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ASSOCIATION OF GROWTH HORMONE (GH), INSULIN-LIKE GROWTH FACTOR 2 (IGF2) AND PROGESTERONE RECEPTOR (PGR) GENES WITH SOME PRODUCTIVE TRAITS IN GABALI RABBITS

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Abstract: The objectives of the present study were 1) to evaluate the polymorphism of growth hormone (GH), insulin-like growth factor 2 (IGF2) and progesterone receptor (PGR) genes in Sinai Gabali rabbits, and 2) to assess their associations with growth, litter size and milk production traits in Sinai Gabali rabbits. The C>T, A>Del and A>G single nucleotide polymorphisms of GH, IGF2 and PGR genes were genotyped by polymerase chain reaction-restriction fragment length polymorphism using BstUI, HpyF31 and BsaI restriction enzymes, respectively. The C/T genotype of GH gene recorded the heaviest body weights for body weight (BW) at 8 wk (1190.22±19.29 g) and 12 wk of age (1842.46±30.19 g) and recorded the largest litter size at birth (LSB: 7.37±0.12 kits) traits. The Del/Del genotype of IGF2 gene showed the superiority over the other genotypes for BW at 4 wk (507.17±8.87 g), 8 wk (1239.39±14.0 g), and 12 wk of age (1950.15±18.1 g), as well as for daily weight gain from 4 to 8 wk (26.05±0.37 g/d), and from 8 to 12 wk of age (25.48±0.56 g/d) traits. The G/G genotype of the PGR gene showed superiority for LSB (7.51±0.13 kits) and litter size at weaning (6.53±0.14 kits) traits over the other genotypes. Regarding milk yield traits; the C/C, A/A and A/A genotypes of GH, IGF2 and PGR genes yielded more milk compared to the other genotypes. The means of total milk yield in 28 d for these genotypes were 2936±29 g, 2921±43 g and 2930±35 g, respectively. Thus, GH, IGF2 and PGR genes might be useful for marker-assisted selection programmes for improvement of rabbit growth, litter size and milk yield traits.

Key Words: gene polymorphism, growth, litter size, milk yield, PCR, RFLP, rabbits.

INTRODUCTION

Rabbits are an important and economic source of meat due to their prolificacy, fast growth and high fecundity (Cartuche *et al.*, 2014). Gabali rabbits are characterised by high resistance to diseases and high tolerance to harsh climatic conditions in comparison with other exotic breeds (Khalil and Baselga, 2002). Gabali rabbits are medium-sized and used mainly for meat; the animals are yellowish-brown with black hairs spread all over the body with soft fur (Afifi, 2002). There are two distinct populations of Gabali rabbits in Egypt; the first one originated in the western desert of the north Mediterranean coast, while the second originated in the Sinai Peninsula. However, the two Gabali populations are acclimatised to the desert conditions; the Sinai Gabali rabbits in our study are characterised by low lactating abilities, which may be a reason for low litter size and weight at weaning (Khalil, 1999). Nevertheless, some trials were conducted for the improvement of productive traits of Egyptian Gabali rabbits by crossbreeding with Californian rabbits (Gad, 1998), New Zealand White (Khalil and Afifi, 2000) and with V-line rabbits (Iraqi *et al.*, 2010a), but this important breed still needs more genetic improvement, using an effective selection tool such as the marker-assisted selection method.

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Molecular techniques are useful tools that assist and complement traditional breeding programmes in farm animals. The use of these techniques accelerates the genetic progress and improves the accuracy of selection, especially in the case of Sinai Gabali rabbits, which showed moderate genetic diversity based on microsatellite (Fouzia *et al.*, 2017; Badr *et al.*, 2019; Rabie, 2019), single nucleotide polymorphisms (SNPs) (Abd El-Ghany, 2015; El-Aksher *et al.*, 2017; Rabie, 2019) and random amplification of polymorphic DNA (RAPD) (Galal *et al.*, 2013; Badr *et al.*, 2016; Mohamed and Abdelfatah, 2018) molecular markers. The somatotrophic axis growth hormone-insulin-like growth factor (GH-IGF) is a key regulator of postnatal growth and metabolism, affecting the growth processes, lactation, mammary gland development and fertility (Renaville *et al.*, 2002; Lucy, 2008; Bagnicka *et al.*, 2010; Mullen *et al.*, 2011; Abdel-Kafy *et al.*, 2014; Abdel-Kafy *et al.*, 2015). The rabbit GH gene is located on Chromosome 19: 48.725.660-48.727.208 reverse strand (Source: UniProtKB/ Swiss Prot; Acc:P46407). Mammalian growth hormone (GH) and Insulin-like growth factor 2 (IGF2) play an important role in the control of reproduction processes such as ovarian folliculogenesis, ovarian oogenesis and corpus luteum function (Hull and Harvey, 2002; Ola *et al.*, 2008). Therefore, GH and IGF2 were considered as candidate genes for the identification of molecular markers associated with growth, litter size and milk yield traits in livestock animals. Progesterone receptor gene (PGR) codes for a protein, and this protein interacts with the progesterone hormone in the establishment and maintenance of pregnancy (Conneely *et al.,* 2002). The PGR gene is located on chromosome 1: 115,601,359-115,672,617 forward strand (Source: UniProtKB/Swiss Prot; Acc:P06186). Progesterone contributes to the release of mature oocytes, implantation and maintenance of pregnancy by suppression of myometrial contractility and by promoting uterine growth (Peiró *et al.*, 2010). Progesterone plays a vital role in the mammogenesis and lactogenesis process. It increases both the size and number of milk ducts. Progesterone inhibits the prolactin receptors and other such prolactin actions including transcription, and mRNA translation for milk proteins (Husvéth, 2011). Progesterone concentration was found to have reduced postpartum in high yielding dairy cattle and this reduction was attributed to the increased metabolic clearance of progesterone and to the increased energy intake by caloric deficits (Reksen *et al.*, 2002). The promoter region of the PGR gene includes an SNP $G>A_{2464}$ that showed that there were some differences in the early embryo survival and development at 3rd d of gestation between two rabbit lines selected by uterine capacity (Peiró *et al.*, 2008; Argente *et al.*, 2010). Therefore, PGR was considered as a candidate gene for the identification of molecular markers associated with litter size and milk yield traits in livestock animals.

Studies concerning the molecular associations of the candidate genes with the production and reproduction traits in rabbits are scarce. Few studies have investigated the relationship of these genes with growth traits (Fontanesi *et al.*, 2008, 2012a,b; Abdel-Kafy *et al.*, 2014, 2015), milk yield traits (Abdel-Kafy *et al.*, 2014, 2015) and litter size traits (Peiró *et al.*, 2008; Merchán *et al.*, 2009; García *et al.*, 2010; Abdel-Kafy *et al.*, 2015; El-Aksher *et al.*, 2017). Therefore, the objectives of the present study were 1) to evaluate the polymorphism of GH, IGF2, PGR genes in Sinai Gabali rabbits, and 2) to investigate the associations of the previous three genes with growth, litter size and milk yield traits in Sinai Gabali rabbits. To our knowledge, there is as yet no existing study that has evaluated the association between the PGR gene and rabbit milk yield.

MATERIALS AND METHODS

Animals and studied traits

Animals used in the present study belonged to the Egyptian Sinai Gabali rabbit breed. Their founders pertain to the stock of wild rabbits bought from Bedouins living in Northern Sinai in Egypt (Iraqi *et al.*, 2010b). These rabbits were housed in the rabbitry of the experimental farm of the Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. The rabbits were reared in a one-floor farm oriented from east to west and the breeding cages were arranged in flat-deck batteries. The temperature ranged from 15 to 35°C, with a relative humidity from 30 to 70%, and the photoperiod was 16L:8D. Intensive reproduction rhythm was followed (mating 24 h after kindling) using natural mating. At the farm, matings were carried out at random within purebreds but always taking care to avoid full and half sibs as well as parent-offspring matings. The breeding animals consisted of 60 Gabali rabbit does and 20 bucks; each buck was allowed to mate with three does. At the moment of parturition, the doe weight at kindling (DWK) was measured, litters born were examined and the litter size at birth (LSB) was recorded within 12 h after kindling, and the litters were checked every morning to remove the dead kits. Weaning of kits took place on day 28

post-kindling and the litter size at weaning (LSW) was recorded. All animals were kept in the same farm under the same environmental conditions throughout every stage of production. The rabbits were fed *ad libitum* on a pelleted commercial diet containing around 16% crude protein and 14% crude fibre during the growing period from weaning till the starting point of production at 19 wk of age, and a diet containing around 18% crude protein, 12 % crude fibre during production with a digestible energy of 2500 kcal/kg. Milk yield of each doe was recorded eight times during the suckling period, twice within a week, from the 3rd to the 28th d after kindling, using the weight-suckle-weight method described by (McNitt and Lukefahr, 1990). Weekly milk yield was calculated as the mean of milk amount obtained at this week multiplied by seven. Total milk yield during the first three weeks of lactation up to day 21 (MY21d) and from kindling up to the 28th day of lactation (TMY) were calculated. Post-weaning, body weights (BW) were recorded at 4th, 8th and 12th wk from kindling (BW4, BW8 and BW12, respectively). The daily weight gains (DG) were calculated during the intervals from weaning (4 wk) to 8 wk and from 8 to 12 wk of age as the difference of weights divided by 28 (DG4-8 and DG8-12, respectively).

Data on 285 weaned rabbits fathered by 30 bucks and mothered by 73 does were used to study the associations between GH and IGF2 genes and growth traits. Data collected on 218 litters produced from 125 does fathered by 69 bucks and mothered by 110 does were used to study the associations between PGR gene and the litter and milk production traits. Approximately 3-5 mL of venous blood samples were collected randomly from the rabbit's ear vein and transferred into tubes containing ethylenediamine tetraacetic acid as anticoagulant.

DNA extraction and genotyping

DNA was extracted from rabbit blood using Gene JET genomic DNA purification kit following the manufacturer's protocol (Fermentas, #K0721). The concentration and 260/280 ratio of the extracted DNA were measured using the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Average concentrations were 50 ng/µL, whereas a ratio higher than 1.8 was recorded for all the DNA samples. Polymerase chain reaction (PCR) was used to amplify 231 bp of GH (Part of 5'-UTR, exon 1 and part of intron 1), 136 bp of IGF2 (Part of exon 1 and part of intron 1; EMBL accession numbers: HE608866 and HE608867) and 558 bp of PGR (part of promoter region) genes using the previously published primers and annealing temperatures (Fontanesi *et al.*, 2012a; Fontanesi *et al.*, 2012b; Peiro *et al.*, 2008, respectively), as shown in Table 1. PCR reaction was carried out in 25 µL containing 5 µL of the DNA template, 10 pmol of each primer and 12.5 µL of Dream Taq Green PCR master mix (Fermentas, #K1071) and nuclease free water up to 25 µL. The cycling protocol was 95°C for 5 min; 35 cycles at 95°C for 30 s, annealing temperatures were 58, 57 and 66°C for GH, IGF2 and PGR genes respectively for 30 s, 72°C for

Table 1: Primer sequence and position and mutation points for GH, IGF2 and PGR genes on the rabbit (*Oryctolagus cuniculus*) genome.

Amplified gene	Primer sequence	Primer position	Mutation position
GH (231 bp)	F 5'-GTATAGTGGGATGGGGTTGG-3'	5'-UTR between - 138 and -119 bp between 48.727.346 and 48.727.327 of chromosome 19 reverse strand	C>T SNP at -78 bp (at 48.727.286 of chromosome 19 reverse strand)
	R 5'-TTACGCTCCCATTCAGAAGC-3'	Intron one between 64 and 83 bp between 1 48.727.135 and 48.727.116 of reverse strand	
IGF2 (136 bp)	F 5'-GGACACCCTCCAGTTTGTGT-3'	exon one between 114 and 133 bp	A>Del at 61 bp of intron 1
	R 5'-CAGCAGGTGTTCCGCAAG-3'	Intron one between 75 and 92 bp	
PGR (558 bp)	F 5'-GAAGCAGGTCATGTCGATTGGAG-3'	Promotor region between -1020 and -998 bp between 115.600.339 and 115.600.361 of forward strand	A>G SNP at -885 bp (at 115.600.474 of chromosome 1 forward strand)
	R 5'-CGCCTCTGGTGCCAAGTCTC-3'	Promotor region between -482and -463 between 115.600.877 and 115.600.896 of chromosome 1 forward strand	

GH: Growth hormone gene; IGF2: Insulin-like growth factor 2; PRG: Progesterone receptor.

30 s; final extension at 72°C for 10 min. Each amplicon was digested by a specific restriction enzyme by incubation for 15 min at 37°C; Bsh1236I (BstUI), DdeI (HpyF31) and Eco31I (BsaI) for GH, IGF2 and PGR genes respectively. Restriction Fragment Length Polymorphism (RFLP) was carried out in reaction volume 40 µL consisting of: 20 µL of PCR product, 14 µL of distilled water, 5 µL of 10× G buffer and 1 µL of restriction enzyme Fast Digest (Fermentas, Vilnius, Lithuania). The restriction fragments were subjected to electrophoresis in 3.5% Agarose gel stained with ethidium bromide and visualised under UV trans-illuminator.

Statistical analysis

The genotypic and allelic frequencies, observed and expected heterozygosity and Hardy-Weinberg equilibrium for the three studied genes (GH, IGF2 and PGR) were calculated using GENALEX software version 6.5 (Peakall and Smouse, 2012).

For the association between GH and IGF2 genes and growth traits (body weight and daily gain), the following mixed model was used:

$$Y_{ijklm}=PO_i+S_j+LSB_k+Genotype_l+a_m+e_{ijklm} \quad \text{(Model 1)}$$

where Y_{ijklm} corresponds to the record of the m^{th} weaned rabbit. PO_i is the fixed effect of the parity order class (2 levels: where class 1 includes parity orders 1, 2 and 3 and class 2 includes parities 4, 5, 6); S_j is the fixed effect of sex (2 levels: males and females); LSB_k is the fixed effect of the litter size at birth at which the m^{th} kit was kindled (4 levels); $Genotype_l$ is the fixed effect of the i^{th} genotype with three levels (C/C, C/T and T/T for GH gene; A/A, A/Del and Del/Del for IGF2 gene); a_m is a random additive effect of m^{th} weaned rabbit; e_{ijklm} and is the residual of the model.

For the association between GH, IGF2 and PGR genes and milk production traits (MY21d and TMY), the following mixed model was used:

$$Y_{ijk}=PO_i+\beta_1\times LSB_j+\beta_2\times DWK_j+Genotype_j+a_j+e_{ijk} \quad \text{(Model 2)}$$

where Y_{ijk} corresponds to the record of the j^{th} rabbit doe. PO_i is the fixed effect of the parity order class (2 levels: where class 1 includes parity orders 1, 2 and 3 and class 2 includes parities 4, 5, 6); β_1 is the regression coefficient of the trait on litter size at birth (LSB_j); β_2 is the regression coefficient of the trait on doe weight at kindling (DWK_j); $Genotype_j$ is the fixed effect of the i^{th} genotype of the j^{th} rabbit doe with three levels (C/C, C/T and T/T for GH gene; A/A, A/Del and Del/Del for IGF2 gene; A/A, A/G and G/G for PGR gene); a_j is a random additive effect of j^{th} rabbit doe; e_{ijk} and is the residual of the model.

Table 2: Genotypic and allele frequencies, observed (HO) and expected (He) heterozygosity, effective number of alleles (Ne) and chi-square test (χ^2) of HWE for GH, IGF2 and PGR genes.

Gene	No		Genotype frequency			Allele frequency		Heterozygosity		
								Ne	Ho	He
			C/C	C/T	T/T	C	T			
GH	285	Observed	0.432	0.396	0.172	0.630	0.370	1.874	0.396	0.466
		Expected	0.397	0.466	0.137	χ^2=6.386*				
IGF2	285		A/A	A/Del	Del/Del	A	Del			
		Observed	0.133	0.488	0.379	0.377	0.623	1.886	0.488	0.470
		Expected	0.142	0.470	0.388	χ^2=0.413^{NS}				
PRG	218		A/A	A/G	G/G	A	G			
		Observed	0.211	0.445	0.344	0.433	0.567	1.965	0.445	0.491
		Expected	0.188	0.491	0.321	χ^2=1.929^{NS}				

HWE: Hardy-Weinberg equilibrium; GH: Growth hormone gene; IGF2: Insulin-like growth factor 2; PRG: Progesterone receptor; *Effect significantly different from 0, $P<0.05$. ^{NS}Not significant.

For the association between PGR gene and litter size traits (LSB and LSW), the same model as Model 2 was used, excluding the regression coefficient of the trait on litter size at birth ($\beta_1 \times LSB_i$).

In a previous step, the additive and residual variances were obtained by REML method using the remlf90 program, then they were used in the blupf90 program (Misztal *et al.*, 2002) to obtain the estimates of the fixed effects, as well as the (co)variance matrix of the errors. The significant differences between the generalised least square means (GLM) for the different genotypes of the three genes were checked to detect the association with these genes and the studied traits.

The additive and dominance effects were estimated according to (Russo *et al.*, 2008). Additive genetic effect (a) was estimated as half of the difference between values of the two homozygous groups, for instance, for IGF2 gene the additive effect was calculated as a=½ (AA–DelDel). The dominance effect (d) was calculated as the difference between the values of the heterozygous group and the average of the values of the two homozygous groups: d=ADel–½(AA+DelDel).

RESULTS

Polymorphism in the GH, IGF2 and PGR genes in Egyptian Gabali rabbits

Three different genotypes were obtained for each of the three studied genes in Gabali rabbits. For GH gene, T/T genotype for the undigested fragment of 231 bp, C/C for the digested one (169 and 62 bp), and the heterozygous C/T genotype for 231, 169 and 62 bp fragments. For IGF2 gene, A/A genotype was recorded for the undigested fragment of 136 bp, Del/Del genotype for the digested one (103 and 33 bp), and the heterozygous A/Del genotype for 136, 103 and 33 bp fragments. Finally, PGR gene, the undigested 558 bp fragment indicated A/A genotype, while the digested one (416 and 142 bp) indicated G/G genotype and the heterozygous individuals (558, 416 and 142 bp) indicated G/A genotype, as shown in Figure 1, Figure 2 and Figure 3.

The alleles and genotypes frequency and the observed and expected heterozygosity of GH, IGF2 and PGR genes are shown in Table 2. The C/C homozygote genotype of GH showed the highest frequency (0.432). On the other hand, IGF2 and PGR showed the highest frequency for the heterozygote genotypes (A/Del=0.488 and A/G=0.445). Chi-square (χ^2) value indicated that the studied rabbit

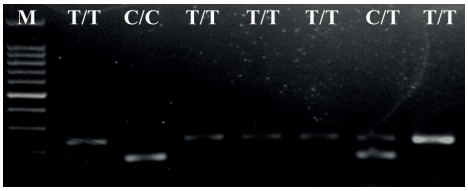

Figure 1: Gel electrophoresis showing the PCR-RFLP products of the SNP identified in the rabbit Growth hormone (GH) gene. The genotypes are indicated at the top of each lane. M is DNA molecular marker.

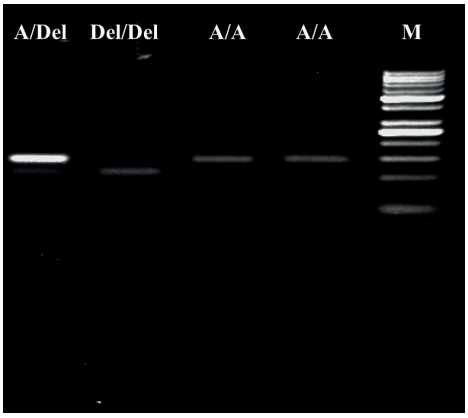

Figure 2: Gel electrophoresis showing the PCR-RFLP products of the indel identified in the rabbit Insulin-like growth factor 2 (IGF2) genes. The genotypes are indicated at the top of each lane. M is DNA molecular marker.

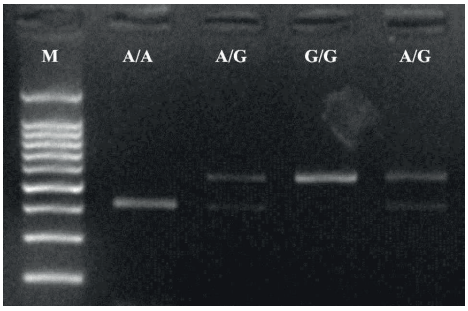

Figure 3: Gel electrophoresis showing the PCR-RFLP products of the SNP identified in the rabbit Progesterone receptor (PGR) gene. The genotypes are indicated at the top of each lane. M is DNA molecular markers.

Table 3: Generalised least square means±standard errors for the effect of growth hormone gene (GH) polymorphism on growth traits of Gabali rabbits.

Trait	C/C N=131	C/T N=98	T/T N=56	Additive effect	Dominance effect
BW4 (g)	488.4±9.5	488.7±10.1	487.3±11.3	–0.57	0.85
BW8 (g)	1162^{a}±18	1190^{b}±19	1157^{a}±22	–2.03	30.72
BW12 (g)	1788^{a}±28.62	1842^{b}±30	1795^{a}±34.14	3.60	50.87
DG4-8 (g/d)	23.93±0.46	24.94±0.50	23.80±0.58	–0.065	1.075
DG8-12 (g/d)	22.38±0.69	23.33±0.73	22.75±0.84	0.185	0.765

BW: Body weight at 4, 8 and 12 wk.; DG: Daily weight gain; Means within row not sharing superscript are significant (P<0.05).

population significantly deviated from Hardy-Weinberg equilibrium at the GH locus, while it followed the Hardy-Weinberg equilibrium at IGF2 and PGR loci.

Association of GH, IGF2 and PGR genes with productive traits in Gabali rabbits

Association analysis between the genotypes of GH and IGF2 genes with the growth traits of our Gabali rabbits are shown in Tables 3 and 4. Our results indicated that the C/T genotype of GH gene was significantly associated with BW8 (1190±19 g) and BW12 (1843±30 g). The IGF2 indel (c.156+61delA) showed a highly significant effect on all studied growth traits (BW4, BW8, BW12, DG4-8 and DG8-12) with rabbits carrying Del/Del genotype achieving higher BW4, BW8 and BW12, and recorded the highest DG4-8 and DG8-12. Our results showed that the estimated dominant genetic effects of GH gene were higher than the additive genetic effects. An opposite trend was recorded for IGF2 in our study, where the estimated additive genetic effects were higher than dominance genetic effects.

The effects of GH and IGF2 genes' polymorphism on the economically important traits of Gabali rabbits, such as litter size and milk yield traits, are shown in Tables 5, 6. The GH gene showed a significant effect on LSB and TMY traits, with the C/T genotype showing the highest LSB (7.37±0.12), while the highest TMY (2936±29) was recorded for the C/C genotype. The IGF2 gene mutation in the present study showed a highly significant effect on TMY, with A/A genotype recording the highest TMY (2921±43 g). The G>A_{2464} SNP of PGR gene in our study showed a significant effect on LSB, LSW and TMY, with the genotype G/G recording the highest LSB (7.51±0.13) and LSW (6.53±0.14) and A/A genotype recording the highest TMY (2930±35), as shown in Table 7.

DISCUSSION

In the present study, we investigated the effects of GH, IGF2 and PGR genes on the economically important traits of our Egyptian Gabali rabbits. Our results showed that the highest frequency of GH was recorded for C/C genotype, while the heterozygote genotypes A/Del and A/G of IGF2 and PGR genes recorded the highest frequency. This trend

Table 4: Generalised least square means±standard errors for the effect of Insulin-like growth factor 2 (IGF2) polymorphism on growth traits of Gabali rabbits.

Trait	A/A N=49	A/Del N=123	Del/Del N=113	Additive effect	Dominance effect
BW4 (g)	462.7^{a}±11.5	477.4^{a}±8.7	507.2^{b}±8.9	–22.24	–7.51
BW8 (g)	1023^{a}±19	1134^{b}±14	1239^{c}±14	–108.155	2.895
BW12 (g)	1584^{a}±25	1750^{b}±18	1950^{c}±18	–182.91	–16.82
DG4-8 (g/d)	19.94^{a}±0.51	23.39^{ab}±0.37	26.05^{b}±0.37	–3.055	0.395
DG8-12 (g/d)	20.13^{a}±0.79	21.98^{ab}±0.56	25.48^{b}±0.56	–2.675	–0.825

BW: Body weight at 4, 8 and 12 wks.; DG: Daily weight gain; Means within row not sharing superscripts are significant (P<0.05).

Table 5: Generalised least square means±standard errors for the effect of growth hormone gene (GH) polymorphism on litter and milk production traits of Gabali rabbits.

Trait	C/C N=80	C/T N=93	T/T N=45	Additive effect	Dominance effect
LSB	6.15^{a}±0.13	7.37^{b}±0.12	6.29^{a}±0.17	–0.54	–0.68
LSW	5.48±0.14	6.33±0.14	5.38±0.18	–0.475	–0.375
MY21d (g)	2310±23	2172±23	2260±29	44.175	93.685
TMY (g)	2936^{b}±29	2767^{a}±28	2875^{b}±35	53.94	115.12

LSB: Litter size at birth; LSW: Litter size at weaning; MY21d: Milk yield up to 21 d of lactation; TMY: Total milk yield; Means within row not sharing superscripts are significant (P<0.05).

was in agreement with Amalianingsih and Brahmantiyo (2014), who recorded a frequency of 0.818 for C/C genotype of GH gene in Rex rabbits. In contrast, Abdel-Kafy *et al.* (2015) recorded the highest frequency of GH gene for the heterozygote genotype (C/T=0.550) in APRI rabbits. El-Aksher *et al.* (2017) reported a similar trend to our results, where the A/G genotypes of PGR gene recorded the highest frequency across three Egyptian rabbit populations (M-line, V-line and Gabali).

For the association studies, our results indicated that the C/T genotype of GH gene recorded the highest and significant values for BW8 and BW12. Fontanesi *et al.* (2012a) and Abdel-Kafy *et al.* (2015) recorded similar trends, where the C/T genotype showed the highest BW8 and BW10 respectively. The IGF2 indel (c.156+61delA) of our study showed a highly significant effect on BW4, BW8, BW12, DG4-8 and DG8-12 traits. Our results were consistent with Fontanesi *et al.* (2012b) and Abdel-Kafy *et al.* (2014), who found a significant effect of IGF2 indel on rabbit finishing weight at 70 d, with the highest weight recorded for Del/Del genotype. The GH genotypes of our study showed a significant effect on LSB and TMY traits, with the highest LSB recorded for C/T genotype, while the highest TMY was recorded for C/C genotype. Our results were in agreement with Zhang *et al.* (2011) who found a significant association between GH gene with prolificacy and superovulation response in goat breeds, where Matou does with AB or CC genotypes recorded larger litter sizes than those with AA and CD. Moreover, the crucial role of GH gene in follicular development and oogenesis has been confirmed in other animal species (Sirotkin *et al.*, 2003; Silva *et al.*, 2009). On the other hand, Abdel-Kafy *et al.* (2015) found a non-significant effect of GH on both LSB and LSW. The significant effect of GH on milk yield was recorded by Abdel-Kafy *et al.* (2015), who found a significant effect of GH on milk yield at 7th and 14th d of kindling, with C/T genotype yielding the highest amount of milk in the Egyptian APRI rabbit breed.

The IGF2 indel (c.156+61delA) of the present study showed a highly significant effect on TMY, with A/A genotype recording the highest TMY. Our results were consistent with those of Bagnicka *et al.* (2010), who found that the IGF2 gene had a significant effect on daily milk yield in Polish Holstein-Friesian cattle, with the most frequent haplotype (TT/GG) showing a superiority in daily milk yield. In contrast, Abdel-Kafy *et al.* (2014) found a non-significant effect of IGF2 on milk yield traits of APRI rabbits. Although IGF2 has an important role in the control of ovarian function, it showed a non-significant effect on rabbit litter size traits in our study. In contrast to our results, Abdel-Kafy *et al.* (2014) found

Table 6: Generalised least square means±standard errors for the effect of Insulin-like growth factor 2 (IGF2) polymorphism on litter and milk production traits of Gabali rabbits.

Trait	A/A N=30	A/Del N=74	Del/Del N=114	Additive effect	Dominance effect
LSB	6.22±0.22	6.25±0.15	7.12±0.12	–0.45	–0.42
LSW	5.34±0.23	5.58±0.16	6.11±0.13	–0.385	–0.145
MY21d (g)	2299±36	2287±24	2196±21	51.47	39.76
TMY (g)	2921^{b}±43	2907^{b}±30	2797^{a}±26	61.805	47.515

LSB: Litter size at birth; LSW: Litter size at weaning; MY21d: Milk yield up to 21 d of lactation; TMY: Total milk yield; Means within row not sharing superscripts are significant (P<0.05).

Table 7: Generalised least square means ± standard errors for the effect of Progesterone receptor (PGR) polymorphism on litter and milk production traits of Gabali rabbits.

	A/A	A/G	G/G	Additive	Dominance
Trait	N=46	N=97	N=75	effect	effect
LSB	6.19^{a}±0.17	6.31^{a}±0.12	7.51^{b}±0.13	–0.66	–0.54
LSW	5.60^{ab}±0.18	5.38^{a}±0.13	6.53^{b}±0.14	–0.465	–0.685
MY21d (g)	2308±29	2264±22	2170±25	69.2	25.3
TMY (g)	2930^{b}±35	2882^{b}±27	2765^{a}±30	82.64	34.36

LSB: Litter size at birth; LSW: Litter size at weaning; MY21d: Milk yield up to 21 d of lactation; TMY: Total milk yield; Means within row not sharing any alphabets are significant (P<0.05).

a significant effect of IGF2 on rabbit litter size trait, with Del/Del genotype recording the highest LSB. Moreover, Buys *et al.* (2006) found that IGF2 had a significant effect on prolificacy of large White and Landrace sow breeds, where those sows which inherited the paternal G allele from heterozygous bucks had higher and significant numbers of total kits born per litter, kits born alive per litter, and higher numbers of kits weaned. Thus, our results confirm the important role of the somatotrophic axis (GH-IGF) on animal reproduction and milk production traits.

Our study showed that the $G>A_{2464}$ SNP of PGR gene recorded a significant effect on LSB, LSW and TMY traits, with the G/G genotype showing the highest LSB and LSW, while the highest TMY was recorded for A/A genotype. The current results were consistent with Peiró *et al.* (2008), who found that the G/G genotype had 0.5 kits and 0.5 implanted embryos more than the A/A genotype. Moreover, in Jining Grey goats, Wang *et al.* (2009) found a significant effect of PGR gene on litter size of Jining Grey Goats, where the does with genotype B/B had 0.52 (P<0.05) and 0.98 (P<0.001) kids more than those with genotype A/B or A/A, respectively. Moreover, the does with genotype A/B had 0.46 (P<0.05) kids more than those with genotype A/A. To our knowledge, there is no existing study that has evaluated the association between PGR gene and rabbit milk yield. Previous studies had shown that the milk yield of Norwegian cattle was inversely related to progesterone hormone levels during the first and third luteal phases postpartum (Reksen *et al.*, 2002).

Although our results had shown that there was an association between the GH, IGF2 and PGR genes with growth, litter size and milk yield traits, it is unknown whether the current DNA polymorphisms of these genes are the causative variant or just molecular markers linked with the real mutation. Therefore, further studies such as linkage analysis and functional genomics are necessary to confirm the current relationship between the studied traits with these genetic variants.

CONCLUSION

The C/T and Del/Del genotypes of GH and IGF2 genes might be useful for marker-assisted selection programmes for improvement of rabbit growth. The C/T and G/G genotypes of GH and PRG genes might be useful for marker-assisted selection programmes for improvement of rabbit litter size at birth. The C/C, A/A and A/A genotypes of GH, IGF2 and PGR genes might be useful for marker-assisted selection programmes for improvement of rabbit milk yield traits.

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Statement of animal rights: The current work was approved by the Committee of Animal Care and Welfare, Benha University, Egypt. As such, the research was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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