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Identification of molecular markers in GDF9gene of Egyptian goat breeds

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Abstract. The aim of this study was to investigate the presence of polymorphism in growth differentiation factor 9 (GDF9) genes and their possible association with litter size in two more prolific goat breeds in Egypt.The does were selected according to their litter size trait, using the pedigree records. Each doe was assembled into two groups: single birth (SB, n = 30) and multiple births (MB, n = 30). 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 monoomorphic of GDF9 in Zaraibi MB does. The mutations in the GDF9 gene associate with fecundity were identified only in investigated MB Egyptian goat breeds. Alignment of the tested alleles with copracircus 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.The study indicates the possibilities of using these markers for selection for high prolificacy in Egyptian goats.

Keywords. GDF9 – Prolificacy – Goat – Fecundity genes – Polymorphism.

Identification de marqueurs moléculaires dans les gènes GDF9 et FSHβ de races de chèvres égyptiennes

Résumé. *L'objectif de cette étude était d'étudier la présence d'un polymorphisme dans le gène du facteur de différenciation de croissance 9 (GDF9) et leur association possible avec la taille de la portée dans deux races de chèvres plus prolifiques en Égypte. Les biches ont été sélectionnées en fonction de leur trait de taille de portée, en utilisant les enregistrements de pedigree. Chaque race a été assemblée en deux groupes: naissance unique (SB, n = 30) et naissances multiples (MB, n = 30). Chez les chèvres Baladi, le schéma de restriction polymorphe indique la présence d'une bande de 710 pb parmi toutes les bés SB et de trois bandes de 710 à 600 pb et 100 pb chez les chèvres B. Chez les chèvres Zaraibi, le schéma de restriction polymorphe indique la présence d'une SB et deux bandes avec 600 pb et 100 pb pour MB. Ces résultats ont montré que la présence de GDF9 polymorphe chez la chèvre Baladi et de GDF9 monomorphe chez Zaraibi MB le fait. Les mutations du gène GDF9 associées à la fécondité ont été identifiées uniquement chez les races de chèvres égyptiennes MB examinées. L'alignement des allèles testés avec la séquence capDFhircus GDF9 d'une banque de gènes a montré une transition lors de naissances multiples de CCGAGG à GTTCAT et de TT à AG dans les régions de 52 à 57 et de 61 à 62, respectivement. L'étude indique les possibilités d'utilisation de ces marqueurs pour la sélection à haute prolificité chez les chèvres égyptiennes.*

Mots-clés. *GDF9 – Prolificacy – Chèvre – Gènes de fécondité – Polymorphisme.*

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 effi-

ciency (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 GDF9gene 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 and 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).

2. PCR amplification of GDF9 gene

Two pairs of primers were designed to amplify axon 1 and flanking of goat GDF9 gene 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:5'-AATTGAACCTAGCCCACCCAC-3' and 5'-AGCCTACATCAACCCATGAGGC-3'. 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.

3. Polymorphism detection and genotyping

Sixty PCR products of partial GDF9 gene (exon 1) were digested using Msp1 restriction enzyme (Germany ER0541) according to the manufacturer's instructions. A final reaction volume of 32 µl contained 10 µl PCR product, 18 µl H2O free of nuclease, 2 µl of 10× buffer, and 2 µl (5 units) of restriction enzyme. The final volume of the mixture was mixed gently and spun down for a few seconds and then incubated for 18 h at 37 °C in water bath and stopped at 65 °C for 10 min. Restriction digestion products were checked by electrophoresis using 3% agarose gel in 1× TAE buffer and staining with ethidium bromide. The 100-bp ladder was used as a molecular size marker.

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 (Figs. 1 and 2).

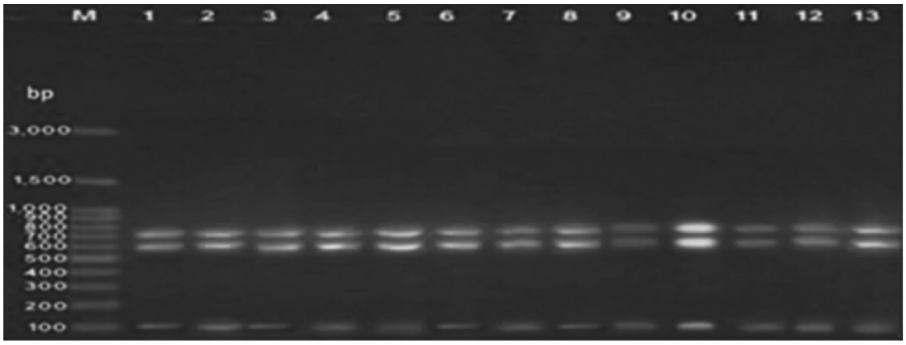

Fig. 1. Lanes (1-13) represents the PCR productsof GDF9 gene of MB Baladi goat breed.

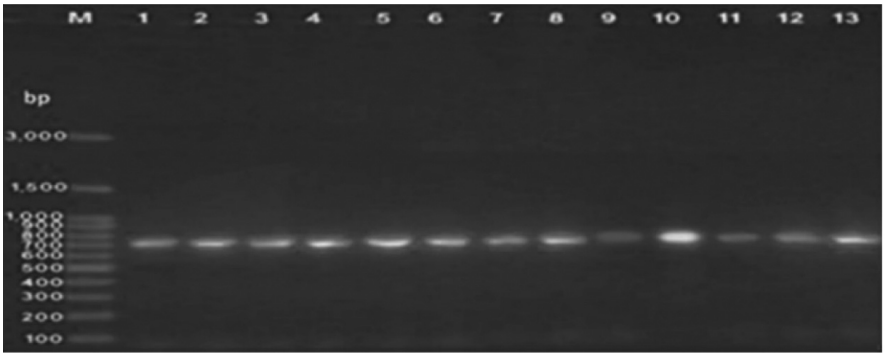

Fig. 2. Lanes (1-13) represents the PCR products of GDF9 gene of SB Baladi goat breed.

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 (Figs. 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. Mutations in fecundity genes GDF-9 and BMP-15 have important economic values in sheep and goat breeding (Hanrahan *et al*., 2004). Noshahr and Rafat (2014) reported that the presence of one copy of mutant GDF9 gene increase fecundity rate in sheep.

2. Sequence Analysis

Alignment 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. 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

Amino acid substitutions were detected and repeated in higher and lower litter size animals, which can be used as marker-assisted selection for litter size trait in the goat breeds under study.

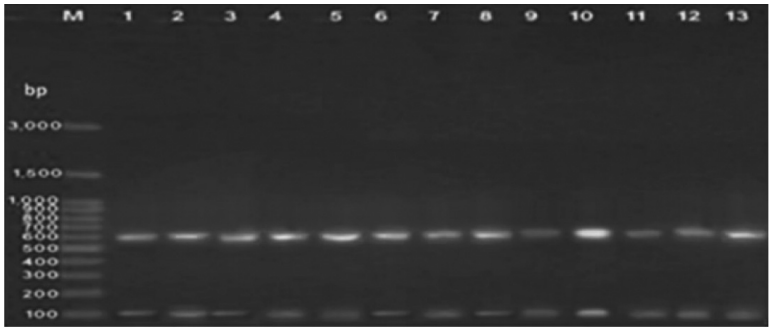

Fig. 3. Lanes (1-13) represents the PCR products of GDF9 gene of MB Zaraibi goat breed.

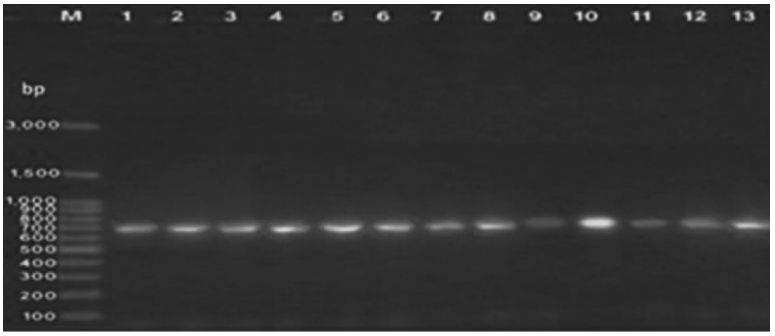

Fig. 4. Lanes (1-13) represents the PCR products of GDF9 gene of SB Zaraibi goat breed.

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