci., 11: 132-139.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heat shock protein 70 (*Hsp70*) is one of HSPs family and its size is 68-73 kDa[1]. It is encoded by a single exon of the *Hsp70* gene, the open reading frame to this gene is 1926 bp approximately. The protein comprises 641 amino acids[2]. The work of *Hsp70* is molecular chaperones and cell preservation against heat shock, able of denaturing proteins[3], it's one of the most important roles is prevent aggregation of the partially folded proteins remodel folding pathways and regulate activity of proteins[4]. Expressed in response to heat stress and environmental stress[5], *hsp70* gene was considered as the ideal biological marker for measuring heat stress in animals[6]. Polymorphism of *hsp70* gene explains the differences between individuals in the tolerance of stress conditions such as heat stress, polymorphism produced by genetic mutations which can be a useful to resist heat stress[7]. Nucleotide polymorphisms in the *hsp70* gene promoter region may affect stress tolerance and haplotypes of *Hsp70* were associate with bulls' fertility and heat tolerance[8,9], the difference in reproductive performance was also associated with polymorphism of *hsp70 gene*[10]. The *Hsp70* is available in spermatozoa of mature bulls[11]. Although the origin of the Holstein cattle is the cold regions (Europe), can be bred and preserve in tropical and sup-tropical environment like Iraq[12], *Hsp70* playing a pivotal function in environmental and heat stress adaptation[13-14], because it is playing important roles in essential cellular processes and also related to stress[15]. The main objective of this study was to identify the manipulation of genotype (molecular changing) to capable with harsh environment (high temperature). Therefore, polymorphisms of the *hsp70* gene of the Holstein bulls born in Iraq were identified. Since there have been no studies and the lack of information dealing with this subject, this study aimed to identify polymorphism of *Hsp70* gene in Holstein bulls which are born in Iraq.

MATERIALS AND METHODS

Animals and semen collection: The study was carried out during 1st November, 2015 to 31th January, 2016. Twenty nine Holstein bulls born in Iraq of known fertility, 2.5-3.5 years old, back to Artificial Insemination Center of Abou-Ghareeb Baghdad, Iraq were used in the present study. Semen was collected from all bulls by using the artificial vagina method.

DNA extraction: The DNA was extracted from semen samples using Chelex-100® (Sigma Aldrich, USA) as already described by Walsh *et al.*[16] and modified by Manuja *et al.*[17]. Concentration and purity of DNA was estimated by using Nano-drop system (Nano Drop thermo scientific 200, USA). To determine the best purity, with an estimate 260/280 ratio, which should be 1.8, that indicate the purity of the DNA[18].

PCR amplification: Amplifications were performed in 25 µL reactions (Table 1). According to First BASE Laboratories/Malaysia, primer was used to amplify conserved region within the HSP70 promotor, *Hsp70*-F: ATGGCGAAAAACATGGCTATCGGC, *hsp70*-R: CTAATCCACCTCCTCAATGGTGGGGCC, First BASE Laboratories/Malaysia, the PCR (A-2040-1 My Genie/Gradient Thermal Bioneer Korea) amplification cycling protocols of PCR amplification are summarized in Table 2. The PCR product was detected on 1.5% ethidium bromide stained agarose gel. PCR product size was 1926 bp (Fig. 1).

Sequencing: The PCR products purification, then sequenced in First BASE Laboratories/Malaysia. Nucleotide sequence alignments and comparisons were done using the Geneious version 10.1.3 software. BLAST analysis were carried out on website http://www.ncbi.nlm.nih.gov., Multiple Sequence Alignment were carried out on website http://www.ebi.ac.uk/Tools/msa/clustalo/ and compared with same gene in Gene Bank accession number AY662497.1.

RESULTS AND DISCUSSION

Results of sequencing and the multiple sequence alignment were compared with heat shock protein *Hsp70* gene of the Holstein bulls in the gene bank. Three new haplotypes were detected and classified as three groups.

First group, the haplotype G1, is the closest haplotype to heat shock protein *hsp70* gene in the Holstein bulls in the

Table 1: Components of PCR reactions

First BASE laboratories (2016)	
Components	Amount (µL)
Water, nuclease free	9.5
2X PCR master mix	12.5
Forward primer (10 µM)	1.0
Reverse primer (10 µM)	1.0
DNA template (75 ng)	1.0
Total	25.0

Table 2: Cycling protocol of PCR amplification

Cycle steps	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 min	1
Denaturation	94	30 sec	30
Annealing	61	30 sec	
Extension	72	2 min	
Final extension	72	10 min	1

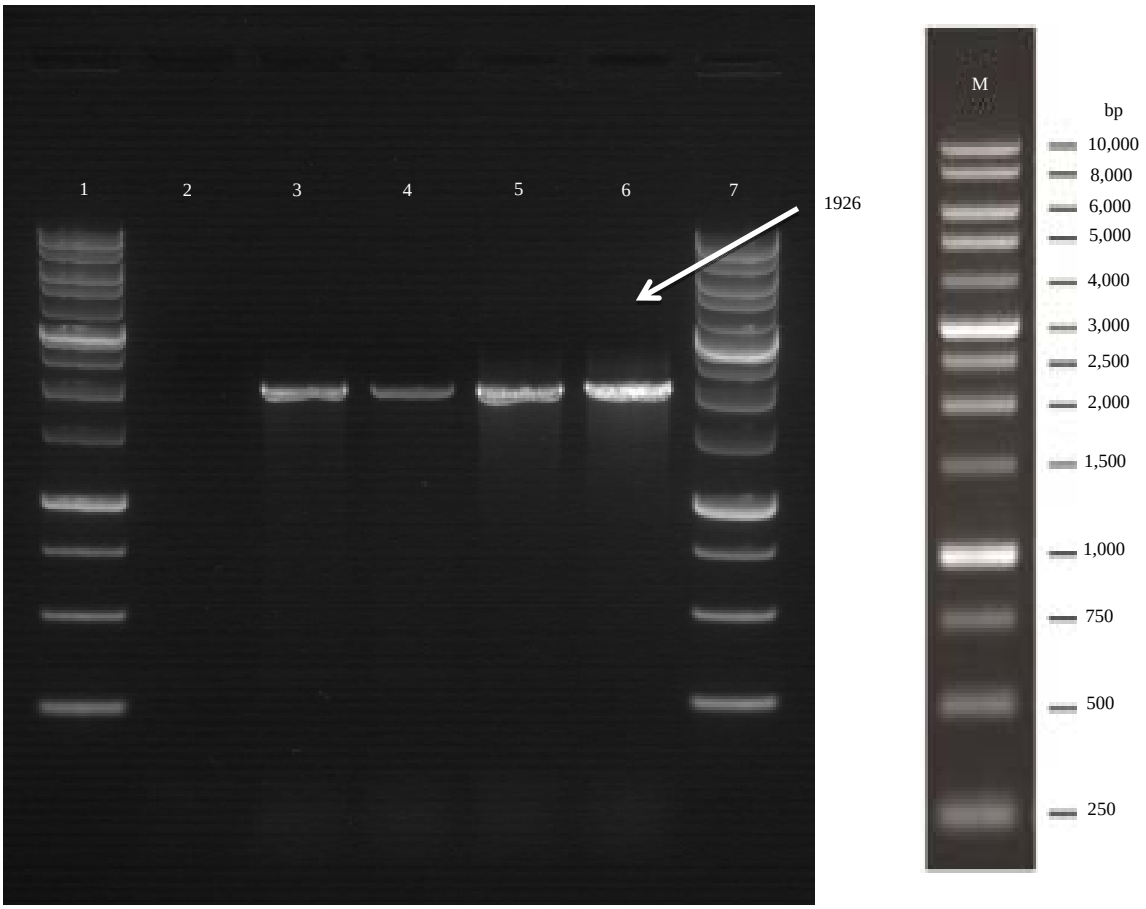

Fig. 1: Gel electrophoresis of PCR products of *Hsp70* gene

Sample 1 and 7: DNA marker, Sample 2: -ve is no-template control (water to replace DNA template), Sample 3-6: DNA template (*Hsp70* gene), A total 75 ng of DNA sample was used in one 25 µL PCR reaction. only 3 µL of PCR product was run on 1% TAE agarose of PCR product was run on 1% TAE agarose gel at 100 V, 60 min

gene bank. This haplotype was exposed to genetic mutations in nucleotides at positions, 6 (C<G), 174 (C<G), 282 (A<G) and 1339 (T<C). All mutations are silent that did not encode a new amino acid. Fifteen Bulls were found in this group.

Second group, the haplotype G2, in comparison with *Hsp70* gene in the Holstein bulls in the gene bank and first group. Nucleotides were substituted in eight different positions, 114 (G<A), 174 (C<G). 282 (A<G) ,1339 (T<C), 1451 (C<A), 1590 (A<G), 1695 (C<T) and 1719 (G<T). These genetic mutations are all silent except the mutation at the position 1451 (missense mutation). It encoded for new amino acid (Aspartic acid instead of alanine). The change nucleotide cytosine (C) instead of adenine (A) led to a change code GCC to GAC which encode a new amino acid[19]. Six bulls were found in this group.

Third group, the haplotypeG3, in comparison with *Hsp70* gene in the Holstein bulls in the gene bank and other groups, in this group nucleotides were substituted for each position 114 (G<A), 174 (C<G), 282 (A<G), 1339 (T<C), 1590 (A<G), 1695 (C<T) and 1719 (G<T). All mutations are silent. Eight bulls were found in this group.

The results of the Multiple Sequence Alignment (MSA) of *hsp70* gene in the bulls (Fig. 2) showed that the convergence ratio of G1, G2 and G3 haplotypes were 99.73, 99.51 and 99.56%, respectively.

These results showed silent mutations in different positions of *hsp70* gene, although it does not encode a new amino acid[20] but it may influence the relevant protein through change in transcription and may impact the precision or efficiency of splicing of mRNA or control of transcripts[21,22]. On the other hand the missense mutation that occurred in haplotype G2 may change protein properties due to differences between amino acids, aspartic and alanine[23].

These mutations may effect on protein folding and stability[24], protein function[25] and protein-protein intractions[26].

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AY662497.1      ATGGCGAAAAACATGGCTATCGGCATCGACCTGGGCACCACCTACTCCTGCGTAGGGGTG 60
                ----------------------------------------TACTCGTGCGTAGGGGTG 18
                ----------------------------------------TACTCCTGCGTAGGGGTG 18
                ----------------------------------------TACTCCTGCGTAGGGGTG 18
                                                        ***** ************

AY662497.1      TTCCAGCACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACCCCCAGC 120
G1              TTCCAGCACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACCCCCAGC 78
G2              TTCCAGCACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACCCCCAGC 78
G3              TTCCAGCACGGCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACCCCCAGC 78
                ************************************************************

AY662497.1      TACGTGGCCTTCACCGATACCGAGCGGCTCATCGGCGATGCGGCCAAGAGCCAGGTGGCG 180
G1              TACGTGGCCTTCACCGATACCGAGCGGCTCATCGGCGATGCGGCCAAGAGCCAGGTGGCG 138
G2              TACGTGGCCTTCACCGATACCGAGCGGCTCATCGGAGATGCGGCCAAGAGCCAGGTGGCG 138
G3              TACGTGGCCTTCACCGATACCGAGCGGCTCATCGGAGATGCGGCCAAGAGCCAGGTGGCG 138
                ********************************* ************************

AY662497.1      CTGAACCCGCAGAACACGGTGTTCGACGCGAAGCGGCTGATCGGCCGCAAGTTCGGAGAC 240
G1              CTGAACCCGCAGAACACGGTGTTCGACGCGAAGCGGCTGATCGGCCGCAAGTTCGGAGAC 198
G2              CTGAACCCGCAGAACACGGTGTTCGACGCGAAGCGGCTGATCGGCCGCAAGTTCGGAGAC 198
G3              CTGAACCCGCAGAACACGGTGTTCGACGCGAAGCGGCTGATCGGCCGCAAGTTCGGAGAC 198
                ********************************* ************************

AY662497.1      CCGGTGGTGCAGTCGGACATGAAGCACTGGCCTTTCCGCGTCATCAACGACGGAGACAAG 300
G1              CCGGTGGTGCAGTCGGACATGAAGCACTGGCCTTTCCGCGTCATCAACGACGGAGACAAG 258
G2              CCGGTGGTGCAGTCGGACATGAAGCACTGGCCTTTCCGCGTCATCAACGACGGAGACAAG 258
G3              CCGGTGGTGCAGTCGGACATGAAGCACTGGCCTTTCCGCGTCATCAACGACGGAGACAAG 258
                ************************************************************

AY662497.1      CCTAAGGTGCAGGTGAGCTACAAAGGGGAGACCAAGGCGTTCTACCCGGAGGAGATCTCG 360
G1              CCTAAGGTGCAGGTGAGCTACAAGGGGGAGACCAAGGCGTTCTACCCGGAGGAGATCTCG 318
G2              CCTAAGGTGCAGGTGAGCTACAAGGGGGAGACCAAGGCGTTCTACCCGGAGGAGATCTCG 318
G3              CCTAAGGTGCAGGTGAGCTACAAGGGGGAGACCAAGGCGTTCTACCCGGAGGAGATCTCG 318
                ********************* ************************************

AY662497.1      TCGATGGTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCCACCCGGTGACC 420
G1              TCGATGGTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCCACCCGGTGACC 378
G2              TCGATGGTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCCACCCGGTGACC 378
G3              TCGATGGTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCCACCCGGTGACC 378
                ************************************************************

AY662497.1      AACGCGGTGATCACCGTGCCGGCCTACTTCAACGACTCGCAGCGGCAGGCCACCAAGGAC 480
G1              AACGCGGTGATCACCGTGCCGGCCTACTTCAACGACTCGCAGCGGCAGGCCACCAAGGAC 438
G2              AACGCGGTGATCACCGTGCCGGCCTACTTCAACGACTCGCAGCGGCAGGCCACCAAGGAC 438
G3              AACGCGGTGATCACCGTGCCGGCCTACTTCAACGACTCGCAGCGGCAGGCCACCAAGGAC 438
                ************************************************************

AY662497.1      GCGGGGGTGATCGCGGGGCTGAACGTGCTGAGGATCATCAACGAGCCCACGGCCGCCGCC 540
G1              GCGGGGGTGATCGCGGGGCTGAACGTGCTGAGGATCATCAACGAGCCCACGGCCGCCGCC 498
G2              GCGGGGGTGATCGCGGGGCTGAACGTGCTGAGGATCATCAACGAGCCCACGGCCGCCGCC 498
G3              GCGGGGGTGATCGCGGGGCTGAACGTGCTGAGGATCATCAACGAGCCCACGGCCGCCGCC 498
                ************************************************************

AY662497.1      ATCGCCTACGGCCTGGACAGGACGGGCAAGGGGGAGCGCAACGTGCTCATCTTTGATCTG 600
G1              ATCGCCTACGGCCTGGACAGGACGGGCAAGGGGGAGCGCAACGTGCTCATCTTTGATCTG 558
G2              ATCGCCTACGGCCTGGACAGGACGGGCAAGGGGGAGCGCAACGTGCTCATCTTTGATCTG 558
G3              ATCGCCTACGGCCTGGACAGGACGGGCAAGGGGGAGCGCAACGTGCTCATCTTTGATCTG 558
                ************************************************************

AY662497.1      GGAGGGGGCACGTTCGACGTGTCCATCCTGACGATCGACGACGGCATCTTCGAGGTGAAG 660
G1              GGAGGGGGCACGTTCGACGTGTCCATCCTGACGATCGACGACGGCATCTTCGAGGTGAAG 618
G2              GGAGGGGGCACGTTCGACGTGTCCATCCTGACGATCGACGACGGCATCTTCGAGGTGAAG 618
G3              GGAGGGGGCACGTTCGACGTGTCCATCCTGACGATCGACGACGGCATCTTCGAGGTGAAG 618
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Fig. 2: Continue

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AY662497.1      GCCACGGCCGGGGACACGCACCTGGGCGGGGAGGACTTCGACAACAGGCTGGTGAACCAC 720
G1              GCCACGGCCGGGGACACGCACCTGGGCGGGGAGGACTTCGACAACAGGCTGGTGAACCAC 678
G2              GCCACGGCCGGGGACACGCACCTGGGCGGGGAGGACTTCGACAACAGGCTGGTGAACCAC 678
G3              GCCACGGCCGGGGACACGCACCTGGGCGGGGAGGACTTCGACAACAGGCTGGTGAACCAC 678
                ************************************************************

AY662497.1      TTCGTGGAGGAGTTCAAGAGGAAGCACAAGAAGGACATCAGCCAGAACAAGCGGGCCGTG 780
G1              TTCGTGGAGGAGTTCAAGAGGAAGCACAAGAAGGACATCAGCCAGAACAAGCGGGCCGTG 738
G2              TTCGTGGAGGAGTTCAAGAGGAAGCACAAGAAGGACATCAGCCAGAACAAGCGGGCCGTG 738
G3              TTCGTGGAGGAGTTCAAGAGGAAGCACAAGAAGGACATCAGCCAGAACAAGCGGGCCGTG 738
                ************************************************************

AY662497.1      AGGCGGCTGCGCACCGCATGCGAGCGGGCCAAGAGAACCTTGTCGTCCAGCACCCAGGCC 840
G1              AGGCGGCTGCGCACCGCATGCGAGCGGGCCAAGAGAACCTTGTCGTCCAGCACCCAGGCC 798
G2              AGGCGGCTGCGCACCGCATGCGAGCGGGCCAAGAGAACCTTGTCGTCCAGCACCCAGGCC 798
G3              AGGCGGCTGCGCACCGCATGCGAGCGGGCCAAGAGAACCTTGTCGTCCAGCACCCAGGCC 798
                ************************************************************

AY662497.1      AGCCTGGAGATCGACTCCCTGTTCGAGGGCATCGACTTCTACACGTCCATCACCAGGGCG 900
G1              AGCCTGGAGATCGACTCCCTGTTCGAGGGCATCGACTTCTACACGTCCATCACCAGGGCG 858
G2              AGCCTGGAGATCGACTCCCTGTTCGAGGGCATCGACTTCTACACGTCCATCACCAGGGCG 858
G3              AGCCTGGAGATCGACTCCCTGTTCGAGGGCATCGACTTCTACACGTCCATCACCAGGGCG 858
                ************************************************************

AY662497.1      CGGTTCGAGGAGCTGTGCTCCGACCTGTTCCGGAGCACCCTGGAGCCCGTGGAGAAGGCG 960
G1              CGGTTCGAGGAGCTGTGCTCCGACCTGTTCCGGAGCACCCTGGAGCCCGTGGAGAAGGCG 918
G2              CGGTTCGAGGAGCTGTGCTCCGACCTGTTCCGGAGCACCCTGGAGCCCGTGGAGAAGGCG 918
G3              CGGTTCGAGGAGCTGTGCTCCGACCTGTTCCGGAGCACCCTGGAGCCCGTGGAGAAGGCG 918
                ************************************************************

AY662497.1      CTACGCGACGCCAAGCTGGACAAGGCGCAGATCCACGACCTGGTCCTGGTGGGGGGCTCC 1020
G1              CTACGCGACGCCAAGCTGGACAAGGCGCAGATCCACGACCTGGTCCTGGTGGGGGGCTCC 978
G2              CTACGCGACGCCAAGCTGGACAAGGCGCAGATCCACGACCTGGTCCTGGTGGGGGGCTCC 978
G3              CTACGCGACGCCAAGCTGGACAAGGCGCAGATCCACGACCTGGTCCTGGTGGGGGGCTCC 978
                ************************************************************

AY662497.1      ACCCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGACTTCTTCAACGGGCGCGACCTCAAC 1080
G1              ACCCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGACTTCTTCAACGGGCGCGACCTCAAC 1038
G2              ACCCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGACTTCTTCAACGGGCGCGACCTCAAC 1038
G3              ACCCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGACTTCTTCAACGGGCGCGACCTCAAC 1038
                ************************************************************

AY662497.1      AAGAGCATCAACCCCGACGAGGCGGTGGCGTACGGGGCGGCGGTGCAGGCGGCCATCCTG 1140
G1              AAGAGCATCAACCCCGACGAGGCGGTGGCGTACGGGGCGGCGGTGCAGGCGGCCATCCTG 1098
G2              AAGAGCATCAACCCCGACGAGGCGGTGGCGTACGGGGCGGCGGTGCAGGCGGCCATCCTG 1098
G3              AAGAGCATCAACCCCGACGAGGCGGTGGCGTACGGGGCGGCGGTGCAGGCGGCCATCCTG 1098
                ************************************************************

AY662497.1      ATGGGGGACAAGTCGGAGAACGTGCAGGACCTGCTGTTGCTGGACGTGGCTCCCCTGTCG 1200
G1              ATGGGGGACAAGTCGGAGAACGTGCAGGACCTGCTGTTGCTGGACGTGGCTCCCCTGTCG 1158
G2              ATGGGGGACAAGTCGGAGAACGTGCAGGACCTGCTGTTGCTGGACGTGGCTCCCCTGTCG 1158
G3              ATGGGGGACAAGTCGGAGAACGTGCAGGACCTGCTGTTGCTGGACGTGGCTCCCCTGTCG 1158
                ************************************************************

AY662497.1      CTGGGACTGGAGACGGCCGGAGGCGTGATGACCGCCCTGATCAAGCGCAACTCCACCATC 1260
G1              CTGGGACTGGAGACGGCCGGAGGCGTGATGACCGCCCTGATCAAGCGCAACTCCACCATC 1218
G2              CTGGGACTGGAGACGGCCGGAGGCGTGATGACCGCCCTGATCAAGCGCAACTCCACCATC 1218
G3              CTGGGACTGGAGACGGCCGGAGGCGTGATGACCGCCCTGATCAAGCGCAACTCCACCATC 1218
                ************************************************************

AY662497.1      CCCACGAAGCAGACGCAGATCTTCACCACCTACTCGGACAACCAGCCGGGCGTGCTGATC 1320
G1              CCCACGAAGCAGACGCAGATCTTCACCACCTACTCGGACAACCAGCCGGGCGTGCTGATC 1278
G2              CCCACGAAGCAGACGCAGATCTTCACCACCTACTCGGACAACCAGCCGGGCGTGCTGATC 1278
G3              CCCACGAAGCAGACGCAGATCTTCACCACCTACTCGGACAACCAGCCGGGCGTGCTGATC 1278
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AY662497.1      CAGGTGTACGAGGGCGAGAGGGCCATGACGCGGGACAACAACCTGCTGGGGCGCTTCGAG 1380
G1              CAGGTGTACGAGGGCGAGAGGGCCATGACGCGGGACAACAACCTGCTGGGGCGCTTCGAG 1338
G2              CAGGTGTACGAGGGCGAGAGGGCCATGACGCGGGACAACAACCTGCTGGGGCGCTTCGAG 1338
G3              CAGGTGTACGAGGGCGAGAGGGCCATGACGCGGGACAACAACCTGCTGGGGCGCTTCGAG 1338
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Fig. 2: Continue

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AY662497.1      TTGAGCGGCATCCCGCCGGCCCCGCGGGGGGTGCCCCAGATCGAGGTGACCTTCGACATC 1440
G1              CTGAGCGGCATCCCGCCGGCCCCGCGGGGGGTGCCCCAGATCGAGGTGACCTTCGACATC 1398
G2              CTGAGCGGCATCCCGCCGGCCCCGCGGGGGGTGCCCCAGATCGAGGTGACCTTCGACATC 1398
G3              CTGAGCGGCATCCCGCCGGCCCCGCGGGGGGTGCCCCAGATCGAGGTGACCTTCGACATC 1398
                ************************************************************

AY662497.1      GACGCCAATGGCATCCTGAACGTCACGGCCACGGACAAGAGCACGGGCAAGGCCAACAAG 1500
G1              GACGCCAATGGCATCCTGAACGTCACGGCCACGGACAAGAGCACGGGCAAGGCCAACAAG 1458
G2              GACGCCAATGGCATCCTGAACGTCACGGCCACGGACAAGAGCACGGGCAAGGACAACAAG 1458
G3              GACGCCAATGGCATCCTGAACGTCACGGCCACGGACAAGAGCACGGGCAAGGCCAACAAG 1458
                ************************************************** *******

AY662497.1      ATCACCATCACCAACGACAAGGGCCGGCTGAGCAAGGAGGAGATCGAGCGCATGGTGCAG 1560
G1              ATCACCATCACCAACGACAAGGGCCGGCTGAGCAAGGAGGAGATCGAGCGCATGGTGCAG 1518
G2              ATCACCATCACCAACGACAAGGGCCGGCTGAGCAAGGAGGAGATCGAGCGCATGGTGCAG 1518
G3              ATCACCATCACCAACGACAAGGGCCGGCTGAGCAAGGAGGAGATCGAGCGCATGGTGCAG 1518
                ************************************************************

AY662497.1      GAGGCGGAAAAGTACAAGGCGGAGGACGAGGTCCAGCGCGAGAGGGTGTCTGCCAAGAAC 1620
G1              GAGGCGGAAAAGTACAAGGCGGAGGACGAGGTCCAGCGCGAGAGGGTGTCTGCCAAGAAC 1578
G2              GAGGCGGAAAAGTACAAGGCGGAGGACGAGGTCCAGCGCGAGAGGGTGTCTGCCAAGAAC 1578
G3              GAGGCGGAAAAGTACAAGGCGGAGGACGAGGTCCAGCGCGAGAGGGTGTCTGCCAAGAAC 1578
                ************************************************************

AY662497.1      GCGCTGGAGTCATACGCCTTCAACATGAAGAGCGCCGTGGAGGATGAGGGGCTGAAGGGC 1680
G1              GCGCTGGAGTCATACGCCTTCAACATGAAGAGCGCCGTGGAGGATGAGGGGCTGAAGGGC 1638
G2              GCGCTGGAGTCGTACGCCTTCAACATGAAGAGCGCCGTGGAGGATGAGGGGCTGAAGGGC 1638
G3              GCGCTGGAGTCGTACGCCTTCAACATGAAGAGCGCCGTGGAGGATGAGGGGCTGAAGGGC 1638
                *********** ************************************************

AY662497.1      AAGATCAGCGAGGCGGACAAGAAGAAGGTGCTGGACAAGTGCCAGGAGGTGATTTCCTGG 1740
G1              AAGATCAGCGAGGCGGACAAGAAGAAGGTGCTGGACAAGTGCCAGGAGGTGATTTCCTGG 1698
G2              AAGATCAGCGAGGCGGACAAGAAGAAGGTGCTGGACAAGTGCCAGGAGGTGATTTCTTGG 1698
G3              AAGATCAGCGAGGCGGACAAGAAGAAGGTGCTGGACAAGTGCCAGGAGGTGATTTCTTGG 1698
                ************************************************************ ***

AY662497.1      CTGGACGCCAACACCTTGGCGGAGAAGGACGAGTTTGAGCACAAGAGGAAGGAGCTGGAG 1800
G1              CTGGACGCCAACACCTTGGCGGAGAAGGACGAGTTTGAGCACAAGAGGAAGGAGCTGGAG 1758
G2              CTGGACGCCAACACCTTGGCTGAGAAGGACGAGTTTGAGCACAAGAGGAAGGAGCTGGAG 1758
G3              CTGGACGCCAACACCTTGGCTGAGAAGGACGAGTTTGAGCACAAGAGGAAGGAGCTGGAG 1758
                ******************* ************************************

AY662497.1      CAGGTGTGTAACCCCATCATCAGCAGACTGTACCAGGGGGCGGGCGGCCCCGGGGCTGGC 1860
G1              CAGGTGTGTAACCCCATCATCAGCAGACTGTACCAGGGGGCGGGCGGCCCCGGGGCTGGC 1818
G2              CAGGTGTGTAACCCCATCATCAGCAGACTGTACCAGGGGGCGGGCGGCCCCGGGGCTGGC 1818
G3              CAGGTGTGTAACCCCATCATCAGCAGACTGTACCAGGGGGCGGGCGGCCCCGGGGCTGGC 1818
                ************************************************************

AY662497.1      GGCTTTGGGGCTCAGGGCCCTAAAGGGGGCTCTGGGTCTGGCCCCACCATTGAGGAGGTG 1920
G1              GGCTT---------------------------------------------------------- 1823
G2              GGCTT---------------------------------------------------------- 1823
G3              GGCTT---------------------------------------------------------- 1823
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Fig. 2: Identified *Hsp70* gene in Iraqi Holstein bulls and the same gene in GenBank
CLUSTAL Omega (1.2.4) multiple sequence alignment

The result agree with Li *et al.*[27], Sodhi *et al.*[28], Kerekoppa *et al.*[29] and Bhat *et al.*[3] on the possibility of polymorphism of *hsp70* gene in cattle.

While the results indicate that the Iraqi Holstein bulls have new haplotypes of *hsp70* in comparison with that recorded in GenBank. The haplotypes of this gene identified from Holstein cattle in Japan and Poland were identical accession number AY662497.1[30,31]. Similar results were found in Italian[6] and Chinese Holstein[32]. However, polymorphism of crossing Indian cattle with Holstein disagreed with this result[33].

There is no study in line with the results of this study on the location of mutations and perhaps the reason is that this study is the first of its kind on the *hsp70* gene polymorphism in the Holstein bulls in Iraq.

These results might indicate that the Iraqi Holstein bulls have adapted significantly to resist heat stress conditions through changes in the polymorphism of the *Hsp70* gene, that's because the mutations in *Hsp70* gene could be used as marker for heat stress tolerances[27] and assisted selection for resistant to heat stress in the breeding[34] but this needs to be further studied to determine the association between *Hsp70* gene polymorphism in semen and its characteristics in Holstein bulls born in Iraq.

CONCLUSION

This study showed three new haplotypes of the *Hsp70* gene in the Holstein bulls born in Iraq as a result of mutations in different positions, where Holstein can modify their genotypes of *hsp70* gene to adapting surrounding environmental conditions, even it was originated in different environments. These changes did not affect the animal fitness.

SIGNIFICANCE STATEMENTS

This study discovers that the *Hsp70* gene can manipulate by mutations (silent or missense) according to the environment conditions. This study will help the researcher to uncover the polymorphisms of *hsp70* gene in Holstein bulls born in Iraq and helps to study the relationship between *hsp70* gene polymorphism and semen quality as a guide to the selection of bulls.

ACKNOWLEDGMENTS

The authors would like to thank Prof. Dr: Asaad Y. Ayied, Department of Animal Production, College of Agriculture, University of Basrah, Iraq. For his scientific assistance in completing this paper.

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