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Detection of (FecB) Gene Polymorphism in Local Sheep Breed at Different Area of Iraq

Ahmed H. Al-Thabhawee[1], Hayder M. Samaka[1*] and Hayder M. Kadhim[2]
[1] Faculty of Veterinary Medicine, University of Kufa, Iraq, [2] Al-Najaf Veterinary Hospital, Central Laboratory, Najaf, Iraq

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***Corresponding author:**
Email address:
haydarm.ali@uokufa.edu.iq

Abstract

The rate of ovulation has largely influenced by both genetic and environmental factors. Currently, "the Booroola FecB gene" considered as a guide to improve the twin productivity in sheep flocks meanwhile retain the important other features desired in the flock.

This study intended to detect the mutation in FecB gene using PCR-RFLP screening method by using specific primers designed to introduce a point mutation in PCR product of FecB gene. A thirty, local sheep breed from the middle and south part of Iraq were used in this study. FecB gene was detected by forced digestion of PCR products using Eco471 (AvaII) digestion enzyme.

The results of this study revealed the lack of (190bp band wild type) gene mutation in all samples of the study that is meant local Iraqi sheep breed are non-carrier for polymorphism genetic factor. However, future study is highly recommended with a large number of "local sheep" for better understanding of this feature.

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Introduction

The total sheep population of Iraq in 1999 was about 9.7million (Al-Salihi, 2012), and it contribute largest to the total meat production in Iraq. An Awassi are of the main sheep breeds (Epstein, 1985). Litter size and lamb growth are important economic traits in sheep breeding and reproduction (Galal *et al.*, 2005). Several studies have indicated that the ovulation rate can be genetically regulated by a set of genes, called Fecundity genes (Al-Barzinji and Othman, 2013; Davis *et al.*, 1982). The Booroola gene was first reported by researchers in Australia and New Zealand (Piper and Bindon, 1982; Davis *et al.*, 1982)

in Merinos. The term "Booroola" was first discovered and taken from the name of the ranch in Australia, where sheep carrying the single gene for prolificacy. Now, Booroola refers to a gene called (Fecb) gene (Fec from fecundity). It acts independent of the breed or type of sheep that it is contained it. The Booroola gene (Fecb) was the first major gene for prolificacy identified in Booroola Merino sheep (Chu *et al.*, 2007). The effect of the gene is additive for ovulation rate, with the ovulation rate of heterozygous ewes increased by about 1.65, and homozygous ewes by about 3.30 (Davis, 1999). Also, one copy of the (b) allele increases the ovulation rate by 1.2 ova shed per ewe ovulating and the lambing rate by 0.6 lambs born per ewe lambing (Piper *et al.*, 1985). The "Fecb" locus is situated in the region of ovine chromosome 6. This chromosome contains bone morphogenetic protein receptor (BMPR type IB) gene and transforming growth factor β (TGFβ) genes family (Mulsant *et al.*, 2001; Wilson *et al.*, 2001). They are obligatory for folliculogenesis through their ability to stimulate granulose cells proliferation and modulate FSH dependent cyto-differentiation (Kumar *et al.*, 2013). The "Fecb" gene can be transferred into any breed of sheep.

So far, the most numerous breed of sheep containing the "Fecb" gene is the Booroola Merino, this breed was developed in Australia and nowadays is also bred in New Zealand. The main objective of the present research was to explore the Fecb gene polymorphism in local Iraqi sheep breeds using the "PCR-RFLP" technique.

Material and methods

During April 2012 till June 2012, a total of 30 blood samples from local breed's sheep, (8 twins producing female, 16 single lambs producing female and 6 males) collected randomly from different part of the middle and south of Iraq. Blood samples were collected by disposable syringe from the jugular vein into EDTA tubes. Genomic DNA extracted from blood samples using Geneaid™, the DNA Isolation Kit (Geneaid Biotech Ltd, Taiwan) and stored at -20˚C.

A region of *Fecb* gene was amplified by using a set of forward and reverse primers (Wilson *et al*, 2001); Primer sequences were as follows: forward (5`-CCA GAG GAC AAT AGC AAA GCA AA-3`) and reverse (5`- CAA GAT GTT TTC ATG CCT CAT CAA CAG GTC -3`) (BiCorp, Canada). The Polymerase chain reactions were carried out in a volume of 25µl, containing 5ul ovine genomic DNA,12.5µl KAPA2G Robust HotStart ReadyMix® (KAPABIOSYSTEM, South Africa) and 1.25µl (10 pmol/µl) for each forward and reverse primers. The reaction mixtures were subjected to the following thermal cycling parameters in a TECHNE TC-300 (Bibby Scientific, USA): denaturation at 94˚C for 5min, followed by 35 cycles of denaturation at 94˚C for 30s, annealing at 60˚C for 30s, extension at 72˚C for 30 seconds, with a final extension at 72˚C for 5 min. PCR products were digested by Eco471 (AvaII) digestion enzyme from (US-biological, USA). The digestion reaction mixture contain 10µl of PCR product, 18µl nuclease free water, 2µl of 10X restriction enzyme buffer and 2µl of E0374-15(AvaII) restriction enzyme. The final volume mixed gently and spins down for few seconds then incubated for 16 hours at 37˚C in UltraCruz™ Hotplate (Santa-Cruz biotechnology, USA) as manufactures recommended. The resulting products were separated by electrophoresis on a 2% agarose-ethidium bromide gel in 1X TBE buffer at 75 Volts for 75 min. The

bands visualized in Gel documentation system (Vision-SCIE-PLAS. UK) and data analyzed by Gene tool analysis software (SynGen, UK).
PCR product of the *Fecb* gene produced a 190bp band. After digestion with AvaII restriction enzyme, the *Fecb* gene homozygous carriers should produce a 160bp fragment (FceBB), whereas non-carrier products remain uncut, at 190bp (Fec++), whereas heterozygote should produce both 160 and 190bp bands (*FecB*+) (Davis *et. al,* 2002).

Results

The results of the FecB gene PCR products showed typical 190bp amplicon for all samples (Figure 1). The PCR-RFLP patterns after "Ava II" digestion revealed only one band of 190bp product (wild type form) in all tested animals (Figure 2), and none of them were carrying gene mutation form.

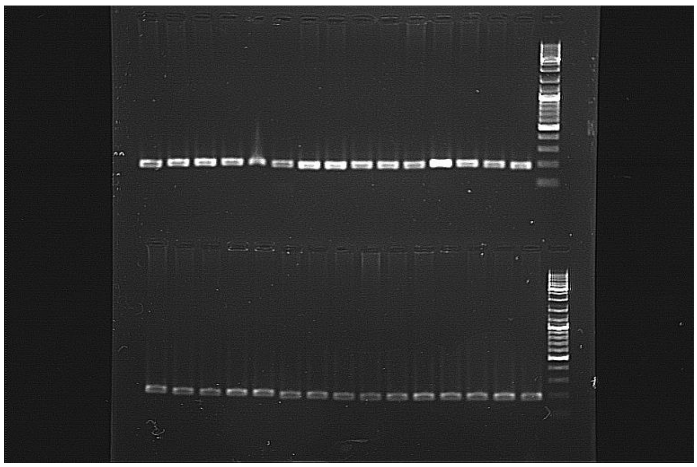

Figure 1: The PCR product of Fecb gene of local Iraqi breed blood samples. The PCR product is 190bp.

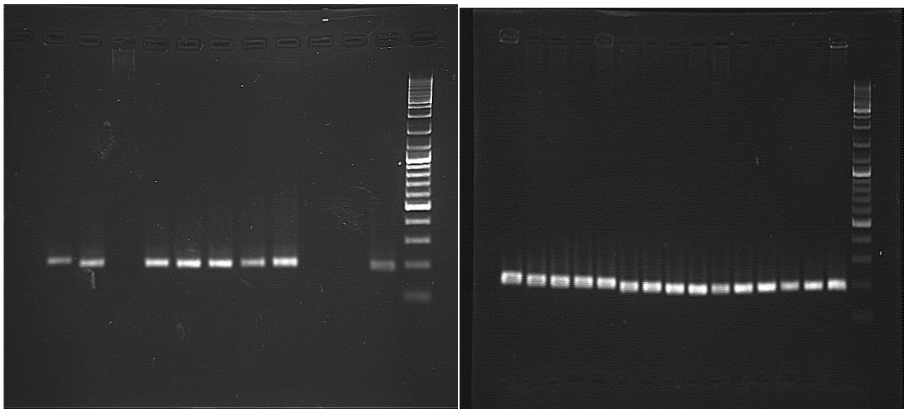

Figure 2: Image of PCR product of the FecB mutation of the Fecb gene digested with Ava II restriction enzyme, all samples showed wild-type allele (++) with 190bp.

Discussion

The BMPR-1B gene had two alleles, the wild type nucleotide (non-carrier) and the mutant nucleotide (carrier). The presence of the nucleotide in wild type sheep codes for glutamine amino acid. However, the presence of G replaces this amino acid with arginine (Souza *et al*, 2001). A mutant BMPR-1B gene and number of copies of the mutation, lead to increase the ovulation rate; the litter size and ovulation rate (Fabre *et al.*, 2006)
The PCR-RFLP revealed only one band of 190bp product in all samples that have been tested. However, the tested animals showed wild type non-carrier of the FecB mutation in the BMPR-1B gene, these results are in agreement with the findings of other studies previously reported. They found that digestion of the FecB gene with AvaII restriction enzyme gave a non-carrier wild type form (Fec++) in all tested animals from different sheep breeds in Middle East area (Al-Barzinji and Othman, 2013; Nejhad *et al.*, 2012; Abouheif *et al.*, 2011; Abulyazid *et al.*, 2011; Moradband *et al.*, 2011; Kasiriyan *et al*., 2009; El-Hanafy *et al.*, 2009; Ghaffari *et al.*, 2009; Kumar *et al.*, 2006). On the other hand, the FecB mutations have been reported in other Asian areas and the most prolific sheep breeds are Indian Garole and Indonesian Javanese (Davis *et al.*, 2002), Small-tailed Han and Hu sheep of China (Davis *et al.*, 2006) as well as the Australian Booroola Merino (Souza *et al.*, 2001).
In conclusion, this study indicates that Booroola gene polymorphism is not related to local breeds of Iraqi sheep. In addition, this breed is not susceptible genetically for twins and this explain the low litter size in Iraqi sheep that related for existence of wild type pattern of FecB gene. More studies recommended with a large number of "local sheep samples" for better understanding of this feature.

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