Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Association of the *HSPA2* Gene with some Reproductive Traits in Holstein Friesian Cattle Reared in Egypt

Menna N. Eid[1], Ahmed S. A. Sosa[2], Hassan R. H. Darwish[3], Karima Gh. M. Mahmoud[2], Mahmoud E.A. Abou El-Roos[1] and Al-Shimaa Al-H. H. El-Naby[1]

[1] *Theriogenology Department, Faculty of Veterinary Medicine, Benha University, Egypt.*
[2] *Animal Reproduction and Artificial Insemination Department, Veterinary Research Institute, National Research Centre, Dokki, 12622 Giza, Egypt.*
[3] *Cell Biology Department, Institute of Biotechnology Research, National Research Centre, Dokki, Giza 12622, Egypt.*

Abstract

POLYMORPHISMS of the *HSPA2* gene; a member of the bovine HSP 70 genes family, have been associated with heat tolerance and reproductive performance in cattle. This study aims to determine the role of genetic variation in the *HSPA2* gene in Holstein cattle fertility. A total of 366 cows were classified into anestrum (37.7%) and fertile (62.3%) groups. Reproductive and productive data were collected from farm records including age at first calving (AFC), calving interval (CI), calf birth weight, milk yield, and days of milking. The genomic DNA of the cows was isolated, and PCR-SSCP was conducted to detect three fragments of the *HSPA2* gene; 208, 317, and 393 bp. *HSPA2* F1 revealed three different patterns, *HSPA2* F2 was monomorphic, and *HSPA2* F3 showed only two patterns. Sequence analysis of *HSPA2* F1 revealed three genotypes (CC, CT, and TT) with only one SNP (C/T) as a transition single base substitution mutation located at 10:76649474 with the ID rs377789074. Meanwhile, *HSPA2* F3 showed two genotypes (CC and CT) with a synonymous SNP (C/T) located at 10:76680875 with the ID rs132895070. The CT genotype of *HSPA2* F1 and *HSPA2* F3 genes was associated with a shorter calving interval and heavier calf birth weight in both fertile and anestrum cattle. Our findings suggest that genetic variations in the *HSPA2* gene could be utilized as a molecular marker for genetic selection to enhance reproductive performance in both fertile and anestrum Holstein cows raised in Egypt.

Keywords: *HSPA2* gene, genotyping, SNPs, fertility, cattle.

Introduction

Cattle, as multi-purpose animals, hold significant importance in Egypt and many other countries due to their role as milk and meat producers (FAO, 2022). Its breeding is directly related to reproductive performance to achieve economic and productive efficiency [1]. Stress, a general term for the overall negative impact of various factors, leads to a broad sense of physical issues in the cattle breeding industry.

Cattle farms constantly deal with challenges caused by thermal stress, which leads to decreased animal growth, immunity, fertility, and milk production. The combination of heat and humidity, known as environmental-induced thermal stress, significantly affects the health and productivity of farm animals [2-4]. Animals have evolved wide adaptive strategies to survive in unfavorable climatic conditions. Their ability to reproduce and withstand harsh weather measures their adaptability. An animal's success in a particular environment is influenced by a variety of factors, including anatomical, physiological, behavioral, morphological, biochemical, cellular, and molecular characteristics [5, 6].

Living organisms respond to stresses at the cellular level by rapidly increasing the production of stress proteins such as heat shock proteins (HSPs). HSPs are highly conserved proteins found in animal cells and play a key role in maintaining cellular balance under various environmental stresses, such as extreme temperatures, drought, salinity, and exposure to heavy metals [7]. These proteins act as molecular chaperones and help eukaryotic cells survive in response to high ambient temperatures [8]. HSPs form large protein families and are categorized based on their molecular weight and amino acid sequences. There are six major HSP families,

*Corresponding authors: Ahmed S. A. Sosa, E-mail: ahmedsosa87@yahoo.com, Tel.: 00201015154261
(Received 20 October 2024, accepted 27 November 2024)
DOI: 10.21608/EJVS.2024.329734.2442

including HSP100, HSP90, HSP70, HSP60, HSP40, and other small HSPs. Among all HSPs, the HSP70 is the most abundant, largest, and conserved protein family through evolution [9, 10]. HSP70, also known as the HS 70 kDa protein, is an essential ATP-dependent protein involved in various cellular processes. It plays a crucial role in the proper folding of newly synthesized proteins under normal conditions and in stabilizing or refolding misfolded proteins into biologically active states [11, 12].

The HSP70 gene family in cattle comprised 17 genes spread across 12 chromosomes. Five genes are intronless, while twelve have multiple exons. These genes vary widely in nucleotide length, ranging from 1911 to 54,017 base pairs [10]. Among the HSP70 genes, HSPA13 exists in the microsome and HSPA9 in the mitochondria. Six genes, namely HSPA1B, HSPA8, HSPA12A, HSPA14, HSPBP1, and HSPH1, are found in the cytoplasm, while HSPA5 and HYOU1 are in the endoplasmic reticulum. The largest number of genes (7), including HSPA1A, HSPA1L, HSPA2, HSPA4, HSPA4L, HSPA6, and HSPA12B, are localized in both the nucleus and the cytosol [10, 11, 13]. In terms of gene positioning, three genes (HSPA1A, HSPA1B, and HSPA1L) are located on chromosome 23, while HSPA2, HSPA6, and HSPA8 are positioned on chromosomes 10, 3, and 15, respectively [4, 10, 14]. The HSP70 gene diversity is commonly utilized for studying thermotolerance traits in cattle [15-21].

Genetic polymorphism in the HSPA1A gene has been linked to improved fertility in cattle and serves as a useful predictor of calving rates in Brahman cattle. Additionally, variations in the promoter region of HSPA1A at positions 1117G/A, 1125A/C, and 1128 G/T are associated with reproductive and productive traits, such as higher pregnancy rates, parity, milk production, days of milking, increased calf weaning weights, and enhanced fertility scores in crossbred Brahman cattle [17, 22-25]. On the other hand, little is known about the molecular mechanisms and polymorphism of the HSPA2 gene linked to fertility in Holstein cattle. Hence, this study aims to investigate whether the polymorphism in the HSPA2 gene can affect various phenotype traits including age at first calving, calving interval, calf birth weight, milk yield, and days of milking in Holstein cows reared under Egyptian conditions.

Material and Methods

Ethics statement

The study protocol was performed for cows under the Faculty of Veterinary Medicine, Benha University ethical standards with an approval number: BUFVTM 19-09-23.

Animals and samples

A total of 366 Holstein Friesian (HF) cows were used in this study. Animals belong to the Animal Production Research Institute in Kafr El-Sheikh Governorate. The animals, aged 6-10 years, were raised under consistent weather and nutritional conditions. The cows were housed in barns, given approximately 3.5 kg of commercial concentrate daily, and provided with grass and water on demand. Animals were divided into two groups: normal cyclic fertile (n=228, 62.3%) and anestrum for 3-12 months postpartum (n=138, 37.7%). Age at 1st calving (AFC), calving interval (CI), calf birth weight, milk yield, and days of milking data for the animals under the study were obtained from the farm records. Animal data consisted of an average from several prior pregnancies. Each animal underwent four rectal and B-mode ultrasound examinations over three months to ascertain its ovarian state and cyclicity. Follicles smaller than 1 mm served as indicators of ovarian inactivity. Blood samples were collected from the jugular vein of animals using vacutainer blood tubes containing EDTA and stored at -20 °C until used for DNA isolation.

DNA isolation and polymerase chain reaction amplification

The genomic DNA was isolated from blood samples using a blood DNA preparation - column Kit (Jena Bioscience, Jena, Germany) following the manufacturer's instructions. The NanoDrop 1000 UV-Vis Spectrophotometer (Thermo-Fisher Scientific, Wilmington, DE, USA) was used to measure the concentration and quality of DNA. DNA concentrations of the samples ranged from 1125 - 935 ng/ µl. The purity and integrity of DNA were appropriate, and the A260/A280 ratio was 1.90.

Three sets of primers were designed using Primer3 software version 4.0 (https://primer3.ut.ee/) to amplify the 3 regions of the HSPA2 gene. The details of the primers, annealing temperature, and expected product sizes are summarized in PCR conditions that were optimized for each primer set. PCR was carried out on approximately 100 ng of genomic DNA in a 25 µL reaction volume. The PCR reaction mixture consisted of 200 µM of each dNTP, 10X Taq polymerase assay buffer, 1 U of Taq polymerase enzyme, and 20 pM of each primer. The thermocycler conditions began with an initial denaturation at 95 °C for 4 minutes, followed by 33 cycles with denaturation at 94 °C for 30 seconds, with varying annealing temperatures based on a specific pair of primers (Table 1), and an extension at 72 °C for 30 seconds, followed by a final extension at 72 °C for 7 minutes. The PCR products were electrophoresed at 100 V in a 1.7% agarose gel in 1X TBE buffer containing 0.5 µg/mL ethidium bromide along with a DNA molecular size marker. The gels were visualized and documented using a gel documentation system (Gel Doc 1000, Bio-Rad, USA).

Single-strand conformation polymorphism (SSCP) technique

SSCP analysis was carried out on PCR products (208, 317, and 393 bp) of the HSPA2 genes to study the genetic variations in these segments. The PCR-SSCP technique was performed following the methods described by [26-28]. To summarize, 5.00 µl of the PCR products were mixed with 45 µl of denaturing solution, which consisted of 98% formamide, 20mM EDTA (pH 8.0), 0.05% xylene cyanol, 0.05% bromophenol blue, and 28 µl of ddH2O. The denaturation was performed at 95°C for 10 min, then snap cooling for 5 min on ice. The denatured samples were then loaded onto an 11% polyacrylamide gel containing (29:1 acrylamide: bis-acrylamide), 10 ml of 1xTBE buffer (Tris-base, Boric acid, and EDTA), 2.5 ml glycerol, 17.5 ml of deionized water, 400µl of APS solution (ammonium persulfate), and 40µl of TEMED (N, N, N′, N′-Tetramethyl ethylene diamine). Electrophoresis was carried out in 0.9x TBE buffer at 4°C, 160V, and 65 mA for 18 hours. Ethidium bromide (0.5µg/ml) staining was used to visualize the isolated DNA fragments on the polyacrylamide gels, which were then photographed using the Bio-Rad Gel-Doc System, USA.

Sequencing analysis

The PCR purification spin procedure (QIAGEN) and QIAquick PCR purification kit (QIAGEN) were used to purify the DNA fragments obtained from PCR reactions. The purified samples were then prepared for sequencing using an ABI PRISM 3730XL analyzer from Macrogen in Seoul, South Korea. The BLAST program [29] was used for sequence identification and confirmation during the sequencing analysis and alignments. For sequence assembly, alignment, and SNP detection, the Bioedit (7.0.5.3) software [30] and CodonCode Aligner 10.0.2 (CodonCode Corp., Dedham, MA, USA) were employed.

Statistical analysis

A two-way analysis of variance was performed on the data using the general linear model and the XLSTAT software [31]. Genotype and animal status were the primary determinants. Age at first calving, calving interval, calf birth weight, milk output, and days of milking were the traits that were evaluated.

The following model was employed:

$$Yijk= \mu + Si + Gj + SGij + eijk.$$

Where:

Y_{ijk}: The kth observation of the j^{th} genotype inside the i^{th} animal status.

µ: Overall mean.

S_i: Effect of the i^{th} animal status.

G_j: Effect of the j^{th} genotype

SG_{ij}: Interaction between the i^{th} animal status and the j^{th} genotype.

e_{ijk}: The random error.

All data are reported using the least square means (LSM) ± standard errors (SE). Duncan's multiple range test was used to separate mean values once significance was determined [32]. A significant threshold of 5% was selected.

Results

PCR-SSCP analysis of three fragments of the bovine *HSPA2* gene was carried out on the genomic DNA of 366 cows to determine the genetic variability in the *HSPA2* gene. The PCR amplification had been detected at 208, 317, and 393 bp for *HSPA2* F1, *HSPA2* F2, and *HSPA2* F3, respectively (Fig.1). The PCR-SSCP analysis revealed three different patterns in *HSPA2* F1 and *HSPA2* F2 showed a monomorphic pattern, while *HSPA2* F3 showed only two patterns (Fig.2). The sequence analysis of the different three patterns of fragment 1 confirmed our results and the sequence of the amplicon showed three different genotypes (CC, CT, and TT) with only one SNP as a transition single base substitution mutation located at 10:76649474 as ten refers to the chromosome number 10, and Consensus nucleotide C changed to the mutant nucleotide T; (C/T) with identification number (ID) rs377789074 as an untranslated region SNP (UTR) mutation. HSPA2 fragment 3 showed only two genotypes (CC and CT) with only one synonymous SNP located at 10:76680875 (C/T) with ID rs132895070 (Figs. 3, 4, and 5).

In the two cow groups, fertile and anestrum, the CC genotype was the most frequent at 54%, while CT occurred at a frequency of 5%, and TT at 41% in *HSPA2* fragment 1. In fragment 3, the frequency was 90% for CC, 10% for CT, and TT was absent. Holstein Friesian cattle populations under study were found to be in Hardy-Weinberg equilibrium, as determined by a Chi-square test. The genotype, allele frequencies, and Hardy-Weinberg equilibrium are presented in Tables (2 and 3).

According to data from the *HSPA2* fragment 1 gene, there are no significant differences in CI and calf birth weight between fertile and anestrum cows. However, anestrum cows had significantly lower milk yield during lactation and fewer days of milking compared to fertile cows (Table 4 and 5). The fertile animals had lower AFC than the anestrum group. The CT genotype was associated with the shortest CI, the highest calf birth weight and the longest AFC. The CT genotype had lower milk yield and days of milking. There was an interaction between the animal status and genotype, as indicated in (Table 4). In fertile HF cattle, the AFC significantly increased with the CT genotype, while it significantly decreased in anestrum cows. Additionally, the lower CI (383.00 ± 41.28 days) was associated with the CT genotype in anestrum and fertile HF cattle. Moreover, a higher calf birth weight (37.66 ± 1.67 kg) was also associated with the CT genotype compared to other observed genotypes in both anestrum and fertile animals. Furthermore, there

were significantly lower milk yields and days of milking in the CT genotype in fertile and anestrum cows.

According to data from *HSPA2* fragment 3, the CT genotype is significantly associated with the shortest CI and the highest calf birth weight (Table 5). The CT genotype and its interaction with the animal status were consistent with the genotypes, and animal status did not influence the significance of the data, as the CT genotype was associated with the shortest CI and the highest calf birth weight in both fertile and anestrum animals. The CT genotype was associated with lower AFC, milk yield, and days of milking in anestrum cows.

Discussion

With the rising temperatures and scarcity of water resources worldwide due to climate change, farmers are seeking livestock species with genetic traits that make them better adapted to environmental stress. By selecting animals based on their genetic adaptability, farmers hope to improve sustainability in livestock systems and ensure a stable food supply for the growing global population [5, 18, 20, 21]. Our study hypothesized that genetic variation in the *HSPA2* gene that plays a significant role in thermotolerance is linked to reproductive traits such as calving interval, age at first calving, and calf birth weight. However, our focus was on Holstein Friesian cattle raised under Egyptian conditions to examine the impact of *HSPA2* gene polymorphisms on some productive and reproductive performance in fertile and anestrum cattle.

In the current investigation, two SNP loci were recorded, the first in the UTR region of Exon1 C160T while the other within the coding region of *HSPA2* in Exon 3 was identified as C224T. Similarly, Onasanya et al, [21] identified four SNP loci within the coding region of HSP genes. Three of these were transitions (T134C in White Fulani, A208G in Ambala, G90A in Sokoto Gudali), while one was a transversion (C197T in Red Bororo), indicating gene polymorphism among Nigerian native cattle breeds. These findings are consistent with previous reports by Lamb et al, [33], who identified eight SNPs in different cattle breeds, and Bhat et al, [18], who reported two SNPs (G>T and G>C) at site 149 in the Indian Tharparkar breed. Suhendro et al, [8] found that Bali cattle raised at higher altitudes had a polymorphic SNP g.-69T>G of the *HSPA1A* gene. The different genotypes of the *HSPA1A* gene were significantly associated with physiological responses and heat tolerance coefficient. Their findings suggest that the GG genotype could be used as a marker for selecting Bali cattle with low physiological attributes. Moreover, Verma et al, [34] reported polymorphisms (two SNP loci) of the HSPB8 gene in the Indian Sahiwal breed. Additionally, Li et al, [35] identified five novel SNPs within the coding region of HSP70 in Chinese Holstein cattle, while Deb et al, [36] found four SNPs in Frieswal crossbred cattle. Sodhi et al, [12] reported high variability of HSP70 (54 SNPs) in three breeds of Indian Zebu and riverine Buffalo. Moreover, Rosenkrans et al, [24, 25] found associations between SNPs at *HSPA* and the reproductive characteristics of animals, including calving traits in cattle.

The coding sequence of *HSPA* was screened and a transversion mutation (SNP) was found, resulting in changes to the amino acid sequence. The GG genotype in mutation G1128T was more frequent than the TT genotype [24]. Previous studies have shown that the polymorphism in the coding sequence of *HSPA* in Bos Taurus and Bos indicus resulted in changes to the amino acids, which could potentially alter gene expression [12, 17, 37].

Our study revealed that there is a connection between the CT genotype in *HSPA2* and a decrease in milk yield and the number of days of milking. In this respect, Deb et al, [36] examined the promoter variants of the *HSPA* gene in Frieswal crossbred cattle and found that the *HSPA* gene is polymorphic and could potentially be used to select dairy cattle with higher milk production and better tolerance to heat. Specifically, the CC genotypes of the *HSPA* gene were found to be associated with a significant increase in heat tolerance coefficient for C-genotypic variants. This can be explained by the fact that heat stress leads to a negative energy balance, which affects the normal secretory functions of dairy cattle, resulting in reduced milk production. Under heat stress, mammary glands produce more (HSPs) to protect and maintain their cells [38]. In our study, we found that the CT genotype of the *HSPA2* gene has a positive impact on reproductive traits in cattle. Cattle with the CT genotype showed lower CI and higher calf birth weights. However, among fertile animals, the CT genotype was negatively associated with age at first calving. The change in the genotype-phenotype relationship could be influenced by the number and status of fertile and anestrus animals in our study. Furthermore, to confirm and strengthen these results, our future studies on genetic variations should involve larger animal populations.

Conclusion

The CT genotype of the *HSPA2* gene might serve as a genetic marker for reduced calving interval and higher birth weight in fertile and anestrum cattle groups. However, the CT genotype was associated with lower age at first calving in anestrum than fertile cows. Additional research is necessary to confirm these results across diverse populations with a large number of animals.

Acknowledgments

Authors want to present great appreciation for the all staff members of Animal Reproduction & AI Department, Veterinary Research Institute, National Research Centre and Cell Biology Department, Institute of Biotechnology Research Center for their valuable help and guidance Theriogenology Department, Faculty of Veterinary Medicine, Benha University, Egypt.

Funding statement

This study didn't receive any funding support.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

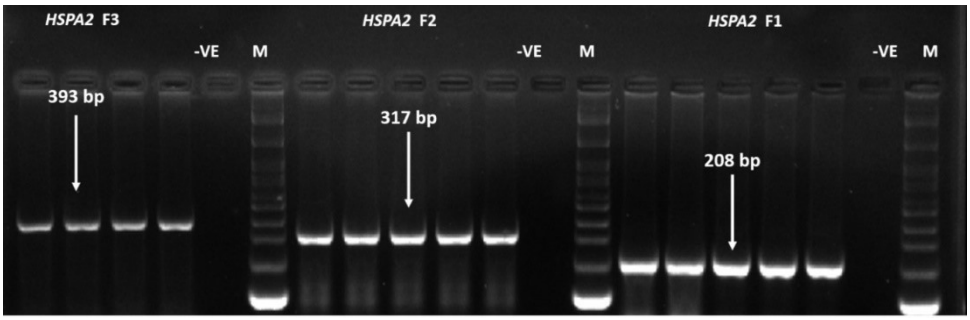

Fig. 1. Ethidium bromide-stained gel of PCR amplification for 3 fragments of *HSPA2* gene. M: 100 -bp ladder marker, -ve: control negative and PCR product of 208, 317, and 393 bp.

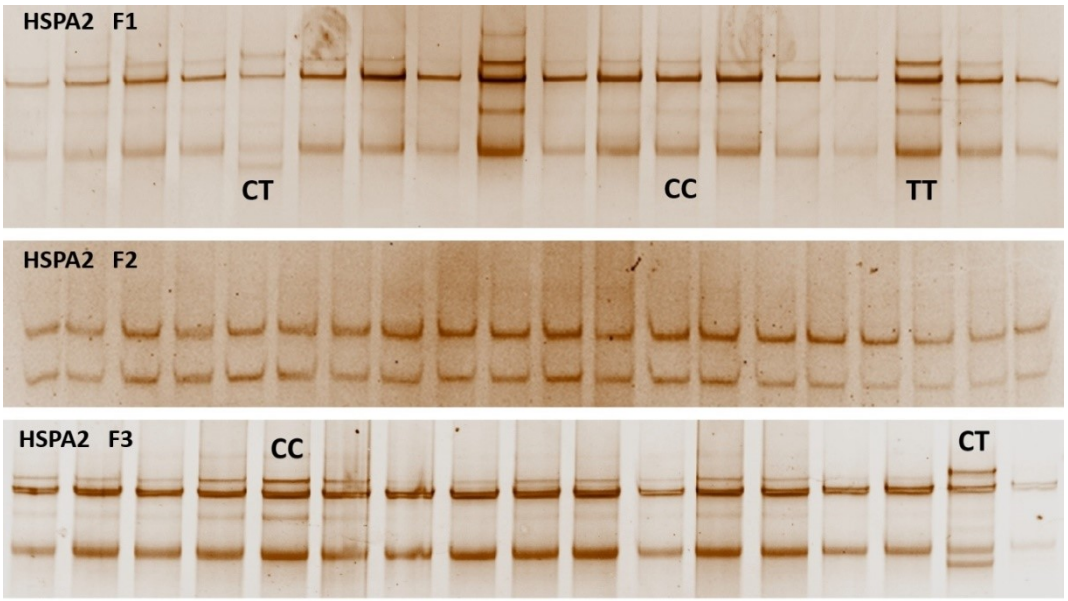

Fig. 2. Ethidium bromide-stained 11% polyacrylamide gel of *HSPA2* three primers PCR products showing three different SSCP patterns in *HSPA2* F1, monomorphic HSPA2 F2, and two SSCP patterns in *HSPA2* F3.

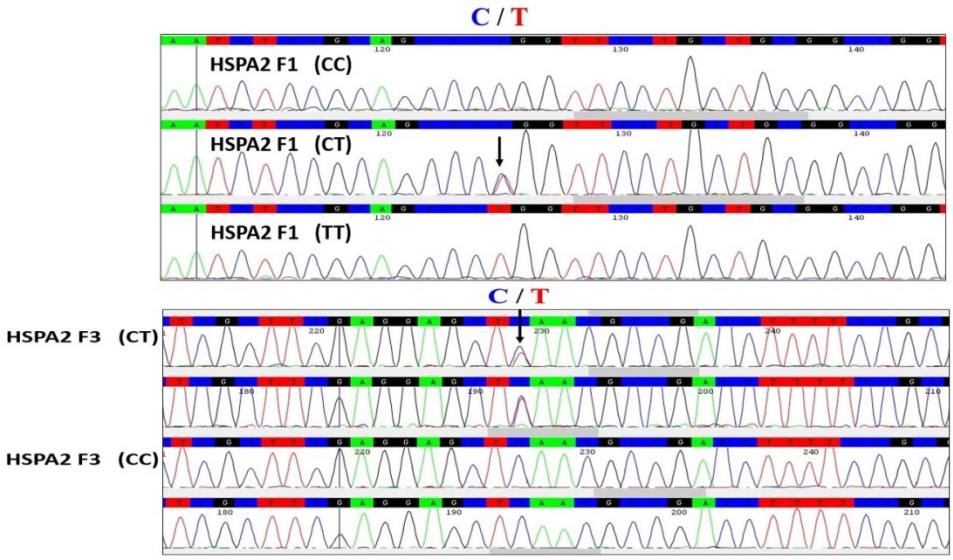

Fig. 3. Sequence analysis of the *HSPA2* gene showed the 2 SNPs as a transition single base substitution mutation; C/T.

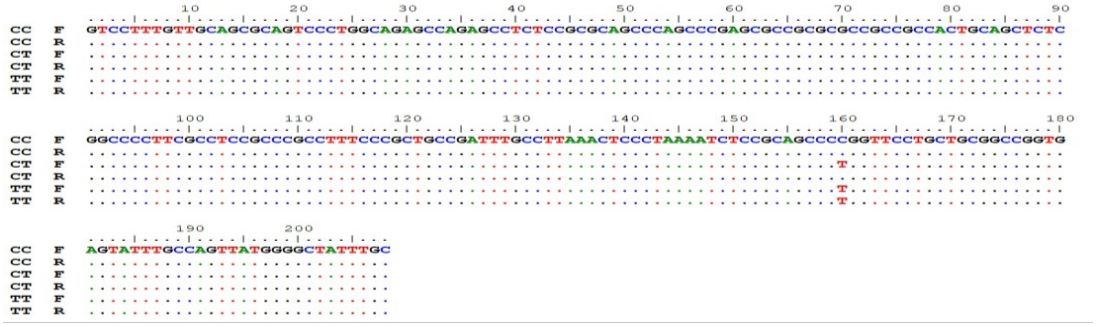

Fig. 4. Sequence Alignment of different genotypes of the *HSPA2 F1* gene.

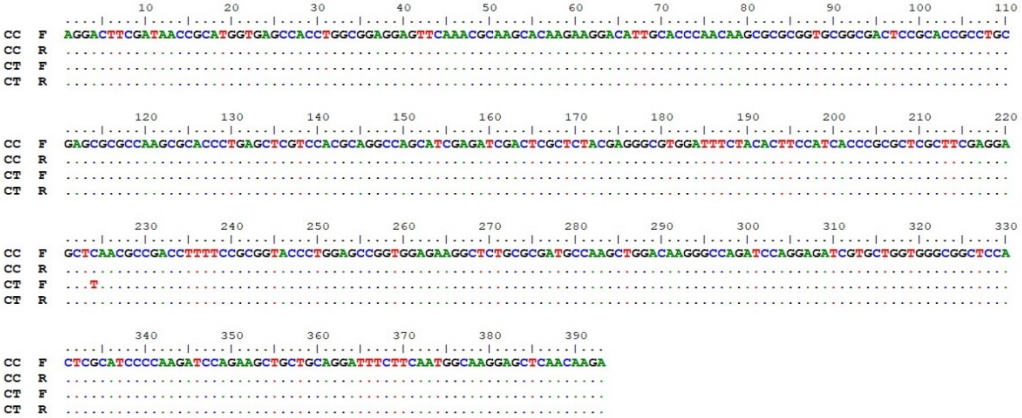

Fig.5. Sequence Alignment of different genotypes of the *HSPA2* F3 gene.

TABLE 1. *HSPA2* gene Primer Details.

Gene name		Sequence 5\ to 3\	PCR Product size	Annealing temperature (°C)
***HSPA2* Fragment 1**	**F**	GTCCTTTGTTGCAGCGCAGTC	**208 bp**	**56**
	R	GCAAATAGCCCCATAACTGGCA		
***HSPA2* Fragment 2**	**F**	AGGAGCCTGCTTCTATCACCTA	**317 bp**	**61**
	R	TCCGTTTTCCCTGCTTGTTTTAC		
***HSPA2* Fragment 3**	**F**	AGGACTTCGATAACCGCATGG	**393 bp**	**63**
	R	TCTTGTTGAGCTCCTTGCCA		

TABLE 2. Frequency of genotypes and alleles for *HSPA2* gene fragment 1 in the population of Holstein-Friesian cattle.

Status	No.	Observed Genotype			Expected Genotype			Allele Frequencies		Hardy-Weinberg equilibrium	
		CC	CT	TT	CC	CT	TT	C	T	x^2 *test*	*P value*
Fertile	228	0.55	0.05	0.40	0.34	0.48	0.18	0.58	0.42	27.58	0.0001
Anestrum	138	0.52	0.04	0.44	0.29	0.50	0.21	0.54	0.46	31.72	0.0001
Total	366	0.54	0.05	0.41	0.32	0.48	0.20	0.57	0.43	46.83	0.0001

TABLE 3. Frequency of genotypes and alleles *HSPA2* gene fragment 3 in the population of Holstein-Friesian cattle.

Status	No.	Observed Genotype			Expected Genotype			Allele Frequencies		Hardy-Weinberg equilibrium	
		CC	CT	TT	CC	CT	TT	C	T	x^2 *test*	*P value*
Fertile	228	0.90	0.10	0.00	0.90	0.10	0.00	0.95	0.05	9.30	0.0023
Anestrum	138	0.91	0.09	0.00	0.92	0.08	0.00	0.96	0.04	24.53	0.0001
Total	366	0.90	0.10	0.00	0.90	0.10	0.00	0.95	0.05	0.017	0.8963

TABLE 4. Least square means ± standard error (SE) of reproductive and productive traits of *HSPA2* fragment 1 gene derived from fertile and anestrum groups.

	Age at 1st calving (month)	Calving Interval (days)	Calf Birth Weight (Kg)	Milk Yield (Kg)	Days of Milking (days)
Animal Status					
Fertile	33.52± 0.45 ^{a}	451.78 ± 10.80 ^{a}	30.20 ± 0.43 ^{a}	2952.07 ± 81.64 ^{a}	342.43 ± 7.99 ^{a}
Anestrum	35.92± 0.64 ^{b}	470.64 ± 15.21 ^{a}	29.17 ± 0.61 ^{a}	2498.22 ± 114.9 ^{b}	279.65 ± 11.2 ^{b}
Probability	**0.002**	**0.371**	**0.468**	**0.021**	**< 0.001**
Genotype HSPA2 fragment 1					
CC	34.61 ± 0.35 ^{b}	483.64 ± 8.23 ^{a}	28.90 ± 0.33 ^{b}	2880.32 ± 62.60 ^{a}	317.26 ± 6.13 ^{a}
CT	37.50 ± 1.06 ^{a}	396.50 ± 25.28 ^{b}	32.41 ± 1.02 ^{a}	1908.58 ± 65.75 ^{c}	229.54 ± 18.7 ^{b}
TT	34.21 ± 0.36 ^{b}	481.53 ± 8.70 ^{a}	28.45 ± 0.35 ^{b}	2594.53 ± 191.0 ^{b}	314.32 ± 6.44 ^{a}
Probability	**0.015**	**0.004**	**0.001**	**< 0.0001**	**< 0.0001**
Animal Status * Genotype HSPA2 fragment 1					
Fertile * CC	34.45 ± 0.39 ^{bc}	463.95 ± 9.23 ^{bc}	29.50 ± 0.37 ^{b}	3193.72 ± 69.74 ^{a}	347.85 ± 6.83 ^{a}
Fertile * CT	44.00 ± 1.23 ^{a}	410.00 ± 29.19 ^{c}	27.16 ± 1.18 ^{b}	1585.91 ± 220.5 ^{c}	205.58 ± 21.6 ^{c}
Fertile * TT	35.20 ± 0.45 ^{b}	487.40 ± 10.65 ^{ab}	28.74 ± 0.43 ^{b}	2732.56 ± 80.53 ^{ab}	329.88 ± 7.89 ^{a}
Anestrum * CC	34.77 ± 0.58 ^{bc}	503.33 ± 13.76 ^{a}	28.36 ± 0.56 ^{b}	2566.92 ± 103.9 ^{b}	286.68 ± 10.2 ^{b}
Anestrum * CT	31.00 ± 1.74 ^{c}	383.00 ± 41.28 ^{c}	37.66 ± 1.67 ^{a}	2231.25 ± 311. 9 ^{b}	253.50 ± 30.5 ^{bc}
Anestrum * TT	33.22 ± 0.58 ^{c}	475.66 ± 13.76 ^{abc}	28.17 ± 0.56 ^{b}	2456.50 ± 103.9 ^{b}	298.77 ± 10.2 ^{b}
Probability	**0.0001**	**0.005**	**0.0001**	**0.003**	**0.012**

S.O.V source of variation ** a...c. Means within trait, followed by different superscripts, differ significantly (Duncan, 1955*).

TABLE 5. Least square means ±standard error (SE) of reproductive and productive traits of *HSPA2* fragment 3 gene derived from fertile and anestrum groups.

	Age at 1st calving (month)	Calving Interval (days)	Calf Birth Weight (Kg)	Milk Yield (Kg)	Days of Milking (days)
Animal Status					
Fertile	33.52± 0.45 [a]	451.78 ± 10.80 [a]	30.20 ± 0.43 [a]	2952.07 ± 81.64 [a]	342.43 ± 7.99 [a]
Anestrum	35.92± 0.64 [b]	470.64 ± 15.21 [a]	29.17 ± 0.61 [a]	2498.22 ± 114.9 [b]	279.65 ± 11.2 [b]
Probability	0.002	0.371	0.468	0.021	< 0.001
Genotype *HSPA2* fragment 3					
CC	34.54 ± 0.27 [a]	484.95 ± 6.15 [a]	28.49 ± 0.25 [b]	2701.84 ± 48.93 [a]	311.96 ± 4.74 [a]
CT	34.62 ± 0.79 [a]	412.12 ± 17.85 [b]	32.49 ± 0.72 [a]	2757.90 ± 141.9 [a]	309.76 ± 13.7 [a]
Probability	0.925	0.000	0.000	0.709	0.880
Animal Status * Genotype *HSPA2* fragment 3					
Fertile * CC	35.10 ± 0.32 [a]	475.84 ± 7.17 [a]	28.75 ± 0.29 [b]	2869.52 ± 57.07 [b]	330.31 ± 5.53 [a]
Fertile * CT	36.75 ± 0.92 [a]	426.75 ± 20.61 [b]	31.67 ± 0.84 [a]	3335.13 ± 163.9 [a]	353.98 ± 15.8 [a]
Anestrum * CC	34.00 ± 0.44 [b]	494.05 ± 9.99 [a]	28.22± 0.41 [b]	2534.15 ± 79.51 [c]	293.61 ± 7.70 [b]
Anestrum * CT	32.50 ± 1.30 [b]	397.50 ± 29.15 [b]	33.31 ± 1.19 [a]	2180.68 ± 231.8 [c]	265.54 ± 22.4 [b]
Probability	0.062	0.210	0.158	0.007	0.076

S.O.V source of variation ** a...c. Means within trait, followed by different superscripts, differ significantly (Duncan, 1955*).

References

1. Key, N., Sneeringer, S. & Marquardt, D. Climate change, heat stress, and US dairy production. *USDA-ERS Economic Research Report*, **175**, (2014).
2. Berman, A. Invited review: Are adaptations present to support dairy cattle productivity in warm climates? *Journal of Dairy Science*, **94** (5), 2147-2158. (2011).
3. Skliarov, P., Kornienko, V., Midyk, S. & Mylostyvyi, R. Impaired reproductive performance of dairy cows under heat stress. *Agriculturae Conspectus Scientificus*, **87** (2), 85-92. (2022).
4. Saeed, A., Wajid, A., Abbas, K., Ayub, G., Din, A. M., Ain, Q., Zahoor ,S., Ali, A., Babar, M., & Hussain, T. Novel polymorphisms in complete coding region of heat shock protein 70.1 gene in subtropically adapted red Sindhi cattle breed. *International Journal of Agriculture & Biology*, **26**,555–560 (2021).
5. Abdelnour, S. A., Abd El-Hack, M. E., Khafaga, A. F., Arif, M., Taha, A. E. & Noreldin, A. E. Stress biomarkers and proteomics alteration to thermal stress in ruminants: A review. *Journal of Thermal Biology*, **79**, 120-134. (2019).
6. Das, R., Sailo, L., Verma, N., Bharti, P., Saikia, J. & Kumar, R. Impact of heat stress on health and performance of dairy animals: A review. *Veterinary World*, **9** (3), 260(2016).
7. Yer, E. N., Baloglu, M. C. & Ayan, S. Identification and expression profiling of all Hsp family member genes under salinity stress in different poplar clones. *Gene*, **678**, 324-336. (2018).
8. Suhendro, I., Jakaria, J., Priyanto, R., Manalu, W., & Noor, R. R. The Association of Single Nucleotide Polymorphism-69T> G HSPA1A Gene with Bali Cattle Heat Tolerance. *Tropical Animal Science Journal*, **45** (4), 429-435. (2022).
9. Si, F. L., Qiao, L., He, Q. Y., Zhou, Y., Yan, Z. T., & Chen, B. HSP superfamily of genes in the malaria vector Anopheles sinensis: diversity, phylogenetics and association with pyrethroid resistance. *Malaria Journal*, **18**, 1-15. (2019).
10. Tripathy, K., Sodhi, M., Kataria, R. S., Chopra, M., & Mukesh, M. In silico analysis of HSP70 gene family in bovine genome. *Biochemical Genetics*, **59**, 134-158. (2021).
11. Craig, E. A. Hsp70 at the membrane: driving protein translocation. *BMC Biology*, **16**, 1-11. (2018).
12. Sodhi, M., Mukesh, M., Kishore, A., Mishra, B. P., Kataria, R. S., & Joshi, B. K. Novel polymorphisms in UTR and coding region of inducible heat shock protein 70.1 gene in tropically adapted Indian zebu cattle (Bos indicus) and riverine buffalo (Bubalus bubalis). *Gene*, **527**(2), 606-615. (2013)
13. Young, J. C. Mechanisms of the Hsp70 chaperone system. *Biochemistry and Cell Biology*, **88** (2), 291-300. (2010).
14. Gallagher, D. S., Grosz, M. D., Womack, J. E., & Skow, L. C. Chromosomal localization of HSP70 genes in cattle. *Mammalian Genome*, **4**, 388-390. (1993)
15. Abbas, Z., Hu, L., Fang, H., Sammad, A., Kang, L., Brito, L. F., & Wang, Y. Association analysis of polymorphisms in the 5′ flanking region of the HSP70 gene with blood biochemical parameters of lactating Holstein cows under heat and cold stress. *Animals*, **10** (11), 2016 (2020).
16. Badri, T., Alsiddig, M., Lian, L., Cai, Y., & Wang, G. Single nucleotide polymorphisms in HSP70–1 gene associated with cellular heat tolerance in Chinese Holstein cows. *Animal Gene*, **20**, 200114 (2021).
17. Basiricò, L., Morera, P., Primi, V., Lacetera, N., Nardone, A., & Bernabucci, U. Cellular thermotolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows. *Cell Stress and Chaperones*, **16** (4), 441-448.2011) .
18. Bhat, S., Kumar, P., Kashyap, N., Deshmukh, B., Dige, M. S., Bhushan, B. & Singh, G. Effect of heat shock protein 70 polymorphism on thermotolerance in Tharparkar cattle. *Veterinary World*, **9** (2), 113(2016).
19. Dikmen, S. E. R. D. A. L., Wang, X. Z., Ortega, M. S., Cole, J. B., Null, D. J., & Hansen, P. J. Single nucleotide polymorphisms associated with thermoregulation in lactating dairy cows exposed to heat stress. *Journal of Animal Breeding and Genetics*, **132** (6), 409-419(2015) .
20. Mkize, L. S., & Zishiri, O. T. Novel single nucleotide polymorphisms in the heat shock protein 70.1 gene in South African Nguni crossbred cattle. *Tropical Animal Health and Production*, **52** (2), 893-901. (2020).
21. Onasanya, G. O., Msalya, G. M., Thiruvenkadan, A. K., Sreekumar, C., Tirumurugaan, G. K., Fafiolu, A. O., & Okpeku, M. Heterozygous single-nucleotide polymorphism genotypes at heat shock protein 70 gene potentially influence thermo-tolerance among four zebu breeds of Nigeria. *Frontiers in Genetics*, **12**, 642213. (2021).
22. Archana, P. R., Aleena, J., Pragna, P., Vidya, M. K., Niyas, A. P. A., Bagath, M., & Bhatta, R. Role of heat shock proteins in livestock adaptation to heat stress. *Journal of Dairy, Veterinary & Animal Research*, **5** (1), 00127. (2017).
23. Hassan, F. U., Nawaz, A., Rehman, M. S., Ali, M. A., Dilshad, S. M., & Yang, C. Prospects of HSP70 as a genetic marker for thermo-tolerance and immuno-modulation in animals under climate change scenario. *Animal Nutrition*, **5** (4), 340-350. (2019).
24. Rosenkrans Jr, C., Banks, A., Reiter, S., & Looper, M. Calving traits of crossbred Brahman cows are associated with Heat Shock Protein 70 genetic polymorphisms. *Animal Reproduction Science*, **119** (3-4), 178-182. (2010).
25. Said, S., & Putra, W. P. B. Novel single nucleotide polymorphisms (SNPs) in the 5'UTR of bovine heat shock protein 70 (bHsp70) gene and its association with service per conception (S/C) of Pasundan cattle. *Biodiversitas Journal of Biological Diversity*, **19** (5), 1622-1625. (2018).
26. Gasser, R. B., Hu, M., Chilton, N. B., Campbell, B. E., Jex, A. J., Otranto, D., & Zhu, X. Single-strand conformation

polymorphism (SSCP) for the analysis of genetic variation. *Nature Protocols*, **1** (6), 3121-3128. (2006).
27. Sosa, A. S., Mahmoud, K. G. M., Kandiel, M. M., Eldebaky, H. A., Nawito, M. F., & Abou El-Roos, M. E. Genetic characterization of FSH beta-subunit gene and its association with buffalo fertility. *Asian Pacific Journal of Reproduction*, **6** (5), 193-196(2017).
28. Mahmoud, K. G., Sakr, A. M., Ibrahim, S. R., Sosa, A. S., Hasanain, M. H., & Nawito, M. F. GnRHR gene polymorphism and its correlation with semen quality in Buffalo bulls (Bubalus bubalis). *Iraqi Journal of Veterinary Sciences*, **35** (2), 381-386. (2021).
29. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. Basic local alignment search tool. *Journal of Molecular Biology*, **215** (3), 403-410. (1990).
30. Hall, T. A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**(41), 95-98. (1999).
31. XLSTAT, Addinsoft. Data analysis and statistics with Microsoft Excel Paris France MacOS. (2019).
32. Duncan, D. B. Multiple range and multiple F tests. *Biometrics*, **11** (1), 1-42. (1955).
33. Lamb, M., Okimoto, R., Brown, M., Brown Jr, H., Johnson, Z., & Rosenkrans Jr, C. Relationship between single nucleotide polymorphisms in the bovine heat shock protein 70 gene and milk characteristics of beef cows. *Journal of Animal Science*, **85** (4),1-7 (2007).
34. Verma, N., Gupta, I. D., Verma, A., Kumar, R., Das, R., & MR, V. Novel SNPs in HSPB8 gene and their association with heat tolerance traits in Sahiwal indigenous cattle. *Tropical Animal Health and Production*, **48**, 175-180. (2016).
35. Li, Q., Han, J., Du, F., Ju, Z., Huang, J., Wang, J., & Zhong, J. Novel SNPs in HSP70A1A gene and the association of polymorphisms with thermo tolerance traits and tissue specific expression in Chinese Holstein cattle. Molecular *Biology Reports*, **38**, 2657-2663 (2011).
36. Deb, R., Sajjanar, B., Singh, U., Kumar, S., Brahmane, M. P., Singh, R., & Sharma, A. Promoter variants at AP2 box region of Hsp70. 1 affect thermal stress response and milk production traits in Frieswal cross bred cattle. *Gene*, **532** (2), 230-235 (2013).
37. Huang, P., Lu, C., Li, J., Xu, J., Liu, Z., Wang, Q., & Cai, Y. Mutations in HSP70-2 gene change the susceptibility to clinical mastitis in Chinese Holstein. *Gene*, **559** (1), 62-72 (2015).
38. Al Reyad, M., Sarker, M. A. H., Uddin, M. E., Habib, R., & Rashid, M. H. U. Effect of heat stress on milk production and its composition of Holstein Friesian crossbred dairy cows. *Asian Journal of Medical and Biological Research*, **2** (2), 190-195 (2016).

ارتباط جين بروتين الصدمة الحراري A2 ببعض الصفات التناسلية في أبقار الهولشتاين فريزيان المرباه في مصر

منة الله نادي عيد [1] ، أحمد سيد عبدالرحيم محمد سوسة [2]، حسن رمضان حسن درويش[3]، كريمة غنيمي محمد محمود[2]، محمود السيد عابد أبوالروس[1] و الشيماء الحسيني حسب النبي[1]

[1] قسم التوليد والتناسل والتلقيح الاصطناعي – كلية الطب البيطري – جامعة بنها- القليوبية- مصر.

[2] قسم التكاثر في الحيوان والتلقيح الاصطناعي- معهد البحوث البيطرية- المركز القومي للبحوث – الدقي- مصر

[3] قسم بيولوجيا الخلية - معهد بحوث التقنيات الحيوية - المركز القومي للبحوث – الدقي- مصر

الملخص

ارتبطت تعددات أشكال جين *HSPA2*، وهو أحد أفراد عائلة جينات HSP 70 في الأبقار، بتحمل الحرارة والأداء التناسلي. تهدف هذه الدراسة إلى تحديد دور التباين الوراثي في جين *HSPA2* في خصوبة أبقار الهولشتاين. تم تصنيف إجمالي 366 بقرة إلى مجموعتين: حيوانات ذات تأخر في دورة الشبق (37.7%) وأخري خصبة (62.3%). تم جمع البيانات التناسلية والإنتاجية من سجلات المزرعة وتشمل (العمرعند الولادة الأولى (AFC) والفترة بين الولادات (CI) ووزن العجل عند الولادة وإنتاج الحليب وأيام الحلب .تم عزل الحمض النووي الجينومي للأبقار، وأجريت PCR-SSCP للكشف عن ثلاثة أجزاء من جين*HSPA2* ؛ 208و317 و393 زوجًا قاعديا .كشف HSPA2 F1 عن ثلاثة أنماط مختلفة، وكان *HSPA2* F2 أحادي الشكل، وأظهر *HSPA2* F3 نمطين فقط. كشف تحليل تسلسل *HSPA2* F1 عن ثلاثة أنماط جينية (CC وCT وTT) مع طفرة استبدال قاعدة واحدة انتقالية (C/T) تقع عند 10:76649474 مع رقم تعريفي rs377789074. وفي الوقت نفسه، أظهر HSPA2 F3 نمطين جينيين (CC وCT) مع مع طفرة استبدال (C/T) تقع عند 10:76680875 ورقم تعريفي rs132895070. ارتبط النمط الجيني CT لجينات *HSPA2* F1 و*HSPA2* F3 بفترة ولادة أقصر ووزن عجل أثقل عند الولادة في كل من الأبقار الخصبة وغير الخصبة. وتشير نتائج الدراسة إلى أنه يمكن استخدام التباينات الجينية في جين HSPA2 كعلامة جزيئية للاختيار الجيني لتعزيز الأداء التناسلي في كل من الأبقار الهولشتاين الخصبة وغير الخصبة التي يتم تربيتها في مصر.

الكلمات الدالة: جين بروتين الصدمة الحرارية A2، تحديد النمط الجيني، تعدد أشكال النيكليوتيدات المفردة، الخصوبة، الأبقار.