



The relationship of polymorphism of the SPEF2 gene to individual and mass motility in the Iraqi Holstein bulls

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Abstract

This study aimed to link the genetic variants of the sperm tail gene SPEF2 and both individual and mass motility to evaluate semen in the Iraqi Holstein bulls. This study was conducted in the College of Agriculture, University of Basrah, and the fields of the Artificial Insemination Centre of the Department of Livestock in the Abu Ghraib area/Ministry of Agriculture (25 km west of Baghdad), for the period from 11/15/2022 until 4/15/2023. The sequencing results were compared with the highest match rate in NCBI due to the occurrence of several different mutations, and the highest percentage of matching was with Chinese bulls that belong to the classification *Bos taurus* (Accession No. KF733182). The study's results clearly indicate a significant correlation between the polymorphism of the SBF2 gene and the rate of motility (individual and mass) in the Iraqi Holstein bulls. Therefore, this gene may be considered a molecular marker for selecting highly fertile bulls.

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Introduction

Holstein bulls are of great economic importance due to their important role in sustaining livestock, so studies are still underway to try to develop their production and increase their fertility (Mohammed *Et Al.*, 2020) ^[24]. Bull fertility is an important economic trait in sustainable livestock production. Although bulls produce large amounts of sperm, some bulls may still suffer from low fertility, which causes significant economic losses in the livestock industry. (Ugur *et al.*, 2022) ^[37] As there are fundamental differences in fertility among bulls, these differences cause significant economic losses. Bulls that suffer from infertility or lack of fertility delay pregnancy and can then cost producers large sums of money without any economic return and cause economic losses that threaten the sustainability of livestock (Kastelic 2013) ^[16]. On the other hand, fertile bulls and semen quality are of vital importance to the livestock industry, as infertile males can cost producers large sums of money without any economic return (Thundathil and Kastelic 2008) ^[34]. Semen characteristics have a moderate heritability coefficient, so there is a possibility of selection on the basis of semen characteristics (Gebreyesus *et al.*, 2021) ^[7], several studies (Habib *et al.*, 2017; Habib *et al.*, 2018) ^[11, 12] have indicated that there are many genes that can be molecular markers in semen. Modiba *et al.* (2022) ^[23] indicated that the SPEF2 gene is one of the candidate genes to be molecular markers associated with semen quality that may directly affect semen characteristics in cattle and therefore can be considered one of the molecular markers in selecting male cattle. The SPEF2 gene is one of the important genes that is directly related to the fertility of farm animals (Guo *et al.*, 2014) ^[8], due to its work in producing a protein that contributes to the normal growth of the sperm tail and thus has a direct effect on sperm motility (Liu *et al.*, 2020; Tu *et al.*, 2020) ^[20, 36], poor sperm motility is associated with decreased fertility in Holstein (Ramirez *et al.*, 2023) ^[31]. Individual sperm motility depends on the structure and function of the tail, as poor motility is significantly linked to genetic defects (Gupta *et al.*, 2012) ^[9]. On the other hand, individual sperm movement is affected by several factors, including the season (Marai *et al.*, 2010) ^[22], breed, and age of the animal (Kiani *et al.*, 2014) ^[18], Low mass motility in buffalo bulls is an indicator of low sperm concentration and poor percentage of individual sperm motility (Muvhali *et al.*, 2022) ^[26], this demonstrates the

link between individual and mass motility. Because non-genetic factors (such as motility) are a very important indicator for measuring semen quality (Ramajayan *et al.*, 2023) [30], this study aimed to link the genetic variants of the SBF2 gene and both individual and mass motility to evaluate semen in the Iraqi Holstein bulls.

Methods and materials

This study was conducted in the College of Agriculture, University of Basra, and in the fields of the Artificial Insemination Centre of the Department of Livestock in the Abu Ghraib area/Ministry of Agriculture (25 km west of Baghdad), for the period from 11/15/2022 until 4/15/2023. 7 Holstein bulls were used, ranging in age between 3 and 4 years, all animals were healthy, disease-free, and under constant veterinary supervision. The semen was collected using an artificial vagina.

Dna extraction:

DNA was extracted according to Tomazic *et al.*, (2021) [35] using the Chelex 100 kit and according to the method recommended by the manufacturer. DNA concentration and purity were estimated using Nano drop.

The amplification of pcr:

The amplification process was carried out according to the method mentioned by Kõressaar *et al.*, (2018) [19], as the sample size for the PCR reaction was 25 microliters consists of 9.5 microliters of distilled water, 12.5 microliters of Master Max, 1.0 microliters of template DNA (75 ng), 1.0 µl first primer (10 µM), and 1.0 µl reverse primer. As for the primer, it was forward : F- CAGGCGATTTCAACTCCCT reverse R- CCTCTCACCGAGCCACTTTT

R. The amplification conditions are shown in Table 1.

Table 1: PCR Program Used For Amplification

Number of Cycles	Time	(C °)Temp	Cycle step
1	5 min	98	Initial Denaturation
35	15	98	Denaturation
	30	55	Extension
	30	72	Final Extension
1	5 دقيقة	72	النهائي (الاستطالة) (التعديد)

The analysis of sequences:

Sequence analysis was performed at the first BASE laboratory in Malaysia, BLAST analysis and Multiple Sequence Alignment were performed (Boratyn *et al.* 2019) [4]. The sequencing results were compared with the highest match rate in NCBI to detect the potential molecular change. To reveal the 3D structure of the protein, the Swiss model was used (Waterhouse *et al.*, 2018) [38].

Estimating individual and mass motility:

The percentage of movement in each of the months December, January, February, March, may and forgetfulness, may was estimated as follows: The percentage of individual motility was estimated based on the method mentioned by Thomas (2021) [33]. The percentage of mass sperm motility was estimated based on what was indicated by Amare and Mekuriaw (2012) [32].

Statistical Analysis:

The statistical analysis process was conducted using the

SPSS (V.27) program (IBM 2020) [15].

Results and discussion

The results of DNA extraction showed that the purity ratio (measured by the Nano Drop device) represented by the ratio A280 / A260 was 1.8 (or close to it) for all study samples (Iraqi Holstein bulls), which is consistent with what was indicated by (Habib *et al.*, 2022 and Hindash and Hindash 2022 and Lutz *et al.*, 2023) [10, 14] as an indication of the best purity.

As for the Iraqi Holstein bulls, the PCR amplification product package for the SPEF2 gene was 5338 pb in size, as shown in Figure (1) which represents the electrophoresis of the PCR product for the gene, and this is consistent with what was indicated by (Nikitkina *et al.*, (2021) [28] about the size of the gene in Holstein bulls.

When performing the BLAST consensus analysis of the sequences obtained in the current study, it can be noted that the SPEF2 gene in bulls The Iraqi Holstein did not match any other record in the gene bank by 100%, which means obtaining new genetic formations for this gene due to the occurrence of a number of different mutations, and the highest percentage of matching was with the Chinese bulls that follow the classification Bos taurus (accession number KF733182) and the percentage of matching reached 99.83%. The reason for these sequences being completely new may be that this gene has not been previously diagnosed and studied in Iraq. When conducting the multiple sequence alignment analysis (MSA) (Appendix 3), two different genetic formations were obtained for the SPEF2 gene as a result of the occurrence of different mutations, noting that these two genetic formations did not match completely (100%) between them as a result of the occurrence of different mutations between them despite the presence of common mutations in both of them. These genetic formations were registered in the gene bank (GenBank under the accession numbers AA: LC754316 (4 animals) and LC754317: BB (3 animals). These results are consistent with what was indicated Both Nikitkina *et al.*, (2021) [28] and Mukherjee *et al.*, (2023) [25] reported on the possibility of genetic polymorphisms for this gene.

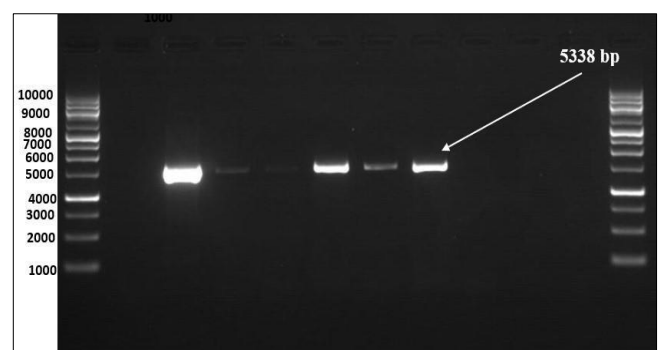


Fig 1: Electrophoresis of the PCR Product of the sperm tail gene Spef2 In Iraqi Holstein Bulls

The results in Table (2) indicate a significant superiority ($P \leq 0.05$) in the percentage of the collective movement rate between the two genetic formations obtained for the SPEF2 gene in fresh semen during the study months, as the genetic formation BB significantly outperformed the genetic formation AA, as the collective movement rate for the two genetic formations reached 40.64 and 16.91, respectively. These results are consistent with what Aboud and Laith

(2023) indicated about the existence of significant differences between the genetic formations in the collective movement rate of sperm for the INHBA gene in Iraqi Holstein bulls, as the collective movement is directly related to the ability of sperm to fertilize, and this is consistent with what Ramirez-Diaz *et al.*, (2023) ^[31] indicated about the important role played by many genes associated with sperm movement in affecting the fertility of semen in Italian Holstein bulls. There were also significant differences ($P \leq 0.05$) between the months of collection for the BB and AA genotypes, as the averages of the overlap between the months were 43.33, 42.22, 45.00, 40.55, 36.11, and 36.66 in months 12, 1, 2, 3, 4, and 5, respectively, for the BB genotype, and 13.33, 14.58, 20.83, 17.91, 13.33, and 21.45 in months 12, 1, 2, 3, 4, and 5 for the AA genotype, respectively. These results are consistent with what was indicated by Barbas *et al.* (2023) ^[3] about the significant differences in the characteristics of fresh semen between the months of collection, as an increase in the rate of collective movement was observed in February. Perhaps the importance of movement in general and collective movement in particular of semen is not only because it is important in the process of completing the fertilization process, but it may extend to the direct and moral impact in increasing the sperm's ability to survive for a longer period, which highlights its great impact on fertility (Prajapati *et al.*, 2022) ^[29]. The results in Table (3) indicate that there are significant differences ($P \leq 0.05$) in the percentage of individual movement rate for both genetic formations obtained for the SPEF2 gene and for all months of the study, as the genetic formation BB significantly outperformed the genetic formation AA, as the individual movement rate for the two genetic formations reached 61.48 and 32.34, respectively. These results are consistent with what was indicated by Hernawati *et al.*, (2021) ^[13] and Ding *et al.*, (2022) ^[5] about the significant association between the multiple genetic formations of the genes responsible for the individual movement of sperm in fresh semen in Iraqi Holstein bulls and Chinese bulls. There are also significant differences ($P \leq 0.05$) between the months of collection for the two genetic formations BB and AA, as the averages of the overlap between the months reached 65.00, 63.88, 68.88, 61.66, 52.77, 56.66 and in months 12, 1, 2, 3, 4 and 5 respectively in the genetic formation BB, and reached 26.66, 25.20, 36.04, 34.37, 32.00 and 39.79 in months 1, 2, 3, 4 and 5 in the genetic formation AA respectively. These results are consistent with what was indicated by Al-Dean and Saleh (2022) ^[2] about the existence of significant differences in the individual movement rate between the genetic formations in Iraqi Holstein bulls when they studied the PIT-1 gene, and other than that, it is a basis for selecting bulls with high fertility. In addition to the above, we can note from the table that the overall average of the individual movement rate in February was significantly higher ($P \leq 0.05$) than the rest of the months, which is consistent with what Netherton *et al.*, (2022) ^[27] indicated about the significant differences in semen characteristics between months, as he summarized that the months in which temperatures are high decrease the semen characteristics in Holstein bulls, and this may be due to the bulls being exposed to heat stress. On the other hand, studies have indicated that there are close significant associations between individual movement and sperm survival rates, as well as a decrease in the rate of acrosome abnormalities, and thus an increase in bull fertility (Kemal *et al.*, 2022 and Egyptien *et al.*, 2023) ^[17, 6].

Table 2: Effect of SPEF2 genotypes on mass motility of fresh semen in Holstein bulls Year Genotype Average Month

General average	Polymorphism		Month
	BB	AA	
16.03± ^{bc} 26.19	43.33	13.33	12
15.03± ^{bc} 26.42	42.22	14.58	1
13.29± ^a 31.19	45.00	20.83	2
13.02± ^{ab} 27.61	40.55	17.91	3
14.07± ^c 23.09	36.11	13.33	4
9.02± ^{ab} 27.97	36.66	21.45	5
	5.14± ^a 40.64	7.93± ^b 16.91	متوسط التشكل الوراثي

Table 3: Effect of SPEF2 genotypes on individual motility of fresh semen in Holstein bulls Year Genotype Average Month

general average s	polymorphism		Months
	BB	AA	
20.82± ^b 43.09	65.00	26.66	12
21.20± ^b 41.78	63.88	25.20	1
18.08± ^a 50.11	68.88	36.04	2
15.85± ^{ab} 46.07	61.66	34.37	3
14.77± ^b 42.57	56.66	32.00	4
9.36± ^b 45.35	52.77	39.79	5
	7.04± ^a 61.48	10.17± ^b 32.34	متوسط التشكل الوراثي

Conclusion

The results of the study clearly indicate that there is a significant correlation between the polymorphism of the SPEF2 gene and the rate of motility (individual and mass) in the semen of the Iraqi Holstein bulls. Therefore, it is possible that this gene is considered a molecular marker for the selection of highly fertile males.

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