Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Comparative analysis of IGFBP-3 gene sequence in Egyptian sheep, cattle and buffalo

Ahmed Abdel-kader Saleh (Elemlak1339@Gmail.Com)
Southwest University https://orcid.org/0000-0003-1314-8066
Amr M.A. Rashad
Alexandria University
Nada. N.A.M. Hassanine
Alexandria University
Mahmoud A. Sharabi
Alexandria University
Yongju Zhao
Southwest University

Research note

Keywords: DNA sequencing, Farm animals, IGFBP-3, RFLP.

Posted Date: August 24th, 2019

DOI: https://doi.org/10.21203/rs.2.13545/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published on September 23rd, 2019. See the published version at https://doi.org/10.1186/s13104-019-4657-6.

Abstract

Objective: A total of 205 animals from four Egyptian livestock species were used in this study to detect polymorphism and perform comparative analysis for IGFBP-3 gene using DNA sequencing and (PCR-RFLP). Results: The digestion of 654 bp with HaeIII restriction enzyme yielded a single restriction pattern of eight fragments in sheep revealing the absence of polymorphism, while in cattle 3 genotypes were identified; (AA), (AB), and (BB). Moreover, one genotype (AA) only was found in buffalo using HealII and MspI restriction enzymes, separately. Digestion profile for a goat with HaeIII revealed only one pattern for three DNA fragments, which means the absence of polymorphism within the IGFBP-3 gene in the tested goats. The nucleotide sequencing analysis indicated similarity percentages in IGFBP-3 gene fragments of 77, 30 and 58% between "sheep and cattle ", "sheep and buffalo ", and "cattle and buffalo", respectively.

Background

There are inherent limitations in most animal species in Egypt, especially farm animals, which were not characterized or determined as a genetic analysis, whether at the level of genes, QTL, or whole genome, so through this modest attempt we try to open a window to study these species, starting from some genes that have an effective effect on the economic traits such as IGFBP-3. On the other side, to the best of our knowledge, no studies are, yet, available on comparisons between IGFBP-3 gene in domestic sheep, goat, cattle and buffalo species of Egypt[1, 2]. Worth mention, in the current study, sample sizes are limited for some species (only 12 samples from buffalo and 18 samples from cattle).

Additionally, the low heritability estimates and the subsequent slow genetic improvement via traditional selection enhanced adopting molecular genetic techniques to achieve remarkable improvements through discovering candidate genes that have significant influences on such traits [2, 3].

IGFBPs or as they call it (Insulin-like Growth Factor Binding Proteins) belongs to an important family of at least (6) homologous proteins that bind IGFs and modulate many of their important biological effects [4]. Therefore, IGFBP-3 is recommended as markers for different body functions [5-8] such as growth, body weight, reproduction, immunity, metabolism and energy balance….etc. IGFBP-3 gene is the main responsible for the multiple and necessary effects of (IGF) [9-11].

The importance of the current study is due to the need of Egyptian breeds from different species to determination and characterization for candidate genes which have an important effect on the economic traits including IGFBP-3 gene. The objectives of this study were to detect polymorphism in IGFBP-3 gene in Egyptian sheep, Falahy goats, Egyptian cattle and El-Beheiry buffaloes using a comparative sequence analysis (DNA sequencing) and PCR-RFLP techniques on samples of these species.

Methods

Animals

Samples of Egyptian farm animal species, namely cattle (n=18; Egyptian cattle), buffaloes (n=12; El-Beheiry breed), sheep (n=150; 50 Rahmani, 50 Barki, and 50 their crosses) and goats (n=25; Falahi breed) were obtained from four different geographical regions at the northern coast of Egypt. Namely; Baltim farm, Kafr El-Sheikh Province (GPS: 31.579900, 31.174533) - Baltim, Al Burlos, Kafr El-Sheikh province (GPS: 31.580019, 31.174490) - Department of

Animal and Fish Production, Faculty of Agriculture, Alexandria University (GPS: 31.206208, 29.919704) - Matrouh Province (GPS: 31.336924, 27.205762) respectively. Blood samples of 5 ml each were collected from the jugular vein, using venojects, treated with 0.5 ml of 2.7% EDTA (**Spark, UK**) as an anticoagulant, kept in an icebox and transferred immediately to the lab. All procedures and experimental protocols conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, Federation of Animal Science Societies (FASS, 2010).

DNA extraction and amplification

Genomic DNA has extracted from blood samples with **QIAGEN (QIAGEN GmbH, Hilden Germany)** according to the manufacturer's instructions. The isolated DNAs were separated by electrophoresis on 0.8% agarose (**Bioshop, Germany**) in 0.5 X TBE buffer prepared according to **Sambrook *et al*** [12] and contained 0.5 µg/ml ethidium bromide (**Sigma, Germany)**. The electrophoresis run was performed using apparatus with power supply (**Biometra, USA**) and visualized by UV trans-illuminator and Gel documentation system (**Gel Doc.Alpha-chem.Imager, USA**).

For Egyptian sheep and local cattle and buffaloes, 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 using primer AASN-P1 ; (F: 5-CCAAGC GTG AGA CAG AAT AC-3),(R:5-AGG AGG GAT AGG AGC AAG AT-3) [9, 13, 14]. PCR for Falahy goats was performed to amplify a 316 bp fragment from exon 2 of the IGFBP-3 gene using the primer as described by **Liu *et al*** [15], AASN-P2; (F:5'-GAA ATG GCA GTG AGT CGG-3'), (R:5'-TGG GCT CTT GAG TAA TGG TG-3').

The amplification was performed using (**iQ SYBR Green Supermix, USA**), 10 p.mol of each primer and 100 µg of genomic DNA under the following amplification conditions: 94°C/5 min, followed by 35 cycles of 94°C/1 min, 60°C/1 min, 72°C/1 min and a final extension step at 72°C/2 min for (AASN-P1) primer. As for (AASN-P2) primer, the conditions were 94°C/5 min, followed by 34 cycles of 94°C/1 min, 63°C/1 min, 72°C/1 min and a final extension step at 72°C/2 min. The amplification was carried out using a DNA Thermo-cycler Gene Amp 6700 (**Applied Bio-system, USA**).

Nucleotide sequence analysis

Automated DNA sequence analysis was carried out on both strands by the DNA sequencing service lab of Korean Research Institute of Bioscience and Biotechnology with an ABI Prism 3100 apparatus. Database similarity searches were performed with the NCBI/BLAST/blast network service at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). The resulted sequences were analyzed using MEGA 6 v.4, Finch T.V 1.01, and Blast 2.0 software to detect Single Nucleotide Polymorphism (SNPs) between sequences. The sequences were deposited in GenBank **(Accession Numbers: MG738673.1, MG738674.1, MG738671.1 and MG738672.1** for cattle, buffalo, sheep, and goat, respectively). Analysis of translated protein of IGFBP-3 gene sequences of tested animals were generated by **ExPASy** program (http://web.expasy.org/translate).

Restriction fragment length polymorphism (RFLP) The RFLP was used to detect genotyping differences between and within sheep groups and local cattle, buffalo, and goat using the PCR of target genes. The PCR amplicons of the IGFBP-3 gene were digested with *HaeIII* for all tested animals also, *TaqI* was used for buffalo only beside *HaeIII*, separately (**Jena Bioscience, Germany**). Defining restrictions sites before digestion with restriction enzymes was achieved by NEB cutter program (http://www.labtools.us/nebcutter-v2-0)[16]. The RFLP-PCR reaction volume was 25 µl, consisted of 12µl H_2O, 2µl 10X *HaeIII* buffer (**Jena Bioscience, Germany**), 1µl (5 unit/ul) restriction enzyme in addition to 10µl amplified DNA. All reactions were incubated at 37°C for 16 hours. Twenty µl

of each reaction were separated by electrophoresis on 3 % agarose gel and visualized by UV trans-illuminator and gel documentation system (**Gel Doc**. **Alpha-chem**. **Imager**, **USA**).

Results And Discussion

Amplification, Manipulation, and digestion

This research note concerns mainly the differentiation between and within different species in IGFBP-3 gene also, take a spotlight on the association between polymorphisms of IGFBP-3 and some economical traits in different farm animal. PCR amplification for tested animals produced an amplified 654 bp fragment of Egyptian sheep IGFBP-3 gene comprised of part of exon 2, complete intron 2, exon 3 and a part of intron 3 **(Fig. 1A)**, an amplified 651 bp fragment of Egyptian cattle **(Fig. 1B)** and an amplified 655 bp fragment of Egyptian buffalo **(Fig. 1C)**, while the amplified 316 bp fragment of Egyptian goat IGFBP-3 gene was comprised of part of exon 2 **(Fig. 1D)**. The PCR products of IGFBP-3 gene obtained for sheep after digestion with *HaeIII* **(Fig. 2A)** showed a digestion profile revealing one pattern only for eight DNA fragments sized 201, 201, 87, 67, 57, 18, 16 and 7 bp and indicating absence of a polymorphism in sheep IGFBP-3 gene. The restriction fragments with sizes; 18, 16 and 7 bp were not seen on the gel.

The present results agree with those of **Kumar *et al* [9]** who studied the genetic diversity among Indian breeds of sheep; Marwari, Mandya, Madras, Red Muzaffarnagari and Banur based on sequencing and digestion profile by *HaeIII* of IGFBP-3 gene and reported that; the digestion profile revealed only one pattern with eight DNA fragments sized 201, 201, 87, 67, 56, 19, 16 and 7 bp for the tested animals and, consequently, no polymorphism was detected [9]. Also, **Choudhary [17]** reported that; all sheep possess intact *HaeIII* restriction site (GG↓CC) at the base number 300 of IGFBP-3 gene sequence indicating also, an absence of polymorphism at this site [17].

For Egyptian cattle, three genotypes were identified: (1) Lanes 1 and 2; homozygous (AA) genotype with 8 restricted fragments at 200, 164, 153, 57, 36, 18, 16 and 8 bp, (2) Lanes 3 and 4; heterozygous (AB) genotype with 5 restricted fragments at 200, 195, 164, 57 and 35 bp and (3) Lanes 5 and 6; homozygous (BB) genotype with 8 restricted fragments at 195, 164, 153, 57, 36, 18, 16 and 8 bp **(Fig. 2B)**. The restriction fragments with sizes; 18, 16 and 8 bp were not seen on the gel. The polymorphism in cattle was due to C→A (GG↓CC to GG AC) transition in exon (3) of the gene at the 451st base position of sequence for allele (B), while was due to (GG↓CC to GC GG) transition in exon (3) of the gene at the 456th base position of cattle sequence for allele (A). These results were in agreement with results of **Shukla [18]; Kumar *et al*. [9], and Choudhary [17]** [9, 17, 18] who detected three genotypes identified in Jersey and exotic Holstein Friesian cattle.

As for the buffalo, the presence of a *TaqI* site characterized by a single homozygous genotype and a non-polymorphic *MspI* site possessing two fragments of sizes 415 and 240 bp were observed **(Supplementary Fig. 1A)**. The *MspI* site (C↓CGG) on 655 bp fragment is located at intron (2). As for *HaeIII* restriction enzyme, all screened buffaloes showed only one genotype (AA) with restriction fragments of sizes 201, 165, 154, 56, 36, 19, 16 and 8 bp **(Supplementary Fig. 1B)** were in accordance with those reported by **Choudhary [22]** on six breeds of buffalo, though the sizes of restriction fragments were different (201, 165, 154, 56, 36, 19, 16 and 8 bp) [22]. The above results mean lack of detected polymorphism among studied buffalo breeds of with respect to IGFBP-3 gene. Similarly, results of **Padma *et al* [19]** on 157 Indian Murrah, Surti, Jaffarabadi and Nagpuri riverine buffaloes [19].

Finally, the PCR profiles of tested goats IGFBP-3 gene digested with *HaeIII* revealed one pattern only for three DNA fragments sized 263, 58 and 8 bp **(Supplementary Fig. 2)**, the restriction fragment with size 8 bp was not seen on

the gel. However, the study of **Lan *et al* [5]** on goats detected polymorphisms in IGFBP-3 gene by PCR-SSCP and DNA sequencing methods. Though the association of the *HaeIII* and *XspI* PCR-RFLPs for goat IGFBP-3 locus with milk traits were analyzed, the significant statistical results were not found [5, 20]. [5] [20].

(Supplementary Fig. 3) shows the diagrammatic representation of exon-intron regions of the tested animals in different species and restriction enzymes sites (*HaeIII* and *TaqI*) on the amplified IGFBP-3 gene fragments.

Nucleotide sequence comparison

Nucleotide sequencing of the amplified fragments of IGFBP-3 gene of sheep, goats, cattle and buffaloes were submitted to the NCBI GenBank (Accession no. **MG738671.1**, **MG738672.1**, **MG738673.1** and **MG738674.1,** respectively) **(Supplementary Fig. 4)**. The nucleotide sequence analysis performed by MEGA-6 VERSION 4 (http://en.bio-soft.net/tree/MEGA.html) indicated that the similarity percent of IGFBP-3 gene fragment between (sheep and cattle was 77 %), between (sheep and buffalo was 30%), while between (cattle and buffalo was 58%) **(Supplementary Fig. 5)**. Based on respective IGFBP-3 gene sequences, the phylogenetic tree was constructed among sheep, cattle and buffalo showing their relative distance **(Supplementary Fig. 6)**.

Protein sequence comparison

The partial part of exon 2, complete intron 2, exon 3 and a part of intron 3 present in the sequence of the amplified IGFPB-3 gene fragments of tested animals as generated by ExPASy program (http://web.expasy.org/translate) and the comparison of amino acids obtained by MEGA-6 VERSION 4 are in **(Supplementary Fig. 7)**. The protein sequence of sheep is different from that of cattle and buffalo by 18 amino acids. The display of amino acids **(Table 1)** accounted for approximately 70 % similarity in their sequence between sheep groups and bovine species vs. cattle and buffalo. However, the study of **Kumar *et al* [9]** indicated approximately 93% similarity in the amino acid sequence for sheep with cattle and buffalo [9].

Limitations

The current work requires to more investigation on IGFBP-3 gene on all Egyptian farm animals especially for local cattle with measure the levels of economic traits in different species and try to build a link between the differentiation in the gene and their performance. Additionally, this trend requires more investigating in many other genes which have a necessary effect on the performance of farm animals and increasing the samples of cattle and buffalo, also we can add other farm animals such as pigs.

Declarations

- **Ethics approval and consent to participate**

All procedures and experimental protocols were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, Federation of Animal Science Societies (FASS, 2010). FASS, 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. https://www.aaalac.org/about/Ag_Guide_3rd_ed.pdf.

- **Consent for publication**

"Not applicable"

- **Availability of data and material**

All data generated or analyzed during this study are included in this manuscript and its supplementary information files.

- **Competing interests**

Dear Editor-in-Chief of BMC Research Notes Journal:

1. The authors have no conflicts of interest to declare.
2. This work is original and never published before.
3. We have no conflict with any other scientist.

((I declare that the authors have no competing interests as defined by BioMed Central, or other interests that might be perceived to influence the results and discussion reported in this paper)).

- **Funding**

Authors give their gratitude to the financial support of China Scholarship Council (CSC No. 2017GBJ001932)/ China, and Faculty of Agriculture / Alexandria University/ Egypt.

- **Authors' contributions**

The work presented here was carried out in collaboration between all authors. M.SH, Y.Z, and A.S defined the research theme. A.R, N.H and A.S designed methods and experiments, A.S and N.H carried out the laboratory experiments, A.S, Y.Z and A.R analyzed the data, A.S, M.SH, Y.Z and A.R interpreted the results and wrote the paper. A.S co-worked on associated data collection and their interpretation. All authors reviewed the manuscript.

- **Acknowledgements**

The first author gives his gratitude to Mr Nashat Abdel-baky for his help in getting the samples from the northeast coast of Egypt.

- **Authors' information**
- **(A. S);** PhD student at Southwest University/ China, also, he is an assistant lecturer at Faculty of Agriculture, Alexandria University/ Egypt.
- **(A. M);** Teacher at the Faculty of Agriculture, Alexandria University/ Egypt.
- **(N. H);** Master student at the College of Veterinary Medicine, Southwest University/ China.
- **(M. SH);** Professor at the Faculty of Agriculture, Alexandria University/ Egypt.
- **(Y. Z);** Professor and Dean of Animal Science and Technology College/ Southwest University/China.

References

1. Kosgey IS, Van Arendonk JA, Baker RL: **Economic values for traits in breeding objectives for sheep in the tropics: impact of tangible and intangible benefits**. Live Prod Sci 2004, **88**:143-160.
2. Sambrook J, Fritschi E, Maniatis T: **Molecular cloning: a laboratory manual. 2nd d. New york**. In.: cold spring harbour laboratory, cold spring laboratory press; 1989.
3. Van Arendonk J, Tier B, Kinghorn BP: **Use of multiple genetic markers in prediction of breeding values**. Genet 1994, **137**:319-329.
4. Hastie PM, Onagbesan O, Haresign W: **Co-expression of messenger ribonucleic acids encoding IGF-I, IGF-II, type I and II IGF receptors and IGF-binding proteins (IGFBP-1 to-6) during follicular development in the ovary of seasonally anoestrous ewes**. Anim Repro Sci 2004, **84**:93-105.
5. Lan X, Pan C, Chen H, Lei C, Liu S, Zhang Y, Min L, Yu J, Li J, Zhao M: **The HaeIII and XspI PCR-RFLPs detecting polymorphisms at the goat IGFBP-3 locus**. Smal Rumin Resear 2007, **73**:283-286.
6. Monniaux D, Pisselet C: **Control of proliferation and differentiation of ovine granulosa cells by insulin-like growth factor-I and follicle-stimulating hormone in vitro**. Biolo Repro 1992, **46**:109-119.
7. Kim J, Yoon D, Park B, Kim L, Na K, Choi J, Cho C, Lee H, Chung E, Sang B: **Identification of novel SNPs in bovine insulin-like growth factor binding protein-3 (IGFBP3) gene**. Asian-Aust J Anim Sci 2005, **18** :3-7.
8. Khalid M, Haresign W: **Relationships between concentrations of ovarian steroids, insulin-like growth factor-1 and IGF-binding proteins during follicular development in the ewe**. Anim Repro Sci 1996, **41** :119-129.
9. Kumar P, Choudhary V, Kumar KG, Bhattacharya T, Bhushan B, Sharma A, Mishra A: **Nucleotide sequencing and DNA polymorphism studies on IGFBP-3 gene in sheep and its comparison with cattle and buffalo**. Smal Rumin Res 2006, **64**:285-292.
10. Spicer L, Echternkamp S: **The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals**. Dom Anim Endocri 1995, **12**:223-245.
11. Besnard N, Pisselet C, Monniaux D, Locatelli A, Benne F, Gasser F, Hatey F, Monget P: **Expression of messenger ribonucleic acids of insulin-like growth factor binding proteins-2,-4, and-5 in the ovine ovary: localization and**

changes during growth and atresia of antral follicles. Biolo Repro 1996, **55**:1356-1367.

12. Sambrook J, Fritsch E: **Maniatis. 1989. Molecular cloning: a laboratory manual**. In.: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; 1997.
13. Darwish H, El-Shorbagy H, Abou-Eisha A, El-Din A, Farag I: **New polymorphism in the 5' flanking region of IGF-1 gene and its association with wool traits in Egyptian Barki sheep**. J Genet Engine Biotech 2017, **15**:437-441.
14. Maciulla J, Zhang H, DeNise S: **A novel polymorphism in the bovine insulin-like growth factor binding protein-3 (IGFBP3) gene**. Anim Genet 1997, **28**:375-375.
15. Liu H, Liu C, Yang G, Li H, Dai J, Cong Y, Li X: **DNA polymorphism of insulin-like growth factor-binding protein-3 gene and its association with cashmere traits in cashmere goats**. Asian-Australasian J Anim Sci 2012, **25**:1515.
16. Vincze T, Posfai J, Roberts RJ: **NEBcutter: a program to cleave DNA with restriction enzymes**. Nucleic Acids Res 2003, **31**:3688-3691.
17. Choudhary V, Kumar P, Bhattacharya T, Bhushan B, Sharma A, Shukla A: **DNA polymorphism of insulin-like growth factor-binding protein-3 gene and its association with birth weight and body weight in cattle**. J Anim breed genet2007, **124**:29-34.
18. Shukla A: **PCR-RFLP studies on insulin-like growth factor binding protein 3 (IGFBP-3) gene in cattle. MV Sc**. thesis submitted to the Indian Veterinary Research Institute (Deemed University), Izatnagar, Bareilly, UP, India; 2001.
19. Padma B, Kumar P, Choudhary V, Dhara S, Mishra A, Bhattacharya T, Bhushan B, Sharma A: **Nucleotide sequencing and PCR-RFLP of insulin-like growth factor binding protein-3 gene in riverine buffalo (Bubalus bubalis)**. ASIAN Australasian J Anim Sci 2004, **17**:910-913.
20. Sharma A, Dutt G, Jayakumar S, Saroha V, Dixit S: **Sequence characterization and genetic variability analysis of GHR, IGF1, and IGFBP-3 genes in nine Indian goat breeds**. J Appli Anim Res 2014, **42**:361-365.

Tables

Table 1. The different amino acids in sheep as compared with cattle and buffalo, which obtained from a part of exon 2, complete exon 3 for each species.

10	8	7	6	5	4	3	2	1	No.
K lysine	T Threonine	Q Glutamine	L Leucine	L Leucine	Q Glutamine	Q Glutamine	S Serine	D Aspartic	Sheep
S Serine	G Glycine	S Serine	S Serine	Y Tyrosine	S Serine	S Serine	Q Glutamine	T Threonine	Cattle & Buffalo
18	17	16	15	14	13	12	11	10	No.
Q Glutamine	L Leucine	F Phenylalanine	R Arginine	L Leucine	C Cysteine	A Alanine	L Leucine	Q Glutamine	Sheep
P Proline	R Arginine	S Serine	S Serine	R Arginine	Y Tyrosine	P Proline	C Cysteine	S Serine	Cattle & Buffalo

Figures

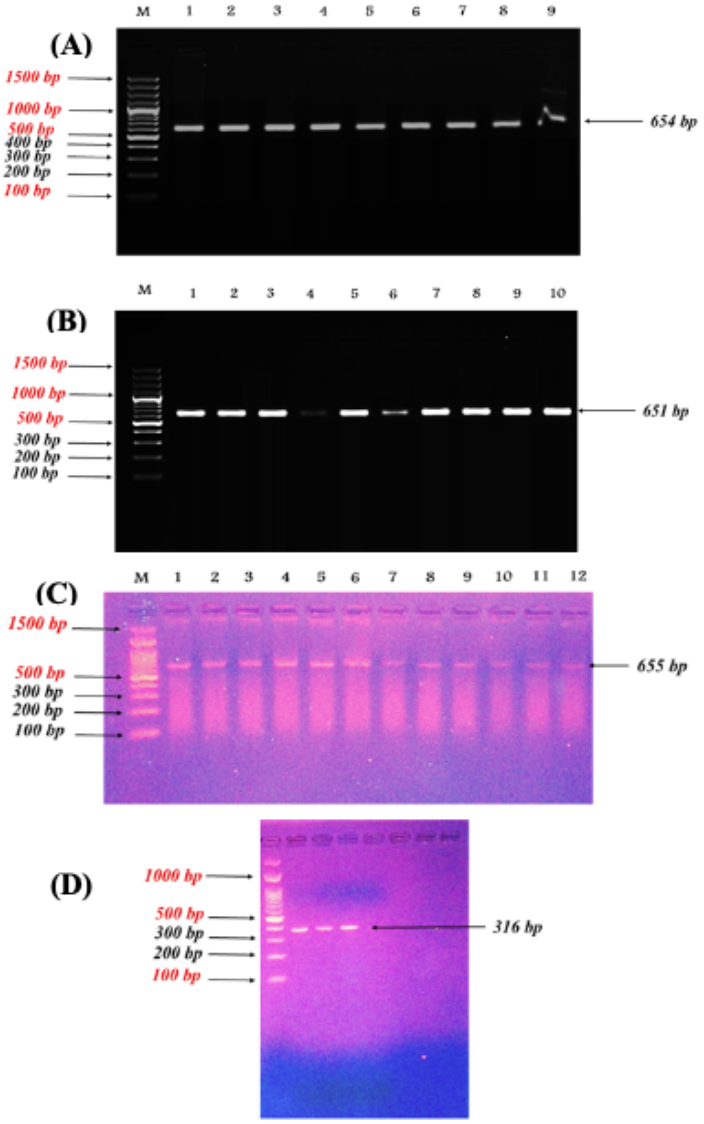
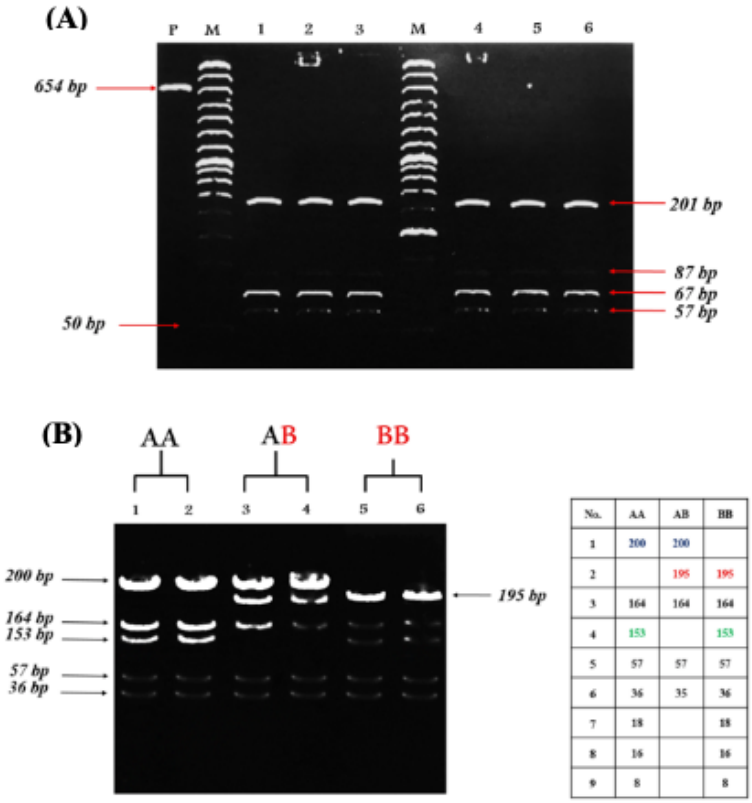


Figure 1

PCR amplification of IGFBP-3 gene for Egyptian sheep (A), Egyptian Cattle (B), Egyptian Buffalo (C) and Egyptian goat (D). M;100 bp DNA ladder.



No.	AA	AB	BB
1	200	200	
2		195	195
3	164	164	164
4	153		153
5	57	57	57
6	36	35	36
7	18		18
8	16		16
9	8		8

Figure 2

(A)-The PCR products of the IGFBP-3 gene from genomic DNA of tested breeds digested by HaeIII. Lanes: 1 and 2; Rahmani, lanes: 3 and 4; Barki, and lanes: 5 and 6; Rahmani X Barki crosses. M, 50 bp DNA ladder, P; PCR product for IGFBP-3. The digestion with HaeIII revealing a single pattern only for 8 DNA fragments sized 201, 201, 87, 67, 57, 18, 16 and 7 bp and indicating absence of a polymorphism in tested sheep breeds for IGFBP-3 gene. The restriction fragments with sizes; 18, 16 and 7 bp were not seen on the gel. (B)- HaeIII restriction pattern of Egyptian cattle IGFBP-3 gene. Lane M: 100-bp ladder marker. Lanes 1 and 2: Homozygous (AA) genotype with 8 restricted fragments at 200, 164, 153, 57, 36, 18, 16 and 8 bp. Lanes 3 and 4: Heterozygous (AB) genotype with 5 restricted fragments at 200, 195, 164, 57 and 35 bp. Lanes 5and 6: Homozygous (BB) genotype with 8 restricted fragments at 195, 164, 153, 57, 36, 18, 16 and 8 bp. The restriction fragments with sizes 18, 16 and 8 bp have not been seen on the gel.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pdf
- supplement2.pdf