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Research Article

Genetic Improvement of Litter Size in Four Goat Breeds in Egypt Using Polymorphism in Bone Morphogenetic Protein 15 Gene

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Abstract | Goats are preferred as a source of good quality meat and milk. Their production efficiency can be improved and increased by introducing highly fecundity goats. The aim of this study was to investigate the polymorphism of *BMP15* fecundity gene by PCR-RFLP and single nucleotide polymorphism (SNP) in four goat breeds in Egypt (Zaraibi, Baladi, Damascus, and Alpine) and their association with litter size. The genomic DNA was extracted from the whole blood, and 141 base pair fragment encoding *BMP15* exon 2 was amplified, purified and sequenced. The sequencing data analysis revealed four SNPs for high litter size does and three SNPs in low litter size. Three SNPs in *BMP15* exon 2 at nucleotide position 757 A>C, 762 G>A and 769 G>C in Alpine, Zaraibi, and Baladi respectively. These nucleotide changes associated with amino acid substitution. Furthermore, one novel non-synonymous mutation has been detected in all higher litter size studied goat breeds and lead to change amino acid where Glutamic acid>Glutamine. The SNPs and amino acid substitutions were detected and repeated in higher and lower litter size animals, which can be used as marker-assisted selection for both traits in the four goat breeds under study.

Keywords | Goat, Breeds, SNPs, *BMP15* gene, Reproduction

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INTRODUCTION

Goats are most domestic species that are widely spread all over the world and have an important economic role in developing countries (Adriana et al., 2010). In Egypt, goats are preferred small ruminant used as a source of meat and milk for the farmer. They are distributed in the country, especially in the Nile River Valley and Delta region (Galal et al., 2005). Increasing productivity of goats will contribute to improve the standard of rural people living (Adu et al., 1988).Several authors reported different figures for litter size in different goat breeds (Abdel-Raheem 1998; Campbell, 1994; Song et al., 2006). Hamed et al. (2009) showed that selection would lead to improving goat litter size at birth.

Three fecundity genes were identified in sheep, one of these genes is Bone morphogenetic protein 15 (*BMP15)* which is a member of the *TGF-β* superfamily, also known as *FecX* or *GDF9B* (Galloway et al., 2000). The *BMP15* gene is an ovary-derived growth factor that is essential for follicular development (Hanrahan et al., 2004). This gene plays an important role in female fertility through promoting the growth and maturation of ovary, promoting granulosa cells mitosis, prevention of granulosa cell apoptosis, regulation of the sensitivity of granulosa cells to follicle-stimulating hormone (FSH) action, contributing in determination of the number of eggs that are ovulated, promotion of developmental competence of oocytes and kit ligand expression stimulation (Juengel et al., 2002; McNatty et al., 2003; Moore et al., 2003; Shimasaki et al., 2004; Persani et al., 2014). Moreover, ewes with two inactive copies of the

BMP15 gene (homozygous for this mutation) are infertile with primary ovarian failure. While, ewes with a single inactive *BMP15* (heterozygous for mutations) are fertile and the effects of these mutations on ovulation rate are additive (Davis et al., 1991; 1993; Hanrahan et al., 2004).

Goat breeds in Egypt, lacking the molecular characterization required for establishing adequate utilization of genetic variation in the development of its production. However, the identification of polymorphism and DNA markers associated with reproductive traits could be used as marker-assisted selection (MAS) which lead to genetic improvement to increase litter size and reproduction efficiency (Ghaffari et al., 2009).

The aim of this study is to screen for *BMP15* gene polymorphism by PCR-RFLP and single Nucleotide Polymorphism (SNP) using DNA sequence analysis in four goat breeds in Egypt (Zaraibi, Baladi, Damascus, and Alpine) and their association with litter size.

MATERIALS AND METHODS

Sample Collection and Genomic DNA Extraction

Blood samples were collected from the jugular vein of fifty does from the four breeds (14/ Baladi, 15/ Zaraibi, 11/ Damascuse and 10/Alpine). The Four goat breeds were obtained from Sakha Animal Production Research Station, Kafr El Sheikh governorate, Egypt. Approximately 10 ml of blood was collected and stored at -20° C before further processing. All selected does were healthy with low (less than two kids/litter) or high (two or more kids/litter) litter size. Genomic DNA was extracted using QIAamp DNA Blood Mini Kit (QIAGEN, Germany) according to manufacturer's instruction.

PCR Amplification of *BMP15*

The 141 bp fragment encoding *BMP15* (exon 2) was amplified using primer 5'- CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3' and 5'- GATGCAATACTGCCTGCTTG- 3' (Kasiriyan et al., 2011). PCR amplification was performed in a total reaction volume of 50 µl which containing 4 µl genomic DNA, 5 µl 10X buffer, 1 µl dNTPs mix (Thermo scientific, Lithuania), 1 µl of each primer (50 nm) (Invitrogen, USA), 0.6 µl of Taq DNA polymerase 500U (Thermo scientific, Lithuania) and 38.4 µl dH_2O. The amplification reaction conditions was carried out using 35 cycles at 94°C for 5 min, followed by 45 sec, 62°C for 40 sec, 72°C for 45 sec, followed by 72°C for 10 min. The PCR product of each sample and 100 bp DNA ladder (Thermo scientific, Lithuania) were loaded in 2% agarose gel stained with ethidium bromide. The electrophoresis was carried out and the electrophoresis gel was visualized and photographed using gel documentation system (InGenius, Syngene Bio Imaging, USA).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

All PCR products of *BMP15* gene were digested with *HinfI* restriction enzyme (Promega, USA). The PCR-RFLP was carried out in reaction volume 20 µl consist of: 10 µl PCR product, 2 µl Restriction Enzyme 10X Buffer, 0.2 Acetylated BSA, 10µg/µl, 7.3 µl dH_2O and 0.5 µl of HinfI restriction enzyme 10u/µl. The reaction was incubated at 37°C for 1hr and the products were separated by 3% agarose gel stained with ethidium bromide and visualized on UV transilluminator.

PCR Purification for DNA Sequencing and Data Analysis

The PCR products were purified and sent for sequencing (3PCR products/each breed) in Invitrogen Company, Germany. The obtained sequences were analyzed using Bio Edit (7.1.3) program to detect Single Nucleotide Polymorphisms (SNPs) from the aligned sequences and their translated amino acid. Sequence alignments were compared with *Capra hircus* breed Black Bengal *BMP15* gene that available in the Gene Bank database (accession number gb|EU888137.1|).

RESULTS

Amplified Fragment of *Bmp15* Gene

The PCR amplification *BMP15* gene for fifty does which have at least one record for litter size, were screened for *BMP15* mutations in the present study. PCR amplification of *BMP15* gene yielded a fragment of 141 bp as shown in Figure 1.

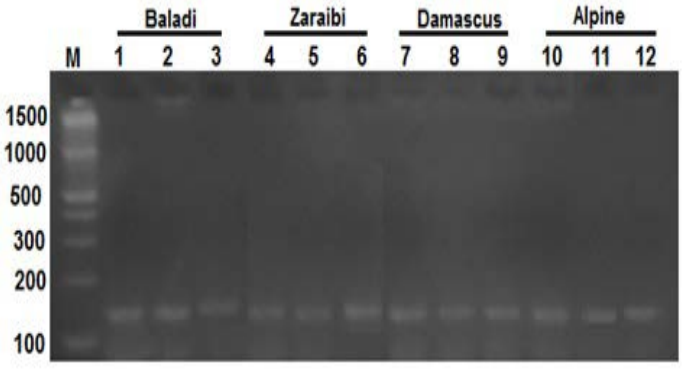

Figure 1: Lane 1-12 represents the PCR product of *BMP15* gene (141-bp) of four goat breeds and Lane (M) is a DNA marker

Genotyping of *Bmp15* gene Using RFLP Technique

Analysis of *HinfI*-PCR-RFLP of *BMP15* (141-bp) indicate presence of one uncut monomorphic band among all studied animals (higher and lower litter size).

Table 1: Single nucleotide polymorphism and amino acid variations of four goat breeds under study.

SNPs	Litter size	Amino acid changed in studied Goat breeds			
		D	B	A	Z
751 G>T and 752 G>C	Low	251 Gly>Cys	251 Gly>Ala	-	-
752 G>C	High	-	251 Gly>Ala	-	251 Gly>Ala
	Low	-	-	-	-
757 A>C	High	-	252 lys> Gln	252 lys> Gln	252 lys> Gln
762 G>T	Low	-	-	254 Glu>Asp	-
760 G>C and 762G>A	High	254 Glu>Gln	254 Glu>Gln	254 Glu>Gln	254 Glu>Gln
769 G>A and 769 G>C	Low	257 Glu>Gln	257 Glu> lys	257 Glu> lys	-
769 G>C	High	-	257 Glu>Gln	257 Glu>Gln	257 Glu>Gln
805 G>C	In all goat breeds (low and high)	269 Glu>Gln	269 Glu>Gln	269 Glu>Gln	269 Glu>Gln

Glu= Glutamic acid, Gln= Glutamine, Gly= Glycine, Cys= Cysteine, lys=Lysine Asp=Aspartic acid and Ala= Alanine. Z for Zaraibi, B for Baladi, A for Alpine and D for Damascus.

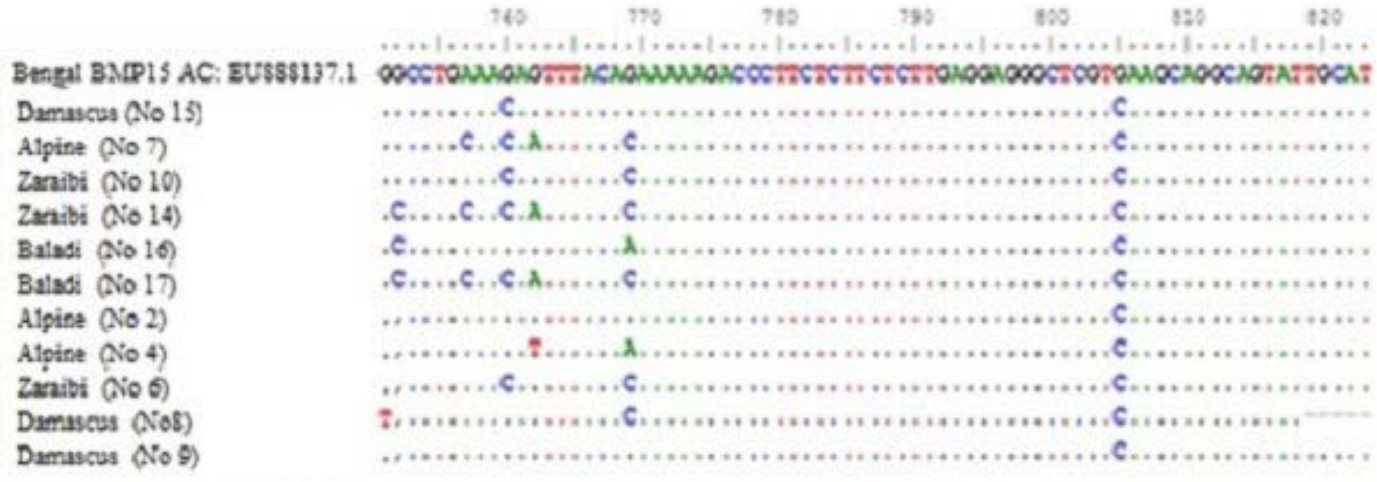

Figure 2: Nucleotide alignment showing SNPs in four goat breeds under study. Does No. (10Z, 6Z, 14Z, 17B, 7A, 15D and 2A) are high litter size. Does No. (8D, 9D, 16B and 4A) are lowest litter size. Z for Zaraibi, B for Baladi, A for Alpine and D for Damascus.

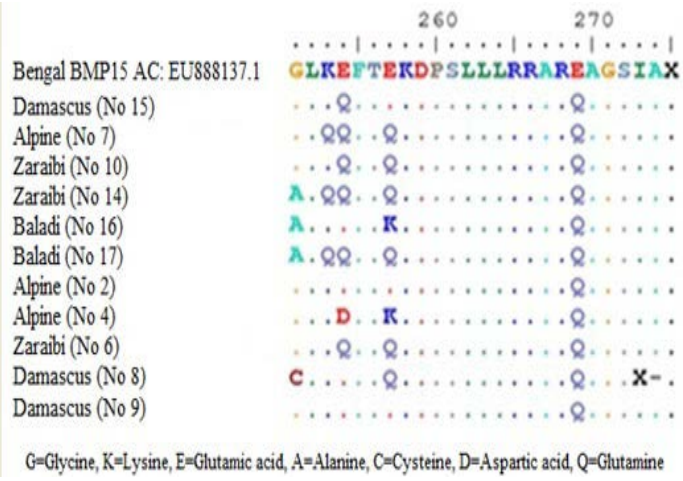

Figure 3: Amino acid alignment showing altered amino acid in four goat breeds under study. Does No (10Z, 6Z, 14Z, 17B, 7A, 15D and 2A) are high litter size. Does No (8D, 9D, 16B and 4A) are low litter size. Z for Zaraibi, B for Baladi, A for Alpine and D for Damascus.

DNA Sequencing, Screening of SNPs and Changed Amino Acids

Sequence analysis of *BMP15* (141 pb) of 11 females represent the four goat breeds (seven high and four low) and their alignment was shown in Figure 2. The changed amino acids were presented in Figure 3. In the present study, the *BMP15* mutation was investigated in the some Egyptian goat breeds. Sequence alignment of all does indicate the existence of seven SNPs among the studied goat breeds having high or low litter size (Table 1).

The results revealed that, there were three SNPs in Zaraibi does number 6, 14 and 10 of high litter size (4, 4 and 3 kids/litter) respectively. There were two SNPs at nucleotide number 760 and 769 G>C and the two corresponding amino acid number 254 and 257 Glu>Gln (Figure 3 and Table 1). These two SNPs can be used as a marker-assisted selection for improvement of litter size in Zaraibi breed.

Moreover, in the Baladi doe number 17 (with 3 kids) has five SNPs at nucleotide number 752 G >C, 757 A>C, 760 G>C, 762 G>A, and 769 G>C showed in Table (1) and the four corresponding amino acids were changed, amino acid number 251 Gly>Ala, 252 Lys>Gln, 254 and 257 Glu> Gln (Figure 3). Also, the Alpine doe number 7 (with 2 kids) had the same previous four SNPs except SNP no. 752. These four SNPs can be used as a marker-assisted selection for high litter size in Baladi and Alpine breeds. In Damascus breed, doe number 15 (with 2 kids) has only one SNP at nucleotide number 760 G>C which leads to change its corresponding amino acids 254 Glu>Gln (Figure 3). From the obtained results there are many SNPs which can use as a marker assisted selection for improving litter size. The SNP in nucleotide 760 is common in all studied breeds (Table 1).

On the other hand, in low litter size does there is many SNPs within each breed. Nucleotides number 752 and 769 in Baladi doe number 16 changed from G>C and G>A and lead to change amino acid 251 Gly>Ala and 257 Glu>Lys. In Alpine breed, SNPs number 762 G>T and 769 G>A were detected in doe number 4 and lead to change translated amino acid 254 Glu>Asp and 257 Glu>Lys (Table I). While, Damascus doe number 8 showed two SNPs, 751 G>T and 769 G>C in Table 1 which lead to change their corresponding amino acids from 251 Gly>Cys and 257 Glu>Gln respectively (Figure 3).

DISCUSSION

PCR-RFLP is a rapid, simple and exact technique for single nucleotide polymorphism (SNP) genotyping. This approach has been used previously to genotype prolific sheep and goat by several research groups (Souza et al., 2001; Davis et al., 2002; Kumar et al., 2006; Guan et al., 2007; Polley et al., 2009).

In our study, PCR-RFLP and SNP approach were used to detect polymorphism in *BMP15*. PCR-RFLP where used *HinfI* restriction enzyme to digest the amplified fragment but this enzyme doesn't digest the DNA amplified fragment and no differences showed between does under study. Similarly, Shokrollahi, (2015) reported that all studied individuals of Markhoz goats showed wild type allele (uncut) with 141 bp in length by using *HinfI* restriction enzyme. Seven different SNPs has been detected in does under study. About three SNPs for high litter size does and three for low litter size and one SNP as specific for local Egyptian breeds. So, SNPs which were detected in *BMP15* can be used as marker assisted selection (MAS) for high litter size as well as low litter size doe within the breeds. Where, nucleotides number 760 G>C in all high litter size does for all breeds under study (Zaraibi, Baladi, Alpine and Damascus). Furthermore, nucleotide number 757 A>C, 762 G>A, and 769 G>C in high litter size does for three breeds (Alpine, Zaraibi and Baladi). From all results, SNPs considered as a marker assisted selection (MAS) for *BMP15* gene which may be improving the litter size in these goat breeds. Moreover, these SNPs affected on amino acids translation where nucleotides number 757, 762 and 769 changed in (Zaraibi, Alpine, Baladi and Damascus) high litter size does lead to change in their corresponding amino acids 253, 254 and 269. Similarly, Nawaz et al. (2013) identified six novel polymorphic sites of *BMP15* gene in two goat breeds (Teddy and Beetal) of Pakistan and concluded that *BMP15* gene is the major gene which increases the fecundity in Teddy goat. Several studies reported that the variations in *BMP15* genes improve the ruminant reproduction and increase the sheep breeding system (Galloway et al., 2000; Hanrahan et al., 2004; McNatty et al., 2005). Another study indicated that *BMP15* is the major gene affected prolificacy in Jining Gray goat breed where they detected two point mutations linked with higher prolificacy (Chu et al., 2007).

Not only select high litter size but also we investigated low litter size does within three breeds. Baladi breeds doe number 16 (low litter size) nucleotide number 769 changed from G>A while in high litter size doe (number 17) the same nucleotide (769) changed from G>C with change in amino acid translation 257 Glu>Lys (Doe number 16), so these SNPs can be consider as MAS for low litter size within Baladi breeds.

Within Alpine breed (does number 7 and 4) nucleotide number 762 changed G>A in high litter size does and changed C>T in low litter size doe. Also, Damascus breed (doe) number 8 nucleotide number 751 and 769 changed G>T and G>C in low litter size only while nucleotide number 760 (doe) number 15) changed G>C in high litter size. Also, these SNPs leads to change their corresponding amino acids. So, SNPs detected in *BMP15* can be used as marker assisted selection (MAS) for low litter size. The substitutions of the amino acid might lead to a change in protein structure, thus affecting the signaling pathway-during follicle differentiation and ovulation. Additionally, Cai-lan et al. (2007) reported that exon 2 of *BMP15* have a mutation in two nucleotides lead to changes two amino acids in high prolificacy breed (Jining Gray goat) and low prolificacy breed (Inner Mongolia Cashmere).

CONCLUSION

We concluded that, there one main novel SNP present in all high litter size of four breeds and three main novel SNPs detected in does (high litter size) of three breeds (Zaraibi, Alpine and Baladi) leads to change their corre-

sponding amino acids. So, we suggest that the obtained SNPs in *BMP15* can be used as MAS for both high and low litter sizes in goats under study.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally to carry out this work.

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