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Genetic Polymorphism Detection in Four Genes in Egyptian and Saudi Sheep Breeds

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Abstract: This work aimed to study the genetic polymorphism in Booroola gene (FecB), Bone morphogenetic protein-15 (BMP-15), Myostatin (MSTN) and Ghrelin genes and its effects on the production. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect polymorphisms of three candidate genes in five Egyptian and Saudi sheep breeds (Barki, Ossimi, Rahmani, Najdi and Harri) to detect the genotype of FecB, BMP-15, MSTN genes and their allele frequencies. Genotypes were determined by *PCR* amplification followed by single nucleotide polymorphism (*SNP*) anthor method for ghrelin gene (Barki, Rahmani, Saidi, Najdi and Harri). Results showed that the polymorphism frequencies of *FecB* gene with *Avall* digestion are significantly imbalanced in five breeds. The Najdi, Harri, Barki, Rahmani and Ossimi sheeps were all homozygous carriers (BB). PCR-RFLP with *HinfI* digestion was used to investigate the genotyping of $FecX^G$ loci in exon 2 BMP-15 genes. The genotype frequencies for ++ were: 0.32, 0.30, 0.29 from Barki, Ossimi, Rahmani, respectively, while +G genotype were 0.68, 0.70 and 0.71 after digestion with *HinfI*. Frequencies of allele + were 0.66, 0.65 and 0.64 in Barki, Ossimi, Rahmani, breeds in Egypt, while 0.34, 0.35 and 0.36 for allele G in Barki, Ossimi, Rahmani, breeds. BMP-15 gene was found to be monomorphic in Najdi and Harri Saudi sheep breeds. Myostatin gene was found to be monomorphic with *HaeIII* digestion in all sheep breeds. All Egyptian and Saudi samples in myostatin locus were digested by *HaeIII* enzyme and showed the mm genotype, all of them were monomorphic. The sequence analysis of ghrelin amplified PCR product indicated three nucleotide substitutions (T to C; A to G and A to C) at nt 38 and nt49, nt 108, nt 60 and nt 71 in Rahmani. The six different detected variables were present in the Saidi sheep breed at different frequencies with a majority of five transversion and one transition (G to T, A to T, A to T, G to C, G to T) at nt 14, nt 56, nt 58, nt 67, nt 60 and 71, respectively. This is one of the first studies on polymorphism of *BMP-15*, myostatin and *ghrelin* genes in Egyptian and Saudi sheep breeds.

Key words: Sheep · RFLP · SNP · Fecundity Genes · Meat Genes

INTRODUCTION

There are 629 sheep breeds (*Ovis aries*) currently maintained in the 52 European countries and 233 maintained in the Asian and Pacific countries [1]. In many developing countries, there are a number of indigenous herds that represent unique lines. They were classified on the bases of morphological characteristics and were given local names [2]. Sheep contribute 6% of the total red meat production in Egypt. The total sheep population in Egypt is 4,200,000 heads. Rahmani, Ossimi and Barki, are of the main sheep breeds in Egypt with a population of 990,000, 514,000 and 470,000 respectively [2].

At present two major genes affecting fecundity (FecB and BMP-15) had been discovered in different breeds of sheep. FecB gene or the Booroola gene (FecB) was the first major gene for prolificacy identified in sheep. The FecB is an autosomal gene in sheep with a large effect on ovulation rate and consequently, litter size. This locus is situated in the region of ovine chromosome 6 corresponding to the human chromosome 4q22-23 that contains the bone morphogenetic protein receptor IB (BMPR-IB) gene, which encodes a member of the transforming growth factor β (TGFβ) receptor family [3, 4]. Many aspects of the FecB gene, including reproductive endocrinology [5], ovary development [6], litter size,

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organ development and body mass [7] have been studied. This gene has an additive effect on litter size and ovulation rate, but has negative effects on fetal growth and development and body mass during gestation.

One copy of FecB increases ovulation rate by about 1.5 and two copies (homozygous carriers) increase by about 3 [8]. According to increased ovulation rates, these extra ovulations increase litter sizes about 1 and 1.5 respectively. It has been shown that, effect of FecB is caused by a point mutation in position 830 leading to an arginine/glutamine transition (Q249R) in the bone morphogenetic protein 1B receptor (BMPR-1B) expressed in oocytes and granulosa cells [9].

Bone morphogenetic protein 15 (BMP-15) is a growth factor and a member of the TGFβ superfamily that is specifically expressed in oocytes. The sheep BMP-15 gene maps to the X chromosome [10]. Bone morphogenetic protein 15 regulates granulosa cell proliferation and differentiation by promoting granulosa cell mitosis, suppressing follicle-stimulating hormone receptor expression and stimulating kit ligand expression, all of which play a pivotal role in female fertility in mammals [11-16]. The FecX ^{G} mutation (Q239Ter) in the BMP-15 gene was associated with increased ovulation rate and sterility in Cambridge and Belclare sheep [17]. The other one mutation in BMP-15 is FecX^{G} (Galway) mutation. Galway allele corresponds to a C/T transition at nucleotide 718. FecX ^{G} mutation leads up to a premature stop codon at amino acid 239 of unprocessed protein thus no mature protein is produced [18].

Myostatin (*MSTN*) or growth differentiation factor-8 (*GDF-8*): Ovine myostatin gene is located on chromosome 2 and consists of three exons and two introns in all species studied [19]. A novel regulatory mutation causing a hypermuscled phenotype in Belgium Texel sheep strain was identified by Clop *et al*. [20]. Sonstegard *et al*. [21] reported that myostatin is a member of the mammalian growth transforming family (*TGF-beta superfamily*), which plays a role in the regulation of skeletal muscle growth. They are known to block myogenesis, hematogenesis and enhance chondrogenesis as well as epithelial cell differentiation *in vitro*. In mice, null mutants are significantly larger than wild-type animals [22]. Muscular hypertrophy, also known as “double-muscling” in Belgian Blue and Piedmontese cattle breeds was shown to result from mutations in the coding region of the myostatin gene [23, 24]. In sheep, quantitative trait loci (QTL) studies showed that myostatin gene had a major effect on muscular development in Belgian Texel [25] and on muscling depth in New Zealand Romney sheep [26], Norwegian white sheep [27] and Charollais sheep [28].

Ghrelin gene: Growth hormone secretion by the somatotroph cells depends upon the interaction between hypothalamic regulatory peptides, e.g. growth hormone-releasing hormone and somatostatin [29]. Recently a novel growth hormone- releasing acylated peptide, ghrelin, has been purified and identified in rat stomach [30]. In ruminants, several reports have been published to investigate the physiological characteristics of ghrelin. The direct evidence of ghrelin to secrete growth hormone from anterior pituitary cells in cattle was offered in the *in vitro* study [31]. Sugino *et al*. [32] and Sugino *et al*. [33] showed that a transient surge in plasma ghrelin levels occurred just prior to a scheduled meal and pseudo-feeding in sheep and that this transient surge was modified by the feeding regimen. Ghrelin secretion seems to be regulated by cholinergic neurons of the vagus and that cholinergic activity suppresses ghrelin secretion in sheep [34].

Best to our knowledge this is the first report of investigation of BMP-15, Myostatin and Ghrelin genes in Egyptian and Saudi sheep breeds. The present study aimed to identify genetic polymorphisms and point mutations for some genes associated with economically important productivity traits in Egyptian and Saudi sheep.

MATERIALS AND METHODS

Animals: Whole blood 140 samples were collected from sheep animals belonging to four main sheep breeds reared in Egypt and two Saudi sheep breeds. The blood samples were collected from different farms belonging to Animals Production Institute. The breeds used in this study are, Rahmani (24 from Animal Breeding Research Station in Sero, Domiata), Barki (25 from Animal Breeding Research Station in Borg El-Arab, Alex) Ossimi and Saidi (23 & 25 from Animal Breeding Research Station in Seds, Bani Swif) while the Saudi sheep breeds (21 Najdi and 22 Harri) obtained from the slaughter house in Jadda, Faculty of Science, King Abd El-Aziz University.

DNA Extraction: Genomic DNA was extracted from the whole blood according to the method described by Miller *et al*. [35] with minor modifications. Briefly, 10ml of blood taken on EDTA were mixed with 25ml of cold 2X Sucrose-Triton and 15ml double distilled water. The tubes were placed on ice for 10 min and mixed by inversion several times. After centrifugation, at 5000 rpm for 15min at 4ºC, the pellet was re-suspended by 3ml of nucleic lysis buffer. The content was mixed with 108µl of 20% SDS and 150µl of proteinase K. The tubes were placed in a water bath at 37ºC overnight.

Table 1: The identification of the primer and restriction enzymes.

Gene	The primer sequences	Size	Annealing temperature	Restriction Enzyme	Reference
FecB	CCAGAGGACAATAGCAAAGCAAA	190	60°C	*AvaII*	Davis *et al.*, 2002
	CAAGATGTTTTCATGCCTCATCAACAGGTC	Bp			
BMP-15	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC	141	60°C	*Hinf1*	Hanrahan *et al.*, 2004
(FecX^G)	GATGCAATACTGCCTGCTTG	bp			
Myostatin	CCG GAG AGA CTT TGG GCT TGA	337	59°C	*HaeIII*	Dehnavi *et al.*, 2012
	TCA TGA GCA CCC ACA GCG GTC	bp			
Ghrelin	CCTGCTCTGGATGGACTTGGC	112	59°C	SNP	Tahmoorespur *et al.*,
	GGCTTGGGGCATTTAGGACG	bp			2010

After the incubation, the tube contents were transferred to a 15-ml polypropylene tube and 2 ml of saturated NaCl was added and shaken vigorously for 15s. After centrifuging at 3500 rpm for 15 min at 4ºC, the supernatant was transferred to a clean 15-ml polypropylene tube and mixed with absolute ethanol. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The precipitated DNA was picked up using the heat sealed pasture pipette, then washed twice in 70% ethanol and exposed to air to dry completely.

The DNA was dissolved in 200 µl TE buffer in 1.5-ml Microfuge tube and kept overnight in an incubator at 37ºC. DNA concentration was determined and diluted to the working concentration of 50ng/µl, which is suitable for polymerase chain reaction using NanoDrop1000 Thermo Scientific spectrophotometer.

Polymerase Chain Reaction (PCR): A PCR cocktail consisted of 1.0 mM upper and lower primers (specific for each tested gene (Table 1), 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin (w/v), 0.1 % Triton X-100 and 1.25 units of Taq polymerase. The cocktail was aliquot into PCR tubes with 100 ng of sheep DNA. The reaction was cycled for 1 min. at 94°C, 2 min at an optimized annealing temperature that was determined for each primer (Table 1) and 2 min. at 72°C for 30 cycles. The PCR products were electrophoreses on 2% agarose gel stained with ethidium bromide to test the amplification success.

Restriction Fragment Length Polymorphism (RFLP): The restriction mixture for each sample, 6 µl from the PCR product were mixed with 2 µl (5–10 units) of the appropriate restriction enzyme and the volume was completed to 5 µl by sterile water (Table. 1). This restriction mixture was mixed with PCR product (~25 µl) and incubated overnight at the optimum temperature of the maximum activity for each restriction enzyme.

The restriction fragments were separated after running horizontally in 3% agarose gel supplemented with ethidium bromide, the running buffer was TBE and the running time was 45 minutes. A well for the DNA size marker (100 bp) were run along with the samples. The bands were visualized using U.V. transilluminator and the gel photo was captured using gel documentation system.

Sequence Analysis: The PCR products represent of gherlin gene detected in this study was purified and sequenced by Macrogen Incorporation (Seoul, Korea) to identify the SNPs. Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite.

RESULTS AND DISCUSSION

FecB: The results of FecB gene PCR products electrophoresis (Fig.1) showed that the technique can be used to detect and genotypes FecB gene clearly. There were 115 individuals from five different breeds (Barki, Ossimi, Rahmani, Najdi and Harri) that were screened with the forced PCR-RFLP approach. The forced PCR of the *FecB* gene produced a 190 base pair (bp) band after digestion with *Ava*II.

The Saudi sheep breeds (Najdi and Harri) did not show the FecB mutation for the BMPR-1B gene distributions (Fig. 2). The results revealed the absence of mutant type B nucleotide, indicating that the examined local Najdi and Harri breeds were wild homozygous (+/+) non carrier sheep. Similar results were also reported by Amiri et al. [36], Ghaffari et al. [37] and Irajeyan et al. [38] who found that digestion of FecB gene 190 bp with *AvaII* restriction enzyme resulted in non carrier wild type in all tested individuals from Iranian Lori- Bakhtiari, Shal and Sangsari sheep breeds respectively. The absent of FecB mutation in BMPR-IB gene of two breeds of Saudi sheep (Najdi and Naimi) according to Abouheif *et al.* [39]. and Montgomery *et al*. [40] stated that most prolific sheep breeds had no evidence of FecB mutation for high

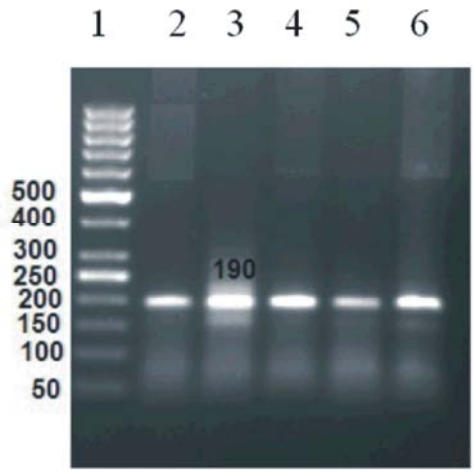

Fig. 1: Ethidium bromide-stained gel of PCR products representing amplification of FecB gene in Egyptian and Saudi sheep breeds. Lane 1. 50-bp ladder marker, Lanes 2-6: 190-bp PCR products amplified from sheep DNA

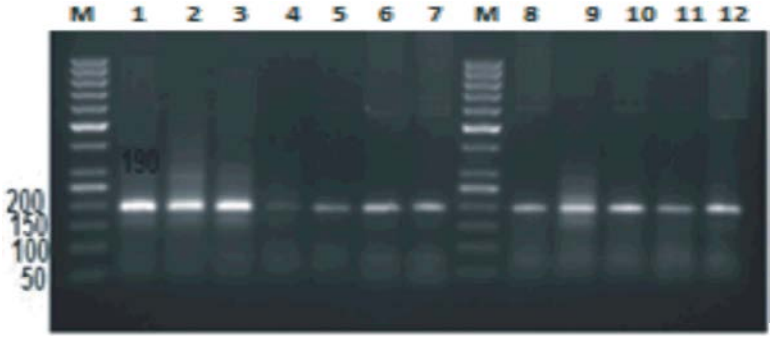

Fig 2: 190-bp PCR amplified Egyptian and Saudi FecB with *AvaII* restriction enzyme. Lane M: 100 bp ladder marker. Lanes 1-12: Restriction digested PCR products from sheep breeds showed one undigested fragment at 190 bp (AA genotype).

ovulation rate and increased litter size. Accordingly, the findings of the present study are in line with those of Guan *et al*. [41] in Chinese sheep who reported that 7 out of 9 sheep breeds were found to be wild type (190bp) in respect to restriction pattern of FecB gene.

The result in five breeds were disagreement with reports in Chinese Merino prolific meat strain have the three different Booroola genotypes (0.51:BB, 0.30:B+ and 0.19: ++) according to Guan *et al*. [41]. Hu were all homozygous for FecB (BB) whereas the other breeds were all of the wild-type (++). In addition, they found that Hu-sheep crossbred progeny had B+ genotype, which exhibited a simple Mendelian pattern of segregation when they were backcrossed. It has been well known that Hu and Chinese Merino prolific meat sheep were highly prolific and Chinese Merino and Romney hills breeds were more prolific in comparison with many other breeds. Findings concerning Hu sheep that has only a single BB genotype were not in agreement with other reports about Booroola sheep, which have observed three genotypes. The phenomenon probably results from the whole process of Hu sheep establishment, which has resulted in the gene being fixed in the Hu population, as had also been observed in some Garole sheep populations in India [42].

BMP-15: The BMP-15 regulates granulosa cell proliferation and differentiation by promoting granulosa cell mitosis, suppressing follicle-stimulating hormone receptor expression and stimulating kit ligand expression. This protein plays a pivotal role in female fertility in mammals [13, 43].

A primer pair was used to detect SNP of the BMP-15 gene, the primer pair usually produces a 141 bp band [17], the PCR product was then subject to cut with restriction enzyme *HinfI*. The PCR-RFLP technique was used to identify the genetic polymorphism of BMP-15 gene in three native Egyptian and two Saudi sheep breeds;

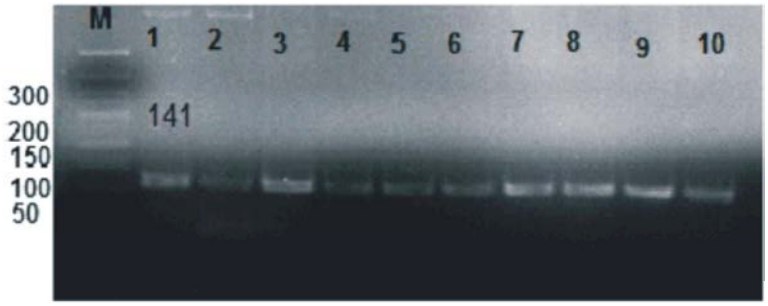

Fig 3: Ethidium bromide-stained gel of PCR products representing amplification of BMP-15 gene in Egyptian and Saudi sheep breeds. Lane M. 100-bp ladder marker Lanes 1-10: 141bp.

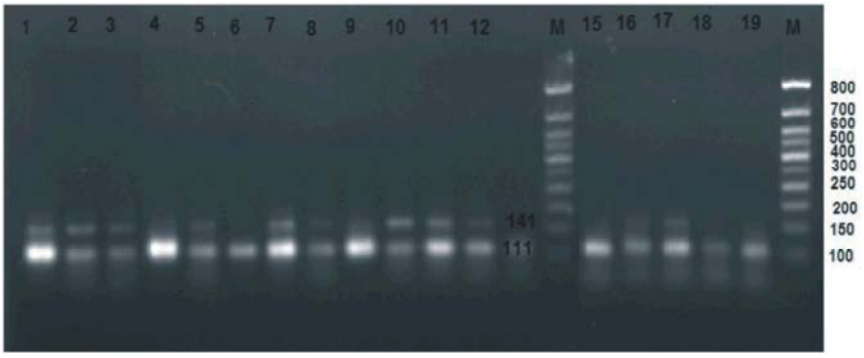

Fig 4: PCR product of the *FecX*^{G} mutation of the *BMP-15* gene digested with *Hin*f I in Egyptian and Saudi sheep breeds. M: lanes 14,20 DNA marker. The wild-type allele (+) is 111 bp and the mutant allele (*G*) is 141 bp. Lanes 1,2,3,5,7,10,11,17(141bp-111bp) *G*+ genotype; lanes 4,6,8,9,12,13,15,16,18,19(111bp-111) ++genotype.

Table 2: Allele and genotypes frequencies of BMP-15 in test sheep breeds

		Genotype frequencies			Allele frequencies	
Breeds	No. of animals	++	+G	GG	+	G
Barki	25	0.32(8)	0.68(17)	0.00	0.66	0.34
Ossimi	23	0.30(7)	0.70(16)	0.00	0.65	0.35
Rahmani	24	0.29(7)	0.71(17)	0.00	0.64	0.36
Najdi	21	0.00	0.00	0.00	0.00	0.00
Harri	22	0.00	0.00	0.00	0.00	0.00

namely Barki, Rahmani, Ossimi, Najdi and Harri, respectively. The primer used in this study flanked a 141-bp fragment (Fig. 3) of BMP-15gene including exon 2 and partially sequences of introns 1 and 2.

In the present study, PCR-RFLP with *HinfI* digestion was used to investigate the genotyping of FecX ^{G} loci in exon 2 BMP-15 genes; the study revealed that the genotyping of BMP-15 gene by agarose gel electrophoresis revealed two genotypes, ++ (111 bp/111 bp) and *G*+ (141 bp/ 111 bp) after digestion with *HinfI* enzyme (Fig.4). The basic finding of the current study was found the polymorphism at the loci of FecX ^{G} *in* exon 2 and partially sequences of introns 1 and 2 in Egyptian sheep only. All sheep were polymorphic for exon 2 in BMP-15 gene. The same result was observed by Jamshidi *et al*. [44] in Sangsari sheep breed of Iran.

Chu *et al*. [45] detected two genotypes, ++ (111 bp/111 bp) and *G*+ (141 bp/ 111 bp) in sheep. Forward and reverse sequencing identified the *Hinf I* RFLP polymorphism as a C/T single nucleotide change at position 718 of the *BMP-15* gene. The nucleotide sequence obtained from genotype *G*+ was identical to the wild-type ++, except for a C→T change at nucleotide 718 of the *BMP-15* gene.

The genotype frequencies for ++ were: 0.32, 0.30 and 0.29 from Barki, Ossimi and Rahmani, respectively, while +G genotype was 0.68, 0.70 and 0.71 after digestion with *HinfI* (Table 2). Frequencies of allele + were 0.66, 0.65 and 0.64 in Barki, Ossimi and Rahmani breeds in Egypt, respectively, while 0.34, 0.35 and 0.36 for allele G in Barki, Ossimi and Rahmani breeds in Egypt, respectively (Table 2). The obtained results in our study agree with the

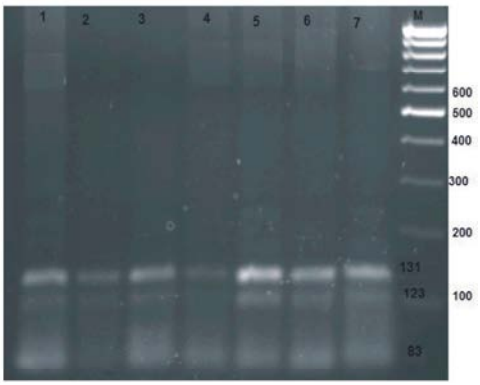

Fig 5: Genotype of MSTN digested with *HaeIII* in Egyptian sheep. Molecular marker was M100.

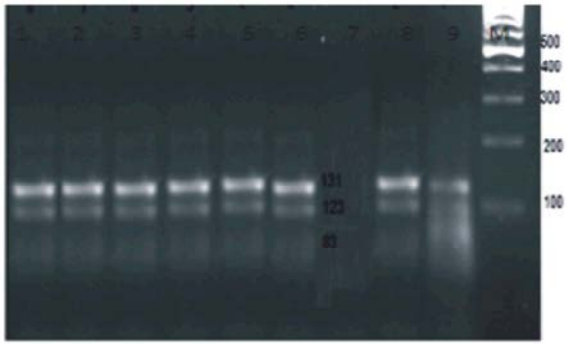

Fig 6: Genotype of MSTN digested with *HaeIII* in Egyptian sheep. Molecular marker was M100.

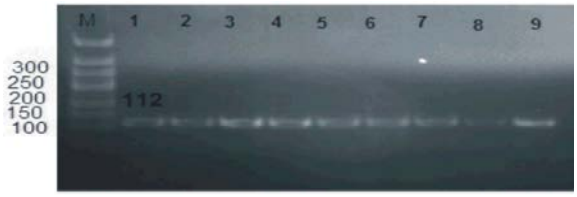

Fig 7: Ethidium bromide-stained gel of PCR products representing amplification of ghrelin gene in Egyptian and Saudi sheep breeds. Lane M: 100-bp ladder marker. Lanes 1-9: 112-bp PCR products.

previously obtained alleles frequencies of genotypes *G*+, ++ and *GG* were 0.60, 0.40 and 0.00, respectively, according to Chu *et al*. [45].

Moradband *et al*. [46] showed that the investigated mutations of $FecX^G$ and *FecB* that have a major effect on litter size were not present in Iranian Baluchi sheep. These results are in agreement with reports in Romanov, Finn, East Friesian, Tees water, Blue face Leicester, D'Man, Chios, Mountain sheep, German White-headed Mutton, Lleyn, Loa, Galician, Barbados Blackbelly [47], Mulpura sheep [48], Suffolk, Dorset, Charolais, In contrast, the presence of *FecB* mutation is reported in several sheep breeds such as Chinese Hu and Han sheep breeds [47,49], Australian Booroola Merino [4], Indian Garole [42] and Javanese sheep of Indonesia [42].

Ovine Myostatin (MSTN) Gene: In our study, the genotyping of MSTN gene were done using PCR-RFLPs methodology. A 337 bp fragment for exon 3 of MSTN locus was amplified, then this fragment was digested with the restriction enzyme *HaeIII*, the digested products were characterized after running on horizontal agarose gel electrophoresis. The *HaeIII* digests the m allele, but not M allele, digestion of the m allele produced three fragments of 83, 123 and 131 bp (Fig. 5). All samples genotyped for all the Egyptian and Saudi breeds showed the mm genotype. As a result, all of them were monomorphic for the m allele (Fig. 6). The obtained results in our study agree with Dehnavi *et al*. [50] who found that all samples from Iranian Zel sheep breed showed *mm* genotype, while disagreement with the previously obtained alleles in Iranian Sanjabi sheep breed [51].

Moreover, Soufy *et al*. [52], observed polymorphism for MSTN gene in Iranian Sanjabi sheep and native kermanian cattle. Han *et al*. [53] suggested that *MSTN* affect other physiological activities during embryonic development, which may explain the abnormal gender ratio observed here. The actual mechanism by which this could occur is however unclear. Furthermore, in transgenic mice with muscle-specific "over expression" of myostatin, muscle mass differences were only detected in male progeny and not females [54]. This suggests that in the female transgenic mice, there were gender-specific mechanisms that could override the effects of myostatin on muscle mass. The putative embryonic roles of myostatin may therefore be gender-specific. This inconsistency may be ascribed to breed differences, mating strategies, population and sampling size, environmental factors, geographical position effect and frequency distribution of genetic variants.

Ghrelin Gene: The desirable fragment of the ghrelin (112 bp of exon1) gene was amplified from ovine genomic DNA then sequenced to identify the polymorphism in exon1 to the ovine ghrelin gene (Fig. 7).

Totally 30 samples from three Egyptian sheep breeds (Barki, Rhmani and Saidi) and two Saudi sheep breeds (Najdi and Harri) were analyzed as a candidate gene for growth traits. These data were compared with available sequence in the international Gene-bank. The sequences were concatenated and the gab-containing sites were deleted so that 560 bp were left for analysis in each breed. The data showed base frequencies of A = 21.43 %, C = 34.82 %, G = 25.89% and T = 17.85 % of nucleotides.

CCTGCTCTGGATGGACTTGGCCATGGCGGGCTCCAGCTTTCTGAGCCCTG
AACATCAGAAACTGCAGGTGAGATGCCACCCCAGGAGCCCCA**CGTCCTAA**
ATGCCCCAAGCC

Fig 8: The sequence analysis of sheep ghrelin gene amplified fragment

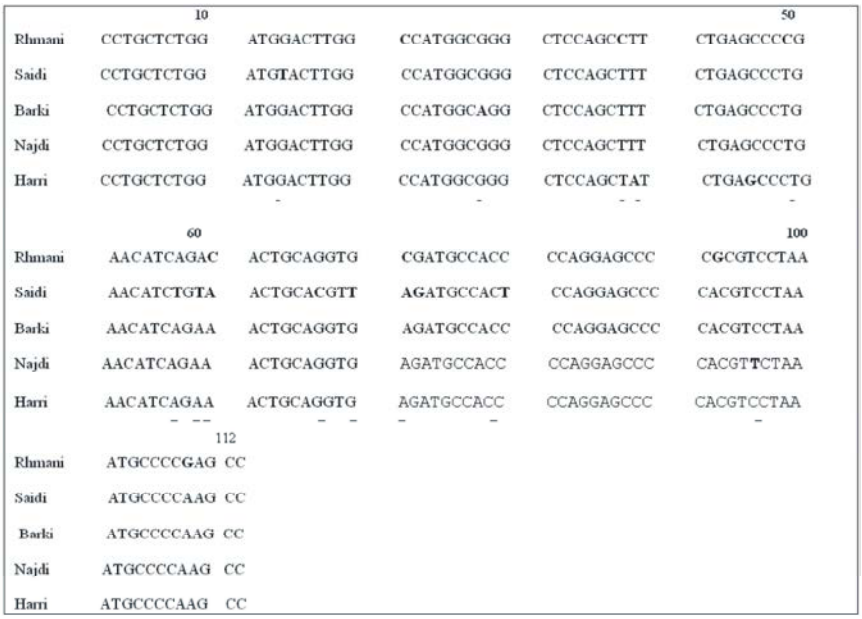

Fig 9: Sequence alignment of sheep ghrelin gene and published sequence.

Nucleotide substitutions are generally considered in terms of changes within the two structural classes of nucleotides (purines and pyrimidines), that is, in terms of transitions and transversions. 552 were constant and 13 were variables. This fragment showed 14 substitutions (6 transversions and 8 transitions) in this studied animals.

Two-way sequence analysis of *ghrelin* amplified PCR product of Egyptian sheep DNA was conducted from animals representing the five variables in Rahmani breed. The sequence analysis indicated three nucleotide substitutions (T to C; A to G and A to C) at nt 38 and nt49, nt 108, nt 60 and nt 71 in Rahmani (Fig. 8).

The six different detected variables were present in the Saidi sheep breed at different frequencies with a majority of five transversion and one transition (G to T, A to T, A to T, G to C, G to T) at nt 14, nt 56, nt 58, nt 67, nt 60 and 71, respectively (Fig. 9).

Molecules differing by as little as a single base substitution should have different conformers under non-denaturing conditions and migrate differently. Therefore, those differences can be detected as a shift in the electrophoresis mobility [55].

Sherman *et al*. [56] found the A/G SNP in GHRL (accession No. AY455980) was also evaluated for its affects on growth, feed efficiency and carcass merit. The GHRL SNP showed minor associations with the feed efficiency traits ($P < 0.10$) but not with the BW of the animals. The frequency of the G allele was low in the population (0.12) and so there were only 8 animals with the GG genotype. It was not possible to examine dominance effects of the alleles, but there was an allele substitution effect of -0.18 kg/d on RFI ($P = 0.083$). A role for GHRL in feed efficiency is very likely because it has been shown to play roles in determining if fat or carbohydrates are the metabolic substrate used for maintenance of energy balance as seen in GHRL knockout mice [57]. This SNP also showed minor associations with carcass merit including positive effects on LMY, yield grade and quality grade in animals that were AA homozygous. This effect on yield of the animals is consistent with the role of GHRL in promoting the release of GH and therefore to initiate growth [30]. In addition to the role of GHRL in GH release, it has also been shown to play important roles in the stimulation of appetite and feeding activity through interactions with peptides such as NPY Jarkovska *et al.* [58]. Although this SNP is in intron 3 of the GHRL gene and is therefore not amino acid changing, it could be in linkage disequilibrium with another SNP in the GHRL gene with greater effects on the traits.

CONCLUSION

This study has highlighted the importance of further investigation for the gene(s) influencing reproductive sheep breeds in Egypt and Saudi. The absence of FecB mutation in the studied animals indicates high possibility of the absence of the Boroola gene mutation in three Egyptian and two Saudi sheep breeds. Further studies regarding other genes which may influence fecundity of the Egyptian and Saudi sheep should be carried out to determine the type and mode of inheritance of such genes.

Furthermore, results showed that PCR-RFLP technique is appropriate tools for screening myostatin loci in sheep breeds. This is one of the first studies on polymorphism of myostatin and ghrelin loci in Egyptian and Saudi sheep breeds Although myostatin locus was monomorphic in the herd, but results showed acceptable polymorphism for ghrelin loci, which may open interesting prospects for future selection programs, especially using marker-assisted selection for improving weight gain and meat quality. Results also confirmed that *PCR-RFLP* and *SNPs* are appropriate tools for evaluating genetic variability.

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