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DETERMINATION OF *FECX, FECB* AND *FECG^H* MUTATIONS IN IRANIAN *ARABIC* SHEEP

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ABSTRACT

Genetic markers are one of the best methods to evaluate animal genetics potential. *FecX, FecB* and *FecG* genes have belonged to *TGFβ* superfamily. These genes have additive effect on litter size and ovulation rate in sheep. The aim of the present study was determination of *FecX, FecB* and *FecG^H* mutations in Iranian *Arabic* sheep. In this study 100 blood samples were collected of prolific sheep of *Arabic* breeds. DNA of blood samples was extracted by modified salting out method. Site of mutation was amplified using specific primers and PCR products were digested with special restricted enzymes for *FecX, FecB* and *FecG^H* mutations. Results have showed no mutations of *FecX, FecB* and *FecG^H* mutation were detected in Iranian *Arabic* sheep breed. Therefore, *FecX, FecB* and *FecG^H* mutations did not cause of prolificacy in this breed and further research is required to evaluate the relationship of the fecundity genes with litter size and ovulation rate in this breed.

INTRODUCTION

In recent years, many studies focused on the genetics of prolificacy in sheep (Ovis aries). three major genes: *BMP15, BMPR1B* and *FecG*, which have been shown to have enhancing effects on ovulation rate and litter size (1). All these three Fecundity genes belong to the transforming growth factor β (*TGFβ*) gene superfamily (2).

Bone morphogenetic protein 15 (BMP-15) is a growth factor that is specifically expressed in oocytes. The sheep *BMP-15* (*FecX*) gene maps to the X chromosome (3). FecX regulates granulosa cell proliferation and differentiation by promoting granulose

cell mitosis, suppressing follicle-stimulating hormone receptor expression, and stimulating kit ligand expression, all of these affects play a pivotal role in female fertility (4). The FecX gene have $FecX^{I}$, $FecX^{H}$, $FecX^{B}$ and $FecX^{G}$ mutants (3, 5, 6). The homozygote for the four mutations in ewes is infertile whereas the heterozygous individuals have a greater ovulation rate size (7, 6).

The sheep *GDF9* (Growth Differentiation Factor 9) gene maps to chromosome 5 and contains 2 exons (8). Eight single nucleotide polymorphisms (SNPs) have been identified so far in sheep *GDF9*, labeled G1–G8. The G8 mutation, also indicated as $FecG^{H}$ (High Fertility), causes increased ovulation rate in heterozygous ewes, while homozygous ewes are sterile (6, 9).

The fecB gene which called bone morphogenetic protein receptor 1B (BMPR1B) Located on ovine chromosome 6 corresponding to the human chromosome 4q22-23 (10). The A to G transition at nucleotide position 746 of the cDNA sequence induces a nonsynonymous substitution of glutamine with an arginine corresponding to position 249 of the mature protein (11, 12). Based on the segregation of the ovulation rate in Merino and Romney flocks, the genotypes in the ewes have been classified as homozygous noncarrier (*FecB+/FecB+*) with ovulation rate of 2 or less, heterozygous carriers (*FecBB/FecB+*) with ovulation rate of 3–4 and homozygous carriers (*FecBB/FecBB*) with more than five ovulations per estrous cycle (13). This increased ovulation rate of *FecBB* carriers is associated with a precocious maturation of a large number of antral follicles that ovulate at a smaller size than non-carrier follicles (14).

Arabic sheep are one the most prolific sheep breed in the Khuzestan province in south western of Iran. This sheep breed are the most important sources of meat in the area. average live weight of adult ranges between 45 to 55 kg. the mean litter size for this breed is 1.5. Characterization of fecundity genes in this breed can help improvement breeding program Therefore, the aim of the present study was identification of *FecX,* *FecB* and $FecG^{H}$ mutations in this sheep breed.

MATERIAL AND METHODS

Samples and breeds

The analysis was carried out on a samples of 100 prolific sheep of *Arabic* breeds. Blood samples were collected into a 5 ml EDTA contained vacutainer tube and transferred to laboratory for DNA extraction within 2 hours. Total DNA extractions were made with a modified salting out method and isopropanol precipitation (15) from whole fresh blood. DNA samples were quantified using Biophotometer (Eppendorf) and stored in −20°C in aliquots.

The amplification of the loci was carried out in a total volume of 25 µl reaction (16). The reaction contained the following constituents: 100 ng of DNA used as a template, 1X PCR Buffer, 0.2 mM of each dNTP, 0.5 µM of each primer, 1 unit of Taq Polymerase and 1.5 mM of MgCl2. PCR amplifications were performed in a Gradient Thermocycler (Bioer Xp cycler) by an initial denaturation of 5 min at 95°C, followed by 35 cycles of 30 seconds of denaturation at 94°C, 30 seconds of annealing based on table 1, 30 seconds of extension at 72°C and a final extension of 10 min at 72°C. The amplified products were electrophoresed in 2% agarose gele and the DNA bands were visualized by ethidium bromide staining technique.

All primers were synthesized by TAG Copenhagen Co., Ltd (Denmark). The restriction endonucleases (RE) and other reagents were purchased from Fermentas Co, Ltd. The primers sequence, annealing temperature and RE are described in Table 1.

Table 1, Primer sequences, Annealing temperature and Restriction enzyme used for

Mutation	Restriction enzyme	Restriction site	wild-type fragment size bp	Mutant fragment size bp	Refference
FecX^{B}	*Dde*I	C/TTAG	Cleaved (122 and 31)	Uncleaved 153	Hanrahan et al. 2004
FecX^{G}	*Hin*fI	G/ACT	Cleaved (112 and 29)	Uncleaved 141	Hanrahan et al. 2004
FecX^{H}	*Spe*I	A/CTAGT	Uncleaved 240	Cleaved (218 and 22)	Galloway et al., 2000

PCR-RFLP

Restriction enzyme	Annealing Temperature (˚C)	Primer Sequence (5′→3′)	Gene
*Spe*I	55	TATTTCAATGACACTCAGAG GAGCAATGATCCAGTGATCCCA	*FecX*^{H}
*Xba*I	55	GAAGTAACCAGTGTTCCCTCCACCCTTTTCT CATGATTGGGAGAATTGAGACC	*FecX*^{I}
*Hin*fI	63	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC GATGCAATACTGCCTGCTTG	*FecX*^{G}
*Dde*I	64	GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA TTCTTGGGAAACCTGAGCTAGC	*FecX*^{B}
AvaII	60	CCAGAGGACAATAGCAAAGCAAA CAAGATGTTTTCATGCCTCATCAACAGGTC	*FecB*
DdeI	62	CTTTAGTCAGCTGAAGTGGGACAAC ATGGATGATGTTCTGCACCATGGTGTGAACCTGA	*FecG*^{H}

According to (6), (17) and (3), forced PCR-RFLP was used to detect the mutations of *FecX (FecX*^{B}*, FecX*^{G}*, FecX*^{H}*, FecX*^{I}*), FecB* and *FecG*^{H}. The mutations, restriction enzymes, restriction sites, wild type and mutant fragments size are shown in Table 2.

FecX^{I}	*Xba*I	T/CTAGA	Uncleaved 154	Cleaved (124 and 30)	Galloway et al. 2000
FecB	A*va*II	G/GACC	Uncleaved 190	Cleaved (160 and 30)	Davis et al. 2002
FecG^{H}	D*de*I	C/TTAG	Cleaved	Uncleaved	Hanrahan et al. 2004

Table 2, The mutations, restriction enzymes, restriction sites, wild type and mutant fragments size

RESULTS

The electrophoresis Results of forced PCR-RFLP are showed in Figs. 1, 2, 3, 4, 5 and 6, respectively. The mutations of *FecX, FecB* and *FecG*^{H} gene were tested in samples from the arabic sheep breeds. All of the 100 individuals were wild homozygote for *FecX, FecB* and *FecG*^{H}. None of the samples carried the mutation in *FecX, FecB* and *FecG*^{H} genes.

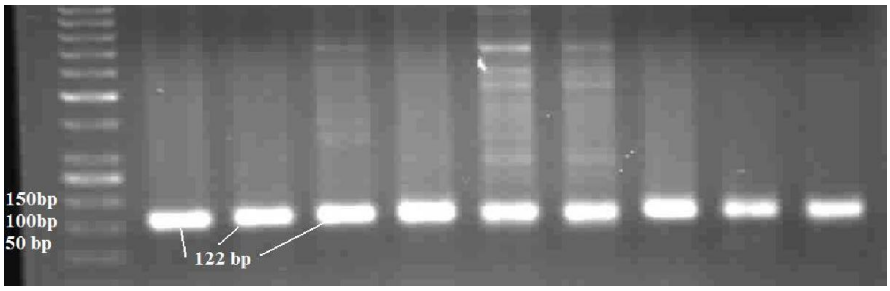

Fig. 1. Image of PCR product of the *FecX*^{B} mutation of the *BMP-15* gene (153 bp) digested with D*de*I. M= 50 bp DNA marker. The wild-type allele is 122 bp.

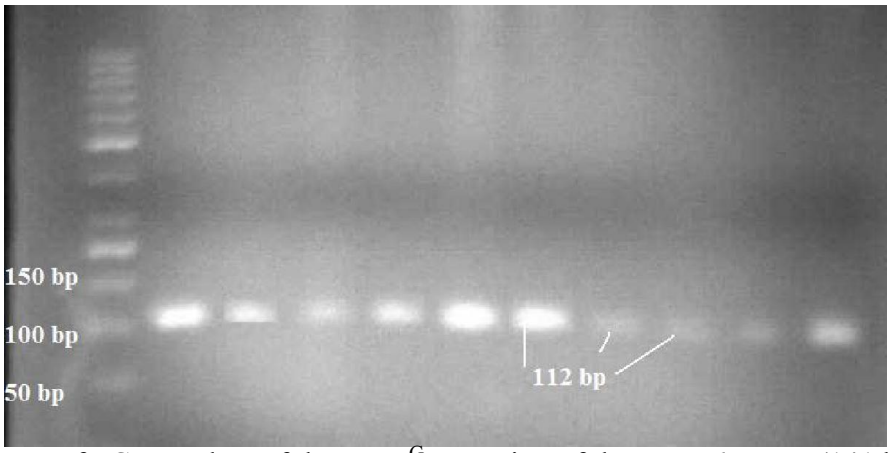

Fig. 2. Image of PCR product of the FecX^{G} mutation of the *BMP-15* gene (141 bp) digested with H*inf*I. M= 50 bp DNA marker. The wild-type allele is 112 bp but the mutation type remains uncleaved.

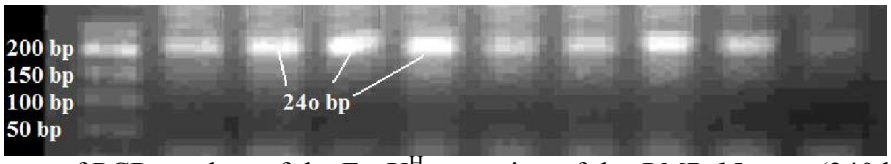

Fig. 3. Image of PCR product of the $FecX^{H}$ mutation of the *BMP-15* gene (240 bp) digested with S*pe*I. M= 50 bp DNA marker. The wild-type allele is 240 bp.

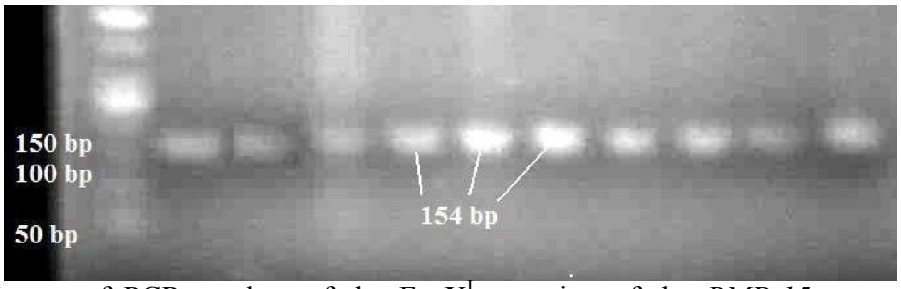

Fig. 4. Image of PCR product of the $FecX^{I}$ mutation of the *BMP-15* gene (154 bp) digested by X*ba*I. M= 50 bp DNA marker. The wild-type allele is 154 bp but the mutation type remained cleaved by X*ba*I.

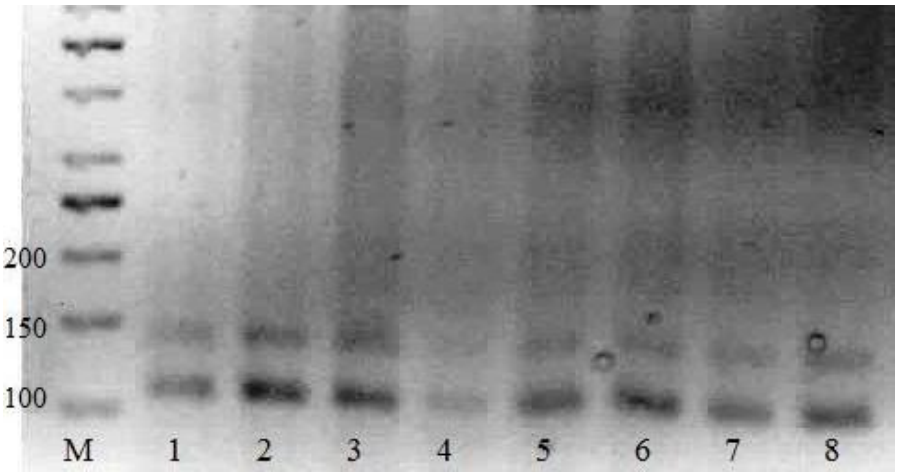

Fig .5 .Agarose gele electrophoretogram for *GDF 9*mutation loci product digested by *Dde*I showing genotypes. M: 50 bp DNA Marker. Lanes 1-8 represent different digestion products of samples from Arabic sheep.

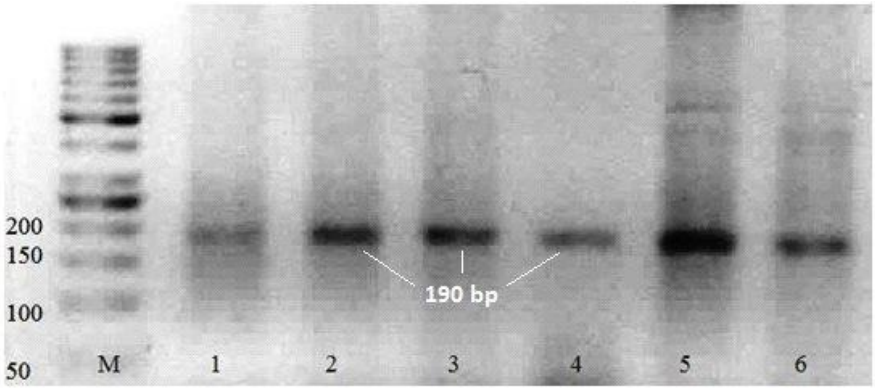

Fig .6 .Agarose gel electrophoresis(2%) of allele specific BMPRIB PCR products digested by A*va*II showing genotypes. M: 50 bp DNA Marker ،Lanes 1-6 represent different digestion products of samples from Arabic sheep breed.

DISCUSSIONS

Sheep is a small and litter-bearing animal, and ideally suited as a model organism for studying on fecundity genes. The *FecX, FecB* and *FecG* genes have been found to be closely associated with prolificacy in sheep (18, 6, 3, 19).

BMP15 is located in the X chromosome. Four mutations in this gene affecting prolificacy have been described. They originate either nonconservative aminoacidic substitutions ($FecX^B$ and $FecX^I$) or premature stop codons ($FecX^G$ and $FecX^H$) having a dosage-dependent effect, While ovulation rates are highly increased in the heterozygotes, the homozygotes show a primary ovarian failure resulting in complete sterility (3, 6, 20).

It has been reported that the litter size and ovulation rate in sheep increase with number of mutations in *FecB* gene. Ewes inheriting one copy of the Booroola gene from either of parents produced about 1.5 extra eggs and gave birth to about 1.0 extra lamb per lambing. Homozygous carriers produced about 3.0 extra eggs resulting in about 1.5 extra lambs per lambing (2). This increase in ovulation rate of *FecBB* carriers is associated with a precocious maturation of a large number of antral follicles that ovulate at a smaller size than non-carrier follicles (14).

The $FecG^H$ (G8) mutation in *GDF9* gene causes an amino acid substitution that has been reported to be associated with increased prolificacy in Belclare and Cambridge sheep (6).

Ovulation rates in *GDF9* and *BMP15* mutants are high in the heterozygotes. The homozygotes, however, have small, flattened streak ovaries with follicles that do not develop up to the primary stage (6, 9, 20) resulting in complete sterility in these animals (6, 20, 19).

The *FecB* mutations have been reported in some of the world's most prolific sheep breeds viz. Australian Booroola Merino (11), Indian Garole, Indonesian Javanese (17), Small-tailed Han and Hu sheep of China (5).

In the present study, we utilized *FecX (FecX^{B}, FecX^{G}, FecX^{H}, FecX^{I}), FecB* and *FecG^{H}* as candidates, but did not find any polymorphism of genes in Arabic sheep.

The studies showed that maybe *FecX, FecB* and *FecG^{H}* were not the only reason responsible for the high prolificacy of sheep (5, 21).

Several investigations show that the *FecBB* allele is absent in low prolific sheep breeds (7, 22), but it is also absent in many prolific sheep, such as Olkuska, Thoka and Woodlands breeds (1). The absence of the currently known prolificacy genotypes in these Iranian sheep breeds implies the possibility that these important mutations affecting prolificacy may be introduced in these breeds by genetic introgression. Indeed genetic introgression can be very beneficial because it allows the introduction of a genotype selectively advantageous in a breed already adapted to the environment in which it is reared (23, 22). Two examples among many, i.e. the *FecB* mutation has been introgressed from Garole sheep into Deccani and Bannur sheep, improving the reproductive performance of local non-prolific breeds (24) and the crossbreeding of Garole×Malpura allowed the introgression of the *FecB* genotype carried by Garole sheep into the non-prolific Malpura, improving the mean litter size of the crossbreds (25).

The incorporation of a major gene for prolificacy into a flock can be achieved using marker- assisted selection, artificial insemination and embryo transfer programmes (1). All of this information can be used for the improvement of Iranian sheep breeding to be applied in those areas of the country where environmental conditions allow taking advantage from the improvement of prolificacy.

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