Research Article

Association of CAST P392 (T>T) Gene Polymorphism with Sperm Quality Traits Following Cryopreservation in Fallow Deer (*Dama dama*)

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Abstract | Genetic polymorphisms play a critical role in determining semen quality and cryotolerance, particularly in wildlife species such as fallow deer (*Dama dama*), where reproductive efficiency is essential for conservation and breeding programs.This study was conducted from 1 January 2024 to 1 October 2025 in collaboration with several wildlife reserves, including Babylon Nature Reserve (Al-Mahawil District), Al-Mustaqbal Private University Reserve (Babylon Governorate), Al-Suqour Reserve (Al-Diwaniyah Governorate), Al-Abbas Shrine Nurseries (Karbala Governorate), and Mohammed Al-Jawad Reserve (Baghdad Governorate). A total of 51 male fallow deer (*Dama dama*) were included in the experiment. The study aimed to investigate the relationship between genetic polymorphism of the CAST P392 (T>T) gene and selected semen parameters under cryopreservation conditions. The results demonstrated that CAST P392 (T>T) polymorphism significantly influenced sperm characteristics. Group motility showed significant differences ($P \leq 0.05$) between CT and CC genotypes throughout the cryopreservation period (days 1–14). Individual motility exhibited significant differences ($P \leq 0.05$) on days 2, 4, 5, 6, 9, 10, and 12. Sperm viability differed significantly ($P \leq 0.05$) on days 1, 2, 3, 4, 12, and 13. Additionally, sperm morphology showed significant variation ($P \leq 0.05$) on days 3, 4, 5, 6, 7, 9, 10, 12, and 13. These findings suggest that CAST P392 (T>T) gene polymorphism is associated with variations in sperm quality under cryopreservation and may serve as a useful genetic marker for improving reproductive performance in fallow deer.

Keywords | CASTP392 (T>T) gene, cryopreservation, fallow deer, genetic, polymorphisms, sperm parameters

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INTRODUCTION

Understanding genetic diversity and its impact on reproductive traits is essential for the conservation and sustainable management of wildlife species such as fallow deer (*Dama dama*). Previous genetic studies on European and Persian fallow deer subspecies have highlighted the importance of maintaining genetic purity and lineage integrity, particularly in light of declining populations in certain regions. Analyses using microsatellite loci have revealed both monomorphic and polymorphic patterns, reflecting varying levels of genetic diversity among populations (Garcia, 2012). Additionally, the genetic history of fallow deer has been described as complex, with significant changes in genetic diversity requiring multiple generations to become evident (Cronin *et al.*, 2006, 2009;

Al-Salman and Al-Gharawi, 2019).

Semen evaluation plays a crucial role in assessing male fertility and ensuring the success of breeding and conservation programs. Key parameters such as semen volume, concentration, motility, morphology, and viability are widely used to determine reproductive potential. In wildlife species, seasonal variations and physiological conditions significantly influence semen quality, as demonstrated in related ungulates such as mountain gazelle, where notable differences in sperm characteristics have been observed across breeding and non-breeding seasons (Zghairand and Hassooni, 2021; Herrera-Sánchez *et al*., 2023). Various laboratory techniques, including sperm concentration assessment, viability testing, and membrane integrity assays such as the hypo-osmotic swelling test (HOST), have been employed to evaluate semen quality in different species.

Studies on fallow deer semen have shown that sperm morphology and structure are generally consistent with those of other ungulates, although variations may occur among individuals and across breeding seasons (Gosch *et al*., 1989). Furthermore, artificial insemination using cryopreserved semen has demonstrated promising reproductive outcomes in related deer species, emphasizing the importance of semen quality in achieving successful fertilization (Jabbour *et al*., 1993).

Despite these advances, limited information is available regarding the relationship between genetic polymorphisms and semen quality under cryopreservation conditions in fallow deer. Therefore, the present study aims to investigate the association between CAST P392 (T>T) gene polymorphism and selected sperm parameters under cryopreservation conditions in fallow deer (*Dama dama*).

MATERIALS AND METHODS

Study animals

All study protocols and animal handling procedures were carried out in line with international standards. This study was conducted from 1 January 2024 to 1 October 2025 in collaboration with several wildlife reserves, including Babylon Nature Reserve (Al-Mahawil District), Al-Mustaqbal Private University Reserve (Babylon Governorate), Al-Suqour Reserve (Al-Diwaniyah Governorate), Al-Abbas Shrine Nurseries (Karbala Governorate), and Mohammed Al-Jawad Reserve (Baghdad Governorate). A total of 51 male fallow deer (*Dama dama*) were included in the study. All animals were clinically evaluated to ensure good physical condition and absence of infectious and reproductive diseases. The study aimed to investigate the relationship between CAST P392 (T>T) gene polymorphism and selected sperm parameters under cryopreservation conditions.

Eight adult males were selected for semen collection from private reserves located in Diwaniyah, Hillah, Karbala, and Baghdad. Each male was housed with approximately ten females in enclosures measuring 7 × 7 m. Animals were fed green fodder (alfalfa) along with a concentrate diet at 2% of their live body weight. Salt licks and water were provided ad libitum.

Semen collection

Given the semi-domesticated nature of fallow deer and the aggressive behavior of dominant males, animals were physically restrained by three trained personnel using nets and ropes. Following restraint, each animal was allowed to rest for approximately three minutes to reduce stress. The external preputial orifice and surrounding area were carefully shaved using scissors, cleaned with a diluted disinfectant solution, and dried with sterile wipes (Figure 1). Semen collection was then performed using an electroejaculator (MiniTube, Germany) equipped with a probe designed for small ruminants (Figures 2 and 3).

Figure 1: Shaving the external preputial orifice and the surrounding area.

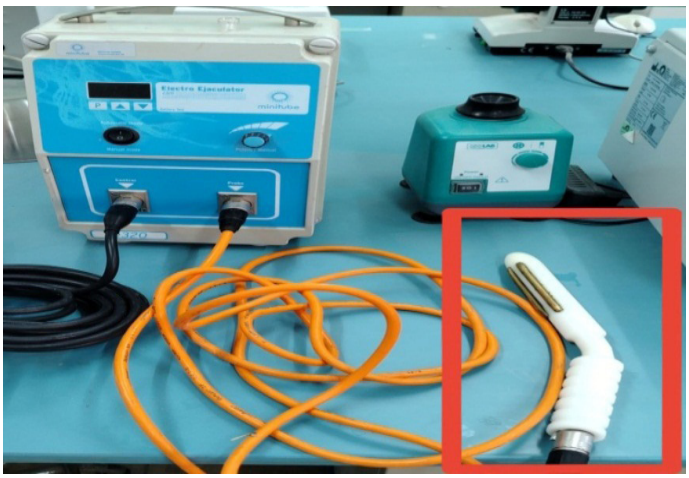

Figure 2: The probe prepared for small ruminants.

Figure 3: Placing the nail in the exit hole of the male fallow deer.

Semen collection was performed after proper restraint and preparation of the animals. Following immobilization by trained assistants, the probe of the electroejaculator was lubricated and carefully inserted into the rectum of the animal. The electroejaculator was then activated (Figure 4). To minimize stress and reduce the risk of shock, the device was switched on and off three times before continuous stimulation was applied during the fourth cycle until ejaculation occurred (Figure 5), following the procedure described by Baiee *et al*. (2018).

Study parameters

Immediately after collection, semen samples were subjected to gross evaluation, including assessment of volume, color, and consistency. A field laboratory setup was used for initial semen analysis prior to cooling and cryopreservation. Fresh samples were evaluated for wave motion, mass motility, progressive motility, and sperm viability. Sperm concentration was not assessed in freshly collected semen in accordance with the experimental design of the study. Subsequently, semen samples were stored under refrigeration at 4±1 °C for varying time intervals prior to further processing.

Figure 4: Electric ejector device.

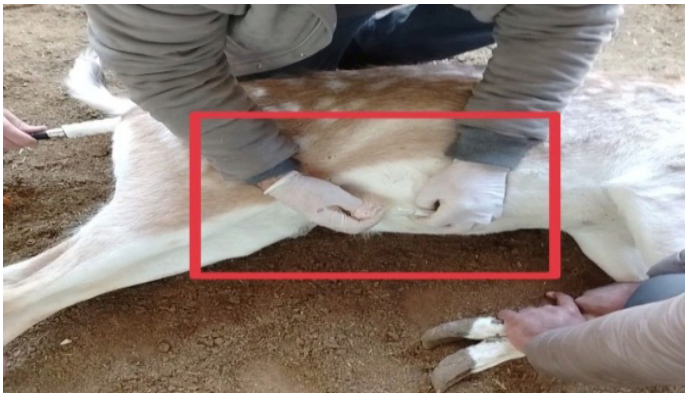

Figure 5: Collecting semen using an electric ejaculation device.

Statistical analysis

A completely randomized design (CRD) was employed to evaluate the effects of different treatments on the studied traits. Differences among group means were assessed using Duncan's multiple range test at a significance level of $P \leq 0.05$ (Duncan, 1955). All statistical analyses were performed using SPSS software (SPSS, 2018).

RESULTS AND DISCUSSION

The results presented in Tables 1 and 2 demonstrated that CAST P392 gene polymorphism significantly affected several sperm traits under cryopreservation conditions. Total motility showed significant differences ($P \leq 0.05$) between CC and CT genotypes across most storage days (days 1–11 and day 14). Specifically, total motility values for CC and CT genotypes were 92.21 and 94.00 (day 1), 90.02 and 93.42 (day 2), 85.75 and 90.71 (day 3), 77.18 and 84.35 (day 4), 69.62 and 73.00 (day 5), 60.67 and 66.42 (day 6), 50.29 and 60.71 (day 7), and 34.48 and 46.71 (day 8), respectively. On later days, values declined to 26.13 and 34.35 (day 9), 21.24 and 27.42 (day 10), 15.75 and 23.00 (day 11), and 5.78 and 3.14 (day 14), respectively (Table 1).

In contrast, no significant differences were observed in total motility between genotypes on days 12 and 13, where values were 12.94 and 13.14 (day 12) and 8.27 and 9.28 (day 13) for CC and CT genotypes, respectively (Table 1).

Regarding individual motility, significant differences ($P \leq 0.05$) were detected on days 2, 4, 5, 6, 9, 10, and 12. The corresponding values for CC and CT genotypes were 81.35 and 78.14 (day 2), 64.43 and 58.57 (day 4), 51.56 and 49.28 (day 5), 46.45 and 42.71 (day 6), 14.18 and 17.00 (day 9), 13.78 and 15.71 (day 10), and 10.00 and 8.71 (day 12), respectively (Table 2). These findings are consistent with previous studies, including Khashan (2020), who reported improved sperm viability and motility in preserved semen of Iraqi goitered gazelle, and Mardenli *et al*. (2020), who observed similar trends in local goats and sheep.

Table 1: Relationship between CAST P392 (T>T) gene polymorphism and total sperm motility (%) under cryopreservation conditions in fallow deer (*Dama dama*) (mean ± standard error).

Genotype	Cryopreservation periods (Days 1-7)						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
CC	92.21 ± 0.31^{b}	90.02 ± 0.09^{b}	85.75 ± 0.44^{b}	77.18 ± 0.72^{b}	69.62 ± 0.31^{b}	60.67 ± 0.74^{b}	50.29 ± 0.72^{b}
CT	94.00 ± 0.20^{a}	93.42 ± 0.44^{a}	90.71 ± 0.88^{a}	84.35 ± 1.24^{a}	73.00 ± 1.52^{a}	66.42 ± 1.77^{a}	60.71 ± 1.65^{a}
Sig.	*	*	*	*	*	*	
Genotype	**Cryopreservation periods (Days 8-14)**						
	Day 8	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
CC	34.48 ± 0.78^{b}	26.13 ± 0.52^{b}	21.24 ± 0.51^{b}	15.75 ± 0.41^{b}	12.94 ± 0.32	8.27 ± 0.54	5.78 ± 0.22^{a}
CT	46.71 ± 2.87^{a}	34.35 ± 1.16^{a}	27.42 ± 1.15^{a}	23.00 ± 1.37^{a}	13.14 ± 0.17	9.28 ± 0.50	3.14 ± 0.17^{b}
Sig.	*	*	*	*	N.S	N.S	*

Different superscript letters (a, b) within the same column indicate significant differences (P ≤ 0.05). * = Significant difference (P ≤ 0.05). N.S = Not significant

Table 2: Relationship between genetic polymorphisms of the CASTP gene (T>T, Gene392) and individual sperm movement (%) under cryopreservation conditions in fallow deer (mean ± standard error).

Genotype	Cryopreservation periods (Days 1-7)						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
CC	86.10 ± 0.39	81.35 ± 0.23^{a}	71.94 ± 0.38	64.43 ± 0.46^{a}	51.56 ± 0.42^{a}	46.45 ± 0.46^{a}	32.27 ± 0.48
CT	85.00 ± 1.04	78.14 ± 1.32^{b}	68.16 ± 1.51	58.57 ± 1.77^{b}	49.28 ± 0.88^{b}	42.71 ± 1.49^{b}	31.64 ± 0.68
Sig.	N.S	*	N.S	*	*	*	N.S
Genotype	**Cryopreservation periods (Days 8-14)**						
	Day 8	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
CC	22.43 ± 0.38	14.18 ± 0.51^{b}	13.78 ± 0.19^{b}	13.00 ± 0.27	10.00 ± 0.12^{a}	5.56 ± 0.25	3.18 ± 0.21
CT	22.42 ± 0.84	17.00 ± 0.66^{a}	15.71 ± 0.28^{a}	12.10 ± 0.55	8.71 ± 0.28^{b}	5.92 ± 0.38	2.71 ± 0.28
Sig.	N.S	*	*	N.S	*	N.S	N.S

Different superscript letters (a, b) within the same column indicate significant differences (P ≤ 0.05). * = Significant difference (P ≤ 0.05). N.S = Not significant.

Table 3: Relationship between genetic polymorphisms of the CASTP gene (T>T, Gene392) and sperm viability (%) under cryopreservation conditions in fallow deer (mean ± standard error).

Genotype	Cryopreservation periods (Days 1-7)						
	Day 1	**Day 1**	**Day 1**	**Day 1**	**Day 1**	**Day 1**	**Day 1**
CC	94.63 ± 0.14^{b}	94.67 ± 0.24^{b}	91.67 ± 0.27^{b}	88.83 ± 0.31^{b}	84.24 ± 0.55	80.62 ± 0.24	74.28 ± 0.36
CT	97.42 ± 0.13^{a}	95.71 ± 0.33^{a}	95.00 ± 0.41^{a}	92.42 ± 0.08^{a}	84.83 ± 0.56	81.85 ± 0.87	73.92 ± 0.99
Sig.	*	*	*	*	N.S	N.S	N.S
Genotype	**Cryopreservation periods (Days 8-14)**						
	Day 8	**Day 8**	**Day 8**	**Day 8**	**Day 8**	**Day 8**	**Day 8**
CC	61.44 ± 0.38	56.01 ± 0.42	47.24 ± 0.32	38.04 ± 0.41	29.86 ± 0.23^{b}	27.41 ± 0.59^{b}	20.40 ± 0.39
CT	62.05 ± 1.45	56.57 ± 0.17	48.42 ± 0.76	39.71 ± 0.83	32.85 ± 0.54^{a}	29.94 ± 0.96^{a}	21.50 ± 0.35
Sig.	N.S	N.S	N.S	N.S	*	*	N.S

Different superscript letters (a, b) within the same column indicate significant differences (P ≤ 0.05). * = Significant difference (P ≤ 0.05). N.S = Not significant.

However, no significant differences were found between genotypes in individual motility on days 1, 3, 7, 8, 11, 13, and 14 under cryopreservation conditions (Table 2).

The results of the current study, presented in Tables 3 and 4, revealed significant differences (P ≤ 0.05) between the reproductive traits associated with the mutation at site 392 of the studied segment of the *CASTP* gene. These differences were observed in sperm vitality under cryopreservation conditions on the first, second, third, fourth, twelfth, and thirteenth days. The values for the CC and CT genotypes on the first day were 94.63 and 97.42, respectively; on the second day 94.67 and 95.71; on the third day 91.67 and 95.00, on the fourth day 88.83 and

92.42, on the twelfth day 29.86 and 32.85, and on the thirteenth day 27.41 and 29.94, respectively. These findings are consistent with those reported by Khashan (2020), who observed similar trends in the Iraqi goitered gazelle under cryopreservation conditions, particularly in semen quality parameters such as acrosome integrity during the early storage period.

In contrast, the results in Tables 3 and 4 indicated no significant differences between genotypes for sperm vitality on the fifth, sixth, seventh, eighth, ninth, tenth, eleventh, and fourteenth days. The observed values remained relatively close between CC and CT genotypes during these periods. These findings also align with those of Khashan (2020), who reported a gradual decline in semen quality after extended cryopreservation in the Iraqi goitered gazelle.

The variation observed in sperm characteristics under cooling and cryopreservation conditions can be attributed to physiological and biochemical alterations occurring in reproductive cells during storage. These processes may lead to changes in membrane integrity, metabolic activity, and cellular stability, ultimately affecting sperm quality. It has been reported that microbial balance and physiological homeostasis play an important role in maintaining cellular efficiency and biological functions (Al-Salhi *et al*., 2022). Furthermore, the application of biological preparations has been shown to improve several physiological and hematological parameters associated with animal performance (Al-Salhi and Al-Shatty, 2023). Additionally, sample handling procedures, particularly freezing and repeated thawing cycles, may directly influence biochemical stability and the reliability of evaluated traits (Al-Salhi, 2025). Therefore, the observed differences in the present study may be attributed to the interaction between genetic variation and environmental stressors associated with cryopreservation.

Regarding sperm morphology, the results in Tables 3 and 4 demonstrated significant differences ($P \leq 0.05$) between CC and CT genotypes on the third, fourth, fifth, sixth, seventh, ninth, tenth, twelfth, and thirteenth days under cryopreservation conditions. The recorded values for CC and CT genotypes were as follows: third day (83.58, 87.00), fourth day (77.43, 79.71), fifth day (68.90, 71.28), sixth day (63.72, 69.35), seventh day (58.51, 61.50), ninth day (56.48, 45.78), tenth day (52.86, 55.57), twelfth day (52.51, 49.00), and thirteenth day (43.48, 38.85), respectively (Table 4).

However, no significant differences were observed between genotypes on the first, second, eighth, eleventh, and fourteenth days. These findings are generally consistent with the results of Khashan (2020), who reported similar trends in sperm morphological stability during early and late stages of cryopreservation in the Iraqi goitered gazelle.

The observed variations in sperm vitality and morphology may also be influenced by cellular defense mechanisms and the ability of cells to withstand environmental stress during preservation. Recent studies have highlighted the role of natural bioactive compounds with antimicrobial and protective properties in reducing cellular damage and improving biological stability (Al-Salhi *et al*., 2025). In addition, natural disinfectants and environmentally derived compounds have been reported to enhance biological efficiency and reduce oxidative stress-related damage (Naser *et al*., 2025). Moreover, improving the immune status of animals has been associated with enhanced physiological performance and increased cellular resilience, which may indirectly contribute to improved reproductive traits (Al-Salhi, 2026). Therefore, the differences observed in this study may result from complex interactions between genetic factors, cellular protection mechanisms, and environmental stress conditions during cryopreservation.

Table 4: Relationship between genetic polymorphisms of the CASTP gene (T>T, Gene392) and sperm morphology (%) under cryopreservation conditions in fallow deer (mean ± standard error).

Genotype	Cryopreservation periods (Days 1-7)						
	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
CC	92.33 ± 0.37	89.18 ± 0.27	83.58 ± 0.48^{b}	77.43 ± 0.43^{b}	68.90 ± 0.33^{b}	63.72 ± 0.50^{b}	58.51 ± 0.49^{b}
CT	92.92 ± 1.16	89.85 ± 0.64	87.00 ± 0.41^{a}	79.71 ± 0.30^{a}	71.28 ± 0.34^{a}	69.35 ± 0.32^{a}	61.50 ± 0.14^{a}
Sig.	N.S	N.S	*	*	*	*	*
Genotype	**Cryopreservation periods (Days 8-14)**						
	Day 8	Day 8	Day 8	Day 8	Day 8	Day 8	Day 8
CC	57.24 ± 0.24	56.48 ± 0.27^{a}	52.86 ± 0.39^{b}	52.48 ± 0.36	52.51 ± 0.45^{a}	43.48 ± 0.36^{a}	42.10 ± 0.33
CT	56.71 ± 0.57	54.78 ± 0.89^{b}	55.57 ± 0.72^{a}	53.64 ± 0.62	49.00 ± 0.38^{b}	38.85 ± 0.42^{b}	40.85 ± 0.76
Sig.	N.S	*	*	N.S	*	*	N.S

Different superscript letters (a, b) within the same column indicate significant differences ($P \leq 0.05$). * = Significant difference ($P \leq 0.05$). N.S = Not significant.

CONCLUSIONS

This study represents the first investigation in Iraq to characterize sperm parameters in brown deer, including detailed assessment of their morphological features. The analyzed gene exhibited genetic variation among the CC, CT, TT, and TG genotypes. A clear association was observed between these genetic variants and reproductive traits. In addition, differences in the genetic configurations of the CASTP392 (T>T) gene were found to significantly influence key reproductive characteristics, indicating its potential role in regulating sperm quality traits.

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NOVELTY STATEMENT

This study provides a foundation for future research on the Iraqi fallow deer (Dama dama), a species classified as endangered, and highlights new opportunities for further scientific investigation.

AUTHOR'S CONTRIBUTION

HFMAl-H: Contribute to conducting direct research work and laboratory tests. HAA-B: Conceptualized the research idea and guided the overall study design. AAA: Contributing to conducting research work in several reserves and field follow-up throughout the duration of the experiment.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in the preparation, analysis, writing, or editing of this manuscript. All research design, data collection, analysis, and interpretation were conducted solely by the authors.

Conflict of interest

The authors have declared no conflict of interest.

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