Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Allelic polymorphism in exon 1 of *GDF9* and exon 2 of *BMP15* genes and its impact on litter size at lambing in Iran-Black sheep

Vahideh Rezaei, Ali Esmailizadeh*, Ahmad Ayatollahi Mehrgardi, Mostafa Dehghani Qanatqestani

Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

Abstract The main purpose of this study was to assess the presence of the previously reported single nucleotide polymorphisms (SNPs) in the sheep growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (*BMP15*) genes and their association with litter size at lambing in Iran-Black sheep breed. Blood samples were taken from 120 Iran-Black ewes. DNA extraction was conducted using a modified salting-out method. DNA fragments with sizes of 462bp and 141bp for the *GDF9* and *BMP15* genes were amplified using PCR with specific primers, respectively. The PCR-RFLP approach was adopted for detecting the genotypes. The results indicated that the SNP in the exon 2 of *BMP15* is a monomorphic locus in Iran-Black sheep. However, the substitution of G to A nucleotide was determined in the *GDF9* locus. Digestion of the 462bp PCR product from exon 1 of the *GDF9* using the *HhaI* restriction enzyme produced fragments of 52, 156, and 254bp. However, DNA fragments containing the A nucleotide yielded only two fragments (52 and 410bp). The heterozygous animals for this mutation in *GDF9* locus had fragments of all four sizes (52, 156, 254, and 410bp). The frequency (0.75) of the wild type allele (+) in *GDF9* locus was higher than the frequency (0.25) of mutant allele (G). The observed frequencies for the GG, G+ and ++ genotypes were 0.05, 0.40 and 0.55, respectively. The association results indicated that the mutation of *GDF9* gene has a substantial impact on lambing rate and the Iran-Black ewes with the GG and G+ genotypes had higher lambing rate than those with the ++ genotype. Thus, a gene assisted selection program to improve lambing rate in this breed can be designed based on the *GDF9* gene mutation.

Keywords: *BMP15*, *GDF9*, Iran-Black sheep, lambing rate

Paper type: Research Paper

*Corresponding author,
E-mail address:
aliesmaili@uk.ac.ir

Received: 25 Nov. 2019,
Accepted: 11 Nov. 2020,
Published online: 01 Dec. 2020.

Introduction

Molecular genetics tools have provided new opportunities to enhance the breeding programs in livestock by allowing the use of DNA markers to identify genes or genomic regions that control traits of interest. The obvious first application of these methods was to discover the genetic basis and develop genetic tests for single gene defects (Dekkers, 2012). For quantitative traits, these advances promised the identification of quantitative trait loci (QTL) and the development of DNA tests that could be done on all selection candidates at an early age to help inform selection decision through

marker-assisted selection (MAS), i.e. selection on a combination of information derived from genetic markers associated with QTL and the traditional phenotypic information (Smith and Simpson, 1986). The use of DNA markers to define the genetic makeup (genotype) and predict the performance of an animal is a powerful aid to animal breeding. Molecular techniques allow detecting variation or polymorphisms among individuals in the population for specific regions of the DNA. These polymorphisms can be used to build up genetic maps and to evaluate differences between markers in the expression of particular traits in a family that might indicate a direct effect of these differences in terms of genetic determination on the trait (Montaldo and Meza-Herrera, 1998). Linkage analysis, association analysis and analysis of gene function can be used to determine which polymorphisms are useful markers for desirable traits (Beuzen et al., 2000). Another application of the molecular genetics methods is assessing the level of diversity in the livestock populations. Genetic diversity can be defined as the set of differences between species, breeds within species and individuals within breeds present in their DNA or observed in animals as a consequence (Woolliams and Oldenbroek, 2018). Thus, it is necessary to preserve species and breeds genetic resources, prioritizing selection strategies for the present-day demands, such as adaptations to the changing environments, enhanced immune responses or consumer demands. Other important reasons to preserve genetic diversity in livestock populations are the insurance against the losses of genetic resources from local adapted populations with socio-economic, cultural, historic and ecological value (Eusebi et al., 2020). Accordingly, genetic diversity analysis has been essential to reconstruct genetic events that shaped the diversity parameters of current populations, including ancestry, prehistoric and historical migrations, admixture events and genetic isolation (Caballero and Toro, 2000).

The genetic diversity of the world's livestock populations is decreasing, both within and across breeds (Eusebi et al., 2020). Conservation of livestock genetic variability is thus important, especially when considering possible future changes in production environments. The Iranian native sheep breeds are fat tailed, carpet-wool breeds adapted to different regions (Farid et al., 1977). Hence, identifying an adaptive breed may promote economic situation of a society. Iran-Black, a crossbred sheep, developed by crossing Baluchi ewes with Chios rams in 1975 at the Abbasabad sheep breeding station situated in Razavi Khorasan province, Iran, is well-adapted to the harsh climate conditions (Rashidi, 2013). At the Abbasabad sheep breeding station, two main breeding objectives were designed, improvement of the sheep production efficiency and the dissemination of genetically superior animals into the local flocks. The profitability of sheep breeding systems is substantially affected by reproductive performance (Matos et al., 1997). However, the most important factor influencing the overall sheep productivity is the ewe production efficiency. The number of lambs weaned and weight of lambs weaned per ewe per year have been proposed to be the most economically important traits (Snyman et al., 1997; Duguma et al., 2002); thus litter size at lambing might be a useful trait for improving the reproductive efficiency.

Variation in litter size at lambing is controlled by both genetic and environmental factors (Hanrahan et al., 2004). The families of transforming growth factor-β (TGF-β) proteins are the most important growth factors in the ovary and are responsible for growth and differentiation of early ovarian follicles. Three related oocyte-derived members of the TGF-β superfamily, namely GDF9, BMP15 and BMPR-IB, have been shown to be essential for follicular growth and ovulation (Hanrahan et al., 2004). In sheep, three prolificacy loci have been discovered, namely bone morphogenetic protein receptor type 1B (*BMPR1B*; or activin-like kinase 6, *ALK6*), known as FecB (Booroola) on chromosome 6 (Souza et al., 2001); growth differentiation factor 9 (*GDF9*), known as FecG on chromosome 5 (Hanrahan et al., 2004); and bone morphogenetic protein 15 (*BMP15*) known as FecX on chromosome X (Galloway et al., 2000; Hanrahan et al., 2004). The full length coding sequence of 1179 nucleotides of the BMP15 gene is contained in two exons, separated by an intron of about 5.4 kb, and encodes a prepropeptide of 393 amino acid residues. The active mature peptide is 125 amino acids long (Galloway et al., 2000). Six different mutations have been identified in the BMP15 gene, each having a major effect on sheep prolificacy. These mutations included FecXI in Inverdale breed (Galloway et al., 2000), FecXH in Hanna breed (Galloway et al., 2000), FecXL in Lacaune (Bodin et al., 2003, 2007), FecXG in Galway (Hanrahan et al., 2004), FecXB in Belclare (Hanrahan et al., 2004) and a 17bp deletion of the functional gene (FecXR) in Rasa Aragonesa sheep breed (Monteagudo et al., 2009). Ewes with two inactive copies of the BMP15 gene (homozygous animals) are sterile (Galloway et al., 2000; Hanrahan et al., 2004). Ewes with a

single inactive *BMP15* gene (heterozygous animals) are fertile and have an increased ovulation rate and a higher incidence of twin or triplet births (Davis et al., 2002, 2005; Galloway et al., 2000; Hanrahan et al., 2004).

The *GDF9* gene spans about 2.5 kilobases (kb) and contains 2 exons separated by a single 1126-base pair (bp) intron and encodes a prepropeptide of 453 amino acid residues. The active mature peptide is 135 amino acids long (Bodensteiner et al., 1999). Eight different point mutations (G1-G8) have been identified in the *GDF9* gene. Three out of the eight polymorphisms are nucleotide changes that do not result in an altered amino acid (G2, G3 and G5). The five remaining nucleotide changes, G1, G4, G6, G7, and G8, give rise to amino acid changes. The G1 arginine to histidine change at amino acid residue 87 in exon 1 substitutes one basic charged polar group with another and occurs at a position before the furin cleavage site for the mature peptide, so is unlikely to affect the activity of the mature protein (Hanrahan et al., 2004).

In the present study, a candidate gene approach was used to identify the basis for genetic variation in the litter size at lambing rate in Iran-Black sheep. The main purpose of this study was to assess the presence of the previously reported single nucleotide polymorphisms (SNPs) in the sheep, *GDF9* and *BMP15* genes, and their association with litter size at lambing in this breed.

Materials and methods

Sample collection and DNA isolation

Jugular blood samples (2 mL per ewe) from 120 Iran-Black sheep were collected into the blood collection tubes containing EDTA as anticoagulant, and transferred to the Molecular Genetics Laboratory, Department of Animal science, Shahid Bahonar University of Kerman, Iran, using cooling chain and stored at -20°C for further analysis. Six clotted blood samples, not suitable for DNA extraction, were discarded. Genomic DNA was isolated by a modified salting-out procedure described by Miller et al. (1988) and the DNA samples were dissolved in Elution buffer (pH= 8.0) and stored at -20 °C for further analysis. The quantity and quality of the extracted DNA were checked by spectrophotometer and %1 agarose gel electrophoreses. DNA samples were adjusted to a concentration of 50 ng/µL and exactly 2 µL of the DNA samples were used as template for polymerase chain reaction (PCR). Primers were synthesized by CinnaGen Company (Iran) based on the sequences described by Hanrahan et al. (2004) and the amplification procedure was carried out based on the method described by Davis et al. (2002).

Single nucleotide polymorphism detection assays

A primer pair was used to perform genotyping on exon 1 of *GDF9* gene in Iran-Black sheep by PCR-RFLP. The primers amplified a 462-bp fragment and were designed based on Hanrahan et al. (2004) as follow:

Forward primer:
5'-GAAGACTGGTATGGGGAAATG
Reverse primer:
5'-CTACACACCTCCAATCTGCTC

A primer pair was also used to perform genotyping on exon 2 of *BMP15* gene in IRAN-Back sheep. The primers amplified a 141bp fragment and were designed based on Hanrahan et al. (2004) as shown below:

Forward primer:
5'-CACTGTCTTCTTGTTACTGT
Reverse primer:
5'-GATGCAATACTGCCTGCTT

Total volume of 25 µL of each PCR reaction contained 2 µL of genomic DNA (50 ng/µL) , 1.5 mM MgCl2, 1 µL of each primer, 2.5 µL 10X PCR buffer, 0.5 µL dNTPs, 1unit Taq DNA Polymerase, 16 µL distilled water. The amplification conditions for primers of the *GDF9* gene were as follow:

The amplification reaction was carried out using 35 cycles at 94°C for 5 min, followed by 94°C for 45 s, 58°C for 40 s, 72°C for 1 minute and final extension at 72°C for 10 min. The 462bp PCR products were digested at 37°C for 16 h with *Hhal* restriction enzyme.

The amplification conditions for primers of the *BMP15* gene were as follows:

The amplification reaction was carried out using 35 cycles at 94°C for 5 min, followed by 45 s, 63°C for 40 s, 72˚ C for 60 s, followed by 72°C for 10 min. The 141bp PCR products were digested at 37˚ C for 8h with *Hinf* I restriction enzyme.

The obtained fragments were separated by electrophoresis on 3% agarose for 80 min. The gels were stained with ethidium bromide and the bands were visualized under the gel documentation system.

Statistical analysis

The POPGENE software (https://sites.ualberta.ca/~

fyeh/popgene.html) was used to calculate the population genetics parameters. The following fixed effects model was employed for analysis of lambing rate, and the least squares means were used for multiple comparisons in litter size among different genotypes.

$$y_{ijkl} = \mu + A_i + B_j + G_k + P_l + e_{ijkl}$$

where, y_{ijkl} is the phenotypic value of the litter size at lambing, μ is the population mean, A_i is the fixed effect of i^{th} ewe lambing year, B_j is the fixed effect of j^{th} ewe birth type (i = 1, 2, 3), G_k is the fixed effect of the k^{th} genotype, P_l is the fixed effect of the l^{th} parity (l = 1, 2, 3, 4) and e_{ijk} is the random residual effect of each observation. Analysis was performed using the GLM procedure (SAS, 2002).

Results and discussion

The total litter weight weaned per breeding ewe has been regarded as an important economic trait in sheep genetic improvement programs (Snowder, 2008; Esmailizadeh et al., 2011). Although the reproductive traits are considered as quantitative traits with basically a polygenic inheritance, it has been shown that most of these traits in sheep are controlled by major effect genes (Hanrahan et al., 2004; Esmailizadeh, 2010; Esmailizadeh, 2014; Nosrati et al., 2019). Thus, the discovery of genes with major effects on ovulation rate and consequently the number of lambs at birth has been the focus of many studies. Three important candidate genes identified for reproductive performance in sheep are *GDF9*, *BMP15*, and BMPIR-1B. We characterized the mutations of G1 in *GDF9* and B2 in *BMP15* and their association with prolificacy in Iran-Black sheep, a synthetic breed resulted by crossing the Iranian local Baluchi ewes and Chios rams originated from Chios Island in Greece. This breed was developed since 1975 to increase the reproduction efficiency with the particular emphasis on wool production and environmental adaptation.

Quality of the extracted DNA and PCR results

The quality of DNA extracted from 500 µL of blood by using the salting-out was determined using electrophoresis on 0.1 agar gel (Figure 1). High concentrations of DNA with high quality suitable for our aim were obtained for all of the 114 samples.

The PCR reactions for identifying the mutation of G1 in *GDF9* were performed utilizing a pair of primers (Hanrahan et al., 2004) made by Cinnagen Company, Iran. Following the PCR reactions, 5 µL of the PCR products with 1 µL of Loading Buffer were placed on 1.6% agar gel for 45 minutes, stained by Ethidium Bromide, and the accuracy of PCR reaction was verified and the amplification of a 462bp fragment of exon 1 in *GDF9* was confirmed (Figure 2).

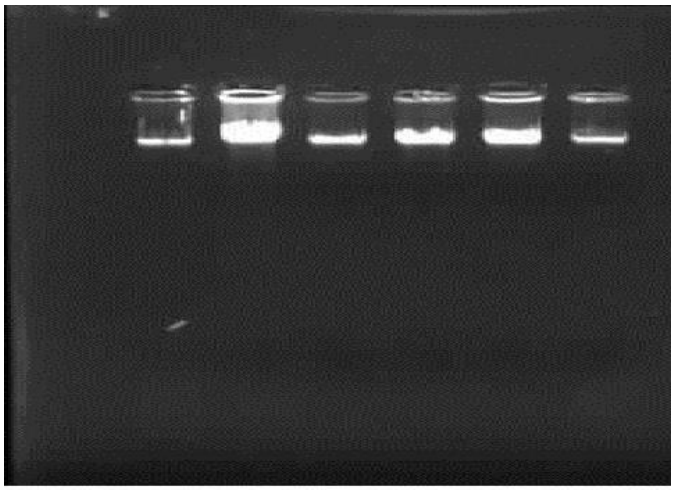

Figure 1. Quality determination of extracted DNA using salting-out method on 0.1 agarose gel

The PCR products were digested by *Hhal* enzyme (Fermentas Company, USA) and were electrophoresed in 3% agarose gel with 100 voltages for 80 minutes to determine the genotypes. The *Hhal* enzyme had the ability to identify four nucleotides with the sequence of

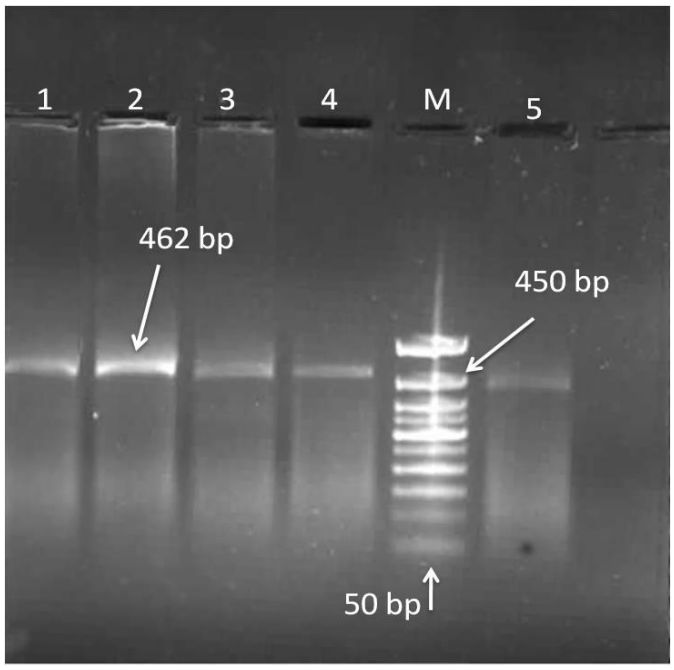

Figure 2. The amplification of 462bp fragment of exon 1 in *GDF9* using PCR. M: 50bp marker for molecular weight determination of the samples.

GCGC in DNA string. In the wild allele, there were two fragment sites on two spots of the amplified segment of the exon 1 in *GDF9*. However, in the mutated allele, due to the replacement of the nucleotide G with A, the enzyme could only identify one spot. Based on the enzyme digestion results for *GDF9* by Hanrahan et al. (2004), if there is a change of G to A, the wild genotype (++) will produce three fragments with sizes of 254, 156 and 52bp. If such a change occurs in the mutated allele (G), two fragments with sizes of 52 and 410bp will be produced. Heterozygous genotype (G/+) in both types of allele will make four fragments with sizes of 254, 156, 410, and 52bp. In our study, similar band patterns to those of Hanrahan et al. (2004) were observed (Table 1) and all of the three expected genotypes (+/+), (G/+), and (G/G) were observed in the samples (Figure 3). The obtained pattern of digestion of PCR products in *GDF9* by *Hhal* enzyme matches with the patterns reported by others (Hanrahan et al., 2004; Michailidis et al., 2008; Moradband et al., 2011).

Concerning the mutation of B2 in *BMP15,* as noted by Hanrahan et al. (2004), the digestion of PCR products by *Hinf* I for the wild homozygous individuals (+/+) will lead to cutting 141bp segment and creating 111 and 30bp segments while for the heterozygous genotype the 141 and 11bp segments are obtained, and for the homozygous mutant genotype (B/B) only the 141bp segment is acquired. In our study, all of the PCR products digested by the *Hinf* I only showed 111 and 30bp segments (Figure 4) implying that the exon 2 in *BMP15* is a monomorphic locus in Iran-Black sheep. These findings agreed with the report of Michailidis et al. (2008) and Moradband et al. (2011) which found that there was no mutation of *BMP15* in the Chios and Baluchi sheep, the breeds used in the crossbreeding program to create the Iran-Black breed.

The level of heterozygosity is the most common criterion of gene diversity in a population that is normally reported as observed heterozygosity (H_O), expected heterozygosity (H_E) and unbiased heterozygosity (H_{Nei}). Shannon information index (I) is also one of the criteria

Table 1. Number of bands resulted from enzyme digestion, and the size of each band in diverse genotypes in exon 1 in *GDF9* gene in Iran-Black sheep

	Genotype		
	G/G	G/+	+/+
Fragment Size (bp)	410	410	-
	-	254	254
	-	156	156
	52	52	52

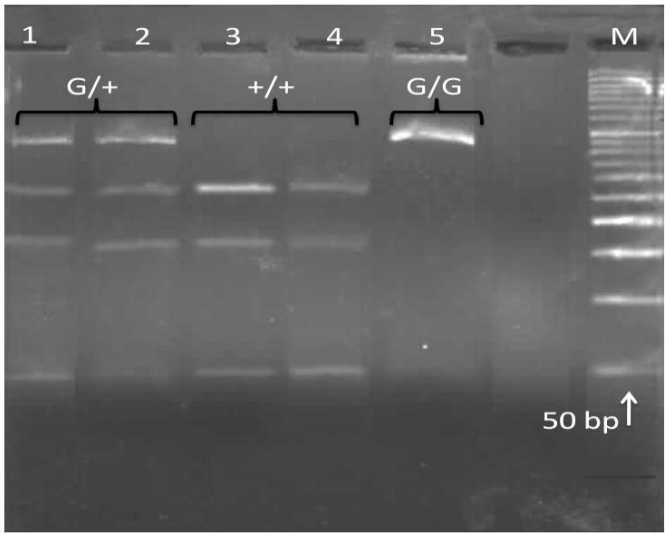

Figure 3. Digestion of PCR products of exon 1 in *GDF9* by using *Hhal* enzyme in Iran-Black sheep. Numbers 1 and 2 are heterozygous genotype (G/+), numbers 3 and 4 are wild homozygous genotype (+/+), and number 5 is homozygous mutant genotype (G/G). M indicates 50bp for molecular weight determination of the bands.

that is utilized for gene diversity and its high value is an indicator of high gene diversity in the intended population. In the present study, Shannon information index was 0.51 and the observed heterozygosity was calculated as 0.38 indicating a relatively high diversity in this population. Other criteria such as the number of effective alleles (Ne) and the number of actual alleles (Na) were 1.6 and 2.0, respectively.

The frequencies of the mutant and the wild alleles

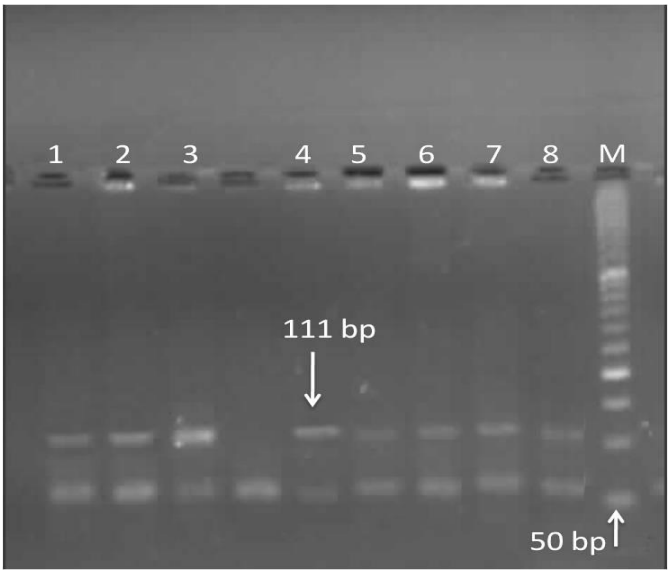

Figure 4. The digestion of PCR product of exon 2 in *BMP15* using *Hinf* I enzyme in Iran-Black sheep. The lanes 1 to 8 are wild homozygous genotype (+/+). M is DNA ladder M50.

were estimated as 0.25 and 0.75 leading to the 0.05, 0.40 and 0.55 frequencies for the GG, G+ and ++ genotypes, respectively. The Chi-squared test was used to test the Hardy-Weinberg equilibrium for the exon 1 in *GDF9* and the results showed that expected genotypic frequencies were not significantly deferent form those of the observed values indicating that the studied population is in the Hardy-Weinberg equilibrium for this locus.

Based on the statistical association results, the effect of *GDF9* genotype on litter size at lambing was significant (Table 2). The ewes with the GG and G+ genotypes had higher lambing rate than those with the ++ genotype. These results agreed with others (Hanrahan et al., 2004; Moradband et al., 2011) that reported the *GDF9* gene in the state of heterozygous improved the ovulation rate and consequently the lambing rate. Mutation of G1 (change of arginine to histidine) that happens in amino acid number 87 in exon 1 of the *GDF9* gene in which one polar group is replaced by another group affects the performance of the complete protein. We found that the ewes carrying at least one mutant allele in the *GDF9* gene produce more lambs than those of the wild type. When we modeled both the additive and dominance gene effects for the *GDF9* gene only additive effect was significant for litter size at lambing implying that only one copy of the mutant allele can increase the litter size. Several published findings (Hanrahan et al., 2004; Juengel et al., 2004 ; Liao et al., 2004; Davis et al., 2005) reported that the *GDF9* homozygous state tends to cause infertility while Silva et al. (2011) reported a novel *GDF9* mutation, a substitution of phenylalanine with cysteine at position 345 (F345C) (FecG^{E}). In contrast to previously reported mutations in both *GDF9* and *BMP15*, the F345C mutation in *GDF9* did not cause sterility in the homozygous individuals, but rather significantly higher prolificacy compared to heterozygous individuals (Silva et al., 2011). *GDF9* and *BMP15* are known to influence ovulation rate in a dose-responsive manner (Moore et al., 2004), indicating that the F345C variant has not completely lost its biological function. The effect of ewe lambing year on litter size was not significant but the birth type of the ewe had a significant effect on the ewe lambing rate (Table 2). The ewes born as triplets tended to have higher (P<0.001) lambing rate when compared with those born as singleton and twin (Table 2). The litter size at birth in the multiparous ewes was lower (P<0.01) than the primiparous ewes (Table 2). These finding is in agreement with those of Mishra et al. (2009) and Barzegari et al. (2010) who reported that the litter size at lambing at the first parity was lower than at the second and third parity.

Genetic variation in ovulation rate in sheep has been widely documented and the evidence shows substantial differences among breeds and in a number of cases exceptional variation within breeds/strains (Bindon et al., 1996; Hanrahan et al., 2004). The genetic variation within breeds/strains can be explained by segregation of a gene with a large effect on ovarian function. This hypothesis provided an explanation for high prolificacy of Booroola sheep (Davis et al., 1982; Piper and Bindon, 1982). Subsequently, putative major genes were invoked to explain the increased litter size and/or ovulation rate in a variety of breeds/strains (Hanrahan et al., 2004). Based on the lambing rate records in Iran-Black sheep provided herein, it is obvious that there is relatively a high variation in lambing performance amongst individuals in this synthetic sheep. The environmental effects such as ewe parity and year were accountable for this variation. However, there was significant genetic variation as the ewes born as twins and triplets tended to have higher lambing rate with extreme variation among individuals implying that major genes affecting the ovulation rate may segregate in this population. Our molecular dissection of litter size at lambing revealed that some of this genetic variation can only be attributed to the sequence variation in the *GDF9* gene rather than the *BMP15* gene. Thus, a gene-assisted selection program to improve the lambing rate in this breed can be designed based on the *GDF9* gene mutation.

Table 2. The least squares means (S.E.) of lambing rate for different genotypes, ewe birth type and different ewe parity

Ewe genotype	Lambing rate	Ewe birth type	Lambing rate	Ewe parity	Lambing rate
G/G (6)[1]	1.88 (0.16) [a]	Singleton (45)[1]	1.60 (0.09) [b]	1 (81)[1]	1.47 (0.10) [b]
G/+ (45)	1.89 (0.09) [a]	Twin (62)	1.69 (0.07) [b]	2 (66)	1.88 (0.11) [a]
+/+ (63)	1.65 (0.09) [b]	Triplet (4)	2.15 (0.21) [a]	3 (38)	2.01 (0.12) [a]
-	-	-	-	4 (20)	1.89 (0.15) [a]

[a,b]: Within columns, mean lambing rates, followed by SE in *parentheses*, with common letter do not differ (P>0.05).
[1]*Numbers* in *parentheses* indicate the *number of individuals used for the analyses in each group.*

Acknowledgements

This study was supported by Shahid Bahonar University of Kerman, Kerman, Iran. The authors wish to thank Mr. M. Jafari (the director of the Abbasabad sheep breeding station, Razavi Khorasan province, Iran) for his cooperation in collecting sheep blood samples and providing the lambing records.

References

Barzegari, A., Atashpaz. S., Ghabili, K., Nemati, Z., Rustaei, M. Azarbaijani, R., 2010. Polymorphisms in *GDF9* and *BMP15* associated with fertility and ovulation rate in Moghani and Ghezel sheep in Iran. *Reproduction in Domestic Animals* 45, 666–669.

Beuzen, N.D., Stear, M.J., Chang, K.C., 2000. Molecular markers and their use in animal breeding. *The Veterinary Journal* 160, 42-52.

Bindon, B.M., Piper, L.R., Hilliard, M.A., 1996. Reproductive physiology and endocrinology of prolific sheep. In: Fahmy, M.H. (Ed.), Prolific Sheep. CAB International, Wallingford, UK pp. 453–470.

Bodensteiner, K.J., Clay, C.M., Moeller, C.L., Sawyer H.R., 1999. Molecular cloning of the ovine growth/differentiation factor-9 gene and expression of growth/differentiation factor-9 in ovine and bovine ovaries. *Biology* of *Reproduction* 60, 381-386.

Bodin, L., Di Pasquale, E., Fabre, S., Bontoux, M., Monget, P., Persani, L., Mulsant, P., 2007. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology* 148, 393-400.

Bodin, L., Lecerf, F. Pisselet, C., SanCristobal, M., Bibe, B., Mulsant, P., 2003. How many mutations are associated with increased ovulation rate and litter size in progeny of Lacaune meat sheep? Proceedings of the 3rd International Workshop on Major Genes and QTL in Sheep and Goats. INRA, Toulouse, France.

Caballero, A., Toro, M.A., 2000. Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genetics Research* 75, 331–343.

Davis, G.H., 2005. Major genes affecting ovulation rate in sheep. *Genetics Selection Evolution* 37, S11-S23.

Davis, G.H., Galloway, S. M., Ross, I.K., Gregan, S.M., Ward, J., Nimbkar, B.V., Ghalsasi, P.M., Nimbkar, C., Gray, G.D., Subandriyo, Inounu, I., Tiesnamurti, B., Martyniuk, E., Eythorsdottir, E., Mulsant, P., Lecerf, F., Hanrahan, J.P., Bradford, G.E. and Wilson, T., 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. *Biology of Reproduction* 66, 1869-1874.

Davis, G.H., Montgomery, G.W., Allison, A.J., Kelly, R.W., Bray, A.R., 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. *New Zealand Journal of Agricultural Research* 25, 525–529.

Dekkers, J.C.M., 2012. Application of genomics tools to animal breeding. *Current Genomics* 13, 207–212.

Duguma, G., Schoeman, S.J., Cloete, S.W.P., Jordaan, G.F., 2002. Genetic and environmental parameters for ewe productivity in Merinos. *South African Journal of Animal Science* 32, 154-159.

Esmailizadeh, A., 2010. A partial genome scan to identify quantitative trait loci affecting birthweight in Kermani sheep. *Small Ruminant Research* 94, 73–78.

Esmailizadeh, A., 2014. Detection of chromosomal segments underlying scrotal circumference in ram lambs and age at onset of puberty in ewe lambs. *Animal Production Science 55*, 1018-1024.

Esmailizadeh, A., Miraei-Ashtiani, S.R., Mokhtari, M.S., Asadi Fozi, M., 2011. Growth performance of crossbred lambs and productivity of Kurdi ewes as affected by the sire breed under extensive production system. *Journal of Agricultural Science and Technology* 13, 701–708.

Eusebi, P.G., Martinez, A., Cortes, O., 2020. Genomic tools for effective conservation of livestock breed diversity. *Diversity 12,* 8.

Farid, A., Makarechian, M., Sefidbakht, N., 1977. Crossbreeding of Iranian fat-tailed sheep: lamb performance of Karakul, Mehraban and Naeini breeds. *Journal of Animal Science* 44, 542-548.

Galloway, S.M., McNatty, K.P., Cambridge, L.M., Laitinen, M.P., Jennife, J.L., Jokiranta, S., 2000. Mutations in an oocyte-derived growth factor gene (*BMP15*) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genetics* 25, 279-283.

Hanrahan, J.P., Gregan, S.M., Mulsant, P., Mullen, M., Davis, G.H., Powell, R., Galloway, S.M., 2004. Mutations in the genes for oocyte-derived growth factors *GDF9* and *BMP15* are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biology of Reproduction* 70,900–909.

Juengel, J.L., Hudson, N.L., Whiting, L., McNatty, K.P., 2004. Effects of immunization against bone morpHogenetic protein 15 and growth differentiation factor 9 on ovulation rate ,fertilization, and pregnancy in ewes. *Biology of Reproduction* 7, 557-561.

Liao, W.X., Moore, R.K., Shimasaki, S., 2004. Functional and molecular characterization of naturally occurring mutations in the oocytesecreted factors bone morphogenetic protein-15 and growth and differentiation factor-9. *Journal of Biological Chemistry* 17, 17391-17396.

Matos, C.A.P., Thomas, D.L., Gianola, D., Perez-Enciso, M., Young, L.D., 1997. Genetic analysis of discrete reproductive traits in sheep using linear and nonlinear models: II. Goodness of fit and predictive ability. *Journal of Animal Science* 75, 88-94.

Michailidis, G., Avdi, M., Pappa V., 2008. Reproductive performance and investigation of BMPR-IB and BMP-15 gene mutations in Greek Chios and Florina sheep breeds. *Archiva Zootechnica* 11, 24-31.

Miller, S.A, Dykes, D.D, Polesky, H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*earch 16, 1215.

Mishra, A.K., Arora, A.L., Kumar, S., Prince., L.L, 2009. Studies on effect of Booroola (FecB) genotype on lifetime ewes' productivity efficiency, litter size and number of weaned lambs in Garole×Malpura sheep. *Animal Reproduction Science* 113, 293–298.

Montaldo, H.H., Meza-Herrera, C.A., 1998. Use of molecular markers and major genes in the genetic improvement of livestock. *Electronic Journal of Biotechnology* 1, 1-7.

Monteagudo, L.V., Ponz, R., Tejedor, M.T., Laviña, A., Sierra, I., 2009. A 17bp deletion in the bone morphogenetic protein 15 (*BMP15*) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. *Animal Reproduction Science* 110, 139- 146.

Moore, R.K., Erickson, G.F., Shimasaki, S., 2004. Are BMP-15 and GDF-9 primary determinants of ovulation quota in mammals? *Trends in Endocrinology and Metabolism* 15, 356–361.

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

Nosrati, M., Asadollahpour Nanaei, H., Amiri Ghanatsaman, Z., Esmailizadeh, A., 2019. Whole genome sequence analysis to detect signatures of positive selection for high fecundity in sheep. *Reproduction in Domestic Animals* 54, 358–364.

Piper, L.R, Bindon, B.M., 1982. Genetic segregation for fecundity in Booroola Merino sheep. In: Barton, R.A., Smith, W.C. (Eds.), Proceedings of the World Congress on Sheep and Beef Cattle Breeding, Palmerston North, New Zealand, pp. 395–400.

Rashidi, A., 2013. Genetic parameter estimates of body weight traits in Iran-Black sheep. *Journal of Livestock Science and Technologies* 1, 50-56.

SAS, 2002. SAS User's Guide: Statistics. Version 9.1. SAS Institute Inc., Cary, North Carolina, 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. , 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. *Animal Genetics* 42, 89–92.

Smith, C., Simpson, S.P., 1986. The use of genetic polymorphisms in livestock improvement. *Journal of Animal Breeding and Genetics* 103, 205–217.

Snowder, G.D., 2008. Genetic improvement of overall reproductive success in sheep: A review. *Asociacion Latinoamericana de Produccion Animal* 16, 32–40.

Snyman, M.A., Olivier, J.J., Erasmus, G.J., Van Wyk, J.B., 1997. Genetic parameter estimates for total weight of lamb weaned in Afrino and Merino sheep. *Livestock Production Science* 48, 111-116.

Souza, C.J.H., MacDougall, C., Campbell, B.K., McNeilly, A.S., Baird, D.T., 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *Journal of Endocrinology* 169, R1-R6.

Woolliams, J.A., Oldenbroek, J.K., 2018. Genetic diversity issues in animal populations in the genomic era. In: Oldenbroek, J.K. (Ed.), Genomic Management of Animal Genetic Resources, 1st ed., Wageningen Academic Publisher, Gelderland, The Netherlands, pp. 13–47.