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GENETIC BIODIVERSITY STUDIES ON IGFBP-3 GENE IN EGYPTIAN SHEEP BREEDS**

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Abstract : The insulin-like growth factor binding protein-3 (IGFBP-3) gene is a structural gene associated with the growth and development of the animals. The present investigation was carried out to study DNA polymorphism by PCR-RFLP of IGFBP-3 gene in four Egyptian local sheep breeds and its comparison with Indian sheep breeds. Genomic DNA was isolated from a total of 20 animals from four Egyptian breeds 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 as compared to earlier reports of 651 bp in cattle and 655 bp in buffalo. On digestion of 654 bp 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 breeds studied revealing absence of polymorphism in those four Egyptian sheep breeds. On comparison, this fragment was monomorphic in Indian sheep breeds and buffalo ,while it was reported to be polymorphic in cattle .

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

Introduction and literature review

Sheep contribute 6% of the total red meet produced 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 *(Galal et al., 2005*). 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 (*Galal et al., 2005*). Barki breed is well adapted to desert conditions (*Aboul Naga, 1976*), 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. Also, 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 (*Aboul Naga and El Shobokshy, 1981*). 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. 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.

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 (*Crawford and Littlejohn, 1998 and Kumar et al., 2006*[b]).

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) (Bale and Conover, 1992). IGF-I and IGF-II are couple of hormones involved in the process of mammalian growth and regenerative processes besides having active role in mammary gland development (*Hossner et al., 1997)*. The bovine IGFBP-3 gene has been cloned and characterized and itsmRNAis 1.65 kb in length (Spratt et al., 1991). The total length of gene is 8.9 kb having five exons (*Martin and Baxter, 1992*). Polymorphic studies and nucleotide sequencing of IGFBP-3 gene have been reported in cattle (*Maciulla et al., 1997; Haegeman et al., 1999; Shukla, 2001; Sun, 2002*) and buffalo (*Padma et al., 2004*). No such information is available in sheep so far, however, mRNA expression of IGFBP-3 gene has been studied (*Hastie et al., 2004*) and the same group have submitted mRNA partial sequence to GenBank (accession no. AF327651). Considering the importance of Egyptian sheep in meat production, the present study was undertaken to find out polymorphism, if any,

of the Egyptian sheep IGFBP-3 gene and compare it with those of Indian sheep breeds, cattle and buffalo.

Materials and Methods

***DNA extraction*:**

The present study was conducted on a total of 20 animals belonging to four Egyptian sheep breeds viz. Rahmani (5), Ossimi (5), Awassi (5) and Barki (5) maintained at Animal Production Research Station, Borg EL-Arab, Alexandria, Egypt. Approximately, 10ml venous blood was collected from each animal using 0.5 ml of 2.7% EDTA as an anticoagulant. Genomic DNA was isolated from blood using QIAamp DNA extraction kit (QIAGEN GmbH, Hilden Germany) 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. The poor quality DNA was re-extracted with phenol–chloroform.

***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 a set of forward (P3: 5`- CCA AGC GTG AGA CAG AAT AC-3`) and reverse (P4: 5`-AGG AGG GAT AGG AGC AAG AT-3`) primers (Maciulla et al., 1997). For amplification, 25 ul of PCR reaction was prepared by adding 10pM of each primer, 100_M 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 a pre-programmed thermal cycler (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 restriction enzyme digested PCR products were separated by 12% PAGE gel and stained with ethidium bromide. The digested products were visualized and documented under gel documentation system (Syngene).

Results of investigations and discussion

Figures (1and 2) represent agarose gel electrophoresis of PCR amplified IGFBP-3gene (654 pb) and *Hae*III digested IGFBP-3 product of Rahmani (R) , Ossimi (O), Awassi (A) and Barki (B) breeds. The amplified 654 bp fragment of sheep IGFBP-3 gene is comprised of last part of exon 2, complete intron 2, exon 3 and a part of intron 3. The exon–intron regions were assigned on the basis of the published reports of this gene in cattle (*Maciulla et al., 1997*).

It is clear from *Hae*III RFLP pattern represented in figure (2) that there were no polymorphism among these four Egyptian sheep breeds in respect to IGFBP-3 gene. 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 visible fragments of sizes 201,201, 87, 67, 57 bp (Fig. 2). This result indicates the homozygosis of this gene in the four breeds studied.

These results confirms those reported earlier by *Kumar et al.* (2006[a]) 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 (The first Five fragments were visible) in all the animals studied revealing absence of polymorphism in these Indian sheep breeds. Also, The present results showed only one genotype (AA) in the four Egyptian sheep breeds studied, similar to the findings reported in buffalo (*Padma et al., 2004*). However, the sizes of restriction fragments were differed as 201, 165, 154, 56, 36, 19, 16 and 8 bp (*Padma et al., 2004*).

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) (*Shukla, 2001; Choudhary, 2004*).

The absence of IGFBP-3 gene polymorphism in sheep and presence in cattle was explained earlier by Choudhary (2004). 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.

Figure 1. PCR amplification of IGFBP-3 gene (654 bp) in Rahmani (R), Ossimi (O), Awassi (A), and Barki (B) Egyptian sheep breeds. Lane M, molecular size marker (50 bp DNA ladder).

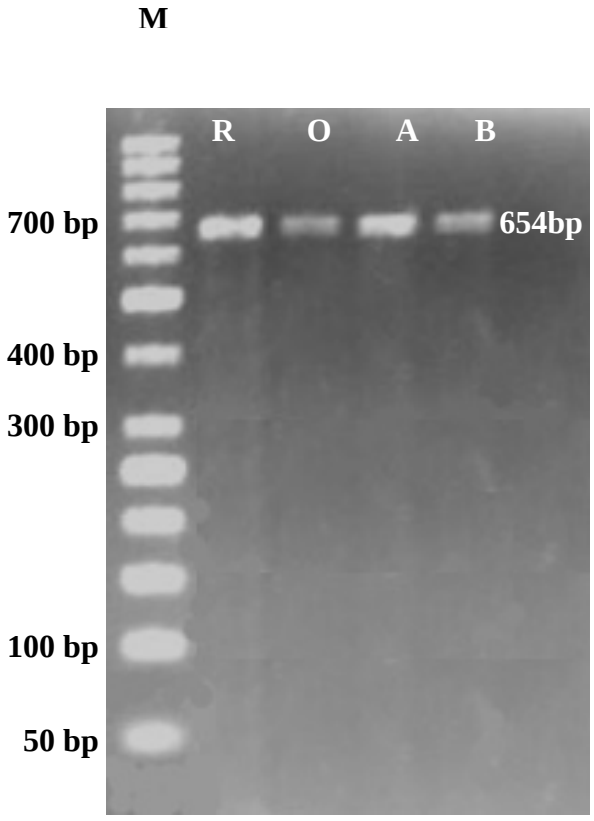
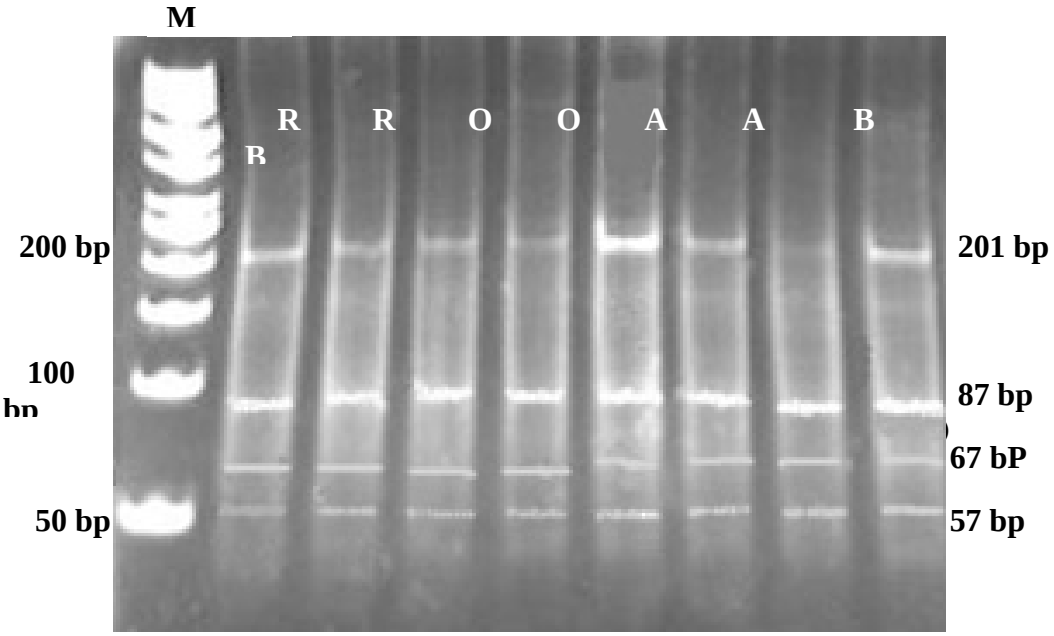


Figure 2 . Representative *Hae*III restriction fragment pattern of 654 bp IGFBP-3 gene in Rahmani (R), Ossimi (O), Awassi (A), and Barki (B) Egyptian sheep breeds. Lane M, molecular size marker (50 bp DNA ladder).



Conclusion

It can be concluded that digestion fragment of IGFBP-3 gene (654 bp) in the four Egyptian sheep breeds 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.

STUDIJE GENETSKOG BIODIVERZITETA NA IGFBP-3 GENU KOD EGIPATSKIH RASA OVACA

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Rezime

Genetski diverzitet domaćih, farmskih životinja je neophodan kako bi se ispunili zahtevi postojećih proizvodnji u različitim sredinama, kako bi se omogućio održivi genetski progres, i olakšalo brzo adaptiranje na promene u odgjivačkim ciljevima. Faktor porasta slična insulinu – gen koji vezuje protein - 3 (IGFBP-3) ke strukturalni gen koji je povezan sa porastom i razvojem životinja. Uzimajući u obzir važnost egipatskih ovaca u proizvodnji mesa, ova studija je realizovana sa ciljem pronalaženja polimorfizma, ukoliko postoje, IGFBP-3 gena kod egipatskih rasa ovaca i poređenja sa onim koji su utvrđeni kod indijskih rasa ovaca, goveda i bivola. Ovo istraživanje je urađeno kako bi se ispitao DNK polimorfizam i to PCR-RFLP gena IGFBP-3 kod četri lokalne egipatske rase ovaca i upoređene sa indijskim rasama ovaca. Genomski DNK je izolovan iz ukupno 20 životinja četri egipatske rase ovaca: Rahmani, Ossimi, Awassi i Barki. Fragment IGFBP-3 gena koji se sastoji od dela eksona 2, kompletnog introna 2, eksona 3 i dela introna 3, je amplifikovan. Amplifikovani fragment je utvrđen u vrednosti od 654 bp kod ovaca u poreženju sa ranijim rezultatima od 651 bp kod goveda i 655 bp kod bivola. Digestijom 654 bp sa *Hae* III restriktivnim enzimom dobijen je pojedinačni restriktivni obrazac pet fragmenata veličina 201, 201, 87, 67, 57 kod svih životinja koje pripadaju egipatskim rasama ovaca koje su ispitivane i otkrivaju odsustvo polimorfizma kod ovih egipatskih rasa ovaca. U poređenju, ovaj fragment je bio monomorfan kod indijskih rasa ovaca i bivola, odnosno polomorfan kod goveda.

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