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Effect of *POU1F1* gene Haplotypes on weights and Milk Production of Awassi sheep

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Abstract:. The aim of the study was to detect polymorphism in the *POU1F1* gene in Iraqi Awassi sheep breed, as well as to establish if haplotype of *POU1F1* gene could be associated with productive traits. This study was carried out at Al-Kafeel station, Karbala city during the period of 01/10/2017 until 01/08/2018. The study included 46 Awassi ewes with their 52 lambs. The laboratory analyses were conducted at the Laboratory of Molecular Genetics, College of Agriculture, University of Basrah. Results showed the successfulness of the PCR amplification process for all six examined fragments of the *POU1F1* gene. Gel electrophoresis was conducted using agarose 2%, the product sizes were 637bp, 789bp, 999bp, 868bp, 1190 bp, and 469bp for the fragments P1, P2, P3, P4, P5, and P6 respectively. The analysis of the nitrogen bases sequences of the *POU1F1* gene for the studied fragments showed a change in 12 different sites of the gene. These changes resulted in 7 haplotypes of H1-H7. The results showed significant influence ($P<0.05$) in haplotypes of the *POU1F1* gene on birth weight . However, haplotypes showed no significant effect on lambs' weights at weaning, six months and daily weight gains during all periods. Statistical analysis showed that different haplotypes of the *POU1F1* gene did not influence on milk productive traits. The examined fragments of the *POU1F1* Awassi gene have been submitted to Gene Bank under the accession numbers (LC469323 to LC469349),

Keywords: Awassi sheep, Haplotypes, *POU1F1* gene, traits production**.**

Introduction

The Polymorphisms of genes at specific sites of the genome has become one of the important signs in the detection of animal characteristics such as productive and physiological traits. Among the genes that have multiple Polymorphisms are *POU1F1*, which is called Factor 1. The POU1F1 gene found in sheep, goats, and cattle on the chromosome (1) at the location 1q21-22 (Woollard *et al.*, 2000).

The gene expression of the *POU1F1* gene occurs mainly in pituitary gland cells and in other tissue cells as mammary gland tissues (Gil-Puig *et al.*, 2002). The *POU1F1* gene consists of six exons and

five introns. The exons encode into a protein of 291 amino acids. POU1F1 gene function and composition are similar with its bovine, human and rat counterparts showing 98.2%, 91.2% and 86.2% respectively (Bastos *et al*., 2006). This gene is called GHF-1, a gene that regulates the gene expression of growth hormone (GH), prolactin hormone (PRL) and thyroid stimulating hormone (TSH-β) (Sun *et al.*, 2002). The researchers found that the polymorphism of *POU1F1* gene is associated with several economic characteristics such as weight at birth, weaning weight, weight gains in sheep (Sadeghi *et al*., 2014; Al-Khuzai, 2018) and in goats (Zhu *et al*., 2019).

Although there is an increasing interest in studying the polymorphism of the POU1F1 gene and its association with specific characters of different animal species in the past years, but the studies on sheep are still few, especially local sheep breeds. Therefore, this study aimed at explaining the effect of genetic polymorphism (haplotypes) of the *POU1F1* gene on the performance of Iraqi Awassi sheep.

Materials and Methods

This study was carried out at AL-Kafeel station, Karbala city during the period of 01/10/2017 until 01/06/2018. The study included 46Al-Awassi ewes and 52 lambs from their births. The laboratory analyses were conducted in the Laboratory of Molecular Genetics, College of Agriculture, University of Basrah.

Daily milk yield recording started from the fourth day after birth until the ewes drying. Every 10 days, the lambs were separated from their dams for 12 hours. Lambs weighed before and after suckling to measure the amount of milk yield. Additionally, after suckling, the ewes were hand milked soon after to get the milk left in their udders and this quantity of milk was recorded. Daily milk yield was considered as twice the morning milk yield.. Calculation of the milk components (fat, protein, and lactose) was performed each time. Milk components was estimated by the Eko-milk analyzer, the Faculty of Veterinary Medicine at Al-Qasim Green University. Lambs weights were taken at birth, weaning, and at 6 months of age using a sensitive electronic balance. Growth rates were calculated as the difference between two different periods divided by the number of days during the same period.

Blood was collected from the jugular vein of ewes and placed in 4 ml test-tubes containing EDTA. (Ethylene diamine tetra acetic acid). DNA Extraction Kit (Genaid, Taiwan) was used to extract the DNA. The concentration and purity of the DNA were confirmed using the nanodrop device. The DNA concentration ranged between 29.3-71.9 ng/ul. The purity OD (optical density) (260/280) was 1.6`-1.92. The DNA extraction process was confirmed by gel electrophoresis using 1% agarose and the Diamond ™ Nucleic Acid Dye (produced by Promega, USA). Six fragments of the *POU1F1* gene were selected, including all six exons as well as parts of the introns. Primers of these fragments (P1-P5) were designed based on the reference copies recorded under the accession numbers (AJ549205, AJ549204, AJ549206, and AJ549207) at the Gene Bank (NCBI). The

fragment (P6) was adopted by Ozmen, *et al*. (2014). Table (1) showed the sequences of the used primers, fragments sizes, and annealing temperature. Primers were manufactured by Bromega Company, USA. PCR product was 25 µl, containing 12.5µl Master Mix, 1µl for each primer forward & reverse, 3 µl DNA template and dd water were added to 25µl. The amplification successfulness was confirmed by migrated PCR products by electrophoresis using 2% agarose and the Diamond ™ Nucleic Acid Dye produced by Promega USA. Twenty microliters of PCR products were sent to Yang Ling Biotechnology Co; Ltd. China to analyze the sequencing of the nitrogen bases of the *POU1F1* gene for the studied fragments of all samples.

The results of the nitrogen bases sequencing of the six studied fragments of the *POU1F1* gene were analyzed by using Bio edit V.7.2.6 software to align the sequences of the fragments, DnaSP V. 6.12 was used to calculate haplotypes and haplotypes frequencies. The haplotypes network for the *POU1F1* gene was constructed using Network V. 5.5 program

The statistical program SPSS (2013) v.23 was used to find the significant differences between the means of the studied traits and the age of dams, sex lambs and birth type were adjusted by Statistical model**:**

Yijklm =µ+ Ai + Sj + Mk + Gl + eijklm Where is:

µ = Overall mean

Yijklm = The value of observation of each trait.

Ai = Effect age of dams (i = 3).

Sj = Effect sex of Lamb (j = 2).

Gm = Effect type of birth (m = 2)

Table (1): Primers of *POU1F1* gene, their sizes and annealing degrees.

Fragment	Primers	Annealing Temperature	Product Size
P1	F: 5'- AGTGAGATCT GAAACGGCCC - 3'	٥٥	637 bp
	R: 5'- ACTATGAGGT GTACGGCATTT - 3'		
P2	F: 5' - AAAACTGGTCAGTCACGCCA- 3'	٦٠	868 bp
	R: 5' – GTATGGAGGCGG GCAATGAA - 3'		
P3	F: 5' - TTCCCAGCAGAGCACTTAACA -3'	٥٨	780 bp
	R: 5' – GTGC TTGTTAACAGCTGTGGGA – 3		
P4	F: 5' – ACCAGGCAATTCTA CACTGAG - 3'	٥٥	1190 bp
	R: 5' –TCTCAATTGGCTCTA TTCATTTTCA -3'		
P5	F: 5'- TCCCTCGGTTGAA TTTGTGCTA -3'	٥٨	999 bp
	R: 5'- TCCA AAGCCTGCAGAGCAAA -3'		
P6*	F: 5'- GTATTGCTGCTAAAG ACGCC -3'	٥٤	469 bp
	R: 5'- GAGG GAAAGATATAGTGAAAGGG -3'		

Gl = Effect haplotype of POU1F1 gene (l=7)

eijmk = The effect of the experimental error which is distributed randomly and naturally and with an average of zero and variation. σ^2e.

Results & Discussion

Amplification and Sequencing of the *POU1F1* gene

Results showed the successfulness of the PCR amplification process for all six examined fragments of the POU1F1 gene. The products sizes were 637bp, 868bp, 789bp, 1190bp, 999bp and 469bp for fragments P1, P2, P3, P4, P5, and P6, (Fig.1). A total of 12 nucleotides polymorphisms (Fig. 3). These changes resulted in 7 haplotypes (H1, H2, H3, H4, H5, H6, and H7), with frequencies of 0.478, 0.282, 0.108, 0.065, 0.022, 0.022 and 0.022, respectively (Table 2).

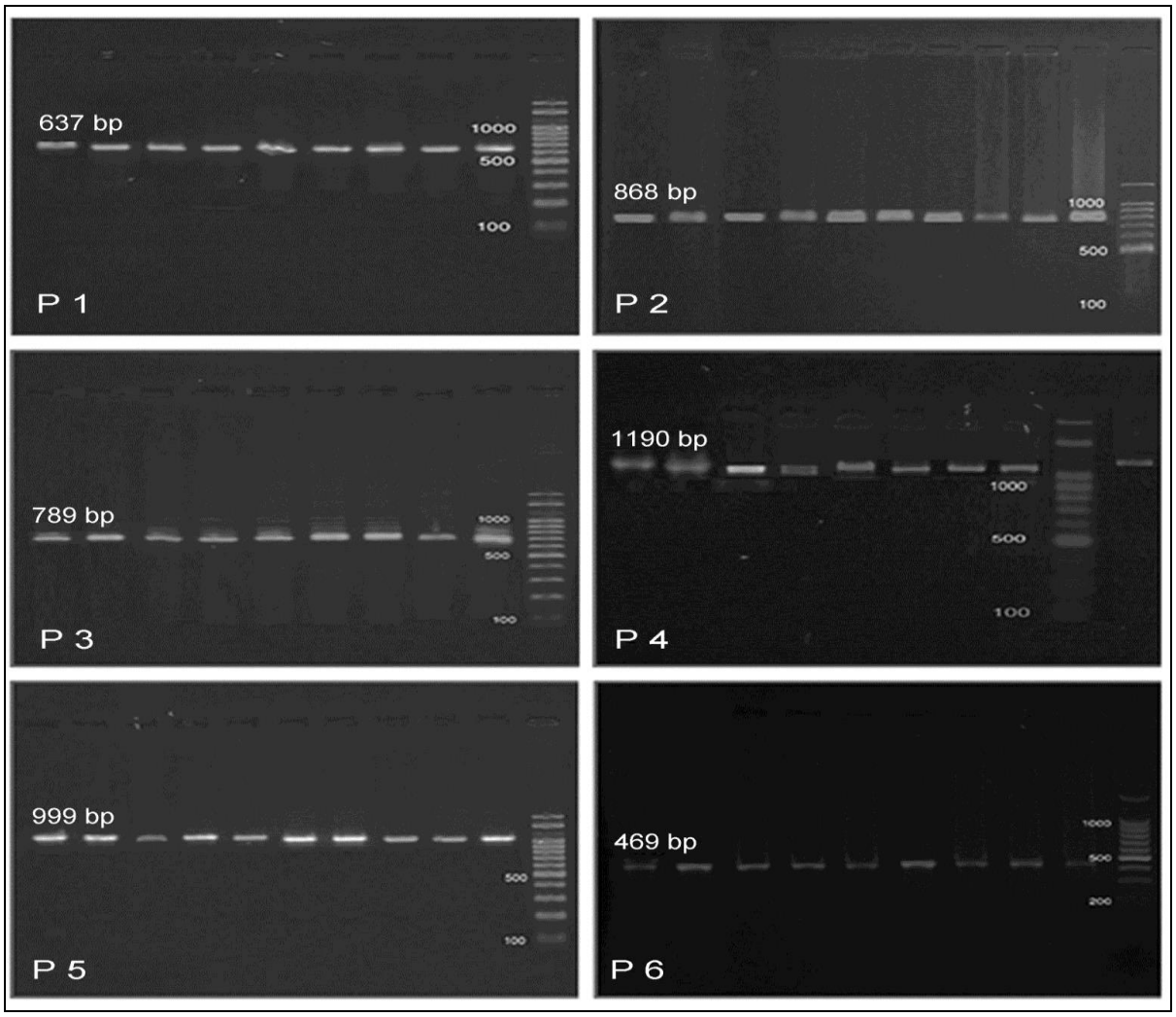

Fig. (1): The amplification results of the examined Fragments of *POU1F1* gene.

Table (2): Haplotypes of the POU1F1 gene.

No.	No. of Animals	Frequency of haplotype	Haplotypes
H1	٢٢	٠.٤٧٨	CGGCAAGAGTAA
H2	١٣	٠.٢٨٢	CGGCACGAATAG
H3	٥	٠.١٠٨	CGGCCAGAATAG
H4	٣	٠.٠٦٥	CGGCCAAGGTAA
H5	١	٠.٠٢٢	CGGCAAGAAGAA
H6	١	٠.٠٢٢	CGGCACGAGGTG
H7	١	٠.٠٢٢	TACGAAGAGTAA
Total	٤٦	١	

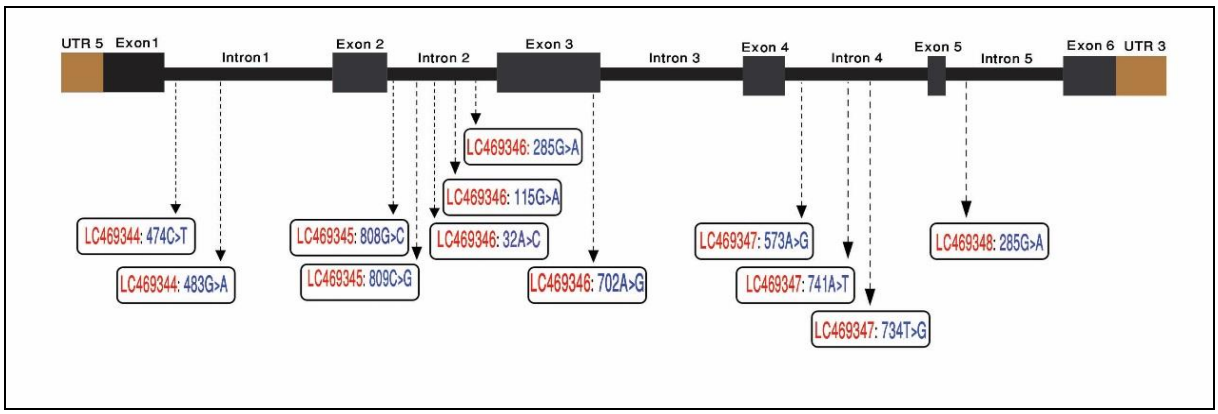

Fig. (3): Changes obtained from analyzing sequences of six different fragments of the gene.

Effect of haplotypes on total and daily weight gain

Table (3) showed the influence of the haplotypes of the *POU1F1* gene on the weight from birth into six month of age and daily weight gains of the lambs. The results showed that birth weight of the haplotype H3 and H4 (4.20 and 4.33 kg) were significantly higher ($P<0.05$) compared to (3.67, 3.70 and 3.50 kg) for haplotypes H1, H2 and other respectively. The present results disagree with Al-Khuzai (2018) in his study on Awassi sheep, and Sadeghi *et al*. (2014) in their study on two breeds of the Iranian sheep indicated there was a significant effect of polymorphism of the *POU1F1* gene on weaning weight., while Zhang *et al*. (2013), Pan *et al*. (2008) and Carrijo *et al*. (2008) found no significant effect for the polymorphism of *POU1F1* gene on birth weight in cattle.

Table (3): Effect of haplotypes of *POU1F1* gene on weights & average daily gain ± standard deviation.

Haplotypes	Nom. of animals	Birth weight (kg)	Weaning weight (kg)	Six months weight (kg)	average daily gain from birth weaning (kg/d)	average daily gain from weaning to six months (kg/d)	average daily gain from birth to six months (kg/d)
H1	٢٢	3.67 ± 0.73 b	15.46 ± 1.44	27.31 ± ٢.٠٧	0.132 ± 0.014	0.129 ± 0.016	0.131 ± 0.01
H2	١٢	٠.٧٤ ± ٣.٧٠ b	١٥.٤٨ ± ١.٣٣	٢٧.١٤ ± ٢.٠٥	٠.١٣٠ ± ٠.٠٠٩	٠.١٢٩ ± ٠.٠١٧	٠.١٣٠ ± ٠.٠٠٩
H3	5	4.20 ± 0.53 a	١٥.٨٤ ± ٢.٣٤	28.16 ± 2.55	± ٠.١٣٢ ٠.٠٢١	٠.١٣٣ ± ٠.٠١٦	٠.١٣٣ ± ٠.٠١١
H4	٣	4.33 ± 0.57 a	15.93 ± 0.50	29.27 ± 1.62	0.136 ± 0.013	0.140 ± 0.012	0.138 ± 0.012
H5, H6 & H7	٣	3.50 ± 1.00 b	15.60 ± 1.92	27.40 ± 2.66	0.134 ± 0.011	0.131 ± 0.010	0.132 ± 0.009

***The means with different letters within the same column differ significantly (P<0.05).**

The results did not show any significant effect of the haplotypes of the *POU1F1* gene on weights at weaning and six months of age. The haplotypes of H1, H2, H3, H4 and other were 15.67, 15.48, 15.84, 15.93 and 15.60 kg and 27.31, 27.14, 28.16, 29.27 and 27.40 and kg at weights of weaning and six months of age, respectively.

The results agreed with Sadeghi, *et al*. (2014) who found no significant effect on the polymorphism of the *POU1F1* gene on lamb weight at weaning for two Iranian sheep breeds). Al-Khuzai (2018) showed a significant effect on the *POU1F1* gene on weaning weight. A study on Korean cattle showed a significant effect of the polymorphism of the *POU1F1* gene (exon 2) on calves weight at six months of age (Seong, *et al*., 2011). The results of table (3) showed no significant differences among the haplotypes of the *POU1F1* gene on the daily weight gains. The daily weight gains from birth to six months of age were 0.131, 0.130, 0.133, 0.138 and 0.132 kg for haplotypes H1, H2, H3, H4 and other. The results disagree with those of Al-Khuzai (2018) on Awassi sheep and Ansary *et al*. (2008) on Iranian sheep who observed significant effect of polymorphism of the POU1F1 gene on daily weight gains of lambs.

Several studies such as Carrijo *et al*. (2008) in Brazilian cattle, Pan *et al*. (2008) in Chinese cattle and Stasio *et al*. (2002) did not observed any effect for the *POU1F1* gene on body weights and daily weight gains.

Effect of haplotypes of the POU1F1 gene on milk production and lactation period.

Daily milk production was not affected by the haplotypes of the *POU1F1* gene (0.813, 0.801, 0.810, 0.852 and 0.773 kg) for haplotypes H1, H2, H3, H4 and other respectively (table 4). The results did not

Table (4): Effect haplotypes of *POU1F1* gene on milk production and lactation period ± standard deviation.

Haplotypes	Number of Dams	Daily milk yield (kg)	First month yield (kg)	Second month yield (kg)	Third month yield (kg)	Total milk yield (kg)	Lactation period (day)
H1	٢٢	0.813 ± 0.114	28.86 ± 3.38	33.05 ± 7.76	19.86 ± 3.57	88.13 ± 14.75	108.00 ± 4.19
H2	١٣	0.801 ± 0.127	27.97 ± 4.39	33.41 ± 8.27	19.64 ± 2.81	85.52 ± 15.20	106.46 ± 3.04
H3	٥	0.810 ± 0.098	28.68 ± 2.72	32.26 ± 9.22	20.46 ± 0.44	87.14 ± 11.86	107.40 ± 1.81
H4	٣	0.852 ± 0.062	30.40 ± 1.73	36.75 ± 6.14	16.90 ± 0.34	93.08 ± 6.25	109.33 ± 1.15
H5, H6 & H7	٣	0.773 ± 0.155	26.63 ± 4.15	32.23 ± 8.45	18.58 ± 3.06	81.77 ± 16.13	105.67 ± 2.06

show any significant differences in monthly and total milk production among the different haplotypes. The total milk production of the haplotypes H1, H2, H3, H4, and others were 88.13, 85.52, 87.14, 93.08 and 81.77 kg, respectively.

In term of the lactation period, the results also showed the absence of a significant effect for haplotypes of the *POU1F1* gene which recorded 108.00, 106.46, 107.40, 109.33 and 105.67 days for the haplotypes H1, H2, H3, H4 and other, respectively. The results of this study agreed with Al-Khuzai (2018) who found no significant effect of the polymorphism of the *POU1F1* gene on milk production for the Iraqi Awassi sheep. The absence of the significant effect of the haplotypes of the *POU1F1* gene on all of daily, monthly and total milk production may due to the fact that the change in the nitrogen bases in the *POU1F1* gene did not alter any of the amino acids for *POU1F1* protein, or due to the need for more animals to ascertain the true effect of the gene. The results of the current study were agreed with Mura *et al.* (2012), who found no effect of the *POU1F1* gene on the amount of milk produced from Sarda sheep in Poland, Daga *et al*. (2013), showed no significant differences between different haplotypes for *POU1F1* gene in milk production and its chemical components fat and protein in Sarda goats in Italy.

Ozmen *et al*. (2014) showed significant differences in the polymorphism of the *POU1F1* gene in the milk production in sheep, there were also significant differences in the chemical components of

the milk (fat, protein, and lactose). While Huang, *et al.* (2008) and Chauhan, *et al*. (2015) found there was a significant effect of the milk production between the polymorphisms of the *POU1F1* gene in cattle.

The presence or absence of the *POU1F1* gene polymorphisms effect in the current study compared to other different studies may belong to the differences in the studied breeds, technique, the studied fragment (exons or introns) from the gene, methods of statistical analysis and the numbers of animals used.

Effect of haplotypes of *POU1F1* gene on chemical milk components.

Table (5) showed no significant effect of the haplotypes of the *POU1F1* gene on chemical milk components (fat, protein, and lactose) during the different months of milk production. The results of the current study agreed with Mura *et al*. (2012) who found no significant effect of polymorphism for the *POU1F1* gene in milk chemical components (fat, protein, and lactose) in Sarda sheep. The results were also agreed with Al- Khuzai, (2018), who showed no significant effect of the different polymorphisms of the *POU1F1* gene on milk chemical components (fat, milk, and lactose). In similar studies on the *POU1F1* gene in Turkish cattle, Aytekin and Boztepe (2013) found no significant effect of the different polymorphisms of the *POU1F1* gene on milk chemical components. While Ozmen *et al.* (2014) obtained three breeds of Turkish sheep effectively on the polymorphisms for *POU1F1* gene on fat and lactose ratio in milk. Other similar studies on goats indicated a significant effect on the polymorphisms of the *POU1F1* gene in milk chemical components (fat, protein, and lactose).

The fragments of the *POU1F1* gene of Awassi sheep breed in this study has been submitted to Gene Bank under the accession numbers LC469323 to LC469349 **(**Table 6).

Table (5): Effect of haplotypes of the *POU1F1* gene on milk components (fat, protein, lactose) ± standard deviation.

Haplotypes	First month			Second month			Third month		
	Lactose (%)	Protein (%)	Fat (%)	Lactose (%)	Protein (%)	Fat (%)	Lactose (%)	Protein (%)	Fat (%)
H1	5.01 ± 0.02	3.95 ± 0.16	٥.٣٢± 0.57	5.14 ± 0.23	4.07 ± 0.19	5.52 ± 0.76	5.20 ± 0.18	4.04 ± 0.12	5.18 ± 0.79
H2	5.10 ± 0.33	4.01 ± 0.22	5.70 ± 0.55	5.27 ± 0.34	4.08 ± 0.20	5.92 ± 0.77	٥.٢٩± ٠.٢٨	4.02 ± 0.2	5.62 ± 0.78
H3	5.07 ± 0.06	4.05 ± 0.12	5.59 ± 0.84	4.98 ± 0.16	3.89 ± 0.13	5.69 ± 0.97	٥.١٠± ٠.٣١	٤.٠٢± ٠.١٨	5.41 ± 0.93
H4	4.99 ± 0.15	4.00 ± 0.05	5.32 ± 0.80	5.23 ± 0.17	4.04 ± 0.13	5.05 ± 0.96	٥.٢٣± ٠.٠٥	٤.٠١± ٠.٠٦	5.17 ± 1.13
H5, H6 & H7	5.04 ± 0.20	3.85 ± 0.23	5.18 ± 0.80	5.20 ± 0.21	4.03 ± 0.14	5.50 ± 0.75	٥.٢١± ٠.٢٧	٤.١٢± ٠.٦٩	5.48 ± 0.98

Table (6): The fragments' sequences submitted to Gene Bank (NCBI).

Fragments	Gene Name	Local on the gene	Accession Numbers
P1	POU1F1	Exon-1 & part of Intron-1	LC469344, LC480423, LC480424
P2	POU1F1	Exon-2 & part of Intron-2	LC469345, LC480425, LC480426
P3	POU1F1	part of Intron2 & Exon-3	LC469346, LC480427, LC480428, LC480429, LC480430
P4	POU1F1	Exon-4 & part of Intron-4	LC469347, LC480431, LC480432, LC480433, LC480434
P5	POU1F1	part of Intron-4 & Exon-5	LC469348, LC480435, LC480436
P6	POU1F1	Exon-6 & part of UTR,3	LC469349

Conclusions

Many of the changes in nitrogen bases found in different sites of *POU1F1* gene there had no association with haplotypes and most of the production traits, such as weights and daily weight gains, with an exception to birth weight, which found the effect of some haplotypes. The results did not show any effect of haplotypes of *POU1F1* gene on milk production, the fragments of the *POU1F1* gene in this study of Awassi sheep breed had been submitted at Gene Bank for the first time in Iraq under the independent accession numbers

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