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A Profile of Single Nucleotide Polymorphisms in Fecundity Genes Among Iranian Sheep Breeds by Using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) Method

Research Article

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ABSTRACT

Ovulation rate and litter size are traits of fecundity and have a very remarkable economic value that leads to an increase in the production of meat, wool and milk. Therefore, exploring on the candidate and functional genes for these traits is very important. Growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15) and bone morphogenetic protein receptor-1B (BMPR1B) are the most important of these genes containing single nucleotide polymorphisms (SNPs) markers that are associated with high fertility in sheeps. The aim of this study was to determine the allelic and genotypic frequencies of seven of these SNPs including; G1, G4, G7 and G8 in *GDF9* gene, B2 and B4 in *BMP15* gene and FecB in *BMPR1B* gene among 532 sheeps from eleven of Iranian native breeds using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. For each mutation region, polymerase chain reactions and digestion reactions with specific endonuclease enzymes, were performed. The results showed that G1 mutation presents among ten breeds except Zandi, G4 mutation in all breeds, and the FecB mutation in two breeds; Kalakuei and Zandi. The G7, G8, B2 and B4 mutations were not observed in any of breeds, and all sheeps were homozygote wild type for these four positions.

KEY WORDS PCR-RFLP, SNP, GDF9, BMP15, BMPR1B, sheep.

INTRODUCTION

The domesticated sheep breeds are probably originated from the wild sheep species such as Asian mouflons (*Ovis orientalis*) and Asiatic urial (*Ovis vignei*), which are still found in Iran National Parks and protected natural areas (Valizadeh, 2010). Iran has 28 native sheep breeds and in terms of breed diversity in sheep, has occupied the first rank in the world (Akbarinejad *et al.* 2014). According to the latest livestock census, conducted in 2017 by Ministry of Jihad-e-Agriculture of Iran (Agricultural Statistics of Iran, 2017), the Iranian sheep population is 47638600 animals that encompass 63.60% of Iranian animals (sheeps/lambs, goats/kids, cattles/calves, buffalos and camels). The meat production trait has taken into account in most previous Iranian sheep breeding program and fertility traits have played the main role to improve lamb and mutton production. Based on previous investigations, different mutations in the transforming growth factor-β (TGFβ) superfamily genes have been reported to affect ovulation rate and litter size in sheep. These genes include bone morphogenetic protein receptor, type 1B (BMPR-1B), bone morphogenetic protein 15 (BMP15), and growth differentiation factor 9 (GDF9) (Jansson, 2014).

The Booroola's ancestor is Garole breed sheep that is indigenous to Bengal area in India (Abraham and Thomas, 2012). The Garole is a small sheep that have a mean litter size of 1.74 to 2.27 (Davis *et al.* 2002). The Booroola phenotype, which increased the litter size, was originally selected from special lines of Australian merino sheep. Booroola fecundity gene is the first major gene for prolificacy identified in sheep (Wilson *et al.* 2001). This gene is located on chromosome 6 of sheep and is effective on the maturation of the granulosa cells, the development of the immature oocyte and its function (Abraham and Thomas, 2012). This gene is known as the activin receptor-like kinase (*ALK6*) and its mutation called FecB. A change from adenine (in wild type) to guanine (in mutant types) at the nucleotide position 746, results in a change from glutamine amino acid (in wild type) to arginine amino acid (in mutant types) at coding region 249 (Mulsant *et al.* 2001). This single autosomal locus can improve the ovulation rate and litter size. The FecB mutation, in addition to the Merino Booroola breed, also presents in some other sheep breeds such as; Hu (Chu *et al.* 2011; Wang *et al.* 2018), Small Tailed Han (Chu *et al.* 2006; Zhang *et al.* 2011) and Bayanbulak in China (Zuo *et al.* 2013), Kendrapada in India (Dash *et al.* 2017) and etc and effects on high fertility in these breeds.

Bone morphogenetic protein 15 (*BMP15*) (*FecX*) gene is located on the chromosome X of sheep and has two exons separated by an intron, which is about 5400 nucleotides. The protein encoded by this gene has 1179 nucleotides and its immature peptide has 393 amino acids. The mature peptide has 125 amino acids (Galloway *et al.* 2000). The gene, which was first found in Romney sheep, is also called *GDF9B* or FecX. This gene, and also *GDF9*, encode separate proteins that express in ovarian tissue. This mechanism is very effective in mammalian fertilization (Hanrahan *et al.* 2004).

Until now, several mutations of this gene including Inverdale (FecX^{I}), Hanna (FecX^{H}), Belclare (FecX^{B}), Galway (FecX^{G}), Lacaune (FecX^{L}), Rasa aragonesa (FecX^{R}), Grivette (FecX^{Gr}) and Olkuska (FecX^{O}) have been identified in different sheep breeds. The first six mutations increase the rate of ovulation in heterozygous individuals (Qiuyue *et al.* 2014). For instance, the estimated effect of FecX^{G} is ranged from 0.77 in Belclare ewes to 1.18 in Cambridge ewes, and for FecX^{B} is 2.38 in Belclare ewes (Hanrahan *et al.* 2004).

Growth differentiation factor 9 (*GDF9*) (*FecG*) is essential for folliculogenesis in sheep ovary and has a highly significant role from the first stage of follicular growth until ovulation. *GDF9* gene is located on chromosome 5 of sheep and has about 2500 nucleotides. Two exons are separated by an intron with 1126 nucleotides. The two exons encode an immature peptide with 453 amino acids. The mature peptide has 135 residues (Hanrahan *et al.* 2004). FecG^{H} or G8 is one of eight mutations (G1 to G8) in this gene that has been identified in Cambridge and Belclare sheeps and led to an increase in ovulation rates in heterozygotes and infertility in mutant homozygotes ewes. The effect of that is 1.79 in Belclare ewes and 2.35 in Cambridge ewes. Up to now, the *FecG^{H}* has been reported in breeds of sheep like as Chios and Karagouniki in Greece too (Liandris *et al.* 2012).

So far, in addition to the *FecG^{H}*, some other mutations in this gene such as; *FecG^{V}* in Ile de France, *FecG^{E}* in Embrapa and *FecG^{T}* in Icelandic Thoka sheep breeds have also been identified that demonstrated an association with high ovulation rate and litter size (Qiuyue *et al.* 2014). The purpose of this study was to investigate the presence or absence of seven SNPs in the three mentioned genes and to determine their allelic and genotypic frequencies among these eleven breeds.

MATERIALS AND METHODS

Blood samples were collected from 532 individuals belong to 11 Iranian native sheep breeds including; Afshari (N=55), Farahani (N=55), Grey Shiraz (N=55), Turki-Ghashghaei (N=55), Naeini (N=48), Kalakuei (N=47), Moghani (N=46), Shal (N=46), Taleshi (N=45), Makuei (N=41) and Zandi (N=39). The detailed information are given in Table 1. The detailed information of SNPs (Table 2) and the position of amino acid changes on amino acid sequences in genes (Figure 1) are given.

The blood samples was collected between 2005 and 2007 according to breed distribution areas over a large geographic area. During sampling, attempts were made to collect animals as unrelative as possible. A modified protocol was applied to isolate genomic DNA from blood samples using GPP kit (Gene Pajoohan Pooya). The extracted DNA was qualified and quantified using NanoDrop (2000) and agarose gel electrophoresis methods. Subsequently, polymerase chain reactions were performed. Some of the primers were deliberately introduced a point mismatch (shown by underlines in Table 3) resulting in PCR products containing restriction site for each endonuclease enzyme (Davis *et al.* 2002; Hanrahan *et al.* 2004). Details of each primer and PCR products (Table 3), PCR reactions (Table 4) and the temperature program for SNPs (Table 5) are given.

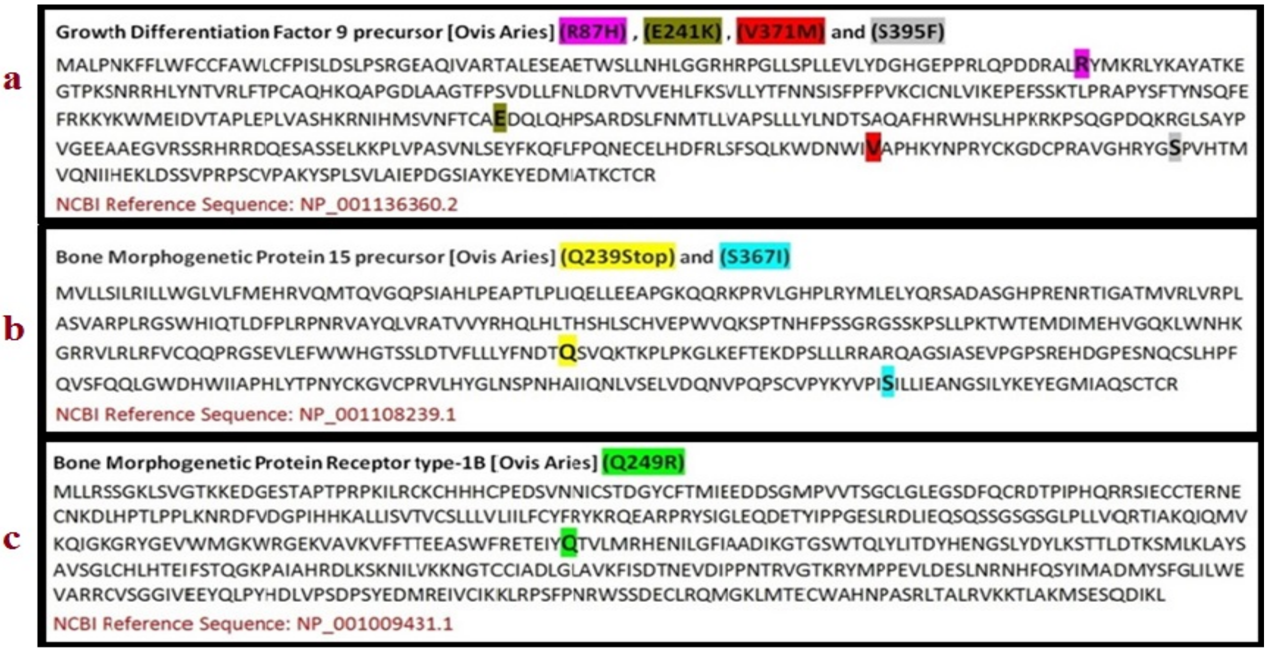
The total volume of each reaction was considered 15 µL and the applied biosystems (AB) thermal cycler (model 9700) was used for amplification.

Table 1 Introduction of eleven breeds in this study

Breed name	Population size	Distribution area (province)	Fecundity rate (%) and reference(s)
Afshari	1150000	Zanjan	10.9% (Akbarinejad *et al.* 20140)
Farahani	1500000	Markazi	3.5% (Tavakolian, 2000), 7.1% (Akbarinejad *et al.* 2014)
Grey Shiraz	700000	Fars	2-3% (Akbarinejad *et al.* 2014)
Kalakuei	190000	Qom, Tehran and markazi	4.1% (Akbarinejad *et al.* 2014), 5-10% (Khaldari, 2014)
Makuei	1400000	North of West Azarbayjan	5% (Saadatnouri and Siahmansour, 2011), 5-10% (Khaldari, 2014), 10.5% (Akbarinejad *et al.* 2014)
Moghani	3500000	Ardebil	5-7% (Khaldari, 2014), 10% (Saadatnouri and Siahmansour, 2011), 16% (Akbarinejad *et al.* 2014)
Naeini	700000	Isfahan	1% (Akbarinejad *et al.* 2014), 1-2% (Khaldari, 2014)
Shal	700000	Qazvin and Alborz	20-30% (Khaldari, 2014), 35% (Saadatnouri and Siahmansour, 2011), 40% (Akbarinejad *et al.* 20140)
Taleshi	470000	Guilan	2.8% [anonym]
Turki-Ghashghaei	700000	Kohgilooye and Boyerahmad and north of Fars	3-4% (Khaldari, 2014)
Zandi	700000	Tehran, Alborz and Qom	5-10% (Akbarinejad *et al.* 2014), 10% (Khaldari, 2014)

Table 2 Informations of the seven single nucleotide polymorphisms (SNPs)

Gene	SNP name	Base change	Base position	Amino acid change	Amino acid position	Reference
GDF9	G1	G→A	260	Arg/R→His/H	87	Hanrahan *et al.* (2004)
	G4	G→A	721	Glu/E→Lys/K	241	
	G7	G→A	1111	Val/V→Met/M	371	
	G8 (FecG^H)	C→T	1184	Ser/S→Phe/F	395	
BMP15	B2 (FecX^G)	C→T	718	Gln/Q→stop codon	239	
	B4 (FecX^B)	G→T	1100	Ser/S→Ile/I	367	
BMPR1B	FecB	A→G	746	Gln/Q→Arg/R	249	Mulsant *et al.* (2001)



Growth Differentiation Factor 9 precursor [Ovis Aries] (R87H) , (E241K) , (V371M) and (S395F)

MALPNKFFLWFCCFAWLCFPISLDSLPSRGEAQIVARTALESEAETWSLLNHLGGRHRPGLLSPLLEVLYDGHGEPPRLQPDDRALRYMKRLYKAYATKE
GTPKSNRRHLYNTVRLFTPCAQHKQAPGDLAAGTFPSVDLLFNLDRVTVVEHLFKSVLLYTFNNSISFPFPVKCICNLVIKEPEFSSKTLPRAPYSFTYNSQFE
FRKKYKWMEIDVTAPLEPLVASHKRNIHMSVNFTCAEDQLQHPSARDSLFNMTLLVAPSLLLYLNDTSAQAFHRWHSLHPKRKPSQGPDQKRGLSAYP
VGEEAAEGVRSSRHRRDQESASSELKKPLVPASVNLSEYFKQFLFPQNECELHDFRLSFSQLKWDNWIVAPHKYNPRYCKGDCPRAVGHRYGSPVHTM
VQNIIHEKLDSSVPRPSCVPAKYSPLSVLAIEPDGSIAYKEYEDMIATKCTCR

NCBI Reference Sequence: NP_001136360.2

Bone Morphogenetic Protein 15 precursor [Ovis Aries] (Q239Stop) and (S367I)

MVLLSILRILLWGLVLFMEHRVQMTQVGQPSIAHLPEAPTLPLIQELLEEAPGKQQRKPRVLGHPLRYMLELYQRSADASGHPRENRTIGATMVRLVRPL
ASVARPLRGSWHIQTLDFPLRPNRVAYQLVRATVVYRHQLHLTHSHLSCHVEPWVQKSPTNHFPSSGRGSSKPSLLPKTWTEMDIMEHVGQKLWNHK
GRRVLRLRFVCQQPRGSEVLEFWWHGTSSLDTVFLLLYFNDTQSVQKTKPLPKGLKEFTEKDPSLLLRRARQAGSIASEVPGPSREHDGPESNQCSLHPF
QVSFQQLGWDHWIIAPHLYTPNYCKGVCPRVLHYGLNSPNHAIIQNLVSELVDQNVPQPSCVPYKYVPISILLIEANGSILYKEYEGMIAQSCTCR

NCBI Reference Sequence: NP_001108239.1

Bone Morphogenetic Protein Receptor type-1B [Ovis Aries] (Q249R)

MLLRSSGKLSVGTKKEDGESTAPTPRPKILRCKCHHHCPEDSVNNICSTDGYCFTMIEEDDSGMPVVTSGCLGLEGSDFQCRDTPIPHQRRSIECCTERNE
CNKDLHPTLPPLKNRDFVDGPIHHKALLISVTVCSLLLVLIILFCYFRYKRQEARPRYSIGLEQDETYIPPGESLRDLIEQSQSSGSGSGLPLLVQRTIAKQIQMV
KQIGKGRYGEVWMGKWRGEKVAVKVFFTTEEASWFRETEIYQTVLMRHENILGFIAADIKGTGSWTQLYLITDYHENGSLYDYLKSTTLDTKSMLKLAYS
AVSGLCHLHTEIFSTQGKPAIAHRDLKSKNILVKKNGTCCIADLGLAVKFISDTNEVDIPPNTRVGTKRYMPPEVLDESLNRNHFQSYIMADMYSFGLILWE
VARRCVSGGIVEEYQLPYHDLVPSDPSYEDMREIVCIKKLRPSFPNRWSSDECLRQMGKLMTECWAHNPASRLTALRVKKTLAKMSESQDIKL

NCBI Reference Sequence: NP_001009431.1

Figure 1 a: four amino acid changes in *GDF9*; b: two amino acid changes in *BMP15* and c: one amino acid change in *BMPR1B*

Table 3 details of primers

Primer	5´-sequence-3´	Size of product	SNP position on PCR product
G1-F	GAAGACTGGTATGGGGAAATG	462 bp	306
G1-R	CCAATCTGCTCCTACACACCT		
G4-F	GGAATATTCACATGTCTGTAAATTTTACATGT**T**CG	161 bp	36
G4-R	GAGGGAATGCCACCTGTGAAAAGCC		
G7-F	GCCTCTGGTTCCAGCTTCAGTC	156 bp	125
G7-R	CAGTATCGAGGGTTGTATTTGTGTGGGGCC**T**		
G8-F	CTTTAGTCAGCTGAAGTGGGACAAC	139 bp	105
G8-R	ATGGATGATGTTCTGCACCATGGTGTGAACC**T**GA		
B2-F	CACTGTCTTCTTGTTACTGTATTTCAATGA**G**AC	141 bp	35
B2-R	GATGCAATACTGCCTGCTTG		
B4-F	GCCTTCCTGTGTCCCTTATAAGTATGTTCCC**C**TTA	153 bp	36
B4-R	TTCTTGGGAAACCTGAGCTAGC		
FecB-F	CCAGAGGACAATAGCAAAGCAAA	190 bp	159
FecB-R	CAAGATGTTTTCATGCCTCATCAACAC**G**GTC		

SNP: single nucleotide polymorphism and PCR: polymerase chain reaction.

Table 4 Polymerase chain reaction reaction components

PCR material	DNA	PCR Buffer	$MgCl_2$	dNTPs	Primer F	Primer R	Taq	ddwater
Initial concentration	20 ng	10 X	100 mM	2 mM	10 pmol	10 pmol	5U	-
Volume used	2.5 µL	1.5 µL	0.3 µL	1.5 µL	1 µL	1 µL	0.3 µL	6.9 µL

Table 5 Polymerase chain reaction (PCR) program in thermal cycler

Step/SNP	G1	G4	G7	G8	B2	B4	FecB
Whole Denaturation	94 °C 5 min	94 °C 5 min	94 °C 5 min	94 °C 5 min	94 °C 5 min	94 °C 5 min	94 °C 5 min
PCR cycles	35	35	35	35	35	35	35
Denaturation	94 °C 1 min	94 °C 30 sec	94 °C 30 sec	94 °C 30 sec	94 °C 30 sec	94 °C 30 sec	94 °C 1 min
Annealing	57 °C 45 sec	63 °C 40 sec	63 °C 40 sec	62 °C 30 sec	63 °C 40 sec	64 °C 40 sec	59 °C 1 min
Extension	72 °C 1 min	72 °C 30 sec	72 °C 30 sec	72 °C 30 sec	72 °C 30 sec	72 °C 30 sec	72 °C 1 min
Final Extension	72 °C 5 min	72 °C 4 min	72 °C 4 min	72 °C 5 min	72 °C 4 min	72 °C 4 min	72 °C 10 min

SNP: single nucleotide polymorphism.

Figure 2 indicates the sequences of seven PCR products. The sequences highlighted in red are annealing sites of the primers, the green nucleotide is the position of mutation and the underlined sequences are cutting sites of each enzyme.

Restriction digestion process was carried out by using a specific restriction enzymes for each mutation. Information of each restriction enzyme and the size of fragments obtained from the products of the enzymes are given in Tables 6 and 7, respectively.

The digested products were electrophoresed in 2% agarose gel and the DNA bands were visualized by ethidium bromide staining technique.

Existence or absence of a restriction site is the source of polymorphism. For genotyping, each band represents an allele having a specific size in base pairs (bp). The data were subjected to appropriate analyses using POPGENE software (ver. 1.31).

RESULTS AND DISCUSSION

A total of 532 individuals from the 11 Iranian sheep breeds were genotyped with the PCR-RFLP approach. The electrophoretic profiles of genomic DNA and PCR products of seven loci are shown in Figures 3 to 10.

Digestion with restriction enzymes in seven loci on agarose geles (2%) have been shown in Figures 11 to 17.

Subsequently, statistical analaysis were carried out. Frequencies of genotypes and alleles for G1 mutation (G→ A), G4 mutation (G→A), FecB mutation (A→G) and other SNPs are given in Tables 8 to 11, respectively.

Due to the fact that polymorphism was observed only in the G1, G4 and *FecB* loci, the Hardy-Weinberg equilibrium for these three positions was examined based on the chi-square test. The HWE test showed that all three polymorphic loci did not deviate from HWE (P>0.05) while considering each breed individually.

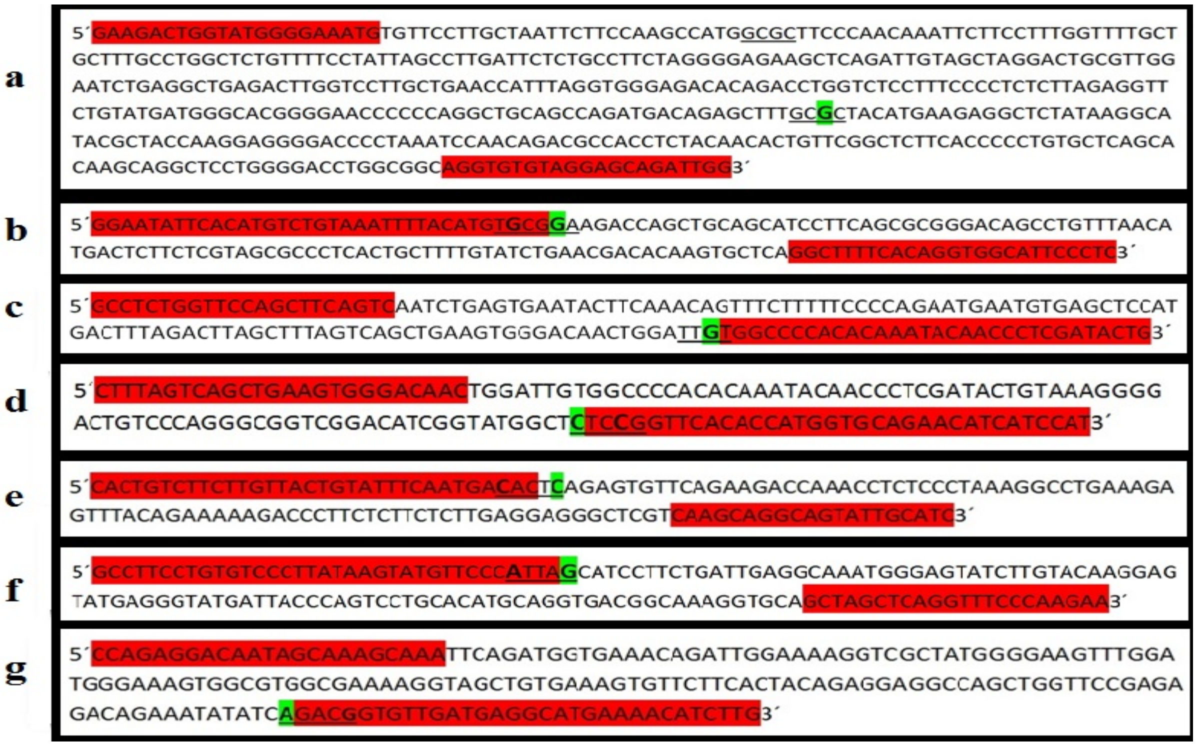


Figure 2 The PCR product sequences
a: 462 bp; b: 161 bp; c: 156 bp and d: 139 bp in *GDF9*; e: 141 bp and f: 153 bp in *BMP15*; g: 190 bp in *BMPR1B*

Table 6 Information of restriction enzymes

Enzyme/SNP	G1	G4	G7	G8	B2	B4	FecB
Name	*HhaI*	*AsuII*	*MseI*	*DdeI*	*Hinf I*	*DdeI*	*AvaII*
Cutting site	GCG.C	TT.CGAA	T.TAA	C.TNAG	G.ANTC	C.TNAG	G.GWCC
Cutting temp	37 °C	37 °C	65 °C	37 °C	37 °C	37 °C	37 °C
Duration time	2:30 h	3 h	3:30 h	3 h	3 h	3 h	3:30 h
Dominant (bp)	254/156/52	161	156	105/34	110/31	121/32	190
Hetero (bp)	410/254/156/52	161/128/33	156/123/33	139/105/34	141/110/31	153/121/32	190/159/31
Recessive (bp)	410/52	128/33	123/33	139	141	153	159/31

Table 7 Digestion reaction components

Material/SNP	G1	G4	G7	G8	B2	B4	FecB
PCR product	2.5 µL	2.5 µL	3 µL	2.5 µL	2.5 µL	2.5 µL	2.5 µL
Digestion buffer	1.5 µL	1.5 µL	3 µL	1.5 µL	1.5 µL	1.5 µL	1.5 µL
Restriction enzyme	0.3 µL	0.25 µL	0.3 µL	0.18 µL	0.25 µL	0.19 µL	0.19 µL
ddwater	10.7 µL	10.75 µL	8.7 µL	10.82 µL	10.75 µL	10.81 µL	10.81 µL

But when all the breeds were combined into one population, deviation from HWE happened only in *FecB* mutation ($P<0.05$). Table 12 showes the p-value of HWE test for each mutation.

Genotyping of G1 showed that there was no recessive homozygote individuals in all breeds. The highest and lowest frequencies of heterozygotes (GA) obtained in Taleshi and Zandi breeds with 0.156 and 0.000, respectively. The mean frequency of heterozygotes in all sheeps as one population was 0.068. The highest and lowest frequency of mutant allele (A) was observed in Taleshi and Zandi breeds with 0.078 and 0.000, respectively. Mean of mutant allele frequency per all animals was 0.034. Further details about this mutation to be extracted from different references are given in Table 13.

The G1 mutation was identified in Cambridge and Belclare sheeps for the first time but without any association with ovulation rate (Hanrahan *et al.* 2004).

With looking at the Table 13, it can be seen that, in other studies such as ours, which aimed only at evaluating allelic and genotypic frequencies, no sample of mutant homozygote is reported.

It indicates that the frequency of this genotype is very low in the main populations of these breeds. In other studies with sheep fecundity records (and probably, the samples have also been selected based on their records), the number of mutant homozygous is low.

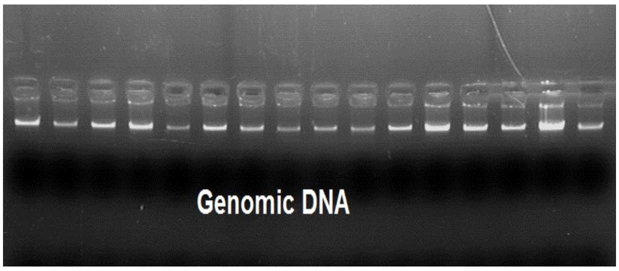


Figure 3 Some of genomic DNA in this study

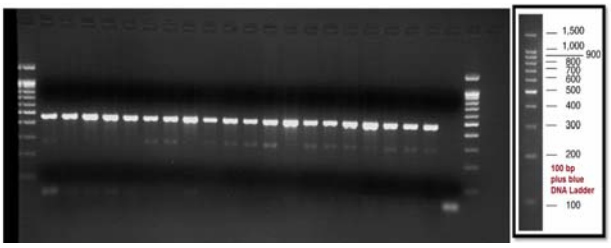


Figure 4 G1 locus; a 462 bp band

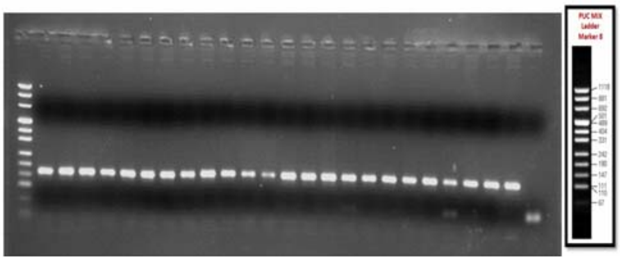


Figure 5 G4 locus; a 161 bp band

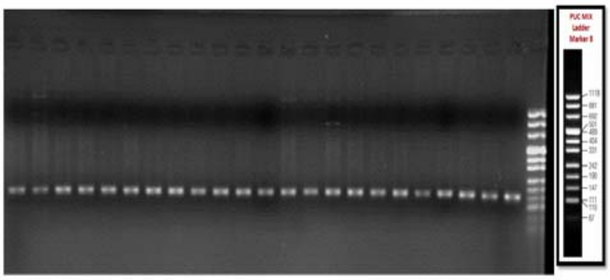


Figure 6 G7 locus; a 156 bp band

The highest frequency of mutant homozygotes has been reported in the Greek Chios breed with 0.087 (Liandris *et al.* 2012). The homozygous (AA) ewes in this breed displayed litter size of 2.25 implying a significant effect of the A allele, as high as 0.33 lambs. The heterozygous ewes showed negative dominance deviation with a statistically significant decrease in litter size of 0.47 lambs.

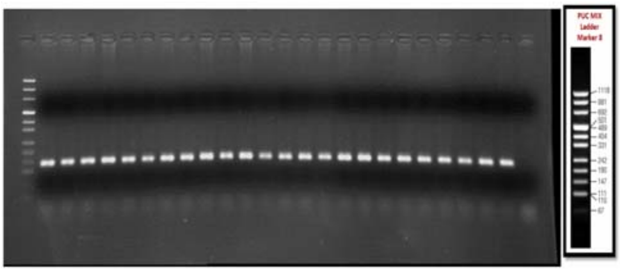


Figure 7 G8 locus; a 139 bp band

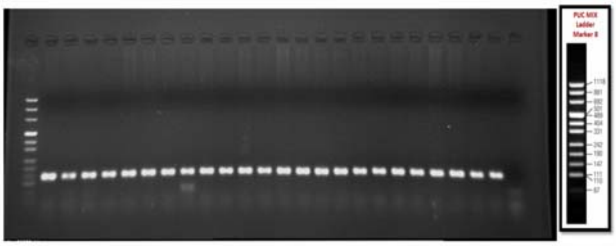


Figure 8 B2 locus; a 141 bp band

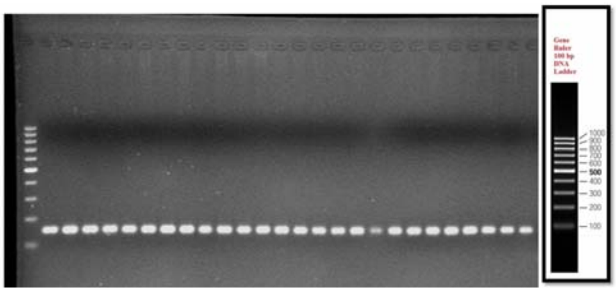


Figure 9 B4 locus; a 153 bp band

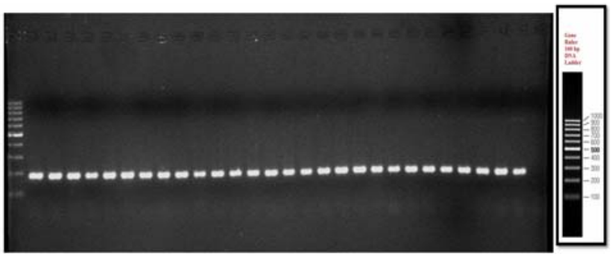


Figure 10 FecB locus; a 190 bp band

The G1 has a major effect on litter size in Baluchi sheep breed of Iran. The heterozygous and wild homozygous ewes had 0.35 and 0.21 more lambs than the homozygous mutant ewes, respectively (Moradband *et al.* 2011). Another report says this mutation leads to increased litter size in heterozygotes (1.56) compared with the wild homozygotes (1.25) in Chilota sheeps in Chile (Paz *et al.* 2015). However, some other studies did not report any association between this mutation and high fertility in sheep.

Genotyping of G4 revealed that there was no recessive homozygote in all studied breeds.

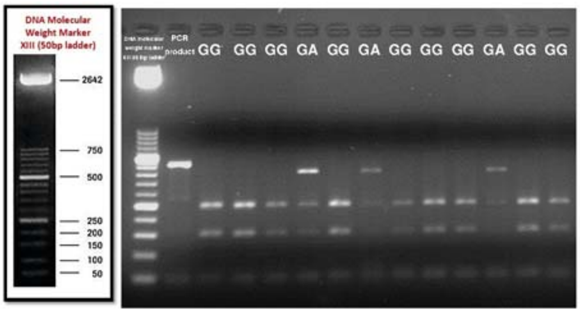


Figure 11 Polymorphism in G1 locus

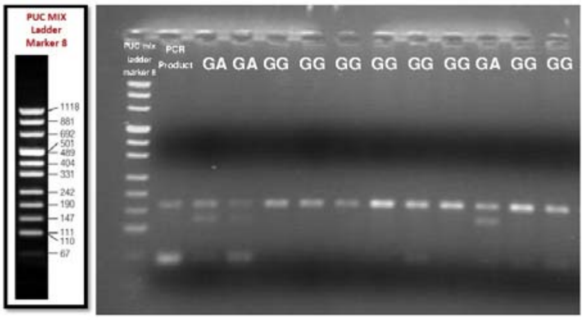


Figure 12 Polymorphism in G4 locus

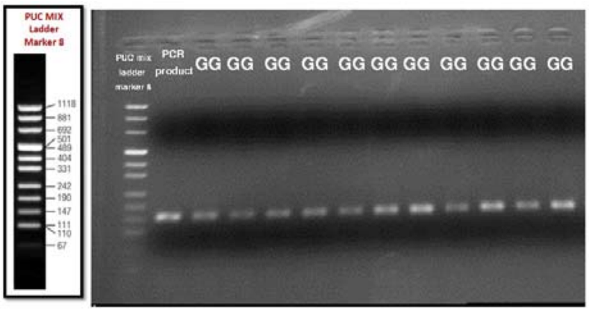


Figure 13 Monomorphism in G7 locus

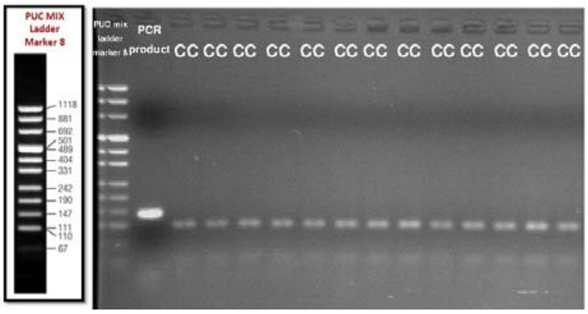


Figure 14 Monomorphism in G8 locus

The highest and lowest frequency of heterozygotes (GA) obtained in Taleshi and Shal breeds with 0.156 and 0.043, respectively. The mean frequency of heterozygotes in all animals was 0.115.

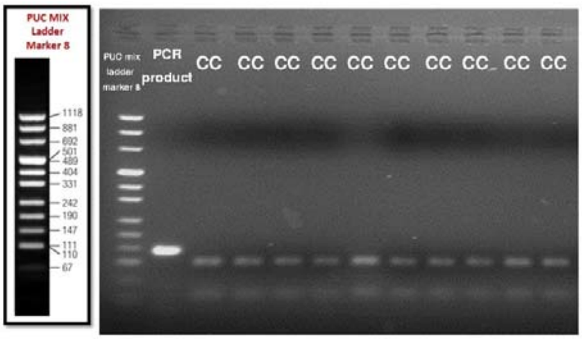


Figure 15 Monomorphism in B2 locus

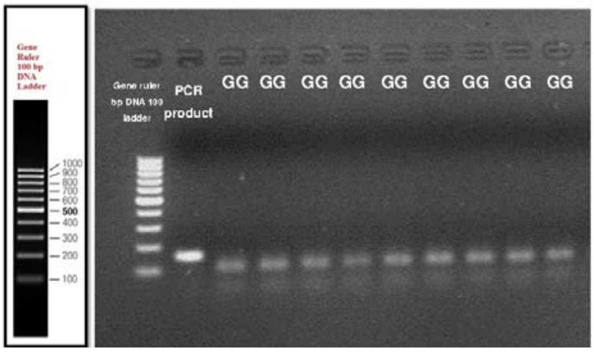


Figure 16 Monomorphism in B4 locus

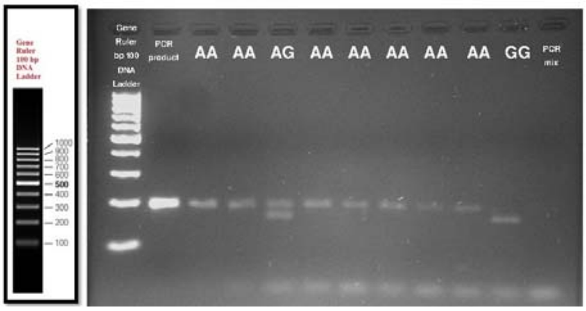


Figure 17 Polymorphism in FecB locus

The highest and lowest frequencies of the mutant allele (A) were observed in Taleshi and Shal breeds with 0.078 and 0.022 respectively. Mean frequency of the mutant allele in all animals was 0.057. This mutation has not been reported in Garole sheep of India (Polley *et al.* 2010) but has been identified in some other breeds around the world. More details are given in Table 14.

For the first time, it had been reported that 13 of 29 Belclare sheep, and 1 of 26 Cambridge sheep, had at least one copy of the G4 mutation with no association to fertility (Hanrahan *et al.* 2004). In Greek Chios sheep breed, the highest litter size (2.17) was observed in heterozygotes and a significant positive dominance deviation as high as 0.61 lambs was obtained (Liandris *et al.* 2012).

Table 8 Frequencies of genotypes and alleles for G1 mutation

Breed (N)	GG (f)	GA (f)	AA (f)	G (f)	A (f)
Taleshi (45)	38 (0.844)	7 (0.156)	0 (0.000)	83 (0.922)	7 (0.078)
Moghani (46)	40 (0.870)	6 (0.130)	0 (0.000)	86 (0.935)	6 (0.065)
Naeini (48)	42 (0.875)	6 (0.125)	0 (0.000)	90 (0.938)	6 (0.062)
Makuei (41)	38 (0.927)	3 (0.073)	0 (0.000)	79 (0.963)	3 (0.037)
Farahani (55)	51 (0.927)	4 (0.073)	0 (0.000)	106 (0.964)	4 (0.036)
Afshari (55)	52 (0.945)	3 (0.055)	0 (0.000)	107 (0.973)	3 (0.027)
Shal (46)	44 (0.957)	2 (0.043)	0 (0.000)	90 (0.978)	2 (0.022)
Grey Shiraz (55)	53 (0.964)	2 (0.036)	0 (0.000)	108 (0.982)	2 (0.018)
Turki-Ghashghaei (55)	53 (0.964)	2 (0.036)	0 (0.000)	108 (0.982)	2 (0.018)
Kalakuei (47)	46 (0.979)	1 (0.021)	0 (0.000)	93 (0.989)	1 (0.011)
Zandi (39)	39 (1.000)	0 (0.000)	0 (0.000)	78 (1.000)	0 (0.000)
Total (532)	496 (0.932)	36 (0.068)	0 (0.000)	1028 (0.966)	36 (0.034)

Table 9 Frequencies of genotypes and alleles for G4 mutation

Breed (N)	GG (f)	GA (f)	AA (f)	G (f)	A (f)
Taleshi (45)	38 (0.844)	7 (0.156)	0 (0.000)	83 (0.922)	7 (0.078)
Moghani (46)	39 (0.848)	7 (0.152)	0 (0.000)	85 (0.924)	7 (0.076)
Turki-Ghashghaei (55)	47 (0.855)	8 (0.145)	0 (0.000)	102 (0.927)	8 (0.073)
Zandi (39)	34 (0.872)	5 (0.128)	0 (0.000)	73 (0.936)	5 (0.064)
Kalakuei (47)	41 (0.872)	6 (0.128)	0 (0.000)	88 (0.936)	6 (0.064)
Naeini (48)	42 (0.875)	6 (0.125)	0 (0.000)	90 (0.938)	6 (0.062)
Makuei (41)	36 (0.878)	5 (0.122)	0 (0.000)	77 (0.939)	5 (0.061)
Afshari (55)	50 (0.909)	5 (0.091)	0 (0.000)	105 (0.955)	5 (0.045)
Farahani (55)	50 (0.909)	5 (0.091)	0 (0.000)	105 (0.955)	5 (0.045)
Grey Shiraz (55)	50 (0.909)	5 (0.091)	0 (0.000)	105 (0.955)	5 (0.045)
Shal (46)	44 (0.957)	2 (0.043)	0 (0.000)	90 (0.978)	2 (0.022)
Total (532)	471 (0.885)	61 (0.115)	0 (0.000)	1003 (0.943)	61 (0.057)

Table 10 Frequencies of genotypes and alleles for FecB mutation

Breed (N)	AA (f)	AG (f)	GG (f)	A (f)	G (f)
Kalakuei (47)	38 (0.809)	8 (0.170)	1 (0.021)	84 (0.894)	10 (0.106)
Zandi (39)	37 (0.949)	2 (0.051)	0 (0.000)	76 (0.974)	2 (0.026)
Afshari (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Farahani (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Grey Shiraz (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Turki-Ghashghaei (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Naeini (48)	48 (1.000)	0 (0.000)	0 (0.000)	96 (1.000)	0 (0.000)
Moghani (46)	46 (1.000)	0 (0.000)	0 (0.000)	92 (1.000)	0 (0.000)
Shal (46)	46 (1.000)	0 (0.000)	0 (0.000)	92 (1.000)	0 (0.000)
Taleshi (45)	45 (1.000)	0 (0.000)	0 (0.000)	90 (1.000)	0 (0.000)
Makuei (41)	41 (1.000)	0 (0.000)	0 (0.000)	82 (1.000)	0 (0.000)
Total (532)	521 (0.979)	10 (0.019)	1 (0.002)	1052 (0.989)	12 (0.011)

Likewise, another report from India, indicates that the average litter size was 1.63, 2.00 and 1.91 for wild homozygous, heterozygous and mutant homozygous of Kendrapada ewes, respectively (Dash *et al*. 2017).

Genotyping of *FecB* revealed that this mutation only finds in Kalakuei and Zandi breeds. The frequencies of wild homozygote (AA), heterozygote (AG) and mutant homozygote (GG) obtained 0.809, 0.170 and 0.021 respectively, for Kalakuei breed and 0.949, 0.051 and 0.000 for Zandi breed.

The frequencies of the wild and mutant alleles were 0.894 and 0.106 respectively, in Kalakuei and 0.974 and 0.026 in Zandi. Mean frequency of the mutant allele in all samples was calculated as 0.011. More details about presence and absence of this mutation in many sheep breeds has been brought in Table 15. As seen in the Table 15, all reports of the occurrence of this mutation in sheeps show a significant relationship between that and higher fertility traits, except for the Bonapala breed of India.

Table 11 Frequencies of genotypes and alleles for G7, G8, B2 and B4 mutations

Breed (N)	Wild (f)	Hetero (f)	Mutant (f)	W Allele (f)	M Allele (f)
Afshari (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Farahani (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Grey Shiraz (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Turki-Ghashghaei (55)	55 (1.000)	0 (0.000)	0 (0.000)	110 (1.000)	0 (0.000)
Naeini (48)	48 (1.000)	0 (0.000)	0 (0.000)	96 (1.000)	0 (0.000)
Kalakuei (47)	47 (1.000)	0 (0.000)	0 (0.000)	94 (1.000)	0 (0.000)
Moghani (46)	46 (1.000)	0 (0.000)	0 (0.000)	92 (1.000)	0 (0.000)
Shal (46)	46 (1.000)	0 (0.000)	0 (0.000)	92 (1.000)	0 (0.000)
Taleshi (45)	45 (1.000)	0 (0.000)	0 (0.000)	90 (1.000)	0 (0.000)
Makuei (41)	41 (1.000)	0 (0.000)	0 (0.000)	82 (1.000)	0 (0.000)
Zandi (39)	39 (1.000)	0 (0.000)	0 (0.000)	78 (1.000)	0 (0.000)
Total (532)	532 (1.000)	0 (0.000)	0 (0.000)	1064 (1.000)	0 (0.000)

Table 12 Hardy-Weinberg equilibrium in three polymorphic sites[1]

Breed	G1	G4	FecB
Kalakuei	1.000^{ns}	0.671^{ns}	0.411^{ns}
Zandi	-	0.704^{ns}	0.908^{ns}
Afshari	0.866^{ns}	0.753^{ns}	-
Taleshi	0.602^{ns}	0.602^{ns}	-
Shal	0.916^{ns}	0.916^{ns}	-
Farahani	0.809^{ns}	0.753^{ns}	-
Moghani	0.667^{ns}	0.607^{ns}	-
Turki-Ghashghaei	0.923^{ns}	0.588^{ns}	-
Grey Shiraz	0.923^{ns}	0.753^{ns}	-
Naeini	0.675^{ns}	0.675^{ns}	-
Makuei	0.844^{ns}	0.712^{ns}	-
Total	0.426^{ns}	0.164^{ns}	0.000^{*}

* ($P<0.05$).

NS: non significant.

In this sheep, although the litter size in mutant homozygotes (1.71) was more than heterozygotes (1.64) and both were more than wild homozygotes (1.50), but the statistical analysis didn’t reveal a significant association (Roy *et al.* 2011). *FecB* mutation showed an additive effect on litter size in Hu sheep in China. Mutant homozygote and heterozygote ewes significantly have 0.713 and 0.419 more lambs than non carrier homozygotes, respectively (Wang *et al.* 2018). In an investigation, this mutation showed an additive effect on ovulation rate and partially dominant effect on litter size in Small Tailed Han sheep of China. In this study, mean of litter size in homozygote mutant and heterozygote ewes was 2.65 and 2.16 respectively, which were significantly more than 1.14 of wild homozygotes (Chu *et al.* 2011). Another report from China clarified a significant effect on litter size in Small Tailed Han, Poll Dorset and their F1 generation, in total (Jia *et al.* 2005). The ewes with BB and B+ genotypes had about 1.04 and 0.74 lambs more than wild non-carriers ewes. Also, the mean of litter size in BB ewes was significantly more than ++ ewes in both breeds. *FecB* mutation also showed a phenotypic effect on litter size in Indian Kendrapada sheep breed.

The average litter size of non-carriers, heterozygotes and homozygote carriers were 1.61, 1.80 and 2.06 respectively (Dash *et al.* 2017). Another report suggests a highly significant difference in the average of litter size in different genotypes of Indonesian fat Tailed sheep (Maskur *et al.* 2016).

Mutant homozygous ewes had the highest average (1.685) compared with heterozygotes (1.455) and wild types (1.145). In Iranian Kalehkoohi sheep, genotypes BB (1.902) and B+ (1.725) had 0.52 and 0.35 lambs, more than ++ type (1.379), respectively and that was a statistically significant difference ($P<0.01$). But no significant difference between BB and B+ genotypes were observed (Mahdavi *et al.* 2014).

Reffering to the Table 15, it can be seen that all breeds that carry the *FecB* allele, are distributed in different parts of Asia (China, Indonesia, India and Iran). Most likely, the Merino Booroola breed in Australia also received this gene from the Indian Garole sheep in the 18th century (Davis *et al.* 2002). The effect of *FecB* is additive for ovulation rate and can increase by about 1.6 corpora lutea per cycle for each copy in Booroola sheep (Wilson *et al.* 2001).

Table 13 The polymorphisms of G1 reported in some studies[1]

	Breed (N)	(A)	(G)	(GG)	(GA)	(AA)	SAWF	Reference
1	Chios (92)	0.240	0.760	0.609	0.304	0.087	Yes	Liandris *et al*. (2012)
2	Araucano Creole (100)	0.220	0.780	0.560	0.440	0.000	-	Paz *et al*. (2014)
3	German Mutton Merino (281)	0.210	0.790	0.612	0.363	0.025	No	Zuo *et al*. (2012)
4	Lori x Romanov (100)	0.200	0.800	0.600	0.400	0.000	-	Pirzadi *et al*. (2014)
5	Ashgar (35)	0.190	0.810	0.630	0.370	0.000	No	Ali *et al*. (2016)
6	Lori (100)	0.188	0.812	0.624	0.376	0.000	No	Zamani *et al*. (2015)
7	Garole (22)	0.180	0.820	0.640	0.360	0.000	-	Polley *et al*. (2010)
8	Baluchi (134)	0.180	0.820	0.720	0.200	0.080	Yes	Moradband *et al*. (2011)
9	Lori (150)	0.165	0.835	0.670	0.330	0.000	-	Nanekarani *et al*. (2016)
10	Ile de France (25)	0.160	0.840	0.680	0.320	0.000	No	Souza *et al*. (2014)
11	Mehraban (115)	0.150	0.850	0.710	0.270	0.020	No	Talebi *et al*. (2018)
12	Corriedale (92)	0.140	0.860	0.730	0.270	0.000	-	Vera *et al*. (2015)
13	Kordi (42)	0.090	0.910	0.810	0.190	0.000	-	Ghaderi *et al*. (2010)
14	Arabic (44)	0.080	0.920	0.840	0.160	0.000	-	Ghaderi *et al*. (2010)
15	Taleshi (45)	0.078	0.922	0.844	0.156	0.000	-	This study
16	Dalagh (100)	0.075	0.925	0.850	0.150	0.000	-	Khanahmadi *et al*. (2014)
17	Moghani (46)	0.065	0.935	0.870	0.130	0.000	-	This study
18	Bayanbulak (120)	0.063	0.937	0.900	0.075	0.025	No	Zuo *et al*. (2013)
19	Naeini (48)	0.062	0.938	0.875	0.125	0.000	-	This study
20	Watish (33)	0.060	0.940	0.880	0.120	0.000	No	Ali *et al*. (2016)
21	Bonpala (97)	0.060	0.940	0.890	0.100	0.010	No	Roy *et al*. (2011)
22	Chilota (57)	0.060	0.940	?	?	?	Yes	Paz *et al*. (2015)
23	Makuei (41)	0.037	0.963	0.927	0.073	0.000	-	This study
24	Farahani (55)	0.036	0.964	0.927	0.073	0.000	-	This study
25	Dubasi (29)	0.030	0.970	0.930	0.070	0.000	No	Ali *et al*. (2016)
26	Hisari (110)	0.030	0.970	0.936	0.064	0.000	-	Bahrami *et al*. (2014)
27	Karagouniki (96)	0.030	0.970	0.948	0.052	0.000	No	Liandris *et al*. (2012)
28	Afshari (55)	0.027	0.973	0.946	0.054	0.000	-	This study
29	Afshari (19)	0.027	0.973	0.947	0.053	0.000	-	Eghbalsaied *et al*. (2014)
30	Shal (46)	0.022	0.978	0.957	0.043	0.000	-	This study
31	Araucana (100)	0.020	0.980	?	?	?	No	Paz *et al*. (2015)
32	Grey Shiraz (55)	0.018	0.982	0.964	0.036	0.000	-	This study
33	Turki-Ghashghaei (55)	0.018	0.982	0.964	0.036	0.000	-	This study
34	Kalakuei (47)	0.011	0.989	0.979	0.021	0.000	-	This study
35	Katahdin (163)	0.000	1.000	1.000	0.000	0.000	-	Escobar-Chaparro *et al*. (2017)
	Dorper (36)	0.000	1.000	1.000	0.000	0.000	-	
	Black belly (17)	0.000	1.000	1.000	0.000	0.000	-	
	Pelibuey (35)	0.000	1.000	1.000	0.000	0.000	-	
	Admixed individuals (55)	0.000	1.000	1.000	0.000	0.000	-	
	Austral (120)	0.000	1.000	1.000	0.000	0.000	No	Paz *et al*. (2015)
	Kendrapada (85)	0.000	1.000	1.000	0.000	0.000	No	Dash *et al*. (2017)
	Shal (8)	0.000	1.000	1.000	0.000	0.000	-	Eghbalsaied *et al*. (2014)
	Zandi (39)	0.000	1.000	1.000	0.000	0.000	-	This study

[1] This table is arranged based on the frequency of the mutant allele, from the highest to the lowest.

SAWF: significant association with fecundity.

BMPRIB gene would be inactivated partially, leading to an advanced differentiation of granulosa cells and also an advanced maturation of ovulatory follicles in homozygous mutant ewes of Australian Booroola merino (Mulsant *et al*. 2001).

Genotyping of G7 revealed that this SNP didn’t occur in any of these eleven breeds and all sheeps have dominant homozygote genotypes. Our report about the absence of this mutation is consistent with several reports of other sheep breeds and of course, is not compatible with a few reports of other breeds. More details about this mutation in several sheep breeds are given in Table 16.

For the first time, It had been reported that at least one copy of G7 mutation presents in 2 of 19 Belclare and 7 of 24 Cambridge sheeps with no linkage to high fertility (Hanrahan *et al*. 2004).

A similar study reported that there was no statistical significant association between mutation and ovulation rate in Finnish Landrace sheep, although a significant association was observed in the Belclare sheep.

Heterozygote ewes had higher ovulation than wild homozygotes (0.17) and ovulation rates in heterozygotes were significantly lower than mean of homozygotes (Mullen and Hanrahan *et al*. 2014).

Table 14 The polymorphisms of G4 reported in some studies[1]

	Breed (N)	(A)	(G)	(GG)	(GA)	(AA)	SAWF	Reference
1	Kendrapada (85)	0.330	0.670	0.580	0.200	0.220	Yes	Dash *et al.* (2017)
2	Kermani (102)	0.240	0.760	0.519	0.481	0.000	-	Khodabakhshzadeh *et al.* (2016)
3	Chios (92)	0.230	0.770	0.576	0.380	0.044	Yes	Liandris *et al.* (2012)
4	Ashgar (10)	0.200	0.800	0.700	0.200	0.100	No	Ali *et al.* (2016)
5	Pelibuey (34)	0.200	0.800	0.620	0.380	0.000	-	Escobar-Chaparro *et al.* (2017)
6	Mehraban (115)	0.180	0.820	0.680	0.290	0.030	No	Talebi *et al.* (2018)
7	Katahdin (94)	0.180	0.820	0.650	0.350	0.000	-	Escobar-Chaparro *et al.* (2017)
8	Dubasi (10)	0.150	0.850	0.800	0.100	0.100	No	Ali *et al.* (2016)
9	Ile de France (25)	0.140	0.860	0.720	0.280	0.000	No	Souza *et al.* (2014)
10	Karagouniki (96)	0.130	0.870	0.802	0.146	0.052	No	Liandris *et al.* (2012)
11	Admixed individuals (44)	0.125	0.875	0.750	0.250	0.000	-	Escobar-Chaparro *et al.* (2017)
12	Dorper (37)	0.120	0.880	0.757	0.243	0.000	-	Escobar-Chaparro *et al.* (2017)
13	Bonpala (97)	0.080	0.920	0.850	0.140	0.010	No	Roy *et al.* (2011)
14	Afshari (19)	0.079	0.921	0.842	0.158	0.000	-	Eghbalsaied *et al.* (2014)
15	Taleshi (45)	0.078	0.922	0.844	0.156	0.000	-	This study
16	Moghani (46)	0.076	0.924	0.848	0.152	0.000	-	This study
17	Turki-Ghashghaei (55)	0.073	0.927	0.855	0.145	0.000	-	This study
18	Zandi (39)	0.064	0.936	0.872	0.128	0.000	-	This study
19	Kalakuei (47)	0.064	0.936	0.872	0.128	0.000	-	This study
20	Shal (8)	0.063	0.937	0.875	0.125	0.000	-	Eghbalsaied *et al.* (2014)
21	Naeini (48)	0.062	0.938	0.875	0.125	0.000	-	This study
22	Makuei (41)	0.061	0.939	0.878	0.122	0.000	-	This study
23	Watish (8)	0.060	0.940	0.880	0.120	0.000	No	Ali *et al.* (2016)
24	Afshari (55)	0.045	0.955	0.909	0.091	0.000	-	This study
25	Farahani (55)	0.045	0.955	0.909	0.091	0.000	-	This study
26	Grey Shiraz (55)	0.045	0.955	0.909	0.091	0.000	-	This study
27	Black Belly (17)	0.030	0.970	0.940	0.060	0.000	-	Escobar-Chaparro *et al.* (2017)
28	Shal (46)	0.022	0.978	0.957	0.043	0.000	-	This study
29	Garole (22)	0.000	1.000	1.000	0.000	0.000	-	Polley *et al.* (2010)

[1] This table is arranged based on the frequency of the mutant allele, from the highest to the lowest.
SAWF: significant association with fecundity.

Based on the estimated breeding values for the Norwegian White sheeps, daughters of homozygous mutant rams produce additional lambs (0.46 to 0.57) compared to daughters of wild homozygous rams. Also, daughters of heterozygous rams produce more lambs (0.20 to 0.25) than daughters of homozygous wild rams (Vage *et al.* 2013). Also mentioned that the average litter size for ewes at first year of age is about 2.061 and at second year is about 2.671, showing that the average litter size is higher at the second year and the genotype showed a significant effect on litter size at both years (Siddiqua, 2013).

In Olkuska sheep, only the presence of one copy of mutant allele, resulted in an increase (0.55 lamb) in litter size. Heterozygous ewes demonstrated high annual lamb production of 346%, with the same for dominant homozygous ewes at 236% .

It is clear from Table 16, that those breeds which have this mutation are natives of the northern Europe or raised in that region.

Genotyping of G8 (FecG^H) revealed that this mutation didn't occur in any of the studied breeds and all sheeps were dominant homozygotes.

Our report about the absence of this mutation is not compatible with some reports of other sheep breeds but is consistent with many reports of other sheep breeds. More details are given in Table 17.

It has been identified that this mutation is essential for folliculogenesis and results in high fertility in heterozygotes and infertility in mutant homozygotes in Cambridge and Belclare sheeps. Estimation of the effect of the mutation on the ovulation rate showed that Belclare ewes had an increase of about 1.79 and Cambridge ewes had about 2.35.

Based on progeny test data in Belclare sheeps, it has been shown that G8 can increase the ovulation rate of about 0.83 in ewe lambs and 1.75 in adult ewes, which both were significant. Overall, the mutation has led to an increase in ovulation rate about 1.39 in both breeds (Hanrahan *et al.* 2004).

Also, another study on Chios breed in Greece reported a statistically positive dominance deviation (0.44 lambs) for this mutation in heterozygous ewes but with no evidence of infertility in homozygous mutant ewes (Liandris *et al.* 2012). It has been suggested that the Lleyn sheep breed is the most likely source of this SNP (and also FecX^G) in Belclare and Cambridge sheeps (Mullen *et al.* 2013).

Genotyping of B2 ($FecX^G$) revealed that this mutation did not occur in any of eleven breeds and all animals were dominant homozygotes. More details are given in Table 18.

In Cambridge and Belclare breeds, the homozygous ewes for this mutation and also, heterozygous ewes for this mutation and B4 simultaneously, were sterile. The mutation significantly increases ovulation rate about 0.77 in Belclare and 1.18 in Cambridge ewes. Based on progeny test data in Belclare sheep, it has been shown that the mutation increases the ovulation rate of about 0.62 in ewe lambs and 0.72 in adult ewes, which both were significant. Overall, this mutation leads to an increase in ovulation rate of about 0.70 in both breeds. The effects of this mutation with B4 was about 0.28 in the Belclare breed (Hanrahan *et al.* 2004). A study on Small Tailed Han sheep showed that the polymorphism of this mutation was not significantly associated with litter size but tended to be associated with that trait.

In Hu sheep, the heterozygous ewes had 0.52 more lambs than the wild genotype ones. In total population, the heterozygous Hu and Small Tailed Han ewes showed 0.30 more lambs than those with wild genotype (Wang *et al.* 2015). Also, based on a study, the heterozygous Small Tailed Han ewes had 0.55 more lambs than wild homozygotes in China (Chu *et al.* 2006).

However, no significant allele effects were obtained in this mutation polymorphisms in Chios and Karagouniki breeds of Greece (Liandris *et al.* 2012). It has been suggested that the Lleyn sheep breed is the most likely source of this mutation (and $FecG^H$) in Belclare and Cambridge sheeps (Mullen *et al.* 2013).

Genotyping of B4 ($FecX^B$) revealed that this SNP was absent in all of the eleven breeds and all sheeps were dominant homozygotes. More details of this mutation in some studies are given in Table 19. This SNP has been identified in Belclare sheepbut not in Cambridge sheep.

Table 15 The polymorphisms of FecB reported in some studies[1]

	Breed (N)	(G)	(A)	(AA)	(AG)	(GG)	SAWF	Reference
1	Hu (12)	1.000	0.000	0.000	0.000	1.000	-	Davis *et al.* (2006)
2	Hu (2021)	0.949	0.051	0.006	0.090	0.904	Yes	Wang *et al.* (2018)
3	Hu (59)	0.898	0.102	0.000	0.203	0.797	Yes	Pan *et al.* (2015)
4	Bonpala (97)	0.865	0.135	0.020	0.230	0.750	No	Roy *et al.* (2011)
5	Hu (35)	0.843	0.157	0.000	0.314	0.686	Yes	Chu *et al.* (2011)
6	Small Tailed Han (154)	0.740	0.260	0.060	0.400	0.540	Yes	Zhang *et al.* (2011)
7	Kendrapada (46)	0.730	0.270	0.110	0.330	0.560	-	Kumar *et al.* (2008)
8	Small Tailed Han (188)	0.730	0.270	0.060	0.420	0.520	Yes	Chu *et al.* (2006)
9	Small Tailed Han (140)	0.725	0.275	0.107	0.336	0.557	Yes	Chu *et al.* (2011)
10	Small Tailed Han (293)	0.677	0.323	0.123	0.399	0.478	Yes	Pan *et al.* (2015)
11	Small Tailed Han (101)	0.663	0.337	0.119	0.436	0.445	Yes	Jia *et al.* (2005)
12	Small Tailed Han (12)	0.630	0.370	0.080	0.580	0.330	-	Davis *et al.* (2006)
13	Garole (22)	0.610	0.390	0.000	0.770	0.230	-	Polley *et al.* (2010)
14	Kendrapada (85)	0.600	0.400	0.153	0.494	0.353	Yes	Dash *et al.* (2017)
15	Small Tailed Han (869)	0.540	0.460	0.020	0.880	0.100	Yes	Wang *et al.* (2015)
16	Hu (761)	0.530	0.470	0.020	0.900	0.080	Yes	Wang *et al.* (2015)
17	Chhotanagpuri (92)	0.413	0.587	0.424	0.326	0.250	Yes	Oraon *et al.* (2016)
18	Small Tailed Han x Poll Dorset (81)	0.395	0.605	0.210	0.790	0.000	Yes	Jia *et al.* (2005)
19	Shahabadi (100)	0.380	0.620	0.240	0.760	0.000	Yes	Debnath and Singh (2014)
20	Kalehkoohi (92)	0.350	0.650	0.424	0.446	0.130	Yes	Mahdavi *et al.* (2014)
21	Cele Black Sheep (274)	0.348	0.652	0.416	0.471	0.113	Yes	Shi *et al.* (2010)
22	Indonesian Fat Tailed Sheep (250)	0.193	0.807	0.672	0.268	0.060	Yes	Maskur *et al.* (2016)
23	Nilagiri (145)	0.140	0.860	0.730	0.260	0.010	-	Sudhakar (2009)
24	Kalakuei (47)	0.106	0.894	0.809	0.170	0.021	-	This study
25	Bayanbulak (120)	0.084	0.916	0.858	0.117	0.025	Yes	Zuo *et al.* (2013)
26	Wadi (30)	0.070	0.930	0.870	0.130	0.000	Yes	Zhang *et al.* (2011)
27	Lori (150)	0.026	0.974	0.948	0.052	0.000	-	Nanekarani *et al.* (2016)
28	Zandi (39)	0.026	0.974	0.949	0.051	0.000	-	This study
29	Poll dorset (79)	0.025	0.975	0.962	0.025	0.013	Yes	Jia *et al.* (2005)

Continued Table 15

30	Finn (38)	0.000	1.000	1.000	0.000	0.000	-	Davis *et al*. (2006)
	Lieyn (16)	0.000	1.000	1.000	0.000	0.000	-	
	D'man (15)	0.000	1.000	1.000	0.000	0.000	-	
	Galician (15)	0.000	1.000	1.000	0.000	0.000	-	
	German white headed mutton (15)	0.000	1.000	1.000	0.000	0.000	-	
	Chios (12)	0.000	1.000	1.000	0.000	0.000	-	
	East Friesian (12)	0.000	1.000	1.000	0.000	0.000	-	
	Teeswater (12)	0.000	1.000	1.000	0.000	0.000	-	
	Blue face Leicester (12)	0.000	1.000	1.000	0.000	0.000	-	
	Loa (12)	0.000	1.000	1.000	0.000	0.000	-	
	Romanov (11)	0.000	1.000	1.000	0.000	0.000	-	
	Tyrolian Mountain Sheep (11)	0.000	1.000	1.000	0.000	0.000	-	
	Mountain Sheep (white) (11)	0.000	1.000	1.000	0.000	0.000	-	
	Mountain Sheep (brown) (8)	0.000	1.000	1.000	0.000	0.000	-	
	Barbados Blackbelly cross (7)	0.000	1.000	1.000	0.000	0.000	-	
	Barbados Blackbelly (2)	0.000	1.000	1.000	0.000	0.000	-	
	St.Croix (2)	0.000	1.000	1.000	0.000	0.000	-	
	Ossimi (20)	0.000	1.000	1.000	0.000	0.000	-	El-Hanafy and El-Saadani (2009)
	Rahmani (20)	0.000	1.000	1.000	0.000	0.000	-	
	Awassi (20)	0.000	1.000	1.000	0.000	0.000	-	
	Barki (20)	0.000	1.000	1.000	0.000	0.000	-	
	Awassi x Barki (20)	0.000	1.000	1.000	0.000	0.000	-	
	Mecheri (62)	0.000	1.000	1.000	0.000	0.000	-	Sudhakar (2009)
	Sicilo-Sarde (51)	0.000	1.000	1.000	0.000	0.000	-	Vacca *et al*. (2010)
	D'man (47)	0.000	1.000	1.000	0.000	0.000	-	
	Barbarine (41)	0.000	1.000	1.000	0.000	0.000	-	
	Noire de Thibar (34)	0.000	1.000	1.000	0.000	0.000	-	
	Queue Fine de L'Ouest (31)	0.000	1.000	1.000	0.000	0.000	-	
	Marwari (20)	0.000	1.000	1.000	0.000	0.000	-	Vivekanand (2010)
	Patanwadi (20)	0.000	1.000	1.000	0.000	0.000	-	
	Dumba (20)	0.000	1.000	1.000	0.000	0.000	-	
	Awassi (50)	0.000	1.000	1.000	0.000	0.000	No	Esen Gursel *et al*. (2011)
	Chios (50)	0.000	1.000	1.000	0.000	0.000	No	
	Imrose (50)	0.000	1.000	1.000	0.000	0.000	No	
	Kivircik (50)	0.000	1.000	1.000	0.000	0.000	No	
	Shandi (35)	0.000	1.000	1.000	0.000	0.000	-	Zhang *et al*. (2011)
	Big Tailed Han (20)	0.000	1.000	1.000	0.000	0.000	-	
	Texel (40)	0.000	1.000	1.000	0.000	0.000	-	Chu *et al*. (2011)
	Chinese Merino (38)	0.000	1.000	1.000	0.000	0.000	-	
	Baluchi (152)	0.000	1.000	1.000	0.000	0.000	No	Moradband *et al*. (2011)
	German Mutto Merino (281)	0.000	1.000	1.000	0.000	0.000	-	Zuo *et al*. (2012)
	Dalagh (100)	0.000	1.000	1.000	0.000	0.000	-	Khatamnejhad and Khanahmadi (2012)
	Hamdani (10)	0.000	1.000	1.000	0.000	0.000	-	Al-Barzinji and Othman (2013)
	Karadi (10)	0.000	1.000	1.000	0.000	0.000	-	
	Arabi (10)	0.000	1.000	1.000	0.000	0.000	-	
	Naeimi (10)	0.000	1.000	1.000	0.000	0.000	-	
	Awassi (10)	0.000	1.000	1.000	0.000	0.000	-	
	Dorper (39)	0.000	1.000	1.000	0.000	0.000	-	Somarny *et al*. (2013)
	Malin (20)	0.000	1.000	1.000	0.000	0.000	-	
	Lori (60)	0.000	1.000	1.000	0.000	0.000	-	Shafieiyan *et al*. (2013)
	Sangsari (150)	0.000	1.000	1.000	0.000	0.000	-	Jamshidi *et al*. (2013)
	Balangir (100)	0.000	1.000	1.000	0.000	0.000	-	Debnath and Singh (2014)
	Bonpala (50)	0.000	1.000	1.000	0.000	0.000	-	
	Finnish Landrace (108)	0.000	1.000	1.000	0.000	0.000	-	Mullen and Hanrahan (2014)
	Awassi (235)	0.000	1.000	1.000	0.000	0.000	-	Jawasreh *et al*. (2014)
	Blackbelly (20)	0.000	1.000	1.000	0.000	0.000	-	Lopez-Ramirez *et al*. (2014)
	Austral (120)	0.000	1.000	1.000	0.000	0.000	-	Paz *et al*. (2015)
	Araucana (100)	0.000	1.000	1.000	0.000	0.000	-	
	Chilota (57)	0.000	1.000	1.000	0.000	0.000	-	
	Barki (20)	0.000	1.000	1.000	0.000	0.000	-	Mahrous *et al*. 2015
	Rahmani (20)	0.000	1.000	1.000	0.000	0.000	-	
	Osseimi (20)	0.000	1.000	1.000	0.000	0.000	-	
	Dorset (48)	0.000	1.000	1.000	0.000	0.000	-	Pan *et al*. (2015)
	Texel (47)	0.000	1.000	1.000	0.000	0.000	-	
	Santa Ines (574)	0.000	1.000	1.000	0.000	0.000	No	Holanda *et al*. (2017)
	Morada Nova (282)	0.000	1.000	1.000	0.000	0.000	No	
	Afshari (55)	0.000	1.000	1.000	0.000	0.000	-	This study
	Farahani (55)	0.000	1.000	1.000	0.000	0.000	-	
	Grey Shiraz (55)	0.000	1.000	1.000	0.000	0.000	-	
	Turki-Ghshghayi (55)	0.000	1.000	1.000	0.000	0.000	-	
	Naeini (48)	0.000	1.000	1.000	0.000	0.000	-	
	Moghani (46)	0.000	1.000	1.000	0.000	0.000	-	
	Shal (46)	0.000	1.000	1.000	0.000	0.000	-	
	Taleshi (45)	0.000	1.000	1.000	0.000	0.000	-	
	Makuei (41)	0.000	1.000	1.000	0.000	0.000	-	

[1] This table is arranged based on the frequency of the mutant allele, from the highest to the lowest.

SAWF: significant association with fecundity.

Table 16 The polymorphisms of G7 reported in some studies[1]

	Breed (N)	(A)	(G)	(GG)	(GA)	(AA)	SAWF	Reference
1	Norwegian White Sheep (853)	0.380	0.620	0.400	0.450	0.150	Yes	Siddiqua (2013)
2	Norwegian White Sheep (403)	0.250	0.750	0.586	0.337	0.077	Yes	Vage *et al*. (2013)
3	Sicilo-Sarde (51)	0.000	1.000	1.000	0.000	0.000	-	Vacca *et al*. (2010)
	D'man (47)	0.000	1.000	1.000	0.000	0.000	-	
	Barbarine (41)	0.000	1.000	1.000	0.000	0.000	-	
	Noire de Thibar (34)	0.000	1.000	1.000	0.000	0.000	-	
	Queue Fine de L'Ouest (31)	0.000	1.000	1.000	0.000	0.000	-	
	Garole (22)	0.000	1.000	1.000	0.000	0.000	-	Polley *et al*. (2010)
	Bonpala (97)	0.000	1.000	1.000	0.000	0.000	No	Roy *et al*. (2011)
	Blackbelly (20)	0.000	1.000	1.000	0.000	0.000	-	Lopez-Ramirez *et al*. (2014)
	Katahdin (163)	0.000	1.000	1.000	0.000	0.000	-	Escobar-Chaparro *et al*. (2017)
	Admixed individuals (55)	0.000	1.000	1.000	0.000	0.000	-	
	Dorper (36)	0.000	1.000	1.000	0.000	0.000	-	
	Pelibuey (35)	0.000	1.000	1.000	0.000	0.000	-	
	Black belly (17)	0.000	1.000	1.000	0.000	0.000	-	
	Kandrapada (85)	0.000	1.000	1.000	0.000	0.000	No	Dash *et al*. (2017)
	Mehraban (16)	0.000	1.000	1.000	0.000	0.000	-	Talebi *et al*. (2018)
	Afshari (55)	0.000	1.000	1.000	0.000	0.000	-	This study
	Farahani (55)	0.000	1.000	1.000	0.000	0.000	-	
	Grey Shiraz (55)	0.000	1.000	1.000	0.000	0.000	-	
	Turki-Ghashghaei (55)	0.000	1.000	1.000	0.000	0.000	-	
	Naeini (48)	0.000	1.000	1.000	0.000	0.000	-	
	Kalakuei (47)	0.000	1.000	1.000	0.000	0.000	-	
	Moghani (46)	0.000	1.000	1.000	0.000	0.000	-	
	Shal (46)	0.000	1.000	1.000	0.000	0.000	-	
	Taleshi (45)	0.000	1.000	1.000	0.000	0.000	-	
	Makuei (41)	0.000	1.000	1.000	0.000	0.000	-	
	Zandi (39)	0.000	1.000	1.000	0.000	0.000	-	

[1] This table is arranged based on the frequency of the mutant allele, from the highest to the lowest.

SAWF: significant association with fecundity.

The mutation significantly increases ovulation rate and its effect was estimated about 2.38. Homozygous mutant ewes and also heterozygous ewes for this mutation and B2 simultaneously, were sterile. Based on progeny test data in Belclare sheep, this SNP increases the ovulation rate of about 0.76 in ewe lambs and 1.11 in adult ewes, which both were significant. Overall, the mutation leads to an increase in ovulation rate of about 0.97 in both breeds. The effect of this mutation with B2 was about 0.28 in the Belclare breed (Hanrahan *et al*. 2004). Based on some finding, the *FecX*^{B} mutation that found among the hyper-prolific ewes in Ireland, has come from a high fertility line that had developed with the use of prolific ewes that choosed from commercial flocks in that country and then used for the genesis of the Belclare sheep breed (Mullen *et al*. 2013). In Greek Chios and Karagouniki breeds, no significant allele effect weas obtained in the polymorphisms analysis of this mutation (Liandris *et al*. 2012).

Survey of linkage between G1 and G4 indicates that all sheeps that were heterozygous by G1, were also heterozygous by G4. On the contrary, sheeps that were heterozygous for G4 did not necessarily had the G1. Hence, the frequency of the G4 was more than the G1. It seems that there is a local haplotype for these SNPs in most breeds. As it is shown in Table 20, Taleshi, Shal and Naeini breeds have the highest linkage and the Zandi breed shows the lowest linkage in the nucleotide position of two mutations. Considering all animals as a population, three local haplotypes were identified. A similar report indicates a high degree of linkage disequilibrium between these SNPs in Mehraban sheep breed that has been confirmed by the high correlation coefficient values (r^2=0.91) (Talebi *et al*. 2018).

Based on the observed polymorphism in the G1, G4 and FecB mutations of *GDF9* and *BMPR1B* genes, all animals used in the present study were classified in six genotypic groups (Table 21).

Table 17 The polymorphisms of G8 (*FecG*^{H}) reported in some studies[1]

	Breed (N)	(T)	(C)	(CC)	(CT)	(TT)	SAWF	Reference
1	Chios (92)	0.290	0.710	0.652	0.109	0.239	Yes	Liandris *et al.* (2012)
2	Karagouniki (96)	0.120	0.880	0.760	0.240	0.000	No	Liandris *et al.* (2012)
3	Ghezel (185)	0.000	1.000	1.000	0.000	0.000	No	Akbarpour *et al.* (2008)
	Cele Black Sheep (274)	0.000	1.000	1.000	0.000	0.000	No	Shi *et al.* (2010)
	Sicilo-Sarde (51)	0.000	1.000	1.000	0.000	0.000	-	Vacca *et al.* (2010)
	D'man (47)	0.000	1.000	1.000	0.000	0.000	-	
	Barbarine (41)	0.000	1.000	1.000	0.000	0.000	-	
	Noire de Thibar (34)	0.000	1.000	1.000	0.000	0.000	-	
	Queue Fine de L'Ouest (31)	0.000	1.000	1.000	0.000	0.000	-	
	Kordi (42)	0.000	1.000	1.000	0.000	0.000	-	Ghaderi *et al.* (2010)
	Arabic (44)	0.000	1.000	1.000	0.000	0.000	-	
	Dumba (20)	0.000	1.000	1.000	0.000	0.000	-	Vivekanand (2010)
	Marwari (20)	0.000	1.000	1.000	0.000	0.000	-	
	Patanwadi (20)	0.000	1.000	1.000	0.000	0.000	-	
	Garole (22)	0.000	1.000	1.000	0.000	0.000	-	Polley *et al.* (2010)
	Small Tailed Han (154)	0.000	1.000	1.000	0.000	0.000	-	Zhang *et al.* (2011)
	Shandi (35)	0.000	1.000	1.000	0.000	0.000	-	
	Wadi (30)	0.000	1.000	1.000	0.000	0.000	-	
	Big Tailed Han (20)	0.000	1.000	1.000	0.000	0.000	-	
	Awassi (50)	0.000	1.000	1.000	0.000	0.000	No	Esen Gursel *et al.* (2011)
	Chios (50)	0.000	1.000	1.000	0.000	0.000	No	
	Imrose (50)	0.000	1.000	1.000	0.000	0.000	No	
	Kivircik (50)	0.000	1.000	1.000	0.000	0.000	No	
	Bonpala (97)	0.000	1.000	1.000	0.000	0.000	No	Roy *et al.* (2011)
	German Mutton Merino (281)	0.000	1.000	1.000	0.000	0.000	No	Zuo *et al.* (2012)
	Dalagh (100)	0.000	1.000	1.000	0.000	0.000	-	Khatamnejhad and Khanahmadi (2012)
	Finnish Landrace (124)	0.000	1.000	1.000	0.000	0.000	-	Mullen *et al.* (2013)
	Galway (41)	0.000	1.000	1.000	0.000	0.000	-	
	Texel (19)	0.000	1.000	1.000	0.000	0.000	-	
	Bayanbulak (120)	0.000	1.000	1.000	0.000	0.000	No	Zuo *et al.* (2013)
	Lori (60)	0.000	1.000	1.000	0.000	0.000	No	Shafieiyan *et al.* (2013)
	Finnish Landrace (108)	0.000	1.000	1.000	0.000	0.000	No	Mullen and Hanrahan (2014)
	Araucano creole (100)	0.000	1.000	1.000	0.000	0.000	-	Paz *et al.* (2014)
	Black Belly (92)	0.000	1.000	1.000	0.000	0.000	-	Lopez-Ramirez *et al.* (2014)
	Afshari (19)	0.000	1.000	1.000	0.000	0.000	No	Eghbalsaied *et al.* (2014)
	Shal (8)	0.000	1.000	1.000	0.000	0.000	No	
	Austral (120)	0.000	1.000	1.000	0.000	0.000	No	Paz *et al.* (2015)
	Araucana (100)	0.000	1.000	1.000	0.000	0.000	No	
	Chilota (57)	0.000	1.000	1.000	0.000	0.000	No	
	Corriedale (92)	0.000	1.000	1.000	0.000	0.000	-	Vera *et al.* (2015)
	Kendrapada (85)	0.000	1.000	1.000	0.000	0.000	No	Dash *et al.* (2017)
	Hu (2021)	0.000	1.000	1.000	0.000	0.000	No	Wang *et al.* (2018)
	Mehraban (16)	0.000	1.000	1.000	0.000	0.000	-	Talebi *et al.* (2018)
	Afshari (55)	0.000	1.000	1.000	0.000	0.000	-	This study
	Farahani (55)	0.000	1.000	1.000	0.000	0.000	-	
	Grey Shiraz (55)	0.000	1.000	1.000	0.000	0.000	-	
	Turki-Ghashghaei (55)	0.000	1.000	1.000	0.000	0.000	-	
	Naeini (48)	0.000	1.000	1.000	0.000	0.000	-	
	Kalakuei (47)	0.000	1.000	1.000	0.000	0.000	-	
	Moghani (46)	0.000	1.000	1.000	0.000	0.000	-	
	Shal (46)	0.000	1.000	1.000	0.000	0.000	-	
	Taleshi (45)	0.000	1.000	1.000	0.000	0.000	-	
	Makuei (41)	0.000	1.000	1.000	0.000	0.000	-	
	Zandi (39)	0.000	1.000	1.000	0.000	0.000	-	

[1] This table is arranged based on the frequency of the mutant allele, from the highest to the lowest.

SAWF: significant association with fecundity.

Table 18 The polymorphisms of B2 ($FecX^G$) reported in some studies[1]

	Breed (N)	(T)	(C)	(CC)	(CT)	(TT)	SAWF	Reference
1	Small Tailed Han (154)	0.360	0.640	0.270	0.700	0.030	-	Zhang *et al*. (2011)
2	Chios (92)	0.350	0.650	0.400	0.500	0.100	No	Liandris *et al*. (2012)
3	Karagouniki (96)	0.300	0.700	0.610	0.170	0.220	No	Liandris *et al*. (2012)
4	Small Tailed Han (188)	0.300	0.700	0.400	0.600	0.000	Yes	Chu *et al*. (2006)
5	Small Tailed Han (869)	0.200	0.800	0.600	0.400	0.000	No	Wang *et al*. (2015)
6	Hu (761)	0.080	0.920	0.830	0.170	0.000	Yes	Wang *et al*. (2015)
7	Kendrapada (46)	0.000	1.000	1.000	0.000	0.000	-	Kumar *et al*. (2008)
	Garole (34)	0.000	1.000	1.000	0.000	0.000	-	
	Malpura (30)	0.000	1.000	1.000	0.000	0.000	-	
	Deccani (15)	0.000	1.000	1.000	0.000	0.000	-	
	Cele Black Sheep (274)	0.000	1.000	1.000	0.000	0.000	No	Shi *et al*. (2010)
	Sicilo-Sarde (51)	0.000	1.000	1.000	0.000	0.000	-	Vacca *et al*. (2010)
	D'man (47)	0.000	1.000	1.000	0.000	0.000	-	
	Barbarine (41)	0.000	1.000	1.000	0.000	0.000	-	
	Noire de Thibar (34)	0.000	1.000	1.000	0.000	0.000	-	
	Queue Fine de L'Ouest (31)	0.000	1.000	1.000	0.000	0.000	-	
	Dumba (20)	0.000	1.000	1.000	0.000	0.000	-	Vivekanand (2010)
	Marwari (20)	0.000	1.000	1.000	0.000	0.000	-	
	Patanwadi (20)	0.000	1.000	1.000	0.000	0.000	-	
	Garole (22)	0.000	1.000	1.000	0.000	0.000	-	Polley *et al*. (2010)
	Baluchi (152)	0.000	1.000	1.000	0.000	0.000	No	Moradband *et al*. (2011)
	Bonpala (97)	0.000	1.000	1.000	0.000	0.000	No	Roy *et al*. (2011)
	Shandi (35)	0.000	1.000	1.000	0.000	0.000	-	Zhang *et al*. (2011)
	Wadi (30)	0.000	1.000	1.000	0.000	0.000	-	
	Big Tailed Han (20)	0.000	1.000	1.000	0.000	0.000	-	
	German Mutton Merino (281)	0.000	1.000	1.000	0.000	0.000	No	Zuo *et al*. (2012)
	Akkaraman (24)	0.000	1.000	1.000	0.000	0.000	-	Karsli *et al*. (2012)
	Daglic (19)	0.000	1.000	1.000	0.000	0.000	-	
	Ivesi (19)	0.000	1.000	1.000	0.000	0.000	-	
	Karakas (19)	0.000	1.000	1.000	0.000	0.000	-	
	Tuj (15)	0.000	1.000	1.000	0.000	0.000	-	
	Finnish Landrace (124)	0.000	1.000	1.000	0.000	0.000	-	Mullen *et al*. (2013)
	Galway (41)	0.000	1.000	1.000	0.000	0.000	-	
	Hiper-Prolific (41)	0.000	1.000	1.000	0.000	0.000	-	
	Texel (19)	0.000	1.000	1.000	0.000	0.000	-	
	Bayanbulak (120)	0.000	1.000	1.000	0.000	0.000	No	Zuo *et al*. (2013)
	Finnish Landrace (108)	0.000	1.000	1.000	0.000	0.000	-	Mullen and Hanrahan (2014)
	Garole (32)	0.000	1.000	1.000	0.000	0.000	-	Kumar *et al*. (2016)
	Austral (120)	0.000	1.000	1.000	0.000	0.000	No	Paz *et al*. (2015)
	Araucana (100)	0.000	1.000	1.000	0.000	0.000	No	
	Chilota (57)	0.000	1.000	1.000	0.000	0.000	No	
	Mehraban (12)	0.000	1.000	1.000	0.000	0.000	-	Ahmadi *et al*. (2016)
	Santa Ines (574)	0.000	1.000	1.000	0.000	0.000	No	Holanda *et al*. (2017)
	Morada Nova (282)	0.000	1.000	1.000	0.000	0.000	No	
	Kendrapada (85)	0.000	1.000	1.000	0.000	0.000	No	Dash *et al*. (2017)
	Barki (68)	0.000	1.000	1.000	0.000	0.000	No	Nagdy *et al*. (2018)
	Ossimi (27)	0.000	1.000	1.000	0.000	0.000	No	
	Rahmani (20)	0.000	1.000	1.000	0.000	0.000	No	
	Afshari (55)	0.000	1.000	1.000	0.000	0.000	-	This study
	Farahani (55)	0.000	1.000	1.000	0.000	0.000	-	
	Grey Shiraz (55)	0.000	1.000	1.000	0.000	0.000	-	
	Turki-Ghashghaei (55)	0.000	1.000	1.000	0.000	0.000	-	
	Naeini (48)	0.000	1.000	1.000	0.000	0.000	-	
	Kalakuei (47)	0.000	1.000	1.000	0.000	0.000	-	
	Moghani (46)	0.000	1.000	1.000	0.000	0.000	-	
	Shal (46)	0.000	1.000	1.000	0.000	0.000	-	
	Taleshi (45)	0.000	1.000	1.000	0.000	0.000	-	
	Makuei (41)	0.000	1.000	1.000	0.000	0.000	-	
	Zandi (39)	0.000	1.000	1.000	0.000	0.000	-	

[1] This table is arranged based on the frequency of the mutant allele, from the highest to the lowest.

SAWF: significant association with fecundity.

Table 19 The polymorphisms of B4 (*FecX*^{B}) reported in some studies[1]

	Breed (N)	(T)	(G)	(GG)	(GT)	(TT)	SAWF	Reference
1	Karagouniki (96)	0.080	0.920	0.840	0.160	0.000	No	Liandris *et al*. (2012)
2	Chios (92)	0.050	0.950	0.910	0.070	0.020	No	Liandris *et al*. (2012)
3	Cele Black Sheep (274)	0.000	1.000	1.000	0.000	0.000	No	Shi *et al*. (2010)
	Sicilo-Sarde (51)	0.000	1.000	1.000	0.000	0.000	-	Vacca *et al*. (2010)
	D'man (47)	0.000	1.000	1.000	0.000	0.000	-	
	Barbarine (41)	0.000	1.000	1.000	0.000	0.000	-	
	Noire de Thibar (34)	0.000	1.000	1.000	0.000	0.000	-	
	Queue Fine de L'Ouest (31)	0.000	1.000	1.000	0.000	0.000	-	
	Garole (22)	0.000	1.000	1.000	0.000	0.000	-	Polley *et al*. (2010)
	Awassi (50)	0.000	1.000	1.000	0.000	0.000	No	Esen Gursel *et al*. (2011)
	Chios (50)	0.000	1.000	1.000	0.000	0.000	No	
	Imrose (50)	0.000	1.000	1.000	0.000	0.000	No	
	Kivircik (50)	0.000	1.000	1.000	0.000	0.000	No	
	Bonpala (97)	0.000	1.000	1.000	0.000	0.000	No	Roy *et al*. (2011)
	Akkaraman (24)	0.000	1.000	1.000	0.000	0.000	-	Karsli *et al*. (2012)
	Daglic (19)	0.000	1.000	1.000	0.000	0.000	-	
	Ivesi (19)	0.000	1.000	1.000	0.000	0.000	-	
	Karakas (19)	0.000	1.000	1.000	0.000	0.000	-	
	Tuj (15)	0.000	1.000	1.000	0.000	0.000	-	
	German Mutton Merino (281)	0.000	1.000	1.000	0.000	0.000	No	Zuo *et al*. (2012)
	Finnish Landrace (124)	0.000	1.000	1.000	0.000	0.000	-	Mullen *et al*. (2013)
	Lleyn (44)	0.000	1.000	1.000	0.000	0.000	-	
	Galway (41)	0.000	1.000	1.000	0.000	0.000	-	
	Texel (19)	0.000	1.000	1.000	0.000	0.000	-	
	Bayanbulak (120)	0.000	1.000	1.000	0.000	0.000	No	Zuo *et al*. (2013)
	Dorper (39)	0.000	1.000	1.000	0.000	0.000	-	Somarny *et al*. (2013)
	Malin (20)	0.000	1.000	1.000	0.000	0.000	-	
	Finnish Landrace (108)	0.000	1.000	1.000	0.000	0.000	-	Mullen and Hanrahan (2014)
	Dalagh (100)	0.000	1.000	1.000	0.000	0.000	-	Khanahmadi *et al*. (2014)
	Austral (120)	0.000	1.000	1.000	0.000	0.000	No	Paz *et al*. (2015)
	Araucana (100)	0.000	1.000	1.000	0.000	0.000	No	
	Chilota (57)	0.000	1.000	1.000	0.000	0.000	No	
	Mehraban (12)	0.000	1.000	1.000	0.000	0.000	-	Ahmadi *et al*. (2016)
	Kendrapada (85)	0.000	1.000	1.000	0.000	0.000	No	Dash *et al*. (2017)
	Hu (2021)	0.000	1.000	1.000	0.000	0.000	No	Wang *et al*. (2018)
	Chios (77)	0.000	1.000	1.000	0.000	0.000	-	Dincel *et al*. (2018)
	Afshari (55)	0.000	1.000	1.000	0.000	0.000	-	This study
	Farahani (55)	0.000	1.000	1.000	0.000	0.000	-	
	Grey Shiraz (55)	0.000	1.000	1.000	0.000	0.000	-	
	Turki-Ghashghaei (55)	0.000	1.000	1.000	0.000	0.000	-	
	Naeini (48)	0.000	1.000	1.000	0.000	0.000	-	
	Kalakuei (47)	0.000	1.000	1.000	0.000	0.000	-	
	Moghani (46)	0.000	1.000	1.000	0.000	0.000	-	
	Shal (46)	0.000	1.000	1.000	0.000	0.000	-	
	Taleshi (45)	0.000	1.000	1.000	0.000	0.000	-	
	Makuei (41)	0.000	1.000	1.000	0.000	0.000	-	
	Zandi (39)	0.000	1.000	1.000	0.000	0.000	-	

[1] This table is arranged based on the frequency of the mutant allele, from the highest to the lowest.
SAWF: significant association with fecundity.

Table 20 G1 and G4 mutations and three observed local haplotypes

Haplotypes	1	2	3	Linkage between G1 and G4 (%)
Nucleotide position	**G1/G4**	**G1/G4**	**G1/G4**	
Breed (N)/genotype	**GG/GG**	**GG/GA**	**GA/GA**	
Taleshi (45)	38	0	7	100%
Naeini (48)	42	0	6	100%
Shal (46)	44	0	2	100%
Moghani (46)	39	1	6	86%
Farahani (55)	50	1	4	80%
Afshari (55)	50	2	3	60%
Makuei (41)	36	2	3	60%
Grey Shiraz (55)	50	3	2	40%
Turki-Ghashghaei (55)	47	6	2	25%
Kalakuei (47)	41	5	1	17%
Zandi (39)	34	5	0	0%
Total (532)	471	25	36	59%

Table 21 Genotypic classification of Iranian sheep breeds based on three point mutations at *GDF9* and *BMPR1B* genes

Genotypic groups	1	2	3	4	5	6
Nucleotide position	**G1/G4/FecB**	**G1/G4/FecB**	**G1/G4/FecB**	**G1/G4/FecB**	**G1/G4/FecB**	**G1/G4/FecB**
Breed (N)/genotype	**GG/GG/AA**	**GA/GA/AA**	**GG/GA/AA**	**GG/GG/AG**	**GG/GA/AG**	**GG/GG/GG**
Afshari (55)	50	3	2	0	0	0
Taleshi (45)	38	7	0	0	0	0
Shal (46)	44	2	0	0	0	0
Farahani (55)	50	4	1	0	0	0
Moghani (46)	39	6	1	0	0	0
Turki-Ghashghaei (55)	47	2	6	0	0	0
Grey shiraz (55)	50	2	3	0	0	0
Kalakuei (47)	32	1	5	8	0	1
Zandi (39)	34	0	3	0	2	0
Naeini (48)	42	6	0	0	0	0
Makuei (41)	36	3	2	0	0	0
Total (532)	462 (%86.84)	36 (%6.77)	23 (%4.32)	8 (%1.50)	2 (%0.38)	1 (%0.19)

CONCLUSION

Fertility is largely influenced by environmental conditions. Having larger populations, minimizing environmental factors, accurate, multiple and seasonal recordings and repeatability estimation are factors that can better determine the association or disassociation between mutants and the reproductive potential of sheeps. In the present study, the presence or absence of seven reported SNPs known as G1, G4, G7 and G8 in *GDF9* gene, B2 and B4 in *BMP15* gene and *FecB* in *BMPR1B* gene were examined among eleven Iranian native sheep breeds that are different in litter size. The results showed that G7, G8, B2, and B4 mutations were not in any of these breeds, and all of the sheeps were monomorphic with dominant homozygote genotype. *FecB* in the two breeds of Kalakuei and Zandi, G1 in all breeds except the Zandi and G4 mutation in all eleven breeds were identified. The absence of reported major mutations (such as G8, B2, B4, and *FecB*), suggests that minor mutations of fecundity genes and their cumulative effects may have pivotal role in monitoring of fertility traits among Iranian sheeps. It is suggested that to achieve a deep insight on how to control the fertility characteristics of the native sheep in Iran, exploring the major genes controlling fecundity trait in Iranian indigenous sheep breeds is performed through whole genome sequencing and SNP calling approaches.

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