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Relationship of polymorphism of the DIO2 gene within exon2 to some growth and prolificacy traits in local black goats

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Abstract

This study was conducted on 40 local goats, aged 2-3 years, during the period from October 15, 2024 to May 15, 2025, in the field of the College of Agriculture, University of Diyala. All molecular genetic analyses were performed in the College of Agriculture laboratory, as well as in the Rukan Al-Rawan (Al-Jadriya) .The aim was to study the relationship between of the DIO2 polymorphism gene and growth traits (birth weight, weaning weight, and total weight gain), as well as its relationship to body dimensions at birth and weaning for dams (does) and prolificacy. The results showed a change from glutamine to arginine, which was considered a missense mutation at the protein level. The results also showed that the genetic polymorphisms of the A9280G of the DIO2 gene, which two genotypes (AA and AG) appeared in the second exon, did not show significant differences between the genetic combinations with regard to the weight and dimensions of the dam body at birth. The same was the case with the growth traits of the kids, as no significant effects were shown in the weight at birth and weaning, the rate of weight gain, and the prolificacy, as the averages were close to each other. While, the results showed significant effects ($P \leq 0.05$) of the two combinations in the traits of chest and abdominal circumference at weaning, with the individuals carrying AA being superior with an average of 45.13 and 48.16 cm compared AG type (42.5 and 45.33 cm) respectively.

Keywords: DIO2 gene, growth traits, local goat

Introduction

The local goat Capra hircus represents one of the most important animal resources in the world due to its productive efficiency in difficult climatic conditions and its ability to exploit poor pastures and transform them into products of nutritional and economic value (1). These animals also possess outstanding genetic capabilities that qualify them to be a primary source of good quality meat, as well as producing milk with high quality characteristics, which makes them an important pillar in food security for small ruminants (2). In Iraq, goats occupy a prominent position in the agricultural sector due to their ability to grow and produce under limited feeding conditions and semi-arid environments, which has led to an increasing interest in raising them in recent years, especially with the possibility of developing and improving breeds through genetic selection programs (3).

Their genetic makeup by increasing the frequency of desirable alleles and providing suitable environmental conditions that ensure the highest possible levels of productive and reproductive performance. It is noteworthy that goats rank third in terms of population size after sheep and cattle in Iraq. Their numbers have declined, reaching 1.6 million head in 2013 (4), whereas the most recent statistics from the Ministry of Agriculture/Animal Resources Directorate for 2022 indicate that the goat population currently stands at 1,360,742 head. Goats are considered among the animals most capable of adapting to harsh environmental conditions, maintaining productivity even under limited grazing resources and low-quality feed, compared with sheep and cattle (5 , 6). Their importance further lies in their production of milk, meat, and hides, in addition to the potential use of goat hair in textile industries (7). In the functional diversity of thyroid-hormone signaling. They contribute significantly to pathways of growth, development, and metabolic processes, and they exhibit cell-specific regulation of thyroid-hormone homeostasis. These enzymes activate or deactivate thyroid hormones by removing a single iodine atom from either the outer or inner ring of the hormone molecule. They are widely distributed across body tissues, and their presence is essential for regulating the intracellular bioactivation of thyroid hormones. Thyroxine (T4) is the primary circulating form produced exclusively by the thyroid gland and is considered a prohormone; however, its biological activity is achieved only after its conversion in tissues to the active form triiodothyronine (T3) or the inactive reverse form (rT3). Three types of iodothyronine deiodinases have been identified—DIO1, DIO2, and DIO3—which, despite being encoded by different genes, share important structural and functional features. The initial deiodination step carried out by these enzymes is crucial for regulating thyroid hormone activity under both normal and pathological conditions that require variation in enzyme distribution and function (8). Among them, DIO2 is regarded as the key enzyme regulating thyroid-hormone synthesis, transport, and action. In sheep and goats, DIO2 gene expression is broadly distributed in the hypothalamus and thyroid gland (9 , 10).

In humans, the DIO2 enzyme is encoded by the DIO2 gene located on the long arm (q) of chromosome 14 at band 24. It is located on chromosome 12 in mice, chromosome 7 in sheep, and chromosome 10 in goats. The gene contains two coding regions (Exon 1 and Exon 2) separated by an intron approximately 7.4 kb in length (11). Therefore, the present study aimed to identify the genetic variants of the DIO2 gene and investigate the distribution and frequencies of genotypes and alleles in the studied population. It further sought to examine the association between the gene's genetic polymorphisms and growth and milk traits, as well as its relationship with thyroid-hormone levels.

Materials and Methods

Sample Collection

A sample of 40 local doe,aged 2-3 years, was collected from the field of the Animal Production Department, College of Agriculture, University of Diyala, from Oc-

tober 15/ 2024 to May 15/ 2025. All molecular genetic analyses were performed in the College of Agriculture laboratory and Rukan Al-Rawan Laboratory.

Blood samples

A 5 ml blood sample was drawn from the jugular vein of 40 local goats. The sample was placed in a tube containing EDTA anticoagulant and stored at -18°C until it was used to determine the genotype of exon 2.

Agarose Gel Electrophoresis

This technique was used to detect the presence of DNA bands. This process was applied after DNA extraction, and to detect the size of the polymerase chain reaction (PCR) product for the exon2 region. The samples were transferred on a 1% agarose gel after dissolving it in 100 ml of TBE solution with the addition of 1.5 microliters of ethidium bromide dye. 3 microliters of the PCR product were taken and loaded into the gel wells and transferred in an electrophoresis device at 50 volts for 50 minutes. After the transfer was completed, the bands were imaged using a Gel Imaging System.

Determining the genotypes of the DIO2 gene

The primer was designed within the exon2 region, with a fragment size of 638 base pairs, using the Geneious Prime program, based on the reference sequence in the Gen Bank/NCBI database, NC-030817, in exon 2:

F: 5' – ACAGAGTAAGCGCAGTAAAGA – 3'

R: 5' –TGCTTCTTCACCTCGAAAAAC – 3'

The reaction mixture was prepared for 40 samples with a final volume of 20 µl, and the appropriate degree of binding was determined for the exon2 (57 °C), which is shown in Table (1).

Table (1): Software used for molecular analysis using PCR technology for the Exon2 to *DIO2* gene

Steps	Temperature	Time	No. of cycles
Initial denaturation	94	5 min.	1 cycle
Denaturation	94	30 sec	35 cycle
Annealing	57	45 sec.	
Extension	72	35 sec.	
Final extension	72	10 min.	1 cycle

Genetic phenotype identification using DNA sequencing

After identifying the Exon2 region segment using PCR, the genetic phenotypes were identified using DNA sequencing. The studied samples (40 samples) were sent to South Korea to identify the genetic phenotypes of the studied segments. Sequence analysis was performed using Sanger sequencing, using BLAST on the NCBI Gene bank website, using bioinformatics software.

Growth traits

The dams weights and body dimensions were recorded at the beginning of the experiment before giving birth using a digital electronic scale, as well as a body measurement kit used to measure the dimensions of their offspring. The weights and body dimensions of the kids were recorded as follows:

1- Birth weight of young calves: Birth weight of young calves was recorded 24 hours after birth using a digital scale.

2- Weaning weight: Weaning weight of young calves was recorded at 60 days of age using a digital scale.

3- Weight gain: Total weight gain was calculated by subtracting weaning weight from birth weight.

Body measurements were taken for newborns before and at weaning as follows (12):

1- Chest circumference: The chest girth was measured using a measuring tape around the chest area directly behind the forelegs and forelegs.

2- Height at withers: The abdominal girth was measured using a measuring tape around the abdominal area directly in front of the hindlegs.

3- Body length: The body length was measured using a measuring tape from the front of the chest to the end of the animal at the hump bone.

4- Wither height: The foreleg height was measured using a sliding ruler, measuring the vertical distance from the ground to the highest point of the shoulder.

5- Rump height: The rump height was measured using a sliding ruler, measuring the vertical distance from the ground to the highest point of the animal's back at the tail.

The fertility rate of mothers was also calculated by dividing the number of offspring born by the number of mothers giving birth to determine the number of offspring per goat.

Statistical Analysis

The data were analyzed statistically using the Statistical Analysis System (SAS) (13) software to study the effect of genetic compositions within the second exon of the DIO2 gene on growth traits, body dimensions, and fertility rates in a sample of local goats. Significant differences between means were compared using the Duncan (14) multinomial test according to the analysis model below:

$$Y_{ijklmn} = \mu + G_i + A_j + B_k + T_l + S_m + e_{ijklmn}$$

Yijk: The observed value n for genotype i, month of birth j, year of birth k, type of birth l, and sex of offspring s.

μ: The overall mean for the trait.

Gi: The effect of the genotype of the A9280G mutation site in the DIO2 gene (AA and AG).

Aj: The effect of the month of birth (11, 12, 1, and 2: for adjustment purposes).

Bk: The effect of the year of birth (2024 and 2025: for adjustment purposes).

Tl: The effect of the type of birth (single and twin: for adjustment purposes).

Sm: The effect of the sex of offspring (male, female: for adjustment purposes).

eijklmn: The random error, which is normally distributed with a mean of zero and a variance of σ2e.

The chi-square test (χ2) was also used to compare the percentages of the distribution of the genotypes for the studied heterogeneity in the studied goat sample according to Mendel's second law.

$$X^2 = \sum \frac{(0-E)^2}{E}$$

The following law was applied to calculate the allelic frequency according to Hardy Weinberg's equilibrium (15).

$$P_A = \frac{2 \times no.Homo + 1 \times Hetro}{2xn}$$

$q_a = 1 - P_A$

Results and Discussion

Genetic phenotypes of the second exon region (Exon 2)

The results of polymerase chain reaction analysis of the second exon region of the DIO2 gene revealed a target fragment size of 638 base pairs (Figure1). DNA sequencing technology was used at the A9280G site, which resulted in a change from the base A, which encodes the amino acid glutamine, to the base G, which encodes the amino acid arginine, resulting in a change in the type of amino acid. This mutation was recorded as a missense mutation. The variation in the sequence of bases led to the two genotypes AA and AG, as shown in Figure (2). The detection of this missense mutation is of biological significance, as it causes a change in the amino acid sequence in the coding region (Exon 2).. This result is consistent with that of Abd Al-Razaq (16), who found that the C478G mutation in the second exon of the studied Awassi sheep samples resulted in a change from the amino acid proline to the amino acid alanine. This result contradicts JianNing et al. (10), who found that the G482C mutation in the second exon of the same gene did not result in a change in the amino acid sequence and therefore did not affect the protein's function.

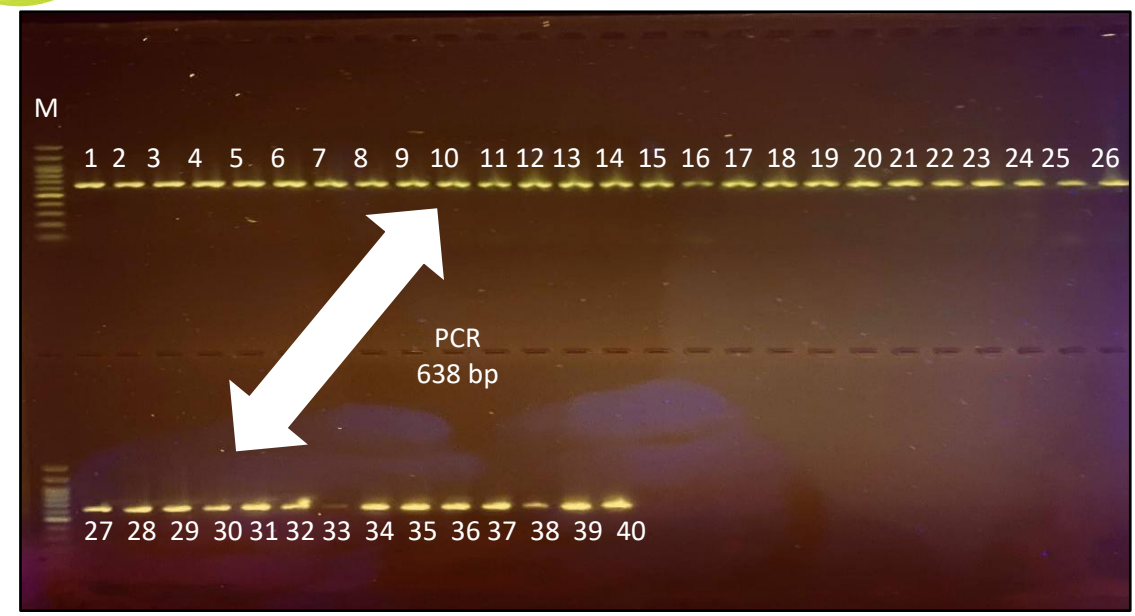

Figure (1): PCR result of the second exon region using 1% acrosome gel for the DIO2 gene using a size marker (100-1500).

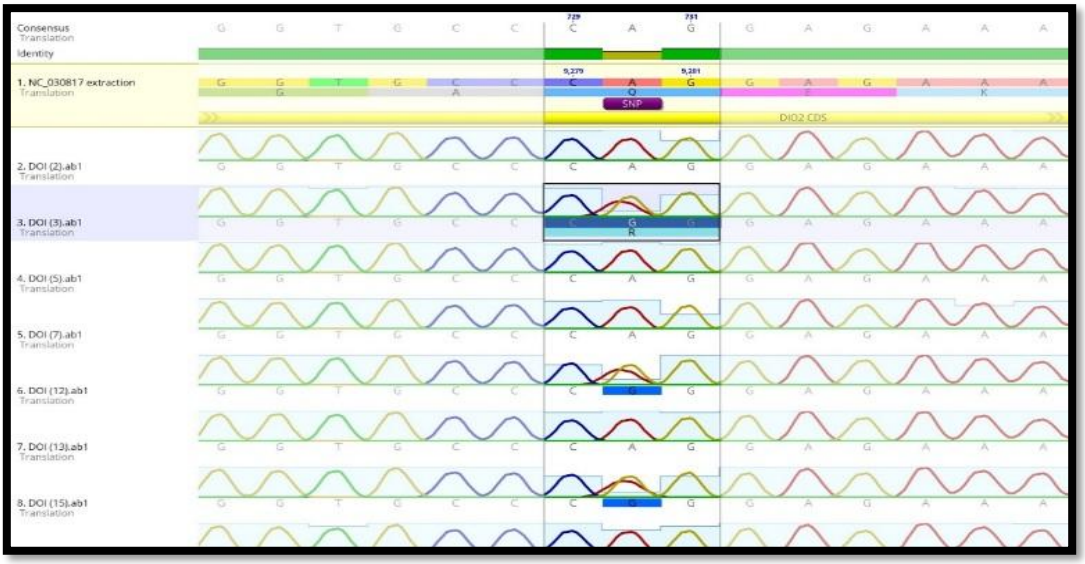

Figure (2): Genetic polymorphisms of the second exon region within the A9280G site

Figure (3) shows the nucleotide sequence of the 638 base pair amplification fragment, indicating the substitution site of the base A for the base G, which caused the conversion of the amino acid glutamine (CAG) to the amino acid arginine (CGG). The registration of this missense mutation is of biological importance as it caused a change in the amino acid sequence in the coding region, which may be reflected in the spatial structure of the protein and its function. This result is consistent with the findings of (16), who found that the mutation C478G in the second exon of the studied samples of Awassi sheep led to a change from the amino acid proline to the amino acid alanine. However, this result contradicts (10), who found that the mutation

G482C in the second exon of the same gene did not lead to a change in the amino acids and therefore does not affect the function of the protein.

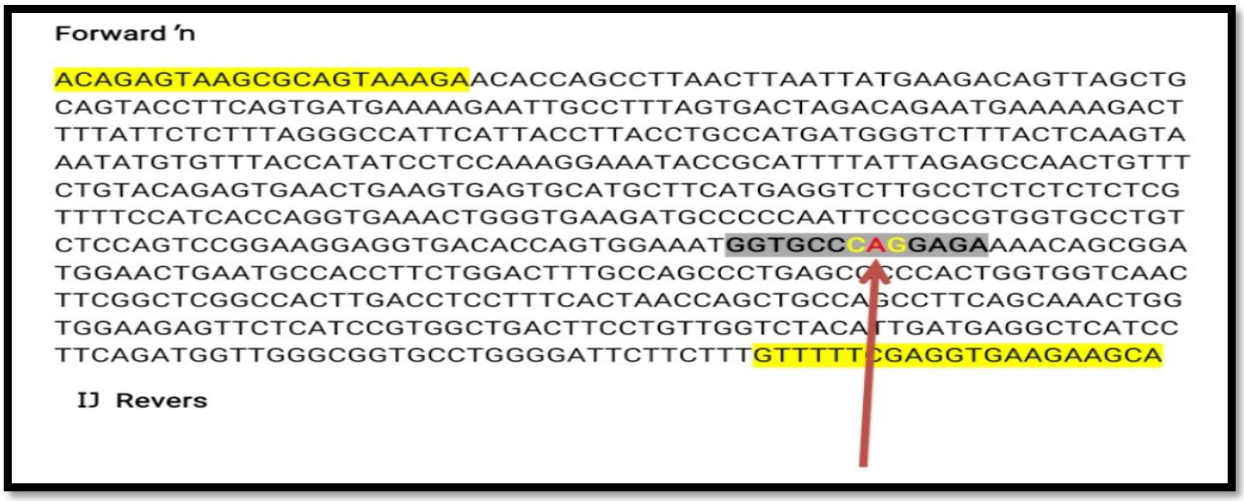

Figure (3): The mutation location in the amplification fragment sequence is 638 base pairs

Relationship of DIO2 genotypes to maternal body weight and dimensions

Table 2 shows that DIO2 genotypes did not significant differences in any of the dams traits. The mean body weight was 35.17 ± 0.79 kg for the AA combination with AG (36.71 ± 0.92 kg), while the body length recorded averages of 66.71 ± 2.04 cm for the AA combination and 65.71 ± 2.54 cm for the AG. The mean height at the withers was 65.81 ± 0.74 cm for the AA combination with 63.28 ± 2.37 cm for the AG. The mean height at the withers recorded averages of 70.28 ± 1.02 and 67.42 ± 1.95 cm for the AA and AG combinations, respectively.

Likewise, no significant differences were recorded between the genotypes, with the averages 79.13 ± 1.05 and 80.14 ± 3.07, 86.21 ± 1.61, and 87.42 ± 5.26 cm for the AA and AG combinations for chest and abdominal circumference, respectively. These results are consistent with what some studies have indicated, indicating that polymorphisms in the DIO2 gene may play a more prominent role in productive traits than in body dimensions (16). Other studies have shown a significant effect of some genes on body dimensions, which may be related to differences between breeds and rearing conditions (17).

Table (2): Relationship between the genotype of the DIO2 gene and the mother's body weight and dimensions

Polymorphism	Number of observations	Mean ± Standard erroe					
		Body weight at birth (kg)	Body length (cm)	Height at withers (cm)	Height at rump (cm)	Chest circumference (cm)	Abdomen circumference (cm)
AA	34	35.17 ±0.79	66.71 ±2.04	65.81 ±0.74	70.28 ±1.02	79.13 ±1.05	86.21 ±1.61
AG	7	36.71 ±0.92	65.71 ±2.54	63.28 ±2.37	67.42 ±1.95	80.14 ±3.07	87.42 ±5.26
Significance Level	---	NS	NS	NS	NS	NS	NS
NS ; ($P \leq 0.05$) *							

The relationship between DIO2 gene polymorphisms and birth, weaning, and weight gain of local goat kids

Table 3 shows that the genotypes of the DIO2 gene did not significant differences in early growth traits. The average birth weight was 3.18 ± 0.11 kg and 3.24 ± 0.15 kg, and the weaning weight was 8.39 ± 0.34 kg and 8.98 ± 0.37 kg for the AA and AG, respectively. While, the weight gain between birth and weaning was 5.18 ± 0.30 kg and 5.70 ± 0.27 kg for the AA and AG genotypes, respectively. These results indicate that genetic variation in Exon 2 of the gene did not significantly affect early growth traits in local goats. The values were very similar between the AA and AG genotypes, which may be attributed to the small sample size for the AG genotype, thus reducing the statistical power to detect significance. Additionally, the gene's role in growth traits may be indirect and related to the regulation of thyroid hormones T3 and T4 and metabolic activity.

This study showed contrasting results when compared to previous studies conducted on sheep. (18) indicated significant variation in lamb birth weights and weight gain rates, while Malewa et al. (19) demonstrated significant differences in growth weights at weaning. Abd Al-Razaq (16) indicated significant differences in both birth weight and weight gain, as well as highly significant differences in weaning weight. This variation may confirm that the effect of the gene differs according to breed and breeding conditions.

Table (3): Relationship between the genetic makeup of the DIO2 gene and birth and weaning weight and rate of weight gain

Polymor-phism	Number of observa-tions	Mean ± Standard erroe				
		Bitth weigh t (kg)	Number of observa-tions	Wean-ing weight (kg)	Number of observa-tions	Weight gain be-tween birth and wean-ing
AA	34	3.18 ±0.11	28	8.39 ±0.34	28	5.18 ±0.30
AG	7	3.24 ±0.15	6	8.98 ±0.37	7	5.70 ±0.27
Significance Level	---	NS		NS		NS
NS ; ($P \leq 0.05$) *						

The relationship between DIO2 gene polymorphisms and kids' body dimensions at birth in local goats

Table 4 indicates that the genotypes of the DIO2 gene did not significant differences in all body measurements at birth for kids. The average body length was 31.03 ± 0.74 cm and 32.83 ± 1.88 cm for the AA and AG genotypes, respectively. The average height at the front was 32.75 ± 0.82 cm for the AA genotype and 31.00 ± 1.54 cm for the AG genotype, while the values were similar at the rump height: 33.73 ± 0.80 cm for the AA genotype and 33.50 ± 1.43 cm for the AG genotype. The average chest circumference was 36.60 ± 0.64 cm for genotype AA and 35.50 ± 1.85 cm for genotype AG. Similarly, abdominal circumference was averaged at 38.03 ± 0.81 cm for offspring of dams with genotype AA, while it was 38.66 ± 3.11 cm for offspring of mothers with genotype AG.

The influence of DIO2 gene may be more pronounced in subsequent growth rate and productivity than in initial morphological measurements at birth, which are more significantly affected by maternal factors, nutrition during pregnancy, and uterine condition. These results contradict studies such as (17) and (16), which showed significant differences in body measurements at birth in Awassi sheep.

Table (4): Relationship between the genotype of the DIO2 gene and the body dimensions of the kid at birth

Polymorphism	Number of observations	Mean ± Standard erroe				
		Body length (cm)	Height at withers (cm)	Height at rump (cm)	Chest circumference (cm)	Abdomen circumference (cm)
AA	33	31.03 ±0.74	32.75 ±0.82	33.73 ±0.80	36.60 ±0.64	38.03 ±0.81
AG	6	32.83 ±1.88	31.00 ±1.54	33.50 ±1.43	35.50 ±1.85	38.66 ±3.11
Significance Level	---	NS	NS	NS	NS	NS
NS ; ($P \leq 0.05$) *						

The relationship between DIO2 gene polymorphisms and weaning kid dimensions in local goats

Table (5) shows that the DIO2 gene genotypes did not exhibit significant differences in most anthropometric measurements at weaning. The mean body length was 42.60 ± 1.07 and 41.83 ± 1.37 cm for the AA and AG genotypes, respectively, while the front height was 39.50 ± 0.82 versus 38.00 ± 1.03 cm for the same genotypes, and the hind height was 42.23 ± 0.88 and 39.83 ± 0.74 cm, respectively. While the results showed significant differences ($P \leq 0.05$) in both chest and abdominal circumference, the AA genotype was superior for both traits compared to the AG genotype, with averages of 45.13 ± 0.94, 42.50 ± 1.71 cm, 48.16 ± 1.12, and 45.33 ± 1.45 cm for the two traits, respectively. This superiority may be attributed to the physiological role of the DIO2 gene in regulating T3 hormone levels, which is responsible for metabolism and energy processes. These processes are reflected in the growth of muscle and fat tissues, which are directly related to chest and abdominal circumference. These are important indicators of the animal's physical condition and future productive capacity. These results were higher than the average chest circumference in studies of some Chinese sheep breeds, and the current values were also higher than those recorded by (17) and (16) for chest and abdominal circumference in their studies of Jordanian and Iraqi Awassi sheep. These differences may be attributed to environmental, management, and healthcare factors such as month of birth, sex, type of birth, season of birth, and maternal weight, all of which affect the aforementioned traits (20).

Table (5): Relationship between the genotype of the DIO2 gene and the body dimensions of the kid at weaning

Polymorphism	Number of observations	Mean ± Standard error				
		Body length (cm)	Height at withers (cm)	Height at rump (cm)	Chest circumference (cm)	Abdomen circumference (cm)
AA	28	42.60 ±1.07	39.50 ±0.82	42.23 ±0.88	45.13 ±0.94 a	48.16 ±1.12 a
AG	6	41.83 ±1.37	38.00 ±1.03	39.88 ±0.74	42.50 ±1.71 b	45.33 ±1.45 b
Significance Level	---	NS	NS	NS	*	*
NS ; ($P \leq 0.05$) *						

The Relationship Between DIO2 Gene Polymorphisms and prolificacy

Table (6) shows the correlation between genotypes and fertility rate at birth. The effects of DIO2 gene genotypes were not statistically significant. The AA genotype recorded a mean of 1.18 ± 0.07, while the mean for the AG genotype was 1.16 ± 0.04.

These results support another study by JianNing et al. (10), which showed no significant relationship between genotypes and birth size in the Small Tail Han sheep breed in China. However, the results of these two studies contradict those of Abd Al-Razaq (16), which revealed a significant effect of genotypes of the same gene. Given that the DIO2 gene may be involved in the seasonal photodynamic control of reproduction, dependent on melatonin hormonal signaling, mammalian reproductive activities are regulated by the day-length rhythm. Revel et al. (21) concluded that reciprocal variations in gene expression of both DIO2 and DIO3 represent a fundamental genetic mechanism within the photochemical molecular chain that contributes to the stimulation of LH melatonin secretion in long-day breeds, which differs from short-day breeds, as shown by Yasuo et al. (22). Although there is no statistical significance in the results of the current study between the two constructs in the levels of T3 and T4, we find a numerical difference in the levels of T3, as the level of T3 for the wild construct AA was higher (1.71 mol/L) than for the hybrid construct (1.67 mol/L). This may mean that the presence of the mutant allele within the studied mutation site (A9280G) caused a decrease in the levels of expression of the DIO2 enzyme,

which was reflected positively in the decrease in the levels of T3 in individuals carrying the AG construct.

Table (6): Relationship between the genetic makeup of the DIO2 gene and the fertility rate (Prolificacy)

Polymorphism	Number of observations	Fertility	
		Litter size	Mean ± Standard erroe
AA	34	40	1.08 ± 0.07
AG	6	7	1.16 ± 0.04
Significance Level	---		NS
NS ; ($\mathbf{P \leq 0.05}$) *			

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