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Relationship of POU1F1 gene polymorphism with some of economical traits in Iraqi awassi ewes

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Abstract
This study was conducted to investigate the relationship of POU1F1 gene polymorphism with total milk production (TMP) and fecundity by using 60 Iraqi Awassi ewes in the First Research Station/College of Agriculture/ AL-Muthanna University. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect polymorphisms of POUIF1gene in this sheep breed. Results of gene which encloses the *EcoR1* endonuclease restriction enzyme showed two genotypes (MM and NN). The frequencies of the M and N alleles differed significantly ($P<0.01$) in the samples, were 0.58 and 0.42 respectively. The results showed the absence of hetero genotype (MN). Results showed no significant effect of genotypes on TMP or lactation period, TMP was 71.59 and 73.52 kg for MM and NN genotypes respectively while the lactation period was 109.74 and 109.14 day for MM and NN genotypes respectively. Litter size was affected significantly ($P<0.01$) by POU1F1 genotype, the highest litter size was in MM group (1.23) while the least litter size was in NN group (1.08).

Keywords: Iraqi Awassi ewes, POU1F1 gene, Total milk production, Litter size

1. Introduction
Sheep milk is characterized by lower cholesterol content, more beneficial composition of fatty acids and better hygiene (lower somatic cell content) compared with cow of buffalo or cow milk [1-3]. Despite the low production of milk and the short lactation period, ewe milk is a major importance in countries where climatic conditions and tradition are not conducive to raising dairy cattle. Many studies refers that the sheep milk quality and components are influenced by breed or genotype and the milk composition varies considerably among breeds of sheep [3]. Genetic variation in ovulation rate in sheep has been widely documented and the evidences show substantial differences among breeds and in a number of cases, exceptional variations within breeds [4]. Litter size is an important economic trait in sheep breeding. Variation in litter size in sheep is controlled by both genetic and environmental factors. Most breeds of domestic sheep have one or two lambs at each lambing, although a small number of breeds consistently have litter sizes of three or more [5]. Attempts to increase litter size by selection within a breed result in slow progress, because the heritability of litter size is low. Therefore, the discovery of major genes (or mutations) with large effects on ovulation rate and thus litter size, has generated considerable interest among sheep breeders and scientists [6]
The *POU1F1* gene (*Pit-1*; Gene ID5449) is a tissue-specific transcriptional factor and belongs to the POU homeodomain family [7]. It regulates GH, prolactin and TSH β-subunit gene expression, and somatotroph, lactotroph, and thyrotrophic development of the anterior pituitary gland and mutations of *POU1F1* account for 7.8% of combined pituitary hormone deficiency (CPHD) and isolated GH deficiency [8, 9]. The earlier published articles reported that genetic polymorphisms of POU1F1 gene were significantly associated with growth, development and lactation in mammalians [10]. In sheep breeds, many studies offered that POU1F1 gene polymorphism is related with many economical traits [11, 12]. Reported that some of mutations in this gene are correlated significantly with performance of sheep. Moreover, polymorphisms in the *POU1F1* gene are associated with important quantitative traits in cattle [13, 14] and in pig [15]. Recently, polymorphisms in the sheep *POU1F1* gene were reported by [16]. All these efforts have been concentrated mainly on mice, humans, cattle, pigs, and sheep. The major aim of this study was to determine the effect of POU1F1 gene polymorphism on milk production and litter size in Awassi ewes and use this information as guidelines or indicators for the management strategies for ewes under the farming conditions for selecting and improving the performance of domestic animals depending on these indicators.

2. Materials and Methods

2.1 Experimental animals and management

Data were made available by the department of animal resources, college of agriculture, University of AL-Muthanna for the period 1/11/2016 to 1/7/2017 on 60 Iraqi Awassi breed ewes selected from the experimental flock reared under extensive conditions. Flock is housed under semi-open sheds and can be fed on the concentrated ration consuming about (500 – 600) gm / head / day, for the period from mating season to the last six weeks of pregnancy. Ration is normally containing 37% yellow corn, 40% wheat bran, 10% hulled barley, 5 – 10% soy bean meal, 1% NaCl and 1% CaCO3. and green roughages such as Alfalfa and clover can be added throughout the season. Annual routinely operations on sheep are dipping and washing with chemicals in order to kill extra parasites so sheep will be ready to mating after hand wool shaving. Sires and dams will be recorded in breed records. Lambs are weighed directly after parturition and tagged with plastic tags. Lambs stays with their dams up to 90 days (weaning age). The health status of the flock must be under regular observations.

2.2 Blood samples and DNA extraction

Blood Samples were withdrawn from all ewes at the same time each of 10 ml from jugular vein. DNA was extracted from blood following the protocol of Sambrook J, Russell [17].

2.3 PCR-RFLP study on POU1F1 gene

One region (508 bp) of the POU1F1 gene was amplified. The primer sequence used to amplify the genes was provided in Table 1. The primer sequences for amplification of POU1F1 gene was designed based on the available sequence of sheep - AJ549207 in Genbank [16]. The restriction enzyme for POU1F1 (AJ549207) gene was chosen by analyzing the DNA sequences using the Gene Tool Lite 1.0 software.

Table 1. Primer sequence and fragment size of POU1F1 gene

Gene	Primer sequence	Fragment size (bp)	Reference
POU1F1	P5 : 5'- ATA CCA GGC AAT TCT ACA CTG – 3' P6 : 5'- GGC CTT GCT TTT CTT TAT AG – 3'	508	[3]

PCR program: The 508 bp fragment of the POU1F1 gene was amplified with the following conditions (Table 2).

Table 2: PCR program for amplification of 508 bp fragment of POU1F1 gene

Step	Process	Temperature	Duration
1	Initial denaturation	95ºC	4 minutes
2	Denaturation	94ºC	45 seconds
3	Annealing	57.5ºC	45 seconds
4	Extension	72ºC	1 minute
5	Go to step 2	34 times	-
6	Final extension	72ºC	10 minutes
7	Refrigeration	4ºC	Forever

2.4 Statistical analysis

Data were analyzed using SAS [18] program according to the following model:

$$Yij = \mu + Ai + eij$$

Where: μ: is an overall means, Ai: Effect of genotype of POU1F1 gene (NN and MM) is a random error.

Estimation of genotype frequencies, the genotypes were assigned on the basis of restriction digestion pattern of the PCR products. The allele and genotype frequencies were calculated by standard formula [19].

$$\text{Genotype frequency} = \frac{\text{Number of individuals of a particular genotype}}{\text{Total number of animals of all genotypes}} \times 100$$

$$\text{Gene frequency} = \frac{2D + H}{2N}$$

Where: D = number of animals homozygous for a particular allele, H = number of heterozygote animals, N = total number of animals, Chi- square test was used to determine the significant differences among phenotypes:

$$X^2 = \sum \frac{(ObservedNo. - ExpectedNo.)^2}{ExpectedNo.}$$

Significant differences among groups were detected by general linear model (GLM) and Duncan's multiple range test [20] was used to compare differences among means.

3. Results and Discussion

Results showed that the nucleotide position 224 of the amplified region has a cleavage site (G|AATTC) for the restriction enzyme, *EcoRI*. The restriction digestion with *EcoRI* produced two fragments of length 224 bp and 284 bp in Awassi sheep breed (Fig.1). The result is partially similar with the results of Ansary [21] and Negahdary [22] who referred to the polymorphism of POU1F1 gene in Baluchi and Makooei Sheep breeds with three genotypes (AA, AG and GG). The result is dissimilar with the results of [23] who referred to the absence of POU1F1 gene polymorphism of Nilagiri and Mecheri sheep breeds.

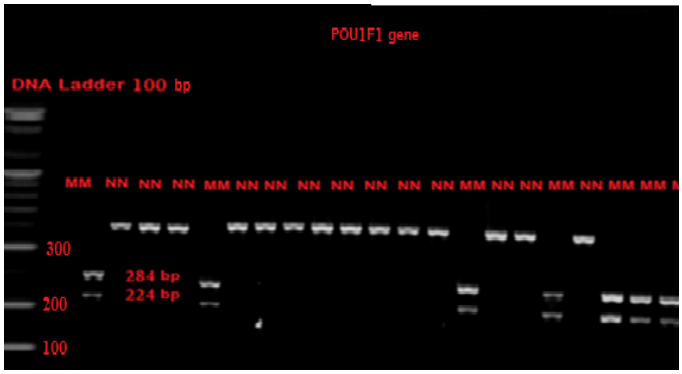

Fig 1: PCR implication of 508 bp fragment of POU1F1 gene

Results showed a significant difference (P<0.01) in the distribution of POU1F1 genotypes MM genotype was found in 35 ewes (58.33%) compared with NN genotype which found in 25 ewes (41.67%) (Table 3). Allele frequency was 0.58 and 0.42 for M and N alleles respectively. Allele frequency of POU1F1 gene is differing according the sheep breed and the volume of sample. The results is accordance with those of Sadeghi [12] and Ozmen [11] who referred to the absence of heterozygous genotype of this gene in both Iranian and Turkish Awassi sheep breeds.

Table 3: Chi square distribution of POU1F1 genotypes in Awassi ewes.

Genotypes	No.	(%)
MM	35	58.33
NN	25	41.67
Total	60	100%
Chi- square (χ^2)	33.327**	
Allele		Frequency
M		0.58
N		0.42
** (P<0.01)		

Table 4: Effect of POU1F1 genotypes on milk production and litter size in Awassi ewes

Genotypes	No.	Means ± SD		
		Total milk production (kg)	Lactation period (day)	Litter size
MM	35	73.52 ± 1.62 a	109.74 ± 0.59 a	1.23 ± 0.04 a
NN	25	71.59 ±1.68a	109.14 ± 0.57 a	1.08 ± 0.02 b
	60	NS	NS	**

NS: no significant, (** P< 0.01).

The statistical results revealed no significant relationship between some of milk traits and genotypes of POU1F1 gene (Table 4). Total milk yield and lactation period increased theoretically in ewes with MM genotype namely, 73.52 kg and 109.74 day respectively compared with the ewes with NN genotypes namely, 71.59 kg and 109.14 day respectively. Many several studies have been done on the association of POU1F1 gene polymorphism and milk traits in animals. Up to now, there were very few reports about POU1F1 gene SNPs and their relationship with milk yield for sheep. The current results is similar with the results of Mura *et al.* [24] who pointed that the POU1F1 genotype is not effect significantly in milk traits in sheep. Dattani [25] and Snabboon *et al.* [9] reported that the absence of significant difference between POU1F1 genotypes resulted from the strong competing between the alleles of this gene and the dominance between the wild allele and mutant allele is not easily detected.

Results showed a significant effect (P<0.01) of POU1F1 genotypes on litter size in Awassi ewes. Litter size increased in ewes with MM genotypes compared with the ewes that carried the NN genotype namely, 1.23 and 1.08 respectively. The results are similar with the past studies which referred to the high effect of POU1F1 polymorphism on reproductive traits in sheep [26-28] indicated that the genotype of this gene is associated with fecundity of sheep and this due to the major control of POU1F1 factor on growth hormone releasing. In addition, Molic *et al.* [1] reported that litter size and fecundity in sheep are affected by many genes effect such as BMP-15, GDF9, FSHRER, Kiss-1, PRLR and INH therefore, if we want to determine the genetic effect on reproductive traits we must investigate of the genotypes of all this genes in animal.

4. Conclusion

In summary, this present study results indicated that the polymorphism of POU1F1 gene was associated with litter size in Awassi ewes. Researchers can use this gene as genetic marker for reproductive traits improving and we can exploit the polymorphism in this gene as a good tool to improve litter size and shorten the breeding process of highly prolific sheep.

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