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

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Introduction

The response of genetic improvement for litter size trait in different livestock species through traditional selection method proved to be time consuming. Molecular genetics as an alternative method could result in increasing accuracy as well as selection response of animals.

Objectives

To investigate the polymorphisms of GDF9 and FSHβ fecundity genes and their possible association with litter size in two Egyptian breeds (Zaraibi and Baladi)

Materials and methods

. Identification of polymorphisms and DNA markers

Blood samples were collected from the jugular vein of Zaraibi (40) and Baladi (40) Egyptian goat breeds. The animals were obtained from Sakha Animal Production Research Station, belonging to Agriculture Research, Egypt. The does were selected according to their litter size trait, using the pedigree records. Each breeds was assembled into two groups; high prolific (two or more kids per litter, n=20) and low prolific (one kid per litter, n=20)

4.1 DNA Extraction

The genomic DNA was extracted from the whole blood, amplified, purified and sequenced.

4.2 Polymerase Chain Reaction (PCR)

The resulted PCR products were digested using restriction enzymes.

Table 2. A list of DNA primers, and restriction enzyme

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

Result and discussion

1. Genotyping of GDF9 gene Using PCR-RFLP Technique

In Baladi goat breed:

Polymorphic restriction pattern indicate presence of three bands with 710 and 600 bp and 100 bp for higher litter size does (MB) (Figures 1) and one band with 710 bp among all SB does (Figure 2)

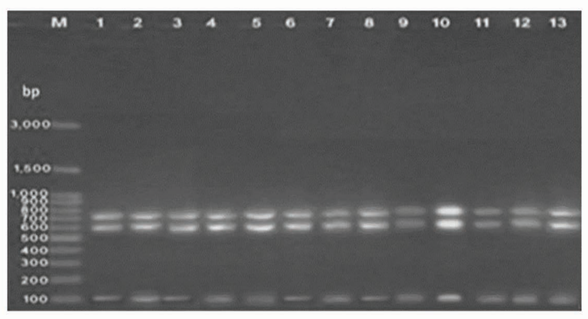

Fig 1. Lanes (1-13) represents the PCR products of GDF9 gene of MB Baladi goat breed

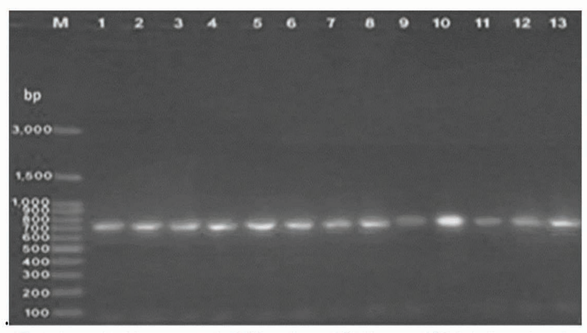

Fig 2. Lanes (1-13) represents the PCR products of GDF9 gene of SB Baladi goat breed

In Zaraibi goats:

Polymorphic restriction pattern indicate presence of two bands with 600 bp and 100 bp for MB does (Figure) and one band with 700bp among all SB does (Figure 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.PCR-RFLP analysis of FSHβ gene revealed that Pst1 restriction did not digest the DNA amplified fragment.

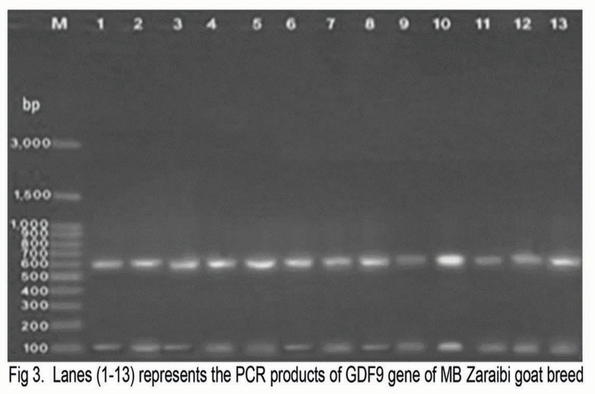

Fig 3. Lanes (1-13) represents the PCR products of GDF9 gene of MB Zaraibi goat breed

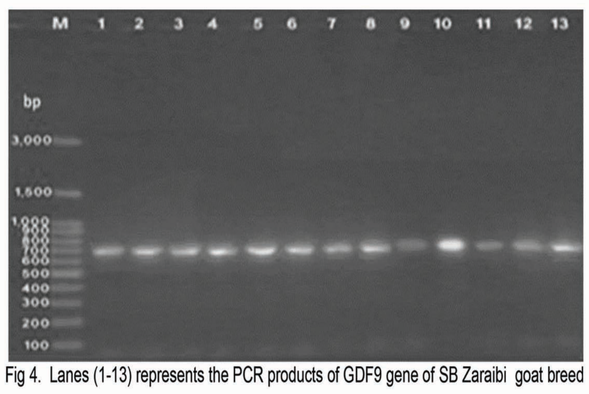

Fig 4. Lanes (1-13) represents the PCR products of GDF9 gene of SB Zaraibi goat breed

2. Sequence Analysis

Alignments 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. Sequence analysis for FSHβ revealed transition for CTGTT to ACAAA in region from 31 10 35 in all prolific samples. These nucleotide changes associated with amino acid substitution.

GDF9 (exon1)208
ACGGATTGGAAGGTCGCTATGGGGAAGTTTTGGATGGGAAAGTGGCGGGCGAAAAGGT
Allele (A) 1
AACGGATTGGAAGGTCGCTATGGGGAAGTTTTGGATGGGAAAGTGGCGTGGCGAAAAGGT
Allele (B) 2
AACGGATTGGAAGGTCGCTATGGGGAAGTTTTGGATGGGAAAGTGGCGTGGCGAAAAGGT
GDF9 (exon1)268

TAGCTGTGAAAGTGTTCTTCACTACAGAGGAGGCCAGCTGGTTCCGAGACAGAAATATATCA

Allele (A)
61TAGCTGTGAAAGTGTTCTTCACTACAGAGGAGGCCAGCTGGTTCCGAGACAGAAATATATCA

Allele (B) 61
TAGCTGTGAAAGTGTTCTTCACTACAGAGGAGGCCAGCTGGTTCCGAGACAGAAATATATCA

GDF9 (exon1)328
TTTCTGTCTCTCGGGACAGGTGGCCTCCTCTGTAGAGAACAACACTTTCACAGCTACCTTTTCG

Allele (A)
121TTTCTGTCTCTCGGGACAGGTGGCCTCCTCTGTAGAGAACAACACTTTCACAGCTACCTTTTCG

Allele (B) 121
TTTCTGTCTCTCGGGACAGGTGGCCTCCTCTGTAGAGAACAACACTTCCACAGCTACCTTTTCG

IV – Conclusions

In conclusion, The GDF9 gene was polymorphic and in agreement with the litter size. Amino acid substitutions were detected and repeated in higher and lower litter size animals. The study indicates the possibilities of using these markers as marker-assisted selection for increase litter size and reproduction efficiency in the Egyptian goat breeds.