Polymorphism of progesterone receptor gene in Moshtohor line rabbits and their parental lines using PCR-RFLP technique

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Abstract

The aim of this study was to detect the polymorphisms in the promoter region of *PGR* gene based on PCR-Restriction Fragment Length Polymorphism technique (PCR-RFLP). Genomic DNA were extracted and amplified from 100 animal belonging to four rabbit populations; an Egyptian synthetic rabbit line named Moshtohor line (M-line) and their parents of Spanish V-line and Sinai Gabali rabbit breed, in addition to using the French Giant Papillon (FGP) as a reference population. PCR-RFLP was applied on 558 bp using the Eco31I restriction endonuclease. PCR-RFLP for *PGR* gene revealed three genotypes of GG, AA and GA, and the genotype of GG yielding two bands of 416 and 142 bp, the genotype AA yielding a single 558 bp band, and the heterozygote genotype GA yielding all the three bands of 558, 416 and 142 bp. The genotypic frequency of GA ranged from 0.680 in V-line to 0.880 in FGP and was higher than AA and GG genotypes in all populations studied. The frequency of A allele was higher than the G allele in all populations except in M-line where G allele recorded the highest allele frequency of 0.540. The highest effective number of alleles (Ne) for SNP of PGR gene was recorded for M-line (1.987), followed by FGP (1.972), while the lowest number was recorded for V-line (1.891). The four populations studied were not in Hardy-Weinberg equilibrium ($P< 0.05$ or $P< 0.001$). The observed (Ho), expected (He) heterozygosity and the polymorphic information content (PIC) averaged 1.943, 0.485 and 0.367, respectively, i.e. the four rabbit populations showed intermediate levels of genetic diversity.

Keywords: Rabbits, PGR gene, SNP, PCR-RFLP.

Introduction

SNPs are used instead of microsatellites in the genetic diversity studies **(Syvänen, 2001).** Whereas most of the RFLP markers were the result of a SNP in a restriction enzyme recognition site, which confirms the importance of SNP markers **(Heaton, 2000; White *et al.*, 2001). Amie Marini *et al.* (2012) and Othman *et al.* (2015)** stated that the genetic polymorphism can be identified by several techniques; one of the most commonly used technique is PCR-RFLP. It is a powerful method for identifying the nucleotide sequence variation in amplified DNA and can detect single base substitutions in the enzymatic restriction sites. Therefore, PCR-RFLP is a simple method for identification of species **(Minarovič *et al.*, 2010),** establishing the genotype structure of the rabbit population and in calculating the allelic frequencies **(Rafayová *et al.*, 2009)**, and identifying the genetic polymorphisms due to the presence of SNP in important genes **(Yang *et al.*, 2013).**

SNPs seem to be appealing markers to apply in the future in farm animal population structure, genetic differentiation and genetic diversity studies because they can easily be used in assessing either the functional or the neutral variation **(Marsjan and Oldenbroek, 2007).** There are few studies concerning the SNP markers in evaluating the rabbit genetic diversity associated with the important traits; one SNP located in the promoter region of *PGR* gene $G>A_{2464}$ **(Peiro *et al.*, 2008; Shevchenko, 2015)**, a SNP located in the *MSTN* gene **(Fontanesi *et al.*, 2008; Rafayová *et al.*, 2009; Shevchenko, 2015)**, a SNP located in the intron 5 of *POU1F1* gene **(Wang *et al.*, 2015),** and a SNP located in the growth hormone receptor gene (GHR) **(Amie Marini *et al.*, 2012; Sahwan *et al.*, 2014; Abdel-Kafy *et al.*, 2015)**.

Genes of *PGR, SCGB1A1, TIMP1* and *IGF1* were analyzed as functional candidate genes for reproductive traits in rabbits **(Peiró *et al.*, 2008; Argente *et al.*, 2010).** The rabbit progesterone receptor (*PGR*) gene mapped on chromosome 1 including five SNPs distributed across the gene; one SNP in the promoter region $G>A_{2464}$, three SNP`s in the 5-UTR exon 1, and a silence SNP in exon 7 **(Peiró *et al.*, 2008)**. *PGR* plays an important role in recognizing the reproductive functions and the maternal characteristics of the females in rabbits **(Gutierrez-Sagal *et al.*, 1993).** The protein encoded by this gene is a nuclear receptor binds specifically to the progesterone hormone and mediates the biological effects to play a central role in establishing the pregnancy to participate in the releasing of mature oocytes, facilitating the implantation and maintaining the pregnancy by promotion of uterine growth and suppression of myometrial contractility **(Graham and Clarke, 1997).**

Nowadays, SNPs and PCR-RFLP are used consecutively in molecular analyses of animal populations. Besides, they are excellent markers for studying polymorphism in candidate genes

responsible for the majority of important traits in the application of marker assisted selection (MAS) or gene-assisted selection to improve the selection response of productive traits in rabbits **(Peiro´ *et al.*, 2008; Abdel-Kafy *et al.*, 2015; Shevchenko, 2015)**. So, the information efficacy from candidate genes in breeding programs has the potential to enhance substantially the accuracy of selection, increasing the selection differences and improving the production performance in rabbits and goats **(Missohou *et al.*, 2006; Fontanesi *et al.*, 2008; Helal *et al.*, 2014).**

The aim of this study was to use the PCR-RFLP as a fast efficacy technique and low cost method to evaluate the polymorphism of SNP G>A_{2464} located in the promoter region of PGR candidate gene in Moshtohor line rabbits as an Egyptian synthetic rabbit line and their parents of Spanish V-line and Gabali rabbits. This technique will be used later in the selection of animals as a marker assisted selection to detect the alleles of desirable traits before the expression of the trait.

Material and Methods

Experimental animal populations

Three rabbit populations namely Moshtohor line (M-line) as a synthetic line and their parents of V-line and Gabali (G) rabbits were used in this study. Also, a French Giant Papillon breed (FGP) was used as a reference breed for normalization of the results. A total of 100 pedigreed rabbits (25 rabbits from each population) were selected from the rabbitry of the Animal Production Department, Faculty of Agriculture, Benha University, Egypt and Inshas rabbitry, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture, Egypt. Moshtohor line was developed by crossing does of Spanish V-line with bucks of Sinai Gabali **(Iraqi *et al.*, 2008).** Spanish V-line is a synthetic maternal line originated in 1983 at the Department of Animal Science, Universitat Politecnica de Valencia, Spain, by crossing animals that were the progeny of four specialized maternal lines and after three generations without selection, the line has been selected for litter size at weaning **(Estany *et al.*, 1989).** The Gabali rabbits are raised by the Egyptian Bedouins in Sinai and the north coast of western desert **(Khalil, 1999; Afifi, 2002; Khalil and Baselga, 2002).** On the other hand, the French Giant Papillon is a foreign breed developed in the Lorraine region of north eastern France in the 19th Century where the breeders at that time raised the Flemish Giant rabbits, the spotted wild rabbits and the French Lop rabbits all together **(Bunnyhugga, 2010)**.

Sampling and genomic DNA extraction

The blood samples were taken from 75 animal belonging to three rabbit populations (25 animal from each population; V-line, Gabali and M-line) along with fur samples from 25 animal of French Giant Papillon breed. The samples were selected from pedigreed animals with the least relationship to decrease the genetic similarity between the genotyped animals.

Genomic DNA was extracted from blood samples belong to V-line, Gabali and M-line rabbits. Approximately 3-5 ml venous blood sample per animal was collected from the rabbit ear vein by 2-gauge 1.5-injection needle into tubes containing EDTA as an anticoagulant. Genomic DNA was extracted from leukocytes using GeneJet Whole Blood Genomic DNA Purification Mini Kit (Cat No. #K0781, Thermo Scientific). For the reference population (French Giant Papillon), DNA was extracted from the fur according to the manufacture of GeneJet Genomic DNA Purification Mini Kit (Cat No. #K0721, Thermo Scientific). The quantity and quality of DNA were determined by spectrophotometer and agarose gel electrophoresis, respectively to be used directly in a variety of downstream applications.

PCR of progesterone receptor gene

PCR of 558 bp of promoter *PGR* gene was performed using two primers designed by **Peiró *et al.* (2006).** The 25 µl PCR reaction mix was prepared in an Eppendorf tube containing 2 µl of template DNA (50 ng/µl), 0.2 µl of Taq DNA polymerase; (AmpliTaq Gold, Applied Biosystems), 1 µl of PGR primers mix (10 pmoles from each primer (Table 1), 2.5 µl of deoxynucleotide triphosphate (2 mM dNTP), 2 µl of (25 mM MgCl2), 5 µl of 5 x reaction buffer $(NH_4)2SO_4$ and 10.3 µl of dH_2O.

Table 1. Primers used for amplification of the PGR gene

Gene	SNP	Primer sequence	Amplified fragment
PGR	G/A_{2646}	PGR- F 5`GAAGCAGGTCATGTCGATTGGAG3` PGR-UTR 5`CGCCTCTGGTGCCAAGTCTC3`	558 bp

The thermal cycler GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, California, USA), was programmed for 35 cycles of PCR. PCR started with 95 °C for 10 min. Each cycle included denaturation step for 30 seconds (95 °C), annealing step for 60 seconds (66 °C) and polymerization step for 90 seconds (72 °C). After 35 cycles, amplification was followed by 15 minutes of elongation (72 °C) and cooling down to 4 °C and storage. The PCR products were electrophoresed on 2 % Agarose gel stained with ethidium bromide to test the amplification success of 558 bp promoter PGR gene.

Restriction Fragment Length Polymorphism (RFLP) for PCR amplicons

The amplicons were digested with Eco31I restriction enzyme, for 8 hours (37 °C) resulting in patterns that could identify and differentiate each of the populations studied. RFLP was carried out in reaction volume 30 µl consisted of: 10 µl of PCR product, 17.5 µl of dH_2O, 2 µl of 10x G buffer and 0.5 µl of Eco31I restriction enzyme (Fermentas). Digestion was stopped by incubation at 65 °C for 20 min. The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gel in 1x TAE for 45 minutes at 120 V using 50 bp promega DNA step ladder (G.152A). Gels were visualized under UV transilluminator and documented in Gel Doc™ XR+ (BIO-RAD).

SNP data and statistical analysis

Allelic and genotypic frequencies were calculated by the standard procedure cited by **Falconer and Mackay (1996).** The statistical analysis was performed by **SAS (2002)** and Duncan multiple range test was used in testing the significance levels of the gene frequency **(Duncan, 1955).** Genetic diversity of SNP G>A_{2464} located in the promoter region of PGR gene was assessed by calculating the effective number of alleles *(Ne*), the observed (*Ho*) and the expected (*He*) heterozygosity using GENALEX version 6.0 **(Peakall and Smouse, 2006)**.

$$Ne = \frac{1}{\sum_{i=1}^{n} p_i^2}$$

$$Ho = \frac{No.\ of \text{ heterozygosity}}{n}$$

$$He = 1 - \sum_{i=1}^{n} p_i^2$$

Where Pi is the frequency of the i^{th} allele, Pj is the frequency of the j^{th} allele and n is the number of alleles.

Hardy-Weinberg equilibrium (HWE) within each population was estimated using Chi-Square test and applying GENEPOP program **(Raymond and Rousset, 1995;** http://genepop.curtin.edu.au/**)**. The polymorphism information content (PIC) was calculated using CERVUS version 3 software **(Kalinowski *et al.*, 2007)**, to be used in SNP (G/A_{2464}) analysis in the four populations studied as:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 \quad p_j^2$$

Results and Discussion

Amplified fragments and genotypes differentiated

As a result of the absence of molecular information about *PGR* gene in the rabbits developed in Egypt, the genetic polymorphism and the diversity of SNP G>A_{2464} located in the promoter region of this gene was done using PCR-RFLP technique. The primers used in this study amplified 558 bp fragments from promoter region of *PGR* gene (Figure 1). These amplified fragments were digested with restriction endonuclease Eco31I. Depending on the presence or absence of the restriction site GGTCTC, three different genotypes were differentiated across the animal populations; AA genotype with one undigested fragment at 558 bp, GG genotype with two digested fragments at 416 and 142 bp and GA genotype with three digested fragments at 558, 416 and 142 bp (Figure 2).

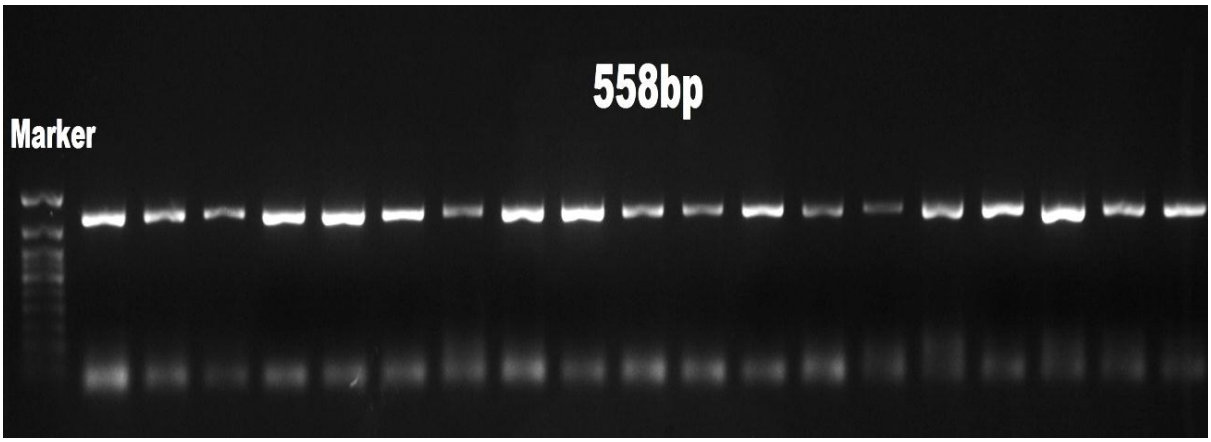

Figure 1. A 558 bp PCR products of progesterone receptor gene.

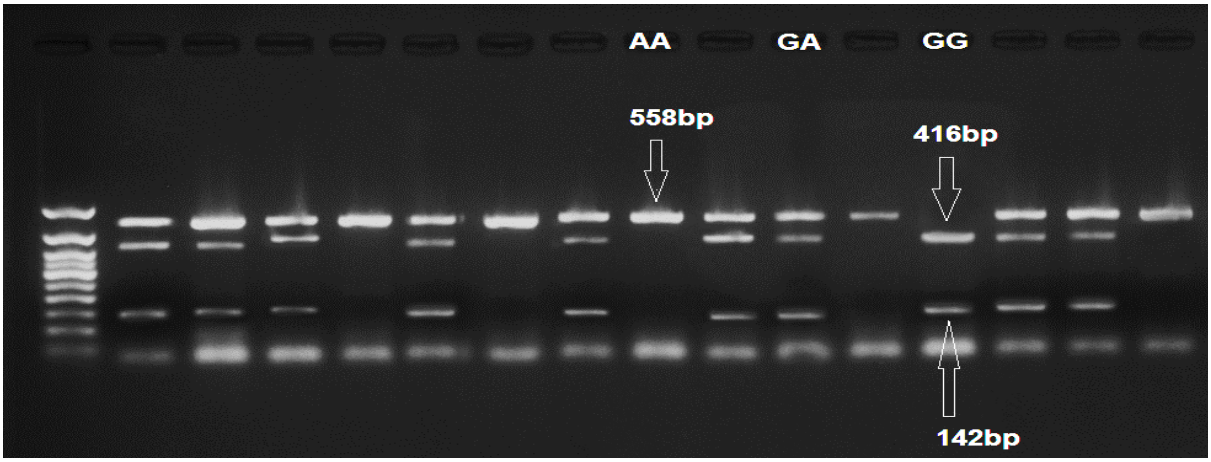

Figure 2. Three separated genotypes with Eco31I digested at 558 bp PCR products of rabbit PGR gene

The genotypic and allelic frequencies in each population

Three genotypes of AA, GG and GA were detected, counted and the allelic, genotypic frequencies of the PGR polymorphisms were estimated by genotyping the three populations of rabbits (V-line, Gabali, M-line) in addition to French Giant Papillon as a reference population. As shown in Table 2, the distribution of AA, GG and GA genotypes for polymorphic variants were 28, 4 and 68% in V-line rabbits, respectively, 24, 4 and 72% in Gabali rabbits, 4, 12 and 84% in M-line rabbits, respectively, while the FGP breed exposed GA=88% and AA=12%. Across the populations, the frequency of GA genotype was high and ranging from 0.68 in V-line to 0.88 in FGP rabbits (P<0.05). For the AA genotype, the highest frequency was recorded in V-line rabbits (0.280) and the lowest frequency was recorded in M-line rabbits (0.040) (P<0.05). For GG genotype, the highest frequency was recorded for M-line (0.120) (P < 0.05) and absented in FGP breed (0.000).

The allelic frequency showed the same trend as the genotypic frequency, where the highest frequency for G allele was recorded by M-line (0.54). The distribution of all genotypic and allelic frequencies showed significance differences across the four populations except the GG genotype showed a significance difference only between M-line, V-line and Gabali rabbits (Table 2). The significant and the highest frequency of GG genotype in M-line rabbits might be caused by selection for the maternal effect applied during the establishment program of the line, indicating that the potentiality of M-line for increasing the uterine capacity and improving litter size traits. **Peiró *et al.* (2006 and 2008)** reported that GG genotype was the most frequent genotype in the line selected to increase uterine capacity and the AA genotype was the most frequent genotype in the line selected to decrease uterine capacity (GG = 0.345, AA = 0.157 & GG = 0.329, AA = 0.171). This trend is similar to that found in M-line. **Shevchenko (2015)** reported an opposite trend where the GG genotype showed lower frequency than the AA genotype (GG = 0.183 and AA = 0.317), i.e. the SNP G/A_{2464} was associated with the reproductive and maternal characteristics in New Zealand White rabbit females.

Table 2. Genotypic and allelic frequency for the SNP (G/ A_{2464}) of *PGR* gene in the four populations studied

	V-line Mean ± SE	Gabali Mean ± SE	M-line Mean ± SE	FGP Mean ± SE	All populations Mean ± SE
Genotypic frequency:					
AA	0.28±0.006^{a}	0.24±0.006^{b}	0.04±0.006^{d}	0.12±0.006^{c}	0.16±0.06
GA	0.68±0.006^{d}	0.72±0.006^{c}	0.84±0.006^{b}	0.88±0.006^{a}	0.77±0.05
GG	0.04±0.006^{b}	0.04±0.006^{b}	0.12±0.006^{a}	0.000	0.07±0.03
Allelic frequency:					
A	0.62±0.006^{a}	0.60±0.006^{b}	0.46±0.006^{d}	0.56±0.006^{c}	0.56±0.04
G	0.38±0.006^{d}	0.40±0.006^{c}	0.54±0.006^{a}	0.44±0.006^{b}	0.44±0.04

The estimate with the same letters in each row are not significantly different (p≤0.05); SE= standard error.

The effective number of alleles and Hardy-Weinberg equilibrium in each population

The effective number of alleles (Ne) for SNP (G/A2464) of PGR gene within each population are presented in Table 3. The highest allelic numbers of *Ne* was obtained for M-line (1.987), followed by FGP with *Ne*= 1.972, While the lowest value was obtained for V-line (*Ne* = 1.891). Chi-Square tests showed that the genotypic frequencies were not in Hardy-Weinberg equilibrium ($P<0.05$ or $P<0.001$) in any of the four rabbit populations.

Table 3. The effective numbers of alleles (*Ne*) and their standard errors (SE) and Hardy-Weinberg equilibrium (HWE) for SNP (G /A $_{2464}$) in each population of rabbits

Population	N	*Ne* ± SE	χ^2 *HWE*
V-line	25	1.891±0.001^{d}	4.9*
Gabali	25	1.923±0.001^{c}	6.3*
M-line	25	1.987±0.001^{a}	11.9 ***
French Giant Papillon	25	1.972±0.001^{b}	15.4 ***
Overall mean ± SE	100	1.943±0.02	

The estimate with the same letters in each column are not significantly different ($p\leq0.05$); χ^2 = Hard-Weinberg equilibrium χ^2 value**;** * = $P < 0.05$, ** = $P < 0.001$**.**

The observed and expected heterozygosity in each population used

The values of observed (*Ho*), expected (*He)* heterozygosity and the polymorphic information content (PIC) are presented in Table 4 to evaluate the genetic diversity of the four rabbit populations. The *Ho* was higher than the *He* in all populations; the cause would be the potential *Ho* population dynamics, selection program and the nature of the sampling process. The four studied rabbit populations averaged intermediate levels of genetic diversity where *Ne* = 1.943, *He* = 0.485 and *PIC* = 0.367. All the values of *He* were above 0.4 and below 0.5 and ranging in size from 0.471 in V-line to 0.497 in M-line, whereas the values of *Ne* ranging in size from 1.891 in V-line to 1.987 in M-line approached to 2.0. These indices were higher than those reported by **Yang *et al.* (2013)** (*Ne* = 1.665, *He* = 0.395 and PIC = 0.320), for SNP G>A $_{214}$ located in exon 1 of *TBC1D1* gene in two rabbit breeds to identify the association between genetic polymorphism of *TBC1D1* gene and body weight (BW). **Wang *et al.* (2015)** reported intermediate level of genetic polymorphism of *He* = 0.472, *Ne* = 1.898 and PIC = 0.361, for a SNP located at 536 bp in intron 5 of *POU1F1* gene in four rabbit breeds.

The *PIC* of this study was higher than that reported by **Rafayova *et al.* (2009)** (*PIC* = 0.3447) for a SNP located at intron 2 of *MSTN* gene in two rabbit lines. According to the classification of *PIC* values (*PIC* < 0.25 = low polymorphism; 0.25< *PIC* <0.50 = intermediate polymorphism; *PIC* > 0.50 = high polymorphism), the populations showed moderate values of polymorphism, i.e. 0.360 for V-line, 0.373 for M-line, and 0.365 for Gabali and 0.371 for Papillon rabbits.

Table 4. The observed (*Ho*), expected (*He*) heterozygosities and the polymorphic information content (PIC) and their standard errors (SE) for the SNP of *PGR* gene (G /A $_{2464}$) in the rabbit populations studied.

Population	*Ho* ± *SE*	*He* ± *SE*	PIC ± *SE*
V-line	0.680±0.006^{d}	0.471±0.003^{b}	0.360±0.003^{b}
Gabali	0.720±0.006^{c}	0.480±0.003^{b}	0.365±0.003^{ab}
M-line	0.840±0.006^{b}	0.497±0.003^{a}	0.373±0.003^{a}
FGP	0.880±0.006^{a}	0.493±0.003^{a}	0.371±0.003^{a}
Overall mean ± SE	0.780±0.05	0.485±0.01	0.367±0.002

The estimate with the same letters in each column are not significantly different ($p\leq0.05$).

Conclusions

1) The *PGR* gene showed that PCR-RFLP technique is an appropriate tool for screening the *PGR* gene and for evaluating the genetic variability in rabbit breeds. This confirmed the fact that *PGR* gene could be used as a candidate gene in the application of marker assisted selection (MAS).
2) The genetic diversity reported higher genetic variation and a selective potentiality in M-line that could be expected to gain more genetic progress in litter size at birth trait; also indicating that M-line minimally affected by some genetic forces which reduce the genetic diversity like selection and genetic drift. Future study is needed to realize the possible association of this SNP with different productive and reproductive traits in our local rabbit populations.
3) The polymorphisms detection using the SNP marker (SNP G>A$_{2464}$) and the possible associations of genetic variation with the reproductive performance may help to find the effective genotypes in selection programs for these traits. Due to the lack in the phenotypic

records in the present study, it was impossible to make the association between different genotypes and the animal performance. However, the present study is the first attempt to investigate the polymorphism of *PGR* gene in Moshtohor line, Spanish V-line, Sinai Gabali breed, and French Giant Papillon rabbits.

References

Abdel-Kafy, E.M., Hussein, Basita A., Abdel-Ghany, Sara M., Gamal El-Din, A.Y. and Badawi, Y.M. (2015). Single nucleotide polymorphisms in growth hormone gene are associated with some performance traits in rabbit. *International Journal of Biology, Pharmacy Alhed Science.* 4(2): 490-504.

Afifi, E.A. (2002). The Gabali rabbits. In Khalil M.H., Baselga M. (Eds). Rabbit Genetic Resources in Mediterranean Countries. *Options Méditerranénnes, Series B, No. 38:51-64.* http://www.iamz.ciheam.org/medrabbit/docs/gabali.pdf

Amie Marini, A.B., Aslinda, K., Hifzan, R.M., Faisal, A.B.M. and Musaddin, K. (2012). HaeIII-RFLP Polymorphism of growth hormone gene in Savanna and Kalahari goats. *Malaysian J. Anim. Sci.* 15:13-19.

Argente, M. J., Merchán, M., Peiró, R., García, M. L., Santacreu, M. A., Folch, J. M. and Blasco, A. (2010). Candidate gene analysis for reproductive traits in two lines of rabbits divergently selected for uterine capacity. *J. Anim. Sci.*,88: 828–836

Bunnyhugga (2010). Giant Papillon - Rabbit breeds library. http://www.bunnyhugga.com/a-to-z/breeds/giant-papillon.html

Duncan, David.B. (1955). Multiple range and multiple F tests. *Biometrics l l*, 1-42.

Estany, J., Baselga, M., Blasco A., Camacho, J. (1989). Mixed model methodology for the estimation of genetic response to selection in litter size of rabbits. *Livestock. Prod. Sci*., (21): 67-76.

Falconer, D. S. and Mackay, T. F. C. (1996). Introduction to quantitative genetics. Longman, Essex, U.K., 4th ed. edition.

Fontanesi, L., Tazzoli, M., Scotti, E., Russo, V. (2008). Analysis of candidate genes for meat production traits in domestic rabbit breeds. *In: 9th World Rabbit Congress, Verona, Italy.* pp. 79-84.

Graham, J.D. and Clarke C.L. (1997). Physiological action of progesterone in target tissues. *Endocr. Rev*., 18(4): 502-519.

Gutierrez-Sagal, R., Perez-Palacios, G., Langley, E., Pasapera, A.M., Castro, I., Cerbón, M.A. (1993). Endometrial expression of progesterone-receptor and uteroglobin genes during early-pregnancy in the rabbit. *Mol. Reprod. Dev*., 34(3): 244–249.

Heaton, M.P. (2000). SNP frequency and haplotype diversity in beef cattle cytokine genes. *VIII Conference of Plant & Animal Genome*. San Diego, CA, USA.

Helal, M. A. Y., Mahboub, H. D., Hemeda, S. A., Ballal, S. S. E., Heikal, H. S. (2014). Polymorphism of bone morphogenetic protein receptor-IB (BMPR-IB) gene with litter size and kids growth of some goat breeds in Egypt. *Alexandria Journal of Veterinary Sciences*. 41 (1): 28-34.

Iraqi, M.M., Afifi, E.A., Baselga, M., Khalil, M. H. and García M. L. (2008). Additive and heterotic components for post-weaning growth traits in a crossing project of V-line with Gabali rabbits in Egypt. *In Proc. 9th World Rabbit Congress, Verona, Italy*.131-136.

Kalinowski, S.T., Taper, M.L. and Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* .16: 1099-1106.

Khalil, M. H. (1999). Rabbit genetic resources of Egypt. *Animal Genetic Resources Information*. 26: 95-111.

Khalil, M. and Baselga, M. (2002). Rabbit Genetic Resources in Mediterranean Countries. *Options Méditerranénnes, Series B, Nº 38*, pp 262, Spain. http://library.wur.nl/WebQuery/clc/1681160

Marsjan, P.A. and Oldenbroek, J.K. (2007). Molecular markers, a tool for exploring genetic diversity. In: Rischkowsky, B., Pilling, D. (eds) *The State of the World`s Animal Genetic Resources for Food and Agriculture, section C, part4, FAO, Rome, Italy*. 359-379**.**

Minarovič, T., Trakovická, A., Rafayová, A., Lieskovská, Z. (2010). Animal species identification by PCR–RFLP of cytochrome b. *Animal Science and Biotechnologies*, 43 (1): 296-299.

Missohou, A., Talaki, E., Laminon, I.M. (2006). Diversity and genetic relationships among seven west African goat breeds. *Asian Australian J. Anim.Sci.*, 19: 1245-1251.

Othman, O.E., Nowier, Amira M., Abd El-Kader, Heba A., Abd El-Aziem, Sekena H. and Youssef, Y.M.K. (2015). Genetic characterization of fibroblast growth factor-5 gene in rabbits. *Global Veterinaria*. 14 (4): 453-458.

Peakall, R. and Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology* Notes. 6: 288- 295.

Peiró, R., Merchán, M., Santacreu, M. A., Argente, M. J., García, M. L., Agea, I., Blasco, A. (2006). Candidate genes for reproductive traits in rabbits: II. Progesterone receptor gene. *Proceeding of 8th World Congress on Genetics Applied to Livestock Production.* 13-18.

Peiro, R., Mercha´n, M., Santacreu, M.A., Argente, M.J., Garcı´a, M.L., Folch,J.M., Blasco, A. (2008). Identification of single-nucleotide polymorphism in the progesterone receptor gene and its association with reproductive traits in rabbits. *Genetics*. 180: 1699–1705.

Rafayová, A., Lieskovska, Z., Trakovicka, A., Kováčik, A. (2009). Detection of MSTN polymorphism in rabbit. *Scientific Papers Animal Science and Biotechnologies. 42(2), 637-641*.

Raymond, M. and Rousset, F. (1995). GENEPOP (version 1.2): population Genetics Software for Exact Tests and Ecumenicism. *J Hered*., 86 (3): 248-249.

Sahwan, Ferial M., El-Sheik, A. I., Sharaf, M.M. and El-Nahas, Abeer F. (2014). Genetic polymorphism in growth hormone receptor gene (*GHR*) and its relationship with growth trait in pure and hybrid rabbit breeds. *Alexandria Journal of Veterinary Sciences*. (43): 45-51.

SAS (2002). SAS Version 9.0 (TS M0), SAS Institute Inc., Cary, NC, USA. http://ftp.sas.com/techsup/download/hotfix/90_updates.html

Shevchenko, E.A. (2015). Molecular genetic analysis as component of organization selection process in rabbit breeding. Розведення і генетика тварин. 50: 177-183.

Syvänen, A.C. (2001). Accessing genetic variation genotyping single nucleotide polymorphisms. *Nature Reviews Genetics*. 2: 930–941.

Wang J., Li, G., Elzo M.A., Yan L., Chen, S., Jia, X., Lai, S. (2015). A novel single nucleotide polymorphism of the *POU1F1* gene associated with meat quality traits in rabbits, *Annals of Animal Science*. 15 (3): 611–620. DOI: 10.1515/aoas-2015-0015

White, P.S., Kwok, P., Oefner, P., Brookes, A.J. (2001). 3rd Int. Meeting on Single Nucleotide Polymorphism and Complex Genome Analysis: SNP's: 'Some Notable Progress'. *Eur. J. Hum. Gen.*, 9: 316-318.

Yang, Z. J., Fu, L., Zhang, G. W., Yang, Y., Chen, S. Y., Wang, J., Lai, S. J. (2013). Identification and association of SNPs in *TBC1D1* gene with growth traits in two rabbit breeds. *Asian-Australasian Journal of Animal Sciences.* 26 (11): 1529.