Polymorphisms Detection in Heat Shock Brotein70 Gene (HSP70) and its Association with Semen Quality in Local Iraqi Goats

Abdulmuttaleb Hazim khaleel [1], Salih Hassan Al-Azzawi[2]

[1,2]Department of Animal Production - College of Agriculture University of Diyala- Iraq

[1]Corresponding author: abdmtaleb85@gmail.com

Article history:

Received: 27 December 2021
Accepted 13 March 2022
Published: 30 June 2022

Keywords: *Breeding value, Dominance variance, alleles substitute, semen, HSP70 gene, local Iraqi goats.*

Abstract

Heat shock protein 70 (HSP70) is a vital gene affecting semen quality traits. The study aimed to estimate the breeding values additive dominance variance of Iraqi black goat semen quality and investigate the effect of allele substitution in heat shock protein 70 gene (HSP70) carpine on some semen quality traits (volume, mass motility, a live sperm, and sperm concentration). DNA isolated from 15 Iraqi black goats was subjected to PCR amplification of the caprine HSP70 gene. Used Single-nucleotide polymorphism (SNP) to detect the variant DNA fragments that were sequenced. Synonymous Genotypes were detected on mutation locus (1528C/T). Results showed three genotypes (CC, CT, TT) frequencies of 0.53, 0.40, and 0.07, respectively, and gene frequency of 0.73 and 0.27 C and T, respectively. The population was in Hardy Weinberg equilibrium. The results showed a high level of concentration sperm for CC and CT genotype compared with TT genotype. The CC genotype was associated with negative B.V for volume ejaculate, mass motility, and live sperm percentages and was positively associated with sperm concentration. CT and TT genotypes showed positive B.V for volume ejaculate, mass motility, and live sperm but negative for sperm concentration. CC and TT genotypes showed negative dominance deviation for all traits studied, while heterozygous genotype CT showed positive dominance deviation for all semen quality traits. The highest genetic variation for live sperm (0.816) but most of this variation is from dominance variance (0.653), followed by Genetic variance for mass motility (0.481), most of it from Additive variance (0.39).

https://dx.doi.org/10.52951/dasj.22140103

Introduction

Domestic goats are essential genetic resources that adapt to local conditions and can be used to provide meat and milk to consumers. Local goat breeds may be used as sires or even dams for crossing with foreign breeds to produce hilly tolerant crosses to harsh environmental conditions and productivity (Al-Qudsi and Al-Rawi,2021; Khalil and Jassim,2018). The new Molecular genetics techniques enable the researchers to use genes of high economic importance found in certain breeds to be transferred to others) Khazraji *et al*., 2020).

Heat shock proteins (HSPs) are a group of proteins produced by the cells in response to stress conditions and are considered one of the most critical responses to stress where HSPs genes are activated (Habib and Saleh, 2019). The HSP70 gene consists of 1926 base pairs encoding the 641 amino acids found in the HSP70 protein of sheep and goats (Gade *et al.*,2010; Pawar *et al*.,2013). HSP70 gene in goats is located on chromosome 23 (Raza *et al*.,2021) HSP70 mitigates the effects of heat stress on cells by mitigating or preventing damage to proteins (Miova *et al*., 2015). HSP70 prevents the harmful effects of heat stress by inhibiting apoptosis, and this is the internal mechanism by which animals adapt to high

environmental temperatures (Mishra and Palai, 2014).

In mice, high expression of HSP70 protein limits testicular degeneration, confirming its protective role against high temperature (Kon and Endoh, 2001). The heat shock proteins HSP70 affect the characteristics of an organism's semen, as they bind to newly formed proteins and assist in the folding and synthesizing of proteins during spermatogenesis (Dun et al., 2012). It helps to deal with the negative effects of various stress conditions, including high temperatures (Gullo and Teoh, 2004), that a polymorphism in the hsp70 gene was associated with the sperm quality of pigs in the hot season (Huang et al., 2002). and the characteristics of bull sperm (Shrum *et al.,* 2010). The polymorphism in the HSP70 gene in bulls semen affects its quality. It is possible its use in artificial insemination programs as a marker that helps select good bulls for insemination (Gafer *et al*., 2015).

Genetic improvement using selection by genetic markers and genome information is primarily based on the additive effect, which plays an essential role in influencing the physiology and evolution of farm animals (Cloete *et al.,* 2004). Understanding the genetic characteristics of a herd requires an analysis of components of Additive variance (VA), which represents breeding value, dominance variance (VD), and supra-dominant variance (VI) (Falconer and Mackay, 1996).

This study aims to estimate the breeding values, calculate the total genetic variance and determine the additive and dominant variability as well as the effect of substitution of alleles and the average effect allele for locus mutation (1528C/T) of the HSP70 gene in Iraqi Bucks and their association with seminal traits (Volume and mass motility, sperm concentration and a live sperm).

Material and Methods

Ethical approval

The Scientific Ethical Committee of the animal production department College of Agriculture University of Diyala, Iraq, approved this study (22/3/2021).

Experimental animals:

This study was conducted in Iraq at the University of Diyala, on 15 Iraqi Bucks average age (1-2) year the average weight of 29 kilograms from 15/6/2021to 15/9/2021. It was placed in a semi-shaded barn, and it was fed a quantity of 500 grams per each Bucks, provide concentrated containing in the rate of 33% of the bran and 65% of barley, and the mixture was supplemented by adding the rate of 1% salt and 1% a mixture of vitamins and minerals, hay as a rough feed available most of the time.

Semen collection

Semen was collected using an artificial vagina in the morning at a rate of six ejaculates per buck tucked biweekly for three months. The semen was contained in a graduated glass tube where the semen volume was measured immediately after collection. Then the glass tube was placed in a water bath (temperature of 37 °C) found in the field laboratory. Mass motility was estimated by placing a drop of semen on a glass slide at 37°C under a microscope at 10× magnification. The wave motion was estimated based on the strength and intensity of moving waves (Walton, 1933). The percentage of live sperm was calculated according to the method of (Swanson and Bearden, 1951). Deads sperm examined under 400× microscopic magnification concentration was calculated according to the method (Salisbury *et al*., 1943).

Laboratory side:

DNA extraction: Blood samples were taken from the jugular vein, the blood was placed in test tubes containing anticoagulant EDTA) to extract DNA according to the (ABIOpure) protocol of the Advance

Scientific Company (ASCO) located in Iraq / Baghdad city (Al- Harithiya) and gene isolation Heat shock (HSP70) for sequence analysis for the period from 9/18/2021to 10/21/2021.

Primers design and the polymerase chain reaction

The studied part was identified by Primers shown in table (1), which were designed by the Advance Scientific Company (ASCO) using the program Primer3 belonging to the National Center for Biotechnology Information (NCBI) were supplied by a Korean company (Macrogen). The molecular detection of the HSP70 gene was carried out using the polymerase chain reaction technique using the reaction mixture consisting of the components detailed in Table (3) according to the program shown in table (2)

Table 1. Primers sequence used in the study

Primers sequence	Primer Name	Start	Stop	Amplicon size (pb)	Gene name	Annealing temp C°
Forward (Sense) 5-GACCTCAACAAGAGCATCAA -3	F	1432	1452	903 pb	HSP70	65
Reverse (Anti Sense) 5-GATCCCAACAGTCTCCATAAC-3	R	2314	2335			

Table 2. The PCR amplification program

Seq	Steps	temperature C^0	Time	Number cycle
1	Initial Denaturation	95	5 minutes	1 Cycle
2	Denaturation	95	30 second	30 Cycle
3	Annealing	65	30 second	
4	Extension	72	30 second	
5	Final Extension	72	7 minutes	1 Cycle
	Hold	10		

Table 3. Components of the polymerase chain reaction mixture

Components used for PCR reaction	volume
Master Mix	12.5µl
Forward primer	1 µl
Reverse primer	1 µl
Nuclease Free Water	7 µl
MgCl2	0.5µl
DNA	3 µl
Total volume	25 µl

Polymerase chain reaction and Electrophoresis:

Alagros Gel electrophoresis was used To check for PCR products. The electrical power for Electrophoresis was turned on at 100 volt / 50 mAmp for 60min. The electrophoresis product was a figure (1). The nucleotide sequence was determined by sending the polymerase chain reaction product to the Korean company (Macrogen) through the Advance Scientific Company.

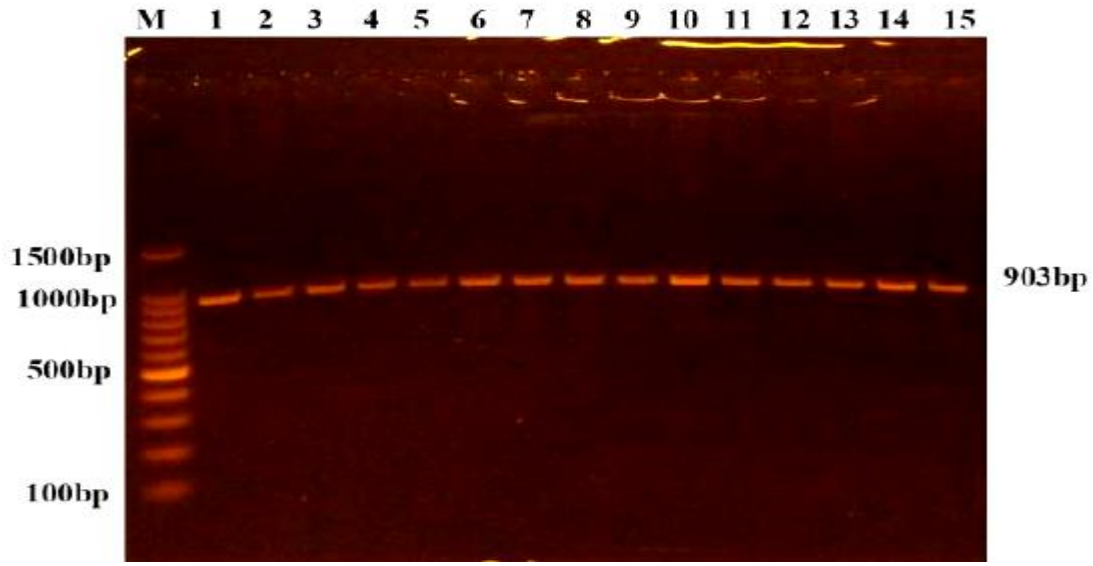

Fig. 1. The Electrophoresis of the product (PCR) of portion (HSP70) gene size 903 bp, which was studied for 15 samples of Iraqi bucks

Statistical analysis:

The genotypic frequencies and allelic frequencies were calculated using the SAS (2012) Version 9.1 program. The genotype distribution in this population was tested for Hardy-Weinberg using the appropriate Chi-square ($\chi 2$) test.

The equations for each calculated value were applied as follows (Falconer and Mackay ، 1996):

1- Average effect of allele C = q [a +d (q – p) = αC

2- Average effect of allele T = - p[a +d (q – p) = αT

3- Effect of substitution of alleles = αC - αT

4- Breeding values : CC = 2 αC ,CT = αC + αT ,TT = 2 αT

5- Dominant deviations : CC = $-2q^2$ d , CT = 2pqd , TT = $2p^2$ d

6- Different variances : VA = 2pq α^2 , VD = 4 $p^2q^2d^2$, VG = VA + VD

Results and Discussion

Percentages of genotypes and allelic in heterogeneity locus of sequences nitrogenous base (C1528T) in HSP70 gene for 15 samples of Iraqi bucks

The analysis using Sequences technology revealed heterogeneity in the gene (HSP70) at the locus (1528 C/T), and the genetic makeup of the experimental animals was determined as shown in table (4). It contained three genotypes CC (Wild), CT (heterozygous), and TT (recessive), as shown in Figure (2). Results showed that the nitrogen base was changed from C to T (1528 C/T). The percentage heterogeneity locus genotypes were 53, 40, and 7% for the genotypes CC, CT, and TT, respectively, with an overall genes frequency of 0.73 for C and 0.27 for the T allele. The values of the chi-square test (x^2) showed that all genotypic frequencies in the population were in Hardy Weinberg equilibrium ($p>0.05$), indicating the selection pressure on this site in the population was not too powerful.

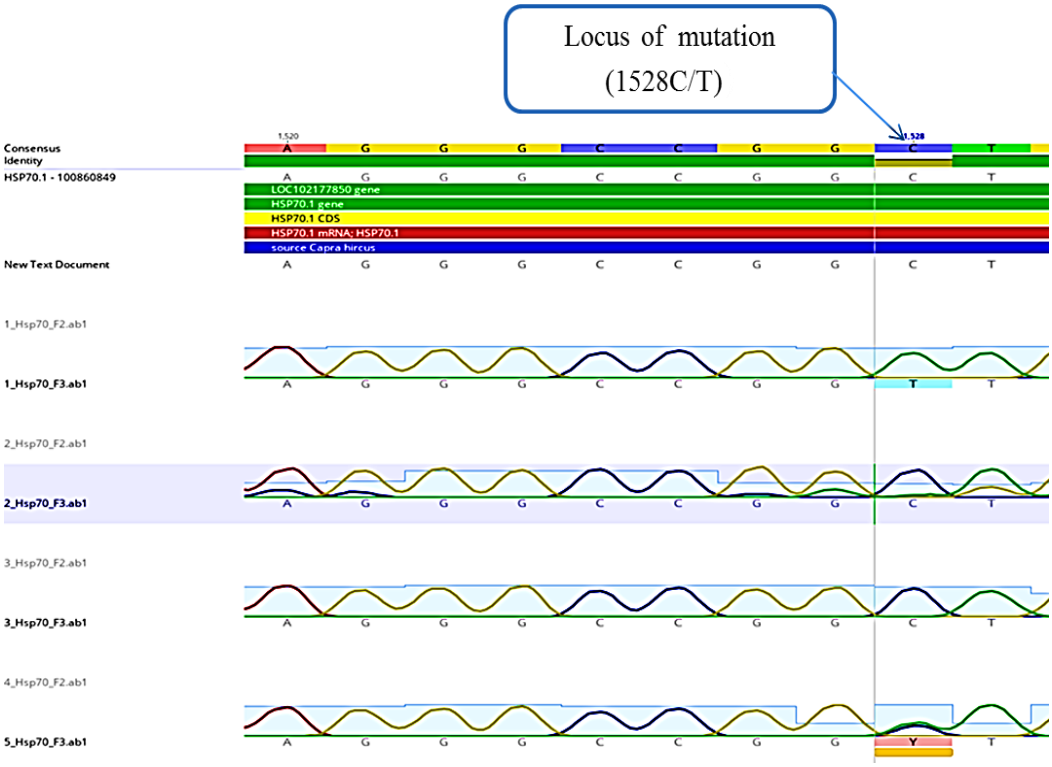

Fig. 2. The location of the variation (1528) in the third segment of the heat shock gene (HSP70) for a sample of local male Iraqi goats, where the letter (Y) symbolizes the fact that the mutation (transition mutation) where the nitrogen base (C) of the type pyrimidine was replaced by a nitrogen base (T) It is also a type of pyrimidine.

Table 4. Number and percentage for the genotype of locus sequences nitrogenous base (1528 C/ T) in gene HSP70 for 15 samples of Iraqi bucks

Genotype	Number	Percentage%
CC	8	53.00
CT	6	40.00
TT	1	7.00
SUM	15	100
Chi-square (χ^2) value	0.038	
Allelic Frequency		
C	0.73	
T	0.27	
NS P>0.05		

There was no difference between the average genotypes of volume ejaculate, mass motility, and live sperm. As for the concentration of sperm, there was a significant difference between the genotypes (Table5).

Table 5. Relationship of genotype (1528 C/T) with some fresh semen traits (mean and standard error) of volume ejaculate, Mass motility, live sperm, and sperm concentration

Trait	Genotype		
	CC	CT	TT
Volume ejuculate	0.9075± 0.032 a	0.945± 0.035 a	0.97± 0.152 a
Mass motility	76.56 ± 1.130 a	77.97± 1.277 a	77.83± 2.891 a
live sperm	82.75± 0.700 a	84.5± 1.051 a	82.15± 2.011 a
Sperm concentration	1.72 ± 0.023 ab	1.77± 0.031 a	1.63± 0.037 b

Different letters within the same row indicate a significant difference between the means at the probability level (0.05).

The results of the mean allele substitution in Table (6) showed that the mean substitution of the allele (T) instead of the allele (C) in this study is (0.034, 0.992, 0.643) for volume ejaculate, mass motility of sperms, and percentage of alive sperms respectively, and mean allele substitution (C) At the allele locus (T) is (- 0.034,-0.992,-0.643) which means that selection towards the allele (T) is essential to improve these traits. But regarding sperm concentration, It was the mean substitution of the allele (C) instead of the allele (T) in this study is (0.0004). The mean allele substitution (T) at the allele locus (C) is (-0.001), which means that selection towards the allele (C) is essential to improve this trait.

Table 6. Mean genetic effect and mean effect of genetic substitution of alleles for semen traits (Volume ejaculate, Mass motility, live sperm, and Sperm concentration) for the (1528 C/ T) locus in the HSP70 gene

Trait	Alleles	Average gene effect	The average effect of gene substitution
Volume ejuculate	C	-0.009	-0.034
	T	0.025	0.034
Mass motility	C	-0.268	-0.992
	T	0.724	0.992
live sperm	C	-0.174	-0.643
	T	0.469	0.643
Sperm Concentration	C	0.0004	0.001
	T	-0.001	-0.001

Effect of the genetic polymorphism of (1528C/T) of genetic variance component shown in Table (7) Bucks with (TT) and (CT) genotypes had higher mean ejaculate volume and Mass motility where the average ejaculate volume was (0.97 and 0.945 ml,) respectively and mass motility (77.97, 77.83%) respectively compared to the (CC) genotype where the average ejaculate volume was (0.9075 ml) and the Mass motility (76.56%). The genotypes (TT) and (CT) with the highest breeding value compared to genotype (CC). Therefore, it is possible to choose the genotype (TT, CT) to improve the characteristics of the semen (ejaculate volume and mass motility) because the breeding values are the value that can be

transferred to future generations (children) (Warmington and Kirton, 1990).

That individuals with the (CT) genotype have the highest mean percentage of live sperms, which was (84.5%) compared to the (CC) and (TT) genotypes, where the average rate of live sperms was (82.75 and 82.15%) respectively and genotype (TT, CT) which is the highest breeding value where it was (0.939 and 0.296) respectively, and that TT genotype is the least dominance deviation compared to genotype (CC, CT) and where the genotype (CT) It had the highest average percentage of live sperms and that its breeding values (0.296) are positive, which can be transmitted to future generations (Warmington and Kirton,1990). where the average effect of the allele and the mean substitution of the allele (T) was positive for these traits compared to the allele (C), which was negative. Therefore, it is possible to select the genotype (CT) to improve the trait (live sperm).

That the genotype (CT) recorded the highest average sperm concentration, which was (1.77×10^9/ml) with a significant difference from the two genotypes (TT, CC), where the average sperm concentration was (1.72 and 1.63×10^9/ml), as for the values of breeding, they were low for all structures, where the highest value was (0.001) for the genotype (CC). For the two genotypes (TT, CT), the Breeding value was (-0.001, -0.002), respectively, and the lowest sovereign deviation was recorded for the genotype (CC). Followed by the two genotypes (TT) and (CT), it was (-0.014, -0.101, 0.037) respectively. The genotype (CT) recorded the highest average sperm concentration. Still, its low breeding value may indicate a low probability of transmission of this trait as it was the average effect of the allele and the mean substitution of the allele (T) is negative for these traits compared to the allele (C), which was positive. Nikbin et al. (2014) reported only one study; two mutations were recorded at the site (74A>C) (ss836187517), and genotypes were AA, AC, CC site and (191C>G) (ss836187518) and genotypes were CC, CG, GG. The C variant allele at the (74A>C) mutation site and the G variant at the (191C>G) mutation site were more frequent. In contrast, the T allele was less frequent in the current study. In the study of Nikbin et al. (2014), it was found at the mutation site (191 C>G) that the pure genotypes (CC, GG) outperformed the heterozygous (CG) in (sperm concentration, mass motility, and live sperm) while the ejection size outperformed the heterozygous (CG) on the pure genotypes. As for the mutation site (74 A>C), the AC genotypes outperformed the pure genotypes (CC, AA) in sperm concentration. As for the (mass movement and live sperm), the heterozygous AC genotypes were the least significant. It is close to the result of the current study, where the genotype CT was significantly better in sperm concentration. In a study by Nikbin et al. (2014), it was found that the C allele of the mutation (191C>G) had a positive effect on sperm concentration, as well as the site of the mutation (74 A>C) .The C allele was the best in increasing the sperm concentration in the semen, and this result is close to the result of the current study, where the C allele was the best in increasing the sperm concentration .This study could be an excellent way to select animals, but more studies are needed to prove this assumption.

Table 7. Breeding value, dominance deviation, and genetic variations genotypes for the (1528 C/T) locus in the HSP70 gene for semen traits (volume ejaculate, mass motility, live sperm, and spermconcentration)

Volume ejaculate							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	8	0.9075	-0.018	-0.001	0.0005	0.00001	0.0005
CT	6	0.945	0.016	0.002			
TT	1	0.97	0.050	-0.007			
Mass motility							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	8	76.56	-0.535	-0.113	0.39	0.0933	0.481
CT	6	77.97	0.456	0.306			
TT	1	77.83	1.448	-0.826			
live sperm							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	8	82.75	-0.347	-0.299	0.163	0.653	0.816
CT	6	84.5	0.296	0.808			
TT	1	82.15	0.939	-2.185			
Sperm concentration							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	8	1.72	0.001	-0.014	0.000001	0.0014	0.001
CT	6	1.77	-0.001	0.037			
TT	1	1.63	-0.002	-0.101			

BV: Breeding value, DD: Dominance deviation, VA: Additive variance, VD: Dominance variance, VG: Genetic variance.

Conclusion

Heat shock protein 70 (HSP70) is a vital gene affecting semen quality traits.The estimate the breeding values additive dominance variance of Iraqi black goat semen quality and investigate the effect of allele substitution in heat shock protein 70 gene (HSP70) carpine on some semen quality traits. Results showed three genotypes (CC, CT, TT).

The results showed a high level of concentration sperm for CC and CT genotype compared with TT genotype. The CC genotype was associated with negative B.V for volume ejaculate, mass motility, and live sperm percentages and was positively associated with sperm concentration.

CT and TT genotypes showed positive B.V for volume ejaculate, mass motility, and live sperm but negative for sperm concentration. CC and TT genotypes showed negative dominance deviation for all traits studied, while heterozygous genotype CT showed positive dominance deviation for all semen quality traits.

Acknowledgments

The authors thank Dr. Bashar Adham (head of the department) and Dr. Zaid Muhammad Mahdi at Diyala University, College of Agriculture, Department of Animal Production, for their assistance.

Conflict of interests

The authors declare that they have no competing interests.

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