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THE ASSOCIATION OF THE POLYMORPHISM AND GENE EXPRESSION OF HEAT SHOCK PROTEIN *HSP70* GENE IN WINTER AND SUMMER IN THE SEMEN OF HOLSTEIN BULLS BORN IN IRAQ

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ABSTRACT

The HSP70 protein considered as a molecular markers to resist various stress conditions, especially high temperatures. Therefore, the high level of gene expression of *hsp70* gene is one of the ways to resist stress conditions, which can vary depending on the polymorphisms. The study aimed to determine the extent of the correlation between gene expression and polymorphisms to *hsp70* gene. The study was conducted in the months: November , December , 2015, January 2016, as the winter season and the months April , May and June, 2016, as the summer season. 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, the General Company for Livestock Services, Semen collected using artificial vagina method, mRNA extraction, Synthesis of mRNA to cDNA and Real-Time Quantitative RT–qPCR were done. The results showed significant differences in the level of gene expression between polymorphisms to the hsp70 gene. In conclusion there is a positive correlation between the gene expression and polymorphisms of hsp70 gene in Holstein bulls born in Iraq .

INTRODUCTION

HSP70 is one of a component of sperm plasma in bulls and sheep (1,2), which increases the secretion of the gonads in different stress situations (3), especially at high temperatures(4). Several studies have shown that there is a significant difference in the level of gene expression of hsp70 proteins between the summer and winter seasons, as the rise in summer temperatures leads to an increase in the level of gene expression of these proteins significantly ($P < 0.05$) compared to the winter (5,6,7).

There is a positive correlation between the high level of gene expression of *hsp 70* gene and the motility of sperm and percentage of live to dead and some characteristics of semen (8,9). On the other hand, gene expression was associated with the genetic factors that have a significant impact in determining the response of individuals to the level of gene expression of *hsp 70* (10,11), in addition, it is positively associated with polymorphisms of *hsp 70* gene (10). Perhaps one of the most important roles of hsp70 protein during stress conditions is its control over the process of reproducing different proteins and post-transcription process of proteins correctly and control the completion of the transcription process, hence, the high level of gene expression of hsp70 in stress conditions is important to complete the process of protein replication correctly (12). The effect of the high level of gene expression of *hsp70* in semen directly influences the subsequent development of embryos by improving the rate of growth of the blastocyst (13,14). Therefor this study aimed to determine the relationship between the polymorphism and the gene expression of the *hsp70* gene in the semen of Holstein bulls born in Iraq in the summer and winter seasons.

METHODS AND MATERIALS

Animals and semen collection

This study was conducted in the months: November , December , 2015, and January 2016, as it was regard as the winter season and the months April , May and June, 2016, as it was regard as the summer season. 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, the General Company for Livestock Services (longitude 44.1922070, latitude 33.3095550 northwest of Baghdad) were used.

Semen collection

Semen collected from all bulls (mature and healthy) by using the artificial vagina method twice a week, early in the morning.

mRNA extraction

RNA extraction was performed from semen samples (5 microliters) depending on the method described by Zhang *et al*., (15), using hot TRIZOL method

Detection and Quantification of mRNA

The quantity and purity of RNA were measured using Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Fisher Scientific, Loughborough, UK). The absorbance of 1µl of RNA at 260nm and 280nm was determined. The purity of RNA was assessed by (A260/A280) ratio, which was above 1.81 for all samples.

Synthesis of mRNA to cDNA

Synthesis of mRNA to cDNA was performed through reverse transcription by reverse transcriptase enzyme as described by Zhang *et al*., (15), according to the method mentioned with kit (AccuPower® RocketScript™ RT PreMix) Bioneer company. The cDNA synthesis was performed in a reaction volume of 20 µl. All reaction mixtures were prepared with ice, the samples were then placed in a 96 Well Thermal Cycler, and cycled at the conditions in (Table 1). The converted cDNA was stored at -20°C and used as a template for PCR amplification. The obtained cDNA was diluted to a final concentration of 25 ng/µl.

Table (1) Program used in cDNA synthesis (temperature and cycles)

Step	Temperature (C°)	Cycle
Annealing primer	°37	10 min
cDNA synthesis	°60	60 min
Heat inactivation	°95	5 min

Real-Time Quantitative RT–qPCR

This reaction was done according to the method described by Zhang *et al*., (15). The SYBR® Premix kit was used, The β-actin gene was amplified as a housekeeping control with primers mentioned in Table 2.

The sample size was 10 microliters containing 6 µL SYBR Green (1x) and 0.5 µl for both the forward and the reverse primer at 10 pCumol and 1 µl of cDNA at 100

β –actin	$^{5-}$ ACCCAGCACAATGAAGATCAA $^{-3}$ (F)	First BASE Laboratories Malaysia (2016)
	$^{5-}$ AACAGTCCGCCTAGAAGCATT $^{-3}$ (R)	
hsp70	$^{5-}$ ATGGCGAAAAACATGGCTATCGGC $^{-3}$ (F)	
	$^{5-}$ CTAATCCACCTCCTCAATGGTGGGGCC3 $^{-3}$ (R)	

ng and then the full volume (2 microliters) distilled water ddH2O. The RT qPCR reaction condition explain in table 3.

Table (2) The primers used to amplify qRT-PCR

Table (3) The RT qPCR reaction condition

Step	Temperature (C°)	time	Cycle
Pre-Denaturation	°95	3 min	1
Denaturation	°95	10 sec	30
Annealing	°57	30 sec	
Extension	°72	30 sec	
Final Extension	°72	10 min	1

A calculation for estimating the efficiency (E) of a real-time PCR assay was performed by Pfaffl (16) as follows :

Efficiency= 10-1/slope -1

The abundance ratio was calculated by the equation of the 'Δ–Δ method' described by Livak and Schmittgen (17). This equation was used for comparing relative abundance results between treatments in real-time PCR and as follows:

Abundance ratio = 2 –[ΔCT sample -ΔCT control

Abundance ratio = 2 –ΔΔCT

The CT raw data were obtained from BIONEER detection system and the calculations were performed by Microsoft Excel®. The data were prepared for statistical analysis (The winter season was adopted as a control group and the calculation of the general average of the winter season and its comparison to the summer season).

The polymorphism of *hsp70* gene

Adopted the haplotypes that describe by Habib *et al.,* (18) as follows :

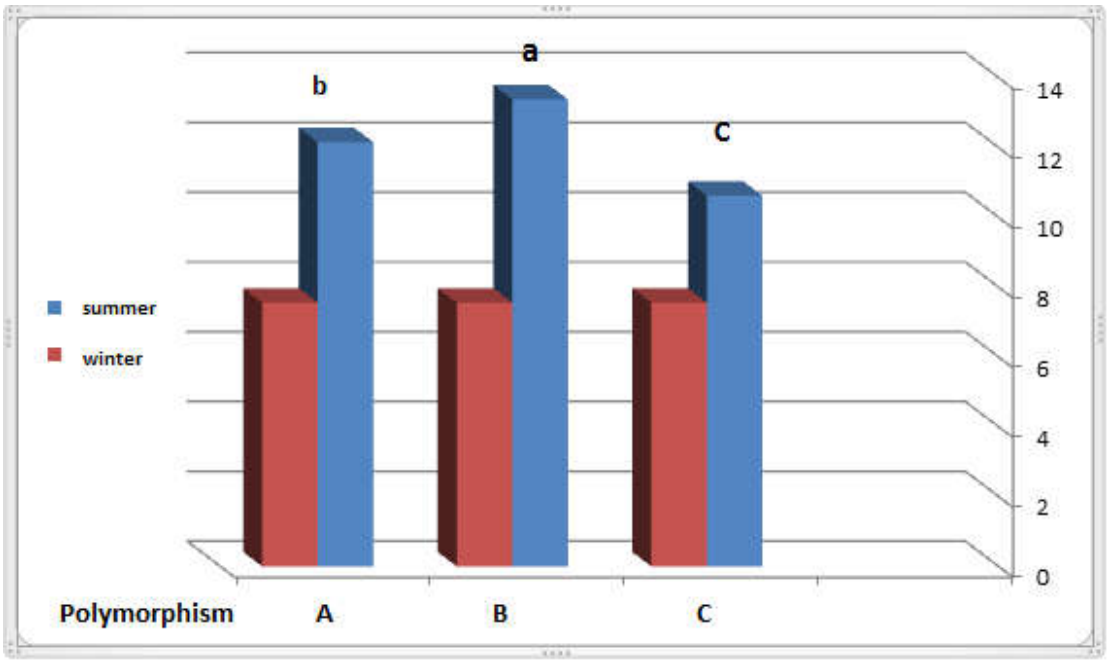
1- First group A (G1) 15 bulls
2- Second group B (G2) 6 bulls
3- Third group C (G3) 8 bulls

RESULTS AND DISCUSSION

The results showed a significant superiority in the level of gene expression of *hsp70* (fig 1) in the summer season compared to the winter season in all groups, in general this results are consistent with those of Kumar *et al.,* (19) indicating a significant increase ($P < 0.05$) in the level of gene expression in the summer season in both cyahual, tharabacar and buffalo cattle. This increase is to enhance the resistance of heat stress caused by high temperature And humidity, *hsp70* gene is one of the most important genes responsible for the tolerance of various stress conditions that directly affect reproduction in the Holstein bulls (20), it consider as a molecular markers to the heat tolerance(21). On the other hands the bulls in the second group genotype B (G2) showed Significantly higher ($P < 0.05$) than genotype C and A in the level of gene expression of heat shock protein *hsp70* gene in the summer season. This superiority Significantly gives an impression of how stable the sperm cells are, Increasing the level of expression of hsp70 positively correlates with sperm cell stability (22), it greatly reduces the effect of heat stress (23), So it has an effective contribution to the survival of cells and living organisms (24, 25), thus it is positively associated with sperm motility (15) and other Characteristics of semen (26). The

most important roles played by the HSP70 with the help of HSP40 proteins is to increase the efficiency of metabolism under different stress conditions as they act directly or indirectly as molecular Chaperone to enhance the utilization of cells from the metabolism under different stress conditions, also reduces apoptosis in the event that the level of gene expression increases in the semen of mammals (27,28). The differences between the polymorphisms of *hsp70* gene may be a sign of the difference in the possibility of tolerance of different stress conditions (especially heat stress) for individuals within a same breed, it has a high heritability to their offspring (29).

The level of gene expression of *hsp70* gene polymorphism in both summer and winter seasons



The horizontal letters mean that there were significant differences at (P <0.05)

CONCLUSION

This study showed a correlation between the *hsp70* gene polymorphism and gene expression in semen of Holstein bulls born in Iraq, gene expression differed in different polymorphism. This link may be refers to the ability of this breed to modify its genes to resist the various environmental changes without any impact on its production.

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