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## Evaluation of polymorphism in BMP-15 gene and the relationship with milk production and reproductive performance in Iraqi awassi ewes

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### Abstract

The study was conducted to investigate the relationship of BMP-15 gene polymorphism with total milk production (TMP), milk components and fecundity by using 60 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 BMP-15 gene in this sheep breed. Results of gene which encloses the *HinfI* endonuclease restriction enzyme show two genotypes (AA and BB). The frequencies of the A and B alleles not differed significantly in the sample (0.48 and 0.52 respectively). The results showed the absence of heterozygous (AB). Results showed a significant difference ( $P < 0.01$ ) between the ewes with AA and BB genotypes on TMP, it was 69.62 and 76.52 kg for AA and BB genotypes respectively while the lactation period was not differed significantly between the AA and BB groups. Lactose percent was affected significantly ( $P < 0.05$ ) by BMP-15 polymorphism, the highest value was in AA group (4.51%) compared with BB (4.41%) while percent of milk fat, milk protein and solid not fat content were not affected significantly by BMP-15 polymorphism. Litter size was affected significantly ( $P < 0.01$ ) by BMP-15 genotype, the highest litter size was in BB group (1.26) while the least litter size was in AA group (1.07).

**Keywords:** Iraqi Awassi ewes, BMP-15 gene, performance

### 1. Introduction

Awassi sheep contribute 58.2% of the total sheep population in Iraq<sup>[1]</sup>. The Awassi is the most numerous and widespread breed of sheep in south west Asia. It's the dominant type in Iraq. Is dual purpose (mutton and wool) sheep breed<sup>[2]</sup>. This breed was widespread because of its good characteristics in regards to meat price and quality, milk quality, validity of wool for the carpet industry and its ability to cope stress of high environmental temperature<sup>[3, 4]</sup>. Last decades, breeding programs based on genetic markers as indirect selection method to improve the quantitative traits which characterizes as a difficult and expensive to measure, exhibit low heritability and are expressed late development. Bone morphogenetic protein 15 (BMP-15) gene is one of genetic markers which coded to produce a growth factor and a member of the TGF super family that is specifically expressed in oocytes. The sheep BMP-15 gene maps to the X chromosome<sup>[5]</sup>. Bone morphogenetic protein 15 regulates granulosa cell proliferation and differentiation by promoting granulosa cell mitosis, suppressing follicle-stimulating hormone receptor expression and stimulating kit ligand expression, all of which play a pivotal role in female fertility in mammals<sup>[6, 7]</sup>. Many studies referred to the relationship of BMP-15 polymorphism with reproductive performance in mammals<sup>[8, 9]</sup>. In sheep,<sup>[10]</sup> proved a strong relationship between the mutations in this gene with twinning rate and considered this gene as a fecundity factor<sup>[11, 12]</sup>. reported that BMP-15 plays a crucial role in follicle stimulating hormone secretion which lead to increase ovulation rate in sheep breeds. The Bmp-15 is responsible for reduce the udder cells apoptosis which lead to longing life and increase the activity of this cells and increase milk production in some of ewes that had mutations in this gene<sup>[13]</sup>. The aim of this paper was to determine the relationship of BMP-15 polymorphism with total milk production and litter size in Awasssi ewes. The study results will act as guidelines for the management strategies for ewes under the farming conditions for selecting and improving the performance of domestic animals depending on this indicators.

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**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% CaCO<sub>3</sub>. 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 Knight [13].

**2.3 PCR-RFLP study on POU1F1 gene**

One region (141 bp) of the BMP-15 gene was amplified. The primer sequences used to amplify the genes are provided in Table 1. The primer sequence for amplification of BMP-15 gene was designed based on the available sequence of sheep and restriction enzyme (*HinfI*) was chosen by analyzing the DNA sequences using the Gene Tool software [14].

**Table 1:** Primer sequence and fragment size of BMP-15 gene.

Gene	Primer sequence	Fragment size (bp)	Reference
BPM-15	5'-CTGTCTTCTTGTTACTGTATTTCAATGAGAC-3' 3'- GATGCAATACTGCCTGCTTG-5'	141 Exone2	[12]

PCR program: The 141 bp fragment of the BMP-15 gene was amplified with the following conditions (Table 2).

**Table 2:** PCR program for amplification of 141 bp fragment of BMP-15 gene [5].

Step	Process	Temperature	Duration
1	Initial denaturation	94	5 minutes
2	Denaturation	94	30 seconds
3	Annealing	62	40 seconds
4	Extension	70	30 second
5	Go to step 2	72	-
6	Final extension	4	10 minutes
7	Refrigeration	94	forever

**2.4 Statistical analysis**

Data were analyzed using SAS [15] program according to the following model:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where:  $\mu$ : is an overall means,  $A_i$ : Effect of genotype of BMP-15 gene (AA and BB),  $e_{ij}$ : 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 [2].

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

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

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:

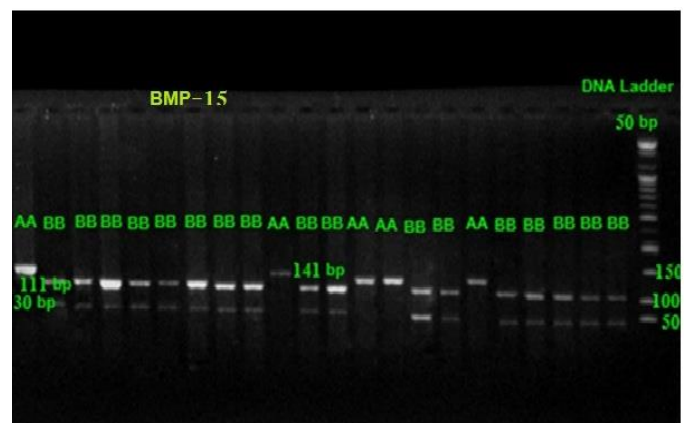
$$\chi^2 = \sum \frac{(\text{ObservedNo.} - \text{ExpectedNo.})^2}{\text{ExpectedNo.}}$$

Significant differences among groups were detected by

general linear model (GLM) and Duncan's multiple range tests [16] was used to compare differences among means.

**3. Results and Discussions**

PCR product of the *Fec<sup>G</sup>* mutation of the BMP-15 gene digested with *HinfI* in local Awassi sheep (fig.1). The wild-type allele (B) was 111 bp and the mutant allele (A) is 141 bp. Single nucleotide mutation (C/T) was identified at position 718 of this gene. The same result was observed by Chu *et al.* [17] who detected two genotypes in sheep.



**Fig 1:** PCR implication of 141 bp fragment of BMP-15 gene

Results showed no significant difference in the distribution of BMP-15 genotypes. AA genotype was found in 29 ewes (48.33%) compared with BB genotype which found in 31 ewes (51.67%) (Table 3). Allele frequency was 0.48 and 0.52 for A and B alleles respectively. The results showed absence of heterozygous genotype (AB) of this gene in this sample of Awassi sheep. The same result was observed by Jamshidi *et al.* [18] Sangsari sheep breed of Iran and [19] in three Egyptian native sheep breeds. The results is dissimilar with the results of Knight [13] who referred to the publicity of A allele compared with B allele.

**Table 3:** Chi square distribution of BMP-15 genotypes in Awassi ewes.

Genotypes	No.	(%)
AA	29	48.33
BB	31	51.67
Total	60	100%
( $\chi^2$ )Chi- square	2.00 NS	
Allele	Frequency	
A	0.48	
B	0.52	

Results represented in Table 4 showed a significant effect ( $P < 0.01$ ) of BMP-15 polymorphism on total milk yield. The highest total milk yield was observed in ewes with AA

genotype (69.62 kg) compared with the ewes that carry BB genotype (76.56kg). Lactation period did not affected significantly by BMP-15 polymorphism. The past studies not gave us an assertive evidence of the relationship of BMP-15 polymorphism with milk traits in sheep therefore, the difference of milk yield may be resulted from the difference of litter size which lead to increase milk yield in ewes with twin lambing compared with single lambing. Litter size increase significantly ( $P < 0.01$ ) in ewes with BB genotype compared with the ewes with AA genotype namely, 1.26 and 1.07 respectively. The results accordance with the results of [11, 12, 20, 21] who referred that the mutations occurs in BMP-15 gene effect directly on litter size and ovulation rate in sheep.

**Table 4:** Effect of BMP-15 polymorphism on milk production and litter size in Awassi ewes

Genotypes	No.	Means $\pm$ SD		
		Total milk yield (kg)	Lactation period (day)	Litter size
AA	29	69.62 $\pm$ 1.37 b	109.37 $\pm$ 0.55 a	1.07 $\pm$ 0.02 b
BB	31	76.56 $\pm$ 1.51 a	109.51 $\pm$ 0.60 a	1.26 $\pm$ 0.05 a
	60	**	NS	**

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

Results showed a significant effect ( $P < 0.05$ ) of BMP-15 polymorphism on lactose ratio (Table 5). The highest lactose content was in the milk of ewes with AA genotype (4.51%) compared with the lowest lactose in milk of ewes with BB genotype and no significant effect of BMP-15 polymorphism on other milk compositions such as fat, protein and solid not fat. The high ratio of lactose in AA group was due to the low

of total milk yield (Table 4). The current result is similar with the most of past studies which referred to negative correlation between milk yield and milk components [22-25]. Reported that milk yield related negatively with fat content and protein in three of European sheep breeds (Tsigai, Valachian and Lacaune).

**Table 5:** Effect of BMP-15 polymorphism on milk components in Awassi ewes

Genotypes	No. of samples	Means $\pm$ SD			
		Fat %	Lactose %	Protein %	SNF %
AA	29 (87)	4.11 $\pm$ 0.52 a	4.51 $\pm$ 0.04 a	5.69 $\pm$ 0.21 a	10.98 $\pm$ 0.26 a
BB	31(93)	4.44 $\pm$ 0.57 a	4.41 $\pm$ 0.04 b	5.37 $\pm$ 0.23 a	10.60 $\pm$ 0.29 a
	180	NS	*	NS	NS

NS: no significant, (\*  $P < 0.05$ ), SNF: Solid not fat.

#### 4. Conclusion

In conclusion, it can be assumed that BMP-15 (exon2) which contained a novel mutation known as  $Fec^G$  in local Awassi sheep breed in Iraq has high variability and the SNP observed in this study are associated with milk yield, lactose percent and litter size. The mapping and linkage characterization of sheep BMP-15 gene needs to be studied in more detail, and the exact mechanism of this gene polymorphism contributing to productive and reproductive performance also requires further investigation.

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