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Genotype frequency of *FecX*^{B} (Belclare) mutation of *BMP15* gene in Chios (Sakiz) sheep

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Abstract

Ovulation rate and litter size, varies between breeds or individulas who belong to the same breed, are evaluated as fertility traits. Fertility traits are important for economic values in animal breeding. However these traits exhibit only in females and they are determined in the later stages of lifes. Hence the genetic perspective is more effective way to confirm the fertility capacities early period of life time. Fecundity genes such as *BMP15* regulate the fertility traits by increasing the ovulation rate due to the occuring mutations. Despite some prolific breed were investigated for *FecX*^{B} mutation; the researches carried out to determine that mutation in Chios sheep breed are limited. The objective of the current study was to estimate the the genotype frequencies of *FecX*^{B} mutation of *BMP15* gene in prolific Chios sheep. We investigated the *FecX*^{B} mutation by PCR-RFLP method in Chios sheep (n=77). According to result, the investigated Chios breed was found monomorphic for *FecX*^{B} mutation. All individuals were digested by DdeI restriction enzyme and showed wild-type genotype and did not carry *FecX*^{B} mutation nin present study. In conclusion it's thought that the high prolificacy in Chios sheep may be based on another region of *BMP15* gene or differ-ent major gene. Thus the effect of other major genes or regions/QTL's should be investigated in future studies with a large number of animals.

Key words: Sheep, fertiliy, mutation, PCR-RFLP, *BMP15* gene, Chios.

Introduction

Reproduction is a complicated function of the body and various type of enviromental and genetic factor affect fertility traits (Drouilhet et al., 2009). Ovulation rate and litter size are important fertility traits for animal breeding because of their financial value and they exhibit a wide range ratio both among different breeds and different individuals in the same breed (Notter, 2008; Fabre et all, 2006). However these traits exhibit only in females and are determined in the advanced years of their lifes (Jansson, 2014). So the genetic perspective on fertility traits is more important for sustainable profitability in sheep breeding (Pramod et al., 2013).

Chios are semi-fat tailed sheep which are originated from Chios Island in Aegean (Hatziminaoglou et al., 1990). As well as their high milk production, they have advanced fertility traits (Theodoridis et al., 2012; Ligda et al., 2000). The litter size of Chios and live-born lamb was found 1.69±0.02 and 1.61±0.02 by Mavrogenis et al. respectively (1985). The avarage litter size was indicated as 1.8-2.2 (Hatziminaoglou et al., 1990); so they are preferred by breeders for economic advantages according to fertility traits.

Fertility traits are regulated by some major genes called fecundity genes (Drouilhet et al., 2009). Piper and Bindon (1980) indicated that the existence of a major gene effects on fertiliy in Booroola Merinos. After the first fecundity gene which effect prolificacy had been

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identified in Booroola Merinos (Piper and Bindon, 1980), various major genes including Growth Differentiation Factor 9 (*GDF9*), Bone Morphogenic Protein 15 (*BMP15/GDF9B*) and Bone Morphogenic Protein Receptor type 1B (*BMPR-1B*) were reported respectively (Kasiriyan et al., 2009). The mutations of these major genes induce an increase in ovulation rate and litter size of some sheep breeds (Davis, 2005). The BMP15 gene (FecX or GDF9B), located regulates the bone morphogenetic protein 15 expression which is essential for sheep fertiliy as an follicle enhancer (Pramod et al., 2013). *BMP15* is a protein, blocks FSH receptor expression in the ovaries. In terms of *BMP15* gene, heterozygote individuals exhibit multiple ovulation, thus it increases the ovulation rate (Jansson et al., 2014). Eight mutations (*Inverdale-FecXI, Hanna-FecXH, Belclare-FecXB, Galway- FecXG, Lacaune-FecXL, Rasa Aragone-sa-FecXR, Grivette-FecXGr, Olkuska-FecXO*) have been identified in the BMP15 gene located on X chromosome (Davis, 2005; Demars et al., 2013; Bodin et al., 2007; Monteagudo et al., 2009). Belclare (FecXB) mutation was first described in profilic Belclare ewes in 1991 by Hanrahan et al. The $FecX^B$ mutation is a G to T change at nucleotide 1100 that is concluded the substitution of serin amino acid (amino acid 99-mature peptide residue) to an isoleucine (Hanrahan et al., 2004). It is pointed out that Belclare mutation significantly increased ovulation rate; the ovulation rate of heterozygote individuals for Belclare mutation were 2,38 ± 0,549. On the other hand, homozygote individuals for Belclare mutation were found steril (Hanrahan et al., 2004).

The $FecX^B$ mutation had been investigated in Malin and Dorper (Wan Somarny et all, 2013); in Iranian Arabic (Mohammadi, 2016); in Barki, Ossemi and Rahmani (Barakat et al., 2017); in Indian Nilagiri (Sithi Marjitha et al., 2015); in Xingjiang Cele Black sheep (Shi et al., 2010); in Lleyn, Texel and Finnish Landrace (Mullen et al., 2013); sheep breed yet. Also it was identified in national sheep breed as Akkaraman, Dağlıç, İvesi, Tuj and Karaman by Karslı et al. (2012). Although the presence and the effects on ovulation rate and litter size of $FecX^B$ mutation were established in Belclare sheep; it was indicated that none of the investigated breed (were mentioned) carried that mutation. Although *FecB, FecXG, FecXI* and *CAST* gene mutation were investigated in Chios sheep (Dinçel et al., 2015); the researches carried out to determine the $FecX^B$ mutation in this prolific breed are limited (Esen Gürsel et al., 2011). The aim of the study was to evalute the genotype frequencies of $FecX^B$ mutation of *BMP15* gene in prolific Chios sheep in order to understand the source of high fertility traits.

Materials and Methods

Animal source and DNA extraction

A total of 77 Chios sheep were investigated in the current study. The ewes from five independent herds were bred according to the Indigenous Genetic Resources Project. For molecular analysis wool samples were collected and cleaned up with ethanol. The genomic DNA was extracted with CTAB (hexa-decyltrimethylammonium bromide) method by Doyle et al. (1987). Clarifications of DNA, consisting the purity and amount, were determined by spectrophotometer (Thermo/NanoDrop-2000C). All samples were kept at -80°C until PCR analysis were performed.

PCR conditions

PCR-RFLP method was used for detection of $FecX^B$ mutation (G to T nucleotide change) in Chios sheep. Primers for target loci of BMP15 gene were designed according to Hanrahan et al (2004). The primers were synthesized as follows: F: 5' GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA and R: 5' TTCTTGGGAAACCTGAGCTAGC (Hanrahan et al., 2004). A total volume of 50 µl PCR reaction mixture contains 1.5 mM MgSO4, 5 µl 10X Buffer, 200 µM dNTP, 10 pM of each primers (forward and reverse) and 1.25 U of Taq DNA polymerase and 100 ng/µl template DNA. The amplification carried out the following protocol: 95°C for 5 min for initial denaturation, followed by 35 cycles of 94°C for 30 s (denaturation), 64°C 40 s (anneling), 72°C for 30 s (extension) and a final extension at 72°C for 5 min (Hanrahan et al., 2004). The 153-bp PCR products of $FecX^B$ were analysed by 3% agarose gel electrophoresis and visualized with ethidium bromide technique by DNr Minilumi.

RFLP (Restriction fragment length polymorphism)

For detection of $FecX^B$ allele, 153-bp PCR products were digested with *DdeI* restriction enzyme as described by Hanrahan et al. (2004). Mutant-type individuals for $FecX^B$ show 153-bp undigested fragment although the wild-type individuals exhibit two fragments as 122-bp and 31-bp (Wan Somarny et al, 2013). Therefore the PCR products (10 µl) were digested with *DdeI* at 20 µl final reaction volume containing 1X NE Buffer 3 and 5 U restriction endonuclease. The RFLP products were seperated by electrophoresis on a 3% agarose gel and monitorized by DNr Minilumi imaging system.

Results

In this study, Chios sheeps were screened for *FecX*^{B} mutation by PCR-RFLP method. The 153-bp DNA fragments were amplified by PCR. The obtained PCR products were digested by DdeI restriction enzyme according to RFLP procedure. Present results revealed that all samples pro-duced single strand with 153-bp weight PCR products (Figure 1). After the amplification, samples were treated with *DdeI* enzyme and as a consequence one genotype was detected with a double band (122 and 31 bp) (Figure 2).

Discussion and conclusions

Wan Soarny et al. (2013) indicated that Malin and Dopper sheep were homozygous non-carrier for *FecX*^{B} mutation as well as it was found in Iranian Arabic sheep breed by Mohammadi et al. (Wan Somarny et al., 2013; Mohammadi et al., 2016). Barakat et al., pointed out that Barki, Ossemi and Rahmani, Egyptian sheep breeds, showed no polymorphism for *FecX*^{B} of *BMP15* gene, similar to data reported by Sithi Marjitha et al. in 2015 (Barakat et al., 2017; Sithi Marjitha et al., 2015). Sithi Marjitha et al. emphasised the absence of *FecX*^{B} mutation in Nilagiri sheep (Sithi Mar-jitha et al., 2015). These findings are in agreement with those reported by Shi et al. (2010) which claimed that no poly-

Figure 1. The PCR product of *FecXB* mutation of *BMP15* gene (3% agarose gel stained with ethidium bromide) (M: Marker; Line 1-7: The 153 bp PCR/amplification product of Chios sheep; Line 8: Negative control).

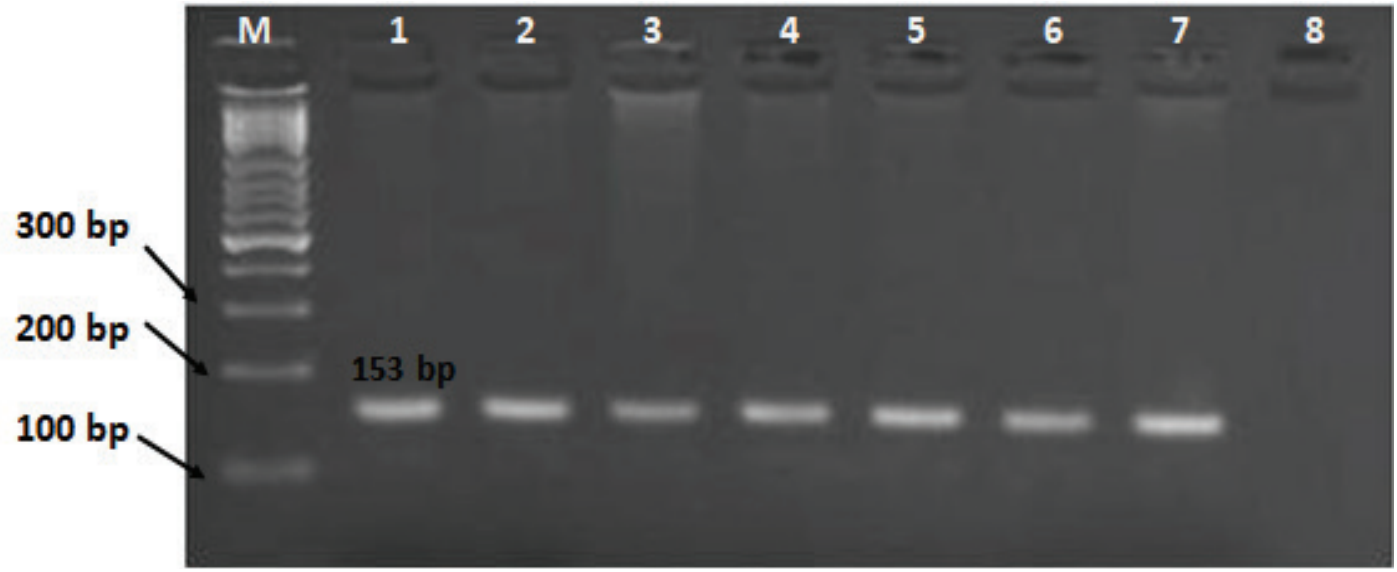
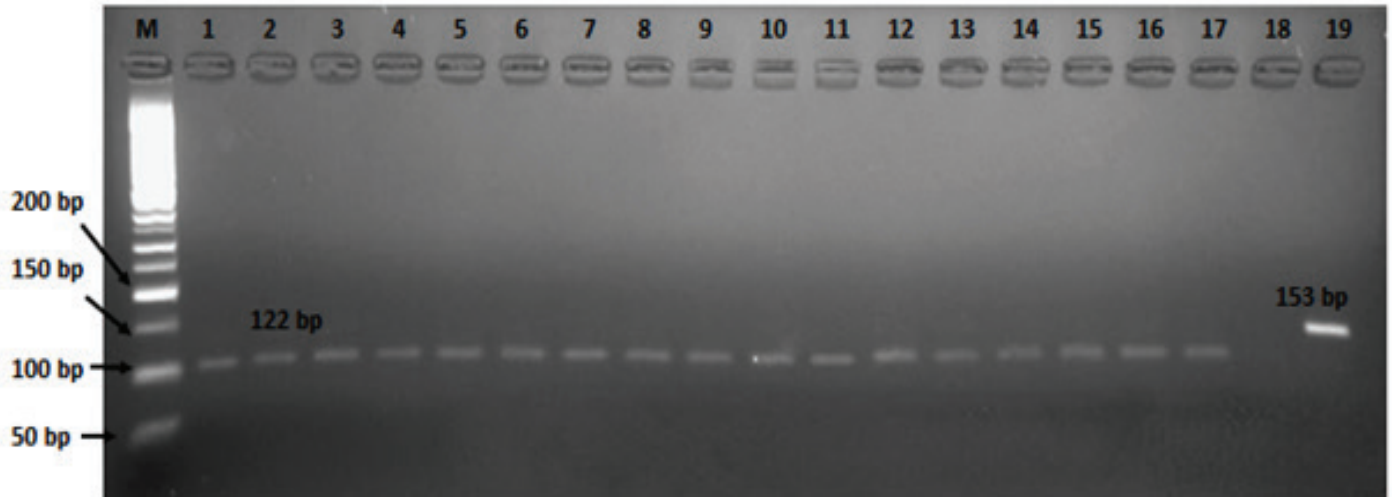


Figure 2. Digested fragments of PCR product by *DdeI* restriction enzyme for *FecX*^{B} mutation of *BMP15* gene (3% agarose gel stained with ethidium bromide) (M: Marker; Line 1-17: The 122 bp RFLP product of Chios sheep; Line 18: Negative control; Line 19: The 153 bp PCR product)



morphism was found in *BMP15* gene (*FecX*^{B}) in Xingjiang Cele black sheep (Shi et al., 2010). Another study which was performed by Karslı et al. (2012) indicated that Akkaraman, Daglıc, Ivesi (Awassi), Tuj and Karakas sheep were found to be monomorfic for *FecX*^{B}. Our results showed that, as demostrated in previous studies performed in different breed, all individuals were found monomor-phic and had wild-type genotype for *FecX*^{B} mutation. In terms of same breed, data obtained from the current study are in close agreement with Esen Gürsel et al. (2011) which were investigated the *BMP15* gene mutaion in Awassi, Chios, Imrose and Kivircik sheep breed. Contrary to results obtained for Belclare breed (Hanrahan et al., 2004), the investigated herd does not carry this mutation. These observations conflict with work of Mullen et al., which reported that hyper-prolific ewes from Irish flocks exhibited *FecX*^{B} mutation (Mullen et al., 2013). As a consequence, it was found that the investigated breed (Chios sheep) did not carry *FecX*^{B} mutation in present study. On possible explanation of the inability to detect the mutation inthis study may be the limited the sample size of herd. Thus further studies should be done with a large number of animals. On the other hand, it was thought that the high prolificacy in Chios sheep may be based on another region of *BMP15* gene or different major gene. Hence the effect of other major genes or regions/QTL's should be investigated in future studies.

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