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Association of Genetic Polymorphisms of *POU1F1* Gene on Twin Production in Egyptian Sheep and Goats

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Abstract

The expression of POU1F1 gene is necessary for the normal survival, differentiation and development of several hormones' secretory cells such as somatotrophs, lactotrophs and thyrotrophs which produce growth hormone, prolactin, and thyrotropin releasing hormone, respectively. Several studies indicated that the POU1F1 gene can act as a potent candidate gene to enhance important traits such as growth, development reproduction and production in different mammals' species. The aim of this investigation was to verify the genetic polymorphisms in POU1F1 gene and to declare their effects on litter size (twin production) in Egyptian sheep and goat breeds. For DNA extraction, blood samples were collected from 139 females (113 ewes/doe at the first, second, third and fourth parity and 26 were a yearling ewe/doe). PCR amplification and PCR-SSCP were carried out using six pairs of primers. The nucleotide sequence genotyping analysis was performed to confirm banding patterns of PCR-SSCP, and to identify associated SNPs with twin production trait. Only primers 5 and 6 detected genetic polymorphisms by PCR-SSCP. The nucleotide sequence analyses between the observed patterns of POUlF1 gene revealed variable nucleotide substitutions at different positions in coding regions on charts. These nucleotide substitutions identified additional POU1F1 alleles and showed further sequence polymorphisms. The sequence analysis showed that "A" allele was more frequent in animals that have high production of offspring. In contrast, the high frequencies of C and T alleles were revealed to induce decrease in prolificacy trait in sheep and goats. The observed different alleles may cause alterations in deduced amino acid sequences that affect litter size and lamb production. The genetic analysis in this study proved that the allele A of POU1F1gene would be considered the favorable gene, and thus could utilized into a breeding program based on gene assisted selection strategy for selection ewes of high prolificacy and twin lambs.

Keywords: POU1F1 gene, PCR- SSCP, Marker assisted selection, Prolificacy, small ruminant.

1. Introduction

The POU domain, class 1, transcription factor 1 (POU1F1) gene (also named Pituitary-specific positive transcription factor 1, PIT-1 or growth hormone factor 1 (GHF-1)) was revealed to be a member of the tissue – specific POU-containing transcription factor family. In ovine and caprine, the POU1F1 genes were all detected to be located on chromosome $1q^{21-22}$ and include six exons involving the POU domain and homeodomain (Woollard *et al.*, 2000). The expression of POU1F1 gene is necessary for the normal survival, differentiation and development of three adenohypophysis cell types including somatotrophs, lactotrophs and thyrotrophs (Li *et al.*, 1990; Simmons *et al.*, 1990). Also, its expression was found to be required for the transcription of the genes encoding growth hormone (GH) (Nelson *et al.*, 1988; Mullis, 2007), prolactin (PRL) (Nelson *et al.*, 1988; Ben-Batalla *et al.*, 2010) and thyroid – stimulating hormone (TSH) (Li *et al.*, 1990). These genes (GH, PRL and TSH) and their coding proteins were observed to be closely related with reproduction in mammals (Hull *et al.*, 2001; Sun *et al.*, 2002; Yasuo *et al.*, 2010). Several studies indicated that the SNPs within POU1F1 gene can act as genetic markers to enhance several important traits such growth, development reproduction and production in different mammals' species. For example, Giordano (2016) observed in humans that the mutations of POU1F1 gene resulted in deficiency in pituitary hormone leading to impairment of growth and development of individuals. Genetic polymorphisms of POU1F1 gene in pig were revealed to have significant effects on litter size as a reproduction trait (Sun *et al.*, 2002).In sheep, four splicing forms of POU1F1 gene had been identified and showed different trans-activation of GH and PRL promoters (Bastos *et al.*, 2006b).Also, Jalil-Sarghale *et al.* (2014)and Ozmen *et al.* (2014) found that the genetic polymorphisms in POU1F1 gene have significant association with milk production and weaning weight traits in some Iranian sheep breeds. In goats, a lot of Single Nucleotide Polymorphisms (SNPs) in POU1F1 gene were observed to be significantly associated with milk performance, cashmere production, growth traits (Lan *et al.*, 2007a; Lan

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et al., 2009; Nazari-Ghadikolaei *et al.*, 2018; Zhu *et al.*, 2019) and litter size rates (Lan *et al.*, 2007a; Feng *et al.*, 2012; Zhu *et al.*, 2019). Based on previous findings, the investigation on exploring potential genetic polymorphisms (SNP -Single Nucleotide Polymorphism-markers) to improve the twin production or enhance the litter size trait in the Egyptian small ruminants is worthy of in-depth study. So far, no researches have detected the SNP polymorphisms of POU1F1 gene in such Egyptian animal species. Therefore, the main aim of this investigation was to verify the genetic polymorphisms in POU1F1 gene (by using PCR- Single Strand Conformation Polymorphism (SSCP) and DNA sequencing) and to declare their effects on litter size (twin production) in Egyptian sheep and goat breeds.

2. Materials and Methods

2.1. Experimental animals

The blood samples were collected from one hundred and thirty-nine healthy females of sheep and goats. The samples were collected from Animal Production Farms belonging to Faculty of Agriculture (Cairo University), New Nubaria Farms belonging to NRC and Governmental Halayieb, Egypt. The sheep females consisted of ninety-five animals (belong to Barki, Osseimi, Rahmani, Saudanez and Awassi breeds) comprised of eighty-three adult ewes and twelve young ewes. On the other hand, the goat females consisted of forty-four animals (belong to Zaraiby, Damascus, Boer, Saanine and Barki breeds) comprised of thirty adult does and fourteen young does. Both the adult ewes and does were at first, second, third and fourth parity.

The animals were not subject to any treatments during this study, and only blood samples were collected from sheep and goats under veterinary supervision. Since the studied animals were a part of the herds of the farms mentioned above at the time of blood collection and were not subject to any treatments, the animals were kept in the same herds after blood sampling for this study.

2.2. Blood sample collection and DNA extraction

The all procedures that carried out on the animals were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Cairo University, Egypt with Permit (Reference) Number: CUFS F Mol. Biol. 50 15. The collection of blood samples had been carried out aseptically from jugular vein of the animal and in vacutainer tubes containing EDTA. Genomic DNA was extracted from white blood cells using salting out method (Aboelenin *et al.*, 2017a; Aboelenin *et al.*, 2017b; Mahrous *et al.*, 2020c)**.** DNA concentration and quality had been assessed, and then stored at -20°C until used into amplification process.

2.3. Primers and PCR amplification

Based on the genomic sequence available in GenBank of POU1F1gene, the following primers sequences (Table1) were designed using Primer-BLAST software (www.ncbi.nlm.nih.gov/tools/primer-blast) based on the sequence of GenBank record NC_019458. The annealing temperature of each pair was optimized using a conventional PCR to exclude the presence of unspecific products or primer dimer, and the PCR products were analyzed by 2% agarose gel electrophoresis as described previously (Madkour *et al.*, 2020; Mahrous *et al.*, 2020a; Mahrous *et al.*, 2020b; Mahrous *et al.*, 2020d; Sroor *et al.*, 2020; Madkour *et al.*, 2021a; Madkour *et al.*, 2021b; Mahrous *et al.*, 2021; Aboelenin *et al.*, 2022; Sroor et al., 2022)#The Polymerase chain reaction had been carried out in 25µl volume involving 2.5 µl of 10 x PCR buffer (50 mmol/L KCL, 10 mmol/L Tris-HCl (pH 8.0), 0.1% Triton X-100) 1.5 mmol/L $MgCl_2$, 200µmol/L each dNTP, 1µmol/L each primer, 50 ng genomic DNA, and 1 U Taq DNA polymerase. The conditions of PCR were denaturation at 94ºC for 6 min; followed by 32 cycles of denaturation at 94ºC for 30s, annealing at 55-62ºC for 30s, extension at 72ºC for 30s; with a final extension at 72ºC for 10 min.

2.4. Single Strand conformational polymorphism (SSCP)

For SSCP analysis, the PCR products were resolved. 10 µl of PCR product were diluted in denaturing solution that consisted of A and B types. “A” type solution included 95% of Formamide, 10 mM NaOH, 0.05% Xylene-Cyanol and 0.05% bromophenol blue. “B” type solution same as “A” solution, plus 20 mM of EDTA (pH 8.0). A 10% SSCP gel mixture (30 ml) was prepared through acrylamide- -bisacrylamide (37.5: 1), TEMED (30 µl) and 10% ammonium persulfate (0.8 ml) in a 1x TBE (90 mM Tris-borate at pH 8.3, 4 mM EDTA), and a voltage of 300 V, running time (6- 8 h) and running temperature at 4ºC. Each PCR reaction had been diluted in denaturing solution, denatured at 95ºC for 5 min, chilled on ice and resolved on non-denaturing polyacrylamide gel. Electrophoresis was performed in a vertical unit (Hoefer Scientist SE600, 160 x 140 x 1 mm) in a 1x TBE buffer. The gels had been stained with 0.1% silver nitrate and visualized through 2% NaOH solution (containing 0.1% formaldehyde). Homozygous and Heterozygous genotypes from different SSCP patterns in different breeds had been photographed and analyzed using Gel Documentation system.

The reactions of PCR were conducted in 25 ul volume containing 0.5 ul of 10 u Mol/1 each primer, 2.5 ul of 10 x PCR buffer [50 mMol/1 KCL, 10 mMol/1 Tris – HCL (pH8.0), 0.1 % TritonX- 100], 1.5 – 1.8ul of 25 mMol/1 Mg Cl_2, 2.0 µl of 2.5 mMol/ 1 each dNTP, 2.0 ul of 50 ng/µl genomic DNA, 1.0 ul of 2.5 U/µl Taq DNA polymerase. The PCR conditions were as follows: initial denaturation at 95ºC for 5 min, followed by 34 cycles of denaturation at 94ºC for 30s, annealing at 55-62 for 30s, extension at 72ºC for 45s; with a final extension at 72 for 10 min.

Table 1. The sequences of primers and product size of PCR amplification of POU1F1 gene.

Primer		Product Size
POU1F1-1-F	GGGCATGGATTGTGTTTGGGTAAG	459 bp
POU1F1-1-R	TTCAAAGCACCCATCCTGACATCTT	
POU1F1-2-F	AAGGCTTTAGTCCACTGTGATG	430 bp
POU1F1-2-R	CACTCTGGTCAAGATTCCCTTTG	
POU1F1-3-F	ACTAAGCTACACTGACTTCTACTTC	470 bp
POU1F1-3-R	GGATTTCTGAGCAGCGTGTAA	
POU1F1-4-F	ATGTTCCTATCTGAATTCTTTTGAC	475 bp
POU1F1-4-R	TTCAACCGAGGGAAACCATT	
POU1F1-5-F	TATAAGTGTAGCCAGACCATT	494 bp
POU1F1-5-R	TCTTCTCCACTAACTTTTAATATC	
POU1F1-6-F	TTTCAGAGTCTTTAGGTTTCCTTTT	456 bp
POU1F1-6-R	AGAGAAACACTTCTGCAAACATTAC	

F stands for forward primer; R stands for reverse primer.

2.5. Sequence analysis

The PCR products of all different patterns forPOU1F1 gene had been purified and sequenced by special Company (Macrogen Incorporation, Seoul, Korea). Sequence analysis and alignment had been performed by cluster wide analysis using Codon Code Aligner Software V. 10.0.2; CodonCode Corporation, Centerville, USA (https://www.codoncode.com/aligner/index.htm).

2.6. Statistical Analysis

The different PCR-SSCP patterns (due to using primers 5 and 6) and allele frequencies (by using primer 5) that were revealed in the present study had been statistically analyzed by one way ANOVA followed by two-way ANOVA. The differences among pattern and allele groups had been determined significantly according to the method of Waller *et al.* (1969). Moreover, the allele frequencies that were identified by using primer 6 had been statistically analyzed using T-Test. The values are expressed as mean±SE. All statements of significance had been based on probability of ($P \leq 0.05$).

3. Results

3.1. Results of PCR amplification of POU1F1 gene:

Genomic DNA of 139 sampled animals had been amplified using six pairs of primers for POU1F1 gene. PCR products were detected by running a 2% Agarose gel electrophoresis (Figures 1 to 6 shows example of 7 genotyped animals). The sizes of PCR products of primers 1, 2, 3, 4, 5 and 6 were 459 bp, 430 bp, 470 bp, 475 bp, 494 bp and 456 bp, respectively. These amplified products were consistent with the target fragments and had good specificity, which could be directly analyzed by SSCP.

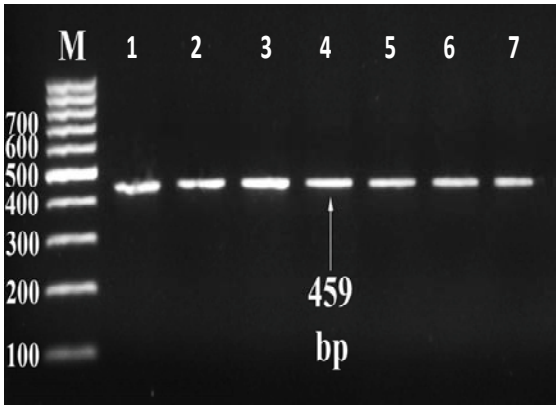

Figure 1. PCR products (Lanes 1-7) of POU1F1 gene at size 459 bp using primer 1. Lane M:100 bp ladder.

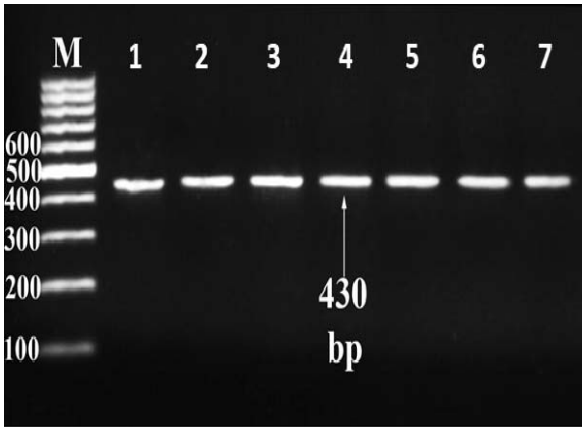

Figure 2. PCR products (Lanes 1-7) of POU1F1 gene at size 430 bp using primer 2. Lane M:100 bp ladder.

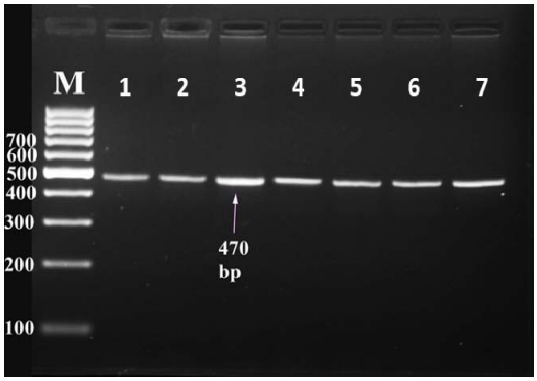

Figure 3. PCR products (Lanes 1-7) of POU1F1 gene at size 470 bp using primer 3. Lane M:100 bp ladder.

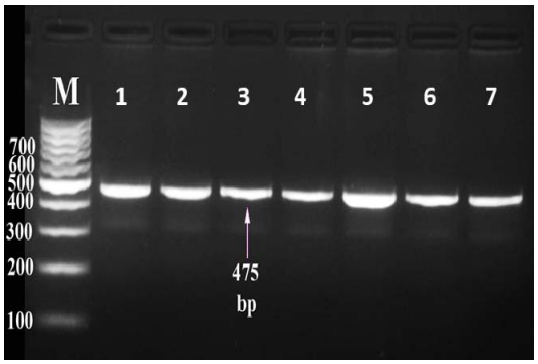

Figure 4. PCR products (Lanes 1-7) of POU1F1 gene at size 475 bp using primer 4. Lane M:100 bp ladder.

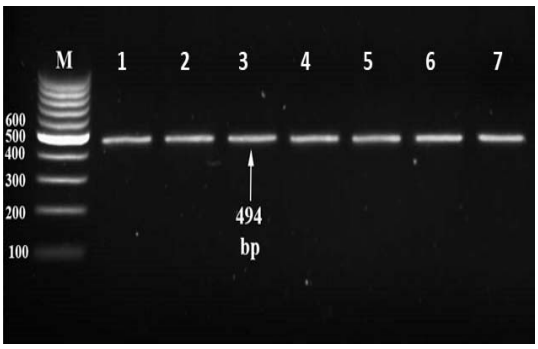

Figure 5. PCR products (Lanes 1-7) of POU1F1 gene at size 494 bp using primer 5. Lane M:100 bp ladder.

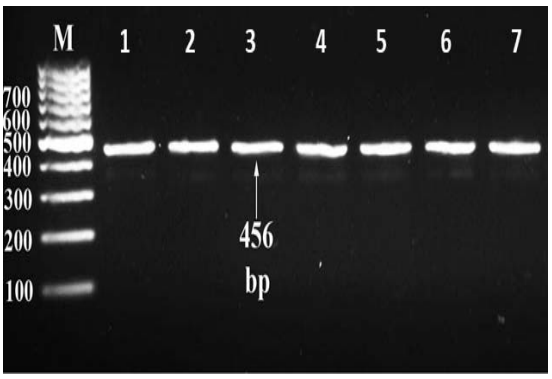

Figure 6. PCR products (Lanes 1-7) of POU1F1 gene at size 456 bp using primer 6. Lane M:100 bp ladder.

3.2. Results of PCR-SSCP analysis:

3.2.1. PCR-SSCP of primers 1,2, 3 and 4 of POU1F1gene:

PCR-SSCP analyses of 459 bp product size (bp) of primer 1 or 430 bp product size of primer 2, or 470 bp of primer 3 and 475 bp of primer 4 were shown in Figures 7, 8, 9 and 10, respectively. No polymorphisms were found and thus all animals (sheep and goats) were monomorphic.

Figure 7. Shows monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 1.

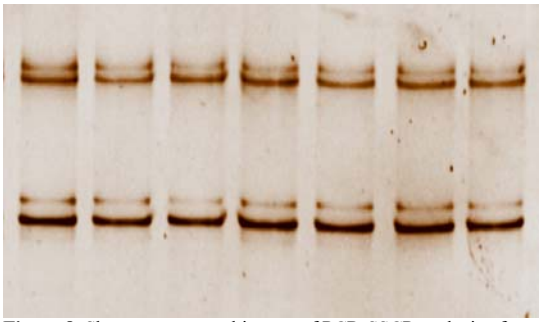

Figure 8. Shows monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 2.

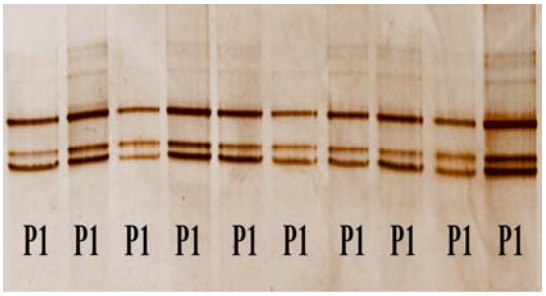

Figure 9. Shows monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 3.

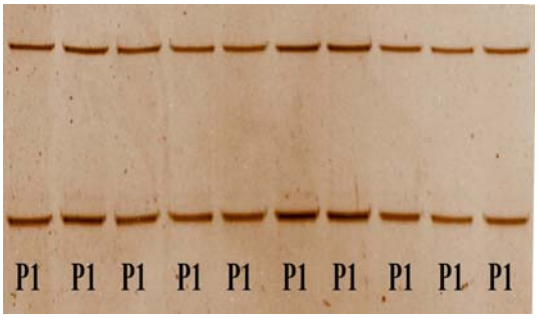

Figure 10. Shows monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 4.

3.2.2. PCR-SSCP of primer 5 of POU1F1 gene:

PCR-SSCP analysis of 494 bp PCR product size was shown in Figure 11 and Table 2. The results detected genetic polymorphisms in all small ruminant breeds. Three banding patterns (P1, P2 and P3 in sheep and P1, P2 and P4 in goats) were identified. The banding patterns of P1 and P2 in both sheep and goats were identical. However, the banding patterns P3 (in sheep) and P4 (in goats) were different.

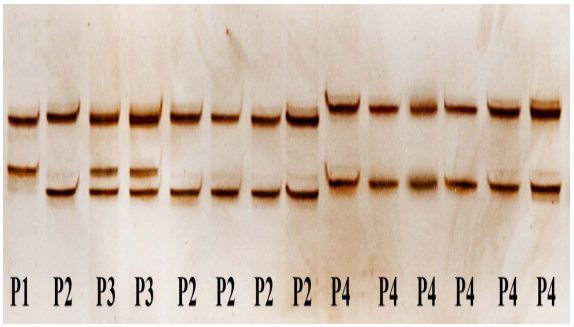

Figure 11. Shows four SSCP different patterns of POU1F1 gene using primer 5.

Table 2. Shows genetic polymorphisms of POU1F1gene using primer 5 and their effects on litter size in different Egyptian sheep and goat breeds:

Species	No. of animals	SSCP patterns (Ps)	No. of animals at age of sexual maturation	No. of mothers	No. of single births (S.b)	No. of twin births (T.b)	% S.b	% T.b	Mean No. of lambing	Mean No. of litter size (Twin production)
		P1	4	34	35	15	1.03	0.44	1.9±0.24a	1.47±0.12ab
Sheep	95	P2	6	42	45	31	1.07	0.74	2.55±0.19ab	1.81±0.1bc
		P3	2	7	6	3	0.86	0.43	1.7±0.47a	1.29±0.28ab
		P1	1	10	13	17	1.3	1.7	4.7±0.49c	3.0±0.38c
Goats	44	P2	1	11	10	14	0.91	1.27	3.45±0.34b	2.18±0.23c
		P4	12	9	6	4	0.67	0.44	1.56±0.47a	1.11±0.3a

Data expressed as mean ± SE. values followed by different superscript letters are significantly different from one another within the same columns ($P \leq 0.05$).

In sheep, pattern 1 (P1) was revealed in 38 females, 34 of them were mothers and the remaining 4 females were at age of sexual maturity, whereas pattern 2 (P2) was observed in 48 animals including 42 mothers and 6 females were at age of sexual maturity. Moreover, pattern 3 was detected in 9 females; these females involved 7 mothers and 2 females were at age of sexual maturity. The results showed that the mothers of P2 had high rates of each litter size and the mean number of lambing in comparison with the mothers of P1 or the mothers of P3. So, this genotype (P2) is considered to be a fecundity gene marker.

In goats, pattern 1 (P1) was found in 11 females, 10 of them were mothers and one female was at age of sexual maturity. Pattern 2 (P2) was detected in 12 females, that included 11 mothers and one female was at age of sexual maturity, whereas pattern 4 (P4) was observed in 21 females involving 9 mothers and 12 females were at age of sexual maturity. The mothers of P1 followed by mothers of P2 had high rates of litter size and increase of mean number of lambing as compared to mothers of P4. The mothers of P1 had the highest rates of each of mean number of lambing and mean number of twin production. So, this genotype (P1) is considered to be a prolificacy gene marker.

3.2.2. PCR-SSCP of primer 6 of POU1F1gene

PCR-SSCP analysis of the 456 bp PCR product size was shown in Figure/Fig. 12 and Table 3. The results detected genetic polymorphisms in sheep and goat breeds. In sheep, the genetic polymorphisms were represented by the presence of 2 banding patterns (P1 and P2). Pattern 1 (P1) was found in 93 animals involving 81 mothers and 12 females were at age of sexual maturity. Pattern 2 (P2) was only identified in two mothers.

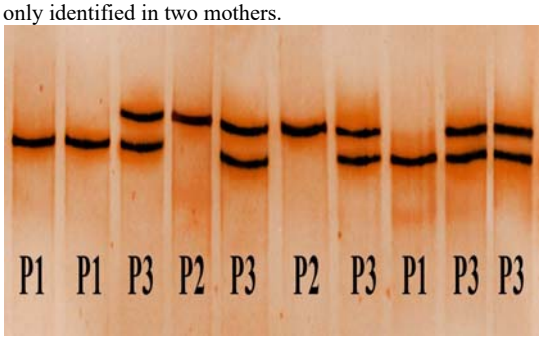

Figure 12. Shows three SSCP different of patterns of POU1F1 gene using primer 6.

Table 3. Shows genetic polymorphisms of POU1F1 gene using primer 6 and their effects on litter size in different Egyptian sheep and goat breeds:

Species	No. of animals	SSCP patterns (Ps)	No. of animals at age of sexual maturation	No. of mothers	No. of single births (S.b)	No. of twin births (T.b)	% S.b	% T.b	Mean No. of lambing	Mean No. of litter size (Twin production)
Sheep	**95**	**P1**	12	81	82	46	1.01	0.57	2.15±0.12a	1.58±0.1ab
		P2	0.0	2	4	3	2.0	1.5	5.0±0.9b	3.5±0.5c
Goats	**44**	**P1**	1	2	3	4	1.5	2.0	5.5±1.4b	3.5±0.5c
		P2	8	25	25	29	1.0	1.16	3.32±0.22ab	2.16±0.14b
		P3	5	3	1	2	0.33	0.67	1.67±0.24a	1.0±0.57a

Data expressed as mean ± SE. values followed by different superscript letters are significantly different from one another within the same columns (P≤0.05).

The mothers of P2 had high rates of each of mean number of litter size and the mean number of lambing as compared to mothers of P1. So, this genotype (P2) is considered to be as a fecundity gene marker. In goats, the genetic polymorphisms were revealed by the presence of three banding patterns (P1, P2 and P3). Pattern 1 and pattern 2 in both goats and sheep were identical. Pattern 1 of goats was observed in three animals including 2 mothers and one female was at age of sexual maturity. Whereas pattern (P2) was shown in 33 females, these females consisted of 25 mothers and 8 animals were at age of sexual maturity. Moreover, Pattern 3 (P3) was found in 8 animals involving 3 mothers and 5 females were at age of sexual maturity. The results indicated that the mothers of P1 followed by the mothers of P2 had high rates of litter size and increase of mean number of lambing in comparison with the mothers of P3. The mothers of pattern 1 had the highest rates of each of litter size and mean number of lambing. So, this genotype (P1) is considered as a prolificacy gene marker.

3.3. Nucleotide sequence analysis of POU1F1 gene:

In the present study, genetic polymorphisms had been identified in POU1F1 gene only by using primers 5 and 6. However, PCR-SSCP analysis showed no genetic polymorphisms by using primers 1, 2 ,3 and 4, where all animals were monomorphic, and nucleotide sequence analyses in these animals were recorded in Figures (13-16).

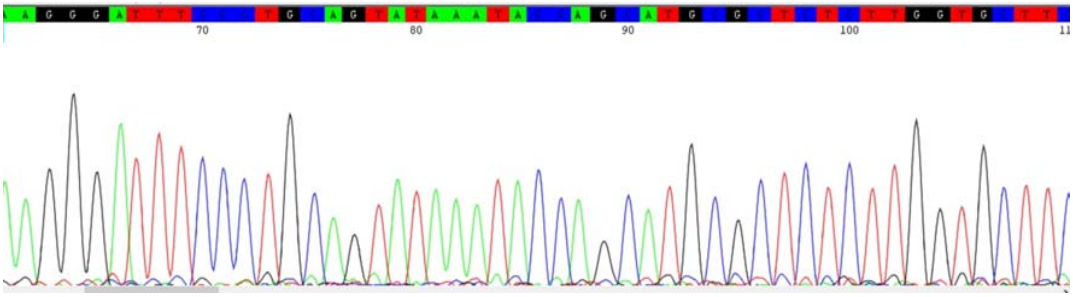

Figure 13. Sequence analysis of monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 1 (Showed no any of SNPs).

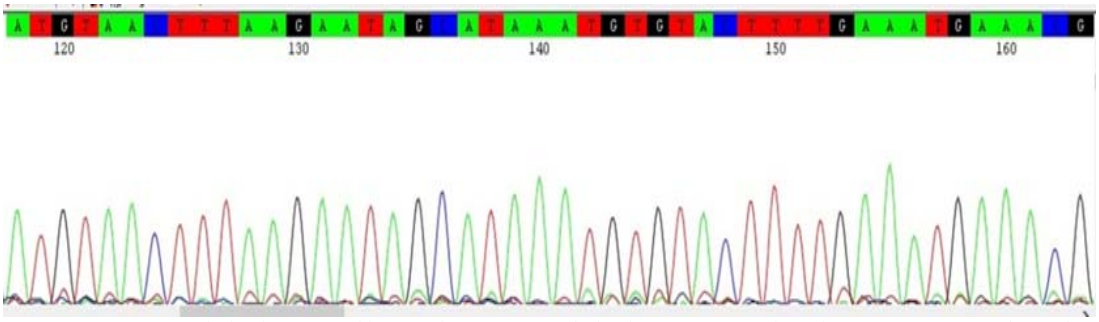

Figure 14. Sequence analysis of monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 2 (no SNPs were detected).

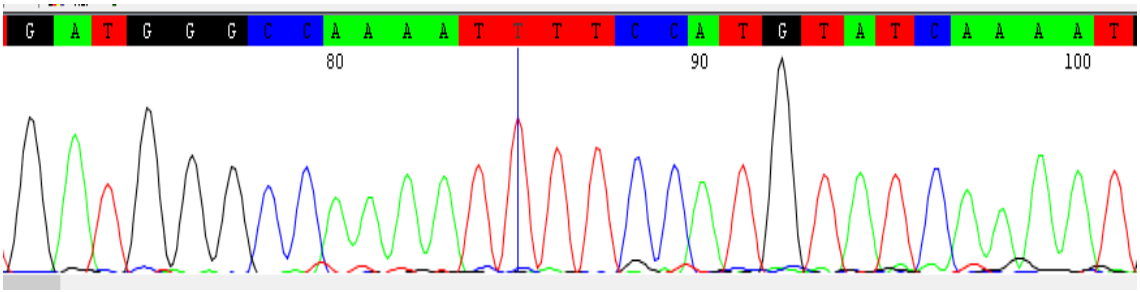

Figure 15. Sequence analysis of monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 3 (Showed no any of SNPs).

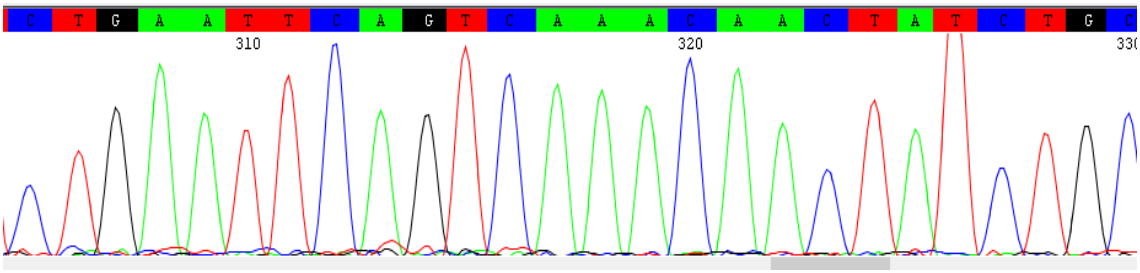

Figure 16. Sequence analysis of monomorphic type of PCR-SSCP analysis of POU1F1 gene using primer 4 (no SNPs were detected).

By using primer 5, the PCR-SSCP results showed four patterns. Nucleotide sequence analysis (Fig.17 and Tables4 and 5) of PCR products detected differences between the four patterns, where several nucleotide substitutions were observed in coding regions on charts in the positions 30, 172, 232 and 257. At position 30, pattern 1 had "A" nucleotide, while patterns 2 and 4 had "G" nucleotide. Whereas at pattern 3, "G" nucleotide was replaced by "A" nucleotide (A/G). In position 172, patterns 1 and 2 were discriminated with "A" nucleotide, while patterns 3 and 4 were discriminated with "C" nucleotide. On the other hand, at position 232, nucleotide sequence analysis showed the presence of "A" nucleotide in patterns 1,2 and 3 and they differed than pattern 4 at the same position, where P4 had "G" nucleotide. Moreover, in position 275, "C" nucleotide was replaced by "T" nucleotide (T/C) in each of patterns 1 and 3; however, "C" nucleotide was found in patterns 2 and 4.

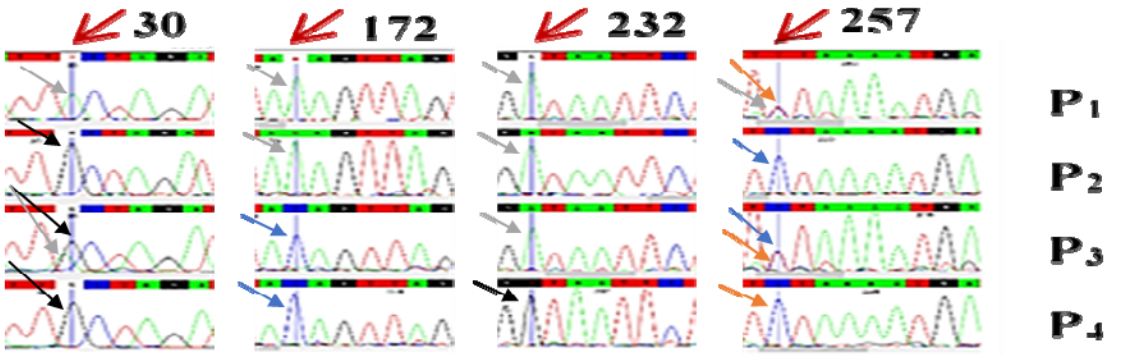

Figure 17. Sequence analysis of PCR-SSCP patterns (P1, P2, P3 and P4) using primer 5. P1 has A, A, A, T nucleotides at the specified positions; P2 has G, A, A, C; P3 has G, G, A, T and P4 has G, C, G, C of POU1F1 gene.

Table 4. Nucleotide sequence analysis of POU1F1 gene using primer 5 showing different alleles and genotypes in Egyptian domestic sheep and goats.

Patterns (P)	Species	Nucleotide sequences in the positions				Genotypes
		30	172	232	257	
P1	Sheep and goats	"A" allele(A/A)	"A" allele(A/A)	"A" allele(A/A)	"T/C" allele	AA, TC
P2	Sheep and goats	"G" allele(G/G)	"A" allele(A/A)	"A" allele(A/A)	"C" allele(C/C)	GG, AA, CC
P3	Sheep	allele "A/G"	"C" allele (C/C)	"A" allele(A/A)	"T/C" allele	AG, CC, AA, TC
P4	Goats	"G" allele(G/G)	"C" allele (C/C)	"G" allele(G/G)	"C" allele(C/C)	GG, CC

Table 5. Nucleotide sequence analyses of POU1F1 gene in Egyptian sheep and goats showing genotype and allele frequencies (using primers 5):

The used primer	Species	T. No. of animals	Type of patterns (P_s) of SSCP analysis	Genotype	No. of animals with SSCP patterns	Genotype frequencies	Allele frequencies
Primer 5	**Sheep**	**95**	P1	AA, TC	AA (29) + TC (9)	AA (0.31) + TC (0.09)	A (0.31±0.14)^a T (0.045± 0.03)^a C (0.045± 0.28)^a
			P2	AA, CC, GG	AA (24) + CC (12) + GG (12)	AA (0.25) + CC (0.126) + GG (0.126)	A (0.25± 0.114)^a C (0.126± 0.096)^a G (0.126± 0.088)^a
			P3	AA, CC, AG, TC	AA (3) + CC (3) +AG (1) + TC (2)	AA (0.03) + CC (0.03) + AG (0.01) + TC (0.02)	A (0.035± 0.02)^a C (0.04± 0.025)^a G (0.005± 0.002)^a T (0.01± 0.007)^a
	Goats	**44**	P1	AA, TC	AA (8) + TC (3)	AA (0.18) + TC (0.07)	A (0.18± 0.091)^a T (0.035± 0.019)^a C (0.035± 0.02)^a
			P2	AA, CC, GG	AA (6) + CC (3) + GG (3)	AA (0.14) + CC (0.07) + GG (0.07)	A (0.14± 0.076)^a C (0.07± 0.042)^a G (0.07± 0.038)^a
			P4	CC, GG	CC (11) + GG (10)	CC (0.25) + GG (0.23)	C (0.25± 0.081)^a G (0.23± 0.79)^a

Data expressed as mean ± SE. values followed by different superscript letters are significantly different from one another within the same columns (P≤0.05).

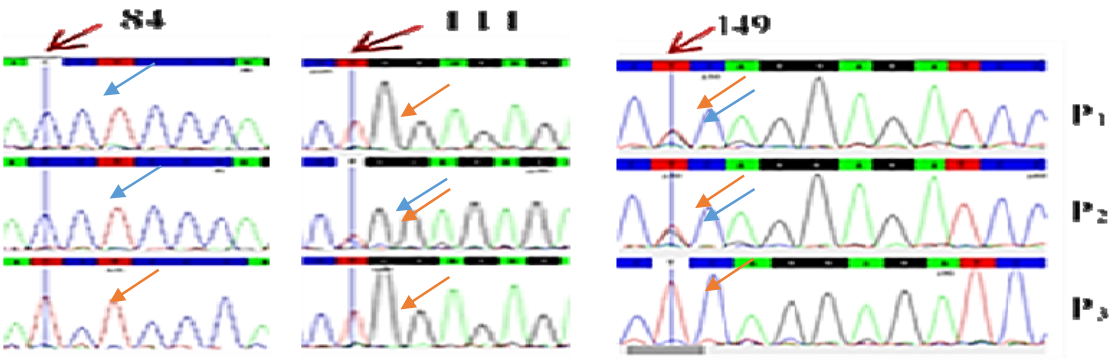

Figure 18. Sequence analysis of PCR-SSCP patterns (P1, P2 and P3), P1 has C, T, T nucleotides at the specified positions; P2 has C, C, T; and P3 has T, T, T of POU1F1 gene using primer 6.

By using primer 6, the PCR-SSCP results clarified 3 patterns. Nucleotide sequence analysis of PCR products (Fig. 18 and Tables6 and 7) identified differences between the three patterns, where several nucleotide substitutions were revealed in coding regions on charts in the positions 84, 111 and 149.

Table 6. Nucleotide sequence analysis of POU1F1 gene using primer 6 showing different alleles and genotypes in Egyptian domestic sheep and goats.

Patterns (P)	Species	Nucleotide sequences in the positions			Genotypes
		84	111	149	
P1	Sheep and goats	"C" allele (C/C)	"T" allele (T/T)	"T/C" allele	CC, TT, TC
P2	Sheep and goats	"C" allele (C/C)	"T/C" allele	"T/C" allele	CC, TC
P3	Goats	"T" allele "T/T"	"T" allele (T/T)	"T" allele (T/T)	TT

At position 84, patterns 1 and 2 were discriminated with "C" nucleotide (C/C), whereas pattern 3 had "T" nucleotide (T/T). Also, at position 111, the sequence analysis showed the presence of "T" nucleotide in each of pattern 1 and pattern 3 (T/T), while in pattern 2, "C" nucleotide was replaced with "T" nucleotide (T/C). Moreover, in position 149 "C" nucleotide was replaced with "T" nucleotide in patterns 1 and 2 (T/C), whereas in the same position (149) pattern 3 was discriminated with "T" nucleotide (T/T).

Table 7. Nucleotide sequence analyses of POU1F1 gene in Egyptian sheep and goats showing genotype and allele frequencies (using primers 6):

The used primer	Species	T. No. of animals	Type of patterns (P_s) of SSCP analysis	Genotype	No. of animals with SSCP patterns	Genotype frequencies	Allele frequencies
Primer 6	**Sheep**	**95**	P1	CC, TT, TC	CC (40) + TT (40) + TC (13)	CC (0.42) + TT (0.42) + TC (0.14)	C (0.49±0.145)^a T (0.49±0.127)^a
			P2	CC, TC	CC (1) + TC (1)	CC (0.01) + TC (0.01)	C (0.015±0.0115)^a T (0.005±0.0052)^a
	Goats	**44**	P1	CC, TT, TC	CC (1) + TT (1) + TC (1)	CC (0.02) + TT (0.02) + TC (0.02)	C (0.03±0.017)^a T (0.03±0.015)^a
			P2	CC, TC	CC (11) + TC (22)	CC (0.25) + TC (0.5)	C (0.5±0.109)^a T (0.25±0.076)^a
			P3	TT	TT (8)	TT (0.18)	T (0.18)

Data expressed as mean ± SE. values followed by different superscript letters are significantly different from one another within the same columns ($P \leq 0.05$).

4. Discussion

The results revealed genetic polymorphisms and different patterns (Polymorphic types) in POU1F1 gene of Egyptian sheep and goats. Moreover, nucleotide sequence analyses between the observed patterns revealed variable nucleotide substitutions causing different alleles (or different genotypes). The genetic analysis in this study noted that the high frequency of A allele (or AA genotype) was more frequent than other alleles or genotypes in animals that have the highest rates of litter size (twin production). In contrast, the high frequencies of C and T alleles or (CC and TT genotypes) were found to be induced the reduction of fecundity rates. Using the PCR-SSCP, PCR-RFLP and DNA sequencing methods, Lan *et al.* (2007c) detected several genetic polymorphisms in nine Chinese domestic caprine breeds. Their results observed four genetic variations, two mutations of them (T-to-G and T-to-C) were detected in the exon 6 and the other remaining two mutations of them (T-to-C) and (G-to-A) were found in the 3`UTR. Also, the using of ALuI PCR-RFLP and nucleotide sequence analysis detected a T to C silent mutation (Ser 279 Ser) in the 174th nucleotide of exon 6. Furthermore, in another study by Lan *et al.* (2007a) on genetic polymorphism in POU1F1 gene of eight Chinese local caprine breeds (Sa, LS, GZ, GB, MT, BJ, GW and LZ) by using DdeI PCR-RFLP and DNA sequence analysis, the results showed two mutations, the first mutation was T to G which located at the 60th base of exon 6 resulting in no amino acid change at the 241st of mature protein. In another study by Lan *et al.* (2007b) a mutation G to A transition was identified at the 92nd position of the 3`UTR region. Also, Lan *et al.* (2009) observed by using PstI PCR-RFLP a mutation T to C transition at position 110 of the 3`UTR region of POU1F1 gene in Inner Mongolia White Cashmere (IMWC) goats. The same authors (Lan *et al.*, 2009) used PCR-SSCP and nucleotide sequence analyses to screen the genetic polymorphism of the exons 1 to 5 and 5`UTR region in mentioned gene. Their results identified different 12 SNPs. In Jining Grey goats, (Feng *et al.*, 2012) showed by using PCR-SSCP, PCR-RFLP and nucleotide sequence analyses, the presence of genetic polymorphisms in POU1F1gene. Six mutations were detected including C 256 T in exon 3, C 53 T and T 123 G in intron 3 and G 682 T (A 228 S), T 723 G and C 837 T in exon 6.

In ovinePOU1F1 gene, (Bastos *et al.*, 2006a) revealed four mutations of 100 Portuguese indigenous "Churro da Terra Quents" sheep, these mutations included one G to A transition at codon 58 (Cys 58 Tyr) in exon 2, two G to A transitions in exon 3 (leading to the changes in amino acids such Gly 89 Asn and Ala 105 Thr), and an "A" to "G" transition in intron 4. Also, in another study, (Bastos *et al.*, 2006b) identified in ovine POU1F1 gene four alternative variants. These variants involved POU1F1-wild –type (wt), POU1F1-β (a 78 bp insert in the trans- activation domain), POU1F-γ (lacking exon 3) and POU1F1- δ (lacking exons 3,4 and 5), respectively.

Concerning the effect of genetic polymorphism of POU1F1 gene on reproductive performance including litter size, the findings of our results revealed that the presence of genetic polymorphisms in POU1F1 gene affected the litter size in Egyptian sheep and goat breeds. Similarly, in Chinese goats, (Lan *et al*., 2007a) observed that the DdeI RFLP Polymorphism T 241 G of POU1F1 gene was significantly (P<0.05) associated with litter size. Also, (Feng *et al*., 2012) identified that the two mutations (C 256 T and G 682 T out of six ones (C 256 T in exon 3, C 53 T and T 123 G in intron 3 and G 682 T (A 228 S), T 723 G and C 837 T in exon 6) in the POU1F1 gene were found to be significantly (P<0.05) affected litter size in Jining Grey goats. In Shaanbei White Cashmere goats (SBWC), (Zhu *et al*., 2019) found by direct DNA sequencing in POU1F1gene three important nucleotide polynorphisms (SNPs), c.682 G>T,c.723 T>G and c.837 >C. Two of these mutations, c.682 G>T and c..837 T>C loci were associated with litter size and the individuals that carry these mutations were found to have more offspring than the individuals that carry other mutation such c.723 T>G.

The association between genetic polymorphism in POU1F1 gene and reproductive traits was explained in previous studies**:**(Sun *et al*., 2002) reported that the polymorphisms in POU1F1 gene were investigated to have significant effects on the levels of both growth hormone (GH) and prolactin (PRL) in the neonates of Chinese Meishan pig. The lower levels of GH and the higher levels of PRL were observed in the neonates with MspI- DD genotype than other genotypes. Their results showed significant correlations between POU1F1 – α mRNA and both GH mRNA and GH plasma concentration levels. As mentioned in the above discussion, (Bastos *et al*., 2006a) detected four variants of POU1F1 ovine gene including POU1F1–wt, POU1F1 – β, POU1F1 γ– and POU1F1 δ. These variants displayed different trans –activation of GH and PRL promoters. The γ and δ variants significantly reduced the trans – activation of GH (P<0.001) and PRL (P<0.05). However, β variant increased 10% in a trans – activation capacity for GH promoter than wt. Moreover, β variant presented 12% in a trans-activation capacity for PRL promoter than those found in wt trans – activation capacity. These results confirm that fertility traits are regulated by several genes in which some SNPs affect the reproduction performance (Farag *et al*., 2018).

5. Conclusion

The present data proved that "A" allele of POU1F1 gene was more frequent in animals that have high production of offspring. In contrast, the high frequencies of C and T alleles were revealed to induce decrease in prolificacy trait in sheep and goats. Therefore, the genetic polymorphisms in POU1F1gene could contribute in performing the successful breeding program. This program could determine the favorable genomic to select a herd with high prolificacy or with greater litter size (twin production).

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Ethics approval and consent to participate

Blood samples were collected form sheep and goats under veterinary supervision and comply with local and international guidelines, international recommendations for the care and use of animals.The Blood samples were collected (after a written approval) from Animal Production Farm in Faculty Agriculture, Cairo University and Nubaria Farm belonging to National Research Centre, in addition to cooperating with the Governmental Halayieb Farm, Egypt). All procedures carried out on the animals were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Cairo University, Egypt with Permit (Reference) Number: CUFS F Mol. Biol. 50 15.

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