Biotechnology in Animal Husbandry 29 (4), p 675-683 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.38
DOI: 10.2298/ BAH1304675A

POLYMORPHISM IN BMP-15 GENE AND ITS ASSOCIATION WITH LITTER SIZE IN ANGLO-NUBIAN GOAT

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Original scientific paper

Abstract: For association between BMP-15 (exon2) gene polymorphisms and litter size trait in Anglo-Nubian goat, PCR-SSCP technique was developed. Twenty-five female Anglo-Nubian goats reared under Egyptian conditions were selected according to their litter size. DNA from blood samples of these animals was extracted to amplify 140-bp of the BMP-15 gene affecting litter size production trait in goats. Based on the breeding value, 25 animals were selected from the highest to the lowest litter size productivity. PCR amplification size of the BMP-15 gene (140-bp) was genotyped in all animals. PCR-SSCP analysis of the BMP-15 gene (140-bp) showed three various genotypes BB, BM and MM with frequencies 0.46, 0.43 and 0.11, respectively. The frequencies of the *B* and *M* alleles were 0.68 and 0.32, respectively. The results indicated that the BB genotype was higher in litter size productivity than the other genotypes with significant differences. The result of this study confirmed that BMP-15 gene may be a strong candidate gene for further applications in marker-assisted selection (MAS) for litter size in goats.

Keywords: Anglo-Nubian goat, Litter size, PCR-SSCP, BMP-15 gene polymorphisms

Introduction

Ovulation rate is determined by a complex exchange of endocrine signals between the pituitary gland and the ovary and paracrine and possibly autocrine signals within ovarian follicles involving the oocyte and its adjacent somatic cells (*Dong et al., 1996; Galloway et al., 2000; Eppig, 2001; Galloway et al., 2002*). BMP 15 gene maps to X chromosome and plays an important role in regulating ovulation rate and oocyte quality. The relative importance of BMP15 in early

follicle development is species- specific and appears to be related to differences between mono-polyovulatory species (*Dube et al., 1998; Grapes and Rothschild, 2002; Moore and Shimasaki, 2005; Silva et al., 2004*)**.** Different mutations in BMP 15 gene in women and ewes have been shown to cause defects in folliculogenesis. Five naturally occurring mutations in exon 2 of the sheep BMP15 gene such as FecXG, FecXB, FecXI, FecXH, FecXL leads to infertility in homozygous ewes due to defects in early folliculogenesis (*Chu et al., 2005; McNatty et al., 2005; Bodin et al., 2007*), where as heterozygous ewes have increased ovulation rate and litter size. Ninety-six percent of the world goat populations are owned by small holders in developing countries with rare genetic sources and improvement programs (*Olivier et al., 2005*). So, it is essential to study the genetics and reproduction in goat breeds using modern genetic methods. One of these methods is marker-assisted selection (MAS) which will be useful for increasing and accelerating the rate of genetic improvement on litter size and encourage its uptake it by commercial goat breeders.

Materials and methods

Animals. Twenty-five female Anglo-Nubian goats kept under Egyptian conditions were chosen according to litter size productivity. Blood samples from these animals were collected by Jugular vein puncture into tubes containing an anticoagulant disodium EDTA. The samples were stored at -20°C until needed for DNA isolation.

DNA isolation. Genomic DNA was isolated from whole blood samples using a commercially available kit (GF-1 Blood DNA extraction kit-Vi Vantis). Genomic DNA was separated on agarose gel electrophoresis using 1% agarose (w/v) in 0.5X TBE buffer. To check genomic DNA bands, the gel was photographed using gel documentation system (Syngene, UK).

PCR amplification and genotyping of BMP-15 gene. A 140-bp fragment of exon2 of BMP-15 gene in 25 female goats was amplified by PCR using forward (5'- CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3') and reverse (5'-GATGCAATACTGCCTGCTTG-3' primers (*Hanrahan et al., 2004*). PCR was performed in a reaction volume of 25 µl using 25 ng of genomic DNA of each sample, 25 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/ µl Taq DNA polymerase (Promega, Germany). Thermal cycling (Autorisierter Thermocycler and Mastercycler Gradient) was carried out by initial denaturation at 94°C for 5 min, followed by 35 cycles each at 94°C for 45 sec, annealing temperature at 62°C for 40 sec, polymerization temperature at 72°C for 45 sec and final extension at 72°C for 10 min, then the

samples were held at 4°C. The amplified DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Single stranded conformational polymorphism (SSCP). Aliquots of 5 µl PCR products were mixed with denaturating solution (98% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue and 10 mM EDTA) and incubated at 98C° for 10 min and then child on ice rapidly. Denaturated DNA was loaded on 10% PAGE gel (10X 10 CM) in 1X TBE buffer and constant voltage 65V for 5 hours. For staining DNA bands and visualizing, the gel was stained with ethidium bromide and photographed by using Gel Documentation system.

Statistical analysis. Data for litter size production in Anglo-Nubian goats was obtained from the farm records to predict breeding value (Table 1). Statistical analysis was performed by SAS version 9.2 (based upon BLUP statistical method) using the following model:

yijk= pi + aj + eijk, where:

pi= Fixed effects (season, year and parity),

aj= Random effect of the ej^{th} does,

eijk= Random error effect.

Table 1. Litter sizes, breeding values (BV) and genotypes of BMP15 gene in experimental animals.

Serial no.	Animal no.	Litter size	BV	Genotype
1	6	2.3	0.2657	BB
2	20	2.5	0.2169	BB
3	22	2.5	0.2153	BM
4	23	1.3	0.2130	BM
5	5	2.0	0.1409	BB
6	14	2.0	0.1153	MM
7	7	1.7	0.1106	BB
8	33	2.0	0.08041	BB
9	32	2.0	0.07218	MM
10	34	2.0	0.03685	BB
11	19	1.0	-0.0097	BB
12	15	1.5	-0.0215	MM
13	11	1.5	-0.0382	BB
14	17	1.5	-0.0545	BB
15	24	1.5	-0.0545	BB
16	26	1.5	-0.0545	BB
17	25	1.0	-0.0716	BB
18	31	1.5	-0.0769	MM
19	12	1.5	-0.112	MM

20	18	1.3	-0.112	MM
21	8	1.3	-0.1245	BB
22	21	1.3	-0.1342	BB
23	9	1.5	-0.1445	BB
24	16	1.3	-0.1925	BB
25	30	2.0	-0.2118	MM

Results and discussion

PCR amplification of the BMP15 (exon2) gene yielded 140-bp in length in all 25 female goats (Figure 1). PCR-SSCP technique was used to identify nucleotide sequence polymorphism by change conformation of alleles within BMP15 (exon2) gene in experimental Anglo-Nubian goat. Characterization and analysis of PCR-SSCP showed two homozygous genotypes: BB genotype in animal numbers 5, 6, 7, 8, 9, 11, 16, 17, 19, 20, 21, 24, 25, 26, 33 and 34 and MM genotype in animal numbers 12, 14, 15, 18, 30, 31 and 32. While, in animal numbers 22 and 23 heterozygous genotype BM was found (Table 1 and Figure 2). The calculated frequencies of BB, BM and MM genotypes were 0.46, 0.43 and 0.11, respectively, and the frequencies of the *B* and *M* alleles were 0.68 and 0.32, respectively (Table 2). The results indicated that the Anglo-Nubian goats with BB genotype had significantly ($p \leq 0.05$) larger size of litter than the goats with other genotypes (BM and MM).

In previous study of BMP15 gene in another goat breed, *Chu et al. (2007)* found two genotypes: AA and AB in Jining Gery goats. Genotype AA was found in low fecundity goat breeds and AB genotype had 1.3 kids more than homozygous AA. In another study on different goat breeds, *Feng et al. (2009)* three genotypes AA, AG and GG in Jining Grey goats were found, while only AA genotype was found in both Liaoning Cashmere and Inner Mongolia Cashmere goats. Boar goat had two genotypes AG and GG, while Angora and Inner Mongolia Cashmere goats had only AA genotype. In a recent study of *Wang et al. (2011)*, three genotypes (AA, BB and AB) were detected in Funiu White goats and their frequency was 0.071, 0.715 and 0.214, respectively. Two genotypes (AB and BB) were detected in Taihang black goats and their frequency was 0.342 and 0.658, respectively. The Funiu white goat with genotype BB had 0.91 or 0.82 kids more than those with AB or AA, respectively. However, these results preliminarily showed that BMP-15 gene is a genetic marker and closely linkage to the litter size trait and consequently, can be used as a marker-assisted selection (MAS) for high litter size productivity in goat.

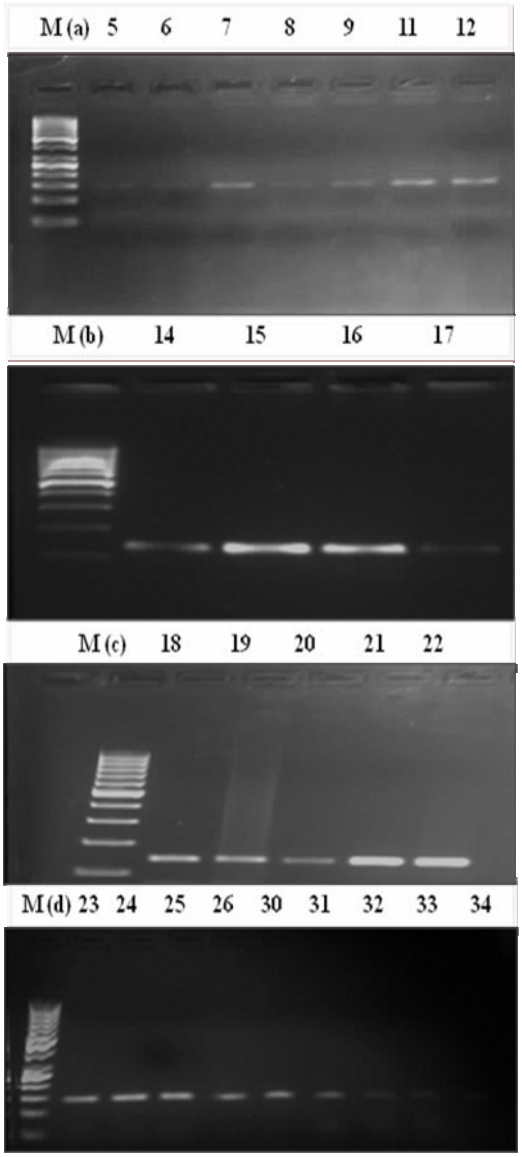

Figure 1. PCR products (140-bp) generated by the BMP15 (exon2) gene primers. Where, lanes: M (a) and M (d) are DNA markers 50-bp, lanes M (b) and M (c) are DNA markers 100-bp and lanes 5-34 are female Anglo-Nubian goats.

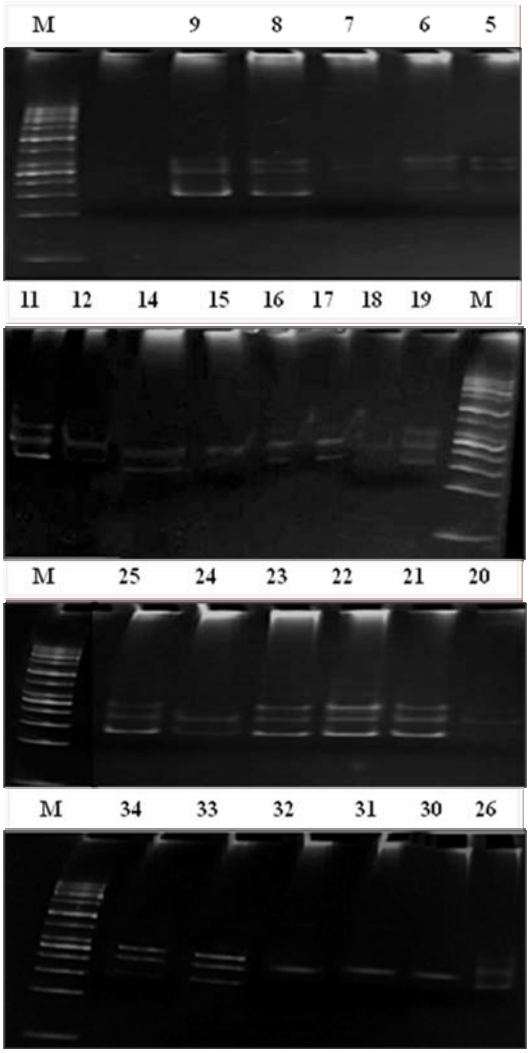

Figure 2: PCR-SSCP analysis of BMP15 gene (140-bp) in 25 animals. Lanes 5, 6, 7, 8, 9, 11, 16, 17, 19, 20, 21, 24, 25, 26, 33 and 34 represent BB genotype, lanes 12, 14, 15, 18, 30, 31 and 32 represent MM genotype, lanes 22 and 23 represent BM genotype and lane M is DNA marker (50-bp).

Table 2. Frequency of genotypes (BB, BM and MM) and alleles (*B* and *M*) in BMP15 locus

Gene	No. of animals	Genotypic frequency			Allelic frequency	
		BB(p^2)	BM(2pq)	MM(q^2)	*B(p)*	*M(q)*
BMP15	25	0.46	0.43	0.11	0.68	0.32

Conclusion

PCR-SSCP technique was developed to associate between BMP-15 (exon2) gene polymorphisms and litter size trait in Anglo-Nubian goat. DNA from blood samples of 25 female Anglo-Nubian goats selected according to their litter size productivity was extracted to amplify 140-bp of the BMP-15 gene. Based on the breeding value, the 25 animals were ordered from the highest to the lowest litter size productivity. PCR-SSCP analysis of the BMP-15 gene (140-bp) showed three different genotypes BB, BM and MM with frequencies 0.46, 0.43 and 0.11, respectively. The results indicated that the BB genotype was higher in litter size productivity than the other genotypes. Consequently, BMP-15 gene can be used as a marker-assisted selection (MAS) to improve litter size production trait in goat.

Acknowledgements

This work was achieved at Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

Polimorfizam BMP - 15 gena i njegova povezanost sa veličinom legla anglo - nubijskih koza

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Rezime

Za povezanost BMP-15 (exon 2) polimorfizma gena i osobine veličine legla anglo-nubijskih koza, razvijena je PCR - SSCP tehnika. Dvadeset pet ženskih grla anglo-nubijskih koza gajenih pod egipatskim uslovima odabrana su prema veličini njihovih legala. DNK iz uzoraka krvi ovih životinja je ekstrahovana da bi se amplifikovala 140-bp u BMP-15 genu koji utiče na veličinu legla kao proizvodnu osobinu u koza. Na osnovu priplodne vrednosti, 25 životinja je izabrano od najveće do najmanje veličine legla, odnosno produktivnosti. PCR amplifikacija veličina BMP-15 gena (140-bp) je genotipizirana kod svih grla. PCR-SSCP analiza BMP-15 gena (140-bp) je pokazala tri različita genotipa BB, BM i MM sa frekvencijama 0.46, 0.43 i 0.11. Frekvencije B i M alela su 0.68 i 0.32,. Rezultati su pokazali da je BB genotip bio bolji sa stanovišta produktivnosti veličine legla od drugih genotipova sa značajnim razlikama. Rezultat ovog

istraživanja je potvrdio da BMP-15 gen može biti jak kandidat gen za dalju primenu u marker asistiranoj selekciji (MAS) na veličinu legla u kozarstvu.

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Received 21 October 2013; accepted for publication 20 December 2013