Polymorphisms GDF9 and FSHβ genes and its association with litter size in Egyptian goat breeds

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Abstract. Phenotypic evaluation and culling of candidate animals for traits by applying traditional animal breeding are usually costly tasks which require considerable time to be carried out. Molecular genetics as an alternative method enables animal breeders to select eligible animals for the desirable trait (s) at their earlier ages. Selection based upon markers could result in increasing accuracy as well as selection response of animals. This research was performed for screening polymorphism of growth differentiation factor 9(GDF9) and follicle stimulating hormone (FSHβ) in two goat breeds Zaraibi and Baladi of Egypt. The both breeds are the more prolific than other breeds of goat found in the country. To find out molecular markers associate with litter size, the selection of animals based on single birth (SB) and multiple births (MB) history were collected. Forty samples were collected from each breed. In Baladi goat breed, polymorphic restriction pattern indicate presence of one band with 710 bp among all SB does and three bands with 710 and 600 bp and 100 bp for MB does .In Zaraibi goats, polymorphic restriction pattern indicate presence of one band with 700bp among all SB does and two bands with 600 bp and 100 bp for MB does. These result showed that presence of polymorphic of GDF9 in Baladi goat and monomorphic of GDF9 in Zaraibi MB does. The mutations in the GDF9 gene associate with fecundity were identified only in investigated MB Egyptian goat breeds. On the other hand, the restriction enzyme which used in PCR-RFLP did not identify any FSHβ gene mutation in exon 1. Aligment of the tested alleles with Capra hircus GDF9 sequence from gene bank showed transition in multiple births does from CCGAGG to GTTCAT and from TT to AG in regions from 52 to 57 and from 61 to 62, respectively. On the other hand, only one transition occurred in FSHβ sequence gene from CTGTT to ACAAA in region from 31 to 35.The study indicates the possibilities of using these markers for selection for high prolificacy in Egyptian goats.

Keywords: GDF9, FSHβ, goat, PCR-RFLP, Prolificacy

I. Introduction

Phenotypic evaluation and culling of candidate animals for traits by applying traditional animal breeding are usually costly tasks which require considerable time to be carried out. The identification of polymorphism and DNA markers associated with reproductive traits could be used as marker-assisted selection which lead to genetic improvement to increase litter size and reproduction efficiency (Ghaffari et al., 2009).Selection based upon markers could result in increasing accuracy as well as selection response of animals (Ji et al., 2003).Therefore, The aim of this study was to investigate the presence of polymorphism in GDF9 and FSHβ genes and their possible association with litter size in the Zaraibi and Baladi Egyptian goat breeds

II – Materials and methods

1. Sample Collection and genomic DNA Extraction

Blood samples were collected from the jugular vein of Zaraibi and Baladi herds kept in Sakha experimental station .The station belongs to Animal Production Research Institute, Agriculture Research Center. The both breeds are the more prolific goat breeds in Egypt the does were assembled into two groups; single birth (SB, n = 30) and multiple births (MB, n = 30). Genomic DNA was extracted from whole blood according the method described by Miller et al. (1988), amplified, purified and sequenced.

2. PCR amplification of GDF9 and FSHβ genes

Two pairs of primers were designed to amplify axon 1 which corresponded to the Gen Bank accession number AF078545, according to Hanrahan et al. (2004). The sequences of the two pairs of primers were as follows:

Table 1. A list of DNA primers, and restriction enzyme (RE)

Gene	Primer sequence	RE	Reference
GDF9	F: (5′-GAA TTG AAC CTA GCC CAC CCA C-3′) R: (5′- AGC CTA CAT CAA CCC ATG AGG C-3′)	Msp1	Galloway et al.,2000
FSHβ	F: (5′ GAT GAA GTC CGT CCA GTT-3′) R: (5′TAG ACC CTC AGG ACC CTC 3′)	Pst1	Davis et al.2002

Polymerase chain reactions were carried out in a 25 µL volume containing approximately 12.5 µl Master Mix (OnePCR™), 1 µl of each primer, 2 µl of genomic DNA (50 ng/µl), and 8.5 µl of sterile deionized water .The amplification reaction conditions was carried out using 35 cycles at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1min, and 72 °C for 2 min, and a final extension step at 72 °C for 10 min using thermal cycler 2720 . PCR products were checked by electrophoresis using 1.8% agarose gel in 1× TAE buffer. The products were then purified using the QIAquick Gel Extraction kit no. 28706 and sequenced by automated DNA sequencing reactions.

III – Results and discussion

1. Genotyping of GDF9 gene Using PCR-RFLP Technique

In Baladi goat breed, polymorphic restriction pattern indicate presence of one band with 710 bp among all SB does and three bands with 710 and 600 bp and 100 bp for MB does (Figures 1and 2).

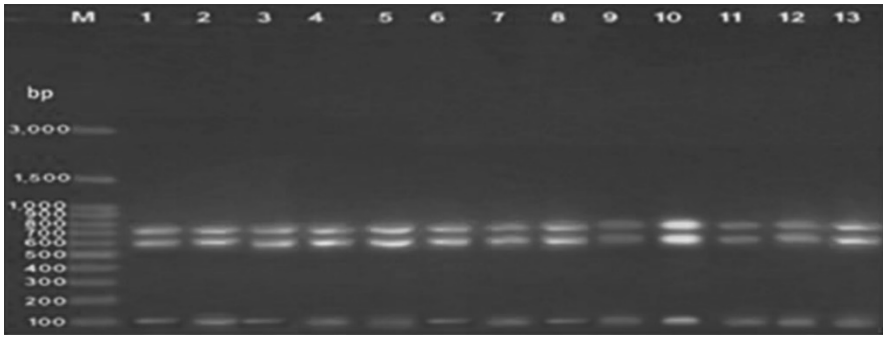

Fig 1. Lanes (1-13) represents the PCR products of GDF9 gene of MB Baladi goat breed

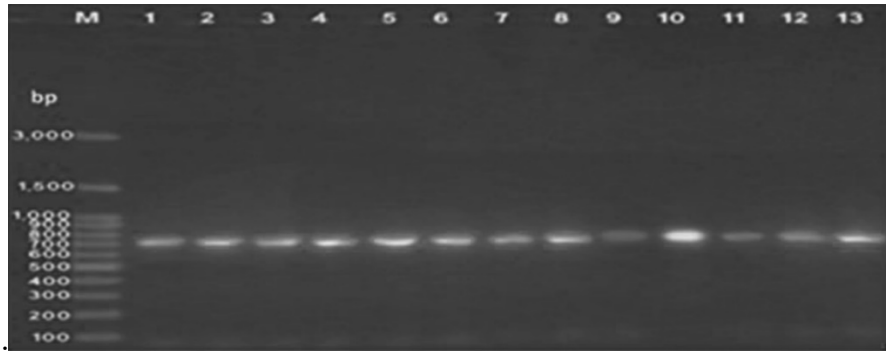

Fig 2. Lanes (1-13) represents the PCR products of GDF9 gene of SB Baladi goat breed

In Zaraibi goats, polymorphic restriction pattern indicate presence of one band with 700bp among all SB does and two bands with 600 bp and 100 bp for MB does (Figures 3 and 4).These result showed that presence of polymorphic of GDF9 in Baladi goat and monomorphic of GDF9 in Zaraibi MB does. The mutations in the GDF9 gene associate with fecundity were identified only in investigated MB Egyptian goat breeds. On the other hand, the restriction enzyme which used in PCR-RFLP did not identify any FSHβ gene mutation in exon 1. Mutations in fecundity genes GDF-9 and BMP-15 have important economic values in sheep and goat breeding (*Hanrahan et al., 2004*). *Noshahr (2014)* reported that the presence of one copy of mutant GDF9 gene increase fecundity rate in sheep.

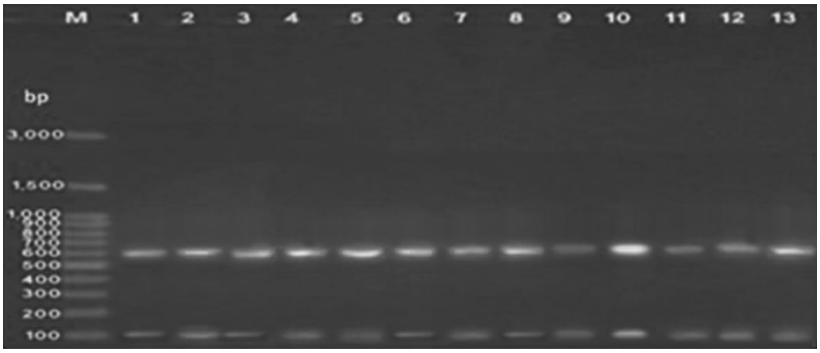

Fig 3. Lanes (1-13) represents the PCR products of GDF9 gene of MB Zaraibi goat breed

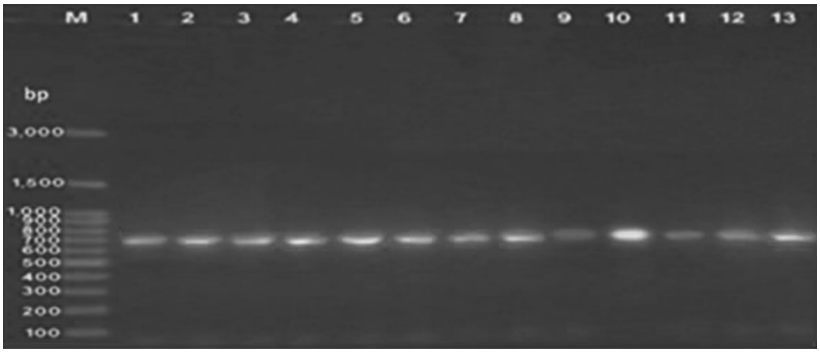

Fig 4. Lanes (1-13) represents the PCR products of GDF9 gene of SB Zaraibi goat breed

2. Sequence Analysis

Aligment of the tested alleles with Capra hircus GDF9 sequence from gene bank showed transition in multiple births does from CCGAGG to GTTCAT and from TT to AG in regions from 52 to 57 and from 61 to 62, respectively. On the other hand, only one transition occurred in FSHβ sequence gene from CTGTT to ACAAA in region from 31 to 35. These nucleotide changes associated with amino acid substitution .Transition from A to G in BMPR-IB has been reported in many breeds **(Chu et al 2007)**.

IV – Conclusions

The study indicates the possibilities of using these markers for selection for high prolificacy in Egyptian goats.

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