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Prolificacy Detection in Egyptian Sheep using RFLP-Specific PCR

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ABSTRACT

Booroola gene (*FecB*) was the first major gene for prolificacy identified in sheep. In this study twenty two (11 twin producing female, 7 single lamb producing female, and 4 male) crossbred sheep were tested for the presence of the FecB mutation of BMPR1B. 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 of the FecB gene, 190 base pair (bp) was amplified using specific primer designed to introduce a point mutation in the resulting PCR products with FecB carrier sheep containing an AvaII restriction site (G|GACC). The FecB DNA test showed that there were no carriers for the FecB mutation in the selected prolific sheep sample.

Key words: Sheep, fecundity, prolificacy, FecB, FecX, PCR

INTRODUCTION

Sheep contribute 6% of the total red meat production in Egypt. 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. Litter size and lamb growth are important economic traits in sheep breeding and reproduction (Galal *et al*., 2005) Genetic variation in ovulation rate in sheep has been widely documented and the evidence shows substantial differences among breeds and in a number of cases exceptional variation 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. This hypothesis provided an explanation for high prolificacy of Booroola sheep (Davis *et al*., 1982; Piper and Bindon 1982). The Booroola gene (*FecB*) was the first major gene for prolificacy identified in Booroola Merino sheep (Chu *et al*. 2007). The *FecB* locus is situated in the region of ovine chromosome 6 corresponding to the human chromosome 4q22-23 that contains the bone morphogenetic protein receptor (*BMPR-IB*) gene, which encodes a member of the transforming growth factor *β* (TGF*β*) receptor family (Mulsant *et al*., 2001; Wilson *et al*., 2001). The FecB locus in sheep increases ovulation rate and litter size (Montgomery *et al*. 1992). A nonconservative substitution (Q249R) in the BMPR-IB coding sequence was associated fully with the hyperprolific phenotype of Booroola ewes (Mulsant *et al*., 2001; Souza *et al*., 2001; Wilson *et al*., 2001). Knowledge of this mutation has prompted researchers to screen other prolific sheep breeds to determine whether this mutation is responsible for their high prolificacy (Davis *et al*., 2002; 2006) these studies, in conclusion, showed that this mutation is present in some breeds while absent in other prolific ones. A DNA Test of *FecB* gene was performed in random samples of five Egyptian breeds namely Rahmani, Ossimi, Awassi, Barki and Awassi x Barki crossbred. The study did not detect the presence of FecB gene in the tested animals (EL-Hanafy and El-Saadani, 2009). The present study is aimed to investigate the presence of

the FecB gene focusing on the prolific sheep, based on the records of the experimental sheep farm, Nuclear Research Center, Egyptian Atomic Energy Authority.

MATERIALS AND METHODS

Animal materials and DNA extraction

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 spectro photometry 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.

PCR- Forced RFLP of FecB gene

A region of FecB gene (190 bp) was amplified by using a set of forward (5`-CCAGAGGACAATAGCAAAGCAAA -3`) and reverse (5`-CAAGATGTTTTCATGCCTCATCAACAG GTC -3`) primers (Wilson *et al*., 2001). The reverse primer was deliberately introduced a point mutation resulting in PCR products with FecB carrier sheep containing an AvaII restriction site (G/GACC), whereas products from noncarriers lacked this site. For amplification, 25 µl of PCR reaction was prepared by adding 25 pM of each primer, 12.5µl Green Taq® PCR Master Mix (www.promega.com), 100 ng DNA template and 0.5 Unit Taq DNA polymerase. 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 60 °C and extension at 72°C each of 30 s, final extension of 5 min at 72 °C and a final denaturation at 99 °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 hours at 37 °C with 10 Units of Ava II 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).

Genotype analysis

The forced PCR of the *FecB* gene produced a 190 base pair (bp) band. After digestion with *Ava*II (Fermentas), the *FecB* gene homozygous carriers had a 160 bp band (BB), the noncarrier had a 190 bp band (++), whereas heterozygotes had both 160 and 190 bp bands (B+).

RESULTS AND DISCUSSION

Fig.1and 2 showed the agarose gel electrophoresis of PCR product (190 pb). When PCR products of animals under study where digested AvaII restriction enzyme, digestions resulted in 190 bp band in all the animals studied revealing the absence of this restriction site of Ava II in those animals.

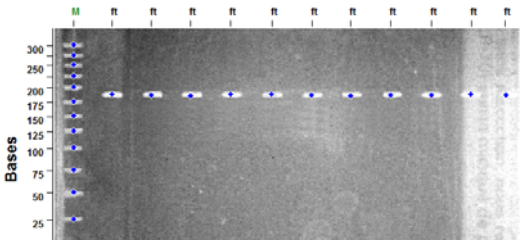

Fig. 1: The PCR product of BMPR-IB of twin producing sheep (ft). The PCR product is 190 bp

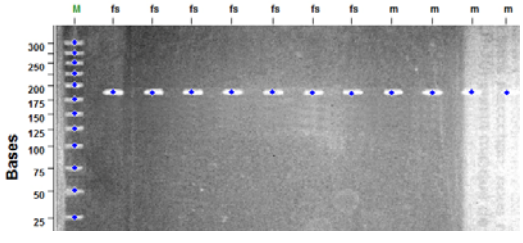

Fig. 2: The PCR product of BMPR-IB of single lamb producing sheep (fs) and male sheep (m) . The PCR product is 190 bp

The results of the present study indicate the absence of the boroola mutation (FecB) in all studied animals. The absence of FecB gene in the studied animals agreed with the results of EL-Hanafy and El-Saadani (2009), who concluded the absence of the gene in five Egyptian breeds. Although, there are about ten breeds in Egypt namely Rahmani, Ossimi, Awassi, Barki, Awassi,Saedi, Farafra, Suhagi, Kanzi, Abudoliek, and manite. The great majority of sheep breeders in Egypt usually cultivate cross-bred sheep as the individuals in the present study. On the other hand, absence of the FecB gene does not necessarily disaffirm the presence fecundity related gene(s). The highly prolific sheep amongst the Egyptian breeds may indicate a presence of a genomic influence. Besides FecB, there are several major genes influencing reproduction in sheep (Davis 2004). The modes of inheritance of the different prolificacy genes include autosomal dominant genes with additive effects on ovulation rate (BMPR-1B), autosomal over-dominant genes with infertility in homozygous females (GDF9), X-linked overdominant genes with infertility in homozygous females (BMP15), and X-linked maternally imprinted genes (FecX2) (Davis 2004). Either of these genes can be present in Egyptian breeds. Nevertheless, if all of these genes are absent in Egyptian local breeds, other molecular techniques can be used to determine the genetic influence on high prolificacy in some Egyptian sheep and study influencing gene(s). In conclusion, this study has highlighted the importance of further investigation for the gene(s) influencing reproductive sheep breeds in Egypt. The absence of FecB mutation in the studied animals indicates high possibility of the absence of the boroola gene mutation in Egyptian sheep. Further studies regarding other genes which may influence fecundity of the Egyptian sheep should be carried out to determine the type and mode of inheritance of such genes.

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ARABIC SUMMARY

الكشف عن الخصوبة في الأغنام المصرية باستخدام تفاعل البلمرة المتسلسل والإنزيمات المحددة

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مورثة البورولا هي أول مورثة يتم تعين العلاقة بينها وبين الخصوبة في الأغنام. في هذه الدراسة تم سحب عينات من ٢٢ من الأغنام الخليط (١١ نعجة تنتج توائم ، ٧ نعجة تنتج حمل واحد ، و أربعة ذكور) تم اختبار وجود مورثة البورولا بها . تم اختيار عينات النعاج المنتجة للتوائم من خلال سجلات مزرعة الأغنام بحيث تكون النعجة قد ولدت توائم في ثلاث مواسم أم الذكور وقد تم اختيارها من مواليد لنعاج منتجة للتوائم. تم اختبار العينات باستخدام تفاعل البلمرة المتسلسل متبوعا بتقطيع الناتج بإنزيم حصري. جميع العينات أعطت قطعة واحدة من الـ DNA ذات حجم ١٩٠ زوج قاعدي (base pair) كناتج من تفاعل البلمرة المتسلسل. عند إخراء تجربة التقطيع الإنزيمي على قطع الـ DNA الناتجة من العينات لم يتم تقطيع أي منها بالإنزيم . نتائج هذه الدراسة ترجح عدم وجود مورثة البورولا في الأغنام المصرية