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ASSOCIATION OF *hsp70* GENE POLYMORPHISM WITH SOME OF FRESH SEMEN CHARACTERS IN SUMMER AND WINTER TO IRAQI HOLSTEIN BULLS

Agricultural Science

Hassan Nima Habib College of Agriculture University of Basrah

Amad Falah. Hassan College of Agriculture University of Basrah

Bassam Yasein Khudaier Asis prof Bassam Yasein Khudaier, College of Veterinary Medicine University of Basrah, Iraq Basrah. - Corresponding Autho

ABSTRACT

Aim: To determine the relationship between the polymorphism of the *hsp*70 gene and some semen characteristics of the Iraqi Holstein bulls.
Methods: The study was carried out during the winter season November, December, 2015, January 2016 and the summer season for the months April, May and June, 2016. Thirty Iraqi Holstein bulls were used back to Artificial Insemination Center of Abou-Ghareeb Baghdad, Iraq. The semen was collected using an artificial vagina. The semen evaluation was then performed and the DNA was extracted then PCR amplified and sequencing.
Result: The results indicate that the haplotype which was exposed to missense mutation compared to the other haplotypes significantly superior in semen characteristics.
Conclusion: Polymorphism of hsp70 gene in bulls semen can be used as a biological marker to resistance of heat stress.

KEYWORDS:

*hsp*70 polymorphism, fresh semen characters, Iraqi Holstein bulls,

Introduction

Heat shock proteins family (HSPs) is a component of seminal plasma, it is a molecular mechanism in the cell to protect it from different stress conditions (Pockley 2001; Rajoriya *et al*., 2014). *HSP70* is a member of this family (Rynkowska *et al*., 2011), an important of the cell's mechanism protein folding and protect cells from heat stress (Morano 2007). *Hsp70* may impact stress tolerance and haplotypes of *hsp70* gene were linked to heat tolerance (Rosenkrans *et al*., 2010; Basirico *et al*., 2011), its affect fertility by affecting the quality of semen (Huang *et al.*, 2000 ; Shrum *et al*., 2010). Polymorphisms of *hsp70* gene associated with fresh semen quality (Nikbin *et al*., 2014). *hsp70* gene polymorphism are expected to be an powerful determinant for heat tolerance in livestock, thus they help greatly in selecting the most tolerant animals for thermal stress conditions (Bhat *et al.*, 2016), as well as considered both mass motility (David *et al.*, 2015), abnormalities sperm (Zodinsanga *et al*., 2015) and live to dead sperm (Saini *et al*., 2016), can give a clear perception of semen fertility and therefore can be elected bulls on the basis of these characters. There are no previous studies of *hsp70* in the Holstein bulls born in Iraq, so there is very little information about the role of *hsp70* gene polymorphism. Therefore, this study aims to determine the relationship between the polymorphism of the *hsp70* gene and some semen characteristics of the Iraqi Holstein bulls.

Methods and Materials

Animals and semen collection

The study was carried out during the winter season November , December , 2015, January 2016 and the summer season for the months April , May and June, 2016. Thirty Holstein bulls born in Iraq of known fertility, 2.5 – 3.5 years old, back to Artificial Insemination Center of Abou-Ghareeb Baghdad, Iraq, the General Company for Livestock Services (longitude 44.1922070, latitude 33.3095550 northwest of Baghdad) were used. Semen collected from all bulls by using the artificial vagina method once a week, early in the morning.

Semen Evaluation

Evaluation of semen was done immediately after collection, each of ejaculate was examined for mass motility, live to dead sperms and abnormalities, mass motility carried out according to (Salisbury *et al.,* 1978), live to dead sperms and abnormalities carried out according to (Herman and Madden 1963).

DNA extraction

DNA was extracted by using Chelex-100® (Sigma Aldrich. USA), according to (Anju *et al*., 2010). Then the purity and concentration of DNA was estimated by using Nano-drop(thermo scientific 200.USA).

PCR amplification

PCR (A-2040-1 Bioneer Korea) amplification was done as described by Habib et al., (2017) with 25 µl reaction (Table 1), using primer Hsp70-F: ATGGCGAAAAACATGGCTATCGGC, Hsp70-R: CTAATCCACCT CCTCAATGGTGGGGCC. The protocol of PCR amplification cycling was demonstrated in table (2), then the product was detected on, 1.5% ethidium bromide, stained agarose gel. The size of product was 1926 bp (fig 1).

Sequencing

Purification and sequencing done at First BASE Laboratories / Malaysia. Multiple Sequence Alignment was carried out on website (http://www.ebi.ac.uk/Tools/msa/clustalo/).

Table (1) PCR Setup

Habib et al., 2017	
Componet	**Amount (µl)**
Water, nuclease free	9.5
2X PCR Master Mix	12.5
Forward primer, 10 µM	1
Reverse primer, 10 µM	1
DNA template (75 ng)	1
TOTAL	25

Table (2) Cycling Protocol of PCR amplification

Cycle step	Temp (° C)	Time	Number of Cycles
Initial Denaturation	95	5 min	1
Denaturation	94	30 s	30
Annealing	61	30 s	
Extension	72	2 min	
Final Extension	72	10 min	1

Statistical analysis

Using the statistical analysis program IBM SPSS version 22 (SPSS 2013) and the application of the general linear model (GLM) at the level of probability (0.05) to test the significance of the differences between the averages studied and based on the formula of the mathematical model:

$Y_{ijkl} = \mu + S_i + G_{.j.} + B.K + e_{ihkl}$

Y_{ijkl}: The value of the viewing of any studied character
μ= General average
S_i= Season Effect (i = 2)
$G_{.j.}$ = Effect of polymorphism (j = 3)

B.K= Bull Effect (K=29)
e_{ihkl}= The effect of experimental error, which is distributed randomly and naturally, with an average of 0 and $\sigma^2 e$

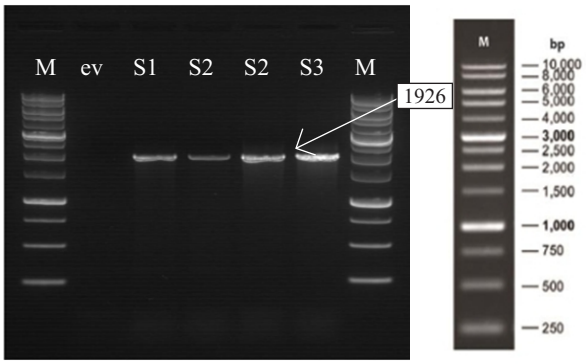

Fig 1: "-ve" is no-template control (water to replace DNA template)
S1 , S2, S3 , S4 : DNA template *hsp*70 gene
M : DNA Marker

A total 75 ng of gDNA sample was used in one 25 ul PCR reaction. Only 3 ul of PCR product was run on 1% TAE agarose gel at 100V, 60 min. (Habib et al., 2017)

Results and Discussion

The results showed three haplotypes of hsp70 gene, as describe by Habib et al., (2017), the differences between them were as follows:

First haplotype A there was one silent mutation in nucleotide at position 6 C < G, did not encoding to a new amino acid. Included 16 bulls.

Second haplotype B have mutations in nucleotide at positions, 114 G <A, 1451 C <A , 1590 A < G, 1695 C < T and 1719 G < T, all mutations are silent except mutation at position 1451 which a missense mutation because of changing code amino acid from Alanine to Aspartic.

Third haplotype C exactly identical with haplotype B except in the absence of a missense mutation in position 1451.

These results are consistent with Cheng et al., (2009) about the possibility of silent mutations in hsp70 gene in Holstein cattle, in the other hand agrees with Maugeri et al., (2010) about the occurrence of a missense mutations in hsp70 gene.

The results are shown in table (3), significantly superior (P <0.05) in winter season in mass motility comparison with summer season, in all haplotypes of hsp70 gene obtained, this is consistent with (Bhakat et al., 2009) about the low quality semen in hot seasons.

On the other hand, the results in table (3) refers that the haplotype B is significantly superior (P <0.05) in the mass motility in comparison with haplotype C and haplotype A, respectively in both seasons, similarity Shrum et al., (2010) which referred to associations between hsp70 gene polymorphism and sperm motility, Perhaps this is because HSP70 work to protect proteins related to respiratory activity and energy level, which directly effect on the sperm motility (Nascimento et al., 2008).

The results are consistent with Hering et al., (2015) Which indicated that mutations significantly affect the rate of mass motility in the semen of the Polish Holstein, and with Zhang et al., (2015) about the association of mutations significantly with semen characteristics such as mass motility in the Quinchuan bulls in China.

Table (3) Association of *hsp70* gene polymorphism with percentage of mass motility in fresh semen of Holstein Bulls born in Iraq during winter and summer seasons

Haplotype	Number of bulls	Mass motility		Average
		Winter	Summer	
haplotype A	16	^{c}64.99 ± 0.61	^{c}60.20 ± 0.32	^{c}62.60 ± 0.22
haplotype B	6	^{a}71.07 ± 0.38	^{a}66.39 ± 0.17	^{a}68.73 ± 0.19
haplotype C	8	^{b}68.86± 0.27	^{b}64.74 ± 0.21	^{b}66.80± 0.17

The vertically different letters mean significant differences at (P <0.05)

The results also indicate in table (4), significantly superior (P <0.05) in winter season in live to dead sperm comparison with summer season, agrees with Rajoriya *et al.*, (2015), who pointed to the significantly superiority (P <0.05) of the live to dead sperm in the winter season compared to the summer season.

further, the results in table (4) refers that significantly superior (P <0.05) of haplotype B in comparison with haplotype C and haplotype A, respectively in the percentage of live to dead sperm in both season of study, as it recorded the highest ratio of live to dead sperm, similarity with Huang *et al*., (2002) and Patterson *et al.,* (2009) reported association between hsp70 gene polymorphism and semen quality, and matched with Nikbin et al., (2014) ; Gafer *et al.,* (2015), who have demonstrated that the polymorphism of hsp70 gene linked with live to dead sperm.

Table (4) Association of hsp70 gene polymorphism with percentage of live to dead sperm in fresh semen of Holstein Bulls born in Iraq during winter and summer seasons

Haplotype	Number of bulls	live to dead		Average
		Summer	Winter	
haplotype A	16	^{c}74.11 ± 0.73	^{c}82.08 ± 0.80	^{c}78.10 ± 0.51
haplotype B	6	^{a}84.45 ± 0.49	^{a}92.69 ± 0.34	^{a}88.57 ± 0.42
haplotype C	8	^{b}81.35 ± 0.40	^{b}89.17± 0.56	^{b}85.26 ± 0.52

The vertically different letters mean significant differences at (P <0.05)
In table (5), the results show a significant differences (P <0.05) in the percentage of abnormalities sperm between the two seasons of the study, the rate of abnormalities sperm in the winter season was significantly lower than the summer season, these results are agreed with (Alam *et al*., 2015), and consistent with Soren *et al.,* (2016) who found that the percentage of abnormalities rate increased significantly (P <0.05) in the summer in Karan Fries (Tharparkar × Holstein Friesian) Bulls.

The differences between the polymorphism, in table (5) showed a significant superiority (P <0.05) of haplotype B that gave the lowest percentage of the abnormalities sperm rate, in comparison with haplotype C and haplotype A, respectively, these results are consistent with Shrum *et al.*, (2010), the polymorphism of the hsp70 gene is closely related to the semen's characteristics in the Holstein bulls in America, and agrees with Gafer *et al.,* (2015), about the polymorphism differences in the semen characteristics of hsp70 gene in bulls.

Table (5) Association of *hsp70* gene polymorphism with percentage of Abnormalities sperm in fresh semen of Holstein Bulls born in Iraq during winter and summer seasons

Haplotype	Number of bulls	Abnormalities		Average
		Summer	Winter	
haplotype A	16	^{c}6.37 ± 0.21	^{c}3.31 ± 0.14	^{c}4.84 ± 0.13
haplotype B	6	^{a}5.30 ± 0.11	^{a}2.08 ± 0.11	^{a}3.69 ± 0.10
haplotype C	8	^{b}5.66 ± 0.13	^{b}2.53 ± 0.16	^{b}4.10 ± 0.09

The vertically different letters mean significant differences at (P <0.05)

In all the results of the present study, we can observe the significantly superiority of haplotypes, which has had more mutations, as we can note that haplotype C was significantly superiority in all studied traits and in both seasons of the study compared to haplotype A, this may be due to the silent mutation, although it does not alter the produced amino acid but might influence the protein by alteration in transcription and impact in the precision or efficiency of splicing of mRNA or transcriptional control (Cartegni *et al*., 2002 and Komar 2007).

On the other hand, it can be observed that haplotype B significantly superiority in all studied traits and in both seasons of the study compared with the haplotype C and A respectively, this can be attributed to the missense mutation, although the number of mutations is close to the haplotype C but also surpasses it, so can be the superiority because of the missense mutation, which resulted from a

change in the amino acid produced from Alanine to Aspartic, which differ in structure (Jeremy *et al.,* 2012), which will result in a change in the properties of the product protein and protein stability and protein-protein interactions (Zhang *et al.,* 2012), thus will affect the protein function (Minde *et al.*, 2011).

The results of the present study are consistent with Li *et al.,* (2011) that mutations in hsp70 gene can be used a as marker assisted selection for anti-heat stress cows in breeding program.

Conclusion

The occurrence of the missense mutation positively affects sperm characteristics. The polymorphism of hsp70 gene in the Iraqi Holstein bulls significantly affected sperm characteristics, can be used as an effective marker to resist heat stress and thus help in the election of bulls for breeding programs and artificial insemination.

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Conflict of Interests:

The authors declare that they have no conflict interests

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