پنجمین کنگره بین المللی و هفدهمین کنگره ملی ژنتیک ایران
5th International & 17th Iranian Genetics Congress
I.G.S. انجمن ژنتیک ایران Iranian Genetics Society
GC17-03970718

بررسی ارتباط ژن‌های FecB و FecG با چندقلوزایی گوسفند عربی

Association of polymorphism in fecundity genes with litter size in Arabi sheep

رضا توحیدی ,علی جوادمنش

Reza Tohidi[1], Ali Javadmanesh[2]

[1] Department of Animal Science, University of Torbat-e Jam, Torbat-e Jam, Iran

e-mail: r.tohidi@tjamcaas.ac.ir

[2]Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

پست الکترونیک مکاتبه کننده: tohidi75@gmail.com

Abstract

Mutations in fecundity genes such as *BMPR1B* (*FecB*) and *GDF9* (*FecG*) are associated with litter size in sheep. Mutations in each of these genes alone increase ovulation. The aim of this study was to evaluate the polymorphism of *FecB* and *FecG* genes in Arabi sheep of Torbat-e Jam region. Blood samples were randomly collected from 25 Arabi ewes. The polymorphic status of the genes was determined using the Tetra-ARMS PCR. All genes were monomorphic and non-carrier. Multiple birth were not observed in these ewes, which could be due to having a wild genotype for the fecundity genes. In order to increase fecundity in this breed, it is necessary to conduct more studies on the hormonal and follicular level.

Keywords: Arabi sheep, fecundity, *FecB*, *FecG*

Introduction

According to previous studies, ovulation rate and litter size in sheep are controlled by a group of genes called fecundity genes. Among these genes, the Booroola gene was reported for the first time in Merino sheep. Recent studies revealed that three genes of *BMPR1B* (*FecB*), *GDF9* (*FecG*) and *BMP15* (*FecX*) have a major effect on sheep fertility (Monteagudo et al., 2009). These three genes belong to the TGFβ family, which are generally called *Fec* genes.

Three methods of HRM, PCR-RFLP and Tetra-ARMS PCR were used in detection of polymorphism in *Fec* genes (Hosseinzadeh et al., 2019). The latter does not require the use of a restriction enzyme and therefore, it is cheaper. Arabi sheep breed are mainly kept in Khuzestan region, but they are reared in other regions such as Khorasan Razavi. The aim of this study was to investigate the polymorphism of *FecB* and *FecG* genes in Arabi sheep of Torbat-e Jam region in Khorasan Razavi province.

Materials and Methods

Blood samples were collected from jugular vein of a total of 25 Arabi ewes. The animals were randomly selected from farmers in Torbat-e Jam area (Paeen Jam). Genomic DNA was extracted using Sinaclon DNA extraction kit (DNP™) according to the manufacturer's instructions. The quality and quantity of DNA was evaluated using agarose gel and spectrophotometer. Two pairs of control (Outer) and specific (Inner) primer were used for *FecB* and *FecG* genes (Table 1). Then, Tetra-Arms PCR was performed according to the following program: 94°C for 4 min, 32 cycles of 30–45 s at 94°C depending upon mutation point, annealing temperature as given in Table 1and 30 s at 72°C, with a final cycle at 72°C for 4 min to complete extension. Finally, the PCR product was electrophoresed on agarose gel and revealed under UV.

Results and Discussion

The results showed that both genes were wild homozygous in all ewes. Only one animal was heterozygous for the *FecG* locus. Since all ewes had single birth, it was consistent with T-ARMS PCR results. Three genotypes of homozygous wild, heterozygous and homozygous mutant were reported for *FecB* and *FecG* loci in Bonpala sheep of India, although most of them were wild homozygotes (Roy et al., 2011). *FecB* mutants showed a high correlation with multiple birth and ovulation rate without increasing in gene expression (Mulsant et al., 2001; Souza et al., 2001; Wilson et al., 2001). The ovulation rate of ewes that received a copy of the Booroola gene from each parent increases by 1.5 times (Fabre et al., 2006). *FecG* gene along with *BMP15* plays an important role in ovulation rate, egg health and pregnancy rate (Juengel et al., 2004).The mutants of both genes together showed a greater effect on the increase of litter size than individual genes (Arnyasi et al., 2004). Mutant homozygous and heterozygous genotypes for the *FecB* locus were observed in the Indian Garole sheep. Furthermore, a polymorphism was reported in an allelic locus for the *FecG* (Polley et al., 2009).

Both gene loci in the present study were wild homozygous. Similar results were observed for Moghani sheep for *FecB* and *FecX* genes (Savar Sofla et al., 2014). The introduction of a mutant carrier individual into the Arabi sheep population may cause increase in ovulation rate. However, *FecG* SNPs did not reveal an association with litter size in Bahmai and Lak Qashqai sheep breeds (Muhaghegh Dolatabady and Habibizad, 2019). Hence, cross-breeding may be considered with caution and more investigations are necessary to find a suitable solution to increase lambing rate.

Table 1. Oligonucleotide sequences for allele specific amplification of *FecB* and *FecG* genes .

Gene	Primer name	Primer sequence	Accession No.
FecB	Outer-F	ACGCACTAACAGTGTGTTGG	AY242067
	Outer-R	GAGAGGAAAGCTAGGAAACCCTG	
	Inner-F	GGTTCCGAGAGACAGAAATATATGG	
	Inner-R	CATGCCTCATCAACACCGTTT	
FecG	Outer-F	CTGCAGCCAGATGACAGAGCTTTTCA	AF312016
	Outer-R	CGTATGCCTTATAGAGCCTCTTCATGTCGC	
	Inner-F	GCCTGGCTCTGTTTTCCTATTAGCCTTG	
	Inner-R	TCTTCTTCCCTCCACCCATTAACCAATC	

References

Arnyasi M, Zsolnai A, Komlosi I, Fesus L, Javor A (2004) Case study of a Hungarian breeding program using imported Booroola rams. Arch Tierz Dummerstorf 47: 359–366. https://doi.org/10.5194/aab-47-359-2004.

Fabre S, Pierre A, Mulsant P, et al (2006) Regulation of ovulation rate in mammals: contribution of sheep genetic models. Reprod Biol Endocrinol 4: 20. https://doi.org/10.1186/1477-7827-4-20.

Hosseinzadeh T, Bahri Binabaj F, Javadmanesh A (2019) Detection of polymorphism in Booroola gene (FecB) in sheep breed in Iran by HRM. The Proceedings of the 4th International Student Biotechnology Congress, Feb 2019, Tehran, Iran.

Juengel JL, Hudson NL, Whiting L, McNatty KP (2004) Effects of immunization against bone morphogenetic protein 15 and growth differentiation factor 9 on ovulation rate, fertilization, and pregnancy in ewes. Biol Reprod 70: 557–561. https://doi.org/10.1095/biolreprod.103.023333.

Monteagudo LV, Ponz R, Tejedor MT, et al (2009) A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. Anim Reprod Sci 110:139–146. https://doi.org/10.1016/j.anireprosci.2008.01.005.

Muhaghegh Dolatabady M, Habibizad, J (2019) Single nucleotide polymorphisms (SNPs) of *GDF9* gene in Bahmaei and Lak Ghashghaei sheep breeds and its association with litter size. Iranian J Appl Anim Sci 9: 427–432.

Mulsant P, Lecerf F, Fabre S, et al (2001) Mutation in bone morphogenetic protein receptor-1B is associated with increased ovulation rate in Booroola Merino ewes. Proc Natl Acad Sci USA 98: 5104–5109. https://doi.org/10.1073/pnas.091577598.

Polley S, De S, Batabyal S, Kaushik R, et al (2009) Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. Small Rumin Res 85: 122–129. https://doi.org/10.1016/j.smallrumres.2009.08.004

Roy J, Polley S, De S, et al (2011) Polymorphism of fecundity genes (FecB, FecX, and FecG) in the Indian Bonpala sheep. Anim Biotechnol 22:151–62. https://doi.org/10.1080/10495398.2011.589239.

Savar Sofla S, Seyedabadi H, Javanrouh Aliabad A (2014) Detection of polymorphism in FecB and BMP15 candidate genes associated with litter size in Moghani flock. Anim Scie J (Pajouhesh & Sazandegi), Abstract in English 106: 86–90. https://doi.org/10.22092/asj.2015.101350.

Souza CJ, MacDougall C, Campbell BK, McNeilly AS, Baird DT (2001) The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. J Endocrinol 169:R1–R6. https://doi.org/10.1677/joe.0.169r001.

Wilson T, Wu XY, Juengel JL, et al (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. https://doi.org/10.1095/biolreprod64.4.1225.