World Rabbit Science

World Rabbit Sci. 2021, 29: 31-40
doi:10.4995/wrs.2021.13001
WRSA, UPV, 2003

ASSOCIATION OF *GH* GENE POLYMORPHISM WITH GROWTH AND SEMEN TRAITS IN RABBITS

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Abstract: Although growth hormone (*GH*) gene mutations are described in several species, the studies concerning their variabilities and associations with economic traits in rabbits are scarce, particularly associations with semen traits. A total of 149 rabbit bucks from five populations (V-line=36, Moshtohor line=28, APRI line=42, cross ½A½M=23, and Gabali=20) were used in the present study to identify polymorphism of c.-78 C>T single nucleotide polymorphism (SNP) of *GH* gene among these populations and to investigate the association of *GH* gene polymorphism with body weight (BW), daily weight gain (DG) and semen traits. DNA was extracted from blood samples for genotyping of c.-78 C>T SNP of *GH* gene based on polymerase chain reaction with the restriction fragment length polymorphism (PCR-RFLP) technique. The genetic diversity of SNP C>T of *GH* gene was assessed in terms of genotypic and allelic frequencies, effective number of alleles *(Ne*), observed (*Ho*) and expected (*He*) heterozygosity, Hardy-Weinberg equilibrium (HWE), reduction in heterozygosity due to inbreeding (F_{IS}) and polymorphism information content (PIC). Three genotypes of TT, CC and TC of PCR product of 231 bp of *GH* gene were detected and all the populations were in HWE in terms of *GH* gene. The highest *Ne* was obtained for the Moshtohor line (1.978), while the lowest allelic numbers were obtained for V-line (1.715) and Gabali breed (1.800). The highest genotype frequency of *GH* gene was 0.48 in TT genotype of V-line, 0.21 in CC genotype of Moshtohor line, 0.67 and 0.56 in TC genotype of ½A½M and Gabali rabbits ($P<0.05$). The highest frequency for C allele was recorded by Moshtohor line (0.45) and the lowest frequency by Gabali (0.32). The genetic diversity scores for *GH* gene were intermediate (*Ho*=0.551, *He*=0.471, *PIC*=0.358). The values of *Ho* ranged from 0.444 in V-line to 0.667 in ½A½M cross, while the values of *He* were 0.425 in V-line and 0.508 in Moshtohor line. The values of *PIC* were moderate and ranged from 0.332 in V-line to 0.375 in M-line. The highest F_{IS} was observed in Moshtohor line (0.042) and the lowest value was observed in ½A½M cross (–0.413). The CT genotype of *GH* gene showed the highest and significant values for body weights at 4, 8, 10 and 12 wk (542, 1131, 1465 and 1861 g) and daily gains at intervals of 4-6 and 8-10 wk (23.1 and 26.5 g). Additionally, the CT genotype recorded the highest and significant values for volume of ejaculate (1.1 mL), sperm motility (57.6%), live sperm (85.6%), normal sperm (93.1%) and sperm concentration in semen (611×10^6/mL), along with the lowest and significant values for dead sperms (14.4%) and abnormal sperms (6.9%).

Key Words: rabbits, growth, semen, *GH* gene, PCR-RFLP.

INTRODUCTION

Growth is the most important trait for evaluating meat producing animals, as feed accounts for 40 to 60% of the total cost (Gidenne *et al.,* 2017). Feed efficiency is mostly expressed as body weight gain and considered an important trait for judging the performance and profitability of rabbit production (Maertens and Gidenne, 2016). Semen quality traits are very important not only because they affect male fertility and prolificacy, but also because they determine the potential fertility of seminal doses when artificial insemination (AI) is used (Khalil *et al.,* 2007;

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https://doi.org/10.4995/wrs.2021.13001

Lavara *et al.,* 2008, 2011). The use of AI is currently widespread as a practice in intensive rabbit farms and has led to an increase in the economic importance of male fertility (Alvariño 2000; Lavara *et al.,* 2013).

Growth hormone (*GH*) gene plays vital roles in postnatal growth, lipid metabolism and muscle mass deposition (Fontanesi *et al.,* 2008, 2012). Moreover, *GH* plays crucial roles in the growth and development of testis and directly affects gametogenesis, steroidogenesis and the division rate of Leydig and Sertoli cells (Zachmann, 1992). The *GH* gene also acts directly and indirectly to promote the development and maturation of spermatogonia and to improve semen volume, sperm morphology and sperm concentration and motility (Tusell *et al.,* 2012). It was reported to be trivial for tissue growth and fat metabolism (Amalianingsih and Brahmantiyo, 2014; Abdel-Kafy *et al.,* 2015; Migdal *et al.,* 2019). Therefore, it has a relevant role in reproduction, lactation and normal body growth in rabbits (Fontanesi *et al.,* 2008, 2012; Tusell *et al.,* 2012; Amalianingsih and Brahmantiyo, 2014; Abdel-Kafy *et al.,* 2015; Migdal *et al.,* 2019). In other studies on bulls, the *GH* gene had a significant effect on the libido score, number of semen doses per collection, post-thaw sperm motility, sperm mass activity, live and dead count, individual fresh sperm motility, total morphological abnormality, head abnormality of sperm, sperm concentration, minor and major defects, scrotal circumference and testicular growth after puberty (Yardibi *et al.,* 2009; Afshari *et al.,* 2011; Pal *et al.,* 2014; Darwish *et al.,* 2016; Amiri *et al.,* 2018). The *GH* gene could therefore be considered as a candidate gene for the identification of molecular markers associated with growth and semen traits in rabbits.

Selection of high fertility bucks is a prerequisite for successful rabbit production and for dissemination of superior genotypes (El-Tarabany *et al.,* 2015). Because of the low values of heritability for semen quality traits, direct selection of these traits is not widely used and is commonly associated with low genetic response (Lavara *et al.,* 2011). So, the candidate genes approach might offer the marker of choice for selecting superior bucks with high quality semen traits. In farm animals, many studies concerning the use of *GH* gene as a molecular marker for semen quality traits have been conducted, such as in dairy bulls (Afshari *et al.,* 2011; Pal *et al.,* 2014; Darwish *et al.,* 2016; Amiri *et al.,* 2018), in buffalo bulls (Darwish *et al.,* 2016), in goat bucks (Nikbin *et al.,* 2018) and in boars (Kmieć *et al.,* 2007). To our knowledge, studies investigating the genetic bases for rabbits semen traits (Lavara *et al.,* 2011) and for the polymorphism and association of *GH* gene with growth and semen traits in rabbits are scarce (Fontanesi *et al.,* 2012; Abdel-Kafy *et al.,* 2015; Migdal *et al.,* 2019). Moreover, no studies have investigated the possible association between *GH* gene polymorphism and semen quality traits in rabbits. So, the objectives of the present study were: 1) to evaluate the polymorphism of *GH* gene in an experiment involved five rabbit populations (V-line, Moshtohor line, APRI line, the cross ½A½M and Gabali); and 2) to investigate the associations of *GH* gene with some growth and semen traits of the bucks belonging to these rabbit populations.

MATERIALS AND METHODS

Animals and origin of populations

In the rabbitry, the breeding animals of V-line, Moshtohor line, APRI line and Gabali rabbits consisted of 60, 45, 30 and 60 does and 20, 15, 10 and 20 bucks, respectively. The ½A½M cross is the first generation resulting from crossing APRI line (15 bucks) with Moshtohor line (45 does). This crossbreeding experiment was conducted during the period from September 2015 until December 2017. Both growth and semen traits were recorded in a total of 149 breeding bucks from five different populations (V-line=36, Moshtohor line=28, A-line=42, ½A½M=23 and Gabali=20).

The origin of the rabbit populations used were as follow: APRI line (A-line) was synthesised by Youssef *et al.* (2008) in the Animal Production Research Institute, Ministry of Agriculture, Egypt by crossing Red Baladi bucks with V-line does. The synthesising pattern of Moshtohor line (M-line) was similar to that for APRI line, but crossing males of Sinai Gabali (G) with V-line females (Iraqi *et al.,* 2008) in the Department of Animal Production, Faculty of Agricultures, Moshtohor, Benha University, Egypt followed by selection for litter weight at weaning and individual weight at 56 d. The V-line is a maternal Spanish line selected for litter size at weaning by the Animal Science Department, Universitat Politècnica de València (UPV), Valencia, Spain and was introduced into Egypt in 1998 (Estany *et al.,* 1989; Khalil and Baselga 2002). The Sinai Gabali breed (G) was raised by the Bedouins in Sinai and is characterised by high resistance

to many diseases and high tolerance for harsh environmental conditions, while Red Baladi is an Egyptian traditional breed that was formed by upgrading of Giant Baladi does with pure Giant Flanders bucks (Khalil and Baselga 2002).

Management and mating system

All the experimental populations were raised in a one floor rabbitry belonging to the Department of Animal Production, Faculty of Agriculture, Moshtohor, Benha University, Egypt. Natural mating avoiding full and half-sib matings as well as parent offspring matings was practised for all rabbit populations. Each buck was allowed to mate with three does, and each doe was transferred to the assigned buck to be mated and returned back again to her own cage. On the 10th day post mating, each doe was palpated to detect pregnancy. Breeding bucks and does were housed individually in flat deck batteries of wire cages and received the standard lighting and ventilation requirements, as well as a vaccination programme. On the 25th day of pregnancy, the nest boxes were supplied with a thick layer of rice straw. The kits were weaned, sexed, ear tagged and transferred to standard progeny wire cages at the 28th days post kindling. The breeding animals were fed on a pelleted commercial ration with 18.01% crude protein, 13.7% crude fibre and 2.5% fat. Egyptian Berseem (*Trifolium alexandrinum*) was offered as a green forage only to the lactating does during winter season in order to enhance their milk production. Growing rabbits and breeding bucks were fed only on a pelleted concentrated diet. Fresh drinking water was available all the time.

Studied traits

Data on growth traits were recorded in the form of body weights (BW) at 4, 6, 8, 10 and 12 weeks and daily weight gains (DWG) during the intervals of 4-6, 6-8, 8-10 and 10-12 wk of age, respectively. Semen data were investigated by collecting the semen once per month using artificial vagina according to Khalil *et al.* (2007). The ages of bucks used ranged from 6 to 12 mo, with an average weight of 3±0.25 kg. The bucks were trained for semen collection using an artificial vagina, where the female rabbit was applied as a teaser. A total number of 1050 ejaculates (V-line=275, M-line=239, A-line=312, ½A½M=144 and Gabali=80) were collected for evaluation of semen quality traits such as ejaculate volume in mL (VE), pH of semen, sperm motility % (MS), live sperm % (LS), dead sperm % (DS), normal sperm % (NS), abnormal sperm % (AS) and sperm concentration (SC). The collected semen was kept in a water bath at 37°C immediately after collection until laboratory evaluation. The volume was measured using a graduated tube. The pH was measured by a pH meter and each ejaculate was evaluated and examined by microscope. Semen was diluted with 2.9% sodium citrate dehydrate solution (37°C) to measure the motility of spermatozoa. Percentages of live, dead and abnormal sperms were recorded as described by Khalil *et al.* (2007). Sperm abnormality was determined by mounting a thin smear of semen sample onto a clean grease-free glass slide and stained with eosin-nigrosin. Using a hand counter under light microscope, 100 sperm cells were counted per slide (×40 magnification). The percentage of normal sperms was predestined, normal sperm as %=100 – abnormal sperm as %. Duplicate smears from each ejaculate were stained with eosin-nigrosin stain. Sperm cell concentration (10^6/mL) was quantified by direct cell count using the improved Neubauer haemocytometer (Khalil *et al.,* 2007).

DNA extraction and polymerase chain reaction amplification

Blood samples were collected for DNA extraction from 149 bucks of the five populations distributed as V-line=36, M-line=28, A-line=42, ½A½M=23 and Gabali=20. Approximately 3-5 mL venous blood was obtained from the rabbit ear vein. Genomic DNA was extracted from leucocytes using the Gene Jet Whole Blood Genomic DNA purification Kit (Fermentas, Waltham, MA, USA). However, the rabbit growth hormone gene is located on chromosome 19: 48.725.660-48.727.208 reverse strand (Source: UniProtKB/ Swiss-Prot; Acc:P46407).

DNA fragment of 231 bp including part of 5'-untranslated region and part of exon 1 of the *GH* gene was amplified using the previously published primers (Fontanesi *et al.,* 2012). According to Fontanesi *et al.* (2012), the primer sequence and polymerase chain reaction with the restriction fragment length polymorphism (PCR-RFLP) assay used in the amplification were: the forward primer 5'- GTATAGTGGGATGGGGTTGG -3' and the reverse primer 5'- TTACGCTCCCATTCAGAAGC -3. 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, Vilnius, Lithuania) 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 with annealing temperature of 58°C for 30 s and 72°C for 30 s; final extension at 72°C for 10 min.

PCR amplicons digestion

For genotyping of the c.-78 C>T single nucleotide polymorphism (SNP) of *GH* gene, the *Bsh1236I* (*BstUI*) restriction enzyme (Fermentas, Vilnius, Lithuania) was used for digestion of the PCR amplicons (Fontanesi *et al.,* 2012). The RFLP reaction was carried out in a total volume of 40 µL consisting of: 20 µL of PCR product, 14 µL of dH_2O, 5 µL of 10× G buffer and 1 µL of restriction enzyme. The reaction was incubated for 15 min at 37°C then subjected to electrophoresis in 3.5% Agarose gel stained with ethidium bromide (Gibco-BRL, Waltham, MA, USA), then photographed with an FX Molecular Imager apparatus (BIO-RAD, Hercules, CA, USA).

Characterising the molecular data in different populations

Genotypic and allelic frequencies were calculated and the genetic diversity for SNP C>T located in the exon 1 region of *GH* gene were assessed in terms of the effective number of alleles (*Ne*) and the observed (*Ho*) and expected (*He*) heterozygosity using GENALEX software, version 6.5 (Peakall and Smouse, 2012). Within each population, Hardy-Weinberg equilibrium (HWE) and the reduction in heterozygosity due to inbreeding (F_{IS}) were estimated using the GENEPOP program, performing the Chi-Square test for each population studied (Raymond, 1995; Kalinowski *et al.,* 2007; http://genepop.curtin.edu.au/). The polymorphic information content (PIC) was calculated using CERVUS software, version 3 (Kalinowski *et al.,* 2007).

Data analysis

For detecting the molecular associations of c.-78 C/T SNP of *GH* gene with growth and semen traits, the following animal models (Model 1 for growth traits and Model 2 for semen traits) were applied using PEST software (Groeneveld, 2006):

$$y=Xb+Z_au_a+e \quad \text{(Model 1)}$$

where y=vector of observed growth trait for the weaned rabbit; b=vector of fixed effects of genetic group of progeny (5 levels), year-season of birth (7 levels), parity (4 levels), the number of kits born alive in which the buck was born (three levels; 1=1-3 kits, 2=4-6 kits and 3=≥7 kits) and genotype for each C/T SNP separately (3 genotypes); X and Z_a=incidence matrices corresponding to the fixed and additive random effects of the rabbit (u_a), respectively, and e=vector of random residual effects.

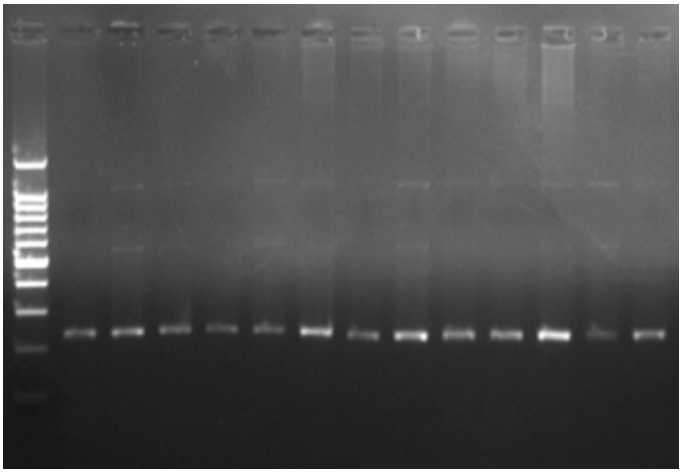
$$y=Xb+Z_au_a+Z_pu_p+e \quad \text{(Model 2)}$$

where y=the vector of observed semen parameter for the buck; b=the vector of fixed effects of genetic groups of the buck (5 levels), year-season of semen collection (10 levels) and genotype for C/T SNP (3 genotypes); u_a=the vector of random additive effect of the bucks and sires and dams of bucks; u_p=the vector of random effects of the permanent non-additive effect of the bucks; X, Z_a and Z_p=the incidence matrices corresponding to the fixed effects, additive genetic effects and permanent environment of the buck, respectively; e=the vector of random residual effects.

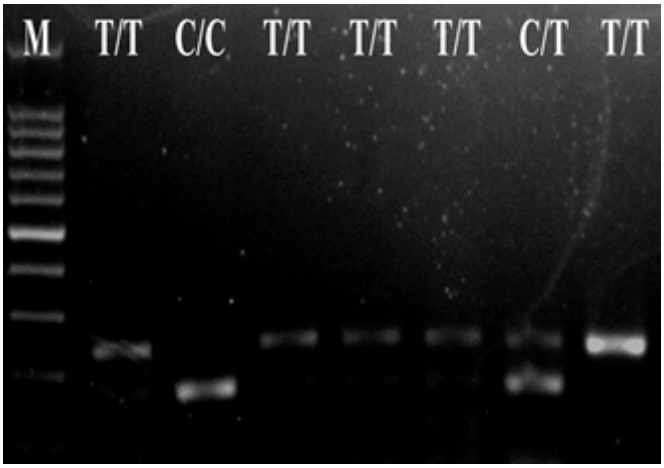
RESULTS AND DISCUSSION

Allele and genotype frequencies of GH gene in each population

Three genotypes of TT, CC and TC of PCR product of 231 bp of *GH* gene were detected by PCR-RFLP analysis in the studied populations (Figure 1). The genotypic and allelic frequencies of the *GH* polymorphisms estimated for each population are shown in Table 1. The frequency of TT genotype of GH gene was significant (P<0.05) and ranged from 0.48 in V-line to 0.28 in ½A½M cross. For CC genotype, the highest and significant frequency (P<0.05) was recorded in Moshtohor line (0.21) and the lowest frequency was recorded in ½A½M cross (0.04). In contrast, the highest and significant frequency of CT genotype (P<0.05) was recorded in ½A½M cross (0.67) and the lowest frequency in V-line (0.45). Across all populations, the CT genotype showed the highest frequency of 0.551, while the TT and CC



PCR products of the *GH* gene



PCR-RFLP products of the C/T SNP identified in *GH* gene.

Figure 1: Gel electrophoresis showing the polymerase chain reaction (PCR) and PCR with the restriction fragment length polymorphism products of the C/T single nucleotide polymorphism (SNP) of GH gene. The genotypes are indicated at the top of each lane. M is 100 bp DNA molecular marker.

genotypes occurred at frequencies of 0.356 and 0.088, respectively. Higher frequency of CT genotype observed in the present study was in accordance with those for Satin (0.455), APRI (0.550), New Zealand White (NZW, 0.643) and Termond White (TW, 0.457) rabbits reported by Amalianingsih and Brahmantiyo (2014), Abdel-Kafy *et al.* (2015), Hristova *et al.* (2018) and Migdal *et al.* (2019), respectively. Hristova *et al.* (2018) reported that the frequency of CC genotype was higher than that of TT genotype (0.300 *vs.* 0.075). Migdal *et al.* (2019) reported that the genotype frequency of GH gene was the highest (54.21%) for TT genotype in all populations studied (NZW, BGG and cross), Migdal *et al.* (2019) reported that the frequency of TT genotypes was the highest (54.21%) in NZW×BGG cross, although they did not identify this genotype at all in BGG, and TT genotypes were rare (3.1%) in the Termond White rabbit population.

The allelic frequency showed the same trend as the genotypic frequency (Table 1), where the highest frequency for C allele was recorded by Moshtohor line (0.45) and the lowest frequency by Gabali (0.32) and T allele showed higher

Table 1: Genotypic and allelic frequencies of the growth hormone gene in different rabbit populations.

		Genotype frequency			Allele frequency	
		TT	*CT*	*CC*		
Population	N	N=54	N=82	N=13	T	C
V-line	36	0.480[d]	0.445[a]	0.070[b]	0.700[d]	0.300[a]
M-line	28	0.320[b]	0.474[b]	0.210[c]	0.550[a]	0.450[d]
A-line	42	0.310[b]	0.615[d]	0.070[b]	0.620[b]	0.380[c]
½A½M	23	0.280[a]	0.667[e]	0.040[a]	0.620[b]	0.380[c]
Gabali	20	0.390[c]	0.556[c]	0.050[a]	0.680[c]	0.320[b]
Standard error	149	0.036	0.014	0.031	0.026	0.026

N: numbers of bucks from different populations; A: APRI line and M: Moshtohor line.
[a,b,c,d,e]Different letters in the same column indicate significant differences at $P<0.05$.

frequency (0.634) than C allele (0.366). Migdal *et al.* (2019) reported that the allele frequencies for *GH* gene were between 0.28 and 0.85 for C allele, and from 0.15 to 0.72 for T allele for all populations studied. Higher frequency of T allele observed in the present study was in accordance with those reported by Abdel-Kafy *et al.* (2015) and Hristova *et al.,* (2018) for APRI (0.540) and NZW rabbits (0.609), respectively. On the contrary, other studies by Amalianingsih and Brahmantiyo (2014) and Migdal *et al.* (2019) reported higher frequency of C allele for CAL (0.625) and Belgian Giant Grey (BGG, 0.850) rabbits, respectively. Fontanesi *et al.* (2008) reported the frequencies of C and T alleles at c.747+34 C>T SNP of *GH* gene to be 0.51 and 0.49, respectively. The frequencies of T and C alleles were reported by Bindu *et al.* (2011) to be 0.43 and 0.57 in a pooled population of NZW and Soviet Chinchilla and their crosses. Abdel-Kafy *et al.* (2015) reported that the observed frequencies in APRI rabbits were 0.540 for allele T and 0.460 for allele C. Hristova *et al.* (2017) in NZW rabbits reported that the allele frequencies were 0.692 for allele C and 0.308 for allele T, while Hristova *et al.* (2018) in outbred rabbits reported that the allele frequencies were 0.613 for allele C and 0.387 for allele T.

The effective numbers of alleles (*Ne*) and chi-square values characterising *GH* gene for each population are presented in Table 2. The highest *Ne* was obtained for Moshtohor line (1.978), while the lowest allelic numbers were obtained for V-line (1.715) and Gabali breed (1.800). The chi-square test showed that the five populations were in Hardy-Weinberg equilibrium (HWE), i.e. the deviation from HWE were not significant and ranged from 0.034 to 3.59 in all studied populations. Similar results were obtained by Abdel-Kafy *et al.* (2015), who found that the chi-square values for *GH* gene in APRI population were not significant. Moreover, Hristova *et al.* (2017) and Migdal *et al.* (2019) confirmed the validity of the HWE for *GH* gene in different populations of rabbits (NZW in Bulgaria and BGG, TW, and a cross of NZW×BGG in Poland).

The observed (Ho) and expected (He) heterozygosities, polymorphic information content (PIC) and fixation index (F_{IS}) in each population

The levels of genetic diversity for *GH* gene presented in Table 3 across the five populations studied were intermediate (*Ho*=0.551, *He*=0.471, *PIC*=0.358). However, the value of *Ho* was higher than the value of *He* in all populations

Table 2: The effective number of alleles (*Ne*) and Chi-square values (χ^2) for Hardy-Weinberg equilibrium (HWE) characterising the growth hormone gene in different rabbit populations.

Population	N	*Ne*	P-value	χ^2 value for HWE
V-line	36	1.715[a]	0.732	0.117[ns]
M-line	28	1.978[d]	0.855	0.034[ns]
A-line	42	1.899[c]	0.126	2.340[ns]
½A½M	23	1.893[c]	0.058	3.590[ns]
Gabali	20	1.800[b]	0.289	1.125[ns]
Standard error	149	0.045	-	-

N: numbers of bucks from different populations; A: APRI line, M: Moshtohor line.
[a,b,c,d]Different letters in the same column indicate significant differences at $P<0.05$; ns: non-significant.

Table 3: The observed (*Ho*) and expected (*He*) heterozygosities, the polymorphic information content (*PIC*), reduction in heterozygosity due to inbreeding (F_{IS}) characterising growth hormone gene in different rabbit populations.

Population	N	*Ho*	*He*	*PIC*	F_{IS}
V-line	36	0.444[a]	0.425[a]	0.332[a]	-0.066
M-line	28	0.474[b]	0.508[d]	0.375[c]	0.042
A-line	42	0.615[d]	0.483[c]	0.360[bc]	-0.300
½A½M	23	0.667[e]	0.483[c]	0.360[bc]	-0.413
Gabali	20	0.556[c]	0.457[b]	0.341[ab]	-0.250
Standard error	149	0.014	0.042	0.006	0.082

N: numbers of bucks from different populations; A: APRI line and M: Moshtohor line.
[a,b,c,d]Different letters in the same column indicate significant differences at $P<0.05$; F_{IS}: reduction in heterozygosity due to inbreeding within each rabbit population.

and the values of *Ho* ranged from 0.444 in V-line to 0.667 in ½A½M cross, while the values of *He* were 0.425 in V-line and 0.508 in Moshtohor line. The highest values of *Ho* over *He* observed in the present study are in agreement with those of Hristova *et al.* (2018), who also reported higher value of *Ho* (0.643) over *He* (0.458) across three NZW rabbit populations. For growth hormone receptor gene (GHR), Gencheva *et al.* (2017), with two populations of NZW and CAL rabbits, found that *Ho* was higher than *He*, resulting in a negative inbreeding coefficient (F_{IS}=–0.174 for NZW rabbits and F_{IS}=–0.027 for CAL rabbits), indicating a sufficient number of heterozygous forms in both groups of rabbits studied.

The *PIC* values were moderate ($0.25<PIC<0.50$) and ranged from 0.332 in V-line to 0.375 in Moshtohor (Table 3). Amalianingsih and Brahmantiyo (2014) reported similar *PIC* values for Rex, Satin and Reza rabbit, at 0.207, 0.375 and 0.373, respectively. The variation between *PIC* values obtained here might be due to the existence of the potential population dynamics, selection programme and the nature of the sampling process. This indicates that C/T SNP of *GH* gene had high degree of genetic information in the rabbit populations studied. For revealing GHR gene polymorphism in three rabbit breeds reared under Egyptian conditions, the 1st exon of the growth hormone receptor gene with expected amplicon size of 263 bp had been detected by Sahwan *et al.* (2014) when investigating the association between growth performance and detected SNP in this locus.

The reduction in heterozygosity (F_{IS}) for each locus across the investigated populations were moderate or low (Table 3). The highest F_{IS} was observed in Moshtohor line (0.042) and the lowest value was observed in ½A½M cross (–0.413). The maintenance of high heterozygosity in heterogeneous populations, despite the presence of narrow inbreeding, allowed expecting a weaker negative effect of inbreeding depression (Tanchev, 2015). In agreement with the present study, Hristova et al. (2018) recorded higher observed heterozygosity than expected for *GH* gene (F_{IS}=–0.317), and therefore negative inbreeding coefficients for outbred NZW rabbits were –0.460 for inbred F_1 and –0.438 for inbred F_2.

Associations of GH gene with rabbit growth traits

To clarify the molecular associations between *GH* gene and growth traits, the generalised least square means for body weights and gains in different genotypes of *GH* gene across all populations were investigated (Table 4). These means are favourable to confirm that there were considerable associations between BW and DWG and genotypes of *GH* gene. Results of El-Sabrout and Aggag (2017a) concluded that the premier step during the biological process of GH is binding with the GH, then activating the expression of insulin-like growth factor 1, which influences the growth of the animal. The CT genotype of *GH* gene showed the highest values ($P<0.05$) in BW at 4, 8, 10 and 12 wk of age (542, 1131, 1465 and 1862 g, respectively) and DWG at 4-6 and 8-10 wk of age (23.1 and 26.5 g, respectively), i.e. the rabbits with TC genotype showed a positive increase in BW at 4, 8, 10 and 12 wk of age relative to TT and CC genotypes. Rabbits of heterozygous genotype (C/T) exhibited heavier body gains compared to rabbits of C/C genotypes. According to Fontanesi *et al.* (2012) and Zhang *et al.* (2012), the CT/GG genotypes should be the most favourable genotypes to be selected. These results were in accordance with Abdel-Kafy *et al.* (2015), who reported that the heterozygote genotype (T/C) was associated significantly with heavy BW at 8 wk (868.6 g) and DWG through a 5-8 wk interval (12.2 g; $P<0.05$). El-Aksher *et al.* (2017) reported significant association between the genotype

Table 4: Generalised least square means (GLM) and their standard errors (SE) for body weight and daily weight gain as affected by single nucleotide polymorphism genotypes of *growth hormone (GH)* gene across all rabbit populations.

	Genotype					
	TT		*CT*		*CC*	
Growth trait	N=54		N=82		N=13	
	GLM	SE	GLM	SE	GLM	SE
Body weight (g):						
4 weeks	485[a]	13	542[b]	24	497[a]	13
6 weeks	777	18	765	32	766	18
8 weeks	1082[ab]	25	1131[a]	47	1071[b]	26
10 weeks	1318[a]	52	1465[b]	97	1351[ab]	54
12 weeks	1651[a]	50	1861[b]	90	1658[a]	51
Daily weight gain (g):						
4-6 weeks	20.8[ab]	1.6	23.1[b]	0.64	19.2[a]	1.6
6-8 weeks	21.9	2.0	19.0	0.83	21.9	2.0
8-10 weeks	17.3[a]	1.8	26.5[b]	1.56	18.4[a]	1.9
10-12 weeks	26.7	2.0	27.8	0.78	24.0	2.0

[a,b]Different letters in the same row indicate significant differences at $P<0.05$.

SNPs of PGR gene and BW for V-line and Sinai Gabali rabbits ($P<0.05$). Likewise, El-Sabrout and Aggag (2017b) investigated significant associations of *GH* gene with BW in V-line and Alexandria rabbits ($P<0.05$). On the contrary, Migdal *et al.* (2019) in BGG breed reported that CC genotype presented the highest and significant weight at birth (87 g) and five weeks (950 g), i.e. GH polymorphisms constitute a good molecular marker for growth in rabbits.

Associations of GH gene with rabbit semen traits

The results of the current study showed that the c.-78 C>T SNP of *GH* gene showed a substantial favourable effect on most of the rabbit semen traits examined. The CT genotype recorded the highest and significant values for the ejaculate volume, (1.1 mL), sperm motility (57.6%), live sperm (85.6%), normal sperm (93.1%) and semen sperm concentration (611×10^6/mL). Moreover, the lowest and significant values for dead sperm (14.4%) and abnormal sperm (6.9%) were recorded for the individuals carrying the CT genotype, as shown in Table 5. On the other hand, both TT and CC genotypes were nearly similar in most semen traits in terms of 0.65 *vs.* 0.7 mL for ejaculate volume, 49.7 *vs.* 50.4% for sperm motility, 80.5 *vs.* 80.7% for live sperm, 19.4 *vs.* 19.3% for dead sperm, 86.6 *vs.* 87.1 for normal sperm, 13.4 *vs.* 12.9% for abnormal sperm, 450×10^6/mL *vs.* 444×10^6/mL for semen sperm concentration.

Table 5: Generalised least square means (GLM) and their standard errors (SE) for semen traits as affected by single nucleotide polymorphism genotypes of growth hormone gene across all rabbit populations.

	Genotype					
	TT		*CT*		*CC*	
	N=54		N=82		N=13	
Semen trait	GLM	SE	GLM	SE	GLM	SE
Volume of ejaculate (mL)	0.65[b]	0.02	1.1[a]	0.03	0.7[b]	0.02
pH of semen	7.5	0.04	7.0	0.07	7.5	0.04
Motility of sperms (%)	49.7[a]	1.1	57.6[b]	2.0	50.4[a]	1.02
Live sperms (%)	80.5[a]	0.6	85.6[b]	1.1	80.7[a]	0.6
Normal sperms (%)	86.6[a]	0.4	93.1[b]	0.7	87.1[a]	0.4
Dead sperms (%)	19.4[b]	0.6	14.4[a]	1.1	19.3[b]	0.6
Abnormal sperms (%)	13.4[b]	0.4	6.9[a]	0.7	12.9[b]	0.6
Semen sperm concentration, $\times10^6$/mL	450[a]	15	611[b]	27	444[a]	13

Number of ejaculates=1050.

[a,b]Different letters in the same row indicate significant differences at $P<0.05$.

Previous studies reported significant associations of *GH* gene with seminal and sexual traits in different farm animals. In this concept, Kmieć *et al.* (2007) in boars stated that the BB genotype of *GH* gene recorded the highest and significant values for ejaculate volume, number and percentage of normal sperm. In dairy bulls, Afshari *et al.* (2011) reported that Iranian Holstein bulls carrying VV genotype of *GH* gene showed the highest concentration of fresh sperm, while the bulls of LL genotype showed the highest percentage of live sperm with the lowest ejaculate volume. Darwish *et al.* (2016) recorded significant and positive associations between the LV genotype of *GH* gene with the ejaculate volume, percentage of individual motility and post-thawing sperm motility in Egyptian cattle and buffalo bulls. Nikbin *et al.* (2018), with four polymorphic SNPs in caprine FSHβ and LHβ in male goats, detected significant associations of the gonadotropin genes with semen quality traits in terms of progressive motility, abnormality of fresh semen, motility, velocity and viability traits of post-thaw semen ($P<0.05$).

CONCLUSIONS

Associations between the CT genotype of *GH* gene and most growth and semen traits were favourable. The c.-78 C>T mutation of *GH* gene constitutes a good candidate gene for increasing selection efficiency for growth and semen traits in rabbits. Further studies are needed to validate the detected associations and to assess the genetic correlation between growth and semen traits in CT-genotyped rabbits using large datasets.

Acknowledgement: The authors are very grateful to the Central Laboratory of the Faculty of Veterinary Medicine, Benha University, Egypt for the help and support in molecular genetic analyses. This work was financially supported by the research project entitled "Genetic improvement of local rabbit breeds by using molecular genetic techniques" from the Scientific Research Fund (SRF), Benha University, Egypt.

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

Conflict of interest: The authors declare that they have no conflict of interest.

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