



## Screening of *IGF-I* and *IGFBP-3* genes polymorphism in popular Goat breeds in Egypt

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### ABSTRACT

The genetic polymorphisms of two functional genes; Insulin-like growth-I (*IGF-I*) and Insulin-like growth factor binding protein-3 (*IGFBP-3*) were investigated in three goat breeds (Barki, Damascus and Zaraibi) by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), for differentiating these breeds. *IGF-I-HaeIII*/RFLP revealed three genotypes (CC, CG and GG) with frequencies; 0.40, 0.10 and 0.50, respectively in Barki. Moreover, the expected heterozygosity ( $H_e$ ) value (0.51) was higher than observed heterozygosity ( $H_o$ ) value (0.10) in Barki. Chi-square ( $\chi^2$ ) value in Barki (13.6) showed a significant deviation from Hardy-Weinberg equilibrium [HWE] ( $P < 0.05$ ). *IGFBP-3-XspI*/RFLP revealed three genotypes (CC, CT and TT), with only CT and TT in all breeds. CT frequency was the highest in Barki (0.80), and TT frequency was the highest in Damascus (0.75).  $H_o$  value was higher than  $H_e$  value in all breeds. Additionally,  $H_o$ ,  $H_e$  and  $\chi^2$  values were the highest in Barki (0.80, 0.49 and 8.26, respectively) and  $\chi^2$  value showed a significant deviation from HWE ( $P < 0.05$ ). The polymorphisms demonstrated in these results are recommended as effective markers for genetic differentiation among goat breeds, opening up interesting prospects for goat breeders for future selection programs.

**Keywords:** Genetic Polymorphism, goat, PCR-RFLP, *IGF-I* gene, *IGFBP-3* gene



### INTRODUCTION

Goats in Egypt are almost 3.13 million goats. They are raised mainly in three regions: the Upper Egypt, Nile Delta and in the desert rangelands [1]. Production systems and breeds in the three zones are different. There are about 1.7 million goats, mainly in mixed flocks, with some goats kept as household animals. In the desert rangelands, 1.4 million sheep and goats are kept in extensive systems [2]. There are five indigenous goat breeds: Baladi (local breed in Delta), Barki or Sahrawi (local breed in Desert), Sinaoy (Bedouin), Saidi and Zaraibi (or Egyptian Nubian). They are duple - purpose animals, with does bred for milk and bucks bred for meat [3]. The former five breeds are pure whereas, the other breeds are mixtures of these breeds, like the Anglonubian breed, coming from crossbreeding of Egyptian Nubian breed with some English breeds. Goat genetic improvement schemes in Egypt have involved crossbreeding trials with foreign breeds like Damascus goats.

Lately, genetic polymorphisms at candidate genes affecting economic traits (like growth, milk yield, meat production and reproductive traits) have stimulated research interest because genetic polymorphisms are well-considered as an aid to genetic selection and to mark evolutionary relationships in different livestock animal breeds [4]. Genetic polymorphism arises from mutation which ranged from change in one nucleotide base (SNP) to variation in several hundred bases [5].

The *IGFs* signaling system, composed of *IGF-I*, *IGF-II*, *IGF-I* receptor, *IGF-II* receptor and six binding proteins (*IGFBP-1-IGFBP-6*), plays a vital role in growth, development, ageing and reproduction. *IGF-I* gene in goat is located on chromosome five and includes six exons and five introns [6]. It exerts a prime function in growth, milk yield, meat production and reproductive traits. Polymorphism in *IGF-I* gene has been studied in different livestock animals such as in cattle [7], sheep [8], goat [9], **chicken** [10] and in swine [11].

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Insulin-like growth factor binding protein-3 (*IGFBP-3*) gene encodes a structural protein, *IGFBP-3*, that binds non-covalently to insulin-like growth factors (*IGFs*) system in circulation and responsible for their multiple influences [12]. Thus, it plays a key function in different body functions such as growth, metabolism, reproduction, controlling body weight, immunity etc. Consequently, *IGFBP-3* gene is well-considered as an effective genetic marker in growth, milk yield and meat production traits [13,14]. *IGFBP-3* gene in goat and cattle is located on chromosome four [15]. The full length of the *IGFBP-3* gene is 8.9 kb and contains five exons [16]. Polymorphism in *IGFBP-3* gene has been studied in different livestock animals such as in sheep [17], buffalo [18], cattle [19] and in goat [20].

The aim of the present study is to screen the genetic polymorphism of two functional genes (*IGF-I* and *IGFBP-3*) in three goat breeds (Barki, Damascus and Zaraibi) via PCR-RFLP method in order to differentiate between these breeds.

## MATERIALS AND METHODS

**Animals and DNA extraction:** A total of 60 healthy does, belonging to three breeds, Barki, Damascus and Zaraibi; 20 samples for each breed. All animals were born and reared in the Agriculture Research Station, belonging to Faculty of Agriculture, Cairo University. Blood samples were collected in tubes containing 2.7% EDTA as anticoagulant and transported to the laboratory under cooled conditions. Genomic DNA was extracted and purified from whole blood collected samples using the salting out technique described by Miller et al. [21]. The DNA concentration was measured using the U.V spectrophotometer at wavelength 260 nm.

**Polymerase Chain Reaction (PCR):** Two pairs of primers were used for amplifying each of *IGF-I* and *IGFBP-3* loci using primers suggested by Liu et al. [22] and Lan et al. [20], respectively. The primer sequences are represented in table (1). Amplification reaction was carried out in a 25  $\mu$ l volume containing 100 ng genomic DNA, forward and reverse primer (both at concentration 10 pmol/ $\mu$ l), 1U *Taq* polymerase, 2.5  $\mu$ l *Taq* polymerase buffer, four dNTPs (each at final concentration of 2.5 mM/ $\mu$ l) and de-ionized double distilled H<sub>2</sub>O up to a total volume of 25  $\mu$ l. Amplification conditions are shown in table (2). The amplicons were analyzed by 1.5 % agarose gel electrophoresis. The gels were stained with ethidium bromide and visualized under ultraviolet light.

**Restriction Fragment Length Polymorphism (RFLP):** It was carried out in 15  $\mu$ l of reaction mixture of each sample containing 5  $\mu$ l of PCR product, 9.5  $\mu$ l of 10 X buffer and 0.5  $\mu$ l of fast restriction enzyme (MBI Fermentas, Germany) specific for each gene (Table 1). The reaction mixture was incubated at 37°C for 10 minutes. Digestion products were separated by electrophoresis on 2.5 % agarose gel, stained with ethidium bromide. The bands were visualized under UV light and the gels were photographed using digital gel documentation system (Bio-Rad, USA).

**Statistical Analysis:** The genotypic and allelic frequencies, the observed and expected heterozygosities and the  $\chi^2$  test for Hardy-Weinberg equilibrium (HWE) were calculated using Pop Gene 32.1 package [23].

## RESULTS AND DISCUSSION

Many different studies detected *IGF-I* gene polymorphisms and proved their association with growth traits among different livestock domestic animals [6,8,9,29,30,31]. For example, Zhang et al. [30] detected a single nucleotide polymorphism (SNP) [G→C] at intron four of the *IGF-I* gene and described its significant association with birth weight, body weight at six and twelve months in Nanjiang Huang goats.

In the current study, *IGF-I*-PCR produced a DNA fragment of 363 bp as shown in figure 1. It includes exon four and part of intron four of *IGF-I* [28]. Digestion of this fragment with the restriction enzyme *HaeIII* produced two alleles; C and G with a complete absence of allele G in both Damascus and Zaraibi breeds. Three genotypes; CC (363 bp), CG (363, 264 and 99 bp) and GG (264 and 99 bp) were found. As shown in table (3), the genotype GG had the highest frequency (0.50) in the Barki breed. Whereas, all goats of both Damascus and Zaraibi breeds showed only CC genotype. The value of expected heterozygosity (0.51) was higher than that of observed heterozygosity (0.10) in the Barki breed. Chi-square ( $\chi^2$ ) value in the Barki breed (13.6) showed a significant deviation from Hardy-Weinberg equilibrium ( $P < 0.05$ ).

These findings were similar to those illustrated previously by Liu et al. [28], who screened this caprine *IGF-I* gene polymorphism in exon four and part of intron four in two Chinese local goat breeds, via gene sequencing and PCR-RFLP. This discovered genetic polymorphism appeared associated with body weight in cashmere goats. However, this association was not significant. In Iranian Markhoz goats, Kurdistani et al. [24]

detected the polymorphism of *IGF-I* gene intron four and its association with growth traits and yearling fleece weight. Likewise, **Sharma *et al.*** [9] investigated the effect of two detected SNPs (g.4700T > C and g.5524C > T) of *IGF-I* gene in Indian Sirohi goats. Furthermore, **Rasouli *et al.*** [25] investigated caprine *IGF-I* gene polymorphism in Iranian Markhoz goats by sequencing and PCR-SSCP. Sequencing results revealed a G to A transition at position 1617 of the *IGF-I* gene (g. 1617 G>A) in the 5' flanking region of the *IGF-I* gene.

Several studies showed the *IGFBP-3* gene polymorphism and illustrated its association with growth traits among various livestock animals, cattle [33,32,19,34], buffalo [35,18], goat [9,25] and sheep [37,17]. In the current study, *IGFBP-3*-PCR produced a DNA fragment of 655 bp as shown in figure 3. It includes a part of intron two, exon three and intron three of *IGFBP-3* gene **Lan *et al.*** [20]. Digestion of this fragment with the restriction enzyme *XspI* produced two alleles, C and T, with a higher frequency of allele T in all the three studied breeds. Three genotypes; CC (655 bp), CT (655, 421 and 234 bp) and TT (421 and 234 bp) were found (Figure 4). As shown in table (4), the uncut CC genotype is absent in all the three breeds and only two genotypes AB and BB were revealed with a higher frequency of CT genotype in both the Barki and Zaraibi (0.80 and 0.55, respectively), and a higher frequency of TT genotype (0.75) in Damascus. The value of observed heterozygosity (Ho) was higher than that of expected heterozygosity (He) in all the three studied breeds. In addition, the values of both Ho and He were the highest in Barki breed (0.80 and 0.49, respectively). Chi-square ( $\chi^2$ ) value was the highest and also showed a significant deviation from Hardy-Weinberg equilibrium ( $P < 0.05$ ) in only the Barki breed.

These findings were in consonance with those previously reported by **Lan *et al.*** [20] who detected four mutations of goat *IGFBP-3* gene in some Chinese dairy goat breeds, by PCR-SSCP and DNA sequencing methods. However, they identified three genotypes, not two as in the current study, by *XspI*/PCR-RFLP, genotype X1X1(655 bp), X1X2 (655, 421 and 234 bp) and X2X2 (421 and 234 bp). This difference in genotypic patterns may be due to breed difference. Similarly, **Sharma**

*et al.* [9] identified Eight SNPs in the exon two of *IGFBP-3* gene indicating the high level of heterozygosity of this fragment. Moreover, **Rasouli *et al.*** [25] investigated goat *IGFBP-3* gene polymorphism in Markhoz goats by gene sequencing and PCR-SSCP, and their relationship with growth traits. Sequencing results revealed T to C transition at position 58 of exon two of the *IGFBP-3* gene.

On the other hand, no polymorphisms were found in a portion of sheep *IGFBP-3* gene including exon two, intron two, exon three and intron three by PCR-RFLP test (**Kumar *et al.*** 17). Moreover, **Shafey *et al.*** [26] found no polymorphism of sheep *IGFBP-3* gene in three Egyptian sheep breeds (Barki, Rahmani and Osseimi), when used *HaeIII*/PCR-RFLP. They found only one restriction pattern including 201, 201, 87, 67, 57, 19, 16 and 7 bp fragments which represent only the allele B. Furthermore, **Ali *et al.*** [ 27] reported the same finding in the four Egyptian sheep breeds (Rahmani, Osseimi, Barki and Awassi) indicating the homozygosity of *IGFBP-3* gene in the four breeds studied.

## CONCLUSION

It is concluded that the *IGF-I* /*HaeIII* and *IGFBP-3*/*XspI* polymorphisms may be utilized as effective markers for genetic differentiation between goat breeds. So, further studies should be made to sequence the amplified DNA fragments 363 and 655 bp of *IGF-I* and *IGFBP-3*, respectively, for more precise differentiation between these breeds at the level of DNA base pair. In addition, *IGF-I* and *IGFBP-3* gene may be potential molecular markers for various economic traits such as growth, reproduction, meat production and milk yield traits. Thus more studies, with large sample size for each goat breed, are needed for the association analysis between caprine *IGF-I* and *IGFBP-3* polymorphisms and these traits.

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**Table 1: Gene, primer sequence (5'→3'), length of PCR product, region and specific restriction enzyme of IGF-I & IGFBP-3 genes**

Gene	primer sequence (5'→3')	PCR product length	region	restriction enzyme
<i>IGF-I</i>	F: CACAGCGTATTATCCCAC R: GACTACTATGAGCCAGAAG	363 bp	Exon 4 & intron 4	<i>HaeIII</i>
<i>IGFBP-3</i>	F: CCA AGC GTG AGA CAG AAT AC R: AGG AGG GAT AGG AGC AAG TT	655 bp	Intron 2, exon 3 & intron 3	<i>XspI</i>

F: forward R: reverse

**Table 2: PCR conditions**

Gene	Primary denaturation in 1 <sup>st</sup> cycle		Denaturation		Annealing		Elongation		Final extension		Number of cycles
	°C	Sec	°C	Sec	°C	Sec	°C	Sec	°C	Sec	
	<i>IGF-I</i>	94	300	94	40	56	35	72	35	72	
<i>IGFBP-3</i>	95	240	94	40	55	35	72	35	72	600	30

**Table 3: Genotype frequency, allele frequency, observed heterozygosity (Ho), expected heterozygosity (He) and  $\chi^2$  values of *IGF-I-HaeIII*/ PCR-RFLP**

Gene / Restriction Enzyme	Breed	Genotype Frequency			Allele Frequency	
		CC	CG	GG	C	G
<i>IGF-I</i>	Barki	0.40	0.10	0.50	0.45	0.55
	Damascus	1.00	0.00	0.00	1.00	0.00
	Zaraibi	1.00	0.00	0.00	1.00	0.00

**Table 4: Genotype frequency, allele frequency, observed heterozygosity (Ho), expected heterozygosity (He) and  $\chi^2$  values of *IGFBP-3-XspI* / PCR-RFLP**

Gene / Restriction Enzyme	Breed	Genotype Frequency			Allele Frequency		Observed Het. (Ho)	Expected Het. (He)	$\chi^2$
		CC	CT	TT	C	T			
<i>IGFBP-3</i>	Barki	0.00	0.80	0.20	0.40	0.60	0.80	0.49	8.26*
	Damascus	0.00	0.25	0.75	0.12	0.88	0.25	0.22	0.32
	Zaraibi	0.00	0.55	0.45	0.27	0.73	0.55	0.41	2.57

(\*) means ( $P \leq 0.05$ )

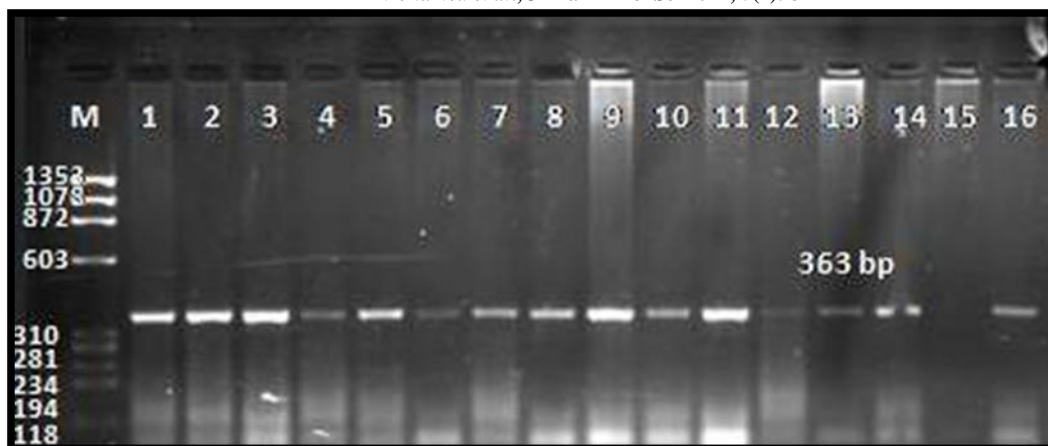


Fig. 1: Agarose gel electrophoresis of *IGF-I* - PCR fragment (363 bp). Lane M,  $\Phi$  DNA ladder. Lanes (1→6) Barki, (7→12) Damascus and Lanes (13→17) Zaraibi breeds.

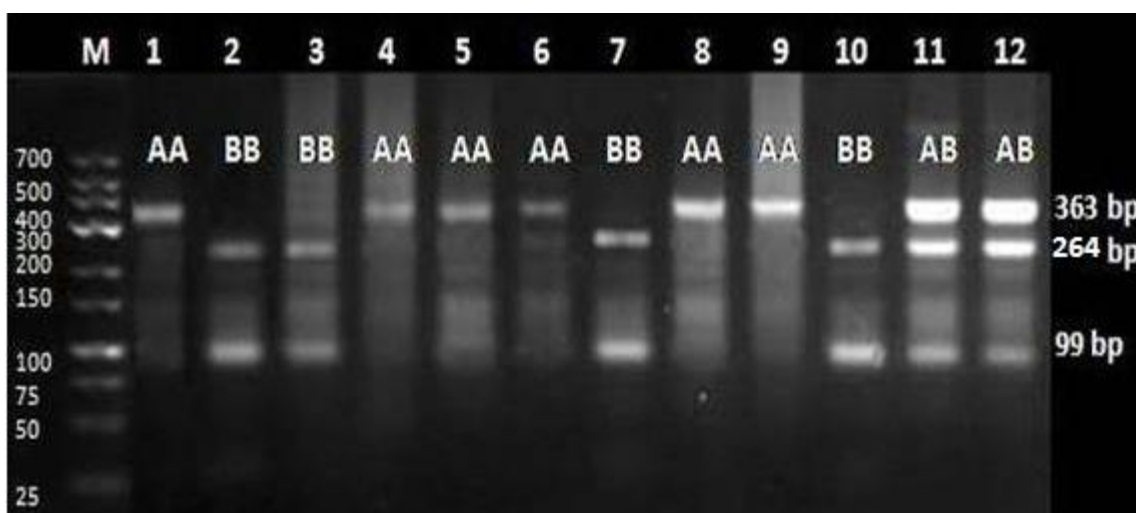


Fig. 2: Agarose gel electrophoresis of *IGF-I* -*HaeIII* / PCR-RFLP fragments. Lane M, 25bp DNA ladder, lanes (1, 4, 5, 6, 8, 9) Genotype CC (363 bp), lanes (11, 12) Genotype CG (363 , 263 and 99bp) and lanes (2, 3, 7, 10) Genotype GG (264 and 99bp).

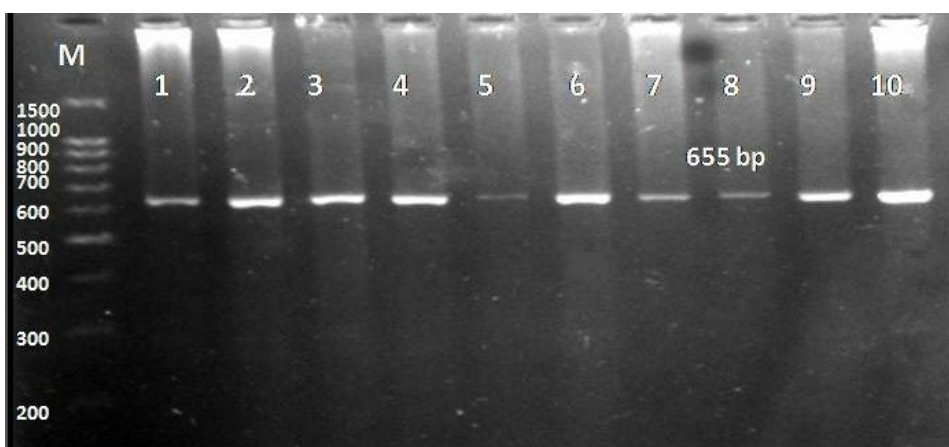
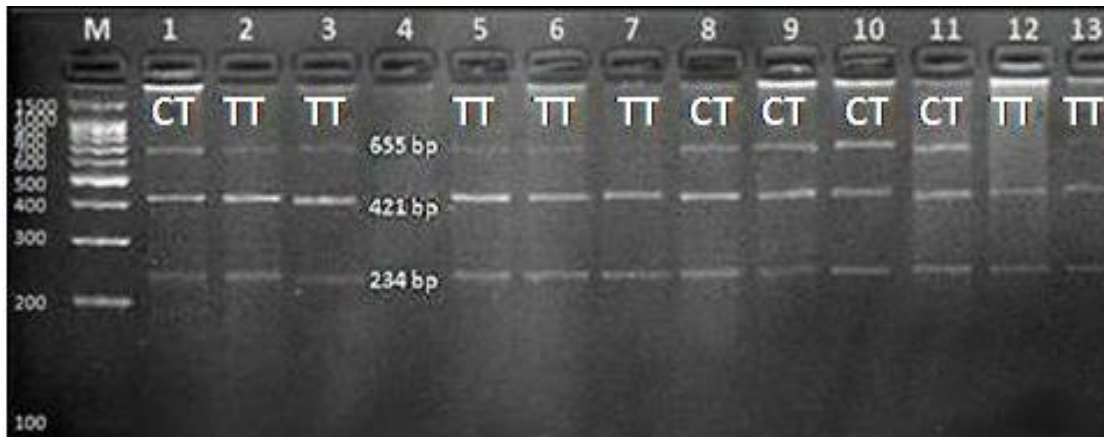


Fig. 3: Agarose gel electrophoresis of *IGFBP-3*- PCR fragment (655 bp). . Lane M, 100 bp DNA ladder. Lanes (1→3) Barki, (4→6) Damascus and Lanes (7→10) Zaraibi breeds.



**Fig. 4:** Agarose gel electrophoresis of *IGFBP-3-XsPI* / *PCR-RFLP* fragments. Lane M, 100 bp DNA ladder, lanes (1, 8, 9, 10, 11) Genotype CT (422 , 366 and 566bp) and lanes (2, 3, 5, 6, 7, 12, 13) Genotype TT (366 and 566bp).

## REFERENCES

1. FAOSTAT (FAO Statistical Yearbook). <http://faostat.fao.org/> (Accessed 2011).
2. Galal S et al. On-station characterization of small ruminant breeds in Egypt. In: L. Iniguez (ed.), Characterization of Small Ruminant Breeds in West Asia and North Africa 2005; 2: 141–193. ICARDA, Aleppo, Syria.
3. Latif MG et al.. Meat production characteristics of Egyptian Baladi and Angora goats. Meat Sci 1987; 20: 211-216.
4. Sodhi M et al. *MspI* allelic pattern of bovine growth hormone gene in Indian Zebu cattle (*Bos indicus*) breeds. Biochem Genet 2007; 45: 145-153.
5. Yahyaoui MH et al. Characterization and genotyping of the caprine  $\kappa$ -casein variants. J Dairy Sci 2003; 86 (8): 2715-2720.
6. Mikawa SG et al. Dynamic aspects in the expression of the goat insulin-like growth factor-I (*IGF-I*) gene: diversity in transcription and post-transcription. Biosci Biotechnol Biochem 1995; 59 (1):87-92.
7. Ge W et al. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. J Anim Sci 2001; 79:1757-1762.
8. Kazemi SM et al.. Study and Identification of Insulin-Like Growth Factor-I Gene Polymorphisms in Zel Sheep Population. Am J Anim Vet Sci 2011; 6 (4): 176-179.
9. Sharma A et al. Novel SNPs in *IGF1*, *GHR* and *IGFBP-3* genes reveal significant association with growth traits in Indian goat breeds. Small Rumin Res 2013; 115 (1): 7-14.
10. Bennett AK et al. Polymorphisms in vitamin D receptor, osteopontin, insulin-like growth factor 1 and insulin, and their associations with bone, egg and growth traits in a layer--broiler cross in chickens. Anim Genet 2006; 37:283-286.
11. Casas E et al. Relationship of growth hormone and insulin-like growth factor-1 genotypes with growth and carcass traits in swine. Anim Genet 1997; 28:88-93.
12. Bale LK, Conover CA. Regulation of insulin-like growth factor binding protein-3 messenger ribonucleic acid expression by insulin-like growth factor I. Endocrinology 1992; 131: 608–614.
13. Thue TD, Buchanan FC. A new polymorphism in the cattle *IGFBP3* gene. Anim Genet 2002; 33 (3): 242.
14. Othman OE et al. Single nucleotide polymorphism in Egyptian cattle insulin-like growth factor binding protein-3 gene. J. Genet Eng Biotechnol 2014; 12: 143–147.
15. Kappes SM et al. A second-generation linkage map of the bovine genome. Genome Res 1997; 7 (3): 235-249.
16. Kim JD et al. Identification of novel SNPs in bovine insulin-like growth factor binding protein-3 (*IGFBP3*) gene. Asian-Aust J Anim Sci 2005; 18(1): 3-7.
17. Kumar P et al. Nucleotide sequencing and DNA polymorphism studies on *IGFBP-3* gene in sheep and its comparison with cattle and buffalo. Small Rumin Res 2006; 64: 285–292.
18. Padma B et al. Nucleotide sequencing and PCR-RFLP of insulin-like growth factor binding protein-3 gene in river buffalo (*Bubalus bubalis*). Asian-Aust J Anim Sci 2004; 17: 910–913.



19. Choudhary V. Molecular studies on leptin and insulin-like growth factors binding protein 3 (*IGFBP-3*) genes in cattle. PhD Thesis, The Indian Veterinary Research Institute (Deemed University), Izatnagar, Bareilly, India, 2004.
20. Lan XY et al. The *HaeIII* and *XspI* PCR-RFLPs detecting polymorphisms at the goat *IGFBP-3* locus. *Small Rumin Res* 2007; 73:283-286.
21. Miller SA et al. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 12-15.
22. Liu WJ et al. The polymorphism of *IGF-I* gene on two goat breeds in China. *J Anim Vet Adv* 2010; 9(4): 790-794.
23. Yeh FC et al. POPGENE, Version 1.31. A Microsoft Window Based Free Ware for Population Genetic Analysis. University of Alberta, Edmonton, 1999.
24. Kurdistani ZK et al. Evaluation of insulin-like growth factor-I gene polymorphism on growth traits and yearling fleece weight in goats. *Small Rumin Res* 2013; 111(1): 10-15.
25. Rasouli S et al. Evaluation of polymorphism in *IGF-I* and *IGFBP-3* genes and their relationship with twinning rate and growth traits in Markhoz goats. *Annal Anim Sci* 2016; DOI: 10.1515/aoas-2016-0020.
26. Shafey HI et al. Genetic Polymorphism of Myostatin and Insulin-Like Growth Factor Binding Protein-3 Genes in Egyptian Sheep Breeds. *Glob Vet* 2014; 13 (3): 419-424.
27. Ali BA et al. Genetic biodiversity studies on *IGFBP-3* gene in Egyptian sheep breeds. *Biotechnol Anim Husb* 2009; 25:101-109.
28. Liu WJ et al. The polymorphism of *IGF-I* gene on two goat breeds in China. *J Anim Vet Adv* 2010; 9 (4): 790-794.
29. Li C et al. Assessment of positional candidate genes *myf5* and *IGF-I* for growth on bovine chromosome 5 in commercial lines of *Bos taurus*. *Anim Sci* 2004; 82:1-7.
30. Zhang C et al. A new single nucleotide polymorphism in the site *IGF-I* gene and its association with growth traits in the Nanjhang Huang goat. *Asian-Aust J Anim Sci* 2008; 21, 1073-1079.
31. Deng C et al. Association of *IGF-I* gene polymorphisms with milk yield and body size in Chinese dairy goats. *Genet Mol Biol* 2010; 33 (2): 266-270.
32. Sun W et al. Polymorphism of insulin-like growth factor binding protein-3 gene and its relationship with beef performance of Qinchuan cattle. *Anim Biotech Bull* 2002; 8: 95-99.
33. Shukla A. PCR-RFLP studies on insulin-like growth factor binding protein-3 (*IGFBP-3*) gene in cattle. MSc Thesis, The Indian Veterinary Research Institute (Deemed University), Izatnagar, Bareilly, UP, India, 2001.
34. Kim J et al. Identification of novel SNPs in bovine insulin-like growth factor binding protein-3 (*IGFBP3*) gene. *Asian-Austral J Anim Sci* 2005; 18 (1): 3-7.
35. Kumar P et al. Buffalo insulin-like growth factor binding protein-3 (*IGFBP-3*) gene polymorphism and its comparison with cattle. *Buffalo J* 2004; 20 (2): 183.
36. Li M et al. Genetic Analysis of *IGFBP-3* Gene and Its Association with Economic Traits in Goats. *Chin J Anim Vet Sci* 2008; 12: 003.
37. EL-Hanafy AA, Salem HH. PCR-RFLP of *IGFBP-3* Gene in Some Egyptian Sheep Breeds. *Am-Eurasian J. Agric. & Environ Sci* 2009; 5 (1): 82-85.