



## TGF-B Super Family Correlation with the Fertility of Iraqi Awassi Ewes

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**Abstract:** This study was conducted in Barakat Abu al Fadhl Al- Abbas station (AS) for raising sheep/a subsidiary of Al- Kafeel Company for General Investment and livestock services in Holy Karbala province and Babylon Province It was undertaken on Awassi sheep for between October, 2013 to May, 2014 to investigate the polymorphism of TGF-B gene and its association with reproductive traits of Awassi sheep. Blood samples were collected from (80) ewes included 35 samples of Awassi ewes producing twins (pure breed), 21 samples of Awassi ewes producing twins lambs (hybrid breed), also 25 samples of Awassi ewes producing single lambs (pure breed), with age (2 .5- 5) years. DNA samples were extracted from each blood sample of sheep. Reference PCR primers were utilized to amplify segments in TGF-B Super Family gene. Then, AFLP technique was performed for each PCR fragment. The results indicated that two genotypes for each of the fragments of TGF-B Super Family gene (AF and FF) were identified. TGF-B Super Family gene revealed two fragments (156 and 245 bp) for FF genotypes and three fragments (156, 254 and 410 bp) for AF genotypes. PCR- AFLP methods detected two genotypes (AF and FF) with predominant of AF genotype in Awassi ewes producing twin, and this study refers to utilizing TGF-B gene as good genetic markers and their correlation with high fertility and twins producing Awassi sheep.

**Keywords:** Fertility traits, Polymorphism, sheep, TGF-B gene.

### Introduction

Awassi is the main sheep breeds in many countries in the middle east. Although this breed is characterized by slow growth, low fertility and low milk production, their ability to survive and reproduce under condition of

drought and extreme climate fluctuations is remarkable (Yousif *et al.*, 2013). Litter size and lamb growth are important economic traits in sheep breeding and reproduction, the ovulation rate can be genetically regulated by

a set of genes, called fecundity gene (Al-Thabthabee *et al.*, 2014). Molecular genetics can overcome these limitations offering new opportunities to the improvement of reproductive traits, as it supplies tools to analyze genetic variability directly at the DNA level with the possibility of detecting the individual genes influencing the reproductive characteristics (Asadpour *et al.*, 2012).

Multiple ovulations in mammals is a complex trait influenced by genetic and environmental factors. Genetic mutation of the first gene growth differentiation factor of TGF- $\beta$  super family (transforming growth factor- $\beta$ ) with major effects on the ovulation rate in sheep was identified (Paulini & Melo, 2011). The TGF- $\beta$  super family contains over 35 members, many of which have shown to be important Role in regulating fertility. TGF- $\beta$  is oocyte specific growth factor that appears to play key roles in granulosa cell development and fertility in most mammalian species (Ghaderi *et al.*, 2010). It has been proposed that TGF- $\beta$  promotes theca cell proliferation as well as decreases steroidogenesis and may, therefore, also prevent theca cells from premature differentiation during folliculogenesis. It was also demonstrated that genetic alterations, e.g., mutations in the TGF- $\beta$  gene lead to infertility in sheep due to ovarian dysfunction (Otsuka *et al.*, 2011). As a result of their role in the folliculogenesis, the availability of TGF- $\beta$  polymorphisms can be very useful in the study of animal reproductive genetics and physiology (Silva *et al.*, 2010). Therefore the aim of this study is to investigate the possibility of an association between the polymorphism of TGF- $\beta$  and reproductive traits in Awassi sheep.

## Materials & Methods

### Animals

Eighty Iraqi sexually mature healthy Awassi sheep were included in this study. These sheep were grazed in the field of the independent department of Holy Al-Hussein in Karbala and Babylon Provinces for a period from October 2013 to May 2014. Sheep divided to 35 samples of pure Awassi ewes producing twins 21 samples of cross Awassi ewes producing twins' lambs, and finale 25 samples of pure Awassi ewes producing single lambs aged (2.5 - 5) years.

### Blood collection

Venous jugular blood samples (4 ml per sheep) were collected from 80 Awassi sheep. The blood samples were placed in EDTA tubes as anticoagulants. Blood samples were transported to the laboratory in an ice box for DNA extraction. Genomic DNA was extracted from the whole blood by by Al-Shuhaib (2017). Then dissolved in TE buffer [10 mmol / l Tris-HCl (pH 8.0), 1 mmol / l EDTA (pH 8.0)] and kept at -20°C for further analysis. Primers and PCR amplification One pair of primer was used to amplify TGF- $\beta$  gene on in the reference gene by Hanrahan *et al.*, (2004). The expected amplification fragment size was 462 bp. The primer sequences used were F:5'-GAA GAC TGG TAT GGG GAA ATG-3' and R5'-CCA ATC TGC TCC TAC ACA CCT-3' (Hanrahan *et al.*, 2004).

Polymerase chain reaction was carried out in 25  $\mu$ l volume containing approximately 2.5  $\mu$ l of 10 x PCR buffer (50 mmol / l KCl, 10 mmol / l Tris-HCl (pH 8.0), 0.1% Triton X-100), 1.5 mmol / l of Mg 12.200  $\mu$ mol / l of each dNTP, 1  $\mu$ mol / l of each primer, 50 ng of genomic DNA and 2 U of Taq DNA polymerase. Amplification conditions were as

follows: initial denaturation at 95°C for 4 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s with a final extension at 72°C for 5 min on Master cycler (Eppendorf AG, Hamburg, Germany) PCR products were electrophoresed on 2% agarose at 75 V for 1 hour and visualized by ethidium bromide. Photos were taken using photo documentation unit.

### AFLP Analysis

Digestion with restriction enzyme used for TGF-B is *Hha* I. Digestion reaction contains 5 µl of PCR product, 5 U appropriate enzyme, 2 µl buffer 10x in 20µl final volume incubated for 3 to 6 h at 37 °C. After digestion with restriction enzymes, all products were separated by using electrophoresis in 2% agarose gel and visualized with ethidium bromide.

### Statistical Analyses

The genotype and allele frequencies of TGF-B gene polymorphisms and G statistic test were used to determine whether the

populations are in Hardy-Weinberg equilibrium using POPGENE32 software 19 (Yeh *et al.* , 1999).

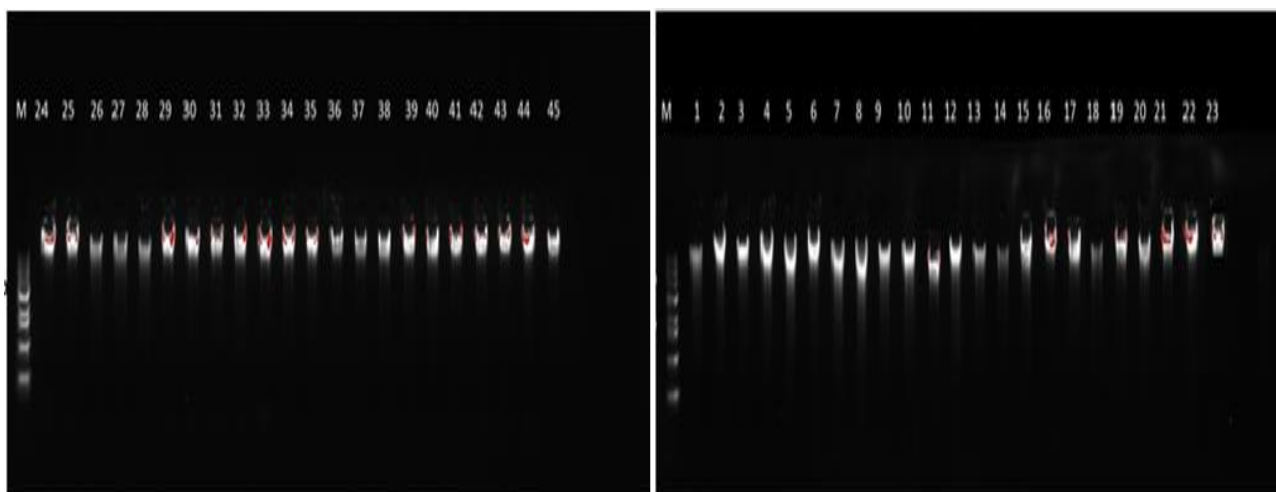
### Results & Discussion

In this study, simple manual procedures are used to isolate DNA, the concentration and purity of the isolated DNA are tested for further molecular analysis, such as PCR (Fig. 1).

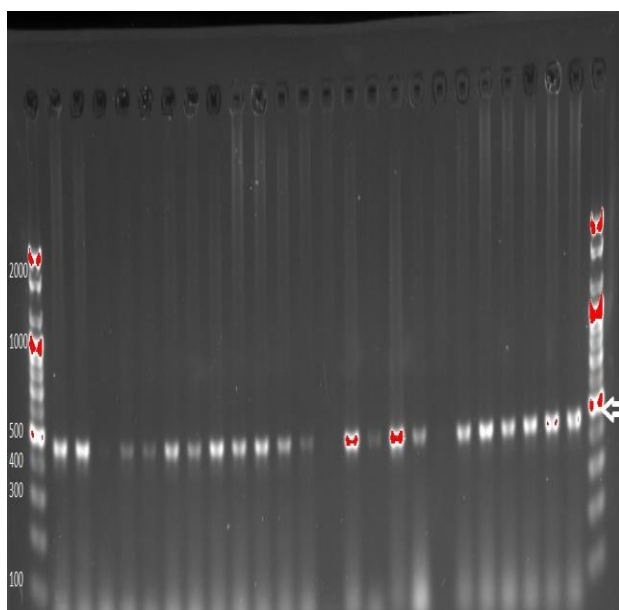
#### PCR Amplified Products of the Exon 1 of TGF-B

A DNA fragment with the size of 462 bp was amplified from exon 1 of TGF-B, all the individuals were generated one obvious band (Fig. 2), indicating the high specificity of the selected specific primers.

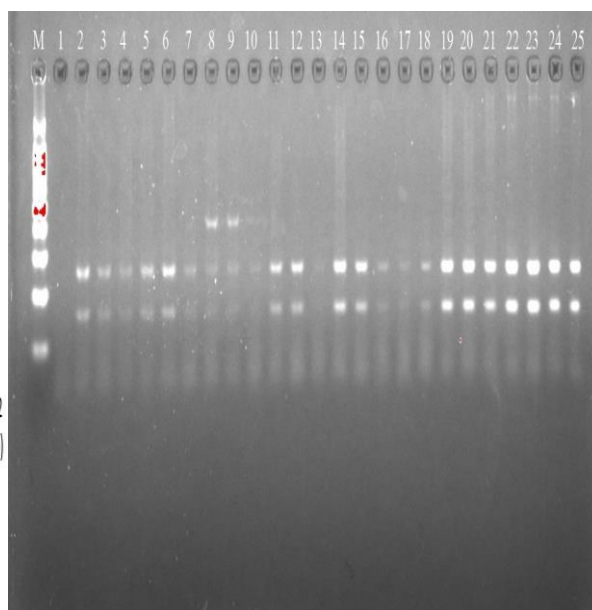
PCR-AFLP method was used to investigate the mutations, which includes digestion with restriction enzymes *Hha* I. TGF-B exon 1 agarose gel electrophoresis reveal variation within this amplicons. Two fragments (156 bp and 245 bp) were produced by the wild type and three fragments (156 bp, 254 bp and 410 bp) by heterozygous (polymorphism) (Fig. 3).



**Fig. (1):** Agarose gel electrophoresis of gDNA extracted using the manual procedure. M; Refers to DNA size marker, lane 1 into lane 23 refers to extracted gDNA samples from Awassi ewes (producing single lambs), lane 24 into lane 45 refers to extracted gDNA samples from pure Awassi ewes producing twins, Electrophoresis conditions: Agarose concentration 0.8%, power applied: 75V (4.1 V / cm), time of run: 1 hr. Staining method; precast ethidium bromide.



**Fig. (2):** Agarose gel electrophoresis of TGF-B exon 1 PCR fragments. M; Refers to DNA size marker, Electrophoresis conditions: Agarose concentration 2 %, power applied: 100V (7V. cm<sup>-1</sup>), time of run: 45 min. Staining method; precast ethidium bromide.



**Fig. (3):** PCR-AFLP analysis of TGF-B gene exon 1 in Awassi sheep using *Hha* I. M; Refers to DNA size marker, lane 1 into lane 80 refers to resulted PCR products were digested with *Hha* I Electrophoresis conditions: Agarose concentration 2 %, power applied: 100V (7V. cm<sup>-1</sup>), time of run: 45 min. Staining method; precast ethidium bromide.

AFLP analysis reveals two genotypes (AF and FF) in all screened TGF-B exon 1 of sheep. The overall ratio of the genotypes AF was the highest (78%) from Awassi ewes producing twin, (100%) from Awassi ewes producing twin (hybrid breed), (19%) from Awassi ewes producing single lambs , while, the genotype FF (21%) from Awassi ewes producing twin and (0%) from Awassi ewes producing twin (hybrid breed) and (80%)

from Awassi ewes producing single lambs (Table 1). Genotype and allele frequencies and Hardy-Weinberg test of TGF-B exon 1 is presented in Table (2). The observed frequencies of A allele were 0.36 and F allele was 0.64 of which the predominant genotype was AF. The  $\chi^2$  test showed that the polymorphism of TGF-B exon 1 in Awassi sheep was at Hardy-Weinberg equilibrium for this locus in the studied population (Table 2).

**Table (1):** Distribution of TGF-B exon 1 polymorphism with different samples of sheep.

. genotype (group)	AF		FF		Total	Chi-square ( $\chi^2$ )
	No.	%	No.	%		
Twin A	27	78.79	7	21.21	34	12.975 **
Twin, Hybrid	20	100	0	0.00	20	15.00 **
Single A	4	19.05	17	80.95	21	13.269 **

\*\* (P<0.01).

**Table (2): The observed, expected, average heterozygosity,  $\chi^2$  test for Hardy-Weinberg equilibrium, and genotype frequencies of TGF-B exon 1 for the *Ovis aries* Awassi breed.**

Obs-Het	Exp-Het	Avr-Het	Nei'-Exp - Het	$\chi^2$	Allele A freq.	Allele F freq.
0.7209	0.4638	0.4611	0.4611	26.8	0.36	0.64

**Abbreviations:** Obs-Het: Observed heterozygosity, Exp-Het: Expected heterozygosity, Avr-Het: Average heterozygosity, Nei-Exp-Het: Nei's expected heterozygosity, freq.: frequency.

In this study, PCR- AFLP method detected two genotypes (AF and FF) with a predominant of AF genotype in producing twins Awassi rams and ewes. TGF-B gene is autosomal that in homozygous state causes sterility and in heterozygous state provides multiple births (Gursel *et al.*, 2011). neutralize TGF-B affects the activity of the *Corpus luteum* and therefore in addition to its impact on follicle growth, its effectiveness was also broadened on the active of ovulation and pregnancy induction (Juengel *et al.*, 2004). An asexual TGF-B gene on chromosome 5 has a mutation that can increase the rate of ovulation in heterozygous ewes and sterility in homozygous ewes (Hanrahan & Owen, 1985). Moreover, partial defects in TGF-B genes were associated with the increased ovulation rate in sheep (Xue-Qin *et al.*, 2009).

Hanrahan *et al.* (2004) reported eight DNA variants in TGF-B of Cambridge and Belclare sheep including from G1 to G8, and showed that Cambridge and Belclare sheep carrying a mutation in the gene (FecGH). Exons 1 and 2 of TGF-B gene increase ovulation rate of heterozygous sheep and the lack of fertility in homozygous sheep. G8 variant also known as FecGH where serine converted to phenylalanine at the base 395 which replaced an uncharged polar amino acid with a nonpolar one at base 77 of the mature coding region and may change the function of TGF-B in sheep (Dutta *et al.*, 2013; López-Ramírez *et*

*al.*, 2014). TGF-B gene variant can be applied in breeding programs by gene-assisted selection (GAS), aiming towards the improvement of sheep reproductive potential and production (Silva *et al.*, 2010). Kolosov Yu *et al.* (2015) determine the TGF-B polymorphism in Russian sheep breeds polymorphism was identified by PCR- RFLP method, and AG and GG genotypes were detected in this study.

## Conclusion

This study refers to utilizing TGF-B gene as good genetic markers and correlation with high fertility and twins producing in Awassi sheep.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

**Ethical approval:** all applicable national and international guidelines for the care and use of animals were followed.

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