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RESEARCH ARTICLE

Polymorphisms of Growth Differentiation Factor 9 (*GDF 9*)and Bone Morphogenetic Protein 15 (*BMP15*) Genes in Barki and Rahmani Sheep Breeds

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Abstract

Blood samples from 100 ewes (Barki and Rhmani breeds, 50 each) were collected from a private farm at Giza Governorate for determination of polymorphisms of bone morphogenetic protein 15 (*BMP15*) and the growth differentiation factor 9 (*GDF9*) genes in the two breeds using PCR-RFLP and DNA sequencing.Restriction analysis of 712 bp*GDF9* gene (amplicon of exon I) using HhaI enzyme revealed 120, 254 and 338 fragments without any differences between the two breeds being tested. *GDF*9 gene exon II amplicon(713 bp) showed similar pattern of restriction in the two tested breeds with 4 bands (54, 62, 137 and 460bp) using HinfI enzyme. The restriction enzymes, HinfI, SpeI and XbaI, failed to digest the amplicons of exons I and II(500 bp, each) of *BMP15* gene in all tested animals of both breeds suggesting absence of polymorphism. This study concluded that there was no polymorphism in the four exons between Barki and Rahmani sheep and the two breeds have the wild genotype with absence of any mutation.

Keywords: Sheep, *GDF9*, *BMP15*, Polymorphism.

Introduction

Small ruminants as sheep play a great role in the socio-cultural, subsistence and socio-economic livelihoods of rural and peri-urban communities [1]**.** Sheep farming is particularly important for small farms because it needs minimum resources and provides good sources of income by selling its products [2]. The main breeds in Egypt are Ossimi, Barki and Rahmani, which are widespread in the middle of Egypt, western Mediterranean coastal region and the north of Nile delta, respectively. In the south of Egypt, Sohagi and Saidi are the most popular breeds [3].

In animal genetics,molecular markers are now considered as a driving force and offer many opportunities to improve reproductive traits [4]. Hence, they provide methods that help for recognition of polymorphism and genetic markers related to reproductive characters [5].

Prolificacy in sheep has been reported to be affected by various major genes, that involves three related oocyte-derived components such as *FecB* (bone morphogenetic protein receptor type 1B) [6]; *FecG* (*GDF9*) and *FecX*(*BMP15*) [7]. Different mutations may occur in these genes resulting in increase or decrease the rate of ovulation, and the impact of these mutations may even reach to the level of infertility in sheep [8].

In mammals, growth differentiation factor played an important action in female reproductive tract, during early folliculogenesis. Chromosome 5 of sheep contains many important genes, one of them is the *FecG* (*GDF9*) gene. The *GDF9* gene length was about 2.5 kb, consisting of 2 exons. The first exon extends 397 nucleotides that translated into the first 132 amino acids, while the second one extends 968 bp and translates the rest of polypeptide chain which containing amino acids from number 133 to 456,

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separated by one base pair 1126 of non-coding sequence [9].

Transforming growth factor β superfamily contains different types of vital proteins as bone morphogenetic proteins which were produced by *FecX*(*BMP15*) gene located on the sex (X) chromosome. It consists of 2 exons with 1179 nucleotide length interrupted by 5.4 kb non-coding sequence, these two exons were translated into nonfunctional polypeptide consist of 393 amino acids, which have been modified to produce the functional one with 125 amino acids only[10].

This study aimed to elucidate the polymorphism in the coding sequence of the *GDF9* gene and the exons I and II of the *BMP15* gene of the two common Egyptian sheep breeds, Barki and Rahmani.

Materials and Methods

Samples

The present study was conducted on 100 adult ewes (twenty months old) of Barki (n=50) and Rahmani (n=50) breedsobtained from a private farm, Giza Governorate, Egypt. The animals were apparently healthy and free from any clinical disorders or diseases. This study was approved by the committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine Zagazig University.

Two mL of blood samples were collected aseptically from each individual's jugular vein in a sterile vacutainer tube EDTA (Qingdao Hiprove Medical Technologies Co., Ltd., China).The samples were transported immediately in an ice box to the laboratory and preserved at -20°C for further processing.

Genomic DNA extraction

Genomic DNA was extracted from such individual blood samples using ZYMO research Kits, (Zymoresearch, Tustin, USA)as stated by the manufacture'sprotocol.Agarose gel (1%) electrophoresis was used to examine the quality and purity of extracted DNA, while Nanodrop (ND1000; NanoDrop Technologies, USA) was used to measure the concentration of DNA[11].

PCR and PCR-RFLP

The PCR reaction was performed in a total volume of 25µLconsisting of: 12 µL master mix (Dream Taq Green 2X, Thermo Fisher Scientific - US), 2 µL DNA, 1µL of both forward and reverse primer (10 Pmole) and 9 µL deionized water. Amplification procedure was carried out in T-professional thermal cycler (Biometra, Germany). Primers for *GDF9* exon I were delivered from a previously published paper [7], whereas the primers for amplification of*BMP15*gene were designed using Primer3 software(http://primer3.ut.ee) using the sequence of *BMP15* gene that available at GenBank (NCBI, https://www.ncbi.nlm.nih.gov/) with accession No. AH009593.2 (Table 1).

Table 1: Oligonucleotide primer sequences for *GDF9* and *BMP15* genes, amplicon sizes and their annealing temperature

Gene	Exons	Primers Sequence (5′-3′)	Amplicon size (bp)	Annealing temperature (°C)
GDF9	Exon 1	GAA TTG AAC CTA GCC CAC CCA C AGC CTA CAT CAA CCC ATG AGG C	712	62
	Exon 2	AGA TTG ATG TGA CGG CTC CT CTC CCA AAG GCA TAG ACA GG	713	60
BMP15	Exon 1	CCT TTG TGG TAG TGG AGC CT TGT GAA GCC TGA CAG AAA ACTG	500	60
	Exon 2	ATG TTG GGC AAA AGC TCT GG AGG CTG AGG GAC ATT CTG AT	500	58

PCR was programmed as follow;initial denaturation at 95°C for 5 min then 35 regular cycles of secondary denaturation for 40 sec at 94°C,annealing asmentioned inTable (1) and extension at 72°C for 40 s then final extension for 9 minutes at72°C [7].

PCR-RFLP methods used in our study were wholly accomplished as described previously [7, 10]**.** PCR products were digested by restriction enzymes for the detection of any mutations. The descriptive data of these enzymes were shown in Table (2).

Table 2: List and description of restriction enzymes used in RFLP-PCR of *GDF9* and *BMP15*

Gene	Enzyme	Restriction site	Incubation period
GDF9 exon I	HhaI	5′...GCGC...3′ 3′...CGCG...5′	37°C /5min
GDF9 exon II	HinfI	5′...GANTC...3′ 3′...CTNAG...5′	
	XbaI	5′...TCTAGA...3′ 3′...AGATCT...5′	
BMP15 exon I, II	HinfI	5′...GANTC...3′ 3′...CTNAG...5′	
	SpeI-HF	5′...ACTAGT...3′ 3′...TGATCA...5′	

The reaction volume of PCR-RFLP was optimized in 10 µL consisting of PCR product (5 µL), Bio Labs 10x NE buffer (1 µL), 0.5 µL restriction enzyme (Bio Labs, UK) and nuclease free water (3.5 µL). The digested fragments were examined through their separation by 1.5-2% agarose gel electrophoresis and the gel was visualized, photographed and examined in a gel documentation system (Bio Doc Analyse, Biometra, Germany).

Sequencing

DNA sequencing of the four exons of *GDF9* and *BMP15*genes produced in both sheep breeds was done in MacroGen Company, South Korea. It was performed in both forward and reverse directions using primers involved in PCR amplificationusing DNA automated sequencer (ABI 3730XL, Applied Biosystem, USA). Obtained sequence data was confirmed by comparing them with the existing nucleotide sequence database with accession numbers AF078545.2 and AH009593.2for *GDF9BMP15* genes, respectively by using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of NCBI. The alignments and assembly of the sequences were performed using the DNA Baser Assembler and CLC Main Workbench 7 programs (https://www.qiagenbioinformatics.com/).

Results

Analysis of amplification and restriction of 712bp fragments of the *GDF9* gene exon I with HhaI enzyme revealed similar results of all tested animals with three short fragments of 120, 254 and 338bp length (Figure 1). Similarly, the sequencing of PCR products from both Barki and Rahmani sheep showed two recognition sites for HhaI, and absence of G1 mutation with homozygous wild type genotype.

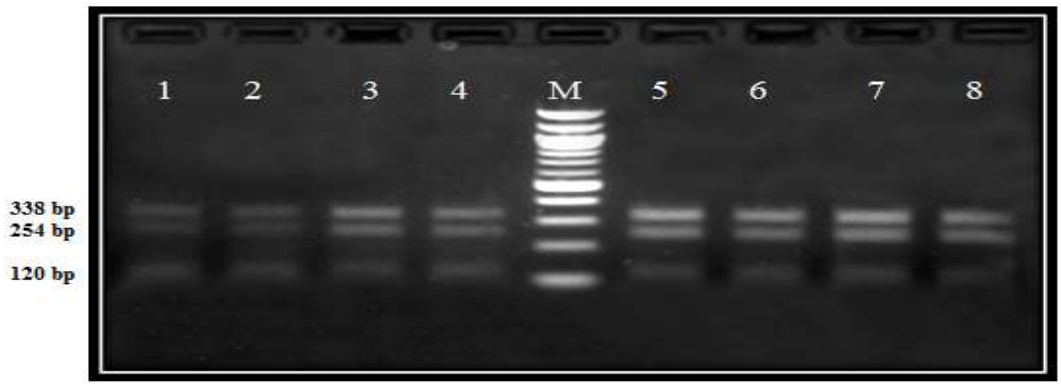

Figure 1:Restriction pattern of exon I of *GDF9* gene after digestion with HhaI. Lanes1-4 represent analysed samples from Barki breed; lanes 5-8 represent those samples from Rahmani sheep; M: molecular weight marker (100 bp).

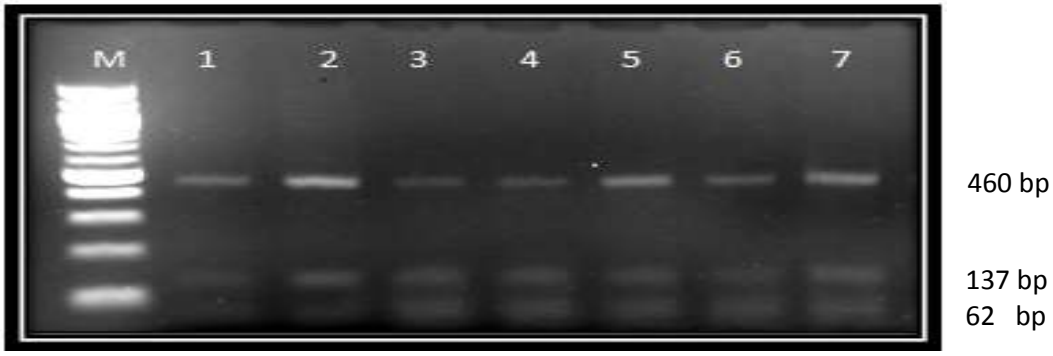

Figure 2:Restriction pattern of exon II of *GDF9* gene after digestion with HinfI in Barki sheep, revealing homozygous wild type genotype. M: molecular weight marker (100 bp).

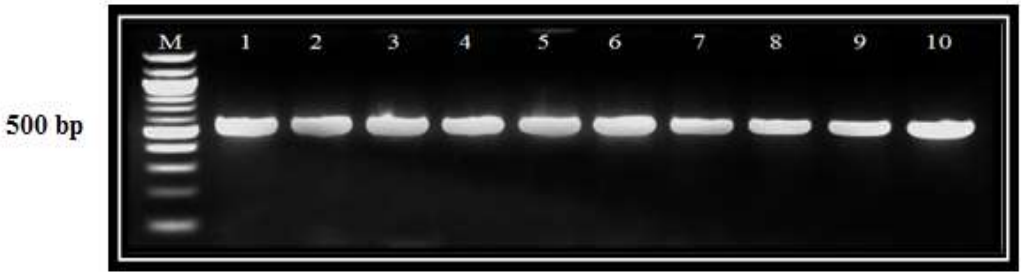

Figure 3:Amplification of exon I, II of *BMP15* gene. Lanes 1-5 represent exon I and Lanes 6-10 represent exon II in Rahmani sheep, M: molecular weight marker (100 bp).

Restriction analysis of 713 bpfrom exon II of the *GDF9* gene with HinfI enzyme revealed our short fragments of 54, 62, 137 and 460bp length (Figure 2) in all animals with no apparent polymorphisms. The sequencing results showed two recognition sites for HinfI and the same genotype with absence of G6, G7 and G8 mutations of *GDF9* gene in both Barki and Rahmani sheep.

Amplification of exons I and II of *BMP15* gene were done using specific primers producing amplicons of 500 bp for both exons (Figure 3). These amplicons were treated with three restriction enzymes, HinfI, SpeI and

XbaI. The three enzymes failed to digest the amplicons of both axons of *BMP15* gene in all analyzed samples of both breeds showing no polymorphism. PCR products were then selected and sequenced in both forward and reverse direction. The obtained sequences were cross-checked to come up with a single consensus sequence for each individual. The sequences confirmed the absence of recognition sites of HinfI, SpeI and XbaI and also showed the same genotype of *BMP15* gene of analyzed samples from Barki and Rahmani sheep.

Discussion

Sheep farming is an important source of income, especially in developing countries. Any breeder's main goal is to achieve the extreme income from sheep farming. Thebreeders use two basic tools for this purpose: selection and breeding. Selection based on phenotype is often inefficient; the select breeding program was the most effective method which used to increase fertility traits according to the genotype of specific animals. Number of candidate genes has been identified affecting fecundity. Different mutations in the genes of *GDF9* and *BMP15* cause an increase in the sheep ovulation rate [12]**.**

GDF9 and *BMP15* genes play an important role in folliculogenesis and ovulation rate control. The different types of *GDF9* and *BMP15* mutations have the greatest affinity to increase proliferation of granulosa cells and decrease the expression levels of FSH receptor and steroid hormones [13] and play an important role by increasing the ovulation rate and lamb size [14, 15].

The amplified products of exon I of *GDF9* gene were 712 bp in length. Restriction digestion of these fragments with HhaI enzyme resulted in three fragments (120, 254 and 338bp). There is no polymorphism was detected in both Barki and Rahmani breeds and they were homozygous wild type genotype (AA). These results agree witha previously published study[16]in which one genotype (AA) in Iranian sheep breeds (Lori-Bakhtyari, Ghezel, Shal, and Afshari) was reported. Additionally,the monomorphic wild type genotype (AA) in Katahdin and Dorper sheep was detected previously [17]. On the other hand,previous reports revealed the presence of polymorphic genotypes (AA, AB and BB) in Sangsari and Baluchi sheep and G1 was found associated with the wild genotype (AA)[8, 18]. Also,Gorlovand coauthors [19]reported the presence of polymorphic genotypes AB and BB with absence of AA genotype in two Russian sheep breeds, Salsk and Volgograd, with the presence of G1mutation in both breeds.

Different types of *GDF9* gene polymorphism were studied using PCR-SSCP technique in Hu, Small tail han, Suffolk and Dorset sheep [20]. The latter study showed presence of AA genotype in the four tested sheep, while genotype AB was found in Dorset, Hu and Suffolk sheep but the genotype BB was detected only in Suffolk sheep. The sequence data showed presence of one SNP (A152G) that changed asparagine amino acid into aspartic acid. Similarly, three different genotypes (AA, AB and BB) were recorded in five breeds of goat; Boer, Jining grey, Beijing native, Wendeng dairy and Liaoning Cashmere due to one SNP (C74T)[21]. Genotypes AB and BB were found in the five tested breeds while AA genotype was found only in Jining Grey goats. The frequencies of the AA, AB and BB genotypes being 0.18, 0.42 and 0.40, respectively.In contrast, our finding mismatched with a previous study [22],which revealed the presence of transition mutation (G to A) in Hisari Sheep with presence of AA and AB genotypes with frequency of 93.64 and 6.36, respectively.

The amplified products of exon II of *GDF9* gene were 713 bp in length. Restriction digestion of these fragments with HinfI enzyme resulted in four fragments (54, 62, 137 and 460). HinfI enzyme did not detect any polymorphism in both Barki and Rahmani breeds and showed absence of G6, G7 and G8 mutations.Our findings matched with Mustafa and coauthors [23] who detected only the wild type genotype (AA) in Lohi sheep and absence of Fec^{G} mutation. Similarly**,** other researchers[24]stated the absence of polymorphism as well as$FecG^{H}$ mutation in Hu sheep, all the animals in this study were wild type genotype (AA). Presence of $FecG^{H}$

mutation was studied in Kivircik, Awassi and Imrose sheep breeds by using PCR –RFLP [25] but the mutation had not been found and all animals were homozygous wild type genotype (AA). Also,another study declared the incidence of the same mutation in *GDF9* gene of Assam hill goat revealing that the wild type genotypes and all the individuals showed (AA) genotype[26].

Contrary to these results,polymorphic genotypes (AA, AB and BB) were found in Cambridge and Belclare Sheep[7]. Previously,polymorphisms were studied in the *GDF9* gene exon II and G7 was not found to be polymorphic with the wild type genotype (AA), whereas G6 was polymorphic in Katahdin and Dorper sheep[17].

The PCR products for both exons of *BMP15* were 500 bp in length. For detection of any mutation different types of restriction enzymes as SpeI, XbaIand HinfI were used. The three enzymes could not cut through the PCR products and all individuals were homozygous with the wild type genotype for the three loci and absent of these mutations. These results were in harmony with a previously published article [27]. Three mutations in Markhoz goat *BMP15* gene with no polymorohism was found in the studied animals which carried the homozygous wild type genotypes (AA) for the three mutations. Additionally $FecX^{B}$, $FecX^{G}$, $FecX^{H}$ and $FecX^{I}$ mutations in Egyptian Sheep breeds including Barki, Osseimi, Rahmani, Saudanez and Awase, and goat breeds involved Zaraibi, Damascus, Boer, Sanan and Barki were studied and the results indicated that all animals had the homozygous wild type genotypes (AA) with no polymorphisms in all these breeds[28]. Polymorphisms of exon II of *BMP15* gene had been reported previously[8] in another sheep breed in which$FecX^{G}$ mutation in all tested sheep ofSangsaribreed carried C to T transition and all was homozygous for this mutation (BB). Similarly,heterozygous (AB) and homozygous mutant (BB) genotypes of $FecX^{G}$ mutation were previously recorded [24]. Moreover, the same results were obtained in another study [29] in which polymorphism of exon I of *BMP15* gene in Iranian Fat-Tailed Sheep was detected. Their study revealed a deletion of 3 bp CTT (656_658del) in exon 1, leading to an amino acid deletion (p.Leu19del) with 0.1 allelic frequency and it was typed as non-synonymous mutation.

Conclusion

This study concluded that there was no polymorphism between the four exons in Barki and Rahmani sheep and the two breeds have the wild genotype with absence of anytype of mutation.

Conflict of interest

The authors have no conflict of interest to declare

References

[1] Galal, S.; Abdel-Rasoul, F.; Anous, M. and Shaat, I. (2005): O-nstation Characterization of Small Ruminant Breeds in Egypt. In: IniguezL. editor. Characterization of Small Ruminant Breeds in West Asia and North Africa.ICARDA,Aleppo, Syria: 141-193.

[2] Naqvi, S.; Sejian, V. and Karim, S. (2013): Effect of feed flushing during summer season on growth, reproductive performance and blood metabolites in Malpura ewes under semiarid tropical environment. Trop anim health prod, 45 (1): 143-148.

[3] ICARDA (International center for agricultural research in the dry areas) (2006):Ivankovic, A.; Dove, P.; Kavar,T.; Caput, P.; B. Mioc; V. Pavic; 1UZ.http:/ /www.icarda.org.

[4] Vignal, A.; Milan, D.; SanCristobal, M. and Eggen, A. (2002): A review on SNP and other types of molecular markers and their use in animal genetics. Genet selevol, 34 (3): 275-305.

[5] Ghaffari, M.; Nejati-Javaremi, A. and Rahimi, G. (2009): Detection of polymorphism in *BMPR-IB* gene associated with twining in Shal sheep using PCR-RFLP method. Int JAgricBiol, 11 (1): 97-99.

[6] Souza, C.J.; MacDougall, C.; Campbell, B.K.; McNeilly, A.S. and Baird, D.T.

(2001): The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (*BMPR1B*) gene. JEndocrinol, 169 (2): R1-R6

[7] Hanrahan, J.; Gregan, S.; Mulsant, P.; Mullen, M.; Davis, G.; Powell, R. and Galloway, S.M.(2004): Mutations in the genes for oocyte-derived growth factors *GDF9* and *BMP15* are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovisaries). Biolreprod, 70(4): 900-909.

[8]Hafezian, S. (2011): Genetic polymorphism *BMP15* and *GDF9* genes in Sangsari sheep of Iran. IntJGenetMolBiol, 3 (1): 31-34.

[9] Silva, J.R.; Van den Hurk, R.; Van Tol, H.T.; Roelen, B.A. and Figueiredo, J.R. (2005): Expression of growth differentiation factor 9 (*GDF9*), bone morphogenetic protein 15 (*BMP15*), and BMP receptors in the ovaries of goats. Molreproddev, 70 (1): 11-19.

[10] Galloway, S.M.; McNatty, K.P.; Cambridge, L.M.; Laitinen, M.P.; Juengel, J.L.; Jokiranta, T.S.;McLaren, R.J.; Luiro, K.; Dodds, K.G.; Montgomery, G.W.; Beattie, A.E.; Davis, G.H. and Ritvos, O.(2000): Mutations in an oocyte-derived growth factor gene (*BMP15*) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat Genet, 25 (3): 279-283.

[11] Boesenberg-Smith, K.A., Pessarakli, M.M. and Wolf, M.D. (2012): Assessment of DNA yield and purity: an overlooked detail of PCR troubleshooting. ClinMicrobiol News, 34 (1): 1-6.

[12] Rout, M.; Panigrahi, S.; Pradhan, S.; Routray, A. and Ranjan Swain, B. (2018): Genetic basis of fecundity in sheep. Pharma Innovation J, 7 (4): 314-316.

[13] Mottershead, D.; Sugimura, S.; Al-Musawi, S.; Li, J.; Richani, D.; White, M. and Mueller, T. (2015):Cumulin, an oocyte-secreted heterodimer of the transforming growth factor-β family, is a potent activator of granulosa cells and improves oocyte quality. J BiolChem, 290 (39): 24007-24020.

[14] Bodin, L.; Di Pasquale, E.; Fabre, S.; Bontoux, M.; Monget, P.; Persani, L. and Mulsant, P. (2007): A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. Endocrinology, 148 (1): 393-400.

[15]Monteagudo, L.; Ponz, R.; Tejedor, M.; Laviña, A. and Sierra, I. (2009): A 17bp deletion in the Bone Morphogenetic Protein 15 (*BMP15*) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. Animreprodsci, 110 (1-2): 139-146.

[16] Eghbalsaied, S.; Khorasgani, F.; Amini, H.; Farahi, M.; Davari, M.; Pirali, A.;Pourali, S.;Vatankhah, M.; Rostami,M. and Atashi,H.(2017): Variant *GDF9* mRNA is likely not the main cause of larger litter size in Iranian Lori-Bakhtyari, Shal, Ghezel, and Afshari sheep breeds. ArchivAnimBreed, 60 (2): 119-129.

[17] Escobar-Chaparro, R.; Guillén, G.; Espejo-Galicia, L.U.; Meza-Villalvazo, V.M.; Peña-Castro, J.M., and Abad-Zavaleta, J. (2017): qPCR and HRM-based diagnosis of SNPs on growth differentiation factor 9 (*GDF9*), a gene associated with sheep (Ovisaries) prolificacy. 3 Biotech, 7 (3): 204-212.

[18] Moradband, F.; Rahimi, G. and Gholizadeh, M. (2011): Association of polymorphisms in fecundity genes of *GDF9*, *BMP15* and *BMP15-1B* with litter size in Iranian Baluchi sheep. Asian-Australas J AnimSci, 24 (9): 1179-1183.

[19] Gorlov, I.; Kolosov, Y.; Shirokova, N.; Getmantseva, L.; Slozhenkina, M.;

Mosolova, N.; Bakoev,N.; Leonova,M.;Kolosov, A. and Zlobina, E. (2018):GDF9 gene polymorphism and its association with litter size in two Russian sheep breeds. RendicontiLincei. ScienzeFisicheNaturali, 29(1): 61-66.

[20] Li, B.; Chu, M. and Wang, J. (2003): PCR-SSCP analysis on growth differentiation factor 9 gene in sheep. ActageneticaSinica, 30 (4): 307-310.

[21] Chu, M.; Wu, Z.; Feng, T.; Cao, G.; Fang, L.; Di, R. Huang, D.W.; Li, X.W.and Li, N. (2011): Polymorphism of *GDF9* gene and its association with litter size in goats.Vet Res Commun, 35 (6), 329-336.

[22] Bahrami, Y.; Bahrami, S.; Mohammadi, H.; Chekani-Azar, V. and Mousavizadeh, S. A. (2014): The Polymorphism of *GDF-9* Gene in Hisari Sheep. Biological Forum, 6 (2): 46-51.

[23] Mustafa, H.; Khan, W.; Kuthu, Z.; Ajmal, A.; Javed, K.; Ain, N. and Zeeshan, M. (2018): Analysis of Fec^G gene in Lohi sheep breed using PCR-RFLP technique. Pure AppBiol, 7 (1): 292-295.

[24] Wang, W.; La, Y.; Zhou, X.; Zhang, X.; Li, F. and Liu, B. (2018): The genetic polymorphisms of TGFβ superfamily genes are associated with litter size in a Chinese indigenous sheep breed (Hu sheep). Animreprodsci, 189 (1): 19-29.

[25] Gursel, E.; Akis, I.;Durak, H.;Mengi, A. and Öztabak, K. (2011): Determination of *BMP-15*, *BMPR-1B* and *GDF-9* gene mutations of the indigenous sheep breeds in Turkey. KafkasÜniv Vet FakDerg, 17 (5):725-729.

[26] Dutta, R.; Das, B.; Laskar, S.; Kalita, D.; Borah, P.; Zaman, G. and Saikia, D. (2013): Polymorphism, sequencing and phylogenetic characterization of growth differentiation factor 9 (*GDF9*) gene in Assam Hill goat. Afr J Biotechnol, 12 (50): 6894-6900.

[27] Shokrollahi, B. (2015):Investigation of *BMP15* gene polymorphisms associated with twining in Markhoz goat. BihareanBiol, 9 (1): 1-4.

[28] Farag, I.; Aboelhassan, D.; Ghaly, I.; Darwish, H.; Darwish, A. and Eshak, M. (2018): Effect of genetic polymorphism of *BMP15* gene on improving twin production in Egyptian small ruminants. Res J AnimVet Sci, 10 (1): 6-14.

[29] Abdoli, R.; Mirhoseini, S.; Hossein-Zadeh, N. and Zamani, P. (2018): Screening for causative mutations of major prolificacy genes in Iranian fat-tailed sheep. IntJFertilSteril, 12(1): 51-55.

الملخص العربي

التعددالمظهري لجيني عامل تمايزالنمو (GDF9) والبروتين عظمى المنشأ (RBM15) في سلالتي الخراف البركي والرحماني

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تم جمع عينات دم من ١٠٠ نعجة (٥٠ للبرقي و ٥٠ للرحماني) من مزرعة بمحافظة الجيزة لتحديد تعدد الأشكال المظهرية لجيني GDF9 و BMP15 في كلا السلالتين و ذلك باستخدام تقنيتي PCR-RFLP وتحديد التتابع الجينى. تم دراسه تعددالأشكال المظهرية باستخدام انزيم القطع HhaIوالذى نتج عنه هضم الحزمه ٧١٢ زوج قاعدى الى ثلاثه اجزاء ١٢٠،٢٥٤،٣٣٨ فى جميع الاغنام ولم يظهر اي اختلاف بين الحيوانات محل الدراسه.كما وجد أنا الاكسون الثانى يتكون من٧١٣ زوج من القواعد و باستخدام انزيم القطع HinfIوالذى نتج عنه هضم الحزمه ٧١٣زوج قاعدى الى اربعه اجزاء (٥٤، ٦٢، ١٣٧،٤٦٠) فى جميع الاغنام ولم يظهر اي اختلاف بين الحيوانات محل الدراسه.اما بالنسبة للجينBMP15, كلا الاكسونين الاول و الثانييتكونا من٥٠٠ زوج من القواعد في كل الاغنام محل الدراسهو لم يقطع اي من الانزيمات المستخدمه في هذه الاكسونات. نستخلص من هذه الدراسة عدم وجود أي فروقات جينية بين السلالاتين محل الدراسة.