IJAS Iranian Journal of Applied Animal Science

Single Nucleotide Polymorphisms (SNPs) of *GDF9* Gene in Bahmaei and Lak Ghashghaei Sheep Breeds and Its Association with Litter Size

Research Article

M. Muhaghegh Dolatabady[1*] and J. Habibizad[1]

[1] Department of Animal Science, Faculty of Agriculture, Yasouj University, Yasouj, Iran

Received on: 5 Sep 2018
Revised on: 21 Nov 2018
Accepted on: 1 Dec 2018
Online Published on: Sep 2019

*Correspondence E-mail: mmuhaghegh@yu.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

Growth differentiation factor 9 (GDF9) belong to the superfamily of transforming growth factor β that is highly expressed in growing ovarian follicles of oocyte, and it has been strongly related to fecundity traits in sheep. Therefore, the *GDF9* gene could serve as a genetic marker for improvement of reproductive performance in sheep. Therefore, the aim of this study was to investigate the fecundity gene of *GDF9* in two local sheep breeds, Bahmaei and Lak Ghashghaei, reared at Kuhgiloyeh and Boyer-Ahmad province of Iran. For this purpose, genomic DNA was extracted from 20 Bahmaei and 20 Lak Ghashghaei (10 single birth, 10 twin births in each breed) multiparous ewes. The entire length of exon 1 and 2 in ovine *GDF9* gene was amplified using three designed primer pairs, and detection of single nucleotide polymorphisms (SNPs) in the amplified fragments was determined by direct DNA sequencing method. The first and third amplified fragments of ovine *GDF9* showed single genotype in two breeds. Sequencing results of the second fragment (P2) discovered 4 SNPs, including 3 SNPs in coding sequence of exon 2 and one novel in intron of *GDF9* gene. No significant association was obtained between combined genotypes of identified SNPs with litter size in both sheep breeds. In general, the two sheep breeds were different in their genetic structure with regard to the *GDF9* gene. Frequency distributions of identified SNPs in exon 2 *GDF9* gene were different in two sheep breeds.

KEY WORDS fecundity gene, litter size, polymorphism, sheep.

INTRODUCTION

In sheep, improvement of reproduction characteristics is one of the main objectives of breeding programs throughout the world. Reproduction traits such as litter size can be genetically regulated not only by many genes with minor effects but also sometimes by major genes with large effects that called fecundity (Fec) genes. To date, three major genes have been identified to influence prolificacy in sheep, which they mainly belong to superfamily of transforming growth factor-beta (TGF-β), namely, growth differentiation factor 9 (*GDF9*), known as *Fec*G (Hanrahan *et al*. 2004); bone morphogenetic protein 15 (*BMP15*), known as *Fec*X (Galloway *et al*. 2000; Hanrahan *et al*. 2004); and bone morphogenetic protein receptor type 1B (*BMPR1B*), known as *Fec*B (Mulsant *et al*. 2001; Souza *et al*. 2001; Wilson *et al*. 2001).

GDF9 is an oocyte-derived component which is necessary for folliculogenesis, oogenesis, and ovulation which play a crucial role in fertility of female (De Castro *et al*. 2016). The gene encodes for ovine GDF9 map on chromosome 5 (Sadighi *et al*. 2002), and spans about 2.5 kilo base pairs (kb) which contain 2 exons interrupted by an intron with 1126 base pairs (bp). The gene encodes for 453 amino acid prepropeptide, while the long mature peptide is 135 amino acids. Single nucleotide polymorphisms (SNPs) on

sheep *GDF9* have been revealed to influence fecundity traits like ovulation rate and litter size. Several different SNPs in *GDF9* have been reported to be associated with fertility in sheep viz. *Fec*G^{H} (G8) mutation in Cambridge and Belclare sheep (Hanrahan *et al*. 2004) and in Sangsari sheep (Kasiriyan *et al*. 2011), *Fec*G^{E} or *Fec*G^{SI} mutation in Santa Ines sheep (Silva *et al*. 2011), *Fec*T^{T} mutation in Thoka sheep (Nicol *et al*. 2009), G1 mutation in Moghani and Ghezel sheep (Barzegari *et al*. 2009), in Garole sheep (Polley *et al*. 2010) and in Bonpala sheep (Roy *et al*. 2011), *Fec*G^{V} mutation in Brazilian sheep (Souza *et al*. 2012; Souza *et al*. 2014), *Fec*G^{I} mutation in Baluchi sheep (Moradband *et al*. 2011) and G7 in Norwegian White Sheep (Souz *et al*. 2014). The *Fec*G^{E} and *Fec*G^{F} mutations have additive effects on ovulation rate and litter size, while *Fec*G^{H}, *Fec*G^{T} and *Fec*G^{V} causes increased ovulation rate and litter size in the heterozygote ewes and infertility in the homozygous carriers (Souza *et al*. 2014).

Generally, most of the Iranian sheep breeds product single lamb per lambing. The Bahmaei and Lak Ghashghaei sheep are two local breeds of Kohkilouеh and Boyer-Ahmad province in Iran, which were reared for meat production. There is no closed nucleus center for breeding of these two breeds in Iran. Furthermore, the Bahmaei and Lak sheep are reared in nomadic conditions, thus, increase the amount of the litter size could provide better economic status for sheep breeder and lead to immigration preventing to the city. The average twin rate in Bahmaei and Lak Ghashghaei sheep breeds have been reported to be less than 10% in nomadic breeding conditions (Khaldari, 2005). To date, little is known about the genetic structure of this breed with regard to the fecundity genes. Therefore, the aim of this study was to identify the genetic variation in the *GDF9* gene and to evaluate their possible associations with litter size in the Bahmaei and Lak Ghashghaei sheep breeds.

MATERIALS AND METHODS

In this study, based on the history of the last lambing, a total of 40 multiparous ewes (20 Bahmaei sheep and 20 Lak Ghashghaei sheep) were selected, including 10 single births and 10 twin births for each breed, at Kohkilouеh and Boyer-Ahmad province of Iran. Selected animals for Bahmaei and Lak breeds were taken from two different flocks at Dehdasht and Gachsaran regions, respectively. Blood samples were collected from the jugular vein using sample tubes containing ethylenediaminetetraacetic acid (EDTA), and genomic DNA was extracted using AccuPrep® genomic DNA extraction kit (BiONEER, South Korea), according to the manufacturer's instructions. To amplify complete region of exon 1 (397 bp) and 2 (1265 bp), three pairs of primer were designed according to ovine *GDF9* sequence (GenBank accession number AF078545) using Primer3plus (Table 1).

Polymerase chain reaction (PCR) was carried out in 25 µL of total volume containing 25 pm of each primer, 200 µ*M* of each dNTP , 1.5 m*M* $MgCl_2$, 2.5 mM10 × buffer, 1.0 U *Taq* DNA polymerase and 50 ng genomic DNA template. The amplification process was carried out by initial denaturation at 94 ˚C for 5 min and then, 35 cycles at 94 ˚C for 45 s, 63 ˚C for 60 s, and 72 ˚C for 30 s followed by 72 ˚C for 7 min. PCR products were checked by electrophoresis using 1.0% agarose gel in 1 × TBE buffer along with DNA ladder. The purified PCR products were commercially sequenced in one direction (two repeats) and the sequences were analyzed using MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/) and FinchTV 1.4.

Statistical analysis

Data analysis was performed using the general linear model (GLM) procedure of SAS (2012).

Mean separation procedures were performed using the least significant difference test.

$$Y_{ijk} = \mu + B_i + G_j + e_{ijk}$$

Where:
Y_{ijk}: individual observation on litter size.
µ: overall population mean.
B_i: effect of breed.
G_j: effect of combined genotypes of identified SNPs.
e_{ijk}: random error.

RESULTS AND DISCUSSION

The entire length of exon 1 and 2 ovine *GDF9* gene was amplified using one and two pairs of primer, respectively. Then, the PCR products were analyzed by Sanger sequencing method. The obtained nucleotide sequences of amplified fragments were aligned and compared using MUSCLE and FinchTV. Multiple sequence alignment showed that obtained sequences of P1 fragment were identical and share 100% homology with ovine *GDF9* gene. It means that there was no SNP in this part of *GDF9* gene in two studied breeds.

The sequence analysis of P2 fragment revealed 4 transition mutations, at positions 3137G/A, 3375C/T, 3381G/A, and 3625G/A (Figure 1) according ovine *GDF9* sequence (GenBank accession number AF078545). The first novel identified SNP (3137G>A) located at intronic part but the remaining mutations were in coding sequence of exon 2 at positions 471, 477 and 721 corresponded to G2, G3 and G4 mutations reported by Hanrahan *et al*. (2004), respectively.

Table 1 Primer sequence, product size and amplified region of ovine *GDF9*

Fragment	Primer sequence	Product size (bp)	Amplified region
G1	5'-aataaatgccaggggaaagg-3' 5'-agcagggccaactcctttat-3'	740	1522-2261
G2	5'-aacaagcctggcaagtgtct-3' 5'-aggcttccttttagggtgga-3'	837	2932-3748
G3	5'-cctccaccctaaaaggaagc-3' 5'-aacatttggccatgaggaag-3'	726	3747-4472

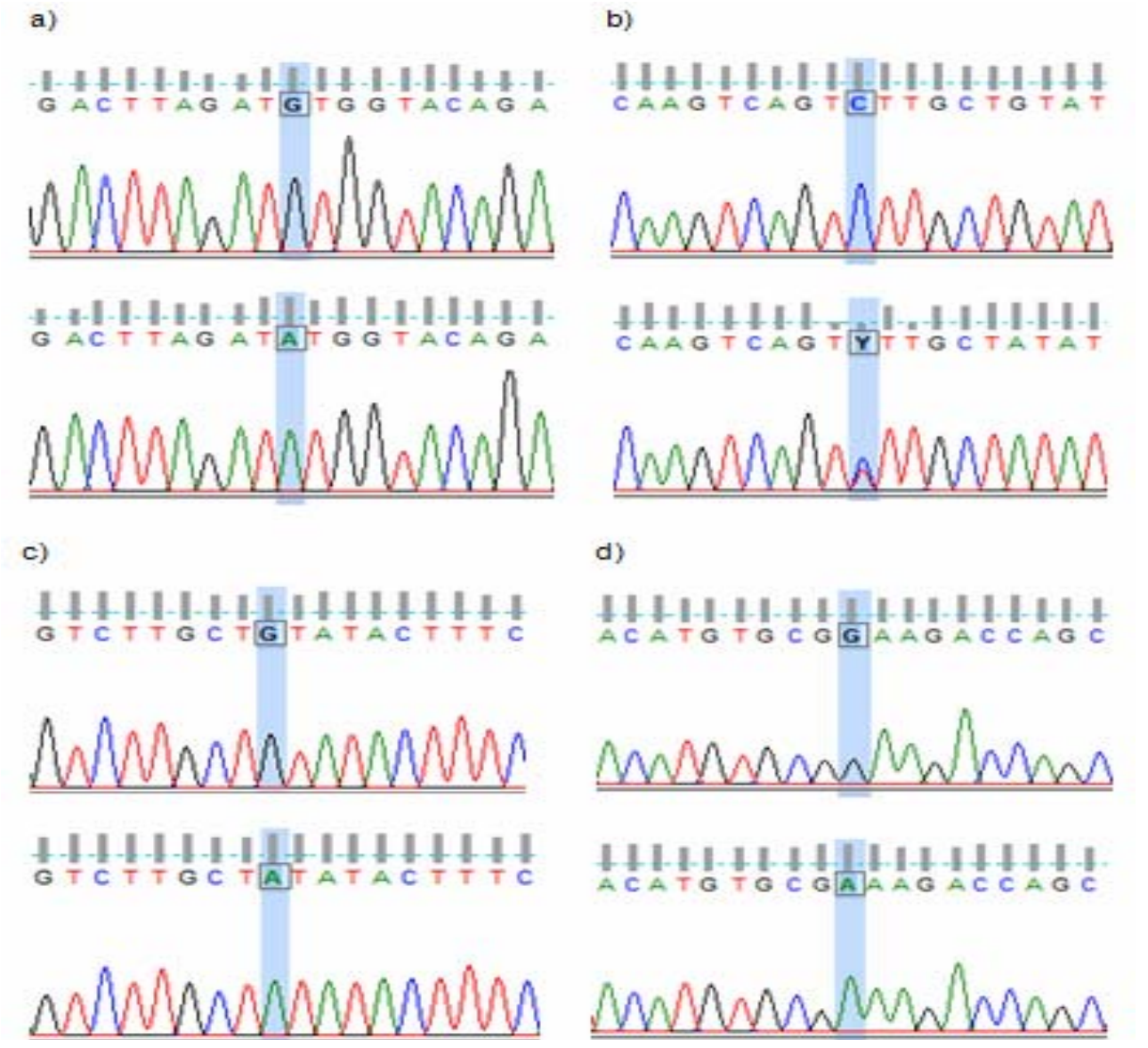

Figure 1 Nucleotide changes of four identified SNPs in GDF9
a) G to A at position 3137 in intron; b) C to T at position 471; c) G to A at position 477 and d) G to A at position 721

SNPs 3137G > A, c477G > A and c721G > A were detected in both breeds while SNP c471C > T was identified only in Bahmaei sheep. In Bahmaei sheep, all three possible genotypes (GG, AG and, AA) were observed for SNPs 3137G > A, c477G > A and c721G > A, while SNP c471C > T revealed wild homozygous (CC) and heterozygous (CT) genotypes. The genetic composition of Lak sheep was different with regard to identified SNPs in P2 fragment. No mutant homozygous genotypes were observed for SNPs 3137G > A, c477G > A and c721G > A in Lak ewes. In addition, all Lak samples were wild homozygous genotype (CC) for SNP c471C > T. An interesting finding of the sequencing results was linkage between mutant alleles of four identified SNPs for P2 fragment in both breeds.

The genotypes and alleles frequencies of four identified SNPs were presented in Table 2. In P3 fragment, that cover second half of exon 2, no genetic variation was found in two breeds. Combined genotype effects of identified SNPs on litter size were not significant; p-values were 0.72 and 0.55 for breed and combined genotype, respectively. Differences between least squares means are given in Table 3. As it shows, mutant homozygous genotypes were not observed in Lak ewes.

In sheep, improvement of fecundity traits by traditional selective breeding has been confirmed to be difficult because of their low heritability. Therefore, use of marker-assisted selection (MAS) along with traditional selection methods could be effective for these traits. To date, several SNPs in fecundity genes have been identified that influence on reproduction traits like ovulation rate and litter size in sheep.

So far, many SNPs have been reported for *GDF9* gene in different sheep breeds throughout the world. For example, eight different SNPs (G1-G8) have been identified in Belclare and Cambridge prolific sheep breeds (Hanrahan *et al*. 2004) which G1 in exon 1, G4, G7 and G8 in exon 2 were the most commonly identified. In our study, four SNPs were detected in two small samples of Bahmaei and Lak sheep. Furthermore, substitutions of A to C in *Fec*T^T allele (Nicol *et al*. 2009), T to G in *Fec*G^E allele (Silva *et al*. 2011), C to T in *Fec*G^V allele (Souza *et al*. 2012), and G to A (Vage *et al*. 2013) were identified in different sheep breeds. In the present study, the three identified SNPs corresponded to G2, G3 and G4 alleles in exon 2 that were discovered in Belclare and Cambridge sheep breeds (Hanrahan *et al*. 2004).

However, based on our results, exon 1 ovine *GDF9* did not show any genetic variation in Bahmaei and Lak sheep, but a novel substitution of G to A was found in intron at position 3137 in Bahmaei ewes.

In *GDF9* gene, as a fecundity gene, some of identified SNPs can increase ovulation rate and litter size, while others have no effect on the ewes' fecundity. In this study, no significant association was found between combined genotypes of identified SNPs and litter size in Bahmaei and Lak Ghashghaei breeds of Iran. However, in Bahmaei sheep, ewes with combined genotypes of AATCAAAA and AGCCAGAG showed lower and higher least square mean for litter size, respectively. The sequencing result of *GDF9* cDNA revealed the G2, G3, and G4 mutations in Iranian Afshari sheep (Eghbalsaied *et al*. 2012) which is in agreement with our results. They also reported that the G4 mutation was only observed in ewes ovaries defined superficially as top quality. No significant difference was found between different genotypes of SNP c477G > A (G3) with litter size of ewes in Laticauda and Bagnolese sheep breeds (Albarella *et al*. 2015). However, with respect to SNP c750G > A, the GG homozygous ewes displayed a significantly higher value of litter size compared with GA heterozygous individuals (2.06 *vs*. 1.75) in Bagnolese sheep (Albarella *et al*. 2015). The SNPs G1, G4 and G8 of the ovine *GDF9* gene were found to be heterozygous with no evidence of infertility for the homozygous individuals in the Chios sheep breed (Liandris *et al*. 2012). Two mutations G1 and G4 were observed in Indian Bonpala sheep but their associations with litter size were not significant (Roy *et al*. 2011). Four SNPs c.471C > T, c.477G > A, c.721G > A and c.978A > G were reported in *GDF9* of Ashgar, Dubasi and Watish sheep breeds in Sudanese, but no significant differences were found in allele or genotype frequencies between the two groups differing in litter size for any of these SNPs (Ali *et al*. 2016). The G1 SNP was found in four fat-tailed sheep breeds of Iran, Afshari, Baluchi, Makui, Mehraban, and it had a litter size of 1.78 ± 0.07 in heterozygous sheep, while in homozygous wild type ewes, the average litter size was 1.16 ± 0.05.

Table 2 Allele and genotype frequencies at four identified mutations in two breeds

SNP	Allele frequency				Genotype frequency					
	Bahmaei		Lak		Bahmaei			Lak		
3137G > A	G	A	G	A	GG	AG	AA	GG	AG	AA
	0.7	0.3	0.875	0.125	0.60	0.20	0.20	0.75	0.25	-
c471C > T	C	T	C	T	CC	CT	CC	CC	CT	TT
	0.925	0.075	10	-	0.85	0.15	0.0	1.00	-	-
c477G > A	G	A	G	A	GG	AG	AA	GG	AG	AA
	0.7	0.3	0.875	0.125	0.60	0.20	0.20	0.75	0.25	-
c721G > A	G	A	G	A	GG	AG	AA	GG	AG	AA
	0.7	0.3	0.875	0.125	0.60	0.20	0.20	0.75	0.25	-

Table 3 Least squares means for litter size of ewes according to combined genotypes of *GDF9* gene

Combined genotype	AACCAAAA	AATCAAAA	AGCCAGAG	GGCCGGGG
Bahmaei	1.50	1.00	1.75	1.5
Lak	-	-	1.40	1.53

None of the individuals analyzed showed homozygote for this SNP (Javanmard *et al*. 2011). The *Fec*G^{V} SNP was reported in Ill de France sheep in Brazil and contributed to an ovulation rate of 2.1 ± 0.1 in homozygous ewes of SNP, while the ovulation rate for ewes without this SNP was 1.2 ± 0.1 (De Soza *et al*. 2012). The *Fec*G^{E} variant was found in Santa Ines sheep.

The homozygous ewes for this SNP had multiple ovulations approximately 96.3%, whereas multiple ovulations in heterozygous and homozygote non carrier's ewes were approximately 31% and 14%, respectively, meaning the SNP has additive action (Silva *et al*. 2011). In another study, significant differences in allelic frequencies of *Fec*G^{E} were not observed between Elite/Superior and Regular/Inferior groups of ewes in Morada Nova sheep, but the effect of *Fec*G^{E} on litter size showed positive influence in profitability in medium to high input farm systems (Lacerda *et al*. 2016). The *Fec*G^{F} (G7) variation was the only polymorphism present on Finnish Landrace sheep. However, no significant effect was found between *Fec*G^{F} and reproduction performance of Finnish Landrace sheep. All ewes in the high selection group were homozygous for *Fec*G^{F}, in the low group but none of the ewes were homozygous for this SNP (Mullen and Hanrahan, 2014). Vage *et al*. (2013) showed that a polymorphism in the *GDF9* that had previously been identified but not considered to have an effect on ovulation rate, is present in Norwegian white sheep and gives an increase in ovulation rate.

CONCLUSION

Polymorphism of *GDF9* gene as a candidate gene for fecundity traits was studied in two sheep breeds of Kohkiloueh and Boyer-Ahmad province of Iran. The results display that genetic structure of two breeds was different with respect to *GDF9* gene. However, three SNPs 3137G > A, c471C > T and c477G > A were observed in two breeds but their genotype frequencies were different. The Lak Ghashghaei ewes were all homozygous wild-type (CC) genotype for SNP c.471C > T, while Bahmaei samples revealed CC and CT genotypes with frequencies of 0.85 and 0.15, respectively. However, no significant association was found between the different combined genotypes of this candidate gene and litter size in both breeds, but further studies with more number of ewes are required to confirm the result of this research.

ACKNOWLEDGEMENT

This study was supported by Yasouj University of Iran. The author thanks from farmer who provided samples for this study.

REFERENCES

Albarella S., Ciotola F., Selvaggi M., Dario C., Licciardi S., Scopino G., Frate D. and Peretti V. (2015). Analysis of major fecundity genes in autochthonous Laticauda and Bagnolese sheep breeds. *Small Rumin. Res*. **133,** 118-122.

Ali A.S., Ibrahim M.T., Mohammed M.M., Elobied A.A. and Lühken G. (2016). Growth differentiation factor 9 gene variants in Sudanese desert sheep ecotypes. *South African J. Anim. Sci*. **46,** 373-379.

Barzegari A., Atashpaz S., Ghabili K., Nemati Z., Rustaei M. and Azarbaijani R. (2009). Polymorphisms in GDF9 and BMP15 associated with fertility and ovulation rate in Moghani and Ghezel sheep in Iran. *Reprod. Domest. Anim*. **45,** 666-669.

De Castro F.C., Cruz M.H. and Leal C.L. (2016). Role of growth differentiation factor 9 and bone morphogenetic protein 15 in ovarian function and their importance in mammalian female fertility-A review. *Asian-Australasian J. Anim. Sci*. **29,** 1065-1074.

De Soza C.J., McNeilly A.S., Benavides M.V., Melo E.D. and Moraes J.C. (2012). Novel GDF9 polymorphism determining higher ovulation. Pp. 87-91 in Proc. Soc. Reprod. Fertil. Annu. Conf., Edinburgh, United Kingdom.

Eghbalsaied S., Ghaedi K., Shahmoradi S., Pirestani A., Amini H., Saiedi T., Nicol L. and McNeilly A. (2012). Presence of SNPs in GDF9 mRNA of Iranian Afshari sheep. *Int. J. Fertil. Steril*. **5,** 225-230.

Galloway S.M., McNatty K.P., Cambridge L.M., Laitinen M.P., Juengel J.L., Jokiranta T.S., McLaren R.J., Luiro K., Dodds K.G., Montgomery G.W., Beattie A.E., Davis G.H. and Ritvos O. (2000). Mutations in an oocyte-derived growth factor gene (BMP-15) cause increased ovulation rate and infertility in a dosage sensitive manner. *Nat. Genet*. **25,** 279-283.

Hanrahan J.P., Gregan S.M., Mulsant P., Mullen M., Davis G.H., Powell R. and Galloway S.M. (2004). Mutations in the genes for oocyte-derived growth factors GDF-9 and BMP-15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biol. Reprod*. **70,** 900-909.

Javanmard A., Azadzadeh N. and Esmailizadeh A.K. (2011). Mutations in bone borphogenetic protein 15 andgrowth differentiation factor 9 genes are associated with increased litter size in fat-tailed sheep breeds. *Vet. Res. Commun*. **35,** 157-167.

Khaldari M. (2005). Principles of Sheep and Goat Husbandry. Jahad Daneshgahi Publication, Tehran, Iran.

Kasiriyan M.M., Hafezian S.H. and Hassani N. (2011). Genetic polymorphism *BMP15* and *GDF9* genes in Sangsari sheep ofIran. *Int. Genet. Mol. Biol*. **3,** 31-34.

Lacerda T.S., Caetano A.R., Faco O., de Faria D.A., McManus C.M., Lobo R.N., de Moraes Silva K. and Paiva S.R. (2016). Single marker assisted selection in Brazilian Morada Nova hair sheep community-based breeding program. *Small Rumin. Res*. **139,** 15-19.

Liandris E., Kominakis A., Andreadou M., Kapeoldassi K., Chadio S., Tsiligianni T., Gazouli M. and Ikonomopoulos I. (2012). Associations between single nucleotide polymer phisms of *GDF9* and *BMP15* genes and litter size in two dairy

sheep breeds of Greece. *Small Rumin. Resh*. **107,** 16-21.

Moradband F., Rahimi G. and Gholizadeh M. (2011). Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-1B with litter size in Iranian Baluchi sheep. *Asian-Australasian J. Anim. Sci.* **24,** 1179-1183.

Mullen M.P. and Hanrahan J.P. (2014). Direct evidence on the contribution of a missense mutation in GDF9 to variation in ovulation rate of Finn sheep. *PloS one*. **9,** e95251.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., Pisselet C., Riquet J., Monniaux D., Callebaut I., Cribiu E., Thimonier J., Teyssier J., Bodin L., Cognie Y., Chitour N. and Elsen J.M. (2001). Mutation in the bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola ewes. *Proc. Natl. Acad. Sci*. USA. **98,** 5104-5109.

Nicol L., Bishop S.C., Pong-Wong R., Bendixen C., Holm E.L., Rhind S.M. and McNeilly A.S. (2009). Homozygosity for a single base-pair mutation in the oocyte specific *GDF9* gene results in sterility in Thoka sheep. *Reproduction*. **138,** 921-933.

Polley S., De S., Brahma B., Mukherjee A., Vinesh P.V., Batabyal S., Arora J.S., Pan S., Samanta A.K., Datta T.K. and Goswami S.L. (2010). Polymorphism of *BMPR1B*, *BMP15* and *GDF9* fecundity genes in prolific Garole sheep. *Trop. Anim. Health Prod*. **42,** 985-993.

Roy J., Polley S., De S., Mukherjee A., Batabyal S., Pan S., Brahma B., Datta T.K. and Goswami S.L. (2011). Polymorphism of fecundity genes (*Fec*B, *Fec*X, and *Fec*G) in the Indian Bonpala sheep. *Anim. Biotechnol.* **22,** 151-162.

Sadighi M., Bodensteiner K.J., Beattie A.E. and Galloway S.M. (2002). Genetic mapping of ovine growth differentiation factor 9 (GDF9) to sheep chromosome 5. *Anim. Genet*. **33,** 244-245.

SAS Institute. (2012). SAS®/STAT Software, Release 8.1. SAS Institute, Inc., Cary, NC. USA.

Silva B.D., Castro E.A., Souza C.J., Paiva S.R., Sartori R., Franco M.M., Azevedo H.C., Silva T.A., Vieira A.M., Neves J.P. and Melo E.O. (2011). A new polymorphism in the growth and differentiation factor 9 (*GDF9*) gene is associated with increased ovulation rate and prolificacy in homozygous sheep. *Anim. Genet.* **42,** 89-92.

Souza C.J., MacDougall C., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. (2001). The Boorola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (*BMPR1B*) gene. *J. Endocrinol*. **169,** 1-6.

Souza C.J., McNeilly A.S., Benavides M.V., Melo E.O. and Moraes J.C. (2014). Mutation in the protease cleavage site of GDF9 increases ovulation rate and litter size in heterozygous ewes and causes infertility in homozygous ewes. *Anim. Genet*. **45,** 732-739.

Souza C.J., McNeilly A.S., Benavides M.V., Melo E.O. and Moraes J.C. (2012). Novel GDF9 polymorphism determining higher ovulation rate and litter size in sheep. Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/72796/1/Souza-SRF-2012O005.pdf.

Vage D.I., Husdal M., Matthew P.K., Klemetsdal G. and Boman I.A. (2013). A missense mutation in growth differentiation factor-9 (GDF-9) is strongly associated with litter size in sheep. *BMC Genet*. **14,** 1-9.

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. (2001). Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB Receptor (Alk-6) that is expressed in both oocytes and granulosa cells. *Biol. Reprod*. **64,** 1225-1235.