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PCR-RFLP of IGFBP-3 Gene in Some Egyptian Sheep Breeds

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Abstract: The present investigation was to study DNA polymorphism of insulin-like growth factor binding protein-3 (IGFBP-3) gene by PCR-RFLP in four Egyptian local sheep breeds. Genomic DNA was isolated from a total of 20 animals from four Egyptian breeds (five each) of sheep namely Rahmani, Ossimi, Awassi, and Barki. A fragment of IGFBP-3 gene, comprising of a part of exon 2, complete intron 2, exon 3, and a part of intron 3, was amplified. The amplified fragment was found to be 654 bp in sheep. On digestion of 654 bp with *Hae* III restriction enzyme yielded single restriction pattern of five fragments of sizes 201, 201, 87, 67, 57 bp in all the animals belonging to the four Egyptian breeds studied revealing absence of polymorphism in those four Egyptian sheep breeds.

Key words: IGFBP-3 gene · PCR-RFLP · Sheep · Polymorphism

INTRODUCTION

Sheep contribute 6% of the total red meet 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 [1]. Rahmani is found in the Northern Delta (middle of Nile Delta), whereas Ossimi and Awassi are found in South of Nile Delta, while Barki is found in the Mediterranean coastal strip west of Alexandria. Egyptian sheep breeds are fat tailed and their body covered with carpet wool. Each breed has its productive characteristics. The mature weight of Rahmani and Ossimi is higher than that of Barki. The fleece weight and quality are higher in Barki than that in Ossimi and Rahmani [1]. Barki breed is well adapted to desert conditions [2], while Ossimi has a wider range of adaptability than Barki. Rahmani is believed to be more resistant/tolerant to internal parasites than other Egyptian breeds. Moreover, the twinning rate is relatively high in Rahmani breed. Although the lactation period is longer in Barki, total yield of milk is about the same in the three breeds [3]. Awassi, a fat-tailed breed, is the most prevalent sheep breed in the Arab Countries. The Awassi sheep breed is common to most of the Middle East Countries including Jordan, Iraq, Syria,Lebanon, Palestine and Egypt. Its extremely hardy sheep breed, well adapted over centuries of use to nomadic and more sedentary rural management especially those related to the scarcity of feed availability and high environmental temperatures, it's a triple purpose animal producing meat, milk and wool, Although Awassi is the natural or basic breed of sheep for production in these areas and a logical choice as the native or basic breed for any genetic improvement because of its apparent adaptation. Awassi population in Egypt is not exactly known.

Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objective [4, 5]. The genetic diversity of indigenous sheep in Egypt in respect to important economic genes has not been sufficiently studied. Genetic characterization and determination of genetic differences between sheep breeds will help in the genetic improvement programs.

Insulin-like growth factor binding protein-3 (IGFBP-3) gene is a structural gene responsible for the multiple effects of insulin-like growth factors (IGFs) [6]. IGF-I and

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IGF-II are couple of hormones involved in the process of mammalian growth and regenerative processes besides having active role in mammary gland development [7]. The bovine IGFBP-3 gene has been cloned and characterized and its mRNA is 1.65 kb in length [8]. The total length of gene is 8.9 kb having five exons [9]. Polymorphic studies and nucleotide sequence analysis of IGFBP-3 gene have been reported in cattle [10, 11, 12, 13] and buffalo [14], but no reports are available in sheep so far. However, mRNA expression of IGFBP-3 gene has been studied in sheep, and mRNA partial sequence was cited in Gen Bank (accession no. AF327651) [15]. Considering the importance of sheep in Egyptian meat production, the present study was undertaken to find out polymorphism, of the IGFBP-3 gene in Egyptian sheep and compare it with those of Indian sheep breeds, cattle and buffalo.

MATERIALS AND METHODS

Animal Materials and Genomic DNA Extraction: The present study was conducted on a total of 20 animals belonging to four Egyptian sheep breeds (five each): Rahmani, Ossimi, Awassi and Barki (maintained at Animal Production Research Station, Borg EL-Arab, Alexandria, Egypt). Approximately, 10 ml venous blood was collected from each animal using 0.5 ml of 2.7% EDTA as an anticoagulant. Genomic DNA was extracted from blood using QIAamp DNA extraction kit (QIAGEN GmbH, Hilden Germany) according to the manufacturer's instructions.

PCR-RFLP of IGFBP-3 Gene: A region of IGFBP-3 gene spanning over a part of exon 2, complete intron 2, exon 3 and a part of intron 3 was amplified by using the forward and reverse primers (5`-3`): CCA AGC GTG AGA CAG AAT AC and AGG AGG GAT AGG AGC AAG AT, respectively [10]. For amplification, 25 µl of PCR reaction was prepared by adding 10pM of each primer, 100µM of each dNTPs, 1.5mM MgCl2, 10× PCR assay buffer, 100 ng DNA template and 0.5 Unit *Taq* DNA polymerase. The amplification was carried out using thermalcycler (Eppendorf Mastercycler) with the following conditions: initial denaturation of 5 min at 94°C followed by 35 cycles of denaturation at 94°C, annealing at 60°C and extension at 72°C each of 1 min and lastly the final extension of 5 min at 72°C. An aliquot of 20 µl of PCR product was digested overnight with 5 Units of *Hae*III restriction enzyme. The PCR products digested by the restriction enzyme were separated by 12% PAGE gel, stained with ethidium bromide and visualized using Gel Doc. System (Syngene).

RESULTS AND DISCUSSION

The Fragment of IGFBP-3 gene (654 bp) has been successfully amplified from the DNA of each sample (20 samples) used in the present study. Figure 1 represent agarose gel electrophoresis of PCR amplified 654 bp fragment of sheep IGFBP-3 gene of Rahmani (R), Ossimi (O), Awassi (A) and Barki (B) breeds. Moreover, PCR amplified products of the IGFBP-3 gene after digested with Hae III was presented in Fig. 2.

Digestion of the PCR product of IGFBP-3 gene from the four breeds with *Hae*III revealed only one type of restriction pattern yielding five fragments with sizes 201,201, 87, 67 and 57 bp (Fig. 2). The results of *Hae*III RFLP pattern represented in Fig. 2 showed that no polymorphism has been detected among these four Egyptian sheep breeds in respect to IGFBP-3 gene. This results indicates the homozygosis of this gene in the four breeds studied.

The obtained results confirmed the reports of Kumar *et al*. [16] where no polymorphism was found in respect to this fragment of IGFBP-3 gene in five Indian breeds of sheep. However, they obtained *Hae*III restriction pattern of eight fragments of sizes 201, 201, 87, 67, 56, 19, 16 and 7 bp in all the animals studied revealing absence of polymorphism in these Indian sheep breeds, Also, The present results which showed no polymorphism in respect

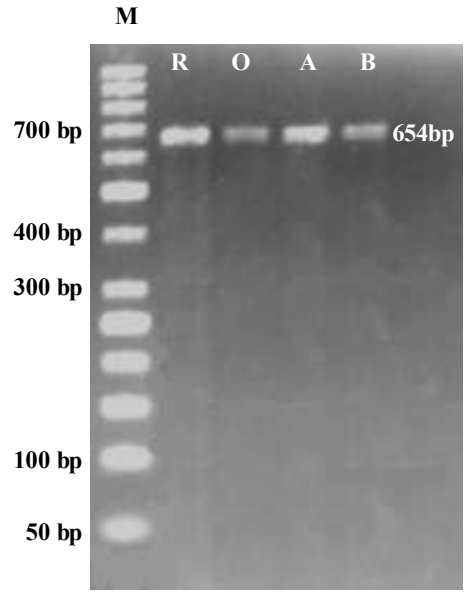

Fig. 1: PCR amplification of IGFBP-3 gene (654 bp) in the four Egyptian sheep breeds: Rahmani (R), Ossimi (O), Awassi (A), and Barki (B) Egyptian sheep breeds. M= DNA ladder with 50 bp

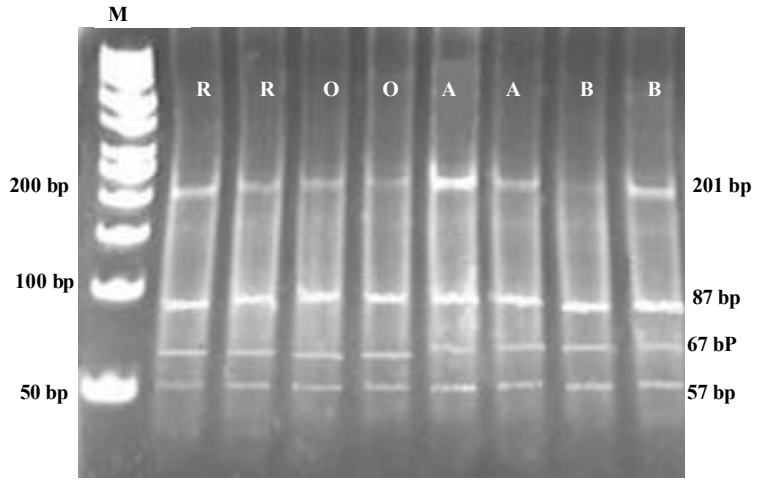

Fig. 2: Representative *Hae*III restriction fragment pattern of 654 bp IGFBP-3 gene in four Egyptian sheep breeds: Rahmani (R), Ossimi (O), Awassi (A), and Barki (B) Egyptian sheep breeds.

to IGFBP-3 gene in the four Egyptian sheep breeds studied, were in accordance with the findings reported in buffalo [14]. However, the sizes of restriction fragments were differed as 201, 165, 154, 56, 36, 19, 16 and 8 bp [14].On the other hand, three genotypes were identified in exotic (Holstein Friesian and Jersey) cattle with restriction fragments of sizes 199, 164, 154, 56, 36, 18, 16 and 8 bp (AA genotype); 215, 164, 154, 56, 36, 18 and 8 bp (BB genotype) and 215, 199, 164, 154, 56, 36, 18, 16 and 8 bp (AB genotype) [12, 17].

The absence of IGFBP-3 gene polymorphism in sheep and its presence in cattle was explained earlier by Choudhary [17]. They reported that All the sheep have intact *Hae*III restriction site (GG ↓ CC) at the base no. 300 indicating the absence of polymorphism at this site. However, the corresponding site in cattle showed polymorphism due to absence of this site. The polymorphism in the cattle was due to C→A (GG ↓ CC to GG AC) transition in intron 2 of the gene at 299th base position of HF sequence, which alters a *Hae*III restriction site.

CONCLUSION

It can be concluded that the fragment with 654 bp of IGFBP-3 gene digested with *Hae* III restriction enzyme yielded single restriction pattern of five fragments of sizes 201, 201, 87, 67, 57 in all the animals belonging to the four Egyptian sheep breeds studied revealing absence of polymorphism in those four Egyptian sheep breeds. On comparison, this fragment was also monomorphic in Indian sheep breeds and buffalo, while it was reported to be polymorphic in cattle. Further studies should be made to sequence the amplified 654 bp fragment of sheep IGFBP-3 gene in the four Egyptian sheep breeds for precious differentiation between these breeds in respect to IGFBP-3 gene at the level of DNA base pair.

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