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Original Article

Genetic variability of myostatin and prolactin genes in popular goat breeds in Egypt

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ABSTRACT

The genetic polymorphisms of two functional genes named: myostatin (*MSTN*) and prolactin (*PRL*) were investigated in three goat breeds (Barki, Damascus and Zaraibi) using Sanger nucleotide sequence and restriction fragment length polymorphism (RFLP) methods, in order to differentiate between these breeds. Nucleotide sequencing of 337 bp *MSTN* gene detected five SNPs in Barki breed, two SNPs in Damascus breed, while the Zaraibi breed did not show any SNPs. Moreover, *MSTN-HaeIII*/PCR-RFLP gave a single Genotype BB was found in all the studied breeds. Meanwhile, Nucleotide sequencing of 196 bp *PRL* gene showed two SNPs in Damascus breed, one SNPs in Zaraibi breed, while the Barki breed did not show any SNPs. Moreover, *PRL-Eco24I*/PCR-RFLP showed three genotypes (AA, AB and BB). The genotype AB showed the maximum frequency in all the studied breeds (0.75, 0.85, and 0.90 for Damascus, Barki and Zaraibi breeds, respectively). Observed heterozygosity (Ho) value was higher than expected heterozygosity (He) value all studied breeds. In addition, the values of both Ho and He were the highest in Zaraibi breed (0.90 and 0.51 respectively). Chi-square (χ^2) value revealed a significant variation Hardy-Weinberg equilibrium ($P < .05$) in the three studied breeds. It is the highest in Zaraibi goats and lowest in Damascus breed. The results demonstrated that the *PRL-Eco24I*/PCR-RFLP polymorphism may be utilized as effective marker for genetic differentiation between goat breeds, but *MSTN-HaeIII*/PCR-RFLP revealed no polymorphism or variation, thus it is not recommended in the selection program. Moreover, these results open up interesting prospects for future selection programs, especially marker assisted selection. In addition, the results established that PCR-RFLP method is a suitable tool for calculating genetic variability.

1. Introduction

Genetic variations at candidate genes touching economic traits (like growth, milk yield, meat production and reproductive traits) have stimulated research interest because they stayed well-considered as an aid to genetic selection and to mark evolutionary relationships in different livestock animal breeds [1]. In this aspect, myostatin (*MSTN*) as well as prolactin (*PRL*) are important potential genes due to their positive effect on growth enactment and meat quality traits.

Myostatin (*MSTN*) gene, also named as growing and differentiation factor-8 (*GDF-8*) gene, is coding for converting growth factor-beta (*TGF-β*) super-family (one of the largest protein groups). This gene was physically mapped to goat chromosome 2q11-q12 [2,3], and consists of two introns and three exons [4]. Additionally, it is a powerful candidate gene, needful for growth and development of domestic animals due to its key function in muscularity, and its potential applications in animal farming [5]. Mutations in *MSTN* gene can quiet its expression or create a non-functional protein, which causes undesired muscularity (i.e. dramatic rise in both muscle fiber quantity [hyperplasia] and mass [hypertrophy]) or the "double-muscling" phenomenon in various species [6], such as dogs [7], pig [8], goat [Boer goat] [9] and, sheep [10].

Prolactin (*PRL*) gene plays a key role in changeable growth, variation and lactation, the hair growth cycle [11]. Many researchers recorded that *PRL* polymorphisms are related to wool or cashmere traits in goat and sheep [12–14]. Others studied the association polymorphism of *PRL* with dairy traits like benefit yield besides

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the yield of protein milk in cattle breeds [15–17]. Furthermore, many researcher suggested the significance of *PRL* gene in improving prolificacy in different sheep breeds [18,19].

Goat remains one of main important livestock kinds and affords a variation of products, such as fiber, milk, meat, and hides. Furthermore, goats are used as a model for biomedical studies [20,21]. Goats in Egypt are almost 3.13 million goats; they are extended essentially in three regions: Upper Egypt, Nile Delta besides in the desert rangelands [22]. There are five indigenous goat breeds: Baladi (local breed in Delta), Barki or Sahrawi (local breed in Desert), Sinaoy (Bedouin), Saidi and Zaraibi (or Egyptian Nubian). They are duple – purpose animals, with does breed intended for milk and bucks bred for meat [23].

The aim of the present study is to screen the genetic polymorphism of two functional genes (*MSTN* and *PRL*) in three goat breeds (Barki, Damascus and Zaraibi) via nucleotide sequence and PCR-RFLP methods in order to differentiate between these breeds.

2. Materials and methods

2.1. Animals

A total of 60 healthy goats, belonging to the three breeds understudy: Barki, Damascus and Zaraibi; 20 samples from each breed. All animals were born and reared in the Agriculture Research Station, belonging to Faculty of Agriculture, Cairo University.

2.2. DNA extraction

Blood samples were collected in tubes containing 0.5 M EDTA as anticoagulant and transported to the laboratory under cooled conditions. Genomic DNA was extracted and purified from whole blood collected samples using the salting out technique described by [24]. The DNA concentration was measured using the U.V spectrophotometer at wavelength 260 nm.

2.3. Polymerase Chain Reaction (PCR)

Two pairs of primers were used for amplifying each of *MSTN* and *PRL* loci using primers suggested by [25,26], respectively. The primer sequences are represented in Table 1. Amplification reaction was carried out in a 25 μl volume containing 100 ng genomic DNA, forward and reverse primer (both at concentration 10 pmol/μl), 1U *Taq* polymerase, 2.5 μl *Taq* polymerase buffer, four dNTPs (each at final concentration of 2.5 mM/μl) and sterile de-ionized double distilled H_2O up to a total volume of 25 μl. Amplification conditions are shown in Table 2. The gels were stained with ethidium bromide and visualized under ultraviolet light.

2.4. Restriction Fragment Length Polymorphism (RFLP)

It was carried out in15 μl of reaction mixture of each sample containing 5 μl of PCR product, 9.5 μl of 10 X buffer and 0.5 μl of fast digest restriction enzyme (MBI fermentas, Germany) specific for each gene (Table 1). The reaction mixture was incubated at 37 °C in water bath for a certain time as demonstrated in Table 1. Digestion products were separated by electrophoresis on 2.5% agarose gel, stained with ethidium bromide. The bands were visualized under UV light and the gels images were captured using digital gel documentation system (Bio-Rad, USA).

2.5. DNA sequencing

The PCR products representing different patterns and alleles of tested genes were purified and sequenced by Macrogen Incorporation (Seoul, South Korea) to identify the SNPs between different patterns and alleles. Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite.

2.6. Statistical analysis

The genotypic and allelic frequencies, the observed and expected heterozygosity and the χ^2 test for Hardy-Weinberg equilibrium (HWE) were calculated using Pop Gene 32.1 package [27].

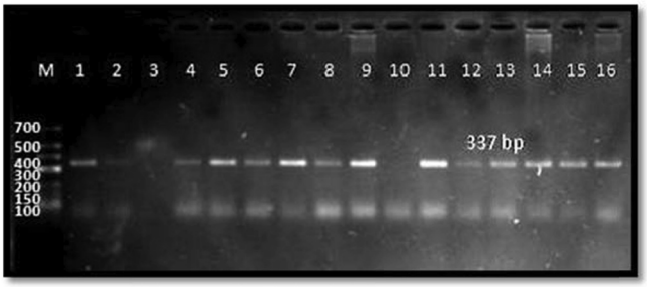

Fig. 1. Agarose gel electrophoresis of *MSTN*-PCR fragment (337 bp). Lane M, 25 bp DNA ladder. Lanes (1, 2, 4, 5, 6), Barki (7, 8, 9, 11), Damascus and Lanes (12, 13, 14, 16) Zaraibi breed.

Table 1
Gene, primer sequence (5′ → 3′), length of PCR product, region and specific restriction enzyme of MSTN *and PRL* genes.

Gene	Primer sequence (5′ → 3′)	PCR product length	Region	Restriction enzyme	Incubation	References
MSTN	F: CCG GAG AGA CTT TGG GCT TGA R: TCA TGA GCA CCC ACA GCG GTC	337 bp	Exon 3	HaeIII	37 °C for 10 minutes	Azari et al. [25]
PRL	F: ATTCCTGGAGCCAAAGAG R: TGTGGGCTTAGCAGTTGT	655 bp	Exon 5	Eco24I	37 °C overnight	Lan et al. [26]

F: forward R: reverse.

Table 2
PCR conditions.

Gene	Primary denaturation in 1st cycle	Denaturation		Annealing		Elongation		Final extension		Number of cycles
	°C/Sec	°C	Sec	°C	Sec	°C	Sec	°C	Sec	
MSTN	94/240	94	60	55.5	35	72	120	72	240	35
PRL	95/300	94	30	5635		72	30	72	600	35

Fig. 2. Agarose gel electrophoresis of MSTN-HaeIII/PCR-RFLP fragments. Lane M, 25 bp DNA ladder, lanes (1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15) Genotype BB (131, 123 and 83 bp).

CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGCTCGTT
ACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATAT
AAGGCCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCATACCCA
TCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGGCCCTTGCTGTACTCCTACAAAGATGT
CTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGAATGATCAAGGA
ACCGCCCTA**GACCGCTGTGGGTGCTCATGA**

Fig. 3a. The sequence analysis of Barki goat *MSTN* amplified fragment. Forward and backward primer with red color.

```
Query  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGCTCGTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTG-TCGTT  59

Query  61   ACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60   ACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGAT  119

Query  121  ATAAGGCCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCATA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  120  ATAAGGCCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCATA  179

Query  181  CCCATCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGGCCCTTGCTGTACTCCTACAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  180  CCCATCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGGCCCTTGCTGTACTCCTACAA  239

Query  241  AGATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGAATGA  300
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| | ||
Sbjct  240  AGATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAAGA  299

Query  301  TCAAGGAACCGCCCTAGACCGCTGTGGGTGCTCATGA  337
||| ||| | |    ||||||||||||||||||||||
Sbjct  300  TCCAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA  337
```

Fig. 3b. Sequence alignment of Barki goat *MSTN* amplified fragment with published sequence.

3. Results and discussion

- *MSTN*-PCR produced a DNA fragment of 337 bp as shown in Fig. 1. Digestion of this fragment with the restriction enzyme *HaeIII* flanked only one cut B allele (131, 123 and 83 bp). The allele B frequency was 1.00 in the three breeds, meaning that only genotype BB was found in all breeds under study (Fig. 2). BB genotypic frequency was 1.00 for all the breeds.
- Sequence examination of the *MSTN* amplicon (amplified PCR product, 337 bp) was conducted, by using each of forward and backward primer. The nucleotide sequence of PCR product for *MSTN* gene in Barki breeds is shown in Fig. 3a
- The sequence alignment of Barki *MSTN* with published sequence (Accession number: KP120861.1, ***Capra hircus***) showed 97% identities with one gap between positions 54 and 56, and eight SNPs; (A/G) transition at position 296, (T/A) transversion at position 298, two (A/C) transitions at positions 303 and 307, two (C/T) transitions at positions 309 and 312, (C/A) transitions at position 313 and (C/G) transversion at position 314 (Fig. 3b).

The nucleotide sequence of PCR product for *MSTN* gene in Damascus breed is presented in Fig. 4a.

The sequence alignment of Damascus *MSTN* with published sequence (Accession number: KP120861.1, *Capra hircus*) showed 99% identities with three SNPs; one (G/A) transition at position 297 and two (A/G) transitions at positions 295 and 298 (Fig. 4b).

The nucleotide sequence of PCR product for *MSTN* gene in Zaraibi breed is shown in Fig. 5a

CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTACCCT
CTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATATCCGGAAA
GGCCAATTACTGCTCCGGAGAAATTGTGAATTTTTATTTAATTTGCAAAAGTATCCTCATACCCA
TCTTGTGCACCAAGCAAACCCCAAAGTGTTCAGCTGTACTCCTACAAAGATGTCTCCAATTAAT
ATGCTATATTTTAATGGCAAAGAACAAATAATATATGGAATGATCAAGGAACCGCCCTA**GACC**
GCTGTGGGTGCTCATGA

Fig. 4a. The sequence examination of Damascus goat *MSTN* amplified fragment. Forward plus reverse primers with red color.

```
Query  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTA  60
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Sbjct  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTA  60

Query  61   CCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATA  120

Query  121  TAAGGCCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCATAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TAAGGCCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCATAC  180

Query  181  CCATCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGGCCCTTGCTGTACTCCTACAAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCATCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGGCCCTTGCTGTACTCCTACAAA  240

Query  241  GATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGAAGAAT  300
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |  ||
Sbjct  241  GATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAAGAT  300

Query  301  TCCAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA  337
            ||||||||||||||||||||||||||||||||||||
Sbjct  301  TCCAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA  337
```

Fig. 4b. Sequence alignment of Damascus goat *MSTN* amplified fragment with published sequence.

– The sequence alignment of Zaraibi *MSTN* with published sequence (Accession number: KP120861.1, *Capra hircus*) showed 96% identities with eleven gaps at positions 122, 123, 124, 125, 126, 151, 152, 167, 168, 169 and 220 (Fig. 5b).

The current results are parallel to those found by [25] in native Iranian Dalagh sheep. A 337 bp part for exon three of *MSTN* locus was magnified and digested via *Hae*III enzyme. This enzyme digested the m allele, nevertheless M allele. Digestion of m allele created three pieces of 83, 123, and 131 bp. Furthermore, all test samples were monomorphs and showed only mm genotype. Similarly, [28] did not find any polymorphism around exon three of *MSTN* gene in Iranian Zel sheep breed.

Inversely, [29] showed the deletion of TTTTA in 5′UTR of caprine myostain gene in different Chinese goat populations via the meth-

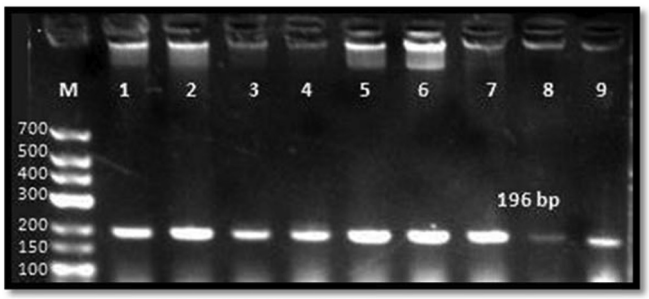

Fig. 6. Agarose gel electrophoresis of *PRL*-PCR fragment (196 bp). Lane M, 100 bp DNA ladder. Lanes (1, 2, 3) Barki, (4, 5, 6) Damascus and Lanes (7, 8, 9) Zaraibi breeds.

CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAAC
TGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATATCCGGAAAGGCCAATTA
CTGCTCCGGAGAAATTGTGAATTTTTATTTAATTTGCAAAAGTATCCTCATACCCATCTTGTGCACCAA
GCAAACCCCAAAGTGTTCAGCCGGCCCTTGCTGTACTCCTACAAAGATGTCTCCAATTAATATGCTATC
ATTTTAATGGCAACAGACATTCACAATAGATAATGGTAGTA **GACCGCTGTGGGTGCTCATGA**

Fig. 5a. The sequence investigation of Zaraibi goat *MSTN* amplified fragment. Forward and backward primers with red color.

```
Query  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTA  60

Query  61   CCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATA  120

Query  121  TCCGGAAAGGCCAATTACTGCTCCGGAGAAATTGTGAATTTTTATTTAATTTGCAAAAGT  180
            |     |||||||||||||||||||||||||  |||||||||||||||   ||||||||||||
Sbjct  121  T-----AAGGCCAATTACTGCTCCGGAGAA--TGTGAATTTTTATT---TTTGCAAAAGT  170

Query  181  ATCCTCATACCCATCTTGTGCACCAAGCAAACCCCAAAGTGTTCAGCCGGCCCTTGCTGT  240
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  171  ATCCTCATACCCATCTTGTGCACCAAGCAAACCCCAAAG-GTTCAGCCGGCCCTTGCTGT  229

Query  241  AGATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAAGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  240  AGATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAAGA  299

Query  301  TCAAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA  337
||||||||||||||||||||||||||||||||||||||
Sbjct  300  TCCAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA  337
```

Fig. 5b. Sequence alignment of Zaraibi goat *MSTN* amplified fragment with published sequence.

Table 3
Genotype frequency, allele frequency, observed heterozygosity (Ho), estimated heterozygosity (He) and χ^2 values of *PRL-Eco24I*/PCR-RFLP.

Gene/restriction enzyme	Breed	Genotype frequency			Allele frequency		Observed Het. (Ho)	Expected Het. (He)	χ^2
		CC	CD	DD	C	D			
PRL	Barki	0.10	0.85	0.05	0.53	0.47	0.85	0.51	9.22[a]
	Damascus	0.25	0.75	0.00	0.62	0.38	0.75	0.48	6.65[a]
	Zaraibi	0.00	0.90	0.10	0.45	0.55	0.90	0.51	12.6[a]

[a] Means ($P \leq 0.05$).

ods of DNA sequencing and PCR-RFLP. They also inferred that the deletion of TTTTA in 5′UTR of caprine *MSTN* gene significantly ($P < .05$ or $P < .01$) influenced the animal body bulk and weightiness from birth to four-month old. They observed that this SNP was conserved in various species and might be a unique mutation in goats. Likewise, [30] indicated that this deletion is found also in different Egyptian sheep breeds (Barki, Rahmani and Osseimi) and not limited to goats.

Contrariwise, [29] showed the deletion of TTTTA in 5′UTR of caprine myostain gene in different Chinese goat populations via the methods of DNA sequencing and PCR-RFLP. They also inferred that the deletion of TTTTA in 5′UTR of caprine *MSTN* gene significantly ($P < .05$ or $P < .01$) influenced the animal body size and weight from birth to four-month old. They observed that this SNP was conserved in various species and might be a unique mutation in goats. Likewise, [30] indicated that this deletion is found also in different Egyptian sheep classes (Barki, Rahmani and Osseimi) and not limited to goats.

Subsequently, [31] tested *MSTN* gene polymorphism as a candidate marker trustworthy for growth in both Boer and Anhui white goats. Three pairs of primers (P1, P2 and P3) were designed to amplify the 5′-UTR and exon one fragments in the *MSTN* gene. The results exhibited two SNPs: DQ167575 g.197G > A and 345A > T and three potent genotypes (AA, BB, and AB) of substitution 197G > A in the 5′-untranslated region was recognized in the two breeds.

The PCR amplification of PRL gene a DNA fragment of 196 bp as viewed in Fig. 6. Digestion of PCR product (196 bp) of *PRL* gene with *Eco*24Irevealed two different alleles, the uncut A allele (196 bp) and the cut B allele (169 and 27 bp). The allele A frequency was (0.53, 0.62 and 0.45) and the allele B frequency was 0.47, 0.38 and 0.55 for Barki, Damascus and Zaraibi, respectively (Table 3).

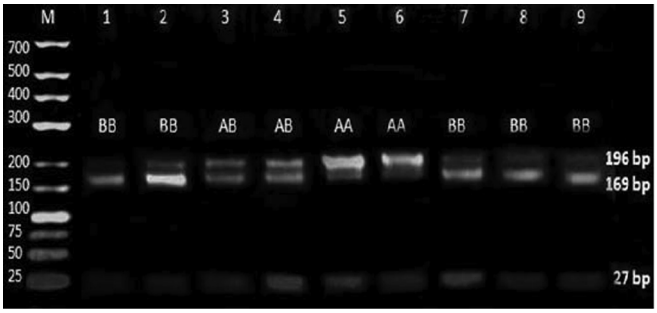

Fig. 7. Agarose gel electrophoresis of *PRL- Eco24I*/PCR-RFLP fragments. Lane M, 25 bp DNA ladder, lanes (5, 6) Genotype AA (196 bp), lanes (3, 4) Genotype AB (196, 169 and 27 bp) and lanes (1, 2, 7, 8, 9) Genotype BB (169 and 27 bp).

Three Genotypes were screened in the three studied goat breeds, AA (196 bp), AB (196, 169 and 27 bp) and BB (169 and 27 bp) (Fig. 7). AA genotypic frequency was 0.10, 0.25 and 0.00, AB genotypic frequency was 0.85, 0.75, and 0.90 and BB genotypic frequency was 0.05, 0.00 and 0.10 for Barki, Damascus and Zaraibi goats, respectively (Table 3).

- The estimates of the observed heterozygosity were 0.85 for Barki goats, 0.75 for Damascus and 0.90 for Zaraibi breeds. Moreover, the estimates of expected heterozygosity were 0.48 for Barki, Damascus and 0.51 for each Barki and Zaraibi breeds, respectively (Table 3).
- *PRL- Eco*24I/RFLP had χ^2 estimates of 9.22, 6.65 and 12.6 for Barki, Damascus and Zaraibi breeds, respectively (Table 3).

Sequence study of the *PRL* amplicon (amplified PCR product, 196 bp) was conducted with forward and inverse primers. The nucleotide sequence of PCR product for *PRL* gene in Barki breeds is presented in Fig. 8a

- The sequence alignment of Barki *PRL* with published sequence (Accession number: NM_001285547.1, ***Capra hircus***) showed 97% identities (Fig. 8b).
- The nucleotide sequence of PCR product for *PRL* gene in Damascus breed is shown in Fig. 9a

The sequence alignment of Damascus *PRL* with published sequence (Accession number: EU256170.1, *Capra hircus*) showed 99% identities with two SNPs; one(T/C) transition at position 177 and other one (G/A) transition at position 178 (Fig. 9b).

The nucleotide sequence of PCR product for *PRL* gene in Zaraibi class is publicized in Fig. 10a.

The sequence alignment of Zaraibi *PRL* with published sequence (Accession number: NM_001285547.1, *Capra hircus*) showed 99% identities with two SNPs; one (T/A) transversion at position 177 (Fig. 10b).

Regarding to polymorphism in *PRL* gene, the present results are in agreement with those reported by [26] in different Chinese indigenous goat breeds by PCR-SSCP and gene sequencing. They detected the X76049: g.576C > A (Pro176Thr) mutation which was established through *Eco*24I PCR-RFLP analysis. In exon five of the caprine *PRL* gene, three different SSCP banding patterns

ATTCCTGGAGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAAACTAAGGATGAAGAGGCACGT
CATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGGGATTCAAGCAAGATTGACACTTACCTTAAGCTCCTGAATTG
CAGAATCATCTACAACAACAACTGCTAAGCCCACA

Fig. 8a. The sequence examination of Barki goat *PRL* amplified fragment. Forward, reverse primers with red color.

(CC, CA and AA) were observed. Moreover, a novel SNP (X76049: g. 576C > A) was recognized, which resulted in an amino acid exchange from Pro (CCC) to Thr (ACC) at position 176 of the protein sequence. As the g.576C > A transversion destroys an *Eco*24I (GRGCY^C) restriction site, the *Eco*24I PCR-RFLP can be used for cost-effective genotyping of the goat *PRL* gene SNP.

```
-   Query  1    ATTCCTGGAGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAA  60
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-   Sbjct  550  ATTCCTGGAGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAA  609
-
-   Query  61   ACTAAGGATGAAGAGGCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGG  120
-                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-   Sbjct  610  ACTAAGGATGAAGAGGCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGG  669
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-                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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-
-   Query  181  AACTGCTAAGCCCACA  196
-
```

Fig. 8b. Sequence alignment of Barki goat *PRL* amplified fragment with published sequence.

ATTCCTGGAGCCAAAGAGACTGAGACCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAAACTAAGGATGAAGAG
GCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGGGATTCAAGCAAGATTGACACTTACCTTAAGCT
CCTGAATTGCAGAATCATCTACAATGACAACTGCTAAGCCCACA

Fig. 9a. The sequence investigation of Damascus goat *PRL* amplified fragment. Forward and backward primers with red color.

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Query  1    ATTCCTGGAGCCAAAGAGACTGAGACCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATTCCTGGAGCCAAAGAGACTGAGACCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAA  60

Query  61   ACTAAGGATGAAGAGGCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ACTAAGGATGAAGAGGCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGG  120

Query  121  GATTCAAGCAAGATTGACACTTACCTTAAGCTCCTGAATTGCAGAATCATCTACAATGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||  ||
Sbjct  121  GATTCAAGCAAGATTGACACTTACCTTAAGCTCCTGAATTGCAGAATCATCTACAACAAC  180

Query  181  AACTGCTAAGCCCACA  196
            ||||||||||||||||
Sbjct  181  AACTGCTAAGCCCACA  196
```

Fig. 9b. Sequence alignment of Damascus goat *PRL* amplified fragment with published sequence.

ATTCCTGGAGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACTCCCATCCCTG
CAAACTAAGGATGAAGAGGCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTG
CGCAGGGATTCAAGCAAGATTGACACTTACCTTAAGCTCCTGAATTGCAGAATCATC
TACAACTACAACTGCTAAGCCCACA

Fig. 10a. The sequence analysis of Zaraibi goat *PRL* amplified fragment. Forward and backward primers with red color.

```
Query  1    ATTCCTGGAGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  ATTCCTGGAGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACTCCCATCCCTGCAA  609

Query  61   ACTAAGGATGAAGAGGCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  610  ACTAAGGATGAAGAGGCACGTCATTCTGCTTTTTACAACCTGCTCCACTGCCTGCGCAGG  669

Query  121  GATTCAAGCAAGATTGACACTTACCTTAAGCTCCTGAATTGCAGAATCATCTACAACTAC  180
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  670  GATTCAAGCAAGATTGACACTTACCTTAAGCTCCTGAATTGCAGAATCATCTACAACAAC  729

Query  181  AACTGCTAAGCCCACA  196
            ||||||||||||||||
Sbjct  730  AACTGCTAAGCCCACA  745
```

Fig. 10b. Sequence alignment of Zaraibi goat *PRL* amplified fragment with published sequence.

Conversely, [32] examined the genetic variant of the *PRL* gene of Indian goats, but no polymorphism was detected. The DNA sequence variations at *PRL* gene were examined in Malabari, Attappady black, Jamnapari and Salem black goat breeds by PCR-RFLP and DNA sequencing techniques. The digestion of PCR product from *PRL* locus with *Rsal* enzyme revealed a lone allele, namely, the allele A (156 bp) in all tested breeds. Subsequent sequencing, product of PCR was initiate to be of 156 bp in length and the BLAST examine at the NCBI site revealed 100; sequence homology with equivalent sequence of ovine mammary gland prolactin mRNA (Ass. X76050.1), caprine mRNA of pituitary prolactin (Accession X76049.1) and mRNA of caprine mammary gland prolactin (Assent number X76048.1).

Due to productivity data lack, in the present study we did not perform an association analysis between the productivity and allele polymorphisms. We expect the productivity on some previous articles which identified the alleles linked with high productivity in other sheep breeds.

4. Conclusion

In the end, this study is considered to be a step advancing for further studies that may add to give additional information about the genetic polymorphism of meat and growth characters of Egyptian goat breeds and the improvement of these economically important traits.

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