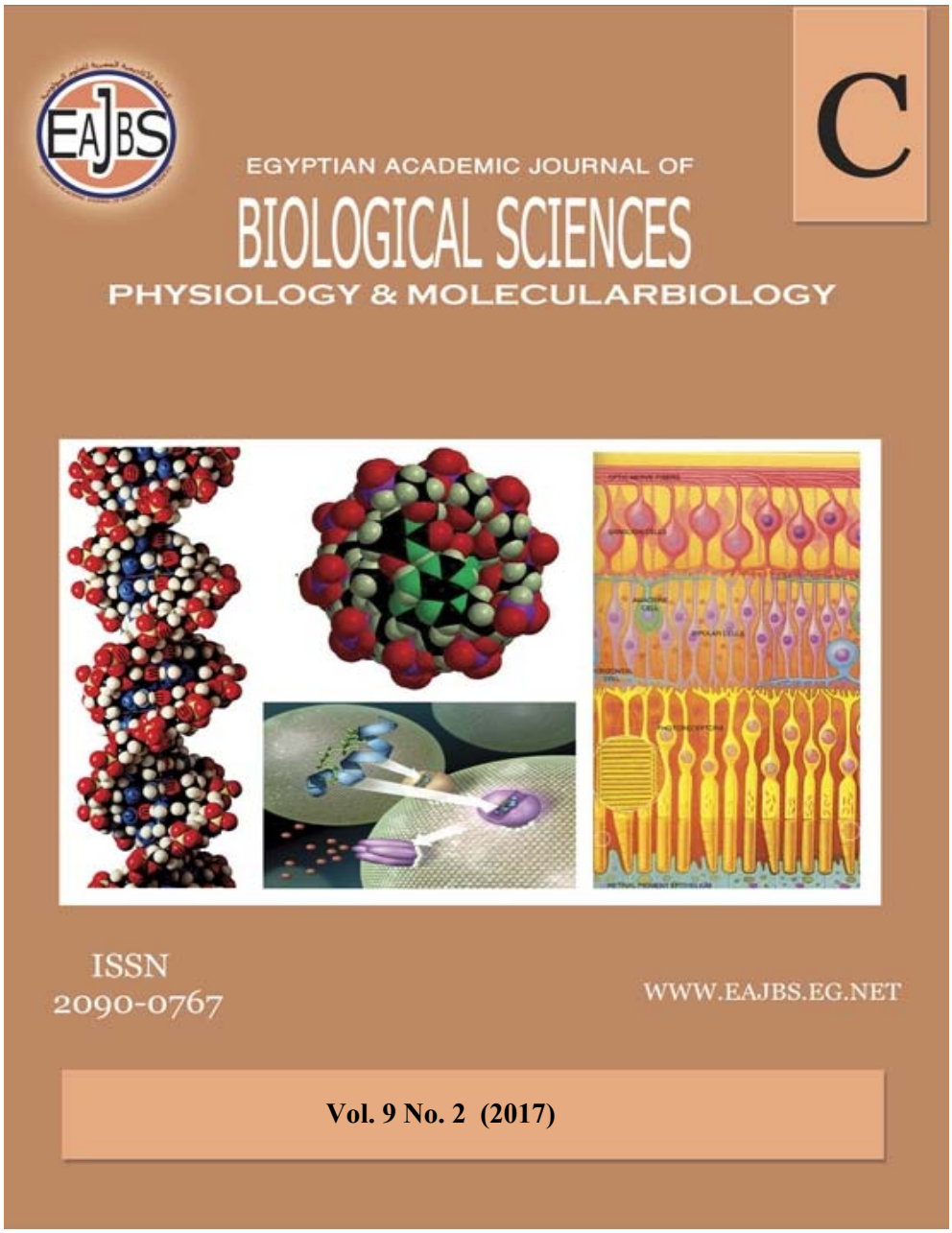

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Physiology & molecular biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidate important biological, chemical, or physical mechanisms of broad physiological significance.

www.eajbs.eg.net

Citation :*Egypt. Acad. J. Biolog. Sci. (C. physiology and Molecular biology) Vol.9(2)pp1-8 (2017)*

Egypt. Acad. J. Biolog. Sci., 9(2): 1 -8 (2017)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
Email: egyptianacademic@yahoo.com

ISSN 2090-0767
www.eajbs.eg.net

Genetic Polymorphisms in Exon-3 region of Growth Hormone Gene in the Egyptian Goat Breeds

Nermin K. El-Halawany [1], Fathy M. Abd-El-Razek [2], Yasmin A. El-Sayed [1], Afaf El-Werdany [1], Abd-El-Monsif A. Shawky [1], Ahmed F. Al-Tohamy [1] and Hamdi Abdel-Shafy [3]

1- Department of Cell Biology, Genetic Engineering Division, National Research Centre, Cairo, Egypt.
2- Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt.
3- Department of Animal Production, Faculty of Agriculture, Cairo University, Cairo, Egypt.
E-mail: yasmin_isd@hotmail.com

ARTICLE INFO

Article History
Received: 5/4/2017
Accepted: 15/5/2017

***Keywords*:**
Egyptian Goat
Growth Hormone gene
Sequencing
Single Nucleotide Polymorphism (SNP)

ABSTRACT

Background: In Egypt, the importance of goats comes from its potential source of meat and milk production. Thus, goat productivity improvement acts as a global strategy to provide Egyptians by important source of protein feeding. Molecular techniques have been employed to analyze growth hormone (GH) gene that promotes goat muscles, bone formation, regulating fat content and other important traits in goat. Our objective was to document and find out an accurate genetic marker sites, single nucleotide polymorphisms (SNPs) in exon-3 region of GH gene in some Egyptian goat breeds. **Methods:** Fifteen blood samples were collected from three Egyptian goat breeds (Baladi, Barki and Zaraibi), DNA was isolated and primer was used to amplify GH gene region corresponding to exon-3 region. Finally, sequencing methodology was applied for PCR products regarding the three goat breeds. **Results:** The results showed four nucleotide substitutions; The first SNP (C→T) was located at nucleotide position 106, the second SNP(G→A) was located at nucleotide position118 and the third SNP (G→A) was located at nucleotide position 128 .Finally, the fourth SNP (T→C) was located at nucleotide position 136. It was found that the SNP (G→A) at nucleotide position 128 caused an amino acid change from Glycine to Serine in the protein sequence of GH gene. **Conclusion:** Our findings demonstrated several genetic variations across exon-3 region of GH gene which can be used as marker in selection of goats with high valued traits.

INTRODUCTION

Goats are the most adaptable domestic animals that can be distributed in different environmental conditions such as arid, humid, tropical, cold, desert and mountain conditions (Kaliber *et al*., 2016). The importance of goats mainly relies on its persistent supplementation of meat, milk, fiber, and skin. Despite they lack genomic research tools as in cattle and sheep, they are considered as an essential animal source for providing row meat and milk products for several industries (Thornton, 2010).

Goat breeds are largely classified according to their geographical location, morphological features and prolific nature.

In Egypt, the most common goat breeds distributed around different regions are Baladi, Barki and Zaraibi breeds (Galal *et al*., 2005). The native Egyptian goat breed located in the Nile Delta and along the Nile is known as Baladi breed. This breed shows highly phenotypic variation among different subpopulations. They are polyestrous animals; kidding all the year. Additionally, they are characterized by good fertility rate with high prolific and high milk yield (Haider and Abdelsalam, 1993). Barki desert goat is the only goat breed raised in the coastal zone of the western desert in Egypt. They are estimated to be 10% of total goat population in Egypt (Haider *et al*., 1994). Zaraibi breed is one of the most important Egyptian goat breeds distributed across the North East of the Nile Delta. They have a great economic value in Egypt due to their highly production of meat and milk as well as their great litter size rate (Marai *et al*., 2002).

In Egypt, most of livestock breeds lack molecular characterization which is required for conserving important genetic resources besides improving animal productivity. This can be achieved by identification and utilization of different genetic variations within and among different animal breeds (Agha *et al*., 2008). Genetic improvement schemes in goat were depended on the selective breeding where the estimated breeding value was derived only from phenotypes. These breeding protocols do not allow for optimal control over precise phenotypic traits resulting in loosing or displacing of many important breeds without knowing their genetic significance (Rout *et al*., 2008). Nowadays, the evaluation of genetic variability in domestic goats is depending on the detection of different genetic markers that are associated with an important economic trait via Marker Assisted Selection (MAS).

Marker Assisted Selection (MAS) involved in the identification of genetic markers (DNA markers) for specific trait that are linked to several Quantitative Trait Loci (QTL) in different genes (Williams, 2005). The genes attributed with different economic traits including growth, reproduction, meat and milk production traits as well as disease resistance trait is known as candidate genes (Supakorn, 2009). These genes were identified by advanced molecular techniques such as Restriction Fragment Length Polymorphism (RFLP), Single Strand Conformation Polymorphism (SSCP) and sequencing techniques (Yang *et al*., 2013). Growth hormone gene is one of the most essential candidate genes which is required for animal's cells activity. It is secreted by somato-tropic cells of the anterior lobe of the pituitary gland. GH gene influences different animal processes such as growth, lactation, reproduction and metabolism (Burton *et al*., 1994). Goat growth hormone gene is encoded by 2500 base pairs (bp). It consists of 5 exons and separated by 4 intervening introns (Kioka *et al.*, 1989).

Many publications have reported that the polymorphisms of this gene have been identified in the promoter regions, UTRs, coding and non-coding regions. Certainly, a few of these polymorphic sites have been precisely characterized for nucleotide and amino acid changes along the GH gene sequence in different goat breeds (Yu *et al*., 2004). The current study concerned with the identification of SNP abundance in exon-3 region of GH gene to identify the nucleotide and amino acid changes across the three Egyptian goat breeds (Baladi, Barki and

Zaraibi) using polymerase chain reaction and sequencing technique.

MATERIALS AND METHODS

Animal population and blood collection: a total of 15 blood samples were collected from three Egyptian goat breeds (Baladi, Barki and Zaraibi) by 5 samples per breed. The three goat breeds were reared in the research farms of the department of Animal Production, Faculty of Agriculture, Cairo University.

Genomic DNA extraction: Genomic DNA was isolated using phenol-chloroform extraction technique (Sambrook and Russell, 2006). The quantity was determined using spectrophotometer and quality of DNA samples were checked on 1.5 % agarose gel electrophoresis. The samples of good quality were used for PCR amplification.

Polymerase Chain Reaction (PCR): PCR reactions were performed in 15 µl reaction mixture containing 1 µl of DNA (50 ng), 7.5 µl Go-Taq green mater mix which consisted of (1X PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTPs and 1 U/µl of *Taq* DNA polymerase). Then, 2µl of a primer mix (10 pmol of each primer) was added where the primer used for exon-3 amplification was previously described by Wickramaratne *et al.,* (2010) in studying GH gene in Osmanabadi and Sangamneri goat breeds.

GH3 F 5'-GGTTCCGAATAAGGCAGTGA-3'
GH3 R 5'-CACCACCACCAACCATCAT -3'

The volume was completed with ddH2O to 15 µl. The thermal cycling conditions (Bio-Rad MJ-mini, USA) included an initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 15s; annealing at 65°C for 30s and extension at 72°C for 45s then a final extension at 72°C for 5 min. The PCR products were detected using 1.5% agarose gel electrophoresis to predict the presence of band which representing the amplified fragment.

DNA sequencing: The amplified fragments of exon-3 region from the three goat breeds were sequenced in ABI 310 automated DNA sequencer (Applied Bio System).

Sequence analysis and single nucleotide polymorphism (SNP) detection: Sequence analysis and alignment of GH gene were carried out using NCBI/BLAST (blastn) https://blast.ncbi.nlm.nih.gov/Blast.cgi against database sequence with the accession number (D00476.1) to identify single nucleotide substitution in the three goat breeds. Additionally, the protein sequence was predicted using EXPASY software https://www.expasy.org/.

RESULTS AND DISCUSSION

Recently, applications of molecular genetics tools allow genotyping of specific genetic loci of multiple genes in different living individuals (Dekkers, 2004). Growth Hormone gene is one of the most important candidate genes which affect a wide variety of physiological parameters. There is evidence of an association between plasma levels of GH gene and its genetic variants in goat (Mousavizadeh *et al.,* 2009). This work was designed for screening of SNPs in exon-3 region of GH gene in three different Egyptian goat breeds.

Target amplification:

The primer used in this study flanked a 449 bp fragment corresponding to exon-3. The amplified fragments obtained from all tested goat animals appeared at 449 bp in 1.5% agarose gel electrophoresis as represented in (Fig. 1).

Sequence analysis:

Sequence data resulted from sequencing of exon-3 fragments was aligned against database sequence with the accession number (D00476.1**)**. The results revealed four nucleotide substitutions in exon-3 region among the three goat breeds as indicated in (Table 1).

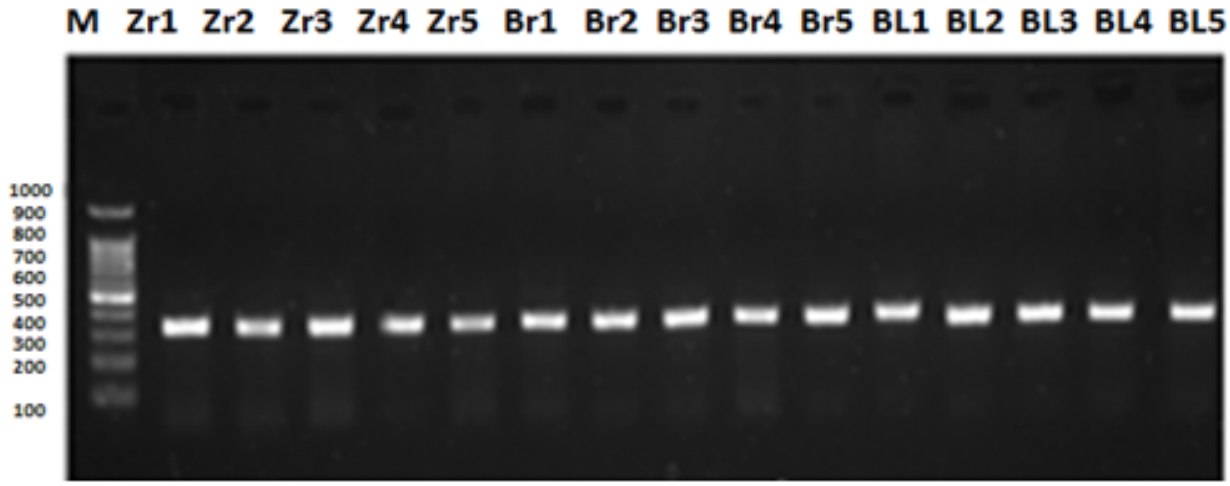

Fig. 1: Amplified PCR products of exon-3 region appeared at 449 bp in the three goat breeds

Table 1: SNP position in exon-3 region of GH gene in the three goat breeds relative to the nucleotide positions in the genomic sequence of GH gene with an accession no: D00476.

Region	Wild type	Mutant type	SNP position in exon-3 region in the three goat breeds (bp)	Nucleotide positions in genomic sequence of GH gene (bp) (accession no: D00476)
Exon-3	C	T	106	1148
Exon-3	G	A	118	1160
Exon-3	G	A	128	1170
Exon-3	T	C	136	1178

Where; bp = base pair

Nucleotide substitutions:

The first nucleotide substitution (C→T) was detected at nucleotide position 106 in exon-3 of GH gene in the three goat breeds as represented in (Fig. 2). The second nucleotide substitution (G→A) was detected at nucleotide position 118 as represented in (Fig. 3).

The third nucleotide substitution (G→A) was detected at nucleotide position 128 as represented in (Fig. 4) while the last nucleotide substitution (T→C) was detected at nucleotide position 136 as represented in (Fig. 5).

The translation of the sequenced segment of exon-3 region of GH gene using EXPASY software refers to a change in the amino acid sequence as a result of the nucleotide substitution (G→A) which is located at nucleotide position 128. This nucleotide substitution caused an amino acid change from Glycine to Serine between G and A alleles as represented in (Fig. 6).

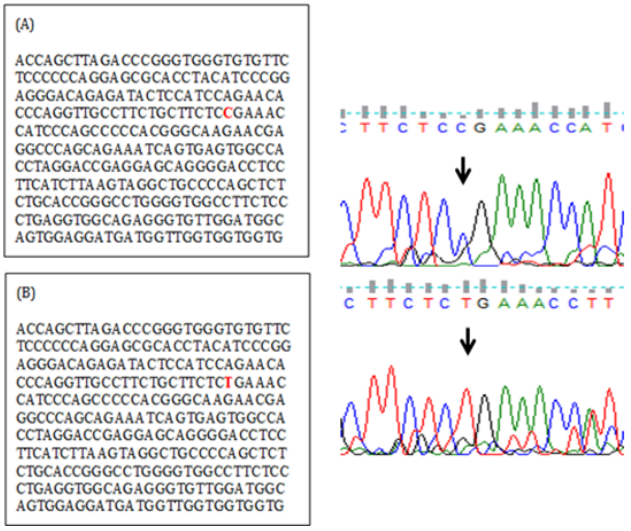

Fig. 2: The first nucleotide substitution (C→T) at nucleotide position 106: (A) Reference sequence with nucleotide C (B) GH sequence with SNP T

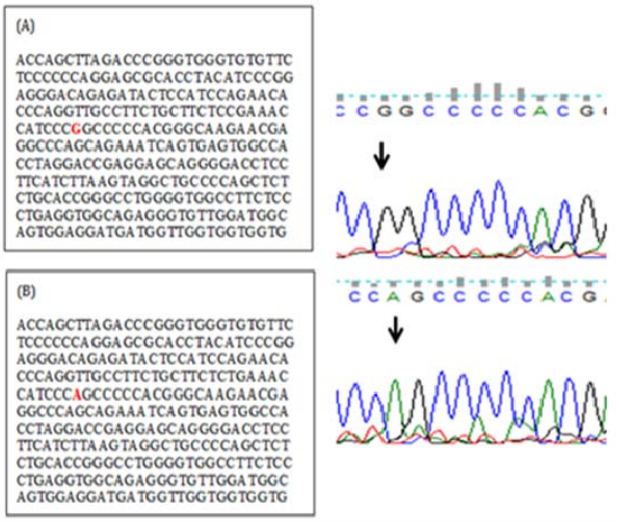

Fig. 3: The second nucleotide substitution (G→A) at nucleotide position 118: (A) Reference sequence with nucleotide G (B) GH sequence with SNP A

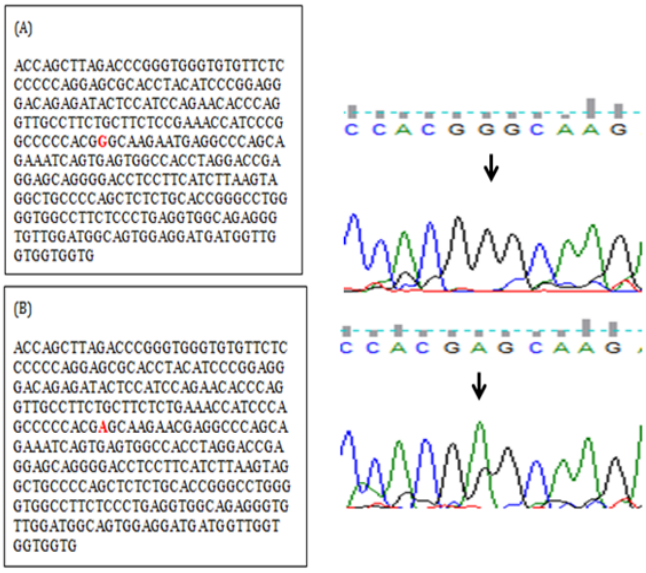

Fig. 4: The third nucleotide substitution (G→A) at nucleotide position 128: (A) Reference sequence with nucleotide G (B) GH sequence with SNP A

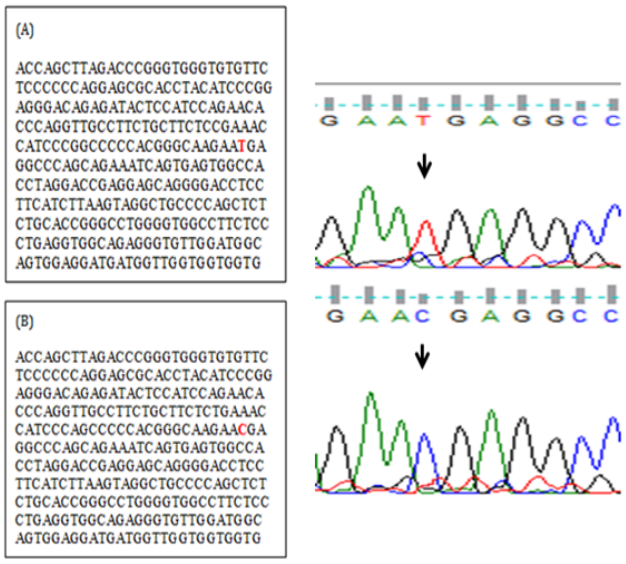

Fig. 5: The last nucleotide substitution (T→C) at nucleotide position 136: (A) Reference sequence with nucleotide T (B) GH sequence with SNP C

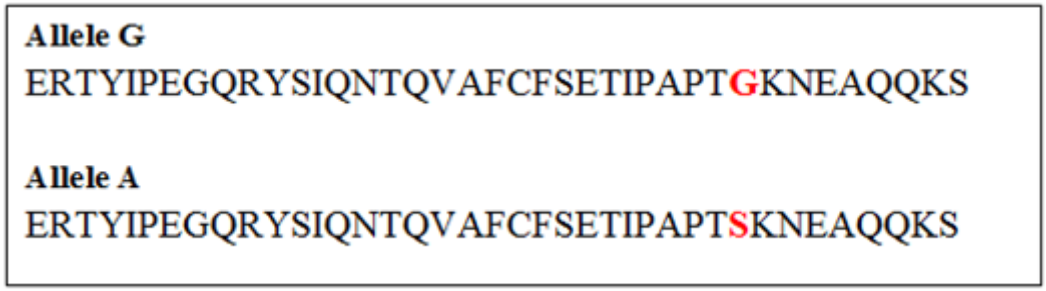

Fig. 6: Amino acid sequence of sequenced segment of exon-3 region of GH gene for alleles G and A changed from Glycine to Serine

In the current study, The location of the first, second, third and fourth SNP were recorded using (nBLAST) tool at nucleotide positions 1148,1160,1170 and 1178 respectively in the genomic sequence of GH gene with the accession no: D00476. The results of the present study agree with Dettori *et al.,* (2013) who investigated the same SNPs in exon -3 region of GH gene in primiparous Sarda goat breed in relation to database sequence with the accession no: D00476. They found that PCR-SSCP of exon-3 showed six different polymorphic patterns that were strongly associated with milk yield and with both fat and protein content. Their results showed that animals with patterns III and IV had the highest milk yield while animals with pattern V with respect to the SNP (T→C) which is located at nucleotide position 1178 was greatly associated with a higher fat and protein percentage than animals with patterns I and IV with a frequency ($P<0.01$).They reported the same amino acid change from Glycine to Serine in the protein sequence of GH gene with regard to nucleotide substitution (G→A) which is located at nucleotide position 1170. They concluded that exon-3 region have the highest proportion of polymorphic loci that can be associated with different traits in goat.

CONCLUSION

In conclusion, new genetic technologies can be applied in improving production traits in livestock species where Marker-Assisted Selection (MAS) allow better selection of heritable traits across several goat breeds. Our study provided useful information regarding the genetic variability in exon-3 region of GH gene in three different breeds of Egyptian goats. The results of this work can be used in a follow up study to associate the discovered SNPs with a desired traits in goat. Due to the limited sample size, further studies are recommended with an adequate sample size.

REFERENCES

Agha, S. H., Pilla, F., Galal, S., Shaat, I., D'andrea, M., Reale, S., Abdelsalam, A. Z. and Li, M. H. (2008): Genetic diversity in Egyptian and Italian goat breeds measured with microsatellite polymorphism. *J Anim Breed Genet,* 125: 194-200.

Burton, J. L., McBride, B. W., Block, E., Glimm, D.R. and Kennelly, J. J.(1994): A review of bovine growth hormone. *Can J Anim Sci*, 74: 167−201.

Dekkers, J. C. (2004): Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. *J Anim Sci,* 82: 313-328.

Dettori, M. L., Rocchigiani, A.M., Luridiana, S., Mura, M.C., Carcangiu, V. and Pazzola, M. (2013): Growth hormone gene variability and its effects on milk traits in primiparous Sarda goats. *J Dairy Res*, 80: 255–262.

Galal, S., Abdel-Rasoul, F., Anous, M.R. and Shaat, I. (2005): On-station characterization of small ruminant breeds in Egypt. In: L. In˜ iguez (ed.), Characterization of Small Ruminant Breeds in West Asia and North Africa. *ICARDA Aleppo Syria*, 2: 141–193.

Haider, A. E. and Abdelsalam, M.M. (1993): Studies on pre-weaning survivability of Baladi and Angora goats and their crosses Egypt. *J Appl Sci*, 8: 266-276.

Haider, A.E., Abdelsalam, M.M, Aboul-Naga, A.M., El-Kimary, I.S. and Eissa, M. (1994): Reproductive performance of desert Barki goats and their crosses with Damascus and Zaraibi breeds under the coastal zone of Western desert in Egypt. *Egypt J Anim Prod*, 31: 99-109.

Kaliber, M., Koluman, N. and Silanikove, N. (2016): Physiological and behavioral basis for the successful adaptation of goats to severe water restriction under hot environmental conditions. *Animal,* 10: 82-88.

Kioka, N., Manabe, E., Abe, M., Hashi, H., Yato, M., Okuno, M., Yamano, Y.,Sakai,H., Komano, T., Utsumi, K. and Iritani, A. (1989): Cloning and sequencing of goat growth hormone gene. *Agric Biol Chem* , 53: 1583-1587.

Marai, I. F. M., Abou-Fandoud, E. I., Daader, A. H. and Abu-Ella , A. A. (2002): Reproductive doe traits of the Nubian (Zaraibi) goats in Egypt. *Small Rumin Res*, 46: 201–205.

Mousavizadeh, A., Abadi, M.M., Torabi, A., Nassiry, M.R., Ghiasi, H. and Koshkoieh, A. E. (2009): Genetic polymorphism at the growth hormone locus in Iranian Talli goats by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). *Iran J Biotechnol,* 7: 51–3.

Rout, P. K., Joshi, M. B., Mandal, A., Laloe, D., Singh, L. and Thangaraj, K. (2008): Microsatellite-based phylogeny of Indian domestic goats*. BMC Genet*, 9-11.

Sambrook, J. and Russell, D. W. (2006): Purification of nucleic acids by extraction with phenol:chloroform. *CSH Protoc,* 2006.

Supakorn, C. (2009): The Important Candidate Genes in Goats - A Review. *Walailak J Sci & Tech*, 6: 17-36.

Thornton, P. K. (2010): Livestock production: recent trends, future prospects. *Philos Trans R Soc Lond B Biol Sci,* 365: 2853-2867.

Wickramaratne, S.H.G., Ulmek, B.R., Dixit, S.P., Kumar, S. and Vyas, M.K. (2010): Use of growth hormone gene polymorphism in selecting Osmanabadi and Sangamneri goats. *Trop Agric Res*, 21: 398-411

Williams, J. L. (2005): The use of marker-assisted selection in animal breeding and biotechnology. *Rev Sci Tech*, 24: 379-91.

Yang, W., Kang, X., Yang, Q., Lin, Y. and Fang, M. (2013): Review on the development of genotyping methods for assessing farm animal diversity. *J Anim Sci Biotechnol,* 4- 2.

Yu, L.M., Jiang, M.L., Qiang, S.G., Jie, P.Q., Wei, S. and Lian, W.G. (2004): Polymorphism analysis of goat growth hormone gene in the 5' regulatory sequence. *Hereditas*, 6: 831-835.

ARABIC SUMMARY

تعدد الأشكال الوراثية فى منطقة إكسون-٣ لجين هرمون النمو في سلالات الماعز المصرية

نيرمين كمال الحلوانى١ ، فتحى محمد عبدالرازق٢، ياسمين احمد السيد١، عفاف الودانى١، عبد المنصف احمد شوقى١، احمد فتحى التهامى١ ، حمدى عبد الشافى٣

١- قسم بيولوجيا الخلية – شعبة الهندسة الوراثية – المركز القومى للبحوث- القاهرة- مصر

٢- قسم الكيمياء- كلية العلوم-جامعة القاهرة- القاهرة- مصر

٣ - قسم الانتاج الحيوانى - كلية الزراعة - جامعة القاهرة - القاهرة- مصر

بريد الكترونى:yasmin_isd@hotmail.com

الخلفية العلمية: يُعد الماعز من اهم مصادر إنتاج اللحوم والألبان فى مصر. وبالتالي، فإن تحسين إنتاجية الماعز هو بمثابة هدف اساسى و حيوي من أجل توفير المزيد من البروتين الغذائى للمصريين. وقد أُستخدمت التقنيات الجزيئية الحديثة لتحليل جين هرمون النمو(GH) والذى له دورا هاما فى تعزيز نمو العضلات ، تكوين العظام، تنظيم محتوى الدهون وغيرها من الصفات الهامة في الماعز. ولذلك فإن الهدف من هذا البحث هو توثيق ومعرفة علامات وراثية دقيقة تعرف بإسم الأشكال النوكليوتيدية المفردة (SNPs) في منطقة إكسون- ٣ فى جين هرمون النمو (GH) في بعض سلالات الماعز المصرية.

المنهجية: تم تجميع خمسة عشر عينة دم من ثلاث سلالات من الماعز المصرية (البلدي و البرقي والزرايبي). وتم عزل و تنقية الحمض النووي و تكبير الجزء الخاص بمنطقة إكسون-٣ عن طريق إجراء تفاعل إنزيم البلمرة المتسلسل بإستخدام البادئة الخاصة لمنطقة إكسون-٣ ثم تم عمل تحليل التتابع النيوكلوتيدى لنواتج تفاعل انزيم البلمرة المتسلسل.

النتائج: أظهرت النتائج وجود أربعة من الطفرات النيوكولتيدية فى منطقة اكسون-٣ حيث وُجدت أول طفرة (C→T) عند الموقع النيوكلوتيدى ١٠٦ وثانى طفرة (G→A) عند الموقع النيوكلوتيدى ١١٨ و وثالث طفرة (G→A)عند الموقع النيوكلوتيدى ١٢٨. و اخر طفرة (T→C) وُجدت عند الموقع النيوكلوتيدى ١٣٦. وقد وُجد أن الطفرة (G→A) عند الموقع النيوكلوتيدى ١٢٨ تسببت في تغيير الحمض الامينى من Glycine إلى Serine في التسلسل البروتينى الخاص بمنطقة إكسون-٣ لجين هرمون النمو.

الخلاصة: أظهرت النتائج التي توصلنا إليها العديد من الإختلافات الجينية فى منطقة إكسون-٣ لجين هرمون النمو و التي يمكن إستخدامها كدلالة جينية في إختيار الماعز ذات الصفات عالية القيمة.