British Biotechnology Journal
3(4): 592-604, 2013

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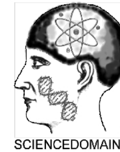

Genetic Characterization of Insulin Growth Factor-1 and Its Receptor Genes in Egyptian Buffalo (*Bubalus bubalis* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEO designed the study, followed up the practical work and wrote the final version of the manuscript. Author MFA managed the analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Author NAAEM performed the practical work. Author KMS followed up the steps of the search. All authors read and approved the final manuscript.

Research Article

Received 13th May 2013
Accepted 30th July 2013
Published 3rd September 2013

ABSTRACT

Aim: The somatotropic axis (SA) comprises genes associated with economically important quantitative traits in livestock like mammary and muscle growth as well as carcass traits. Insulin growth factor-1 (*IGF-1*) and its receptor (*IGF-1R*) are two important genes belonging to the SA. The aim of this study was to evaluate the genetic polymorphism of *IGF1*/*Sna*BI and *IGF-1R/TaqI* restriction sites in Egyptian buffalo.
Methodology: Genomic DNA was extracted from blood samples of 100 healthy buffaloes maintained at the Mahlet Mussa and El-Gmeasa herds from 2010 to 2012. PCR was performed using primers flanking a 250-bp fragment of the regulatory region of the buffalo *IGF-1* gene and a 616-bp fragment of the *IGF-1R* gene encompassing 51-bp from exon 12, 479-bp from intron 12 and 86-bp from exon 13. The PCR-amplified fragments were digested with *Sna*BI (*IGF-1*) and *Taq*I (*IGF-1R*), electrophoresed and analyzed on agarose gels stained with ethidium bromide. The two amplified fragments were also sequenced and aligned with published sequences.
Results: All buffaloes investigated in this study were genotyped *BB* (i.e., negative for the *Sna*BI restriction site at position 224^225 (TAC^GTA) of the *IGF-1* regulatory region) and

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AA (i.e., positive for the *Taq*I restriction site at position 47^48 (T^CGA) of the *IGF-1R* exon 12).

Conclusion: Our Egyptian buffaloes were homozygous BB and AA for *IGF1*/*Sna*BI and *IGF-1R/TaqI* restriction sites, respectively. The nucleotide sequence data were submitted to NCBI/Bankit/GenBank with the accession numbers KC852883 and KC852884 for Egyptian buffalo *IGF-1* and *IGF-1R* genes, respectively. The study of variation in these sequences may be useful in future marker-assisted selection (MAS) and genetic conservation programs.

Keywords: Buffalo; IGF-1; IGF-1R, PCR; RFLP.

1. INTRODUCTION

The improvement of buffalo productivity has been dependent on DNA and genetic markers that are associated with various productivity traits of economic importance to promote more efficient and relatively easy selection of Egyptian buffaloes with an advantage for inheritable traits.

Most productivity traits of economically importance in livestock are quantitative in their nature [1]. The genetic improvement of farm animal productivity has been based on concepts of quantitative genetics, which assume that there are several genes, each of which has a certain effect on a particular quantitative trait. Some traits are controlled by a single gene, but most quantitative traits are usually controlled by multiple genes (polygenic) and influenced by environmental factors [2].

In quantitative genetics, there are a number of single genes associated with mammary or muscle growth, development and function which were studied as excellent candidates for linkage relationships with quantitative traits of economic importance. Among them, a somatotropic axis (SA) contains the most promising candidates [3]. Insulin growth factor-1 (*IGF-1*) and its receptor (*IGF-1R*) genes are two important genes belonging to the somatotropic axis.

IGF-1 gene is localized on chromosome 5 and consists of 6 exons in cattle; it is considered a marker for growth rate and meat production because of its role in cell proliferation and growth. A single nucleotide polymorphism (SNP) was found in the 5′ flanking region of *IGF-1* related to meat production traits like body weight, subcutaneous backfat and longissimus dorsi area. The SNP was identified as a T/C transition, which can be determined by RFLP with *Sna*BI digestion [4].

In *Bos Taurus,* the *IGF-IR* is encoded by a single gene located on chromosome 21 [5]. Whole Genome Shotgun (WGS) libraries [6] reported that *Bos Taurus* chromosome 21 contains coding region of the *IGF-IR* gene and consists of all 21 exons separated by long introns. Digestion of 625-bp PCR product with *Taq*I restriction enzyme determined two different alleles, A and B. The associations of *IGF-1R*/*Taq*I genotypes with milk production and calving interval were reported [7].

Due to the lack of knowledge about the genetic characterization and nucleotide sequences of *IGF-1* and *IGF-1R* genes in Egyptian buffalo, this study aimed to evaluate the genetic polymorphism of *IGF1*/*Sna*BI and *IGF-1R/Taq* in Egyptian buffalo. Nucleotide sequences of

amplified fragments for these two genes were analyzed, aligned with the published sequences and submitted to database of NCBI/Bankit/GenBank.

2. MATERIALS AND METHODS

2.1 Animals

The genomic DNA used in this study was extracted from blood samples of 100 healthy female buffaloes. They were taken from two organized stations of buffaloes; Mahlet Mussa station which lies near to Sakha in Kafer El-Sheakh governorate (60 blood samples) and El-Gmeasa station which lies near to Tanta city in Gharbia governorate (40 blood samples).

2.2 Genomic DNA Extraction

Genomic DNA was extracted from the whole blood of 100 unrelated female Egyptian buffaloes according to established protocol [8] with minor modifications. Briefly, 10 ml of blood taken on EDTA were mixed with 25 ml of cold 2X Sucrose-Triton and 15 ml double distilled water. The tubes were placed on ice for 10 min and mixed by inversion several times. After centrifugation, at 5000 rpm for 15 min at 4ºC, the pellet was re-suspended by 3 ml of nucleic lysis buffer. The content was mixed with 108 µl of 20% SDS and 150 µl of Proteinase K. The tubes were placed in a water bath at 37ºC overnight.

After the incubation, the tube contents were transferred to a 15-ml polypropylene tube and 2 ml of saturated NaCl was added and shaken vigorously for 15 sec. After centrifuging at 3500 rpm for 15 min at 4ºC, the supernatant was transferred to a clean 15-ml polypropylene tube and mixed with absolute ethanol. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The precipitated DNA was picked up using the heat sealed pasture pipette, then washed twice in 70% ethanol and exposed to air to dry completely.

The DNA was dissolved in 200 µl TE buffer in 1.5-ml Microfuge tube and kept overnight in an incubator at 37ºC. DNA concentration was determined and diluted to the working concentration of 50ng/µl, which is suitable for polymerase chain reaction using NanoDrop1000 Thermo Scientific spectrophotometer.

2.3 Polymerase Chain Reaction (PCR)

A PCR cocktail consisted of 1.0 µM upper and lower primers specific for each tested gene (Table 1), 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin (w/v), 0.1 % Triton X-100 and 1.25 units of Taq polymerase. The cocktail was aliquot into PCR tubes with 100 ng of buffalo DNA. The reaction was cycled for 1 min. at 94ºC, 1 min at an optimized annealing temperature that was determined for each primer (Table 1) and 1 min. at 72ºC for 30 cycles. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success.

Table 1. The sequences and information of primers used in this study

Gene	Sequences 5′ -------- 3′	PCR conditions	PCR product size	Restriction enzyme used	References
IGF-1	ATT ACA AAG CTG CCT GCC CC ACC TTA CCC GTA TGA AAG GAA TAT ACG T	94°C 1 min 58°C 1 min 72°C 1 min	250 bp	*Sna*BI	[17]
IGF-1R	CCC AAT GGA TTG ATC CTC ATG T GCT GTG TAG TTC CCT GGG TT	94°C 1 min 56°C 1 min 72°C 1 min	616 bp	*Taq*I	

2.4 Restriction Fragment Length Polymorphism (RFLP)

The PCR products for the two tested genes were digested with the restriction enzyme specific for each gene (Table 1). The restriction mixture for each sample was prepared by adding 2.5 µl of 10× restriction buffer to 10 units of the restriction enzyme and the volume was completed to 5 µl by sterile water. This restriction mixture was mixed with PCR product (~25 µl) and incubated overnight at the optimum temperature of the maximum activity for each restriction enzyme. The digested PCR products were electrophoresed on agarose gels staining with ethidium bromide to detect the different genotypes of the two tested genes.

2.5 Sequence Analysis

The PCR products for each tested gene were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite. Results of endouclease restriction were carried out using FastPCR. The nucleotide sequences of the two tested genes in Egyptian buffalo were submitted to GenBank (NCBI, BankIt).

3. RESULT AND DISCUSSION

Insulin-like growth factor (IGF) is known to affect reproduction, fetal development, growth and lactation in a number of animal species [9,10]. For the critical important role of Insulin-like growth factor genes and their receptors, the genetic characterization and variation of these genes in livestock have been used by breeders to select the best animals in a breed.

Due to the lack of knowledge about the genetic characterization and nucleotide sequences of *IGF-1* and *IGF-1R* in Egyptian buffalo, this study aimed to evaluate the genetic polymorphism of *IGF1*/*Sna*BI and *IGF-1R/Taq* in Egyptian buffalo. Nucleotide sequences of amplified fragments for these two genes were analyzed, aligned with the published sequences and submitted in database of NCBI/Bankit/GenBank.

3.1 *IGF-1* Gene

The primers used in this study flanked a 250-bp fragment located in the regulatory region of the buffalo *IGF-1* gene. The amplified fragments obtained from all tested Egyptian buffalo DNA (100 animals) were 250-bp (Fig. 1).

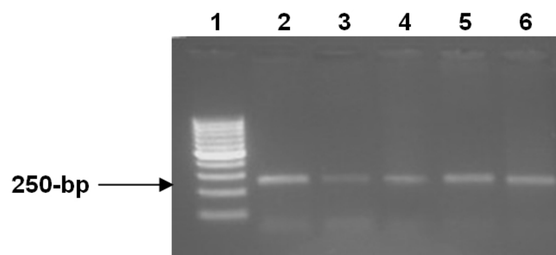

Fig. 1. Ethidium bromide-stained gel of PCR products representing amplification of *IGF-1* gene in Egyptian buffalo

Lane 1: 100-bp ladder marker

Lanes 2-6: 250-bp PCR products amplified from Egyptian buffalo DNA

In order to verify the PCR product, two-way sequence analysis of the *IGF-1* amplified PCR products of buffalo DNA was conducted. The buffalo amplicon obtained was found to be 250 nucleotides (Fig. 2). The Egyptian buffalo *IGF-1* nucleotide sequence was submitted to nucleotide sequences database of NCBI/ Bankit/GenBank and has the accession number **KC852883**

```
ATTACAAAGCTGCCTGCCCCTTTCCAGGTTCTAGGAAATGAGATCATTCCCCTCACTTGGCAACCAGGACGAG
GGGTCATCCCAGCGCTGTCTTCCATTCTAGTTTACCCCAGTCGTTTGAGGGTTAAAATCATAGAGTATGCTTGA
GATGGTCTTTTTTTCATTTCTTGTTTTTTAAATTTTGTGTTGGCTCTGGAATATAAAATTGCTCGCCCATCCTCCA
CGTATATTCCTTTCATACGGGTAAGGT
```

Fig. 2. The sequence analysis of Egyptian buffalo *IGF-1* amplified fragment
The forward and reverse primers are bold

The sequence alignment of Egyptian buffalo *IGF-1* with published sequence (accession number: EU159115.1, *Bubalus bubalis*) showed that our animals possess identities at 99% with one SNP (**T/A**) at position 226 in our sequence (Fig. 3).

These PCR amplified fragments (250-bp) were digested with *Sna*BI endonuclease. Depending on the presence or absence of the restriction site at position 224^225 (TAC^GTA), we can easily differentiate between 3 different genotypes: AA with two digested fragments at 224- and 26-bp, BB with one undigested fragment at 250-bp and AB with three digested fragments at 250-, 224- and 26-bp.

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Query  241  CGGGTAAGGT  250
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Sbjct  278  CGGGTAAGGT  287
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Fig. 3. Sequence alignment of Egyptian buffalo *IGF-1* amplified fragment with published sequence
Substituted nucleotide is bold and underlined

All buffalo animals investigated in this study are genotyped as **BB** where all tested buffalo DNA amplified fragments were treated with *Sna*BI endonuclease and gave one undigested fragment at 250-bp due to the absence of the restriction site at position 224^225.

Insulin-like growth factor I (*IGF-I*) is a 70 amino acid, single chain polypeptide encoded by a single gene [11]. *IGF-1* gene is considered to be a factor that regulates growth,

differentiation and the maintenance of differentiated function in numerous tissues and in specific cell types of mammals through binding to a family of specific membrane-associated glycoprotein receptors [12].

Brief summary of the current state of knowledge about insulin-like growth factors (IGFs) was given by Szewczuk et al. [13]. In cattle the *IGF-1* gene was mapped to chromosome 5 [14]. Based on the chromosome homology between cattle and river buffalo, we expect *IGF-1* gene to be located on the long arm of buffalo chromosome 4 (4q) which resulted from the centric fusion between two acrocentric cattle chromosomes nos. 5 and 28 [15]. Transcripts derived from exons 1 and 2 are alternatively spliced onto exon 3 and finally the mature *IGF-I* is encoded only by parts of exons 3 and 4 [16,17].

IGF-1/*Sna*B1 polymorphism, identified by Ge et al. [4], is a T (allele A) to C (allele B) transition located in the regulatory region of the *IGF-1* gene, which can directly or indirectly affect production traits. In other words, this marker can be affecting phenotypic traits or can be in linkage disequilibrium with polymorphism affecting these traits.

Curi et al. [18] observed two genetic variants (A and B) of the *IGF-1*/*Sna*B1 polymorphism in four cattle breeds. Genotype AA was characterized by the presence of two digested fragments at 226- and 23-bp, while genotype BB was determined by the presence of a single fragment at 249-bp. In the studied samples allele B was found to be fixed in the group of Nellore animals. The frequency of allele B was significantly higher (p=0.05) than that of allele A in all studied groups. The same result related to the presence of allele B with a higher frequency than allele A in different cattle breeds was also reported by Akis et al. [19].

In the present study, All investigated buffaloes are genotyped as **BB** where all tested buffalo DNA amplified fragments at 250-bp located in the regulatory region of buffalo *IGF-1* were treated with *Sna*BI endonuclease and gave one undigested fragment at 250-bp. This results of *IGF-1*/*Sna*B1 polymorphism indicated that the Egyptian buffaloes are genetically closer to the Nellore breed than other cattle breeds like Canchim or Angus breeds.

Associations were studied between *IGF-1* gene polymorphism and selected beef and milk production traits in Polish Holstein-Friesian cattle [20]. The AB genotype was found positively associated with live body weight at slaughter, cold carcass weight as well as weight of meat and fat in valuable cuts. Cows carrying AB genotype yielded daily more milk and more milk fat and milk protein than those of AA and BB genotypes.

The effects of *IGF-1* polymorphism were examined on milk production traits and genetic trends in the Iranian Holstein bulls [21]. A total of 282 bulls were genotyped for *SnaB*I restriction site in the 5' flanking region of *IGF-1* by applying PCR-RFLP method. The allele frequencies for C and T alleles were 0.562 and 0.438, respectively. The results indicated that genetic variants at the 5'-noncoding region of the bovine *IGF-1* gene had a marked effect on estimated breeding values of milk (EBVM) and fat yields (EBVF). The heterozygous bulls (*CT* genotype) had higher EBVM and EBVF than homozygous bulls (p=0.1).

Reyna et al. [22] reported the association between *IGF-1/Sna*BI polymorphism with production traits in Charolais and Beefmaster breeds. A C/A transversion was detected in intron 4, but it was not associated with the growth traits. Also frequencies of alleles and genotypes of *IGF-1/Sna*BI polymorphism were identified by Szewczuk et al. [3]. The frequency of *IGF-1/Sna*BI allele A occurred similar to that of allele B (0.55 and 0.45,

respectively). Cows with *IGF-1*AB genotype yielded more milk, fat and protein compared to other genotypes (p=0.05).

3.2 *IGF-1R* Gene

The primers used in this study (Table 1) flanked a 616-bp fragment consisting of 51-bp from exon 12, 479-bp from intron 12 and 86-bp from exon 13 of the buffalo *IGF-1R* gene. The amplified fragments obtained from all tested buffalo DNA (100 animals) were at 616-bp (Fig. 4).

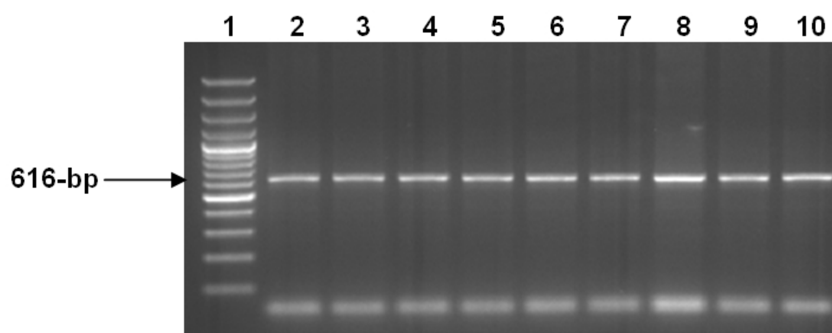

Fig. 4. Ethidium bromide-stained gel of PCR products representing amplification of *IGF-1R* gene in Egyptian buffalo

Lane 1: 100-bp ladder marker.
Lanes 2-10: 616-bp PCR products amplified from Egyptian buffalo DNA.

In order to verify the PCR product, two-way sequence analysis of the *IGF-1R* amplified PCR products of buffalo DNA was conducted. The buffalo amplicon obtained was found to be 616 nucleotides (Fig. 5). The Egyptian buffalo *IGF-1R* nucleotide sequence was submitted to nucleotide sequences database of NCBI/ Bankit/GenBank and has the accession number **KC852884**

CCCAATGGATTGATCCTCATGTATGAAATAAAATATGGATCCCAAGTCGAGGTGAGTCTGGACGCCGAGCACG
TGTGGGTGCATTTCTGTTACAGGTATGGTGTACATTACACCGAGACACAGGGCACCCCCGGTTAGCTGAAGAC
TACACAATGGAATAAGGAGGTGAGTCAGTGAGAACTTAGGTCTTTGTAGCAGCTCTCTGCTCTTTGAGGAGTTT
TCACAGCGTGCAAGGAGCTGGCTTATCCTCACCCGCCAAGAAATTGTTTCTTTACAGAAAGTGATCTTTCAGCC
TAGAGACTGGTCTTAATAACTTAATCTTTCTGAAACACTTTTTGGTTAAAGTAATAAGGTCTGGTTAGGGCTTGA
TGCGCTTTCAGTTCCTGGAGGACTGCCACCACGTAGAGCCGGTGTTTGCCCTTTTCCTGAGAGTCGGCGTCAG
CATCAGCGGGAGGCGAGCGGCCAGGCAGCCACAGCTGTCTGTTTCCAACGTGTGTGGGGAATTGACACCTGT
GTTTTATATCTTCCAGGATCAGCGGGAATGTGTGTCCAGACAGGAGTACAGGAAGTATGGAGGAGCCAAGCTA
AACCGGCTC**AACCCAGGGAACTACACAGC**

Fig. 5. The sequence analysis of Egyptian buffalo *IGF-1R* amplified fragment

The forward and reverse primers are bold

The sequence alignment of Egyptian buffalo *IGF-1R* with published sequence (accession number: JQ924783.1, *Bos taurus*) showed that our animals possess identities at 98% with 13 SNPs, 4 (**C/T**) at positions 66, 132, 227 and 250; 3 (**A/G**) at positions 98, 179 and 453; 2 (**G/A**) at positions 217 and 230; one (**C/A**) at position 149; one (**T/G**) at position 170; one (**G/C**) at position 192 and one (**A/T**) at position 522 in our sequence (Fig. 6).

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Query  1    CCCAATGGATTGATCCTCATGTATGAAATAAAATATGGATCCCAAGTCGAGGTGAGTCTG  60
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Query  61   GACGCCGAGCACGTGTGGGTGCATTTCTGTTACAGGTATGGTGTACATTACACCGAGACA  120
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Query  301  CTGGTCTTAATAACTTAATCTTTCTGAAACACTTTTTGGTTAAAGTAATAAGGTCTGGTT  360
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Sbjct  395  CTGGTCTTAATAACTTAATCTTTCTGAAACACTTTTTGGTTAAAGTAATAAGGTCTGGTT  454

Query  361  AGGGCTTGATGCGCTTTCAGTTCCTGGAGGACTGCCACCACGTAGAGCCGGTGTTTGCCC  420
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Query  421  TTTTCCTGAGAGTCGGCGTCAGCATCAGCGGGAGGCGAGCGGCCAGGCAGCCACAGCTGT  480
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Sbjct  515  TTTTCCTGAGAGTCGGCGTCAGCATCAGCGGGGGGCGAGCGGCCAGGCAGCCACAGCTGT  574

Query  481  CTGTTTCCAACGTGTGTGGGGAATTGACACCTGTGTTTTATATCTTCCAGGATCAGCGGG  540
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Query  541  AATGTGTGTCCAGACAGGAGTACAGGAAGTATGGAGGAGCCAAGCTAAACCGGCTCAACC  600
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Query  601  CAGGGAACTACACAGC  616
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Sbjct  695  CAGGGAACTACACAGC  710
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Fig. 6. Sequence alignment of Egyptian buffalo *IGF-1R* amplified fragment with published sequence

Substituted nucleotides are bold and underlined

These PCR amplified fragments (616-bp) were digested with *Taq***I** endonuclease. Depending on the presence or absence of two restriction sites at positions 47 and 455 (**T^CGA**), we can easily differentiate between 3 different genotypes: AA with two digested fragments at 569- and 47-bp, BB with three digested fragments at 408-, 161- and 47-bp and AB with four digested fragments at 569-, 408-, 161- and 47-bp.

All buffalo animals investigated in this study are genotyped as **AA** where all tested buffalo DNA amplified fragments were digested with *Taq***I** endonuclease and gave two digested fragment at 569- and 47-bp due to the presence of the restriction site at position 47^48 (**T^CGA**) (Figs. 7 and 8).

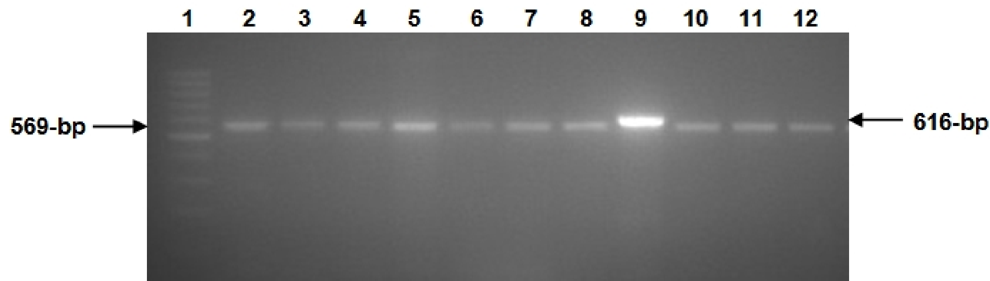

Fig. 7. The electrophoretic pattern obtained after digestion of PCR amplified buffalo *IGF-1R* gene with *Taq*I restriction enzyme

Lane 1: 100-bp ladder marker.
Lanes 2-8 and 10-12: Homozygous AA genotypes showed two digested fragments at 569- and 47-bp (not showed in figure).
Lane: 9: Undigested PCR amplified fragment at 616-bp.

CCCAATGGATTGATCCTCATGTATGAAATAAAATATGGATCCCAAG**T^CGA**GGTGAGTC
TGGACGCCGAGCACGTGTGGGTGCATTTCTGTTACAGGTATGGTGTACATTACACCGA
GACACAGGGCACCCCCGGTTAGCTGAAGACTACACAATGGAATAAGGAGGTGAGTCAG
TGAGAACTTAGGTCTTTGTAGCAGCTCTCTGCTCTTTGAGGAGTTTTCACAGCGTGCAA
GGAGCTGGCTTATCCTCACCCGCCAAGAAATTGTTTCTTTACAGAAAGTGATCTTTCAG
CCTAGAGACTGGTCTTAATAACTTAATCTTTCTGAAACACTTTTTGGTTAAAGTAATAAG
GTCTGGTTAGGGCTTGATGCGCTTTCAGTTCCTGGAGGACTGCCACCACGTAGAGCCG
GTGTTTGCCCTTTTCCTGAGAGTCGGCGTCAGCATCAGCGGGAGGCGAGCGGCCAGG
CAGCCACAGCTGTCTGTTTCCAACGTGTGTGGGGAATTGACACCTGTGTTTTATATCTT
CCAGGATCAGCGGGAATGTGTGTCCAGACAGGAGTACAGGAAGTATGGAGGAGCCAA
GCTAAACCGGCTCAACCCAGGGAACTACACAGC

Fig. 8. Endonuclease restriction of Egyptian buffalo *IGF-1R* amplified fragment using FastPCR

*Restriction site (**T^CGA**) is bold.*

In *Bos Taurus,* the *IGF-IR* is encoded by a single gene located on chromosome 21 [5]. Based on the chromosome homology between cattle and river buffalo, the *IGF-1R* gene is expected to be located on the acrocentric buffalo chromosome 20 [15]. Whole Genome Shotgun (WGS) reported the sequence of *Bos Taurus* chromosome 21 [6]. Total length about 71,6 Mb pairs contains coding region of the *bIGF-1R* gene and consists of all 21 exons separated by long introns, spanning more than 301,2 kbp in length.

Moody et al. [5], by digestion of 625-bp PCR product with *Taq***I** restriction enzyme, revealed a polymorphism in alleles A and B. However, they concluded that usefulness of this polymorphism may be limited by the low B allele frequency and its presence in only *Bos indicus* cattle.

Akis et al. [19] determined the allele and genotype frequencies of *IGF-1R* gene polymorphisms in East Anatolian (EAR) and South Anatolian Red cattle (SAR). In both breeds, high frequencies of allele A of *IGF-1R* related to milk traits were observed. Therefore, no relationship of the polymorphisms with economic traits was observed, as both breeds have respectively low carcass and milk parameters.

The frequency of the two polymorphic variants located in intron 4 of the gene coding for *IGF-1R* in Holstein-Friesian cows were recorded by Szewczuk et al. [23]. The *IGF-1R* gene polymorphism was identified with PCR-RFLP using the *Hinf*I and *Mph*1103I restriction enzymes. For the *IGF-1R/Hinf*I polymorphism, the highest frequency was found for the BB and AB genotypes and the lowest one was for the AA genotype. The frequency of alleles was 0.28 and 0.72 for allele A and B, respectively. Statistical analysis showed that the analyzed polymorphism significantly affected milk yield, milk protein yield (p=0.01) and milk fat yield (p=0.05), favoring the *BB* genotype.

Regarding to *IGF-1R* polymorphisms detected by *Taq*I digestion [3], the frequency of alleles A and B were 0.61 and 0.39, respectively. No significant effects of the *IGF-1R/Taq*I polymorphism on the fat and protein yield and milk fat content were identified. Cows with the *IGF-1RBB/IGF-1AB* genotype combination yielded more milk, fat and protein compared to other combinations (p=0.05).

4. CONCLUSION

Our Egyptian buffaloes were homozygous *BB* and *AA* for *IGF1*/*Sna*BI and *IGF-1R/TaqI* restriction sites, respectively. The nucleotide sequence data were submitted to NCBI/Bankit/GenBank with the accession numbers KC852883 and KC852884 for Egyptian buffalo *IGF-1* and *IGF-1R* genes, respectively. The study of variation in these sequences may be useful in future marker-assisted selection (MAS) and genetic conservation programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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