JJBS

Volume 15, Number 4,December 2022
ISSN 1995-6673
Pages 569 – 577
https://doi.org/10.54319/jjbs/150405

Jordan Journal of Biological Sciences

Genetic Polymorphisms of Inhibin-Ba (Inhβa) Gene and their Association with Twin Production Trait in Egyptian Small Ruminants

Hassan R. Darwish , Mohamad M. Aboelenin *, Kamilia B. Abd El-Aziz, Ibrahim M. Farag

Cell Biology Department, National Research Centre, Giza, Egypt

Received: June 4, 2021; Revised: January 16, 2022; Accepted: February 2, 2022

Abstract

Reproductive traits are regulated by several genes, and genetic polymorphism within these genes which have a role on the reproductive performance could be used in marker assisted selection breeding programs. Several variants of inhibin βA (INHβA) gene affect the litter size in small ruminant. Therefore, different single nucleotide polymorphisms (SNPs) within this gene could be used as a genetic marker to improve the reproductive efficiency (specially litter size) of sheep and goats. The objective of this study is to find out INHβA subunit-encoding gene polymorphisms by employing PCR-SSCP assay and subsequent analyses of nucleotide sequence, as well as to investigate the potential association of these genetic polymorphisms with twin yield in Egyptian goats and sheep breeds. One hundred and thirty-nine females were chosen, 113 of them were mothers at the 1st., 2nd., 3rd and 4th parity, and the remaining 26 females were young at sexual maturity age. Blood samples were collected for genomic DNA extraction and PCR amplification were performed using five specific primers spanning the entire INHβA CDS partial 5`UTR. Four out of the five PCR amplicons displayed different genetic polymorphisms, and the subsequent nucleotide sequence analyses showed many nucleotide substitutions. In sheep, the amplified fragment 1 showed 3 different genotypes A, C and A/C. The sequence analysis for the 3rd amplicon showed two different genotypes, A and G. Also, the 5th amplicon reveals 3 different genotypes, A, G and A/G. In goats, the sequence analysis of the 4th amplified fragment showed different three genotypes, G, A and A/G. The statistical analysis proposed that the genotype AA (in sheep) could be favorable gene markers for twin production. The present study suggested that the genetic polymorphisms of INHβA gene might have effects on fecundity traits in Egyptian small ruminants.

Keywords: INHβA gene, PCR-SSCP, Genetic polymorphism, Twin production, Egyptian Sheep and Goats.

1. Introduction

Egyptian sheep and goats are considered as one of the earliest domesticated farm animals. They have played a crucial part in animal husbandry due to their extensive uses in many disciplines, such as meat, milk, wool, hair and skin production. So, it is important to improve the reproductive traits of such species to meet people's increasing need of different animal products. Also, the enhancement of reproduction performance will ultimately pave the way for the economic benefit of farmers (Tudu *et al.*, 2015; Mishra *et al.*, 2017).

Reproductive traits are polygenic in nature and controlled by many genes (Ray *et al.*, 2016). The identification of important genetic variations of the genes responsible for reproductive traits in farm animal populations will help to reveal marker assisted selection in a more precise manner (Ahlawat *et al.*, 2015). The inhibin beta A subunit encoding gene (INHβA) may be suitable candidate that may affect the litter size in small ruminant species (Zi *et al.*, 2012). The identification of single nucleotide polymorphisms (SNPs) of such gene and studying their association with fecundity traits may be helpful for enhancing the reproductive efficiency (especially litter size) of sheep and goat animals (Chu *et al.*, 2007; Zi *et al.*, 2012). Several studies showed that the inhibin is considered to be a glycoprotein hormone, and it belongs to the superfamily called transforming growth factor-beta (TGF-β). This superfamily and its related cell-surface receptors are important intra-ovarian regulatory factors of ovarian follicular development and ovulation rate (Ling *et al.*, 1985; Rivier *et al.*, 1985; Robertson *et al.*, 1985; Woodruff *et al.*, 1996). Inhibin was found to consist of two subunits, α and β linked by disulfide bonds (Mason *et al.*, 1985). It was revealed to engaged in the regulation, synthesis and secretion of the pituitary follicle-stimulating hormone, steroidogenesis and maturation of follicles (Leyhe *et al.*, 1994; Hiendleder *et al.*, 1996b; Chu *et al.*, 2007). Also, Phillips (2005) reported that the inhibin is expressed in different organs of the body including testis, ovary, uterus in human, pig, sheep and mouse. However, a previous study in sheep, Rodgers *et al.* (1989) observed that follicles are considered to be the major source of inhibin; Moreover, Medan *et al.* (2003) reported that the

* Corresponding author. e-mail: Mohamed2000mail@yahoo.com.

inhibin is a major factor that controls the ovulation rate in domestic and laboratory animals.

INHβA ovine gene was revealed to be located on chromosome 4q26 (Brunner *et al.*, 1995). Some studies showed that INHβA gene has a main role in controlling the reproductive performance in sheep and goats: In sheep, Fleming *et al.* (1992) revealed that Booroola ewes have significant high level of inhibin-βA mRNA in follicles of homozygous FecB gene-carriers as compared to control. Also, in different sheep breeds, Vanmontfort *et al.* (1998) found significant breed differences in circulating β-inhibin concentrations with different reproductive performances. Moreover, Leyhe *et al.* (1994), Hiendleder *et al.* (1996a), Hiendleder *et al.* (1996b) and Chu et al. (2007) reported that in sheep, the polymorphisms of inhibin-βA gene have had obvious significant impact on the litter size in different breeds. In goats, Zi *et al.* (2012) studied cDNA sequences and mRNA expression of INHβA gene in prolific (lezhi black) and nonprolific (Tibetan) goat breeds; and they have analyzed sequences of 1,360 base pairs that encode an assumed peptide of 425 amino acids. The comparison between the two breeds showed three base changes in INHβA (A to G at positions: 318, 831 and 1109), resulting in a substitution of one amino acid (Asp to Gly). These authors suggested that the presence of base changes in INHβA gene that resulted in amino acid substitution might be influencing the regulatory mechanism of differential fertility in these breeds. Since the studies on genetic variations in INHβA of Egyptian small ruminant species were lack, the objective of this study was to identify the polymorphism in INHβA gene and to investigate the association of these SNPs with enhancing litter size in local sheep and goat breeds.

2. Materials and Methods

2.1. Animals

The sheep and goats used in this study included 139 females. These females consisted of 95 of sheep breeds (Barki, Osseimi, Rahmani, Saudanez and Awase) and 44 of goat breeds (Zaraiby, Damascus, Boer, Saanine and Barki). 83 of sheep and 30 of goats were mothers at first, second, third and fourth parity, and they were used for the association of identified genotypes in INHβA gene with the twin production or with the litter size. The remaining 26 females (12 of sheep and 14 of goats) were young and at an age of sexual maturity. For the same reproductive trait (twin production), these young females were conducted to select the animals that carry favorable gene markers (alleles) for their utilizing in successful breeding program. The animal breeds were sourced from Animal Production Farms belonging to Faculty of Agriculture (Cairo University), Nubaria belonging to National Research Centre and Governmental Halayieb, Egypt. 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 samples of blood were collected. Genomic DNA was extracted from the whole blood samples according to the method described previously (Aboelenin *et al.*, 2017a; Aboelenin *et al.*, 2017b; Mahrous *et al.*, 2020c). DNA concentration and quality were assessed and stored at -20°C before use.

2.3. Primers and PCR amplification

Primers sequences were shown in table 1. These primers were cited by Chu *et al.* (2007) and used for identification of genetic polymorphisms in INHβA gene by using PCR-SSCP technique and nucleotide sequence analysis. 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; 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 as follows: firstly, 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.

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

Primer	Primer sequence (5'→3')	Product size	Amplified region	Reference sequence
Primer 1	F: CAGGATGCCCTTGCTTT R: CATCGGGTCTCTTCTTCAA	224 bp	Exon 1 (1351-1574)	U16238
Primer 2	F: CACTTGAAGAAGAGACCCG R: CACCTGATTCCGCGAAC	193 bp	Exon 1 (1553-1745)	
Primer 3	F: GGCACAGCCAGGAAGACG R: CGTATGTCCAGGGAGCTCTTG	335 bp	Exon 2 (398-732)	
Primer 4	F: ATACGGATTGCCTGTG R: CTCACAGTAGTTGGCGT	333 bp	Exon 2 (728-1060)	U16239
Primer 5	F: GCTACCACGCCAACTACTGT R: TCTCTGGACCATCTCGCTC	293 bp	Exon 2 (1038-1330)	

2.4. Single-strand conformational polymorphism (SSCP)

For performing the SSCP analysis, the PCR products had been resolved. 10 ul of PCR product had been diluted in denaturing solution that consisted of A and B types. "A" type solution involved 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). About 10% SSCP gel mixture (30 ml) had been prepared through acrylamide- -bisacrylamide (37.5: 1), TEMED (30 ul) 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.

2.5. Sequence analysis

In order to clarify the nucleotide analysis in the SSCP patterns that were given of tested gene (INHβA) in the present study, PCR product were purified and sequenced by special company (Macrogen Incorporation, Seoul, Korea). Sequence analysis and alignment were performed by cluster wide analysis using CodonCode Aligner software, CodonCode Corporation, USA.

2.6. Statistical analysis

The obtained data for PCR-SSCP patterns had been statistically analyzed by one way ANOVA followed by two-way ANOVA. The differences among pattern groups were determined significantly according to the method of Waller and Duncan (Waller *et al.*, 1969). Also, the allele frequencies that were revealed in this study had been statistically analyzed using T-test.

The values are expressed as mean ± SE. All statements of significance were based on probability of ($P \leq 0.05$).

3. Results

3.1. PCR amplification of INHβA gene:

Genomic DNA of 139 animals of different sheep and goat breeds had been used to amplify the entire CDS and partial 5`UTR of INHβA gene using five primers pairs spanning 1326 bp. PCR amplicons were run on a 2% agarose gel and the five PCR amplicons sizes were 224 bp, 193bp, 335bp, 333bp and 293bp, respectively (Figurers 1 to 5). The first primer has had amplified the first 224 bp stretch of the INHβA gene (1 to 224), the second primer amplified the stretch from 203 to 395, the third one covered the distance from 396 to 730, the fourth one amplified the distance from 726 to 1058 and the fifth primer covered the distance from 1036 to 1328. These amplified products found to be consistent with the target regions with high specificity were used directly in SSCP assay.

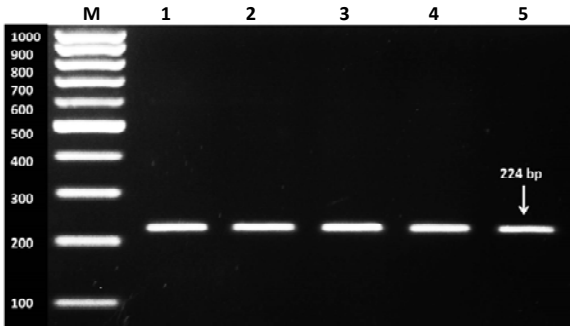

Figure 1. PCR product (lanes 1-5) of primer 1 of INHβA gene at size 224 bp. Lane M: 100 bp ladder.

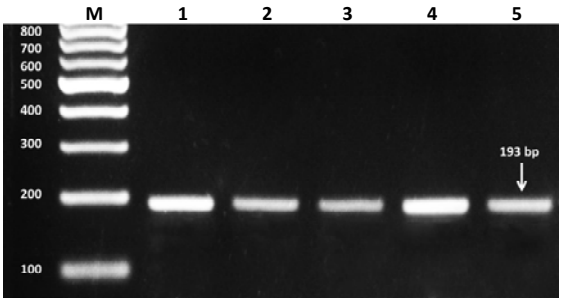

Figure 2. PCR product (lanes 1-5) of primer 2 of INHβA gene at size 193 bp. Lane M: 100 bp ladder.

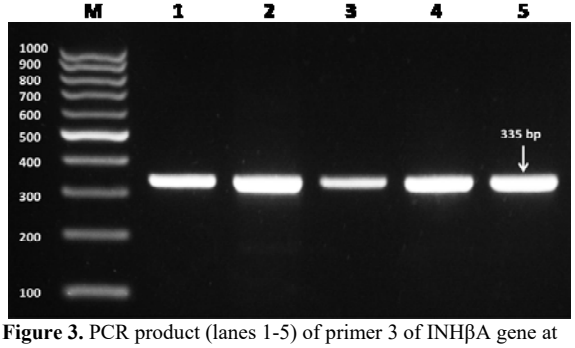

Figure 3. PCR product (lanes 1-5) of primer 3 of INHβA gene at size 335 bp. Lane M: 100 bp ladder.

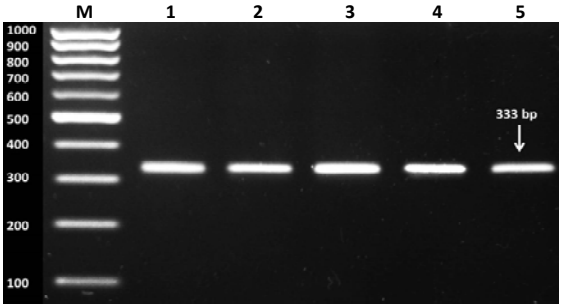

Figure 4. PCR product (lanes 1-5) of primer 4 of INHβA gene at size 333 bp. Lane M: 100 bp ladder.

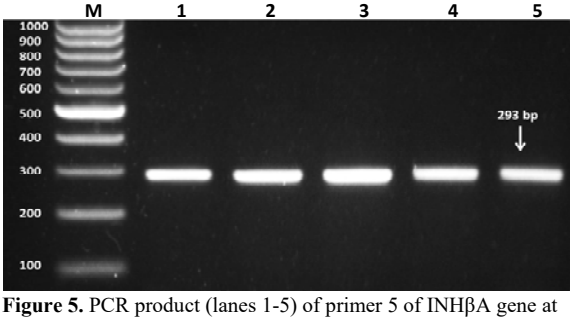

Figure 5. PCR product (lanes 1-5) of primer 5 of INHβA gene at size 293 bp. Lane M: 100 bp ladder.

3.2. PCR-SSCP analysis:

3.2.1. PCR-SSCP of primer 1 of INHβA gene:

PCR-SSCP analyses of the first amplicon (224bp) were shown in Figure 6 and Table 2. The results revealed three different banding patterns (P_1, P_2 and P_3) in sheep. However, in goats, only one pattern was found, and all goats were subjected to pattern (P_1) as that of sheep, and they were monomorphic on gel.

In sheep, 62 animals classified as pattern one, comprise 59 mothers and 3 females were at puberty, whereas 29 animals were classified as pattern two involving 21 mothers and 8 females at puberty. Only 4 animals were classified as pattern three, and consisted of 3 mothers and one female at puberty. Statistical analysis revealed that P1-mothers have high rates of litter size as compared with that of P3, while there is a significant increase in lambing mean number when compared to that of P2 or P_3. Consequently, P1-genotype is deemed to be a prolificacy gene marker.

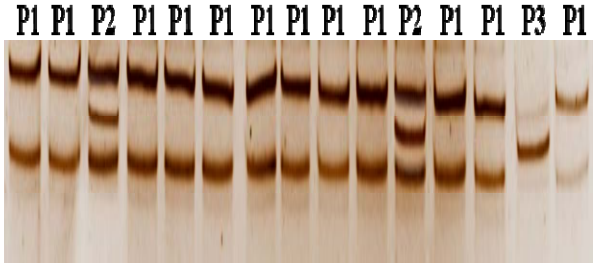

Figure 6. Shows three SSCP different patterns using primer 1 of INHβA gene.

Table 2. Shows genetic polymorphisms (SSCP patterns) of INHβA gene using primer 1 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)	Mean No. of S.b	Mean No. of T.b	Mean No. of lambing	Mean No. of litter size (Twin production)
Sheep	95	P1	3	59	61	42	1.03	0.71	2.45 ± 0.4^{c}	1.74 ± 0.1^{b}
		P2	8	21	22	7	1.04	0.33	1.71 ± 0.14^{b}	1.47 ± 0.24^{ab}
		P3	1	3	3	0.0	1.00	0.00	1.00 ± 0.23^{a}	1.00 ± 0.49^{a}
Goats	44	P1	14	30	29	35	0.97	1.16	3.30 ± 0.12^{d}	2.13 ± 0.32^{c}

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

3.2.2. PCR-SSCP of primer 2 of INHβA gene:

The second fragment (193bp) revealed just one pattern as shown in Figure 7. No polymorphisms were found, and both sheep and goats were monomorphic.

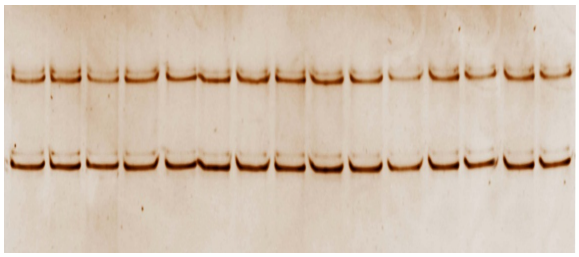

Figure 7. Shows SSCP pattern (monomorphic) using primer 2 of INHβA gene.

3.2.3. PCR-SSCP of primer 3 of INHβA gene:

PCR-SSCP analysis of the third fragment (335 bp) was shown in Figure 8 and Table 3. The results detected polymorphisms in sheep by the presence of two banding patterns, pattern 1 (P_1) and pattern 2 (P_2). However, the banding patterns in all goats were monomorphic, and they were classified as pattern 1 as that of sheep. In sheep, 70 animals were classified as pattern 1, 63 of them were mothers and the remaining 7 animals were at puberty; whereas 25 females including 20 mothers and 5 animals were at puberty were classified as pattern 2. Statistical data showed that P_1-mothers had a significant high rates of litter size and increase of mean number of lambing as compared to P_2-mothers. So, the polymorphism of this site could be considered a prolificacy gene marker.

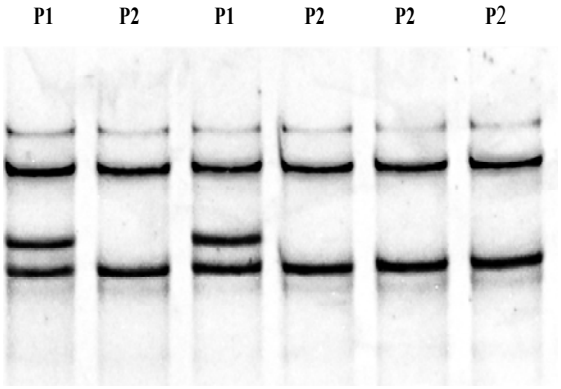

Figure 8. Shows two SSCP different patterns using primer 3 of INHβA gene.

Table 3. Shows genetic polymorphisms (SSCP patterns) of INHβA gene using primer 3 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)	Mean No. of S.b	Mean No. of T.b	Mean No. of lambing	Mean No. of litter size (Twin production)
Sheep	95	P1	7	63	67	44	1.06	0.69	2.46±0.15^{b}	1.75±0.19^{b}
		P2	5	20	19	5	0.95	0.25	1.45±0.16^{a}	1.20±0.7^{a}
Goats	44	P1	14	30	29	35	0.97	1.17	3.30±0.2^{c}	2.14±0.19^{b}

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

3.2.4. PCR-SSCP of primer 4 of INHβA gene:

PCR-SSCP analyses of the fourth fragment of INHβA gene (333 bp) were shown in figure 9 and table 4. Three banding patterns (P_1, P_2 and P_3) were identified in goats. However, in sheep, the genetic polymorphisms were not detected, and all sheep females were subjected to pattern 1 as that of goats and they were monomorphic.

In goats, pattern 1 (P1) was investigated in 32 females, 22 of them were mothers and the remaining 10 females were at puberty, whereas P2 was observed in 10 animals including 6 mothers and 4 females at sexual maturity age. Moreover, pattern 3 (P3) was only detected in two mothers. The results showed that P2 had elevated lambing mean number and high litter size rates when compared to P1-mothers, but no statistical significance was found.

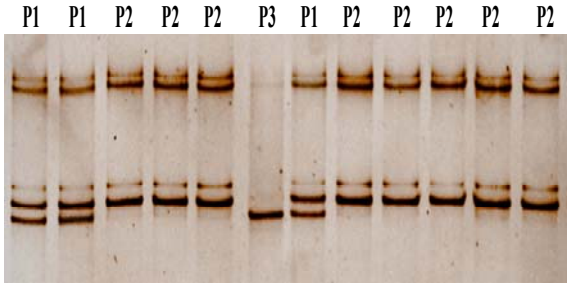

Figure 9. Shows three SSCP different patterns using primer 4 of INHβA gene.

Table 4. Shows genetic polymorphisms (SSCP patterns) of INHβA gene using primer 4 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)	Mean No. of S.b	Mean No. of T.b	Mean No. of lambing	Mean No. of litter size (Twin production)
Sheep	95	P1	12	83	86	49	1.04	0.59	2.22±0.19^{a}	1.63±0.2^{a}
		P1	10	22	24	22	1.09	1.00	3.09±0.27^{ab}	2.09±0.28^{b}
Goats	44	P2	4	6	4	9	0.67	1.50	3.67±0.33^{bc}	2.17±0.18^{b}
		P3	0.0	2	1	4	0.50	2.00	4.50±0.5^{c}	2.5±0.5^{c}

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

3.2.5. PCR-SSCP of primer 5 of INHβA gene:

PCR-SSCP analyses of the fifth fragment of the inhibin beta A gene (293bp) were shown in figure 10 and table 5. The results detected genetic polymorphisms in sheep animals. These polymorphisms were represented by the presence of 3 banding patterns (P_1, P_2 and P_3). However, in goats, the genetic polymorphisms were absent. All goat animals were subjected to pattern 2 (P_2) as in sheep and they were monomorphic. In sheep, pattern 1(P_1) was found only in 3 mothers, while pattern 2 (P_2) was found in 39 animals involving 34 mothers and 5 females at sexual maturity age. Pattern 3 (P_3) was found in 53 females including 46 mothers and 7 females at puberty. The P3-mothers showed high litter size rates, which was insignificant in comparison with the mothers of P1 or P2 genotype. The mothers of pattern 3 showed a statistically significant increase in lambing rate number in comparison with P1-ewes. So, P3 or P2 genotypes could be considered as a potential fecundity gene marker.

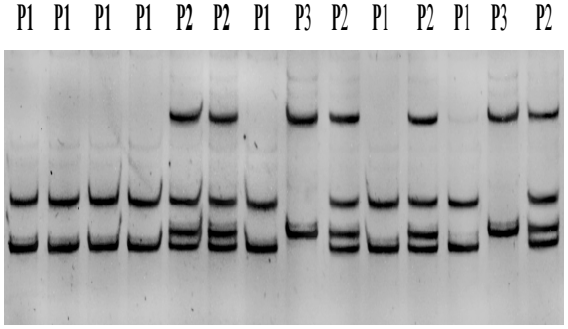

Figure 10. Shows three SSCP different patterns using primer 5 of INHβA gene.

Table 5. Shows genetic polymorphisms (SSCP patterns) of INHβA gene 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)	Mean No. of S.b	Mean No. of T.b	Mean No. of lambing	Mean No. of litter size (Twin production)
Sheep	95	P1	0.0	3	3	0.0	1.00	0.0	1.00±0.57[a]	1.00±0.23[a]
		P2	5	34	40	16	1.18	0.47	2.12±0.15[b]	1.65±0.18[ab]
		P3	7	46	43	33	0.93	0.72	2.37±0.11[b]	1.65±0.17[ab]
Goats	44	P2	14	30	29	35	0.97	1.17	3.30±0.15[c]	2.14±0.18[b]

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

3.3. Nucleotide Sequence Analyses of INHβA Gene:

Genetic polymorphisms in INHβA gene were detected in three amplified fragments (1, 3 and 5) in sheep, while the fourth amplified fragment was polymorphic in goats; on the other hand, no genetic polymorphisms were detected in the amplified fragment 2 in both sheep and goats. The nucleotide sequence analysis of PCR amplicon1 revealed the presence of a SNP in the position 113 (Fig.11). P_1 discriminated with "A" nucleotide (A/A), while P_2 discriminated with "C" nucleotide (C/C). P_3 ewes had both alleles with "C" and "A" nucleotide (A/C). Figure 12 shows sequence analysis of monomorphic type of PCR-SSCP analysis by using primer 2.

The primer 3 showed two patterns (P_1 and P_2) in INHβA gene. Nucleotide sequence analyses of the third amplicon showed differences between the two patterns in position of 157 on charts (Fig.13). In this location, pattern 1 discriminated with "A" nucleotide "(A/A), while P2 had "G" nucleotide (G/G) in the same location on the chart.

There were three different patterns of the fourth amplicon using primer 4 (P1, P2 and P3). Nucleotide sequence analysis identified differences between the three patterns in the position of 123 on charts (Fig.14) where pattern 1 discriminated with nucleotide "G"(G/G), while pattern 2 discriminated with nucleotide "A" (A/A). P3-animals had both alleles (A/G), where they had both G and A nucleotide at the position 123.

Moreover, using primer 5 showed three patterns. Nucleotide sequence analysis proved differences between the three patterns in the position 222 on charts (Fig.15). Pattern 1 discriminated with "G" nucleotide (G/G), while pattern 2 had both alleles with “G” and “A” nucleotides (A/G). Furthermore, pattern 3 was discriminated with "A" nucleotide (A/A).

Table 6 shows the detected alleles and genotypes of INHβA gene in local domestic sheep and goats in Egypt.

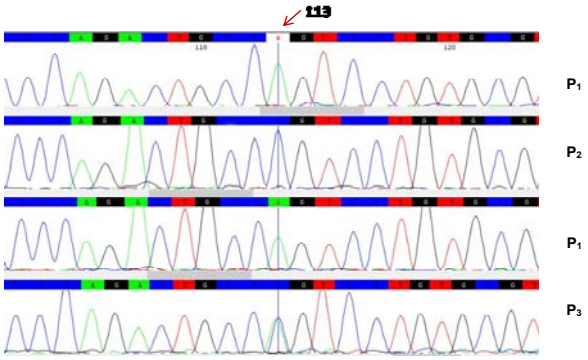

Figure 11. Sequence analysis of PCR-SSCP patterns (P_1, P_2 and P_3) of INHβA gene using primer 1.

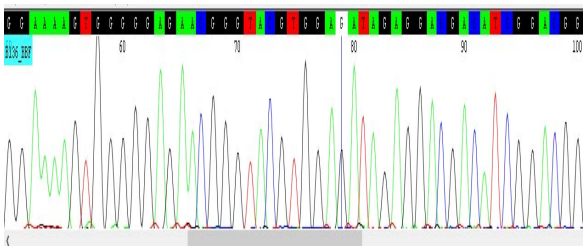

Figure 12. Sequence analysis of monomorphic (showed no any of SNPs) type of PCR-SSCP analysis of INHβA gene using primer 2.

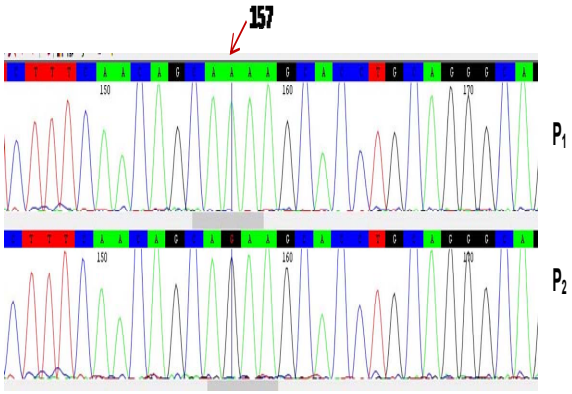

Figure 13. Sequence analysis of PCR-SSCP patterns (P_1 and P_2) of INHβA gene using primer 3.

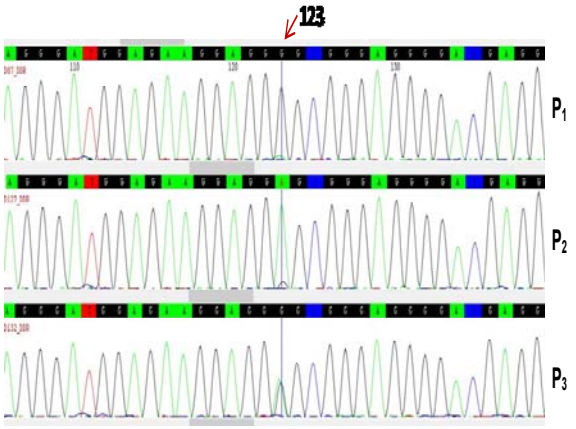

Figure 14. Sequence analysis of PCR-SSCP patterns (P_1, P_2 and P_3) of INHβA gene using primer 4.

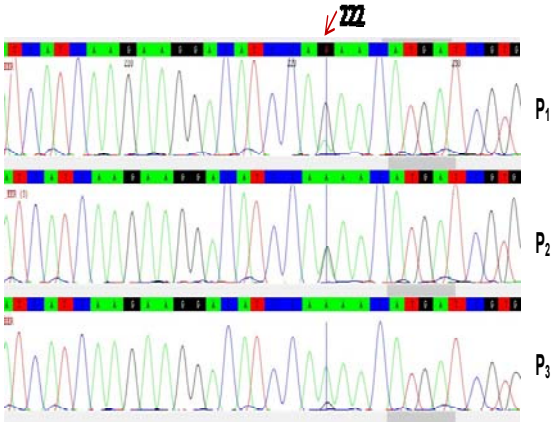

Figure 15. Sequence analysis of PCR-SSCP patterns (P_1, P_2 and P_3) of INHβA gene using primer 5.

Table 6. Nucleotide sequence analysis of INHβA gene in Egyptian sheep and goats showing relative genotype and allele frequencies.

The used primer	Species	No. of animals	Type of patterns (Ps) of SSCP analysis	Genotype	No. of Animals with SSCP pattern	Relative genotype frequency	Relative allele frequency
Primer 1	Sheep	95	P1	AA	62	0.65	A(0.67 ± 0.01)[b] C(0.33 ± 0.09)[a]
			P2	CC	29	0.31	
			P3	AC	4	0.04	
	Goats	44	P1	AA	44	1.00	A(1.00 ± 0.09)[b] C(0.0 ± 0.0)[a]
Primer 3	Sheep	95	P1	AA	70	0.74	A(0.74 ± 0.126)[b] G(0.26 ± 0.118)[a]
			P2	GG	25	0.26	
	Goats	44	P1	AA	44	1.00	A(1.00 ± 0.0009)[b] G(0.0 ± 0.0)[a]
Primer 4	Goats	44	P1	GG	32	0.73	A(0.25 ± 0.12)[a] G(0.75 ± 0.11)[b]
			P2	AA	10	0.23	
			P3	AG	2	0.04	
	Sheep	95	P1	GG	95	1.00	A(0.0 ± 0.0)[a] G(1.00 ± 0.01)[b]
Primer 5	Sheep	95	P1	GG	3	0.03	A(0.76 5± 0.1)[b] G(0.235 ± 0.11)[a]
			P2	AG	39	0.41	
			P3	AA	53	0.56	
	Goats	44	P2	AG	44	1.00	A(50 ± 0.04)[a] G(50 ± 0.04)[a]

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 present results by using SSCP and nucleotide sequence analyses detected different genetic polymorphisms in INHβA gene of Egyptian sheep and goat breeds. Some of these genetic polymorphisms were shown to be associated with increasing the litter size mean number and the lambing mean number in the local sheep and goats. The current results are consistent with that revealed by Chu *et al.* (2007). These authors studied the genetic polymorphisms in INHβA gene in eight sheep breeds, including two high fertile breeds (Small Tail Han and Hu sheep) and six low fertile breeds (Chinese Merino, Corriedale Dorest, German Mutton Merion, South African Mutton Merion, Texel, sheep). Their results determined 21 single nucleotide polymorphisms (SNPs) in the entire coding region and partial 3`UTR. These SNPs formed 12 genotypes and 9 alleles. 17 SNPs of 21 were from Hu breed; this breed is discriminated with early sexual maturity, year-round estrus and high prolificacy. Furthermore, the Small Tail Han KL-genotype mothers had more lambs than those with KK-genotype ewes. Also, the present findings are in coincidence with those revealed in several previous studies on ovine INHβA gene polymorphisms, where Hiendleder *et al.* (1992) observed allelic presence in Merino landschafe, Booroola Merino X Merino landschafe and East friesian Milk sheep (1.9 for A1 and 1.5 for A2). The frequency of alleles was 0.18 (A1) and 0.82 (A2). Moreover, Hiendleder *et al.* (1996b) studied the genetic polymorphism in INHβA gene of five sheep breeds (Merino landschaf, East Friesian Milk sheep,

Rhoenschaf, Romanov and Heidschnucke) with different reproductive performance and compared with wild sheep (O. musimon, O. ammon, O. vignei) breeds; TaqI RFLP were identified. Nucleotide sequence analysis had revealed variable SNPs at positions 21 (A /G), 25 (C/T), 80 (A/G), 119 (C/T), 165 (C/T), 216 (C/T), 260 (A/T), 310 (C/G) and 372 (A/G) between domestic and wild sheep; 3 of them led to changes in the inferred sequences of the amino acid structure. They found that the frequencies of INHβA TaqI alleles significantly differed between breeds, and allele A has a positive effect on the average litter sizes. Moreover, in 4 sheep breeds (East Friesian Milk sheep, Romanov, Merino landschaf and Rhoenschaf), Leyhe *et al.* (1994) observed 2 TaqI alleles of INHβA gene, these breeds were found to be significantly different in TaqI allele frequencies with different reproductive performance. Hiendleder *et al.* (1996a) pointed the different alleles of ovine INHβA gene (by using genomic cloning and comparative sequence analysis) and studied their effects on the litter size in East Friesian and Merino landschaf Milk sheep mothers. They identified that the nucleotide substitution had influenced the fertility rate. On the other hand, Fleming *et al.* (1992) observed high significant levels of INHβA mRNA in follicles that carry FecB gene when comparing with control. Also, Jaeger *et al.* (1994) examined 1,000 lambing records and observed that the genetic polymorphism had clear effect on litter size in sheep, which confirms the fact that reproductive traits are polygenic in nature (Ray *et al.*, 2016; Farag *et al.*, 2018).

5. Conclusions

In conclusion, the present study demonstrated that INHβA is a potent candidate gene that affect fecundity trait in local small ruminants in Egypt. So, the polymorphisms in such genes are favorable genetic markers that could be investigated before starting breeding programs and could be utilized for improving twin production in Egyptian sheep and goat breeds.

Acknowledgements

We acknowledge the financial support of the National Research Center to cover all the materials which was provided as a "Internal project (No. 11020104) and under title": "Use of gene markers and Ca2+ regime for improving production of twins in Egyptian domestic sheep and goats", PI: Dr. Ibrahim Farag.

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, and in cooperating with the Governmental Halayieb Farm, Egypt). The 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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