Association of PRLR, IGF1 and LEP genes polymorphism with milk production and litter size in Egyptian Zaraibi goat

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Research Article

Keywords: PRLR, IGF1, LEP, Goat, Polymorphism, SNP, Milk Production, Litter size

Posted Date: April 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1562772/v1

Abstract

This study aimed to detect specific nucleotide polymorphisms in Prolactin receptor (PRLR), Insulin like growth factor (IGF1) and Leptin (LEP) genes and their correlation with milk production (MP) and litter size (LS) traits in Zaraibi goat. PCR-SSCP products of different patterns of each gene were sequenced and aligned to reveal two single nucleotide polymorphisms (SNPs) (T>C) and (G>A) in 3'UTR of PRLR gene and registered with accession numbers OM418863 for TT and OM418864 for CT, while (G>A) SNP was registered as OM418861 for GG and OM418862 for AG in exon 10. TT, CT, AG and GG genotypes were distributed in the studied animals with frequencies 0.43, 0.57, 0.65 and 0.35, respectively. While alleles C, T, A and G frequencies were 0.28, 0.72, 0.32 and 0.68, respectively. CT and AG genotypes associated significantly (P<0.05) with higher MP and LS respectively. By studying the haplotypes of PRLR, C-A and T-A were associated with the highest and the lowest level of MP respectively. For LS, T-A and C-G showed significant correlation with the highest and the lowest rate respectively. Regarding IGF1 gene, two SNPs were detected; T74C at exon 4 which registered as (OM418860), and combined SNPs as ins. G470, A531G and T534C (PP genotype) at 5' flanking region that registered as (OM418859). For LEP, only one SNP was found in intron 2 (G281A) which submitted to GenBank as (OM418855). All detected SNPs have shown to be involved in regulating the MP or LS as reproductive traits in goat.

Introduction

Goats are among the first domesticated farm animals, due to their great adaptability to different environmental conditions, therefore they can be raised in arid, humid, tropical, cold, desert or mountain conditions (Kaliber et al., 2016). Goats are considered as very useful animals for their good productivity and easy to handle, moreover, they do not compete with man for food. In Egypt, there are five indigenous goat breeds: Baladi (primarily in the delta), Barki (in the west desert), Zaraibi (northeast of the delta), Sinaoy (in Siani peninsula) and Saidi (in Upper Egypt). Zaraibi goat is a dual-purpose animal and considered as being the most promising dairy goat among the local Egyptian breeds due to its high genetic potential for prolificacy and milk production (Dowidar et al., 2018).

Although milk production potential of goat dams is highly associated with their kids' growth and survival, litter size (LS, numbers of kids born per doe) is also a very important factor determining the reproductive efficiency of the farm animals and has a highly significant influence on goat prolificacy (Tesema et al., 2020). For Zaraibi goat, litter size was ranged from 2.1 and 2.14 kids per doe (Abu El-ella et al., 2011 and Aboul-Naga et al., 2012), while total milk yield (TMY) was ranged from 249 to 363.15 kg/h (El-Saied et al., 2007, Abdelhamid et al., 2013).

As milk production and reproduction are complex traits (i.e. controlled by many genes and environmental factors), some nucleotides polymorphism might account for large amounts of genetic variation. Therefore, selection programs using specific genetic markers could be a good strategy for precise and improving genetic changes of these traits (Bhowmik et al., 2019). Genes attributed with different

economic traits including growth, reproduction, meat and milk production traits as well as disease resistance trait is known as candidate genes (Supakorn, 2009).

Prolactin plays an important regulatory function in mammary gland development. It is an anterior pituitary peptide hormone and is essential for reproductive performance. The action of this hormone is mediated by its receptor encoded by prolactin receptor gene (PRLR), which is a member of growth hormone/prolactin receptor gene family (Ahlawat et al., 2015). In goats, PRLR gene was mapped on chromosome 20 and consists of five exons and four introns, encoding the 199-amino-acid for mature protein (Hayes et al., 1996). PRLR is considered as an excellent candidate for linkage analysis of quantitative trait loci (QTL) affecting milk production traits (Shi et al., 2011).

Zhang et al. (2007) sequenced 315 bp fragment of exon 10 of PRLR gene in Chinese goat breeds and revealed one mutation (143A→G), this mutation caused a change in amino acid sequence (Met→Val), and had a significant effect on litter size, where goats with genotype AG had more kids than those with genotype AA. Also, Wu et al. (2014) detected SNPs at exon 10 significantly associated with litter size in Lezhi black goats. In a study using SSCP technique, Ran et al. (2011) revealed significant association between three SNPs in intron 1 and 2 of PRLR gene and litter size in four Chinese goat breeds. In addition, Hou et al. [16] found two SNPs at exon 9 of PRLR gene which caused a change in amino acid sequence Ser485Asn and Val548 Met. These SNPs were significantly associated with litter size and milk production in Boer and Guanzhong goat breeds. Moreover, they detected SNPs in 3’ UTR that showed a significant effect on milk production in the same breed (Hou et al., 2014b).

Insulin-like growth factor 1 (IGF1) is considered as a strong candidate gene for reproductive traits (Gobikrushanth et al., 2018). In goats, IGF1 gene was mapped on chromosome 5 (Naicy et al., 2017), and consists of 1–6 exons in different species (Andrade et al., 2008), that produce a polypeptide of 70 amino acids that are highly conserved (Ge et al., 2001). According to polymorphism studies, Tahmoorespur et al. (2009) found a significant association between polymorphism in 5’ flanking region of ovine IGF-1 and average daily milk yield (ADMY). Moreover, Naicy et al. (2016) detected two SNPs (g.224A > G and g.227C > T) in low prolific Attappady Black and high prolific Malabari goat breeds, where three genotypes (PP, PQ and QR) have been observed. PQ genotype showed (A→G transition at 224th position), while QR genotype showed two SNPs, one SNP as PQ genotype and the other at 227bp (C→T transition). There was a significant association between the three IGF1 genotypes and litter size, but genotypes PQ and QR revealed a greater number of born kids than homozygous PP genotype. In local goat breeds (Zaraibi, Baladi and Barki), Othman et al. (2016) have detected a SNP (C/G) at position 282 bp that has a significant effect on growth trait. Recently, Lestari et al. (2020) identified SNP (g5752G→C) at intron 4 of IGF-I gene in Kejobong goats, where two genotypes GG and CC found to be significantly associated with body weight. In addition, Sebastiano et al. (2020) found two SNPs at 5’ UTR and exon 3 of IGF-1gene showed a significant association with reproductive traits and milk production in Sarda dairy sheep.

Leptin (LEP) is synthesized mainly by adipose tissue; it plays the main role in regulation and control of productive performance of animals (Kumar et al., 2020). LEP is synthesized as a pro hormone (premature

and inactive form of leptin protein), it consists of 167 amino acids, but their mature functional polypeptides consist of 146 amino acids (Marwarha et al., 2012). It is located on chromosome 4 in the ovine genome and consists of three exons and two introns, two exons only are translated into protein (Wallace et al., 2014). Exon1 is a non-coding part (Buchanan et al., 2002).

Singh et al. (2009) found 5 haplotypes in exon 2 and 6 haplotypes in intron 2 of LEP gene in Indian goats. Maitra et al. (2014) found non-synonymous (g.1029T > C) mutation, where amino acid Valine has changed to Alanine in 7 breeds of Indian goat. Another study on exon 3 of LEP gene in Barki sheep found one SNP which has a significant association with milk production (Abousoliman et al., 2020). Therefore, this study was designed to detect SNPs of PRLR, IGF-1 and LEP genes in Zaraibi goat using single-strand conformational polymorphism (PCR–SSCP) analysis and sequencing technique to investigate the potential associations between these polymorphisms with milk production and litter size.

Materials And Methods

Chemicals

All chemicals used were of analytical grade and were purchased from Sigma scientific services co. and Promega (Cairo, Egypt). All reagents were used according to the necessary health and safety procedures. The molecular kits are listed elsewhere.

Animals And Ethical Considerations:

One hundred Zaraibi does (mature goat females) reared in El-Serw experimental station (In Damietta governorate) belonging to Animal Production Research Institute (APRI), Agriculture Research Center (ARC). Does were selected according to their parities (kidding seasons) from 2nd to 5th, and (24–45 kg) body weight at mating. Animals were fed according to Nutrient Requirements of Goat (NRC, 2007). Zaraibi goats were supplied by a basal ration consisting of 25% concentrate feed mixture (CFM) beside 75% fresh berseem (Egyptian clover) during winter feeding, or 50% CFM and 50% berseem hay during summer feeding. Milk yields of all does were recorded during lactation season for daily milk yield (DMY) and total milk yield (TMY) productions. Number of kids born for each doe was recorded for each season to calculate litter size.

Handling and protection of animals used in study were done according to the recommendations of European Union directive 86/609/EEC (Louhimies, 2002), and approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Cairo University, Egypt with Permit Number: CU/I/F/47/18.

DNA Extraction and PCR Amplification

Ten ml of total blood were collected from the jugular vein of each goat using vacuum tubes containing 7.5 mg of EDTA. All blood samples were stored at − 80°C until DNA extraction. Genomic DNA was extracted from all collected blood samples using salting out method as described by (Miller et al., 1988). The concentrations and purity of the extracted DNA were measured using spectrophotometer (Eppendorf Biophotometer plus). PCR Amplification was performed using Bio Rad thermal cycler (model C1000) according to primer condition (Table 1), using primers list for amplification of PRLR, IGF-1 and LEP genes (Table 2). PCR amplicons were electrophoresed in 1% agarose gels, using 1X TBE buffer containing 200 ng/ml of ethidium bromide, then visualized under UV light and photographed by digital camera.

Table 1
PCR conditions of the primer sets of PRLR, IGF-1 and LEP genes

Character	Initial Denaturation	Cycles	Denaturation	Annealing	Extension	Final cycle
For milk production PRLR-M	5 min at 95°C	35 cycles	94°C for 30 s	50°C for 30 s	72°C for 35 s	10 min at 72°C
For litter size PRLR-L	5 min at 94°C	32 cycles	94°C for 30 s	55°C for 30 s	72°C for 30 s	10 min at 72°C
For milk production IGF1-M	5 min at 96°C	35 cycles	94°C for 40 s	50°C for 40 s	72°C for 40 s	10 min at 72°C
For litter size IGF1-L	2 min at 96°C	35 cycles	95°C for 30 s	61°C for 20 s	68°C for 60 s	5 min at 68°C
For milk production LEP-M	2 min at 94°C	35 cycles	94°C for 1 min	55°C for 1 min	72°C for 1 min	15 min at 72°C
For litter size LEP-L	2 min at 95°C	30 cycles	95°C for 1 min	55°C for 1 min	72°C for 1 min	7 min at 72°C

Single Strand Conformation Polymorphisms (SSCP)

SSCP analysis detects the changed migration rate of DNA molecules due to sequence-dependent differential intramolecular folding of ssDNA under non-denaturing gel electrophoresis conditions (Orita et al., 1989). Five microliter aliquot of each amplicon was mixed with 5 µl of T.E. buffer, and 5µl of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue and 0.025% xylene cyanol). After denaturation at 95^0C for 10 min, samples were rapidly cooled on wet ice for 10 min., Electrophoresis was carried out in 20*15 cm, 10% acrylamide: bisacrylamide (29:1) gels (Gasser et al., 2006) at 150 V. for 4

hours then stained with ethidium bromide, visualized under UV light, and photographed by Bio-Rad Laboratories, Hercules, CA, USA.

Table 2
Primers used for amplification of PRLR, IGF1 and LEP genes. (M: Milk production & L: Litter size)

Gene name	Primer name	Sequence (5'-3')	PCR product size	Reference
Prolactin receptor (PRLR)	PRLR-M –F	AGTGAGAGTTATGGAAGGATG	443bp	Hou et al., 2014b
	PRLR-M–R	AAGGTTAAGCAACTGGTCTT		
	PRLR-L –F	AAACCCCCTTGTTCTCTGCTA	315bp	Zhang et al., 2007
	PRLR-L –R	CCCAACCCAACTGGAGTCTGC		
Insulin like growth factor-1 (IGF-1)	IGF1-M –F	GCTGGGTGTAGCAGTGAACA	320bp	Deng et al., 2010
	IGF1-M –R	GTTGCTTCAGAAGCATAACT		
	IGF1-L –F	GGGTATTGCTAGCCAGCTGGT	601bp	Naicy et al., 2016
	IGF1-L –R	CCGGGCATGAAGACACACACAT		
Leptin (LEP)	LEP-M –F	TGGAGTGGCTTGTCATTTCCTTCT	400bp	Singh et al., 2009
	LEP-M –R	GTCCCTGCTTCTGGCCACCTAACT		
	LEP-L –F	AGCAGTCCGTCTCCTCCAAA	152bp	Singh et al., 2009
	LEP-L –R	AGATATTTGGATCACATTTCTG		

Sequence analysis

PCR products representing different SSCP patterns of PRLR, IGF-1and LEP genes were purified and sequenced by automated DNA ABI Prism 3130 Genetic Analyzer using the same sets of primers used for PCR (Sanger et al., 1977). The nucleotide sequences were compared against the corresponding goat gene sequence (GenBank Accession Number: KC109741.1 and EU662222.1 for PRLR, KX432967.1 and KT315919.1 for IGF-1 gene and EU220012.1 and MH716185.1 for LEP) and analyzed by Cluster omega

and Jalview sequence alignment editor program 2.11.1.6. The nucleotide sequences of new gene variants were submitted to NCBI.

Statistical analysis

Analysis of variance and least squares means were calculated using the General Linear Model (GLM) procedure of SAS (2004). Three models were performed; the first one was used to estimate the effect of all parameters (parity, breeding season, PRLR-M genotypes, PRLR-L genotypes and haplotypes PMPL) on ADMY, while the second and third models were explored the effect of same parameters on TMY and litter size, respectively.

$Y_{ijklmn} = \mu + P_i + S_j + PM_k + PL_l + PMPL_m + e_{ijklmn}$

Where,

μ = the overall population mean,

Y = the observed records of ADMY, TMY and LS.

P_i= the fixed effect of i^{th} parity of does (i = 1,……5),

S_j= the fixed effect of j^{th} breeding season (j = Autumn, spring)

PM = the fixed effect of k^{th} PRLR-M genotypes (k = CT, TT)

PL = the fixed effect of l^{th} PRLR-L genotypes (l = AG, GG)

PMPL = the fixed effect of m^{th} haplotypes of PRLR-M and PRLR-L (m = C-A, C-G, T-A, T-G)

e_{ijklmn}= Random error.

Genetic equilibrium was estimated according to the Hardy-Weinberg equilibrium (HWE) and Chi-square test using Michael [40] calculator.

Results

A total 100 Zaraibi does genotyped for polymorphisms in 3'UTR and exon 10 in PRLR, exon 4 and 5' flanking region in IGF-1 and intron 2 and exon 3 in LEP. The PCR products successfully amplified as follow: 443 bp fragment of 3'UTR flanking region 1-443bp and 315 bp of exon10 of PRLR gene flanking region 424–738 bp (GenBank Accession Number: KC109741.1 and EU662222.1 respectively). For IGF-1 gene, 320 bp fragment of exon 4 flanking region 1-320 bp and 601 bp of 5'flanking region 1-601 bp (GenBank Accession Number: KX432967.1 and KT315919.1, respectively) while, 400 bp of intron 2 flanking region 1-400 bp and 152 bp of exon 3 flanking region 779–930 bp of LEP gene (GenBank Accession Number: EU220012.1 and MH716185.1 respectively).

Pcr-sscp Analysis

Regarding PRLR gene, PCR SSCP analysis revealed two genotypes (TT and CT) for 3'UTR region with frequencies 0.43 and 0.57, respectively and allelic frequencies 0.28 and 0.0.72 for C and T alleles, respectively (Fig. 1a) (Table 3). Another two genotypes (AG and GG) were observed at exon 10 with frequencies 0.65 and 0.35, respectively and allelic frequencies 0.32 and 0.68 for A and G alleles, respectively (Fig. 2a) (Table 3). For IGF-1 gene, two genotypes were detected as CC genotype (for exon 4 (Fig. 3a)) and PP (5' flanking region (Fig. 4a)) which contains three SNPs ins. (G) at position 470, A531G and T534C. However, in LEP gene, only AA genotype was observed in intron 2 and there is no variation in exon 3(Fig. 5a).

Genetic equilibrium was estimated based upon the Hardy Weinberg equilibrium and Chi-square test using. The obtained results showed that genotype distributions of two locus in PRLR gene disagree with Hardy-Weinberg equilibrium ($P < 0.05$) for Zaraibi goat breed which prove that the genotypic frequencies had been affected by selection and mutation (Table 3).

Table 3
Genotype and Allelic frequencies of the PRLR locus in Zaraibi goats

	Genotypes frequencies		**Allelic frequencies**			
PRLR-M	CT	TT	C	T	χ^2	P-value
	0.57 (57)	0.43 (43)	0.285	0.725	15.89	P < 0.01
PRLR-L	AG	GG	A	G		
	0.65 (65)	0.35 (35)	0.32	0.68	23.18	P < 0.01

CT, TT, AG and GG genotype frequencies was at the PRLR locus; n = 100 Zaraibi does; genotypes and alleles frequencies were assessed according to Hardy-Weinberg equilibrium (HWE) and χ^2, chi-square value. The number of animals per genotype is indicated in parentheses.

Sequencing Analysis

After sequencing 3'UTR in PRLR gene, one SNP was detected with nucleotide transversion from Thymine (T) to cytosine (C) at position 62 when compared with PRLR gene (accession number: KC109741.1) and indicated in our sequence deposited in the nucleotide database with the accession numbers OM418863 and OM418864 (Fig. 1(b, c). For exon 10, transition from Guanine (G) to Adenine (A) at position 144 was found by comparing it with PRLR gene (acc. No. EU662222.1) and indicated in our sequence deposited in the nucleotide database with the accession numbers (OM418861 and OM418862) this SNP resulted in an amino acid change from Valine (V) to Methionine (M) (Fig. 2 (b, c)).

For IGF1, after comparing nucleotide sequence with IGF1 gene (acc. No.KX432967.1) one point mutation was observed in exon 4 at position 74 from T to C, this SNP submitted to GenBank with accession number (OM418860) which led to change of amino acid from Serine (S) to Proline (P) (Fig. 3b). Another three SNPs (ins. (G) at position 470, A531G and T534C) were found in 5' flanking region by comparing the sequence with acc. No. (KT315919.1) and registered in GenBank with accession number (OM418859) (Fig. 4b).

However, LEP gene had one SNP (G281A) in intron 2 when compared with GenBank (acc.no. EU220012.1) and submitted to GenBank with accession number (OM418855) (Fig. 5 (b, c)). On the other hand, exon 3 didn't has any variation from sequence on GenBank (acc. No. MH716185.1). All previous SNPs have been recorded for the first time in this study and submitted to GenBank as indicated previously.

Effect Of Prlr Genotypes On Milk Production And Ls

As shows in table (4) at 3'UTR of PRLR gene (PRLR-M), transition of (T > C) at position 62 has highly significant effect on ADMY and TMY. CT genotype has higher ADMY (0.86 ± 0.03 kg) and TMY (211.88 ± 6.8 kg) than TT (0.79 ± 0.03 and 182.15 ± 7.6, respectively). However, in exon 10 (PRLR-L), the A > G mutation at position 144 Significantly affected the LS of does, where AG genotype has higher LS (2.10 ± 0.1 kids) than GG genotype (1.79 ± 0.1 kids).

Table 4: Effect of PRLR genotypes on average daily, total milk yield and litter size

Genotypes	Average daily milk yield (kg/ day)	Total milk yield (kg)	Litter size (kids)
PRLR-M			
CT	0.86±0.03 [a]	211.88±6.8 [a]	2.04±0.1[a]
TT	0.79±0.03 [b]	182.15±7.6 [b]	2.10±0.1[a]
PRLR-L			
AG	0.84±0.03 [a]	203.46±7.2 [a]	2.10±0.1[a]
GG	0.83±0.03 [a]	212.46±8.3 [a]	1.79±0.1[b]

Results were expressed as least-squares means (LSM) ± standard error (SE); Mean values marked with the different letter are different (significant, *P* < 0.05).

Table 5: Effect of haplotype on ADMY, TMY and LS

	Average daily milk yield (kg/ day)	Total milk yield (kg)	Litter size (kids)
C-A	0.87 ± 0.03 [a]	220.64 ± 7.0 [a]	2.10 ± 0.1[a]
C-G	0.84 ± 0.03 [ab]	217.56 ± 8.5 [a]	1.76 ± 0.1[b]
T-A	0.73 ± 0.03 [b]	181.75 ± 7.9 [b]	2.11 ± 0.1[a]
T-G	0.78 ± 0.04 [b]	187.21 ± 9.4 [b]	1.84 ± 0.1[b]
Results were expressed as least-squares means (LSM) ± standard error (SE); Mean values marked with the different letter are different (significant, $P < 0.05$).			

For haplotype distribution frequencies, it was found that C-A is the most frequent haplotype by 35%, followed by T-A (30%), then C-G (22%), and T-G is the least frequent (13%). Meanwhile, these haplotypes had significant effect on ADMY, TMY and LS ($P < 0.01$), the results indicated that C-A has the highest level of ADMY and TMY (0.87 ± 0.03 and 220.64 ± 7.0, respectively), while T-A has the lowest level (0.73 ± 0.03 and 181.75 ± 7.9). However, for litter size, T-A has the highest level (2.11 ± 0.1 kids) and C-G was the lowest one (1.76 ± 0.1 kids) (Table 5).

Effect Of Parity And Breeding Season On Admy, Tmy And Ls

The overall means of ADMY, TMY and LS were 0.80 kg, 198.7 kg and1.96 kids, respectively. ADMY, TMY and LS of Zaraibi does were affected significantly by parity ($P < 0.01$), they showed the lowest value at the first parity (0.74 ± 0.03, 179.62 ± 7.3 kg and 1.76 ± 0.1kids, respectively) and increased gradually until highest-level at the 4th parity (0.87 ± 0.04, 214.14 ± 9.4 kg and 2.11 ± 0.1 kids, respectively) (Table 6). Breeding season significantly affected ($P < 0.05$) the ADMY and TMY, where does produced higher amount of milk in Spring than in Autumn.

Table 6
Effect of parity and breeding season on ADMY, TMY and LS

Items	Average daily milk yield (kg/ day)	Total milk yield (kg)	Litter size
Parity			
1	0.74 ± 0.03^{b}	179.62 ± 7.3^{c}	1.76 ± 0.1^{c}
2	0.85 ± 0.03^{a}	201.35 ± 7.9^{ab}	1.85 ± 0.1^{bc}
3	0.86 ± 0.03^{a}	208.31 ± 8.5^{a}	1.99 ± 0.1^{ab}
4	0.87 ± 0.04^{a}	214.14 ± 9.4^{a}	2.11 ± 0.1^{a}
≥ 5	0.78 ± 0.04^{b}	193.03 ± 10.6^{bc}	2.06 ± 0.1^{a}
Breeding season			
Autumn (November)	0.84 ± 0.03^{b}	197.36 ± 8.0^{b}	1.96 ± 0.1^{a}
Spring (March)	0.90 ± 0.03^{a}	201.22 ± 7.1^{a}	1.94 ± 0.1^{a}
Results were expressed as least-squares means (LSM) ± standard error (SE); Mean values marked with the different letter are different (significant, $P < 0.05$).			

Discussion

During recent years, a great interest is focused on using molecular markers in understanding the animal genome and genetic diversity analysis. Molecular markers have been widely used to assess genetic variations since they provide an information on every region of the genome. In addition, polymorphism in the transcription factor binding sites is important, as nucleotide substitutions may change the level of gene expression (Ge et al., 2001). This study evaluated the correlation of some newly detected SNPs in candidate genes (PRLR, IGF1 and LEP) with productive and reproductive traits for Zaraibi goat.

PCR-SSCP and sequence analysis concluded two genotypes TT and CT in 3'UTR of PRLR gene with significant associated with milk production where CT has a greater milk yield. This is in harmony with findings of Hou et al. (2014b), who found that CC and CT genotypes were significantly associated with milk production, while CC has a greater milk yield in Chinese goat breeds.

Regarding exon 10 in PRLR gene, it has two genotypes AG and GG with significant effect on litter size where AG has a stronger effect than GG, these results are in harmony with that reached by Zhang et al. (2007) who noticed FF, FG and GG genotypes with higher litter size for FG does ($P < 0.05$) in Chinese goat breeds. Also, Wu et al. (2014) investigated exon 10 of PRLR gene and found four genotypes AA, AB, AC, and AD with significant effect on litter size in Lezhi black goats, and he has concluded that PRLR gene expression and mutations in exon 10 of PRLR gene may be associated with the reproductive effects of

goat. Hou et al. (2013) screened four novel SNPs g.40452T > C and g.40471G > A mutations were in the intron 2 and g.61677G > A and g.61865G > A mutations were in the exon 9 in PRLR gene with significant association with milk production traits in Saanen and Guanzhong goat breeds. In addition, Gao et al. (2015) studied polymorphisms at exon 10 of PRLR gene and found three genotypes BB, BC and CC in one locus and DE and DD in another locus in Chinese sheep breedss that didn't significantly affect the litter size.

For exon 4 at IGF-1, one genotype CC was found, this result agreed with Deng et al. (2010) who reported a higher significant effect on milk yield for CC genotype than other ones. In the current study, one genotype PP was observed in 5'flanking region of IGF1 locus. Also, Sebastiano et al. (2020) found that AA genotype in exon 3 showed higher milk yield in Sarda dairy sheep. However, in Markhoz goat, Sebastiano et al. (2020) noticed 3 genotypes GG, AG, and AA with significant association with litter size.

In LEP gene, we recorded one genotype AA in intron 2 and we didn't find any variation in exon 3 between Zaraibi does. On the other hand, three genotypes in exon 3 were found by Abousoliman et al. (2020) with significant association with milk yield in Barki sheep. On the other hand, Bhowmik et al. (2019) proved that reproductive traits of cow were associated with T allele of LEP gene, and 3 genotypes CC, CT, TT were reported with significant effect on litter size.

In the present study, ADMY and TMY were significantly affected by parity ($P < 0.01$) and breeding season ($p < 0.05$), where they increased gradually with the increase of parity until the fourth parity. Means of the ADMY and TMY were higher in March (Spring) compared with November (Autumn) this may be due to good nutrition and availability of Egyptian clover. These findings agreed with Hamed et al. (2009) who found that ADMY and TMY of Zaraibi goat were significantly affected by parity. The same results were estimated by Mohamed (2016), who concluded that milk yield increased with raising of parity number. In addition, litter size was significantly affected by parity ($p < 0.05$), where it progressed from first parity until the fourth parity, may be due to the improvement of reproduction efficiency in the goat farm because of the management system permitted to cull does with low litter size. This result agreed with (Hamed et al., 2009) who reported that year and breading season have a significant effect on litter size in Zaraibi goat. On the contrary, breeding season has no significant effect on Zaraibi goat litter size.

These results provide new insights into crucial role of genetics polymorphism on productive and reproductive performances in goat, where genotype/phenotype association has become an important reference to be used in goat breeding programs.

Conclusion

PRLR, IGF-I and LEP could be considered as a potential candidate gene marker for productive and reproductive traits, their polymorphisms were investigated in dairy and prolific Zaraibi goat. All SNP investigated have shown to be involved in regulating the milk production and reproductive activity. So, these SNPs can be used successfully in planning selection programs to improve the productive and reproductive performances in goat and could be identified before using successful breeding program.

Declarations

Authors' contributions: The study conception and design were performed by Akmal Abdel- Rehiem El-Ghor, Ehab Salah Abdel-Aal, Haidan Moustafa El-Shorbagy and Shaimaa Abdel-Azeem Mohamed. Material preparation, data collection and analysis were performed by Shaimaa Abdel-Azeem Mohamed. The first draft of the manuscript was written by Shaimaa Abdel-Azeem Mohamed and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval: All procedures involving animals were in compliance with the recommendations of European Union directive 86/609/EEC (Louhimies, 2002), and ethical approval was granted by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Cairo University, Egypt with Permit Number: CU/I/F/47/18.

Consent to participate and consent for publication: All authors agreed to participate in this work. They also approved the content of the research and the submission of the manuscript to the Veterinary Research Communication journal.

Acknowledgement

This work was supported by Faculty of Science and Cairo University and Sheep & Goat Research Department, Animal Production Research Institute.

Data accessibility

"All data is provided in full in the results section of this paper."

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Department of Zoology, Faculty of Science, Cairo University

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Figures

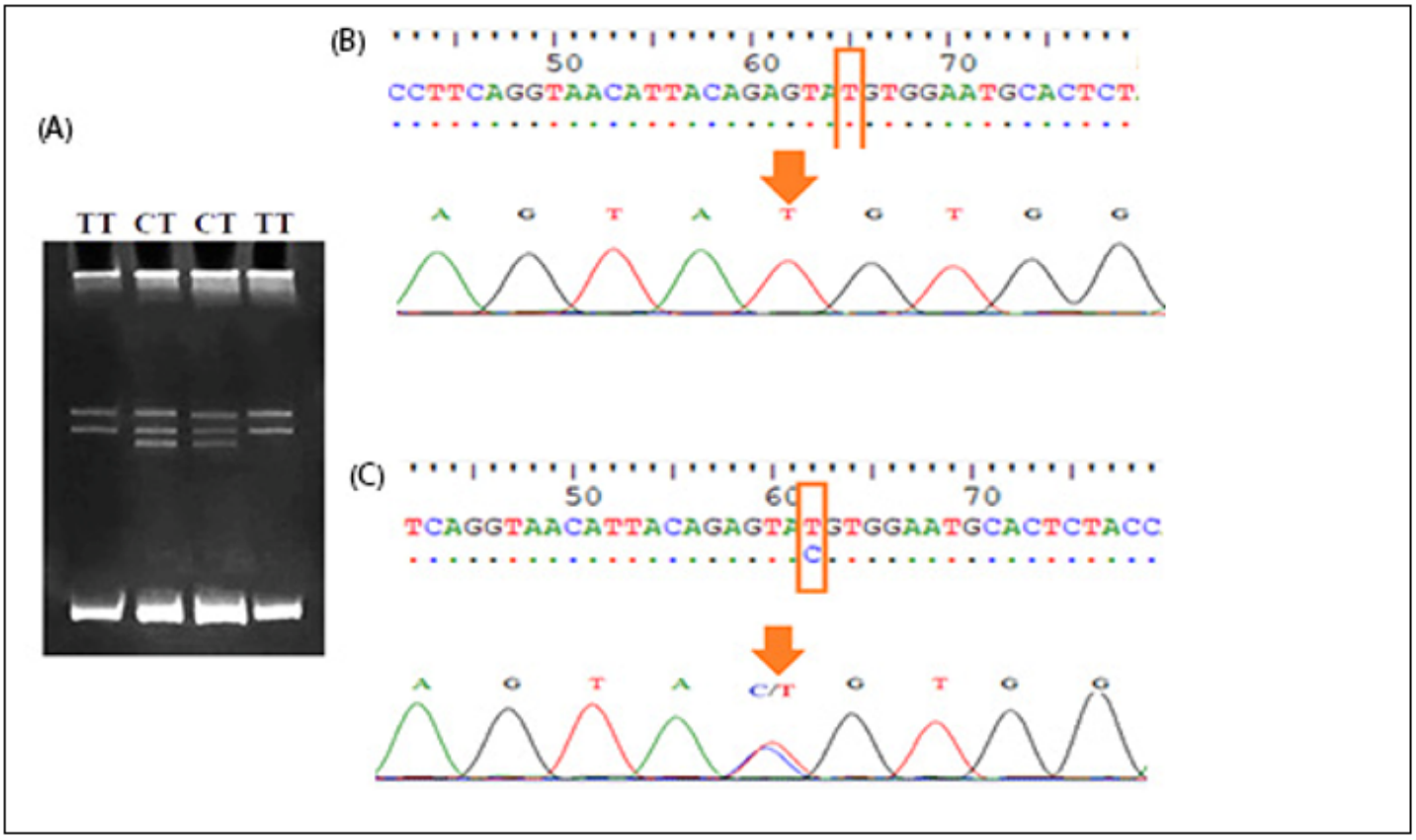

Figure 1

Genotyping of 3’ UTR fragment of PRLR gene. (A) The electrophoretic pattern obtained after SSCP analysis showing homozygous genotype TT (lane 1,4) and heterozygous genotype CT (lane 2,3). (B) DNA sequencing analysis representing TT genotype. (C) DNA sequencing analysis representing CT genotype.

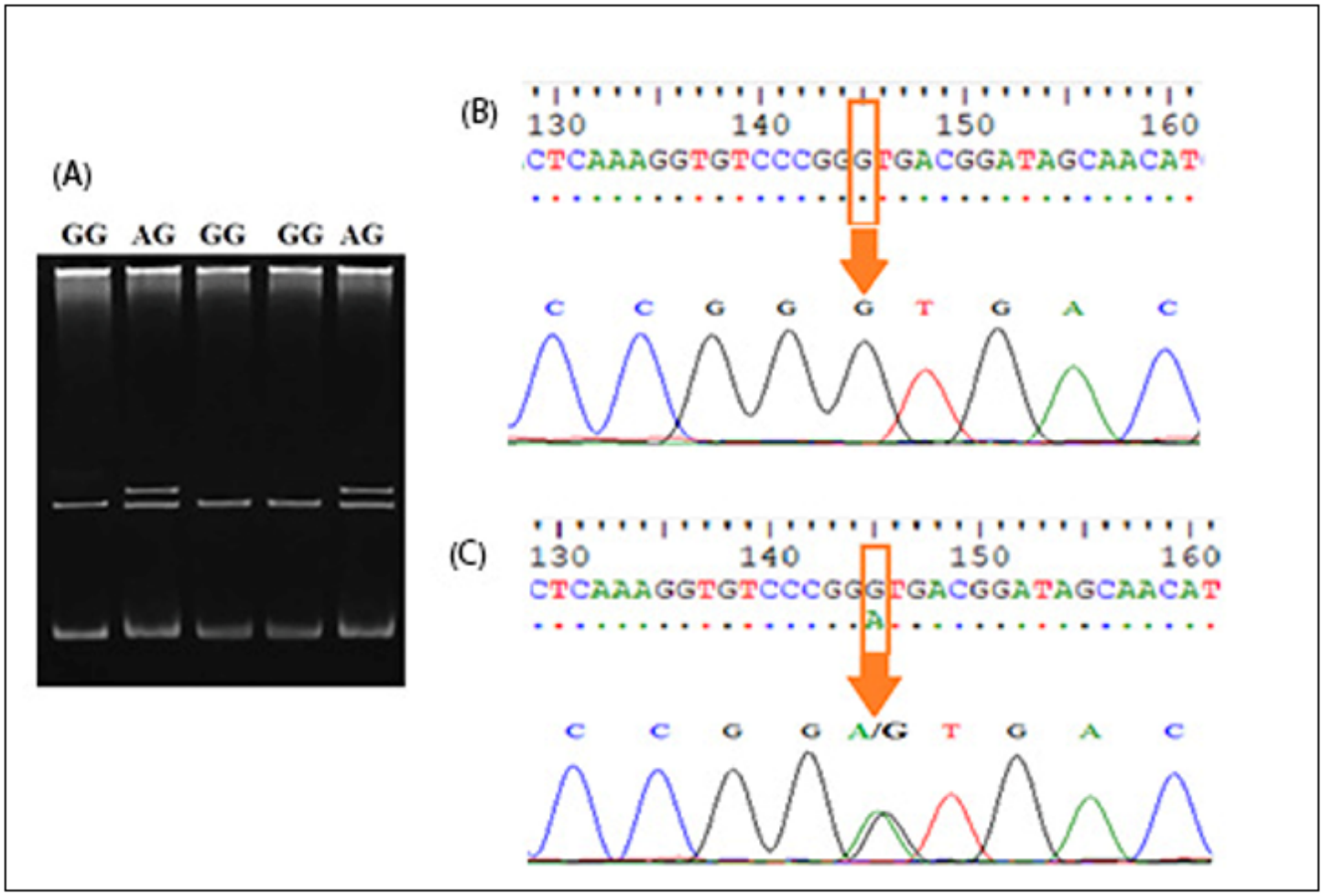

Figure 2

Genotyping of exon 10 region of PRLR gene. (A) The electrophoretic pattern obtained after SSCP analysis showing homozygous genotype GG (lane 1,3, 4) and heterozygous genotype AG (lane 2,5). (B) DNA sequencing analysis representing GG genotype. (C) DNA sequencing analysis representing AG genotype

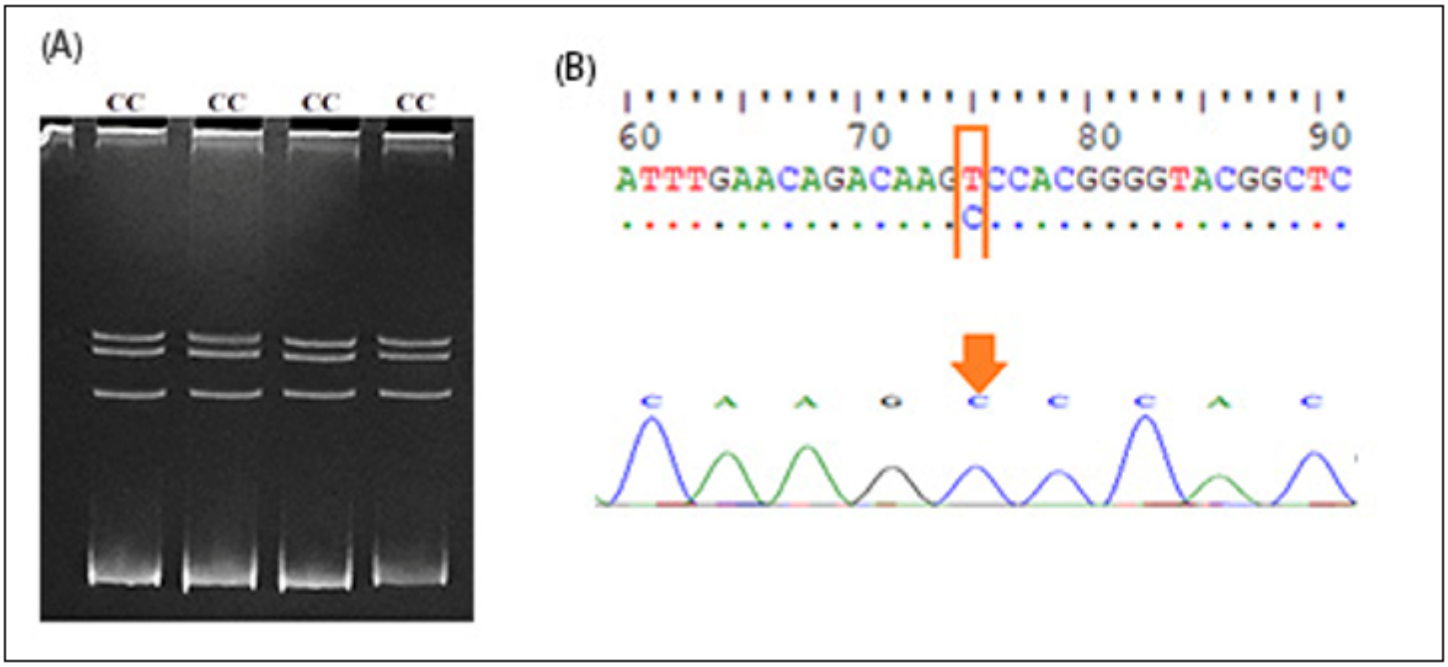

Figure 3

Genotyping of exon 4 of IGF-1 gene. (A) The electrophoretic pattern obtained after SSCP analysis showing homozygous genotype CC (lane 1-4). (B) DNA sequencing analysis representing CC genotype.

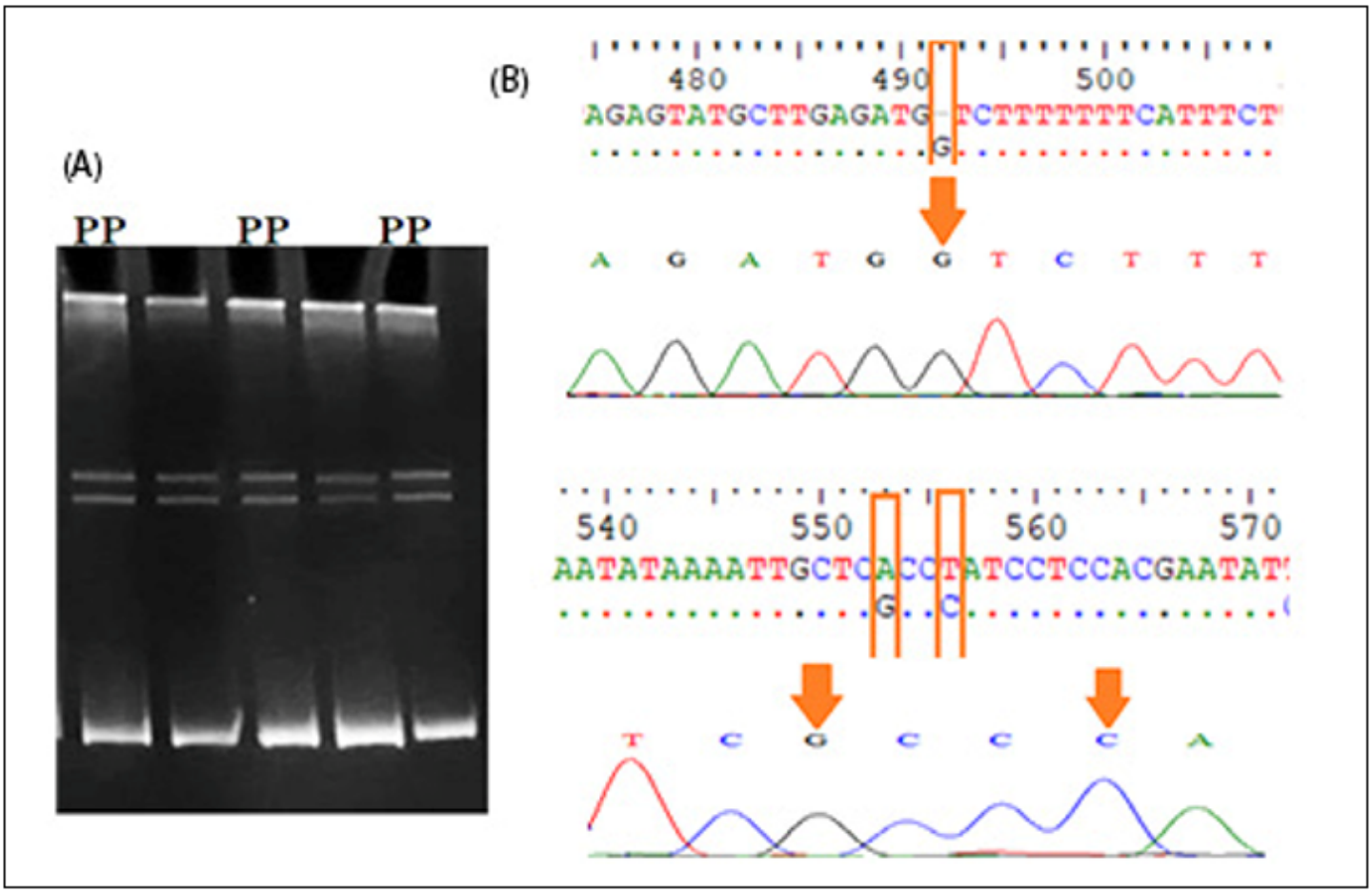

Figure 4

Genotyping of 5’ flanking region of IGF-1 gene. (A) The electrophoretic pattern obtained after SSCP analysis showing homozygous genotype PP (lane 1-3). (B) DNA sequencing analysis representing PP genotype (ins.G at 471, A530G and T533C).

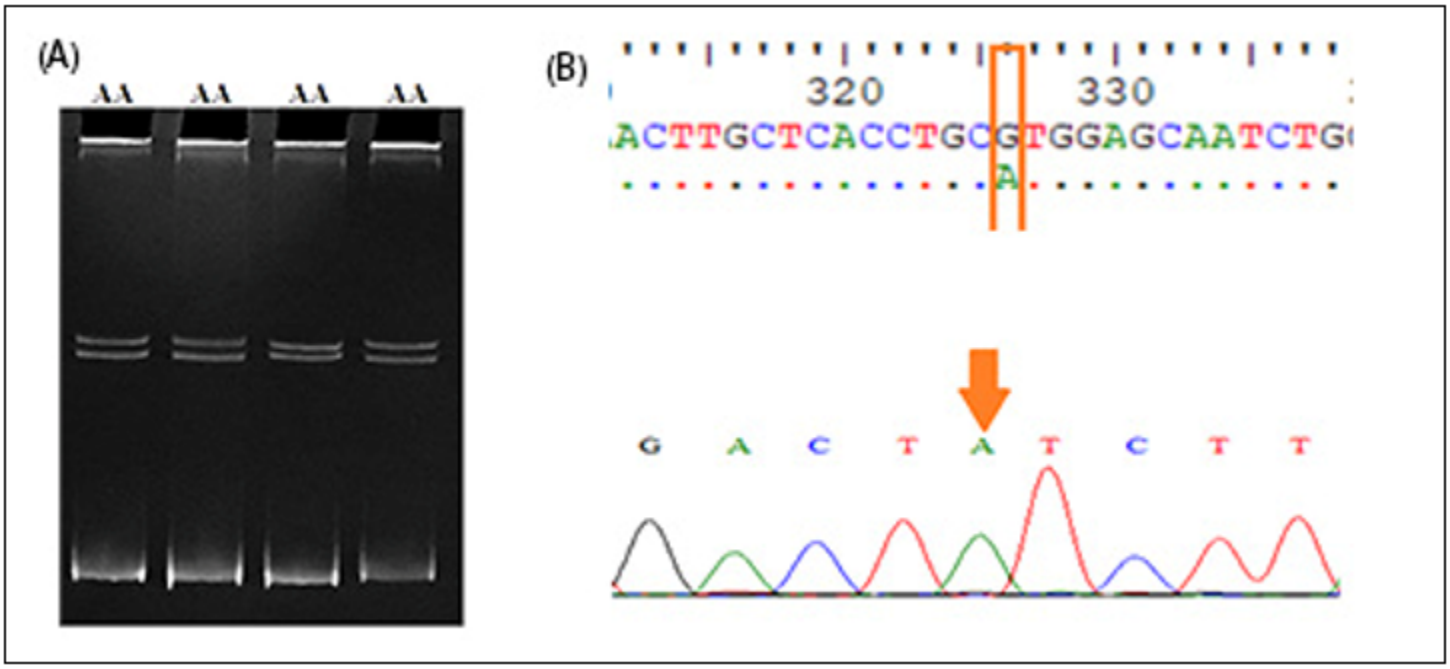

Figure 5

Genotyping of intron 2 region of LEP gene. (A) The electrophoretic pattern obtained after SSCP analysis showing homozygous genotype AA (lane 1-4). (B) DNA sequencing analysis representing AA genotype.