Relationship *GDF9B* (*BMP-15*) Gene Polymorphism with litter size in Iraqi Awassi Sheep

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Abstract

Thirty-two of ewes from Iraqi Awassi breed and thirty-eight of her births used in the present study with the same feeding conditions from Al-Kafeel sheep station, Karbala, molecular analysis were completed in the Molecular Genetics Laboratory at the College of Agriculture / University of Basrah. We investigated fecundity genes-bone morphogenetic protein 15 (*BMP-15*) in order to detect any possible mutations in this gene in iraqi Awassi ewes using DNA sequencing technique. We identified one single nucleotide polymorphism (SNP) from the studied fragment of gene *BMP-15* which is 356bp at (T287C) site, the frequency polymorphisms of this site for CC, CT and TT were 0.59, 0.22 and 0.19, as for the frequency of allele A and B, it was 0.69 and 0.31. The difference of the least squares means for litter size between polymorphisms of *BMP-15* gene CC, CT and TT was not significant ($p>0.05$) with this study, the values of the litter size were recorded 1.16, 1.29 and 1.17 for polymorphism TT, CT and CC respectively.

The results did not show any significant effect ($P<0.05$) of the difference in the *BMP-15* gene polymorphisms at site (T287C) on any of the growth traits as lamb weight at birth (BW), weaning (WW) and average daily gain (ADG). The sequences of *BMP-15* gene were submitted to the GenBank database, the Sequences were recorded under accession numbers LC495449 into LC495469.

Key words:- *BMP-15* gene, Polymorphism, Awassi sheep, litter size

Introductio

Sheep breeding is particularly very important for small farms because it needs low resources and provides good production of income **(**3). Improving the proportion of twins in ruminant animals, especially sheep, is becoming increasingly important, as an increase in the number of births resulting can yield significant gains in profit. Litter size and Ovulation rate are controlled genetically by several genes with minor or major effects, called fertility genes (Fec). (1).

The occurrence of mutations in the *BMP-15* gene substitution the amino acids, which in turn increase the ovulation rate in heterozygous individuals and cause sterility in homozygous ewes. (21). Marker-assisted selection using both BMPR-IB and *BMP-15* genes is warranted to increase litter size in sheep and will be of considerable economic value to sheep producers. (5). The ovulation rate in sheep has been observed to be significantly increased by mutations in a closely linked group of genes. These genes are bone morphogenetic protein receptor type 1B (BMPR-1B), bone morphogenetic protein 15 (*BMP-15*), and growth differentiation factor 9 (GDF9), which are all part of the ovary-derived transforming growth factor-β (TGFβ) superfamily (17). These genes have an important effect on ovulation rate and litter size (11). The BMB15 gene is located on the X chromosome in sheep and goats (12).

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Various mutations in bone morphogenetic protein-15 (*BMP-15*) gene increased ovulation rate & litter size in sheep. (6). Six different point mutations in *BMP-15* gene have been discovered, affecting on ovulation rate or the litter size for several different breeds of sheep are $FecX^G$ and $FecX^B$ (12), $FecX^I$ and $FecX^H$ (9), $FecX^L$ (4), and the $FecX^R$ in Raza Aragoneza breed (17). (25) noted that increasing the gene expression of the *BMP-15* gene leads to a decrease litter size, opposite that to the GFD-9 and BMPB1 genes, whose gene expression increases the litter size in sheep. In a study on Brazilian sheep discovered a new SNP in *BMP-15* gene that increased the ovulation rate and prolificacy of homozygote sheep (23). In a study on Chinese ewes of the Han breed on the second exon from the *BMP-15* gene, (5).found that the heterozygous genotype recorded 2.61 litter size compared to 2.06 for the homozygous genotype. In addition to that, many studies have indicated the effect of the polymorphisms of the *BMB15* gene on increasing the litter size , (6) , (4) , (15),. in sheep (26) , (10) in goats.

The goal of the present study was to search for possible mutations in *BMP-15* gene and associated with increased litter size in Iraqi Awassi sheep.

Materials and methods

Animals

Thirty-tow of ewes from Iraqi Awassi breed and thirty-eight of her births used in the present study *with the same feeding conditions from Al-Kafeel sheep station, Karbala* collected the blood and recorded the litter size, Birth weight (BW), weaning weight(WW) and average daily gain (ADG) , Blood samples from all ewes were collected by Jugular vein

DNA extraction, amplification & Sequencing

molecular analysis were completed in the Molecular Genetics Laboratory at the College of Agriculture / University of Basrah. Genomic DNA was isolated from 100 ul of whole blood from all ewes by using gSYNC^{TM} DNA extraction kit (Taiwan).

Amplification of a 356bp of the selected fragment of the *BMP-15* gene using PCR Technique, using of primers with the following nucleotide sequences: Forward, TTCTCCGTCTAGGGGTATGAG -3'and Reveres, 5' AGGGAACAAGAGCAAAG-

CGTTAGC -3' according to (19) program of PCR was : 95°C for 4 min for initial denaturation , followed by 30 cycles of 95°C for 1 min, 58°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products of *BMP-15* gene were migrated by electrophoresis on a 2% agarose gel in parallel with a 250-bp DNA marker. PCR products send to a company for sequencing analysis by Yang Ling Biotechnology Company ; Itd China.

Polymorphisms analysis

Alignments of all sequences with *BMP-15* gene were performed using BioEdit v7.2.5 (13). Allelic and genotypic frequencies and Hardy-Weinberg homeostasis were estimated using the POPGENE v1.32 program (27).

Protein & Phylogenetic Tree Structure

The three-dimensional figure for the *BMP-15* gene in this study was drawn using the web site for molecular modelling Which is known as EzMol (22). Use the Clustal Omega tool (24) to draw a phylogenetic tree of *BMP-15* gene sequences by use Neighbor-joining tree method (NJT) for comparison with the this study sequences and reference copies from Genbank under accession numbers (LC495456 Iraq "present study", AH009593 New Zealand, JN655672 Pakistan, FJ600402 Indian and KT853038 Mexico)

Statistical analysis

Analysis of the Relationship between *BMP-15* Gene Polymorphisms with litter size and Growth traits was conducted by applying a general linear model (GLM) procedure using SPSS software version 22.

The model was described as:

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Y = μ + a + e

Where:

Y represents the value of Growth traits or litter size,

μ represents the overall mean.

a represents the effect of the Polymorphism.

e represents the random residual error.

Results & Discussion

Amplification, Sequencing and Genetic Polymorphism

The electrophoresis results of the amplification product resulting from the PCR technique showed the success of the amplification process. The size of the studied fragment of the *BMP-15* gene was 356 bp as it appeared in Fig. 1. The products were then sequenced by Yang Ling Biotechnology Company ; Itd China. The Sequences were submitted in Gene Bank under accession numbers (LC495449-LC4954469).

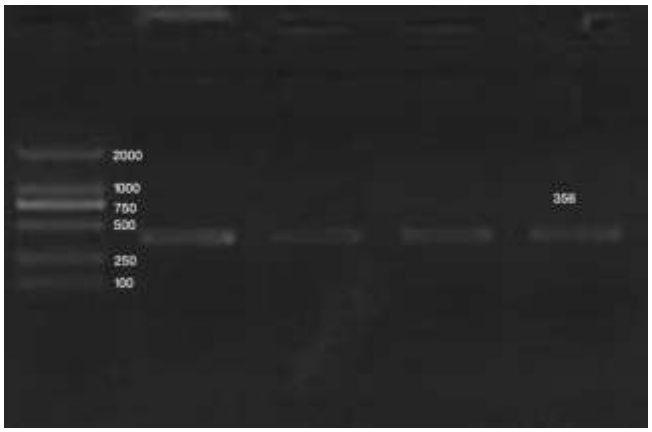

Figure. (1) PCR amplification of the *BMP-15* Gene using (2.5% agarose gel stained with ethidium bromide).

We identified one single nucleotide polymorphism (SNP) from the studied fragment of gene *BMP-15* which is 356bp at (T287C) site . The frequency polymorphisms of CC, CT and TT were 0.59, 0.22 and 0.19, while frequency of allele A and B, it was 0.69 and 0.31. In a study on Ukrainian sheep of the Askanian breed (14) did not find any polymorphism for the same studied fragment of the *BMP-15* gene.

On the other hand (12) found Six different point mutations in *BMP-15* gene have been discovered, affecting on ovulation rate or the litter size for several different breeds of sheep are $FecX^G$ and $FecX^B$, $FecX^I$ and $FecX^H$ (9), $FecX^L$ (4), and the $FecX^R$ in Raza Aragoneza breed (16).

In a study on Watish ewes, (18) did not observe any polymorphisms other than the wild type for the $FecX^B$ and $FecX^H$ alleles, and $FecX^I$ for the *BMP-15* gene. Except for $FecX^G$, which showed a low variation in the polymorphisms, as homozygous polymorphism formed about 0.993 compared to 0.006 for heterozygous polymorphism. A study by (7) was conducted to detect the $FecX^B$ mutation of the *BMP-15* gene by PCR-RFLP method for some Turkish sheep breeds, as the results of this study did not show any polymorphisms other than the wild polymorphism. (19) , (8) in Egypt was not observed by studying the same selected fragment of the second exon of the *BMP-15* gene using the RFLP-PCR technique, It was found that all animals have a single polymorphism (wild polymorphism) and no mutation was detected for the studied fragment.

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Table 1. Genotypes and allele frequencies of the *BMP-15* gene for ewes from Iraqi Awassi breed.

The Gene	Genotypes	Observed numbers	genotype frequencies	expected numbers	X^2
BMP-15	CC	19	0.59	15.82	7.25
	CT	7	0.22	13.35	
	TT	6	0.19	2.82	
Total	32		100	32	
The Gene	Allele		Allele frequencies		
BMP-15	C		0.69		
	T		0.31		

Litter size

The results indicated that among three polymorphisms CC, CT and TT for *BMP-15* gene at (T287C) site no significant correlation between this polymorphisms and litter size. The mean litter size for homozygous polymorphisms CC & TT were recorded 1.16 & 1.17 Respectively, while the heterozygous polymorphism it was recorded 1.29 table (2). The results of our study did not agree with the results of the (2) study that was also conducted in Iraq on the Awassi sheep breed, as the results of their study indicated the presence of significant differences ($p<0.05$) for the homozygous polymorphism AA compared polymorphisms AC and CC in litter size for the litter size recorded 1.389, 1.150 and 1.167 respectively.

(16) indicated, with his study on the second exon of the *BMP-15* gene, he found a new mutation in the form of a deletion of 17 base pairs that led to a termination code, found that ewes with heterozygous genotype gave an increase in the litter size reached 2.66 compared to the average herd Which estimated 1.36, this mutation named is $FecX^R$, which was revealed in this study by the Spanish Rasa Aragonesa breed, this a mutation leads to a complete decrease in the functions of the second exon of *BMP-15* and this effect may be similar to the mutations that lead to a stop codon of the peptide chain of the *BMP-15* protein. (23) discovered a new SNP in *BMP-15* gene that increased the ovulation rate and prolificacy of homozygote sheep. In a study on Chinese ewes of the Han breed on the second exon from the *BMP-15* gene, it was found that the heterozygous genotype recorded 2.61 litter size compared to 2.06 for the homozygous genotype. (5).

In goats, (10) found that mutations were associated with increased litter size in Markhoz goat, the litter size was significantly altered by GDF9 and *BMP-15* genes genotypes.

Table (2) The effect of the Polymorphisms of the *BMP-15* gene on the litter size in Iraqi Awassi sheep

Polymorphisms	Number of Ewes	Number of Births	Litter size
TT	19	22	1.16
TC	7	9	1.29
CC	6	7	1.17
Mean	32	38	N.S

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Growth traits

The results did not show any significant effect (P<0.05) of the difference in the *BMP-15* gene polymorphisms at site (T287C) on any of the growth traits as lamb weight at birth (BW), weaning (WW) and average daily gain (ADG) table (3). The results of our study did not agree with (2) , as weaning weight and the average daily gain from birth to weaning, where the homozygous polymorphism CC was significantly superior (p<0.05) compared to the other polymorphisms CT and TT.

Table (3) The effect of the Polymorphisms of the *BMP-15* gene on the growth traits of Iraqi Awassi sheep.

Production Traits of *BMP-15* gene for Awassi Sheep				
No.	Polymorphisms	Birth Weight (BW) /kg	Weaning Weight (WW) /kg	Daily Average Gain (DAG) /kg
1	TT	3.42 ± 0.16	14.89 ± 0.86	0.127 ± 0.008
2	TC	3.71 ± 0.76	15.11 ± 1.35	0.126 ± 0.014
3	CC	3.83 ± 0.75	15.75 ± 0.76	0.132 ± 0.012
Mean		3.56 ± 0.13	15.10 ± 0.17	0.128 ± 0.010
Significant		N.S	N.S	N.S

Phylogenetic tree & Protein Structure

The results of the comparison of the *BMP-15* gene for phylogenetic tree between different global Breeds with the Awassi sheep breed in the current study showed that there are three main branches. The Awassi sheep breed with a separate main branch, while the remaining branches were divided between New Zealand and Pakistan with one branch, India and Mexico with another main branch figure (2).

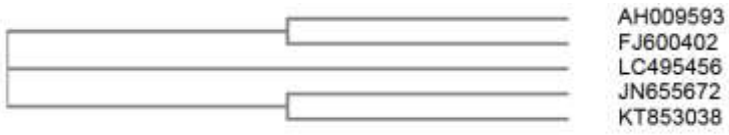

Figure. 2 Phylogenetic tree of *BMP-15* sequences in current subsequence and reference copies for different Breeds in would.

The accession numbers used in the phylogenetic tree design were taken from the Genbank are (LC495456 Iraq "current study", AH009593 New Zealand, JN655672 Pakistan, FJ600402 Indian and KT853038 Mexico)

The absence of the effect of the genetic morphology of the *BMP-15* gene may be attributed to the fact that the site of the mutation that we found in this study is that it occurred in a non-coding site, specifically in the first intron region of *BMP-15 gene*, and this mutation did not change any amino acid from the peptide chain of the PMP15 protein while in a study (21) the mutations for in the *BMP-15* gene substitution the amino acids, which in turn increase the ovulation rate in heterozygous individuals and cause sterility in homozygous ewes. Drawing the three-dimensional shape of the peptide chain of the gene *BMP-15* protein using the EzMol tool as shown in Figure 7.

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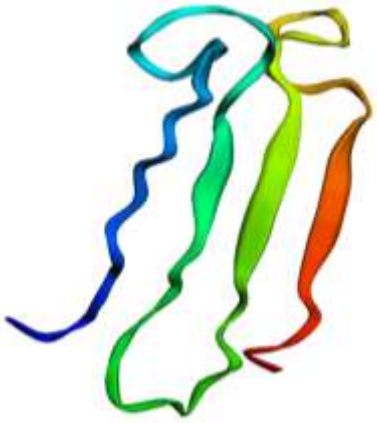

Figure. 3 The three-dimensional shape of the peptide chain of the studied fragment of the BMP-15 gene.

conclusion

Through the above results obtained from the current study, we conclude that the *BMP-15* gene cannot be considered as a selection marker for selecting the best growing animals or the highest litter size.
However, further researches using larger samples and to study regions larger than the BMB15 gene are needed to detect the effects of those mutations on Awassi sheep litter size in Iraq.

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