Research Article

Association Between *FSHR* Gene (T279G) Polymorphism and Biochemical Blood Parameters in Fallow Deer (*Dama dama*)

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Abstract | The present study aimed to investigate the association between the single nucleotide polymorphism (SNP) T279G in the follicle-stimulating hormone receptor (FSHR) gene and selected biochemical blood parameters in fallow deer (*Dama dama*). A total of 51 male fallow deer were included in the study. All animals were clinically examined to ensure good health status and absence of infectious and reproductive diseases. The results revealed significant differences between TT and TG genotypes for cholesterol (CHOL) levels and follicle-stimulating hormone (FSH) concentrations ($P < 0.05$). However, no significant differences were observed between genotypes for other biochemical parameters, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), globulin (GLUB), albumin (ALB), total protein (TP), aspartate transaminase (AST), alanine transaminase (ALT), or hormonal parameters such as luteinizing hormone (LH), testosterone (TEST), and cortisol ($P > 0.05$). The results demonstrated that the T279G polymorphism in the FSHR gene is associated with changes in cholesterol and FSH levels but shows no effect on other biochemical parameters in fallow deer.

Keywords | Biomarkers, Molecular genetics, Genetic variation, Physiological indicators, Wildlife studies

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INTRODUCTION

The number of deer raised on farms worldwide is estimated at approximately 5 million. New Zealand accounts for more than half of the world's deer production (Daszkiewicz *et al*., 2021). Deer farming is primarily important in Europe (Chakanya *et al*., 2016). It is a ruminant animal of the deer family. The male is called a buck, the female is called a sheep, and the young is called a fawn. Deer vary in size from medium to large among different species. In addition to the adult male's length (130–170 cm) and shoulder height (85–95 cm), it weighs 60–85 kg (Jensz and Finley, 2013).

The FSHR gene encodes the receptor protein for follicle-stimulating hormone (FSH). This receptor is a G protein–coupled receptor located on the surface of ovarian granulosa cells, where it binds FSH to regulate follicular development and estrogen production. Primary and multipotent tissue-resident stem cells that express FSHR are found in many adult tissues, including bone marrow and reproductive tissues. They help maintain homeostasis throughout life. Any dysfunction of these stem cells leads to various diseases. They also often transform into cancer stem cells, causing cancer. It is located on chromosome 11 and contains 10 exons in the fallow deer (*Dama dama*) (Al Salman and Al-Gharawi, 2019; Bhartiya and Hiren, 2021).

Studies have investigated the biochemical parameters of deer serum under different environmental and physiological conditions. Milas *et al.* (2004) evaluated serum biochemical parameters in European deer from various regions of Croatia and reported significant variations associated with age, sex, and sampling location. Their findings suggested that nutritional and environmental factors, in addition to age and sex, should be considered when interpreting serum biochemical values in deer. Similarly, in another study blood biochemical parameters in male and female Iraqi goitered gazelles were evaluated (Banana, 2014). The study reported values for glucose, urea, creatinine, bilirubin, cholesterol, triglycerides, high-density lipoprotein, uric acid, lactate dehydrogenase, total protein, and albumin. No significant differences were observed between males and females in most biochemical parameters, although males showed numerically higher values for several traits except glucose and uric acid.

Fallow deer (*Dama dama*) have gained increasing attention because of their economic importance, meat quality, and adaptability to farming systems (Daszkiewicz *et al*., 2021). Moreover, genetic polymorphisms are known to influence productive, physiological, and biochemical traits in animal species (Kareem and Hadi, 2022).

Therefore, the present study aimed to investigate the association between the FSHR gene polymorphism (279 T>G) and selected biochemical blood parameters in fallow deer (*Dama dama*).

MATERIALS AND METHODS

This study was conducted from January 1, 2024, to October 1, 2025, in cooperation with 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).

Fifty-one male fallow deer (*Dama dama*) were used in the experiment. The males were evaluated for their physical appearance and lack of infectious and reproductive diseases. Males are caught using specially prepared and well-sealed nets, without anesthetizing the animal. Blood samples are taken from the animal's jugular vein using sterile 10 ml syringes. After the vein area is cleaned, 8 ml of blood is collected for each animal, as shown in Figure 1.

The collected blood samples were divided into two portions. The first portion (2 mL) was transferred into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and stored under refrigeration until DNA extraction. The second portion (6 mL) was placed into anticoagulant-free gel tubes for serum separation. Samples were centrifuged at 3,000 rpm for 10 min, and the separated serum was carefully collected using sterile capped syringes. Serum samples were then transferred into sterile tubes and stored at −15°C until hormonal and biochemical analyses were performed, according to the methods described by Al-Salhi Al-Shatty (2023) and Al-Salhi (2025).

Figure 1: Collection of blood samples from the jugular vein of a fallow deer (*Dama dama*).

DNA was extracted from deer blood according to the kit instructions provided by the Korean company Geneaid (Figure 2). 200 microliters of blood were taken and placed in a 1.5 ml Eppendorf tube. 200 microliters of PBS (Phosphate buffered saline) were added. 20 microliters of Proteinase K (20 mg/ml) were added. The samples were mixed using a vortex shaker and incubated at room temperature for 2 min. Subsequently, 200 µL of genomic lysis/binding buffer was added, and the mixture was vortexed for 1 min, followed by incubation in a water bath at 55°C for 10 min. Then, 200 µL of absolute ethanol (100%) was added, and the samples were mixed thoroughly for 1 min. A filter tube with a collection tube was prepared, and 600 µL of the mixture was transferred into the filter tube and centrifuged at 14,000 rpm. The filtrate was discarded. Afterwards, 400 µL of Wash Buffer 1 was added and centrifuged at 14,000 rpm, followed by discarding the filtrate. Subsequently, 600 µL of Wash Buffer 2 was added and centrifuged at 14,000 rpm for 3 min, and the filtrate was discarded. The filter tube was then transferred into a new 1.5 mL Eppendorf tube, and 75 µL of preheated elution buffer was added. The samples were centrifuged at 14,000 rpm for 1.5 min to obtain the extracted DNA. Finally, DNA quality was evaluated by electrophoresis using 1% agarose gel.

Amplification of the FSHR gene was performed using specific forward and reverse primers, as shown in Table 1. The PCR reaction mixture and the quantities of its components are presented in Table 2, while the thermal cycling conditions used for DNA amplification are summarized in Table 3.

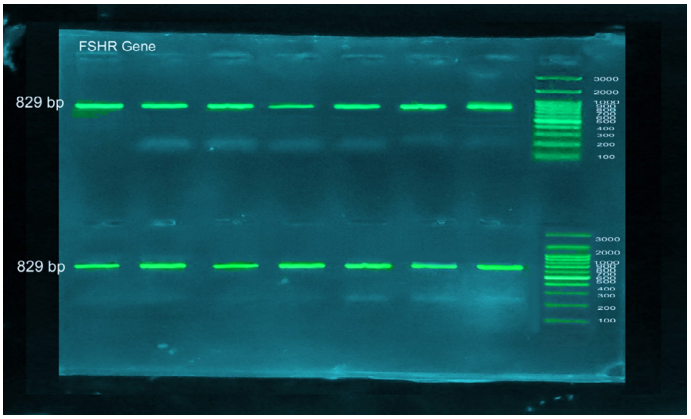

Figure 2: Electrophoresis of DNA extracted from deer blood using 1% agarose gel and voltages of 70 and 65 mA.

Table 1: Primer sequences of the *FSHR* gene.

Gene	Primer name	Sequence	Product size (bp)	Annealing temperature
FSHR Gene	FSHR-F	AGGAGCTTGGT-GATCAACGG	829 bp	15°C
	FSHR-R	AGGTGCCCCTAT-AGCCAGAA		

Table 2: Materials used in PCR purification and their quantities.

Chemical substance	Master mix	DNA template	Primer		Distilled water	Final volume
			Forward	Reverse		
Volume (microliter)	12.50	3	1	1	7.50	25.00

Table 3: PCR DNA program for amplifying the *FSHR* gene.

Stages	Temperature	Time (minutes)	Cycle No.
Initial denaturation	95°C	4	1
Denaturation	95°C	0.30	35
Annealing	55-57 °C	0.30	
Extension	72°C	0.45	
Final extension	72°C	7	1

Amplified PCR products were identified using agarose gel electrophoresis (AGE). A 2% agarose gel was prepared by dissolving 0.3 g agarose in 20 mL of 1× TBE buffer using a microwave oven. GreenViewer DNA stain (ABM, Canada) was used as an ethidium bromide substitute. A 100 bp DNA ladder was loaded into one well as a molecular size marker, while 4 µL of each PCR product was loaded into the remaining wells. Electrophoresis was carried out at 70 V and 65 mA for 35 min. After electrophoresis, the gel was visualized using a gel documentation system to determine the size of the amplified DNA bands.

Statistical analysis

The completely randomized design (CRD) was used to study the effect of different treatments on the studied traits. Significant differences among means were compared using Duncan's multiple range test at a significance level of 0.05 (Duncan, 1955). Statistical analysis was performed using SPSS software (SPSS, 2018).

RESULTS AND DISCUSSION

The results of the present study, shown in Tables 4 and 5, demonstrated no significant differences in most blood biochemical parameters associated with the T279G polymorphism in the follicle-stimulating hormone receptor (FSHR) gene. No significant effects ($P > 0.05$) were observed for high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein (TP), albumin (ALB), globulin (GLOB), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), luteinizing hormone (LH), testosterone (TEST), and cortisol concentrations.

hese findings are consistent with those reported by Scharfe *et al.* (1998) in elk (*Cervoidea*). Blood biochemical parameters may reflect physiological and environmental changes, particularly those related to metabolism, hormonal activity, and nutritional status. The present study indicated physiological stability in fallow deer (*Dama dama*) across the evaluated genotypes. Blood biochemical analysis is considered an important diagnostic tool for evaluating health status and physiological or pathological conditions in

Table 4: Relationship between genetic polymorphism of the FSH receptor gene (FSHR 279 T>G) and blood biochemical parameters in fallow deer (mean ± SE).

Genotype	TG (mg/dL)	CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	ALB (g/dL)	GLUB (g/dL)	TP (g/dL)	AST (U/L)	ALT (U/L)
TT	31.12±1.31	6.51 ±0.34[b]	67.00±2.23	54.48±3.18	7.77±0.099	4.18±0.109	3.26±0.122	151.97±5.06	81.03±4.26
TG	30.77±1.74	10.77±0.69[a]	75.16±1.75	54.38±2.72	8.03±0.11	4.15±0.17	3.23±0.05	141.83±3.98	87.5±2.16
Sig.	N.S	*	N.S	N.S	N.S	N.S	N.S	N.S	N.S

*The different letters indicate the presence of significant differences between the groups. N.S: Indicates no significant differences between the mean coefficients. TG: Triglycerides, CHOL: Cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, ALB: albumin, GLOB: globulin, TP: total protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

Table 5: Relationship between genetic polymorphism of the FSH receptor gene (FSHR 279 T>G) and blood hormone levels in fallow deer (mean ± SE).

Genotype	FSH (mIU/mL)	LH (mIU/mL)	Test (ng/mL)	Cortisol (ng/mL)
TT	0.30±0.00[b]	0.16±0.00	0.38±0.09	75.54±4.50
TG	0.56±0.01[a]	0.14±0.00	0.46±0.08	74.16±6.74
Sig.	*	N.S	N.S	N.S

*The different letters indicate the presence of significant differences between the groups. N.S: Indicates no significant differences between the mean values. FSH: follicle-stimulating hormone, LH: Luteinizing hormone, TEST: testosterone.

animals, including fallow deer. In addition, previous studies Tdemonstrated that improvements in environmental and health conditions may indirectly influence physiological parameters through reduced microbial load (Al-Salhi *et al*., 2025; Naser *et al*., 2025).

The present findings differ from those reported in Iraqi goitered gazelles by Banana (2014). Such variation may be attributed to species differences, body weight, nutritional status, environmental conditions, and genetic background. Similar observations were reported by Cuervo *et al*. (2011), who demonstrated variability in biochemical parameters among threatened deer species, including *Gazella cuvieri*, *Gazella dama*, and *Gazella dorcas*, particularly for cholesterol, total protein, albumin, and lactate dehydrogenase (LDH). Differences in LDH activity may also be associated with skeletal muscle stress and handling during capture.

The present study also showed no significant differences in HDL, LDL, TP, ALB, GLOB, and TG among the studied genotypes. Similar findings have been reported in goats and sheep under experimental conditions (Mardenli *et al*., 2019). These results may indicate that the FSHR gene exerts only an indirect effect on most biochemical traits. Such physiological stability may reflect maintenance of immune and metabolic homeostasis (Al-Salhi, 2026). Likewise, cortisol concentrations did not differ significantly among genotypes, which is in agreement with the findings of Sejian *et al*. (2019) regarding stress adaptation in sheep.

Similarly, AST and ALT activities showed no significant differences between genotypes, suggesting that the studied mutation may not directly influence hepatic enzyme activity. Comparable results were reported by Zhang *et al*. (2020) in goats and sheep.

In contrast, the results presented in Tables 4 and 5 revealed significant differences ($P \leq 0.05$) between genotypes for cholesterol (CHOL) and follicle-stimulating hormone (FSH) concentrations. The TT and TG genotypes showed values of 67.00 and 75.16 for CHOL, respectively, while both genotypes showed similar FSH values. These findings are partially consistent with those reported by Scharfe *et al*. (1998) in elk. Variations in blood biochemical markers may reflect differences in metabolism, endocrine regulation, and nutritional adaptation. However, information regarding the association between FSHR polymorphisms and biochemical traits in fallow deer remains limited.

CONCLUSIONS

This study provides the first insight in Iraq into the association between FSHR (T279G) gene polymorphism and blood biochemical parameters in fallow deer (*Dama dama*). Two genotypes (TT and TG) were identified. Significant differences were observed only in cholesterol (CHOL) and follicle-stimulating hormone (FSH), whereas all other biochemical and hormonal parameters remained unaffected. These findings suggest a limited and selective influence of this polymorphism on physiological traits, supporting its potential relevance in genetic characterization and wildlife genetic studies.

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NOVELTY STATEMENT

This study opens new perspectives for conducting further research on the Iraqi fallow deer (*Dama dama*), an endangered species.

AUTHOR'S CONTRIBUTION

Hydarfalih Mahdi Al-Hassani: Contribute to conducting direct research work and laboratory tests.
HadiAwad Al-Burkat: Conceptualized the research idea and guided the overall study design.
Ali Abdullah Alsaadoon: 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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