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Genetic variability in IGFBP-3 and GH genes and their association with body weight and growth performance at birth, weaning and six-month in sheep

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Abstract

Body weights at birth, weaning and six-month and growth performance in lambs are economic traits that could be strongly used in sheep breeding objectives. Moreover, previous genetic studies recommended strong associations between these economic traits and polymorphisms in several candidate genes in different livestock species. The aim of this investigation was to study the polymorphism in IGFBP-3 and GH genes using DNA sequencing and RFLP, and their association with body weights and growth performance of Rahmani, Barki, Rahmani X Barki cross, Awassi/Awassi X Suffolk cross and Ossimi sheep breeds. Digestion of 654 bp for IGFBP-3/*HaeIII* yielded only one restriction pattern of 8 fragments in all animal groups revealing the absence of polymorphism also, DNA sequencing confirmed that. While, sequencing analysis of the amplified fragment of GH gene revealed polymorphism in nucleotide sequencing, and the amino acid sequences for GH gene of Rahmani were different from those of other breeds in 4 amino acids, while, each of Rahmani X Barki cross and Ossimi amino acid sequences were different in 3 amino acids. The genetic differences in GH gene coincided with differences in birth, weaning and six-month weights of the breeds under study. Thus, the current results suggest that body weights and growth performance of tested sheep breeds at different stages of growth are statistically affected by GH gene which could be considered as a candidate gene for growth in breeding programs.

Keywords: DNA sequencing; IGFBP-3; GH; RFLP; Sheep

Abbreviations: IGFBP-3, Insulin-like Growth Factor Binding Protein-3 gene; GH, Growth hormone gene; RFLP, Restriction fragment length polymorphism; PCR, polymerase chain reaction; IGF, insulin-like growth factor; ADG, average daily gain; MAS, marker-assisted selection.

1. Introduction

Most of the local breeds of farm animals, especially in developing countries, including sheep, suffer from the lack of genetic improvement programmes based on genetic and genomic analysis (Herd et al., 2003; Saleh et al., 2019; Saleh et al., 2020). Meanwhile, efforts in several countries are being made to intensify production systems, primarily through changing productive and reproductive management regimes and by detection of differentiation or mutations in genes that affect growth traits (Guimarães, 2007; Tester and Langridge, 2010; Lewis, 2018; Wall et al., 2018; Saleh et al., 2019; Saleh et al., 2020). Determination and characterization of the genetic differences between and within sheep breeds will help the rapid improvement of their economically important traits (Saleh, 2019a).

Moreover, to realize integrated productivity by the existing animal breeding systems, changes occurred in the growth performance of animals need to be interpreted in light of the correlated alterations in any other performance traits (Herd et al., 2003; Rachid et al., 2019). This could be achieved by utilization of appropriate production indices that incorporate precise measures of all performance traits supported by a chart for the genes controlling their behaviour (Newman, 1994). Additionally, growth traits including; birth, weaning, and six-month weights are extremely important in sheep husbandry (Rasouli et al., 2017). Consumer preference for heavier sheep with less fat has created considerable change within sheep breeding to increase weight and body size. The individuals (lambs) that grow rapidly reach market weight at a younger age, which generally means that those lambs require a limited feeding period and have a low-risk rate of death loss. With the development of biotechnology molecular biology, a more efficient and accurate selection goal could be achieved using molecular tools to explore more genes related to the behaviour of growth traits (An et al., 2011; Saleh et al., 2019).

Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) and Growth Hormone (GH) genes were recently discovered to be involved in the regulation of growth traits including growth rates from birth to weaning and six-month in sheep (Hanrahan et al., 2004; Saleh, 2016; Juengel, 2018; Mazerbourg and Monget, 2018; Saleh et al., 2019). IGFBP-3 and GH genes play a key role in mammalian development, growth control, and reproduction activity as well (Currie et al., 1996; Li et al., 2008; An et al., 2011; Saleh, 2016; Rasouli et al., 2017; Saleh et al., 2019).

IGFBP-3 is a structural unique gene responsible for several effects of IGF. It plays a key role in mammalian development, growth, and reproduction (Mazerbourg and Monget, 2018; Saleh, 2019b). Single-nucleotide polymorphism (SNP) of the IGFBP-3 gene has been discovered and described in cattle and buffalo to be associated with some production traits (Maciulla et al., 1997; Saleh, 2017; Saleh, 2019b; Saleh et al., 2019). On the other side, associations have been made between few numbers of the IGFBPs and follicle status (Besnard et al., 1996; Khalid and Haresign, 1996). Moreover, due to the important role of IGFBP-3 in animal development and growth, it is considered as a candidate gene to be utilized as a marker for production and growth traits in livestock species (Ali et al., 2009; Shafey et al., 2014; Saleh et al., 2019).

The protein encoded by GH gene, found in 1920s is a necessary member of the prolactin/somatotropin family of hormones which play a necessary role in physiological processes (Yousefi et al., 2013). GH affects cell proliferation and growth either directly or indirectly through stimulation of insulin-like growth factor (IGF) (Boyd and Bauman, 1989). In most mammals, GH is the product of a single gene and is generally secreted in a pulsatile manner by the pituitary gland. GH is well documented that GH influences several biological activities (Veldhuis et al., 2001) of sheep such as growth (Breier, 1999; Saleh, 2017), lactation (Baldi, 1999), reproduction (BK et al., 1995; Saleh and Zhao, 2020), metabolism (Bauman, 1999), and pregnancy (Gluckman et al., 1979). GH gene is located on chromosome 17 at the growth hormone locus. Insertion and deletions (*Indels*) or mutations in GH gene lead to differences in growth performance (Pereira et al., 2005). GH gene is a candidate for controlling growth in sheep since it plays a crucial role in development and growth regulation (Boyd and Bauman, 1989). A few genetic investigations on the growth of sheep breeds have concentrated on birth, weaning and yearling weights (Bathaei and Leroy, 1998; Saleh, 2017). Pereira et al., (2005) found a significant effect for the bovine GH genotype on yearling weight. Yousefi et al., (2013) observed a positive correlation between GH and average daily gain (ADG) from weaning to yearling in sheep and used the gene as a candidate for marker-assisted selection (MAS) in different livestock species.

According to several investigations the polymorphism in IGFBP-3 and GH effect on the mammalian growth control, development, and reproduction activity (Currie et al., 1996; Li et al., 2008; An et al., 2011; Saleh, 2016; Rasouli et al., 2017; Saleh et al., 2019; Saleh and Zhao, 2020), thus the aims of this investigation were to detect the genetic polymorphism in IGFBP-3 and GH genes by Restriction fragment length polymorphism (RFLP) and perform DNA sequencing and relating that to the diversity among some Egyptian sheep breeds for birth, weaning and six-month weights and growth performance.

2. Materials and methods

2.1. Animals, sampling and body weights (growth performance)

The blood samples were obtained from five sheep breeds viz; Rahmani (n= 45; 20♂, 25♀), Barki (n= 45; 19♂, 26♀), Rahmani X Barki cross (n= 123; 54♂, 69♀), Awassi/Awassi X Suffolk cross (n= 38; 17♂, 21♀), and Ossimi (n= 35; 16♂, 19♀). The studied sheep breeds were originally from three geographical regions in Egypt as follows; Alexandria city (GPS: 31.206208, 29.919704), Sakha (GPS: 31.087032, 30.948859), and Matrouh governorate (GPS: 31.336924, 27.205762), but raised in north Egypt, Alexandria city "experimental station" (GPS: 31.206208, 29.919704) which they were kept under a semi-intensive system. Additionally, data on the body weight of both sheep sexes were collected concurrently at different three stages of age; at birth, weaning and six-month.

2.2. DNA isolation and amplification

Genomic DNAs were extracted from sheep blood samples with QIAGEN kit (Hilden, Germany). The isolated DNA was separated using Gel electrophoresis system (Biometra, USA) on 0.8-1.2% agarose (B.Shop, Germany) in 0.5 X TBE buffer (Sambrook and Fritsch, 1997) and about 0.5 µg/ml ethidium bromide "C₂₁H₂₀BrN₃" (Sigma, Germany). The electrophoresis run was

performed utilizing Gel electrophoresis system and visualized using the Gel documentation system (Alpha-chem. Imager, USA).

The specificities of the PCR primers targeting IGFBP-3 and GH genes were previously tested by [Kumar et al., \(2002\)](#) and [Yousefi et al., \(2013\)](#) respectively ([Table 1](#)). The amplification was performed using (S. Green Supermix, Germany), ten p.mol of each primer and 80-90 ng of genomic DNA processed under the amplification conditions as shown in ([Table 2](#)). The amplification was carried out utilizing a Thermo-cycler (Gene Amp 6700, Bio-system, USA).

Table 1. The listing primer and sequence (5'→3') of IGFBP-3, and GH genes.

Locus	Method	Primer sequence (5'→3')
IGFBP-3	RFLP & DNA Sequencing	F: 5'- CCAAGCGTGAGACAGAATAC -3` R: 5'-AGGAGGGATAGGAGCAAGAT-3`
GH	DNA Sequencing	F: 5'-GAAACCTCCTTCCTCGCCC-3` R: 5'-CCAGGGTCTAGGAAGGCACA-3`

Table 2. Cycles conditions of PCR

Gene	Denaturation		Annealing		Extension		Final extension		Number of cycles
	°C	Sec	°C	Sec	°C	Sec	°C	Sec	N
IGFBP-3	94	300	54	30	72	60	72	300	30
	°C	Sec	°C	Sec	°C	Sec	°C	Sec	N
GH	94	300	56/58	30	72	45	72	120	35

2.3. Restriction Fragment Length Polymorphism (RFLP)

The RFLP method was utilized to detect the differences in genotypes among the studied sheep breeds, using the PCR of the IGFBP-3 gene. The IGFBP-3 gene products from PCR were digested with the *HaeIII* restriction enzyme (Bio-search Technologies, USA). The PCR-RFLP reaction volume was 25 µl, consisted of 2 µl 10X digestion buffer, 1 µl restriction enzyme, 12 µl H₂O, in addition to 10 µl PCR product. All reactions were incubated at 37°C with *HaeIII* for 16 h. 20 µl of each reaction were separated using the Gel electrophoresis system on 2.5-3 % agarose gel and visualized by gel documentation system. Genotypes were detected by inspecting RFLP patterns.

2.4. Nucleotide sequence analysis

The DNA sequence analysis was achieved on both strands for GH and IGFBP-3 genes by the lab service of Functional Foods & Nutrition (Alexandria, Egypt) with an (ABI Prism 3100

apparatus, USA). Database similarity discoveries were carried out with the blast network service /BLAST at (NCBI) (<http://www.ncbi.nlm.nih.gov>). The sequences were analysed utilizing Finch T.V 1.01, Blast 2.0, and MEGA 6 V.4 software to detect SNPs among the different sequences. The sequences of GH gene for studied animals were deposited in GenBank under Accession Numbers; KP893631.1, KP893632.1, KP893634.1, KP893633.1, and KP893635.1 for Rahmani, Barki, Rahmani X Barki cross, Awassi/Awassi X Suffolk cross, and Ossimi, respectively, as for IGFBP-3, the sequence was deposited in GenBank under Accession Number; (Accession no. MG738671.1) for studied breeds. Analysis of translated protein of GH gene sequences for tested sheep breeds was generated using ExPASy program (<http://web.expasy.org/translate>).

2.5. Statistical Analysis

Data of birth, weaning and six-month weights of the breeds under study were collected and investigated for normality by the Shapiro-Wilk test (SAS, 2009), and results refereed that all data were distributed normally [Shapiro-Wilk test (W) ≥ 0.90]. Also, the GLM procedure of SAS was used to determine the effects of breed on weights according to the following model:

$$Y_{ij} = \mu + B_i + e_{ij}$$

Where: Y_{ij} = animal weight trait, μ = the overall mean, B_i = the fixed effect of i^{th} breed, and e_{ij} = the residual error. Significant differences between means within each weight were tested using least significant difference ($LSD_{0.05}$).

3. Results and Discussion

3.1. Amplification, purification, manipulation, and digestion

This investigation concerns mainly the polymorphism among tested sheep groups for IGFBP-3 and GH genes. It also spotlights the relationship between differentiation of these genes and their association with weights at birth, weaning and six-month. PCR amplification for the tested sheep breeds produced an amplified 654 bp fragment for IGFBP-3 gene (Fig. 1a), and 365 bp for GH gene (Fig. 1b).

3.2. Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) gene

The PCR products of IGFBP-3 gene obtained from experimental sheep were digested with *HaeIII*. Digestion profile revealed one pattern of eight DNA fragments sized 201, 201, 87, 67, 57, 18, 16 and 7 bp for all sheep (Fig. 2a). These results mean that; no polymorphism has been detected within IGFBP-3 gene among the studied sheep breeds. Also, the nucleotide sequencing of the amplified fragment of IGFBP-3 gene of studied sheep breeds with NCBI GenBank (Accession no. MG738671.1) confirmed the same result (Fig. 2b).

Fig. 1. (a) I- Purified PCR product of IGFBP-3 gene from Rahmani (R), Barki (B), Ossimi (O), Rahmani X Barki cross (C), and Awassi (A)/Awassi X Suffolk cross (SC), M; 100 bp DNA ladder and II- Purification of IGFBP-3 gene (654bp) from Awassi X Suffolk cross (SC), M; DNA ladder with 100 bp. (b) PCR amplification of GH gene from genomic DNA of Rahmani (R), Barki (B), Ossimi (O), Awassi (A), Rahmani X Barki cross (C), and Awassi X Suffolk cross (SC), M; 100 bp DNA ladder.

CCAAGCGTGAGACAGAATCCGTAAGAGCTTTTCCTCTTGTTGTTGTGGGGGTGG^{GG^}
^{CC}CACCTGGGCTGGGTATCCAGAGATCACAGGGTCACCATGACTCAAGAGCCCAG
CAGTTACTCCAAACCTGGTGCTGATGCACCAAGCAGCTGTGAGCCCCCTCCTACAGA
AGGGATATTGACCCTCCCCTGTGGCAGAGATCCCTTGAGAATCAGGGTACTGCTCT
TCAGACCTCTGCTGGGCAGAGCAGTGTTCTCCCAAAGCT^{GG^}CCTCTTTTTGTTTAC
TT^{GG^}CCTCTGAGT^{GG^}CCT^{GGCCT}TGTGTGCCCTGTCCCAGTTCTATGGCTTGCCCT
GGGGAATCATGAGAGAGACAGGGGGCTGTGGCTGGCATCTGCATGGGAACGGTGA
CAACTAAATCAGACAAAAGATACTCGAGGAGCACATGGTCAGTTCCCTGGGTGTTA
TGGGGTTTTATCAAACACAGAGTTCCCAGGTAACCCGTCCCTCCTCCCCGGG^{GG^CC}
AGGTTGCCGGGAAATGGAGGACACACTGAAC^{GG^}CCTCAAGTTCCTGAACACACTC
AGCCCCAG^{GG^CC}ATCCACATTCCCAACTGTGACAAGAAGGGCTTACTCGAGGAGG
CACCTAACACGACCTCATCAATTCTCGCTCCTATCGG

Fig. 2. (a) The PCR products of the IGFBP-3 gene from genomic DNA of tested sheep breeds digested by *HaeIII*; Rahmani (R), Barki (B), Ossimi (O), Rahmani X Barki cross (C) and Awassi (A)/Awassi X Suffolk cross (SC). 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 for IGFBP-3 gene. The restriction fragments with sizes; 18, 16 and 7 bp were not seen on the gel. **(b)** A 654 bp sequence of IGFBP-3 gene of studied sheep breeds (NCBI accession no. MG738671.1).

On the other side, genetic differentiation of this gene in livestock species including sheep was under investigation (Liu et al., 2012; Saleh et al., 2019; Sankhyan et al., 2019; Sarmah et al., 2019; Splaine et al., 2019). Li et al., (2008) reported three genotypes of IGFBP-3 among seven populations of goats with significantly high association with weaning weight, body length, rib eye area, heart girth, and body length. Also, Choudhary, (2004) identified three genotypes of IGFBP-3 in exotic Holstein Friesian and Jersey cattle. The polymorphism with respect to this gene in cattle was due to C/A (GGCC to GGAC) transition in intron number 2 of this gene at 299 position bases at the sequence, which alters the *HaeIII* restriction site. (Saleh et al., 2019) confirmed that the digestion of IGFBP-3/*HaeIII* yielded three genotypes for cattle. Sharma et al., (2014) reported that 6 SNPs out of 8 mutations in Indian goats were found to be synonymous in IGFBP-3 but were found to be nonsynonymous in nine goat breeds. This was also in accordance with the findings reported on Chinese goat (Lan et al., 2007a), which indicate that caprine IGFBP-3 gene may have more functionality. Amino acid sequence variation in caprine IGFBP-3 gene may also be functional, so that affected the expression of the enzyme activity of the gene (Sharma et al., 2014).

In the current study, there was no polymorphism found among studied sheep breeds for IGFBP-3 gene. This agrees with the previous study (Saleh et al., 2019) that the digestion of IGFBP-3 with *HaeIII* yielded one RFLP pattern for goats, and for buffalo, one genotype (AA) was found when *HaeIII* and *TaqI* restriction enzymes were used separately, while three genotypes were found for cattle. Also, the current results agree with those of Ali et al., (2009) who reported that the RFLP pattern for IGFBP-3/*HaeIII* obtained for some sheep breeds yielded one pattern of five fragments sized 201, 201, 87, 67, and 57 bp. Also, with Kumar et al., (2006) who studied the genetic diversity among Indian sheep breeds, Mandya, Marwari, Madras, Banur, and Red Muzaffarnagari for the sequence of IGFBP-3 gene digested by *HaeIII* and reported that the digestion profile revealed only one pattern with 8 DNA fragments for all sheep breeds and no polymorphism was detected. Also, these results were in accordance with those reported on six breeds of buffalo. Nevertheless, the fragments sizes were various which indicated a lack of polymorphism detected for the six buffalo breeds with respect to IGFBP-3 gene.

In contrary, Rasouli et al., (2017) discovered that three genotypes (CC, TC, and TT) and a mutation of IGFBP-3 gene "Exon 2/ 316 bp" at position 58, besides three genotypes for IGF-I gene "5' Flanking region/ 249bp" (GG, GA, and AA) and a mutation at position 1617. Their discoveries indicated that different genotypes of these genes had an effect on birth, and six-month weights, but the interactions among different genotypes of both genes were significant for weaning weight, and ADG from birth to weaning. Also, Li et al., (2008) investigated 7 different populations of goats by PCR-RFLP. They found three genotypes (AA, AG, and GG) in the IGFBP-3 gene. The correlation between different genotypes and weaning weight, 10th month body length, rib eye area, 10th heart girth, and 12th month body length were significant ($P<0.05$) and the association was significant for 3rd heart girth and body length at 3rd month ($P<0.01$). The genetic effect of AG genotype was significantly higher than GG genotype for weaning weight and rib-eye area ($P<0.05$) whilst, the above five physical measure traits were ($P<0.01$). Additionally, according to Lan et al., (2007b) there are associations between mutations in IGFBP-3 and weight traits at birth, 6 months, and 12 months and twinning rate in Inner Mongolia White

Cashmere, Xinong Sannen, Laoshan, Guanzhong, Guizhou White, Shaanan White, and Leizhou breeds. They found two SNPs; A>G (position: 78 of intron 2), and G>A (position: 217 of intron 2) of IGFBP-3 gene are possibly associated with production traits ($P>0.05$). Also, two mutations in this gene revealed a significant association with twinning rate ($P<0.05$); C>T (position: 58 of exon 2/ Proline to Serine), and C>G (position: 67 of exon 2 / Arginine to Glutamic).

Worth mentioning, in a study to detect the novel SNPs of IGFBP-3 and their associations with litter size (LS) and weight traits in goat, the genotype was significantly associated with LS ($P<0.05$). However, no significant correlation of SNP with weight traits were detected at ($P>0.05$). While, two mutations in IGFBP3 showed a significant association with fertility traits (Lan et al., 2007a). Association investigations are, thence, warranted to evaluate the necessary role of these SNPs in production traits for sheep populations (Choudhary, 2004).

3.3. Growth hormone (GH) gene

3.3.1. Nucleotide sequence and amino acid sequence comparisons for GH gene

Nucleotide sequencing of the amplified fragment of GH gene of Rahmani, Barki, Rahmani X Barki cross, Awassi/Awassi X Suffolk cross, and Ossimi were submitted to the GenBank "NCBI" under accession numbers: KP893631.1, KP893632.1, KP893634.1, KP893633.1 and KP893635.1, respectively (Fig. 3). Sequencing of the amplified GH gene fragments of the tested sheep breeds was generated by ExPASy program and the nucleotide analysis and percent distances of GH gene fragments for sheep breeds (Fig.4 and Table 3) along with comparisons of amino acids were generated by MEGA 6 V.4 (<http://en.bio-soft.net/tree/MEGA.html>) (Fig. 5).

Table 3. Nucleotide sequence distances, percent similarity (above diagonal), percent distance (below diagonal); of GH gene of tested sheep breeds.

Breeds	Rahmani	Barki	RXB*	Awassi/SC**	Ossimi
Rahmani	-	0.98	0.98	0.98	0.99
Barki	0.0341	-	0.99	0.98	0.97
RXB*	0.0468	0.0149	-	0.97	0.97
Awassi/SC**	0.0307	0.0211	0.0303	-	0.97
Ossimi	0.0339	0.0398	0.0367	0.0465	-

*RXB; Rahmani X Barki cross, **SC; Awassi X Suffolk cross

A	1	gggtggaggc	cgttgggtgg	cactagaagg	cgcagctggc	ctccccgaag	cggcgacact
	61	tcattgacct	caggtacgtc	tccgtcttgt	gcaggtcctt	ccggaagcag	gagagcagac
	121	cgtagtctct	gagcagcgca	tcatcactgc	gcatgtttgt	gtcaaatttg	tcattaggtct
	181	gcttgaggat	ctgcccagcc	cggggggtaa	catcttccag	ctcctgcca	gggagggaga
	241	aacagaaagg	ccgaagggcc	ctgaggagcg	gctccctcct	gcccgtcca	ttttccacc
	301	tcccctatag	gcttggaata	gggcgaggaa	ggaggtttca		
B	1	tagggggagg	gagaaggctg	gaactaaaag	gcgcagctgg	cctccccgaa	gcggcgacac
	61	ttcatgacct	tcaggtacgt	ctccgtcttg	tgcaggtcct	tccggaagca	ggagagcaga
	121	ccgtagtctt	tgagcagcgc	atcatcactg	cgcaggtttg	tgtcaaattt	gtcataggtc
	181	tgetttagga	tctgcccage	ccggggggta	acatcttcca	gctcctgcca	agggagggag
	241	agacagaaag	gccaaaggcc	cctgaggagc	ggctccctcc	tgcccgtccc	attttccacc
	301	ctcccctata	ggcttgagaa	agggcgaggaa	aggaggtttc	a	
C	1	agggggggaa	gaaggctgga	actaaaaggc	gcagctggcc	tccccgaagc	ggcgacactt
	61	catgaccttc	aggtacgtct	ccgtcttgtg	caggtccttc	cggaagcagg	agagcaaacc
	121	gtagtctctt	agcagcgcat	catcactgcg	catgtttgtg	tcaaatttgt	cataggtctg
	181	cttgaggatc	tgcccagccc	ggggggtaac	atcttccagc	tctgccaag	ggagggaaag
	241	acagaaaggc	caaaggcccc	tgaggagcgg	ctccctcctg	cccgctccat	tttccaccct
	301	cccctatagg	cttgagaaag	ggcgaggaaag	gaggtttcaa	tggcgaggaa	ggatgtttca
D	1	agggggggag	gggagtaggc	tggcactaga	aggcgagct	ggcctccccg	aagcggcgac
	61	acttcatgac	cctcaggtac	gtctccgtct	tgtgcaggtc	cttccggaag	caggagagca
	121	gaccgtagtt	cttgagcagc	gcacatcac	tgcgcaggtt	tgtgtcaaat	ttgtcatagg
	181	tctgcttgag	gatctgccc	gcccgggggg	taacatcttc	cagctcctgc	caaggaggag
	241	agagacagag	aggccgaagg	gccctgagga	gcggctccct	cctgcccgtc	ccattttcca
	301	ccctccccta	taggcttgga	gaaggggcag	gaaggaggtt	tca	
E	1	tagggggaga	ggataggtgg	gactaaaagg	ggcagctggc	ctccccgaag	cggcgacact
	61	tcattgacct	caggtacgtc	tccgtcttgt	gcaggtcctt	ccggaagcag	gagagcagac
	121	cgtagtctct	gagcagcgca	tcatcactgc	gcatgtttgt	gtcaaatttg	tcattaggtct
	181	gcttgaggat	ctgcccagcc	cggggggtaa	catcttccag	ctcctgcca	gggagggaga
	241	aacagaaagg	ccgaagggcc	ctgaggagcg	gctccctcct	gcccgtcca	ttttccacc
	301	tcccctatag	gcttggaata	gggcgaggaa	ggaggtttca	a	

Fig. 3. (A) A 365 bp sequence of GH gene of Rahmani breed (NCBI accession no. KP893631.1), (B) A 365 bp sequence of GH gene of Barki breed (NCBI accession no. KP893632.1), (C) A 365bp sequence of GH gene of Rahmani X Barki crossbreed (NCBI accession no. KP893634.1), (D) A 365 bp sequence of GH gene of Awassi/Awassi X Suffolk cross (NCBI accession no. KP893633.1), (E) A 365 bp sequence of GH gene of Ossimi breed (NCBI accession no. KP893635.1).

Fig. 4. Nucleotide sequence comparison of amplified GH gene of tested sheep breeds.

Fig. 5. Comparative analysis of amino acid sequences of GH gene of tested sheep breeds.

The protein sequence of Rahmani breed was different from that of other breeds in four amino acids are Tryptophan, Leucine, Glycine and Arginine instead of Glycine, Lysine, Alanine and Lysine in other breeds, while in Rahmani X Barki cross the protein sequence was different from that of other breeds in three amino acids; Asparagine, Lysine and Lysine, in place of Aspartic, Arginine and Glutamic. Also, Ossimi breed protein sequence was different from that of other breeds sequence in three amino acids are (Arginine), (Glycine), and (Glycine) instead of (Glutamic or Lysine or Proline), (Alanine), and (Arginin). Hence, there was approximately (96.25 %) similarity in the amino sequences among tested sheep breed (Table 4).

Table 4. Dissimilarity between amino acids in protein sequences in tested sheep breeds.

No.	1	2	3	4
1- Rahmani	(W) Tryptophan	(L) Leucine	(G) Glycine	(R) Arginine
O*	(G) Glycine	(K) Lysine	A Alanine	(K) Lysine
2- Barki	(K) Lysine			
O*	(E) Glutamic			
3- RXB**	(N) Asparagine	(K) Lysine	(K) Lysine	
O*	(D) Aspartic	(R) Arginine	(E) Glutamic	
4- Awassi/SC***	(R) Arginine	(E) Glutamic		
O*	(K) Lysine	(K) Lysine		
5- Ossimi	(R) Arginine	(G) Glycine	(G) Glycine	
O*	(E, K, P) Glutamic, Lysine, Proline	(A) Alanine	(R) Arginine	

* **O** = Other studied sheep breeds, ****RXB** = Rahmani X Barki cross, *****SC** = Awassi X Suffolk cross

Growth performance, body weight and body size are important indicators of body growth and some other breeding traits as well (Okoro et al., 2010). Mahrous et al., (2018) studied the polymorphism of GH gene for the three loci; GH-1, -2, and -6 in three goat breeds; Damascus, Barki, and Zaraibi, utilizing RFLP and DNA analyses and confirmed the absence AA of genotype, which all animals had genotypes BB or AB. Worth mentioning, An et al., (2011) when investigated the polymorphisms of GH gene in Boer (BG) and Xinong Saanen (SG) goats found two alleles (A and B) and three genotypes (AA, AB and BB) using SSCP. In addition, a SNP at A73C, was discovered by DNA sequencing. Polymorphisms of this gene was shown to be

associated with growth traits in BG breed and AA genotype was associated with superior growth of animals up to 3 months of age.

3.4. The weight at birth, weaning and six-month

Table 5 shows birth, weaning, and six months weights for Barki, Rahmani, Rahmani X Barki crosses, Awassi/Suffolk crosses and Ossimi. Birth weight of Rahmani X Barki crossbred lambs was the highest ($P < 0.05$) followed by Barki which was higher ($P < 0.05$) than Rahmani, Awassi/ Suffolk crosses and Ossimi lambs. However, Rahmani is known to have the highest twinning rates (Saleh et al., 2020) and, therefore, the highest litter birth weight among all breeds (Marai et al., 2009). Awassi and crossbred lambs growth from birth to weaning were the highest which caused their weaning weights to be significantly higher than that of other breeds. Also, Rahmani lambs achieved relatively high growth rate at the same period which permitted them to catch up with Barki lambs, therefore no significant differences were found between the two breeds but both had higher weaning weights ($P < 0.05$) than Ossimi lambs. At six months of age Awassi/ Suffolk crosses lambs scored the highest weight ($P < 0.05$) indicating good breed mothering ability from birth to weaning and better-growing ability thereafter. Rahmani X Barki cross maintained high weight superiority throughout the period from birth to six months of age above their purebred parents and Ossimi lambs. Rahmani and Barki lambs went side by side for weaning and six-month weights, regardless of the lower birth weight of Rahmani, and both breeds had heavier six-month weight than Ossimi.

Table 5. Mean, standard deviation (SD), coefficient of variation (CV) and range for birth, weaning and six-month weights of sheep breeds under study.

Breeds	Weights (kg)								
	Birth			Weaning			Six month		
	Mean	S.D	C.V	Mean	S.D	C.V	Mean	S.D	C.V
Barki	4.10 ^b	0.38	9.32	19.90 ^b	2.57	12.92	24.05 ^c	3.52	14.65
Rahmani	3.77 ^c	0.48	12.66	19.89 ^b	2.71	13.62	24.00 ^c	3.58	14.93
RXB*	4.40 ^a	0.53	12.06	22.17 ^a	3.16	14.25	27.30 ^b	3.71	13.58
Awassi/SC**	3.50 ^c	0.25	7.15	23.23 ^a	1.74	7.51	31.64 ^a	2.19	6.92
Ossimi	3.69 ^c	0.20	5.45	17.00 ^c	0.96	5.65	21.70 ^d	1.29	5.97
Overall	-	0.41	10.40	-	2.5	11.96	-	3.25	12.16

RXB** = Rahmani X Barki cross, *SC** = Awassi X Suffolk cross, ^{a-c} Means with different letters in the same column within the same factor are significantly different.

3.5. The relationship between growth performance and the prevalence of GH gene genotypes

In the current study, the differentiated amino acid sequences of GH in different studied sheep breeds were accompanied with variation in body weights and growth performance which may suggest a direct influence of GH protein sequence on the growth pattern of sheep. Additionally, [Yousefi et al., \(2013\)](#) reported that; o^{GH} gene has been used as a candidate gene for growth rate in many livestock species.

This is in agreement with [Malewa et al., \(2014\)](#) who reported apparent effects of the genotypic polymorphism for GH gene on growth rate and weaning weight, also, was in agreement with finds of [Kumari et al., \(2014\)](#) who investigated genetic polymorphisms of GH gene at loci A1575G and A781G in nine sheep breeds from different regions in India. Their results revealed that; genetic variation exists at A781G locus which paved a lane for investigation of QTL for growth traits in the tested breeds. Also, in the study of [Gorlov et al., \(2017\)](#), it was confirmed that polymorphism in GH gene was associated with growth traits in Salsk sheep "Russian Federation" and they discovered three genotypes AA, AB and BB, the AB was found to have great effects on weaning and 9 months, body weights, ADG, and slaughter, and carcass weights. [Hua et al., \(2009\)](#) confirmed that the polymorphism of GH gene was as a candidate gene for several growth traits in Boer goat bucks, where two SNPs were discovered for GH gene; A1575G " Leucine¹⁴⁷ " with two genotypes (CC, and CD), and A781G " Serine/Glycine³⁵ " with other two genotypes (AA, and AB). AB genotype resulted in a significant increase in weaning weight ($P=0.014$) and chest girth at birth ($P=0.03$) comparing to AA genotype, while CD genotype contributed to weaning height ($P=0.04$) less than CC genotype. On the contrary, according to a genetic study on GH gene of Zel sheep breed, no significant effect ($P>0.05$) between yearling weights of different GH genotypes was found (Yousefi and Azari, 2012).

The variation between breeds with respect to body weights and growth rates at different stages of maturity under prevalence of different GH genotyping may reflect a relatedness between these differentiation and growth performance of sheep. This should strongly recommend the utilization of sheep GH gene in the primary steps of MAS program with deeper studies to follow, especially for IGFBP-3 where these genes are considered as candidates for controlling growth in sheep, also, they play a necessary role in the regulation of growth and development ([Baldi, 1999](#); [Saleh et al., 2019](#)).

4. Conclusions

Improving sheep production is strongly based on animal genetics as most of the quantitative economic traits have polygenetic nature. Molecular tools provide valuable knowledge that could contribute to enriching the information about genes underlying economical traits. In view of the lack of enough investigations about the genetic polymorphisms of production genes in Egyptian sheep breeds, the aims of this work were to study the differentiation "polymorphisms" in IGFBP-3 and GH genes and their association with several growth traits. The important role of IGFBP-3 and GH genes in growth performance is well recognized, then their polymorphisms and interaction with the growth trait should be the subject of further research. According to the current

study, the probable presence of an association between differentiation in genotypes of GH gene and growth performance also, weight at birth, weaning and six-month could be candidate markers in sheep breeding program for improvement of growth rates.

Declarations

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Ethical approval

The management of the experimental animals was in agreement with the welfare guidelines approved by the Animal and Fish Production Department, College of Agriculture, Alexandria University, Egypt (No. AFP7-AB2-1016). Also, all procedures and experimental protocols were in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, Federation of Animal Science Societies (FASS, 2010) https://www.aaalac.org/about/Ag_Guide_3rd_ed.pdf.

Authors' contributions

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

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Availability of data and material

All data generated or analyzed during this study are included in this manuscript

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Figures

Figure 1

(a) I- Purified PCR product of IGFBP-3 gene from Rahmani (R), Barki (B), Ossimi (O), Rahmani X Barki cross (C), and Awassi (A)/Awassi X Suffolk cross (SC), M; 100 bp DNA ladder and II- Purification of IGFBP-3 gene (654bp) from Awassi X Suffolk cross (SC), M; DNA ladder with 100 bp. (b) PCR amplification of

GH gene from genomic DNA of Rahmani (R), Barki (B), Ossimi (O), Awassi (A), Rahmani X Barki cross (C), and Awassi X Suffolk cross (SC), M;100 bp DNA ladder.

Figure 2

(a) The PCR products of the IGFBP-3 gene from genomic DNA of tested sheep breeds digested by HaeIII; Rahmani (R), Barki (B), Ossimi (O), Rahmani X Barki cross (C) and Awassi (A)/Awassi X Suffolk cross (SC). 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 for IGFBP-3 gene. The restriction fragments with sizes; 18, 16 and 7 bp were not seen on the gel. (b) A 654 bp sequence of IGFBP-3 gene of studied sheep breeds (NCBI accession no. MG738671.1).

A

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1  ggggtggaggc  cgttgggtgg  cactagaagg  cgcagctggc  ctccccgaag  cggcgacact
61  tcatgaccct  caggtacgtc  tccgtcttgt  gcaggtcctt  ccggaagcag  gagagcagac
121 cgtagttctt  gagcagcgca  tcatcactgc  gcatgtttgt  gtcaaatttg  tcataggtct
181 gcttgaggat  ctgccagcc  cggggggtaa  catcttccag  ctctgcca  gggagggaga
241 aacagaaagg  ccgaagggcc  ctgaggagcg  gctccctcct  gcccgctcca  ttttccaccc
301 tcccctatag  gcttggaana  gggcgaggaa  ggaggtttca

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B

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1  tagggggagg  gagaaggctg  gaactaaaag  ggcagctgg  cctccccgaa  gcggcgacac
61  ttcattgacc  tcaggtacgt  ctccgtcttg  tgcaggtcct  tccggaagca  ggagagcaga
121 ccgtagttct  tgagcagcgc  atcatcactg  cgcattgttg  tgtcaaattt  gtcattaggtc
181 tgcttgagg  tctgccagc  ccggggggta  acatcttcca  gctctgcca  agggagggag
241 agacagaaag  gccaaaggcc  cctgaggagc  ggctccctcc  tgcccgctcc  attttccacc
301 ctcccctata  ggcttgagga  agggcgagga  aggaggtttc  a

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C

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1  agggggggaa  gaaggctgga  actaaaaggc  gcagctggcc  tccccgaagc  ggcgacactt
61  catgaccctc  aggtacgtct  ccgtcttgtg  caggtccttc  cggaagcagg  agagcaaac
121 gtagttcttg  agcagcgcat  catcactgcg  catgtttgtg  tcaaattttg  cataggtctg
181 cttgaggatc  tgcccagccc  ggggggtaac  atcttccagc  tctgccaag  ggagggaaag
241 acagaaaggc  caaagggcc  tgaggagcgg  ctccctcctg  cccgctccat  tttccaccct
301 cccctatagg  cttggagaag  ggcgaggaa  gaggtttcaa  tggcgaggaa  ggatgtttca
361 tttttgttc

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D

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1  gaggggggag  gggagtaggc  tggcactaga  aggcgcagct  ggctccccg  aagcggcgac
61  acttcatgac  cctcaggtac  gtctccgtct  tgtgcaggtc  ctccggaag  caggagagca
121 gaccgtagtt  cttgagcagc  gcatcatcac  tgcgcattgt  tgtgtcaaat  ttgtcatagg
181 tctgcttgag  gatctgcca  gccgggggg  taacatcttc  cagctcctgc  caaggagggg
241 agagacagag  aggcgaagg  gccctgagga  gcggctccct  cctgcccgtc  ccattttcca
301 ccctccccta  taggcttgg  gaaggggcag  gaaggaggtt  tca

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E

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1  taggggggaga  ggataggtgg  gactaaaagg  ggcagctggc  ctccccgaag  cggcgacact
61  tcatgaccct  caggtacgtc  tccgtcttgt  gcaggtcctt  ccggaagcag  gagagcagac
121 cgtagttctt  gagcagcgca  tcatcactgc  gcatgtttgt  gtcaaatttg  tcataggtct
181 gcttgaggat  ctgccagcc  cggggggtaa  catcttccag  ctctgcca  gggagggaga
241 aacagaaagg  ccgaagggcc  ctgaggagcg  gctccctcct  gcccgctcca  ttttccaccc
301 tcccctatag  gcttggaana  gggcgaggaa  ggaggtttca  a

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Figure 3

(A) A 365 bp sequence of GH gene of Rahmani breed (NCBI accession no. KP893631.1), (B) A 365 bp sequence of GH gene of Barki breed (NCBI accession no. KP893632.1), (C) A 365bp sequence of GH gene of Rahmani X Barki crossbreed (NCBI accession no. KP893634.1), (D) A 365 bp sequence of GH gene of Awassi/Awassi X Suffolk cross (NCBI accession no. KP893633.1), (E) A 365 bp sequence of GH gene of Ossimi breed (NCBI accession no. KP893635.1).

Figure 4

Nucleotide sequence comparison of amplified GH gene of tested sheep breeds.

Figure 5

Comparative analysis of amino acid sequences of GH gene of tested sheep breeds.