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Polymorphism of Booroola *FecB* Gene in Prolific Individuals from Najdi and Naeimi Sheep Breeds of Saudi Arabia

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Abstract: The present investigation was carried out to examine the presence of Booroola FecB mutation gene in 124 selected prolific ewes from 6 flocks representing two Saudi Arabian local sheep breeds by forced PCR-RFLP method. Genomic DNA was extracted from the blood of 69 Najdi and 55 Naeimi matured ewes with litter size varied from 2.01-2.16 lambs per ewe lambing. Digestion of *FecB* gene 190 base pair with AvaII restriction enzyme resulted in non carrier 190 bp band wild type in all samples which revealed the absence of this mutation in the tested Najdi and Naeimi sheep breeds.

Key words: *FecB* gene, prolificacy, PCR-RFLP method, Najdi ewes, Naeimi ewes, sheep, Saudi Arabia

INTRODUCTION

Sheep occupy a special niche in Saudi Arabian agribusiness economy. The population of sheep is about 7 million head where black Najdi and Naeimi are fat-tailed sheep and considered the breeds of choice (Abdo *et al*., 1989) they are known for their hardiness and adaptability to the prevailing adverse environments. As lamb production is a main source of income in all flocks, increasing the fecundity of local sheep has always been an important breeding goal. Yet, prolificacy of all indigenous sheep breeds are low and is thus a major constraint to increased lamb production (Abouheif and Alsobayel, 1982). Recent developments in molecular biology have opened the possibility of identifying and using genomic variation and major genes for the genetic improvement of fecundity. Mulsant *et al*. (2001) and Wilson *et al*. (2001) reported that a single gene mutation on ovine chromosome 6 corresponding to the human chromosome 4q22-23 was responsible for the high prolificacy in Booroola Merino sheep. This mutation is located in the kinase highly conserved domain of the bone morphogenetic protein receptor 1B (BMPR-1B or activin-like kinase 6, ALK6) and is characterized by precocious differentiation of ovarian follicles, leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles (Souza *et al*., 2003). The significantly increased ovulation of Booroola gene-carrier sheep (FecB) has been shown to increase number of lambs born per ewe therefore, genetic progress of such trait could be rapid, provided that it is possible to identify and directly select for beneficial allele by forced PCR Restriction Fragment Length Polymorphism (RFLP) approach based on the reports described by Souza *et al*. (2001) and Davis *et al*. (2002). These discoveries make it possible to use DNA testing to determine whether other breeds of sheep carry the mutation without the need for the traditional pedigree information. Extensive research has been carried out on different prolific sheep breeds to identify the genes involved in controlling ovulation rate and prolificacy. Consequently, tests on Indian Garole (Pardeshi *et al*., 2005) and Chinese Hu (Guan *et al*., 2007) samples showed that populations of these sheep are homozygous for the BMPR-1B mutation and tests on samples from Indonesia also showed that this mutation was segregating in Javanese sheep (Davis, 2004). This study was undertaken to screen for the presence of the Booroola (*FecB*) gene in Najdi and Naeimi sheep since many random crosses had been attempted with imported sheep from Australia and New Zealand however, this knowledge is necessary before any further attempts to introgress this gene into Saudi Arabian local sheep.

MATERIALS AND METHODS

Sampling and DNA extraction: A total of 124 adult ewes from 6 breeding flocks representing Najdi (n = 69) and Naeimi (n = 55) sheep were selected in this study based on previous history of multiple births; any selected ewe should has at least two lambing record of multiple births. The distribution numbers of the selected ewes with

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Table 1: The distribution numbers of the selected ewes[1] with multiple lambing records within each studied flock of Najdi and Naeimi sheep

Breed	Najdi			Naeimi		
No. of available records	2	3	4	2	3	4
Flock						
1	2	8	1	0	0	0
2	5	10	0	3	14	3
3	7	6	0	4	5	4
4	17	3	4	0	0	0
5	0	0	0	6	4	1
6	5	1	0	7	4	0
Total	36	28	5	20	27	8

[1]with at least 2 lambs per ewe lambing

multiple lambing records within each studied flock of Najdi and Naeimi (local name of Awassi) sheep are shown in Table 1. Approximately 10 mL blood was collected aseptically from the jugular vein of each ewe in EDTA. All blood samples were taken back to the laboratory under low temperature. Genomic DNA was extracted from blood using GFX Kit produced by Amersham Biosciences. The quality of DNA was checked by spectrophotometer taking ratio of optical density value at 260 and 280 nm. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further research. The DNA samples were dissolved in TE buffer (pH 8.0) and stored at -20°C pending analysis.

PCR-Forced RFLP of *FecB* gene: A region of *FecB* gene (190 bp) was amplified by using a set of Forward (F) and Reverse (R) primers (Wilson *et al*., 2001); F, 5'-CCAGA GGACAATAGCAAAGCAAA-3' and R, 5'-CAAGATGT TTTCATGCCTCATCAACAGGTC-3'. The reverse primer was deliberately introduced a point mutation resulting in PCR products with FecB carrier sheep containing an AvaII restriction site (G/GACC) whereas products from non-carrier lacking this site. For amplication, 25 μL of PCR reaction was prepared by adding 10 pM of each primer, 100 μM of each dNTPs, 1.5 mM $MgCl_2$, 10×PCR assay buffer, 100 ng DNA template and 1 unit Taq DNA polymerase. The amplification was carried out using a pre-programmed thermal cycler with the following conditions: initial denaturation of 5 min at 95°C followed by 35 cycles of denaturation at 94°C, annealing at 60°C and extension at 72°C each of 30 sec and lastly the final extension of 5 min at 72°C. DNA tests were carried out using forced PCR-RFLP based on the method described by Davis *et al.* (2002). An aliquot of 10 μL of PCR product was digested for 6 h at 37°C with 10 units of AvaII restriction enzyme. The restriction enzyme digested PCR products were separated by 2% agarose gel and stained with ethidium bromide. The forced PCR of the *FecB* gene produced a 190 bp band. After digestion with AvaII enzyme, the *FecB* gene homozygous carriers had a 160 bp band ($FecB^{BB}$), the non-carrier had a 190 bp band ($FecB^{++}$), whereas heterozygous had both 160 and 190 bp bands ($FecB^{B+}$).

RESULTS AND DISCUSSION

The *BMPR-1B* gene had two alleles, the A wild type nucleotide (non-carrier) and the G mutant nucleotide (carrier). The presence of the A nucleotide in wild type sheep codes for glutamine amino acid but presence of G replaces this amino acid with arginine (Souza *et al*., 2001). A mutant *BMPR-1B* gene increases the ovulation rate; the litter size and ovulation rate increase with number of copies of the mutation (Fabre *et al*., 2006). There are no reports of FecB polymorphism in any of the Middle East fat-tailed sheep breeds until now. However, there was a chance for Najdi and Naeimi sheep to receive the FecB allele from imported Australian Merino crosses in early 1980's at that time they were utilized to improve local breeds for meat purposes. The electrophoretograms of forced PCR-RFLP revealed only one band of 190 bp product in all tested samples. None of the tested individuals carried the FecB mutation in the *BMPR-1B* gene. Therefore, the results revealed the absence of mutant type G nucleotide, indicating that the examined local black Najdi and Naeimi breeds are wild homozygous ($FecB^{+}$/$FecB^{+}$) non-carrier sheep. Therefore this evidence cleared the doubts on probable presence of the gene in these breeds, since all samples were taken from selected prolific individuals within each tested flock. The litter size in black Najdi (Abouheif and Alsobayel, 1982) and Naeimi ewes (Said *et al*., 1999) are very small ranging from 1.11-1.38 and from 1.04-1.08 per ewe lambing, respectively. On the other hand, the mean litter size for the selected individuals of Najdi and Naeimi sheep were 2.16 and 2.01 and the mean numbers of studied lambing records per ewe were 2.57 and 2.83, respectively (Table 1). Genetic variation in litter size in sheep has been widely documented and the evidence showed substantial differences among breeds and in a number of cases exceptional variations within breeds/strains. Prolific sheep are the consequence of different environmental conditions, nutrition, selection and possibly even Quantitative Trait Loci (QTL) or mutant major genes regulating ovulation (Piper *et al*., 1985). Montgomery *et al.* (2001) stated that most prolific sheep breeds had no evidence of FecB mutation for high ovulation rate and increased litter size. Accordingly, the findings of the present study are in line with those of Guan *et al.* (2007) in Chinese sheep who reported that 7 out of 9 sheep breeds studied were found to be wild type (190 bp) in respect to restriction pattern of *FecB* gene.

Similar results were also reported by Amiri *et al.* (2007), Ghaffari *et al.* (2007) and Irajeyan *et al.* (2009) who found that digestion of *FecB* gene 190 bp with AvaII restriction enzyme resulted in non-carrier wild type in all tested individuals from Iranian Lori-Bakhtiari, Shal and Sangsari sheep breeds, respectively. The absent of FecB mutation in *BMPR-1B* gene of five breeds of Egyptian sheep was also reported by El-Hanafy and El-Saadani (2009).

CONCLUSION

The results of the present study based on electrophoretograms of forced PCR-RFLP found no FecB mutation in the Saudi Arabian Najdi and Naeimi sheep. Although, the study is still a preliminary research, there is a probability that the mutant allele was not available in the tested individuals. Therefore, there is a need to undertake a further study on relatively larger sample sizes. A number of other mutant genes affecting lambing rate have been also detected for which the Najdi and Naeimi sheep may be studied.

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