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Genetic Mutations of the Prolactin Gene in Awassi Sheep

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Annotation: The study was conducted on 67 ewes and their offspring at the Khairat Al-Ittihad Sheep and Cattle Breeding Station in Babil Governorate, Iraq, during the period from December 2023 to May 2024. Data were collected from the station's records, including ages, weights, and birth sequences, in addition to laboratory analyses of blood and milk samples. This study aimed to analyze PRL gene mutations in Awassi sheep. DNA was extracted from blood samples using specialized techniques, and the target PRL gene was amplified using polymerase chain reaction (PCR). The results revealed the identification of three mutations in the PRL gene: SNP1: 277 C>T, SNP2: 684 G>T, and SNP3: 737 T>G. The allelic frequencies of the mutations were diverse, with the CC genotype predominating in SNP1, the GT hybrid in SNP2, and the TG hybrid in SNP3.

Keywords: PRL gene mutations, SNP1, SNP2, SNP3, Awassi sheep.

Introduction:

Genetic factors are a major factor influencing milk production in sheep. Different breeds of sheep have different production capacities based on their genetics. Sheep with good genetics and bred to increase milk production typically exhibit higher milk yields than other sheep. Awassi sheep are known for their higher milk production compared to other breeds (McManus *et al.*, 2010). The genotype of sheep plays an important role in determining the composition of milk. Sheep with certain genes can produce milk containing varying proportions of fat, protein, and carbohydrates. Genetics affects not only the quantity of milk but also its quality, as some breeds can produce milk with higher fat or protein content (Barillet, 2007).

The prolactin gene's genotype consists of several exons and introns. Exons contain the sequences that encode the prolactin protein, while introns play a role in regulating gene expression, the gene also includes prolactin regions that regulate when and where the gene is expressed. The prolactin gene typically consists of four to six exons connected by introns. Prolactin acts as a protein hormone secreted from the anterior lobe of the pituitary gland. It plays a key role in stimulating mammary cells to produce milk, in addition to regulating the immune system and promoting cellular growth and development (Souza *et al*., 2001; Spencer and Bazer, 2002).

The prolactin gene is located on chromosome 6 in humans, it consists of approximately 10,000 base pairs, contains five exons and four introns, and encodes the hormone prolactin, which consists of 199 amino acids. Gene expression is regulated by promoter elements that respond to factors such as estrogen and progesterone, which regulate gene expression and prolactin secretion (Bole-Feysot, 1998; Freeman *et al*., 2000).

In Iraqi sheep, especially the Awassi breed, the prolactin gene plays a vital role in regulating milk production and the estrus cycle. Prolactin stimulates milk production in the mammary glands and has multiple effects on the reproductive system and other physiological functions. Prolactin is considered a key hormone for stimulating milk production. Studies have shown that prolactin levels rise significantly during lactation, enhancing milk yield and quality. Prolactin stimulates the mammary glands and stimulates milk production by increasing the expression of genes associated with milk production. This makes Awassi sheep an ideal choice for farmers seeking to improve the productivity of their animals (Al-Samarai and Al-Anbari, 2009; Jawasreh *et al*., 2019).

Prolactin gene expression is regulated by several factors, including stimulating hormones, such as growth hormone and prolactin-releasing hormone (PRH) and inhibitory hormones such as dopamine and somatostatin, which contribute to achieving the physiological balance necessary to ensure optimal sheep performance (Freeman *et al*., 2000). Research indicates that changes in prolactin gene expression can significantly impact milk production, which reinforces the importance of this gene in improving breeding programs and selecting sheep with high milk production efficiency (Al-Samarai and Al-Anbari, 2009; Jawasreh *et al*., 2019). In Iraqi sheep, including the Awassi breed, the prolactin gene plays a central role in controlling milk production and the estrous cycle. Research has shown that prolactin gene expression can vary based on environmental and physiological factors such as nutrition and lighting (Talafha and Ababneh, 2011).

The prolactin gene begins the translation of messenger RNA (mRNA) into prolactin protein in the ribosomes. This process is regulated by several factors, including hormones such as estrogen and progesterone, which enhance prolactin gene expression. Dopamine, which reduces prolactin secretion via D2 receptors located in mammary cells (Freeman *et al*., 2000).

This study aims to identify genetic mutations in the prolactin gene in Awassi sheep.

Materials and Methods:

This study was conducted at the Khairat Al-Ittihad Sheep and Cow Breeding Station, affiliated with the Al-Ittihad Company, in the Shomali District of Babil Governorate, from December 1, 2023 to May 31, 2024, it was conducted. Sixty-seven mother animals and 67 lambs were used for one production season. The parents ages ranged from 2 to 4 years. Data was obtained from the records maintained at the station, including their ages, birth numbers, birth weights, and current birth sequence. The laboratory aspect was also conducted in the station's laboratory.

Blood samples were drawn from the jugular vein in the neck using a 10 ml syringe. A total of 5 ml of blood was drawn for each sample after the blood collection area was cleaned and sterilized with ethyl alcohol. The samples were then emptied into a sterile test tube free of anticoagulants. They were stored at -4°C until laboratory use and DNA extraction.

DNA was extracted from sheep blood samples using a kit provided by the Korean company Geneaid.

Primers for the PRL gene were purchased by the Korean company Macrogen, in the form of a dried powder placed in a special tube labeled with the nitrogenous base sequence. The primers were prepared by adding 300 microliters of distilled water (dd water), to achieve a primer concentration of 100 picomoles. This is considered the stock solution, and 10 microliters were then taken from it. 90 microliters of distilled water (dd water) were added again. This resulted in a primer concentration of 10 picomoles, the concentration required for PCR. Table (1) shows the dilution of the primers and the quantities of distilled water (dd water) added.

Table (1) shows the dilution amounts for the primers.

chemical substance	Master Mix	DNA template	Primers		distilled water	Final volume
			Forward	Reverse		
Volume (microliter)	13	4	1	1	6	25

Table (2) Sequence of PRL gene primers used.

Gen	Primers	Volume/ nucleotide	GC%
PRL -F1	5'- CTGTGTGTGTCTGTGCCTTTCC - 3'	21	52
PRL -R1	5'- CAAAATCCTGGTCCAGGGCAAC - 3'	22	50
PRL -F1	5'- ATGAGTTTGGTCAATTAGGTGGAACAC - 3'	24	50
PRL -R2	5'- ACAAAATCCTGGTCAAGGGCAA - 3'	21	48

Table (3) Stages of PCR technique for PRL gene.

Gen	Stages	Temperatures	Time (min.)	Cycle No.
GDF9	First metamorphosis	95C°	5	1
	The Metamorphosis	95C°	0.30	35
	Adhesion	55 C°	0.45	
	Elongation	72C°	1.00	
	Final elongation	72C°	10	1

Results and Discussion:

The first mutation is SNP1:

The results of the sequencing of the studied PRL gene fragment (773 base pairs) revealed a change at position 277 of the studied region of this gene. The nitrogenous base guanine (C) was changed to the nitrogenous base thymine (T). The individuals carrying the homozygous CC genotype outnumbered the ewes. They accounted for 42 ewes, compared to 21 and 7 ewes with the CT and TT genotypes, respectively. The frequency of the CC, CT, and TT genotypes was 59.70, 29.85, and 10.45 percent, respectively. The frequency of the C and T alleles was 0.75 and 0.25, respectively. The results are consistent with Evlagina *et al*. (2021) in a study that included 248 Russian Lacaune sheep on the Nikolaev farm in Krasnodar region (Table 4).

Table (4) Number and percentages of genotypes for the PRL gene SNP1: 277 C>T.

Genotype	No.	(%)
CC	40	59.70
CT	20	29.85

TT	7	10.45
Total	67	% 100
Chi square (χ^2)	---	43.388 **
Allele	Frequency	
C	0.75	
T	0.25	

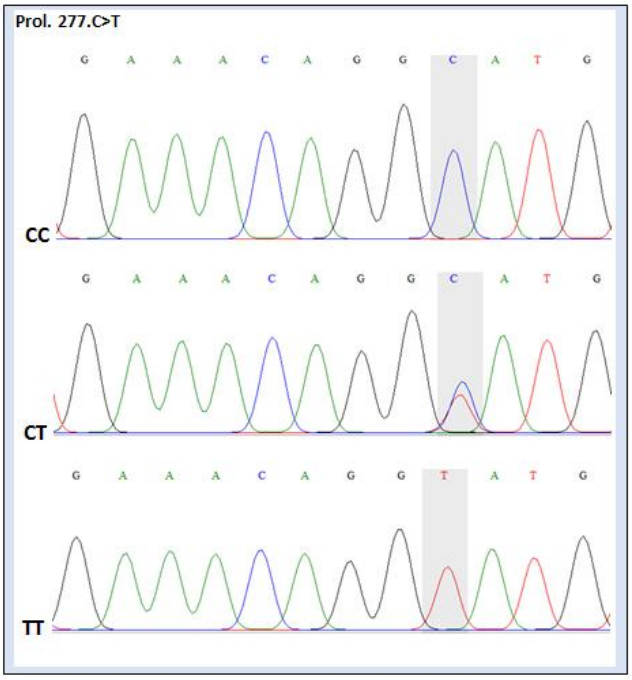

Figure (1) The location of the first mutation 277.C>T from the studied region of the prolactin gene.

The second mutation is SNP2:

As for the second mutation, the sequencing results of the studied segment of the PRL gene showed a size of 773 base pairs. A change was found at position 684 of the studied region of this gene, where the nitrogenous base guanine (G) was changed to the nitrogenous base thymine (T). The individuals carrying the hybrid genotype GT outnumbered the ewes, forming 35 ewes, compared to 25 and 7 ewes with the GG and TT genotypes, respectively. The frequency of the GT, GG, and TT genotypes was 52.24, 37.31, and 10.45 percent, respectively. The frequency of the G and T alleles was 0.63 and 0.37 percent, respectively. In a study Mohamed *et al.* (2020), which was similar to our study, GT (heterozygous) represented 52.24%, GG represented 37.31%, and TT represented 10.45%, with allele frequencies of G = 0.63 and T = 0.37. The distribution is also similar, with heterozygous being the most frequent (~34.7%), followed by GG, then TT (~13.7%), and the allele distribution is also similar (Table 5).

Table (5) Number and percentages of genotypes for the PRL gene SNP2: 684 G>T.

Genotype	**No.**	**(%)**
GG	25	37.31
GT	35	52.24
TT	7	10.45
Total	67	% 100
Chi square (χ^2)	---	10.676 **
Allele	Frequency	
G	0.63	
T	0.37	

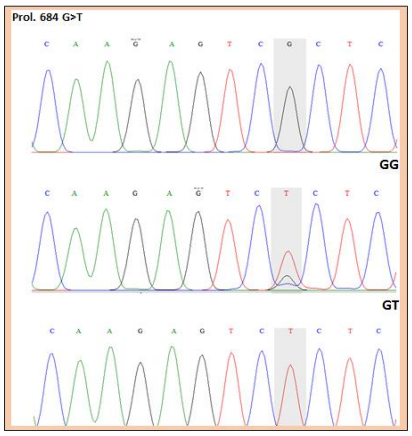

Figure (2) The location of the second mutation 684 G>T from the studied region of the prolactin gene.

The third mutation, SNP3:

This discrepancy in the sequencing results of the studied segment of the PRL gene (773 base pairs) is evident. There was a change at position 737 of the studied region of this gene. The nitrogenous base, guanine (T), was changed to a nitrogenous base, thymine (G). Individuals carrying the GT hybrid genotype outnumbered those carrying the GT hybrid genotype. They accounted for 32 ewes, compared to 26 and 9 ewes carrying the TT and GG genotypes, respectively. The frequency of the GT, GG, and TT genotypes was 47.76, 38.81, and 13.43 percent, respectively. The frequency of the G and T alleles was 0.63 and 0.37 percent, respectively. A study conducted by Al Thuwaini (2021) confirmed the presence of mutations in A and T (Table 6).

Table (6) Number and percentages of genotypes for the PRL gene SNP3: 737 T>G.

Genotype	No.	(%)
TT	26	38.81
TG	32	47.76
GG	9	13.43
Total	67	% 100
Chi square (χ^2)	---	8.761 **
Allele	Frequency	
T	0.63	
G	0.37	

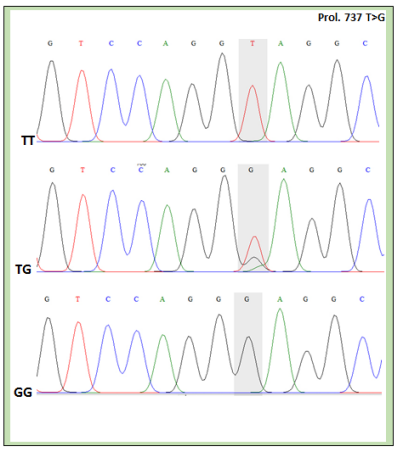

Figure (3) Location of the third mutation Prol. 737 T>G from the studied region of the prolactin gene.

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