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Sequence Assigning of the Growth Differentiation Factor-9 (*GDF9*) Gene in Markhoz Goats

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Abstract: This study was conducted to sequence the Growth Differentiation Factor-9 (*GDF9*) from Transforming Growth Factor beta (*TGFβ*) super family in Markhoz breed of goat. This gene has vital role in ovarian follicle development, ovulation rate and fertility. Blood samples were collected from Sanandaj Markhoz goat breeding station and DNA was extracted using standard phenol chloroform method. The primers for exon 1 and 2 were designed and used for amplification of the gene fragments through the simple PCR procedure. The length of exon 1 and 2 are 463bp and 997bp, respectively. Sequence detection was performed after amplification of the gene segments. A mutation at exon 1 in base No.233 with C¦A type and two mutations at exon 2, one in base No.354 with T¦C type and another in base No.594 with A¦C type were recognized. These mutations were documented and recorded in the NCBI gene bank with number of GU784823.

Key words: *GDF9*, markhoz goats, ovulation rate, PCR, sequencing, *TGFβ*

INTRODUCTION

TGFβ super family is a large group of proteins that consists of more than 35 members (Chang *et al*., 2002). These proteins have large effects on numerous physiological procedures as transcellular ligand before and after birth. Growth differentiation factor-9 (*GDF9*) gene has regulating roles in fertility (Davis, 2005; Hanrahan *et al*., 2004; Kleppe *et al*., 1971; Shimasaki *et al*., 2004; Welt *et al*., 2002). According to Chromosome map, this gene is located on chromosome 5. Protein coding region length for this gene is 1359 base that produces a protein with 318 Amino Acids (Hanrahan *et al*., 2004). Goat *GDF9* gene includes 5508bp that has two exons. This gene identified to has essential role in follicular development in mice for the first time, and lacking of it, results halting the follicular development and infertility in early stages. *GDF9* gene is necessary for fertility, so that infertility take place in carriers of homozygote alleles for this gene in mammals. They also reported that, Cambridge and Belclare sheep with one mutation in *GDF9* gene exon1 and 2, have higher fertility which leads to increased ovulation rate in heterozygote sheep and infertility in homozygote ones (Knight and Glister, 2001). Barzegari *et al*. (2008) studied on *GDF9* and *BMP15* genes in Ghezel and Moghani sheep, have showed that ewes with mutation were infertile and prolific when they are homozygote and heterozygote for those mutations respectively.

The aim of this study was assigning the *GDF9* gene in Markhoz goats.

MATERIALS AND METHODS

This study was performed at Islamic Azad University, Tabriz branch during 2010. Nearly 10cc blood was collected aseptically from the jugular vein of five does and was kept in -20ºC. EDTA used as anticoagulant. Blood samples collected from Markhoz breeding station in Sanandaj, northwest of Iran. DNA extraction conducted using standard Phenol-Chloroform method. The extracted DNA was evaluated via the 0.8% Agarose gel electrophoresis and spectrophotometery technique. Primers for exon 1 and 2 were designed using Oligo5 software (Table 1). These primers can amplify the 463bp and 994bp fragments for exon 1 and 2 respectively. Each of PCR products has 50 µL volume containing 26 µL PCR master kit, 1 µL DNA, 3 µL from both of the forward and reverse primers (with 10pmol density, each) and 17 µL DNAase free water, were prepared and tested with the 1.2% Agarose gel electrophoresis method, and then sequenced by Macrogen Inc. South Korea.

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Table 1: Primers used for exon 1 and 2 of *GDF9* gene amplification

Gene	Primer	September 8, 2011Primer Sequence (5´-3´)	Annealing temperature (ºC)
GDF9 (Exon1)	F1	GAAGACTGGTATGGGGAAATG	61
463bp	R1	CCAATCTGCTCCTACACACCT	
GDF9 (Exon2)	F2	TGGCATTACTGTTGGATTGTTTT	62
994bp	R2	AACGACAGGTACACTTAGTGGC	

RESULTS

Exon 1 and 2 amplification: PCR products were tested using the 1.2% Agarose gel electrophoresis technique and the results showed that desired fragments amplified properly and no unspecific fragments were seen (Fig. 1).

GDF9 sequence: In this research, Markhoz breed *GDF9* gene both exons were sequenced for the first time. The length of exon 1 and 2 assigned as 463bp and 997bp, respectively. Sequence results showed one mutation in exon 1 at nucleotide No. 233 with C→A type and two in exon 2, which are at nucleotide No. 354 with T→C type and at nucleotide No.594 with A → C type (Fig. 2). Inspection of exon 2 sequences and mutations have confirmed Niazi *et al.* (2008) results, which exon two, the registered at NCBI gene bank with FJ472652 number.In exon one the third base of coding codon CTA, changed from C to A with no change in amino acid that is Leucine, so the mutation is silent. In the mutations of is a change in coded amino acid from Valine to Alanine, and in second mutation, the second base in CCG codon changed from A to C so coded amino acid changes from Glutamine to Proline. These results showed that both mutations in exon 2 are missense.

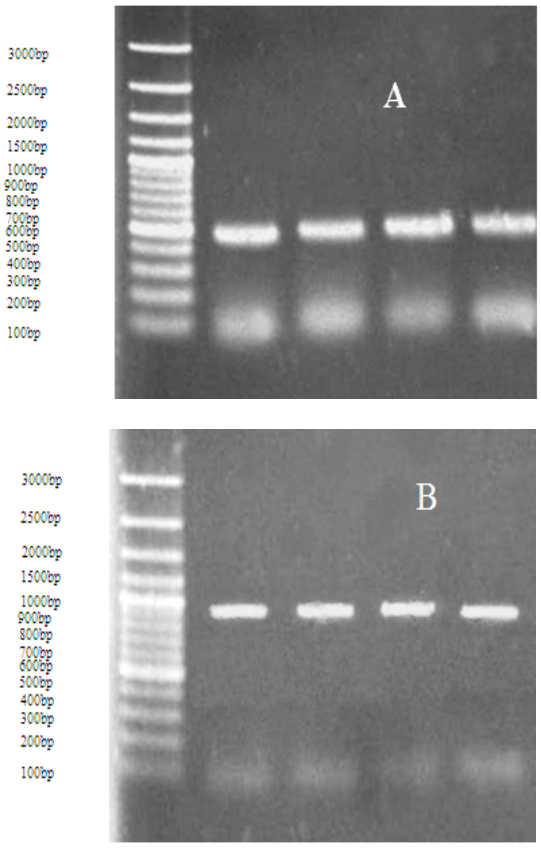

Fig. 1: Electrophoresis images of amplified exon1 (A) and exon2 (B)of *GDF9* gene

DISCUSSION

Several genes are defined to have associations with fecundity in some animals. It is verified that some loci in *BMP15*, *GDF9*, *BMPR-1B*, other genes, and their mutations have major effects in sheep prolificacy (Davis, 2005, 2004). Some of mentioned loci and genes were studied in goats (Arefnezhad *et al*., 2010) and it is determined that their influence in prolificacy of goats were not as significant as of sheep. In this research we sequenced Markhoz *GDF9* gene exons and found three single nucleotide mutations. These mutations need further studies to confirm their relevance with goat fecundity.

With considering, the significance of this gene in fecundity and ovulation rate, consecutive studies of this gene and other candidate genes, the use of techniques such as PCR-RFLP, SSR-PCR or PCR-SSCP look important. It is advised using greater flocks with pedigree. Therefore, detailed comparisons between gene sequences and stated mutations in prolific and non-prolific does by proper technique, propose. Additionally, it is advisable to examine other sections of this gene (for example introns) and to use infertile animals.

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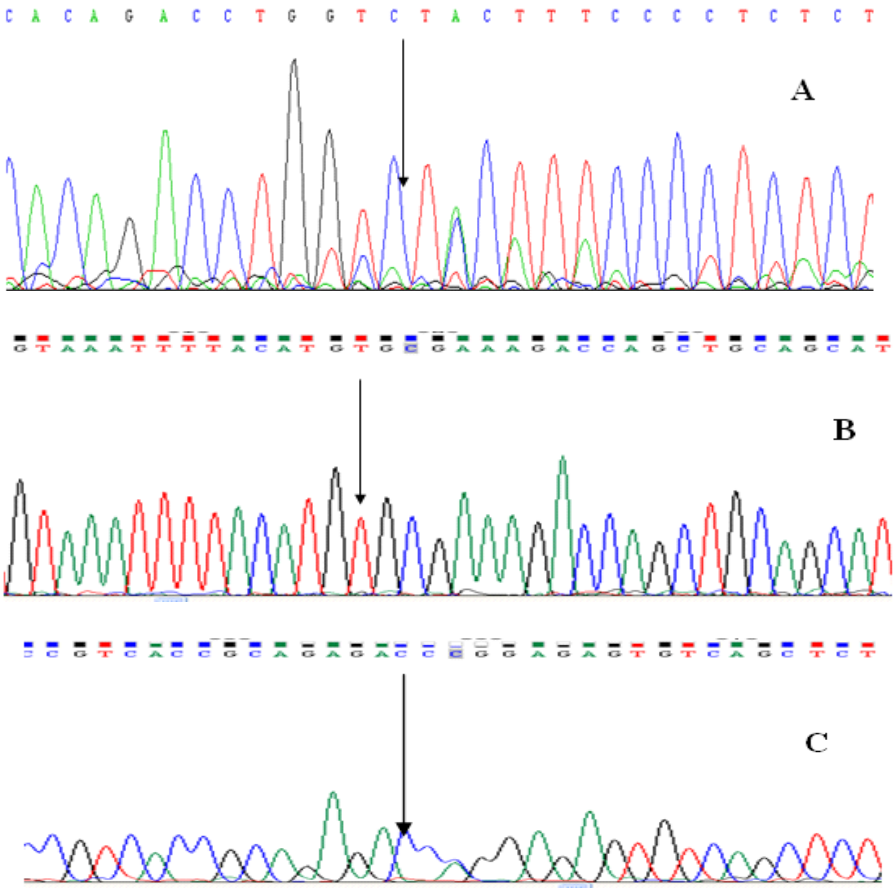

Fig. 2: Mutations in exon1 (A) and 2 (B and C) of *GDF9* gene

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