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FecX[1] gene as a monitor for the production of twins in the Egyptian sheep

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Abstract

Twenty two (11 twin producing female, 7 single lamb producing female, and 4 male) crossbred sheep were tested for the presence of the FecX[1] mutation of BMP15. They were carefully selected from the records of the Egyptian nuclear research center experimental sheep farm. The females were selected for their twin production in three repetitive production cycles while the males were selected for being produced from prolific females as above. Forced restriction PCR the FecX[1] mutations, DNA test for the presence of FecX[1] mutation showed no amplification resulted from DNA samples of the twin producing ewes while two amplified DNA fragments which has a molecular size 154 bp and 120 bp resulted from the single lamb producing females and male sheep under study.

Key words: Sheep, Fecundity, Prolificacy, FecB, FecX, PCR

Introduction

The total sheep population in Egypt is 4,200,000 heads. Rahmani, Ossimi, and Barki, are of the main sheep breeds in Egypt with a population of 990,000, 514,000, and 470,000 respectively. Sheep contribute 6% of the total red meat production in Egypt. Litter size and lamb growth are important economic traits in sheep breeding and reproduction (Galal et al., 2005). Genetic variation in the ovulation rate in sheep has been widely documented and the evidence shows substantial differences among breeds and the number of exceptional variation cases within breeds/strains (Bindon et al., 1996). The latter phenomenon can be explained by segregation of a gene with a large effect on ovarian function. The Inverdale (FecX[1]) locus was identified in a prolific family of Romney sheep (Davis et al., 1995). Genetic mapping for the FecX[1] locus was assigned to the sheep X chromosome by classical segregation analysis (Davis et al., 1991). Mapping studies to locate the gene were concentrated on the X chromosome. The gene was shown to map to the sheep X chromosome within the critical region and no recombinants were observed between the FecX locus and BMP15 (Galloway et al., 2000). The ovine gene was sequenced and shown to be similar to the gene in humans, mice and rats, with a full length sequence of 1179 bp containing two exons. Mutation was found in BMP15 in FecX[1] carriers. In FecX[1] carriers, a single T to A transversion at nucleotide position 92 results in the substitution of a valine with aspartic acid in a highly conserved region of the protein (Galloway et al., 2000). It appears likely that the amino acid change impairs the ability of BMP15 to form dimers and interferes with the biological action of BMP15 in ewes homozygous for the FecX[1] variant (Montgomery et al., 2001).

The present study is aimed to investigate the presence FecX[1] gene mutations focusing on the prolific sheep, based on the records of the experimental sheep farm, Nuclear Research Center, Egyptian Atomic Energy Authority.

Materials and Methods

The present study was conducted on a total of 22 crossbred Egyptian sheep breeds maintained at Experimental Sheep farm, Nuclear Research Center, Egyptian Atomic Energy Authority, Abuzabal , Cairo. Approximately, 1 ml venous blood was collected from each animal in heparinized tubes. Genomic DNA was isolated from blood using Wizard® Genomic DNA Purification Kit (www.promega.com) according to the manufacturer's instructions.

The quality of DNA was checked by spectrophotometry taking ratio of optical density (OD) value at 260 and 280 nm. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further work.

Analysis of samples for FecXI was carried out using the forced RFLP method Primer 12 has been

designed to generate a forced XbaI restriction enzyme site (T|CTAGA) in PCR products from carriers of the FecXI mutation in the BMP15 gene, whereas products from non-carriers of the mutation lack this site. Genomic DNA was amplified using forward primer (GAAGTAACCAGTGTTCCCTCCACCCTTTTCT) and reverse vprimer (CATGATTGGGAGAATTGAGACC) (Davis et al., 2006). For amplification, 25 µl of PCR reaction was prepared by adding 10 pM of each primer, 12.5µl Green Taq® PCR Master Mix (www.promega.com) and 100 ng DNA template. The amplification was carried out using a pre-programmed thermal cycler (Little genius, www.bioer.com.cn) with the following conditions: initial denaturation of 5 min at 94 ◦C followed by 30 cycles of denaturation at 94°C, annealing at 55°C and extension at 72°C each of 2s, final extension of 5 min at 72 °C. DNA tests were carried out using forced PCR RFLP based on the method described by Davis et al. (2002). An aliquot of 10 µl of PCR product was digested for 1 hour at 37°C with 10 Units of XbaI restriction enzyme (www.promega.com). The restriction enzyme digested PCR products were separated by 2% agarose gel. Ethidium bromide was added to the gel during formation and to the running buffer. The digested products were visualized on Biorad trans-illuminator, image was shot using digital camera and the gel was documented using totallab 120 (Nonlinear USA Inc).

The forced PCR of the $FecX^1$ gene produced a 190 bp band. After digestion with XbaI (promega), the $FecX^1$ gene homozygous carriers had a 124 bp band (XX), the noncarrier had a 154 bp band (++), whereas heterozygotes had both 124 and 154 bp bands (X+).

Results and Discussion

As seen in Figures 1 and 2, no PCR product resulted for the twin producing sheep, figure 1, while there where an amplification of two bands of 154, and 120 molecular sizes in the single lamb producing females and male specimens. No new bands were produced when PCR products the single lamb producing females and male specimens were digested with XbaI. These results signifies non-carrier (++) 154 bp band in all the animals studied revealing the absence of this restriction site of XbaI in those animals.

These primer sequences used in this study have been used to test the presence of inverdale gene in 28 sheep breeds from 17 countries. The sheep strains are Javanese, Thoka**,** Woodlands, Olkuska, Lacaune, Belclare, , Cambridge, Romanov (2 strains), Finn (2 strains), East Friesian,Teeswater, Blueface Leicester, Hu, Han, D'Man, Chios, Mountain Sheep (three breeds), German Whiteheaded Mutton, Lleyn, Loa, Galician, Barbados Blackbelly (pure and crossbred) and St. Croix) (Davis et al., 2002, 2006). The absence of PCR product using the primer set in twin producing female sheep indicates the possibility of a mutation in binding sites of the primers sequences. This mutation may be related to the increased fertility in the twin producing sheep in Egypt. Moreover, as notice from the results, the specific primer set used in this study may be used to directly test the fecundity in local Egyptian sheep. On the other hand, as shown in figure 2, the single lamb producing female sheep and male sheep produced two DNA fragments of molecular size 154bp and 120 bp. The 154 bp PCR DNA fragment product is believed to be the expected PCR product of the primers binding site (Galloway et al, 2000; Davis et al., 2002, 2006) while the 120 bp DNA fragment is additional. When the DNA PCR product was digested with XbaI, no digestion was detected. Results suggest the absence of $FecX^1$ mutation the Egyptian local sheep under study.

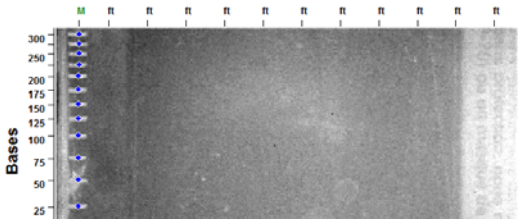

Fig. 1: The PCR product of BMP-15 of twin producing sheep (ft) there are no PCR products ft: female produce twins

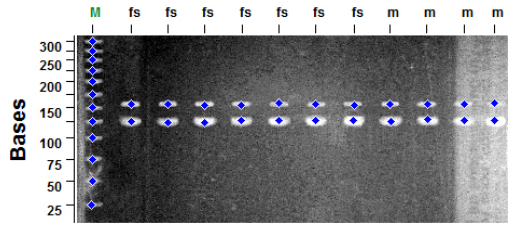

Fig. 2: The PCR product of BMP-15 of single lamb producing sheep (fs) and male sheep (m) with PCR products of 154 bp and 120 bp fs: female produce single; m: male

In conclusion, this study showed, in the twin producing female sheep, a possible presence of a mutation in the binding region of specific primers designed to test the presence of FecX1 in Egyptian sheep. This mutation hindered binding of these primers to their specific site therefore no amplification appeared. It is possible that this result may be used as a test to distinguish the twin producing individuals among the local Egyptian sheep. Further studies may take place to sequence and identify the mutation in this region. On the other hand, the amplification of 120 bp

DNA fragment is unique to the Egyptian sheep thus further studies may be conducted to sequence and study the significance of this DNA fragment.

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