



Global Agronomy Research Journal

The Relationship of polymorphism of the *SPEF2* gene to individual and mass motility in the semen of male Iraqi buffaloes

Olaa Bahaa Salem ¹, Omar Adel Mohammed ², Hassan Nima Habib ^{3*}

¹⁻² Department of Artificial Insemination, Ministry of Agriculture, Baghdad, Iraq

³ Department of animal production, College of Agriculture, University of Basrah, Basrah Iraq

* Corresponding Author: **Hassan Nima Habib**

Article Info

ISSN (online): 3049-0588

Volume: 02

Issue: 05

September - October 2025

Received: 12-08-2025

Accepted: 14-09-2025

Published: 15-10-2025

Page No: 54-58

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 male Iraqi buffaloes. 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 male Indian buffalo (Accession No. XM 025270630). The study's results clearly indicate a significant correlation between the polymorphism of the *SPEF2* gene and the rate of motility (individual and mass) in the semen of male buffalo. Therefore, this gene may be considered a molecular marker for selecting highly fertile males.

DOI: <https://doi.org/10.54660/GARJ.2025.2.5.54-58>

Keywords: *SPEF2* Gene Polymorphism, Semen Motility, Iraqi Buffaloes, Male Fertility, Sperm Quality

Introduction

The Iraqi buffalo has great economic importance, but it suffers from many different problems that have led to a decline in its production for various reasons, the most important of which may be the lack of recent studies that address it (Rossi *et al.*, 2018) ^[27]. The buffalo constitutes an important and effective part of the agricultural sector in particular and the economy in general. The country in general, but their production level does not cover the needs of the local market for many reasons, including mismanagement, poor nutrition, and genetic factors (Al- Rudha, *et al.*, 2021) ^[1]. On the other hand, male fertility 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 (2008 Thundathil and Kastelic) ^[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) ^[5], several studies (Habib *et al.*, 2017; Habib *et al.*, 2018) ^[9-10] ^[17] have indicated that there are many genes that can be molecular markers in semen. Modiba *et al.* (2022) ^[24] 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) ^[6], 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) ^[19, 36], poor sperm motility is associated with decreased fertility in buffaloes (Kumaresan *et al.*, 2023) ^[17]. 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) ^[7]. On the other hand, individual sperm movement is affected by several factors, including the season (Marai *et al.*, 2010) ^[23], breed, and age of the animal (Kiani *et al.*, 2014) ^[14]. Low mass motility in buffalo bulls is an indicator of low sperm concentration and poor percentage of individual sperm motility (Muvhali *et al.*, 2022) ^[25], 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) ^[26], this study aimed to link the genetic

variants of the SBF2 gene and both individual and mass motility to evaluate semen in male Iraqi buffaloes.

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. 8 male buffaloes 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) [5] 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 Köressaar *et al.*, (2018) [15], 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-TAGTAGTAGCGCTGCCTTGG reverse R-TGGAAGATTCATAGCCACTCCTG. The amplification conditions are shown in Table 1.

Table 1: PCR program used for amplification

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

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) [2]. 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, and forgetfulness 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) [31].

Statistical Analysis

The statistical analysis process was conducted using the SPSS (V.27) program (IBM 2020) [13].

Results and Discussion

The results of DNA extraction showed that the percentage of purity (measured by the Nano Drop) represented by the ratio A280/A260 was 1.8 (or close to it), and this is consistent with what was indicated by Habib *et al.*, (2022) [8] and Lutz *et al.*, (2022) [21] as a guide to best purity.

The size of the PCR product for the SPEF2 gene was 5772 pb in male buffalo, as shown in Figure (1), this is consistent with what Deng *et al.*, (2016) [3] indicated about the size of the gene in male buffalo. The results of detecting the sequences obtained in the current study using the BLAST technique showed that the SPEF2 gene in male Iraqi buffaloes does not match 100% with any other record in the GenBank due to the occurrence of a number of different mutations, and that the highest percentage of matching was with male Indian buffaloes (Accession No. XM 025270630), When performing multiple sequence alignment (MSA) analysis, two different polymorphism of the SPEF2 gene were obtained as a result of the occurrence of different mutations. These polymorphisms were registered in GenBank under the accession numbers AA: LC754318 (2 animals) and LC754319: BB (6 animals).

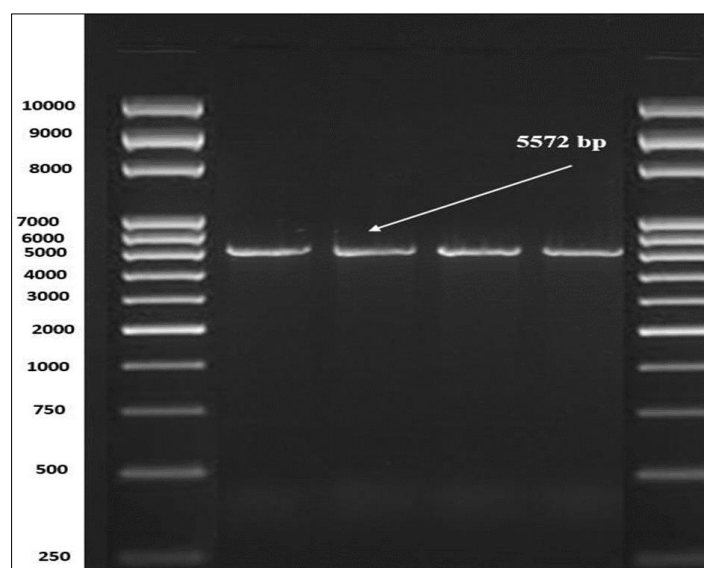


Fig 1: Electrophoresis of the PCR product of the sperm tail gene SPEF2 in male buffalo

The results in Table (2) indicate that there are significant differences ($P \leq 0.05$) in the percentage of individual motility rate for both polymorphism obtained for the SPEF2 gene and for all months of the study, as it reached 63.00, 62.17, 70.17, 66.67, and 54.67 in months 1, 2, and 3. And 4 and 12, respectively, in the polymorphism AA, and reached 41.11, 39.61, 37.50, 42.67, and 43.22 in months 1, 2, 3, 4, and 12, for the polymorphism BB, respectively. These results are consistent with what was indicated by Lu *et al.*, (2022) ^[20] about the presence of significant differences in the rate of individual movement between the ARID4A polymorphism in male Chinese buffalo, these results are also consistent with what was indicated by Rullo *et al.*, (2023) ^[28] and Wang *et al.*, (2023) ^[37] about the existence of significant differences in favour of one of the polymorphism of genes associated with semen characteristics.

The results of the table also indicate that there is a significant superiority ($P \leq 0.05$) for the polymorphism AA, which reached 63.33 compared to the polymorphism BB, which reached 40.82, these results agreed with Eidan and Khudhir (2023) ^[4] about the significant correlation between the polymorphism and the individual motility rate of Holstein bulls for the ATP1A1 gene, as the CC polymorphism showed highly significant superiority for individual motility compared to the CA polymorphism.

These results also agree with Kumaresan *et al.*, (2023) ^[17] about the fertility of male buffaloes being significantly related to the polymorphism of the genes that are molecular markers of male fertility. These results also agreed with what Song *et al.*, (2023) ^[32] pointed out about the presence of significant differences in semen characteristics between the

different polymorphism resulting from genetic mutations, whether these mutations are missense (resulting from a change in the amino acid produced) or silent mutations (did not result from a change in the amino acid produced).

The results in Table (3) indicate that there are significant differences ($P \leq 0.05$) in the percentage of mass motility rate for the two-polymorphism obtained in the current study throughout the months of the study and for both polymorphism, as it reached 39.17, 40.00, 39.17, 37.50, and 39.17 in the months. 1, 2, 3, 4 and 12

respectively in the polymorphism, AA, reached 27.50, 25.00, 26.11, 26.67, and

28.33 in months 1, 2, 3, 4, and 12, respectively. For the BB polymorphism, there was also a significant superiority ($P \leq 0.05$) in the average polymorphism, reaching 39.00 and 26.72 for the two conformations for the AA and BB polymorphism, respectively. These results are consistent with what Mahmoud *et al.*, (2021) ^[22] and Hasanain *et al.*, (2022) ^[11] indicated about the presence of significant differences in the rate of movement between the genetic configurations of the genes associated with semen characteristics in males. Egyptian buffalo.

In addition, we can notice from the table that the overall average of the mass motility rate in months 12 and 1 was significantly higher ($P \leq 0.05$) than in months 2, 3, and 4. This is consistent with what was indicated by Kumar *et al.*, (2019) ^[16] and Hirabhai *et al.*, (2022) ^[12] reported on the significant differences in the quality of fresh semen between months, concluding that the months in which temperatures rise have a decrease in the quality of semen characteristics in male Indian buffaloes.

Table 2: Percentage rate of individual Motility of polymorphism for the months of study

Month	Polymorphism		General average
	AA	BB	
1	63.00	41.11	11.76±46.58
2	62.17	39.61	11.33±45.25
3	70.17	37.50	15.41±45.67
4	66.67	42.67	12.44±48.67
12	54.67	43.22	7.25±46.08
Average of Polymorphism	6.83± ^a 63.33	6.52± ^b 40.82	

Table 3: Percentage rate of mass Motility of polymorphism for the months of study

Month	Polymorphism		General average
	AA	BB	
1	39.17	27.50	7.92±30.42
2	40.00	25.00	8.24±28.75
3	39.17	26.11	7.84±29.37
4	37.50	26.67	8.63±29.38
12	39.17	28.33	7.51±31.04
Average of Polymorphism	2.03± ^a 39.00	6.71± ^b 26.72	

Conclusions

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 male buffaloes. Therefore, it is possible that this gene is considered a molecular marker for the selection of highly fertile males.

References

1. Al-Rudha AM, Khalil NK, Altaai NA. Evaluation of bacterial contaminants and heavy metals in cow and buffalo raw milk sold in Baghdad governorate. Iraqi J Vet Sci. 2021;35:101-5.
2. Boratyn GM, Thierry-Mieg J, Thierry-Mieg D, Busby B, Madden TL. Magic-BLAST, an accurate RNA-seq aligner for long and short reads. BMC Bioinformatics. 2019;20(1):1-19.
3. Deng T, Pang C, Lu X, Zhu P, Duan A, Tan Z, *et al.* De novo transcriptome assembly of the Chinese swamp buffalo by RNA sequencing and SSR marker discovery. PLoS One. 2016;11(1):e0147132.
4. Eidan SM, Khudhir SA. Association between ATP1A1

- gene polymorphisms with semen characteristics in Holstein bulls. *Iraqi J Agric Sci.* 2023;54(2):330-7.
5. Gebreyesus G, Lund MS, Kupisiewicz K, Su G. Genetic parameters of semen quality traits and genetic correlations with service sire nonreturn rate in Nordic Holstein bulls. *J Dairy Sci.* 2021;104(9):10010-9.
 6. Guo F, Yang B, Ju ZH, Wang XG, Qi C, Zhang Y, *et al.* Alternative splicing, promoter methylation, and functional SNPs of sperm flagella 2 gene in testis and mature spermatozoa of Holstein bulls. *Reproduction.* 2014;147(2):241-52.
 7. Gupta S, Handa KK, Kasliwal RR, Bajpai P. A case of Kartagener's syndrome: Importance of early diagnosis and treatment. *Indian J Hum Genet.* 2012;18(2):263.
 8. Habib HN, Al-Rishdy KH, Al-Hellou MF. Molecular description of melatonin receptor 1A gene in Iraqi buffalo. *Iraqi J Vet Sci.* 2022;36(4):905-12.
 9. Habib HN, Hassan AF, Khudaier BY. Molecular detection of polymorphism of heat shock protein 70 (Hsp70) in the semen of Iraqi Holstein bulls. *Asian J Anim Sci.* 2017;11:132-9.
 10. Habib HN, Khudaier BY, Hassan AF. Molecular detection of polymorphism of heat shock protein 70 (HSP70) in the semen of Arabi rams. *Basrah J Vet Res.* 2018;17(3):156-66.
 11. Hasanain MH, Mahmoud KGM, Ahmed YF, Nawito MF, EL-Menoufy AA, Ismail ST. Polymorphism investigation of sex determining factor gene (SRY) and the association with semen criteria with field fertility in Egyptian buffalo bulls. *Egypt J Chem.* 2022;65(4):279-86.
 12. Hirabhai PK, Hirjibhai TP, Hirjibhai SH, Bhagvanbhai VK. Seasonal variation in semen quality and conception rate of Jaffarabadi buffalo bulls (*Bubalus bubalis*) in India. *Buffalo Bull.* 2022;41(3):431-9.
 13. IBM Corp. IBM SPSS Statistics for Windows, Version 27.0 [Computer software]. Armonk, NY: IBM Corp; 2020.
 14. Kiani FA, Yousaf A, Zafar MA, Nawaz M, Akbar Z, Sohoo MR, *et al.* Effect of age on physical characteristics of Kundhi buffalo bull semen. *Int J Curr Microbiol App Sci.* 2014;3(11):445-53.
 15. Kõressaar T, Lepamets M, Kaplinski L, Raime K, Andreson R, Remm M. Primer3_masker: integrating masking of template sequence with primer design software. *Bioinformatics.* 2018;34(11):1937-8.
 16. Kumar N, Ghosh SK, Mohanty TK, Prasad JK, Singh RK, Patel B. Effect of heat stress on physico-morphological characteristics and sperm functions in Murrah buffalo semen. *Indian J Anim Sci.* 2019;89:390-3.
 17. Kumaresan A, Sinha MK, Paul N, Nag P, Ebenezer Samuel King JP, Kumar R, *et al.* Establishment of a repertoire of fertility associated sperm proteins and their differential abundance in buffalo bulls (*Bubalus bubalis*) with contrasting fertility. *Sci Rep.* 2023;13(1):2272.
 18. Li DY, Yang XX, Tu CF, Wang WL, Meng LL, Lu GX, *et al.* Sperm flagellar 2 (SPEF2) is essential for sperm flagellar assembly in humans. *Asian J Androl.* 2022;24(4):359.
 19. Liu C, Lv M, He X, Zhu Y, Amiri-Yekta A, Li W, *et al.* Homozygous mutations in SPEF2 induce multiple morphological abnormalities of the sperm flagella and male infertility. *J Med Genet.* 2020;57(1):31-7.
 20. Lu G, He C, Yang X, Cheng Y, Yang R, Song J, *et al.* Association between single-nucleotide polymorphisms in ARID4A gene and sperm quality of Chinese water buffalo. *Vet Arh.* 2022;92(5):577-90.
 21. Lutz Í, Miranda J, Santana P, Martins T, Ferreira C, Sampaio I, *et al.* Quality analysis of genomic DNA and authentication of fisheries products based on distinct methods of DNA extraction. *PLoS One.* 2023;18(2):e0282369.
 22. Mahmoud KG, Sakr AM, Ibrahim SR, Sosa AS, Hasanain MH, Nawito MF. GnRHR gene polymorphism and its correlation with semen quality in buffalo bulls (*Bubalus bubalis*). *Iraqi J Vet Sci.* 2021;35(2):381-6.
 23. Marai IF, Haebe AAM. Buffaloes' reproductive and productive traits as affected by heat stress. *Trop Subtrop Agroecosyst.* 2010;12(2):193-217.
 24. Modiba MC, Nephawe KA, Mdladla KH, Lu W, Mtileni B. Candidate genes in bull semen production traits: an information approach review. *Vet Sci.* 2022;9(4):155.
 25. Muvhali PT, Bonato M, Malecki IA, Cloete SW. Mass sperm motility is correlated to sperm motility as measured by computer-aided sperm analysis (CASA) technology in farmed ostriches. *Animals.* 2022;12(9):1104.
 26. Ramajayan P, Sivaselvam SN, Karthickeyan SMK, Venkataramanan R, Gopinathan A. Non-genetic effects and repeatability estimates of semen production traits in Murrah buffalo bulls. *Trop Anim Health Prod.* 2023;55(2):73.
 27. Rossi G, Conti L, Al-Fartosi K, Barbari M. Implementation of practical solutions to improve buffalo breeding development in rural areas of South Iraq. *Agron Res.* 2018;16(2):564-73.
 28. Rullo R, Caira S, Nicolae I, Marino F, Addeo F, Scaloni A. A genotyping method for detecting foreign buffalo material in Mozzarella di Bufala Campana cheese using allele-specific and single-tube heminested-polymerase chain reaction. *Foods.* 2023;12(12):2399.
 29. Sironen A, Kotaja N, Mulhern H, Wyatt TA, Sisson JH, Pavlik JA, *et al.* Loss of SPEF2 function in mice results in spermatogenesis defects and primary ciliary dyskinesia. *Biol Reprod.* 2011;85(4):690-701.
 30. Sironen A, Vilkkilä J, Bendixen C, Thomsen B. Infertile Finnish Yorkshire boars carry a full-length LINE-1 retrotransposon within the KPL2 gene. *Mol Genet Genomics.* 2007;278:385-91.
 31. Sisay TA, Amare A, Mekuriaw Z. Quality evaluation of cryopreserved semen used in artificial insemination of cattle in selected districts of Western Gojjam zone of Amhara region, Ethiopia. *J Reprod Infertil.* 2012;3(1):1-7.
 32. Song B, Yang T, Shen Q, Liu Y, Wang C, Li G, *et al.* Novel mutations in DNAH17 cause sperm flagellum defects and their influence on ICSI outcome. *J Assist Reprod Genet.* 2023;40:1-8.
 33. Thomas J. Determining reproductive fertility in herd bulls. [place unknown: publisher unknown]; 2021.
 34. Thundathil JC, Kastelic JP. Breeding soundness evaluation and semen analysis for predicting bull fertility. *Reprod Domest Anim.* 2008;43:368-73.
 35. Tomazic ML, Hamer M, Bustos CP, Arregui M, Ascencio M, Saraullo V, *et al.* Use of Chelex-100 for the molecular diagnosis of five animal pathogens. *Rev FAVE Cienc Vet.* 2021;20(1):11-2.

36. Tu C, Nie H, Meng L, Wang W, Li H, Yuan S, *et al.* Novel mutations in SPEF2 causing different defects between flagella and cilia bridge: the phenotypic link between MMAF and PCD. *Hum Genet.* 2020;139(2):257-71.
37. Wang X, Li Z, Feng T, Luo X, Xue L, Mao C, *et al.* Chromosome-level genome and recombination map of the male buffalo. *Gigascience.* 2023;12:giad063.
38. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, *et al.* SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 2018;46(W1):W296-303.

How to Cite This Article

Salem OB, Mohammed OA, Habib HN. The relationship of polymorphism of the SPEF2 gene to individual and mass motility in the semen of male Iraqi buffaloes. *Glob Agron Res J.* 2025;2(5):54–58. doi:10.54660/GARJ.2025.2.5.54-58

Creative Commons (CC) License

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.