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An investigation of mutations ($FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$) on BMP-15 gene in some local sheep breeds raised in Turkey

Türkiye'de yetiştirilen bazı yerel koyun ırklarında BMP-15 genindeki ($FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$) mutasyonların araştırılması

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ABSTRACT

Genetic mutations on major genes increase ovulation rate and litter size in sheep. Three major genes have been identified belonging to the TGF-β superfamily until now. These genes are *BMPR-IB* (Bone morphogenetic protein receptor IB), *BMP-15* (Bone morphogenetic protein-15) and *GDF9* (Growth differentiation factor 9). Different mutations ($FecX^B$, $FecX^G$, $FecX^I$, $FecX^H$, $FecX^L$ and $FecX^R$) on Bone Morphogenetic Protein-15 gene either increase ovulation rate or cause infertility in sheeps. The purpose of this study was to investigate whether the four different mutations ($FecX^G$, $FecX^I$, $FecX^H$ and $FecX^B$) exist on BMP15 gene in five local sheep breeds reared in Turkey. PCR-RFLP method was used to determine these mutations. A total of 96 blood samples were investigated from Akkaraman (24 samples), Dağlıç (19 samples), İvesi (19 samples), Tuj (15 samples) and Karakaş (19 samples) sheep breeds. To determine $FecX^G$, $FecX^I$, $FecX^H$ and $FecX^B$ mutations, DNA fragments with 141, 154, 240 and 153 bp sizes were PCR amplified by using spesific primers, respectively. PCR products were digested to detect $FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$ point mutation by using *Hin*fI, *Xba*I, *Ahl*I, *Bst*DEI restriction enzymes, respectively. As a result, $FecX^I$, $FecX^H$, $FecX^G$ and $FecX^B$ loci could not be determined. Random selected 96 sheeps from five flocks were monomorph in terms of $FecX^I$, $FecX^H$, $FecX^G$ and $FecX^B$ loci.

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ÖZ

Koyunlarda majör genler üzerindeki genetik mutasyonlar ovulasyon oranını ve bir batında doğan yavru sayısını artırmaktadır. Şu ana kadar TGF-β süper familyasına ait üç majör gen tanımlanmıştır. Bu genler *BMPR-IB* (Bone morphogenetic protein receptor IB), *BMP-15* (Bone morphogenetic protein-15) ve *GDF9* (Growth differentiation factor 9) dur. Koyunlarda Bone Morphogenetic Protein-15 geni üzerindeki farklı mutasyonlar ($FecX^B$, $FecX^G$, $FecX^I$, $FecX^H$, $FecX^L$ ve $FecX^R$) ovulasyon oranını artırmakta ya da kısırlığa neden olmaktadır. Bu çalışmanın amacı Türkiye'de yetiştirilen beş yerel koyun ırkında BMP-15 geninde dört farklı mutasyonun ($FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$) olup olmadığının araştırılmasıdır. Bu mutasyonları belirlemek için PCR-RFLP yöntemi kullanılmıştır. Akkaraman (24 örnek), Dağlıç (19 örnek), İvesi (19 örnek), Tuj (15 örnek) ve Karakaş (19 örnek) koyun ırklarından toplam 96 kan örneği incelenmiştir. $FecX^G$, $FecX^I$, $FecX^H$ ve $FecX^B$ mutasyonlarının belirlenmesi için sırasıyla 141, 154, 240 ve 153 bç büyüklüğündeki DNA fragmentleri spesifik primerler kullanılarak PCR ile çoğaltılmıştır. PCR ürünleri, $FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$ nokta mutasyonlarını belirlemek için sırasıyla *Hin*fI, *Xba*I, *Ahl*I, *Bst*DEI restriksiyon enzimleri kullanılarak kesilmiştir. Sonuç olarak incelenen örneklerde $FecX^I$, $FecX^H$, $FecX^G$ ve $FecX^B$ lokusları tespit edilememiştir. Beş sürüden rasgele seçilen 96 koyun $FecX^I$, $FecX^H$, $FecX^G$ ve $FecX^B$ lokusları bakımından monomorf bulunmuştur.

1. Introduction

Litter size is important economic value in sheep breeding. Productive traits in domestic livestock are generally inherited in a multigenic or quantitative manner. Therefore, responses to selection for reproduction may be slow. Increasing prolificacy in sheep via selection within the available breeds is very slow process. Some major genes are responsible for high prolificacy in sheeps. The use of major genes for reproduction increases response to selection. Therefore the identification and use of

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major genes is required to increase the rate of genetic improvement (Davis et al. 1991).

Major genes are occurred as a result of a mutation in sheeps. Primary mutations which increase ovulation rate in sheep located on Bone Morphogenetic Protein Receptor IB (BMPR-IB), Bone Morphogenetic Protein 15 (BMP-15) and Growth Differentiation Factor 9 (GDF-9) genes. The Booroola gene (FecB) was the first major gene for prolificacy identified in sheep (Davis 2005). One copy of the Booroola gene increases ovulation rate (eggs shed per ewe ovulating) by about 1.5 and two copies by about 3.0. These extra ovulations typically increase litter size (lambs born per ewe lambing) by about 1.0 and 1.5, respectively (Davis et al. 2006).

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) superfamily. They are multifunctional proteins that regulate growth and differentiation in many cell types (Wilson et al. 2001). BMP-15 gene has been mapped for sheep chromosome-X and contains six different mutations. These mutations were called as $FecX^{I}$ (Inverdale), $FecX^{H}$ (Hanna), $FecX^{G}$ (Galway) and $FecX^{B}$ (Belclare), $FecX^{R}$ (Rasa Aragonesa) and $FecX^{L}$ (Lacaune) (Galloway et al. 2000; Hanrahan et al. 2004; Monteagudo et al. 2009). All these mutations show same phenotype; heterozygous animals have higher ovulation rates (+1-1.5) than their wild-type contemporaries. Homozygous carrier ewes are infertile due to arrested follicular development from the primary stage of growth (McNatty et al. 2005; Galloway et al. 2002).

In BMP-15 gene, mutations that are caused amino acid changes summarized in Table 1. $FecX^{R}$ mutation is consisting of deletion 17 base pairs that resulting premature stop codon in Rasa Aragonesa sheeps. The other five loci are consisting of a point mutation. As the molecular basis of these mutations on BMP-15 gene single nucleotide change, one of the most commonly DNA marker method is PCR-RFLP which is used identification of similar point mutation in livestock (Davis et al. 2006; Chu et al. 2007; Ghaffari et al. 2009; Tajangookeh et al. 2009).

Akkaraman, Dağlıç, İvesi, Tuj and Karakaş are raised fat-tailed sheep breeds in Turkey. These sheep breeds are extremely resistant to harsh environmental conditions, but usually give birth to only one or two lamb. The aim of this study is to investigate the presence of the $FecX^{G}$, $FecX^{I}$, $FecX^{H}$ and $FecX^{B}$ mutations in Akkaraman, Dağlıç, İvesi, Tuj and Karakaş sheep breeds.

2. Material and Method

2.1 Material

The blood samples were randomly collected from 96 sheeps belonging to five local Turkish sheep breeds reared different regions of Turkey (Table 2). Blood samples were taken (approximately 10 ml) from the jugular vein, using sample tubes containing EDTA.

2.2. Methods

2.2.1. DNA extraction

The blood samples were kept at -20^{0}C until the isolation of total DNA. Genomic DNA from blood samples was made using method by Miller et al. (1988). Agarose gels and spectrophotometer (NanoDrop-SD 1000) were used to determine DNA quality and quantity. DNA concentration was adjusted to approximately 50 ng/µl before DNA amplification.

2.2.2. PCR conditions

The primer sequences and annealing temperatures were given in Table 3. DNA was amplified initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 sec, annealing (temperatures for each primer pair are shown in Table 3) 40 sec, extension at 72°C for 30 sec, with final extension 72°C for 5 min on Mastercycler 5333 (Eppendorf AG, Germany). The PCR reaction was performed in 10X PCR buffer (containing $(NH_4)_2SO_4$, pH:8.8), 25mM $MgCl_2$, 2,5 mM dNTPs mix, 1 U Taq DNA Polymerase (BIORON), 0,2µM of each primer and 50 ng/µl of template DNA in 50 µl reaction volume. PCR products resolved by electrophoresis on 1.5% agarose gel.

2.2.3. Detection of the FecXH mutation

The 240 base pair (bp) fragments were obtained with PCR procedure. The 240 bp PCR product was digested with AhlI (A/CTAGT) restriction enzyme 3 h at 37°C (10 U AhlI, 2 µl enzyme buffer and 10 µl PCR product) and the products were separated by electrophoresis on a 2% agarose gel and visualised with ethidium bromide. Containing the FecXH mutation products 218 bp and 22 bp fragments, while non-carrier products remained uncut at 240 bp (Hua et al. 2008).

Table 1. Polymorphic sequence variations in BMP-15 gene that affect prolificacy in sheep.

Allele symbol	Base change	Coding residue (aa)	Mature peptide residue (aa)	Amino acid change
$FecX^{I}$	T-A	299	31	Val-Asp
$FecX^{H}$	C-T	291	23	Glu-Stop
$FecX^{G}$	C-T	239	-	Gln-Stop
$FecX^{B}$	G-T	367	99	Ser-Ile
$FecX^{L}$	G-A	321	53	Cys-Tyr
$FecX^{R}$	17bp deletion	-	-	-

Sources: Galloway et al. 2000; Hanrahan et al. 2004; McNatty et al. 2005; Monteagudo et al. 2009.

Table 2. Sample numbers of breeds and obtained places.

Breed	No. samples	Places
Akkaraman	24	Konya Bahri Dağdaş International Anim. Res. Inst.
Dağlıç	19	Seydişehir, Ketenli Town
İvesi	19	Atatürk University, Faculty of Agriculture
Tuj	15	Atatürk University, Faculty of Agriculture
Karakaş	19	Yüzüncü Yıl University, Faculty of Agriculture

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Table 3. Primer sequences and annealing temperatures.

Gene	Mutation	Primer	Primer Sequence(5'- 3')	Annealing temp. (°C)	Reference
BMP15	$FecX^H$	FH	TATTTCAATGACACTCAGAG	55	Hua et al. 2008
		RH	GAGCAATGATCCAGTGATCCCA		
	$FecX^I$	FI	GAAGTAACCAGTGTTCCCTCCACCCTTTTCT	55	Davis et al. 2006
		RI	CATGATTGGGAGAATTGAGACC		
	$FecX^G$	FG	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC	63	Hanrahan et al. 2004
		RG	GATGCAATACTGCCTGCTTG		
	$FecX^B$	FB	GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA	62	Hanrahan et al. 2004
		RB	TTCTTGGGAAACCTGAGCTAGC		

2.2.4. Detection of the FecXI mutation

After PCR procedure with FI and RI primers, 154 bp bands obtained. The 154 bp PCR product was digested with XbaI (T/CTAGA) restriction enzyme 3 h at 37ºC (10 U XbaI, 2 µl enzyme buffer and 10 µl PCR product) and the products were separated by electrophoresis on a 2% agarose gel and visualised with ethidium bromide. After digested with enzyme, products containing the FecXI mutation yield 124 bp and 30 bp fragments, whilst non-carrier products remain uncut at 154 bp (Davis et al. 2006).

2.2.5 Detection of the FecXG mutation

Based on the methods Hanrahan et al. (2004), the 141 bp fragments were obtained by using FG and RG primers with PCR procedure. The 141 bp PCR product was digested with HinfI (G/ACT) restriction enzyme 3 h at 37ºC (10 U HinfI, 2 µl enzyme buffer and 10 µl PCR product) and the products were separated by electrophoresis on a 3.5% agarose gel and visualised with ethidium bromide. The wild type products could be cleaved by HinfI (G/ACT) with a 112 bp and 29 bp fragments; the mutation type remained uncleaved.

2.2.6. Detection of the FecXB mutation

The FecXB mutation was detected using the improved by Hanrahan et al. (2004). The 153 bp PCR product was digested with BstDEI (C/TNAG) restriction enzyme 3 h at 60ºC (10 U BstDEI, 2 µl enzyme buffer and 10 µl PCR product) and the products were separated by electrophoresis on a 3.5% agarose gel and visualised with ethidium bromide. While wild type individuals are digested with 122 bp and 31 bp fragments, the mutation type individuals remain uncut at 153 bp.

3. Results and Discussion

DNA fragments of 141,154, 240 and 153 bp were amplified by PCR for $FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$ point mutations in BMP-15 gene. Subsequently PCR products were digested to determine $FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$ point mutation by using *Hin*fI, *Xba*I, *Ahl*I, *Bst*DEI restriction enzymes, respectively. Consequently, none of the samples carried the $FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$ mutations on BMP15 gene. Agarose gel photographs are shown in Figure 1, Figure 2, Figure 3 and Figure 4. All sheeps were monomorphic for $FecX^G$, $FecX^I$, $FecX^H$, $FecX^B$ point mutations.

Recently, studies related to major genes have have intensified. Many sheep (Davis et al. 2002; Guan et al. 2007; Jamshidi et al. 2009; Kasariyan et al. 2009) and goat (Hua et al. 2008; Polley et al. 2009; Bargazegari et al. 2010) breeds in the world the presence of these genes have been investigated at the molecular level. While studying previously in the only prolific breeds for these genes (Davis et al. 2006; Kumar et al. 2006; Chu et al. 2007), non-prolific breeds have been investigated in

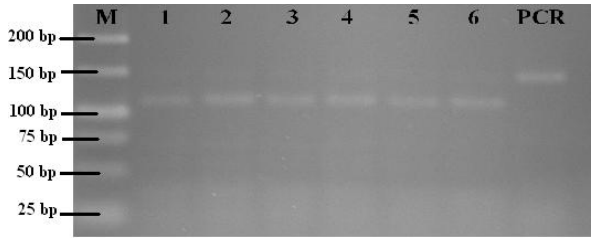

Figure 1. Digestion of PCR products with HinfI restriction enzyme for FecXG mutation (Image of agarose gel 3.5%). Lane 1-6: PCR products with digestion mix., PCR: only PCR product M: DNA Marker (Fermentas-Cat.No: SM1191).

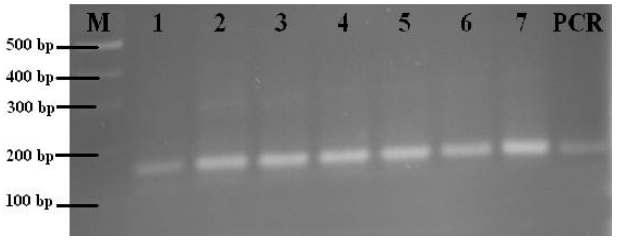

Figure 2. Digestion of PCR products with XbaI restriction enzyme for FecXI mutation (Image of agarose gel 2%). Lane 1-7: PCR products with digestion mix., PCR: only PCR product M: DNA Marker (Bioron-Cat.No: 306005).

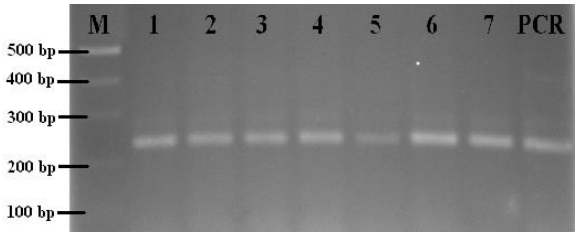

Figure 3. Digestion of PCR products with AhlI restriction enzymes for FecXH mutation (Image of agarose gel 2%). Lane 1-7: PCR products with digestion mix., PCR: only PCR product M: DNA Marker (Bioron-Cat.No: 306005).

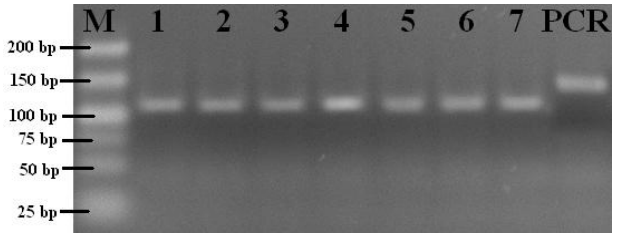

Figure 4. Digestion of PCR products with BstDEI restriction enzyme for FecXB mutation (Image of agarose gel 3.5%). Lane 1-7: PCR products with digestion mix., PCR: only PCR product M: DNA Marker (Fermentas-Cat.No: SM1191).

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recent years (Jamshidi et al. 2009; Kasariyan et al. 2009; Karslı and Balcıoğlu 2010). Kumar et al. (2006) reported that although some individuals single lambing in Garole breed, were found to be carriers FecB. The reason might be death of embryos, non-expression of FecB gene, nutritional factors leading to hormonal imbalances and other unknown environmental factors. Because of worst environmental conditions and the general based on pasture sheep breeding in Turkey, even if local sheeps have low prolificacy should be examined in terms of major genes.

Unlike the Booroola (FecB) mutation, heterozygous in ewes for *BMP-15* mutations increases ovulation rate while homozygous ewes are sterile due to small-undeveloped 'streak' ovaries (Galloway et al. 2000; Hanrahan et al. 2004). These genes should be examined in terms of both cause prolificacy and infertility. Reared in local sheep breeds in Turkey, few studies have done to identify the major genes. Booroola gene was investigated for Sakız (Polat 2006), Akkaraman, Morkaraman, Dağlıç, İvesi, Karakaş and Tuj (Karslı and Balcıoğlu 2010). But Booroola gene couldn't be determined in none of the studies. All studies in literature related to BMP-15 gene were not found in Turkey.

The results of this study are similar with reports $FecX^{I}$ loci in Romanov, Finn, East Friesian, Teeswater, Blueface Leicester, Hu, Han, D'Man, Chios, Mountain sheep, German Whiteheaded Mutton, Lleyn, Loa, Galician, Barbados Blackbelly breeds (Davis et al. 2006), and $FecX^{G}$ loci in Han sheeps (Chu et al. 2007), and $FecX^{I}$ loci in Chios and Florina breeds (Michailidis et al. 2008), and $FecX^{G}$ allele in Sangsari breed (Kasariyan et al. 2009), and $FecX^{I}$ loci in Sangsari breed (Jamshidi et al. 2009), and $FecX^{G}$, $FecX^{I}$, $FecX^{B}$, $FecX^{H}$ loci in D'Man, Queue Fine de L'Ouest, Sicilo-Sarde breeds (Vacca et al. 2010).

Some researchers reported that two samples in St. Croix and Barbados Blacckbelly, 21 samples in Finn (Davis et al. 2006), 25 samples in Sakız and Florina (Michailidis et al. 2008), 150 samples in Sangsari (Jamshidi et al. 2009), and 406 samples in Sakız sheep breeds (Polat 2006), were used. When similar studies examined, major differences were found in respect to number of samples. Increasing the number of samples increases the probability of detection of this gene. But, in such studies pure individuals should be used that representing characteristics of breed.

4. Conclusions

Recently improvements in molecular genetics enables major genes to be used in genetic improvement programmes based on marker assisted selection. PCR based diagnostic techniques are fast and reliable for determining of different point mutations. In order to take advantage of major genes, these genes should be determined. Major genes can be transferred to non-carrier breeds via cross breeding. Thus reproduction performance can be increased. There are no many researches related to major genes in Turkey. Number of samples should be increased to determine major genes. All sheep breeds reared in Turkey should be investigated in terms of major genes, especially BMP-15 ($FecX^{L}$, $FecX^{R}$) and GDF9 major genes. In case of detection of major genes, these genes can be used for increasing of reproduction performance. But, in case it is not detected, local sheep breeds can be cross breeding with carrying major genes breeds.

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