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POLYMORPHISM OF GDF9 (EXON-1) GENE AND ITS ASSOCIATION WITH MILK PRODUCTION AND PROLIFICACY OF AWASSI SHEEP

Fouad Lazim Jasim Al-Khuzai and Jaafar Ramahdan Ahmed

Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.

Abstract

This study was conducted on 50 Awassi ewes in two different location (30 ewes and its progeny in animals farm / Department of Animal Production / College of Agriculture Engineering Sciences / University of Baghdad and 20 ewes and its progeny in the First Research Station for Agriculture College Muthanna University) and the Physiology Laboratory, College of Agriculture, Department of animals Resources and also in Laboratories for Biotechnology and Molecular Genetics Analysis (Advanced Scientific Bureau in Baghdad) for the period from 15/6/2018 to 15/12/2018. The objective to this study was to identify the genotypes for Growth differentiation factor 9-GDF9 gene/exon-1 and the relationship between these genotypes with some productive and reproductive traits of Awassi sheep. The results of this study can be summarized as follows. The percentage of genotype distribution for the GDF9 gene in sample studied ewes were 80.00, 18.00 and 2.00% for the genotypes GG, GA and AA respectively, the differences between these percent were highly significant ($P<0.01$), with an allele frequency of 0.89 and 0.11% for both G and A allele respectively. The effect of GDF9 genotype in total milk production and the lactation period was non-significant, as for the milk components were influenced significantly ($P<0.05$) by the differences between the genotype of the GDF9 gene in fat and solid non-fat percentage favor the heterozygous GA. GDF9 genotype affected significantly ($P<0.05$) on litter size, dams with GA genotype namely, 1.33 ± 0.08 lamb/ewe. We can concluded from the study of the GDF9 gene polymorphism that the use of these markers to put a strategically method of genetically improvement for the sheep to increase of economical income from sheep flock by selection and genotypes crossing which gave a good performance. The application of this study in bigger samples of animals for many productivity seasons may give more accurate results for applying replacement and culling strategy.

Key words **:** *Awassi sheep- GDF9 (Exon-1) gene-Milk production-Prolificacy*.

Introduction

The local sheep characterize decrease in production of meat and milk belonged for different genetic and environment factors and adapted for severe environment conditions on production performance (Ishak and Ajeel, 2013). For improvement and expanding of sheep breeding projects and increasing the number of animals must be use the modern methods for food and improving the genetic and environment conditions (Al- Rawi, 2006) and must be interest in economic traits reproductive efficiency, stay ability, milk production of sheep and lambs growth from the application of selection program (Alkass and Juma, 2005). The importance of pointers for reproductive efficiency in sheep and goats were, (Fertilization %), (Fertility %), Prolificacy, (Twining rate) or Litter size. Improvement of sheep reproductive traits is one of the main objectives of breeding work. Direct selection by fertility is characterized by relatively low efficiency, which is connected on the one hand, with low heritability, on the other hand, with a limited manifestation gender (Kolosov *et al.*, 2015). Modern trends in sheep breeding include the use of new methods based on the application of DNA technologies, thus providing the industry being profitable and competitive (Chu *et al.*, 2007, Karagodina *et al.*, 2014). Marker selection is an important trend in practical genetics (Marker Assisted Selection - MAS), suggesting the use of DNA markers associated with productivity traits (Yang *et al.*, 2010, Klimenko *et al.*, 2014). The DNA marker-based technologies are widely applied in national breeding programs in several countries with developed sheep breeding (Iwanowska *et al.*, 2011, Chu *et al*., 2012). Growth differentiation factor 9 (GDF9) is a growth factor and a member of the transforming growth factor β superfamily that is secreted by oocytes in

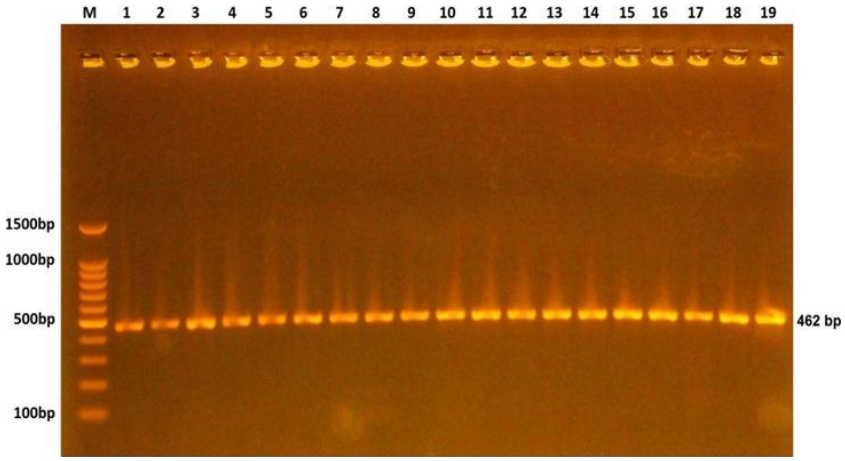

Fig. 1: Amplification of Exon-1 of GDF9 gene (fragment 462bp)

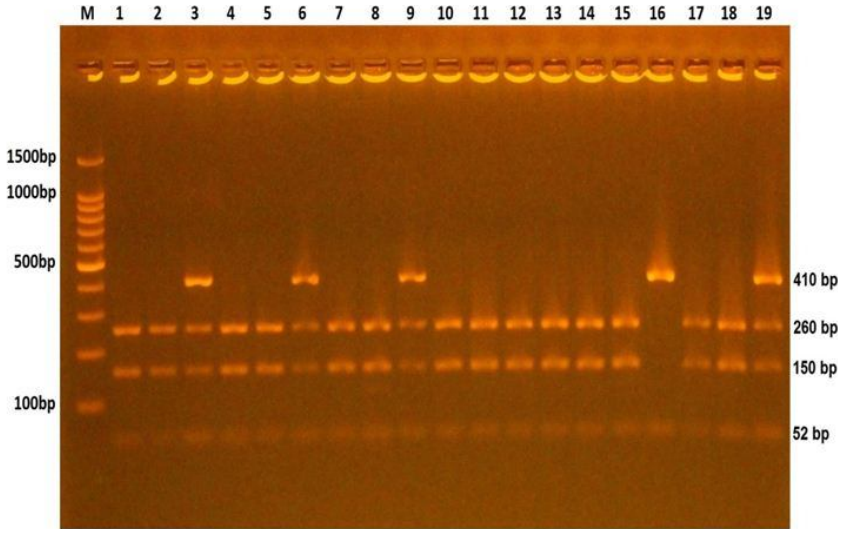

Fig. 2: Determine the genotype of Exon-1of GDF9 gen from restriction enzyme *Hha1*.

growing ovarian follicles, which is essential for growth and differentiation of early ovarian follicles (McPherron *et al*., 1993). The ovine GDF9 gene was determined in the 5th chromosome (Sadighi *et al*, 2002, Hanrahan *et al.,* 2004). The length of the gene is approximately 2.5kb consisting of two exons separated by one intron 1126bp and coding a pro-peptide of 453 amino acids, with the mature peptide being composed of 135 amino acids (Ghaderi *et al.*, 2010). Exon 1 spans 397bp and encodes for amino acids 1-134, while Exon 2 spans 968bp and encodes for amino acids 135-456. The single intron spans 1126bp (Ghaffari *et al.*, 2009).

Materials and Methods

The blood samples were collected from 50 ewes, it was prepared from the jugular vein in the neck of animal and using vacuum tube 10 ml containing EDTA, the blood was divided in two parts (two tubes) the first part for extracted DNA and the second part is for another test, so all tubes of first test put in cool-box containing ice, then transported to the laboratory. DNA was extracted from blood by using of kit of USA PROMEGA Company. DNA extraction is electrophoresis on Agarose 0.5gm add to 50ml of (1X) TPE in beaker with one drop of ethidium bromid stain to determine the quality and quantity of DAN (Sambrook *et al*., 2001). Polymerase chain reaction-(PCR) technique was done to amplification the studied region of Exon-1 of GDF9 gene 462bp with two primers and the sequence of it was shown in (Table 1), in addition to, the other used materials were present in kit of master mix and size use of mixture (PCR) is 25µl, which explained in (Table 2). Temperature and time program of polymerase chain reaction (PCR) stages was shown in (Table 3). To be sure of DNA product was obtained from (PCR) process of all samples, it was loading on Agarose gel (2% buffer TBE) electrophoresis and in markers size 1500bp (100bp) for accuracy of desired fragment that shown in (fig. 1). RFLP technique was done by restriction enzyme *Hha1* with DNA product (fragment 462bp), then products obtained of electrophoresis were isolated on Agarose gel (2% buffer TBE). After that, gel was taken to gel document system took it the photo. After finishing of laboratory works the genotypes of all samples were known and determine the allele frequency, check the Hardy-

Table 1: Primers sequences of Exon-1 of GDF9 gene.

The sequence of primers		Name of gene
F: 5'-GAAGACTGGTATGGGGAAATG -3'	Exon-1	GDF9
R: 5'-CCAATCTGCTCCTTACACACCT-3'		gene

(Bahrami *et al.*, 2014)

Table 2: The used materials for PCR to amplification of Exon-1 GDF9 gene.

Volume (µl)	Reagent
12.5	Profitaq PCR premix kit
1	P_F
1	P_R
8.5	H_2o
2	Template DNA
25µl	Final Volume

Table 3: The used program of polymerase chain reaction (PCR) in this study.

No.	Stages	Temperature	Time	No.of cycles
1	Initial Denaturation	95 C°	5 min.	1
2	Denaturation	95 C°	0.5 min.	30-35
3	Annealing	58 C°	0.5 min.	30-35
4	Extension	72 C°	0.5 min.	30-35
5	Final Extension	72 C°	7 min	1
6	Final incubation	4 C°	10 min	-

(Bahrami *et al.*, 2014)

Table 4: Number and percentage of genotype for exon-1 of GDF9 gene in study Awassi sheep sample.

The percentage %	The number	Genotype
80.00	40	GG : Wild
18.00	9	GA : Hetero
2.00	1	AA : Mutant
100 %	50	Total
81.320 **	-	(X^2) Chi-square
		Allelic frequency
	0.89	G
	0.11	A
	** (P<0.01)	

Table 5: Associated genotype of Exon-1 of GDF9 gene with total milk production and lactation period.

Standard error± Mean		Number of ewes	(Genotype)
Total milk production(kg)	Lactation period (day)		
76.05 ± 1.41a	110.85 ± 0.67a	40	GG
75.78 ± 2.72a	112.22 ± 1.37a	9	GA
NS	NS	Total number 49	Probability
The means with different letters within the same column are significantlybetween them(P<0.05)			

Weinberg equilibrium, observed homozygote (wild) GG, (Mutant) AA, heterozygote GA has been examined and it was done Pop Gene32 software and it was shown in (fig. 2).

Results and Discussion

Electrophoresis of amplified products of Exon-1position of GDF-9 gene confirmed 462bp fragments from amplification. PCR products obtained by using Agarose gel (2%) were electrophoresed that in fig. 1, given an example of the PCR products.

Amplified 462bp fragment of GDF-9 gene wasim pressed restriction enzyme *HhaI*. Restriction enzyme will cut GCG"!C site. About this gene, wild-type allele has 2 cut locals that after digestion created 3 fragments (3 bands) 260, 150 and 52bp. So, a homozygous individual in this case is as GG and with 3 bands. While the mutant type allele has a cut local that after digestion created 2 bands 410 and 52bp. Therefore homozygous individual in this state as AA and has 2 fragments. In animals heterozygous GA a 4 bands or fragments is visible of 410, 260, 150 and 52bp of the digest (Fig. 3). After electrophoresis, digested products of all samples in studied Awassi sheep of three genotypes GG and GA and AA shown 19 samples (14 genotype GG, 4 genotype GA. 1 genotype AA in fig. 2.

Table 4, explain number and percentage of genotypes for Exon-1 of GDF9 in samples of Awassi sheep, it shown that there was high significant differences (P<0.01) between genotypes, which percentage of homozygous (wild) GG were 80%, while percentage of heterozygous GA were 18% and percentage of genotype (mutant) AA were 2%, this was in line with those reported by Bahrami *et al.*, (2014), Kolosov *et al.*, (2015). In their study on Exon-1 of GDF9 gene. They found that wild genotype percentage GG was the greater, followed by heterozygous GA, while the mutant AA was absent.

Table 5, shown there was no significant differences between genotypes of Exon-1 of GDF9 in total milk production and lactation period of ewes, it mean that the formed proteins from gene expression of GDF9 not effect

Table 7: Association genotype of Exon-1 of GDF9 gene in prolificacy of ewes.

Mean ± standard error of prolificacy (lamb)	Lamb number	Ewes number	(Genotype)
1.23 ± 0.07 a	49	40	GG
1.33 ± 0.08 b	12	9	GA
*	61 Total number	49 Total number	Probability
The means with different letters within the same column are significantly between them(P<0.05).			

Table 6: Effect of genotype of Exon-1 of GDF9 gene in milk composition.

Mean = standard error %				Number of samples	(Genotype)
Solid nonfat%	%lactose	Protein %	% Fat		
10.54 ± 0.24 b	4.24 ± 0.04 a	5.61 ± 0.25 a	3.52 ± 0.44 b	40 (120 samples)	GG
11.27 ± 0.27 a	4.36 ± 0.07 a	0.19 ± 5.82a	4.31 ± 0.57a	9 (27 samples)	GA
*	NS	NS	*	49 Total Number (147 Sample)	Probability
The means with different letters within the same column are significantly between them (P<0.05).					

on milk production and lactation period, may be affected on another traits in animals.

Table 6, shown the effect of genotype of Exon-1 of GDF9 gene for studied ewes in milk composition, it explain that there is significant differences ($P<0.05$) between genotypes in percentage of fat and solid nonfat only in milk composition, made up with the heterozygous in two traits.

Table 7, shown that there was significant differences ($P<0.05$) between genotypes in prolificacy of Awassi ewes made up with heterozygous GA and it about 1.33 = 0.08 lamb/ewe. The act of GDF9 gene was very specific in growth of ovarian follicles (Tang *et al.*, 2013). It indicate that the product protein from heterozygous of this gene has more positive effect in increase of ovarian follicles growth and then increase in ovulation rate and then increase in litter size, increase number of lambs, increase in twining % in ewes more than the wild genotype GG and this accept with all done studies on GDF9 gene that ewes heterozygous for most mutations of this gene were more prolificacy from ewes of another genotypes in these mutation (Hanrahan *et al.*, 2004).

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