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Galway Point Mutation (*FecX^G*) in the Bone Morphogenetic Protein 15 Gene (*BMP15*) is Associated with Prolificacy in the Sudanese Desert Sheep Ecotypes

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Abstract

This study tested the association between ***FecX^G*** point mutation located in exon 2 of *BMP15* gene and the prolificacy of Dubasi, Shugor and Watish sheep ecotypes, under dryland farming, Sudan. Blood samples were randomly collected from unrelated 100 ewes (Dubasi; n= 30, Shugor: n= 30, and Watish: n= 40). Bone Morphogenetic protein (*BMP15*) gene was amplified using PCR-RFLP. Two genotypes were found in all studied breeds (heterozygous and wild type). The calculated total genotype frequencies of BB, Bb and bb genotypes were 0.31, 0.69 and 0.00, respectively, while allele frequencies were 0.66 for B and 0.34 for b. Litter size was influenced by the genotypes of *BMP15* gene, parities and subtypes ($p<0.05$), highest for Watish and 4th parity. Alignment of *BMP15* samples along with database reference sequence revealed that most sequence regions were identical except for one variable nucleotide at position 111 bp where a guanine (G) was replaced by adenine (A) in Watish and Shugor samples. All amino acids were the same at residue 275. Watish and Shugor breeds are more related. The study concluded that the presence of one copy of $FecX^G$ point mutation of BMP15 gene increased the litter size by 0.17 lambs in the studied ecotypes.

Keywords: *BMP15 gene, Ewe, Sudanese Sheep, Residue, Wild type, Mutant type, dryland*

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1. Introduction

The sheep Bone Morphogenetic Protein 15 Gene *(BMP15)* gene which belongs to TGFβ (Transforming growth factor-β) [1], is an X-linked gene that had been shown to play a major role in ovarian regulation [2]. *BMP15* gene is essential for the early development of follicles in sheep. It stimulates proliferation and granulosa cells differentiation by suppressing follicle stimulating hormone (FSH), stimulating mitosis, and promoting ligand expression which have a large role in mammalian female fertility [3,4]. It also plays a major role in homeostasis, repairing tissue, embryonic development, apoptosis, neural, heart, cartilage development, and bone formation [4,5]. Sheep Bone Morphogenetic Protein 15 has been mapped on the X chromosome. The whole length of the coding sequence (1179 bp) is contained in two exons separated by an intron (5.4 kb) (Figure S1) and encodes a pre propeptide of 393 amino acids. The active mature peptide is of length of 125 amino acids [6].

Until now, there are eight genetic known mutations with major impacts on litter size and ovulation rate detected in *BMP15* gene (Figure S2). These are; $FecX^L$ in Lacaune sheep, $FecX^B$ in Belclare sheep, $FecX^R$ in Rasa Aragonesa sheep, $FecX^I$ in Inverdale sheep, $FecX^H$ in Hanna sheep, $FecX^{Gr}$ in the Grivette sheep, $FecX^O$ in Olkuska sheep and $FecX^G$ in Cambridge sheep (Galway mutation) [7]. All heterozygous ewes had higher prolificacy than wild type ewes but the mutant type had non additive effect on ovulation rate and the homozygotes were sterile [8,9,10,11]. Because the gene is an X-Linked, males only carry one copy and pass it to all daughters but not to sons [12].

Galway mutation ($FecX^G$) elevates the prolificacy of heterozygotes by about 0.7 lambs per ewe. As carrier rams, as well as non-carrier ewes, need to be maintained in a crossbreeding system. In this study, the Bone Morphogenetic Protein gene in three ecotypes of Sudanese sheep (Dubasi, Shugor and Watish) was scanned for polymorphism aiming to detect any possible mutation(s) and test their association with litter size.

2. Materials and Methods

Ethical approval: Animals Use in Research was according to the committee of the University of Khartoum regulations. The guidelines and regulations set out by the Sudan Veterinary Council were strictly followed during animal handling and sampling.

All experimental protocols used are approved by the University of Khartoum and in accordance with the University of Khartoum Laboratory Authority guidelines.

2.1. Genotypic Detection of *the BMP15* Gene mutations

The present study was conducted in the Molecular Biology and Immunology Unit, Department of Biology, Central laboratory of Veterinary Research, Ministry of Animal Resources and Fisheries.

2.1.1. Animals

One hundred blood samples were collected from unrelated ewes. The ewes belonged to the three ecotypes of Sudanese Desert Sheep: Dubasi (n= 30), Shugor (n= 30), and Watish (n= 40). The parity number and litter size of each ewe were recorded. Blood samples were taken from the jugular vein and placed in 10 ml vacuum blood collection tubes containing EDTA. All samples were directly stored in an ice box and transferred to the laboratory where they were stored at-20˚C till further processing.

2.1.2. DNA Extraction

Genomic DNA extraction was done using the Guanidine Chloride method as described by [13] with slight modifications. Briefly; 3-5 ml blood was gathered in EDTA tubes, then 10 ml red cell lysis buffer (RCLB) was added in a Falcon tube and centrifuged for 5 min at 6000 rpm, this process was repeated until a clear pellet appeared. The supernatant was discarded and 800 µl of white cell lysis buffer (WCLB) and 10 µl of proteinase K (10 mg/ml) plus 1 ml Guanidine Chloride and 300 µl Ammonium Acetate, were added, vortexed and incubated at 37˚C overnight. Then, an equal volume of chloroform was added to the new Falcon tube, mixed and centrifuged at 6000 rpm for 5 mints. The upper layer was gathered into a clean Falcon tube and 10 ml of cold ethanol (95%) was added and incubated at – 20˚C overnight. Then, the sample was centrifuged for 10-15 minutes at 6000 rpm and the supernatant discarded. The pellet was washed with 4 ml of 70% ethanol and centrifuged for 7 min at 12000 rpm, and the supernatant discarded. The preceding steps were repeated till the pellet became clear and was dried for 1-2 hours. Then 100 ml of Tries Acetate (TE) buffer or 20 µl distilled water was added and stored at -20˚C. The quality of the extracted DNA was examined with Agarose gel and visualized using Bio-Rad Gel Documentation 2000 system.

2.1.3. Amplification of Bone Morphogenetic protein 15 gene (*BMP15*)

The amplification reaction was carried out by RFLP-PCR and conditions were as described by [14] (Table 1) using 37 cycles at 95˚ C for 30s at initial denaturation, followed by 94˚ C for 45s as denaturation step, 62˚

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C for 40s (annealing), 72˚ C for 45s (extension), and the final extension at 72˚ C for 10 min. The PCR products (141 bp) were used with 0.5 µl *Hinf*I restriction enzyme. The results were visualized by U.V under gel documentation system.

Table 1. The primers and restriction enzymes

Gene	Point mutation	Primer name	Primer Sequences 5'-3'	PCR Product (bp)	Annealing Temperature	Restriction Enzyme	Reference
BMP15	$FecX^G$	B2-HinfIF	5'-CACTGTCTTCTTGTTACTGT ATTTCAATGAGAC-3'	141 bp	62° C	HinfI	[14]
		B26	5'-GATGCAATACTGCCTG CTTG-3'				

2.1.4. Gene and genotype frequencies estimation

The Gene and genotype frequencies were calculated by counting as described by [15]**.**

2.1.5. Association analyses between genotypes, parity number and Litter size in the studied ecotypes

The association between genotypes of the studied fecundity genes, parity number and litter size in the three sheep types under study was tested using the General linear model (GLM) method, performed by IBM SPSS Statistics version 21 (Statistical Package for the Social Sciences). The chi-square test was used to examine the statistical significance, and a p-value of >0.05 was considered not significant. Analysis of variance and Duncan multiple range test (DMRT) were carried out as appropriate to assess the statistical significance of various factors affecting litter size. The linear model was:

$$Y_{ijk} = \mu + B_i + G_j + P_k + E_{ijk}$$

Where:

Y_{ijk}= The litter size record of the ijkth ewe.

µ = The overall mean.

B_i= Effect of the ith ecotype (i = 1-3).

G_j= Effect of the jth genotype (j = 1-3).

P_k= Effect of the kth parity number (k = 1-4).

E_{ijk}=The random error term.

2.2. Sequencing for the studied *BMP15* gene mutation

The purification and standard sequencing of the PCR products of *BMP15* gene was performed for each genotype by Macrogen Company (Seoul, Korea).

2.3. Bioinformatics Analysis

2.3.1. Sequences similarity and alignment

The DNA Chromatograms were presented by the "Finch TV 1.4.0 software" and DNA cleaning process was done by removing nucleotides with sharp quality (Less than 10). The nucleotides of the *BMP15* gene were blasted against the NCBI databases to get the most similar and identical sequences [16]. Sequences with high identities were gotten from NCBI in the FASTA format and they underwent alignment by "Bio Edit 7.0 software" with the reference sequence [17] which was obtained from NCBI (NC_019484). Then, global phylogenetic trees were designed using "Multiple sequence alignment by software CLUSTALW" to determine the relationship among sheep breeds from different countries. The reference proteins of the studied fecundity genes were obtained from ExPASy-Universal protein resource (Research, 2007). Nucleotide sequence was translated to protein by ExPASy translation tool [18] and subjected to multiple sequence alignment by using Bio Edit 7.0 software.

3. Results

3.1. Allele and Genotype frequencies

3.1.1. Detection of the *FecX^G* Mutation in the *BMP-15* Gene

The PCR-RFLP technique was applied to determine the *BMP15* gene genotypes of sheep samples under study using *HinfI* restriction enzyme. The results of amplification of exon 2 of *BMP15* gene showed amplified fragments of a product size of 141 bp (Figure 1). Only two genotypes were found in all breeds. The wild type animals (BB) had two fragments 111 and 30 bp (Figure 2). The heterozygous animals (Bb) had three fragments of 141, 111 and 30 bp (Figure 3).

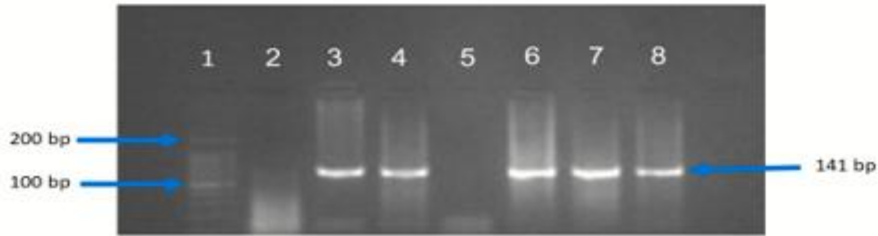

Figure 1. PCR amplification of exon 2 of point mutation *FecX^G^* of *BMP15* gene on 2% agarose gel electrophoresis in samples of Sudan Desert sheep. Lane 1, DNA ladder: MW 100-1500 bp fragments. Lane 2 and 5 negative samples. Lane 3, 4, 6, 7 and 8 showing the typical band size of 141bp corresponding to the molecular size of *BMP15* gene.

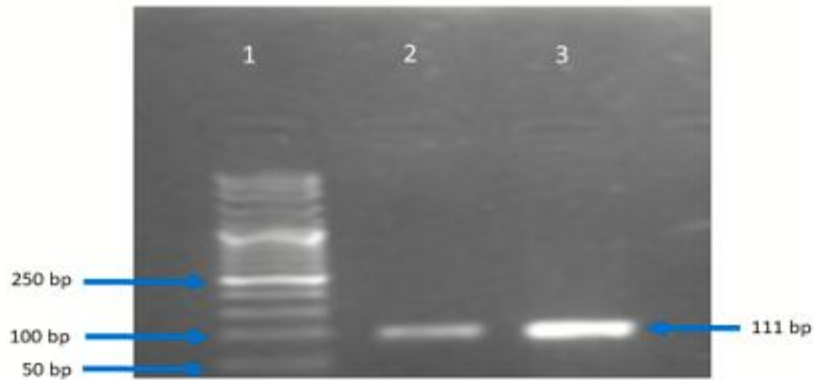

Figure 2. DNA electrophoretic pattern of *FecX^G^* point mutation of the *BMP15* gene amplified after digestion with *HinfI* restriction enzyme. Lane 1, DNA ladder: MW 50-1000 bp fragments, lane 2 and 3 represent the wild type genotype BB (111 and 30bp).

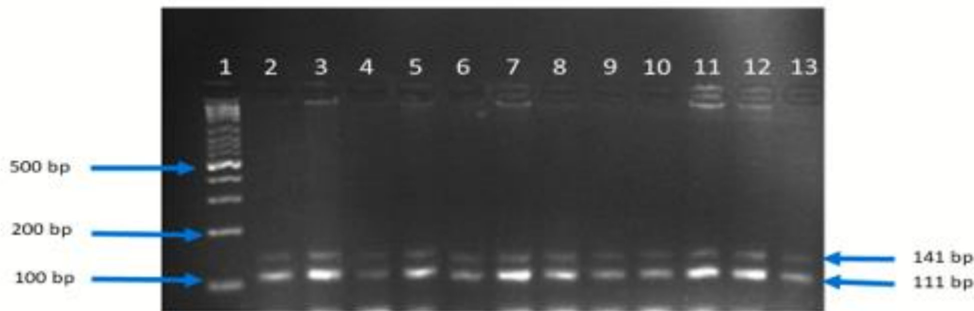

Figure 3. DNA electrophoretic pattern of *FecX^G^*point mutation of the *BMP15* gene amplified after digestion with *HinfI* restriction enzyme. Lane 1, DNA ladder: MW 100-1500 bp fragments, lane 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 represent the heterozygous genotype Bb (141,111 and 30bp).

3.1.2. Genetic variability

The allele and genotype frequencies of *BMP15* gene are presented in Table 2 for *FecX^G^* mutation. The total allele frequencies (B) and (b) were (0.66) and (0.34). The highest frequency of the mutant type allele (b) was found in Shugor (0.39), followed by Watish (0.31) and Dubasi (0.27). The genotypic frequency of the heterozygotes (Bb) among in all three ecotypes was higher than that of the wild type genotype (BB).

The Chi-square test (Table 2) was used to evaluate agreement with Hardy - Weinberg expectations. Both Shugor and Watish in addition to the pooled population of all three types were found to deviate from Hardy-Weinberg equilibrium with regard to the *BMP15* locus. The exception was the Dubasi population which was in HWE.

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Table 2. Allele and genotype frequencies of the point mutation *FecX*^{G}of the *BMP15* gene Dubasi, Shugor and Watish Sudanese sheep breeds:

Breed	No. of Animals	Allele Frequency		Genotype Frequency		HWE			
		BB	Bb	(B)	(b)	BB	Bb	bb	(χ2- value)
Dubasi	20	9	11	0.73	0.27	0.45	0.55	0.00	2.60 NS
Shugor	23	5	18	0.61	0.39	0.22	0.78	0.00	9.30 S
Watish	37	11	26	0.69	0.31	0.30	0.70	0.00	12.37 S
Total	80	25	55	0.66	0.34	0.31	0.69	0.00	21.90 S

NS: No significant deviation from HWE, S: Significant deviation from HWE, (P<0.01)

3.1.3. The association with litter size

Table 3 presents litter size data classified by *BMP15* genotypes and parity number in the three sheep ecotypes under study. The heterozygous (Bb) genotypes lambed 0.17 lambs more than the wild types (BB). Also, the results indicated that the Watish breed litter size was insignificantly higher than that of Shugor and Dubasi. On the other hand, the 2nd and 4th parity exhibited the highest litter size followed by 3rd and 1st parities, but the differences were not significant (Table 4).

Table 3. The association of *BMP15* genotypes and parity number with litter size in Dubasi, Shugor and Watish[1,2]

Item	Type / Number	LS Mean ± SE
Breed	Dubasi	1.270 ± 0.054^a
	Shugor	1.288 ± 0.059^a
	Watish	1.305 ± 0.047^a
Genotype	BB	1.203 ± 0.052^b
	Bb	1.373 ± 0.038^a
Parity Number	1st	1.189 ± 0.053^a
	2nd	1.327 ± 0.053^a
	3rd	1.286 ± 0.058^a
	4th	1.348 ± 0.085^a
Grand mean		1.288 ± 0.033

[1]LS; Litter Size, SE; Standard Error

[2]Means with the same superscripts within each column are not significantly (P>0.05) different.

Table 4. Analysis of variance: The effect of *BMP15* genotypes and parity on litter size of the three breeds[1,2].

Source of variation	DF	SM	MS	F-value	Pr> F
Breed	2	0.053	0.027	0.127*	0.881
Parity	3	0.962	0.321	1.536*	0.206
Genotypes	1	1.564	1.564	7.490**	0.007

[1]SM = Sum of Squares, DF = Degrees of Freedom, MS = Mean of Squares. *P < 0.05, **P < 0.01.

3.2. The DNA Sequencing of *BMP15* gene

Three different samples of DNA that were not used in the restriction enzyme analysis were subjected to sequencing [accession numbers were: E-Dubasi (MN862515); F-Shugor (MN862517); G-Watish (MN862516)]. The results of alignment of *BMP15* samples with database reference sequence showed that most sequence regions were similar and identical except for one variable nucleotide at position 111 bp where a guanine (G) was replaced by adenine (A) in the Sudanese G-Watish and F-Shugor samples (Figure 4). The Sudanese E-Dubasi sample was more closely aligned to the reference sequence and other sequences from database than the G-Watish and F-Shugor samples (Figure 5) and conserved regions were dominant in most of the sequence (Figure 6).

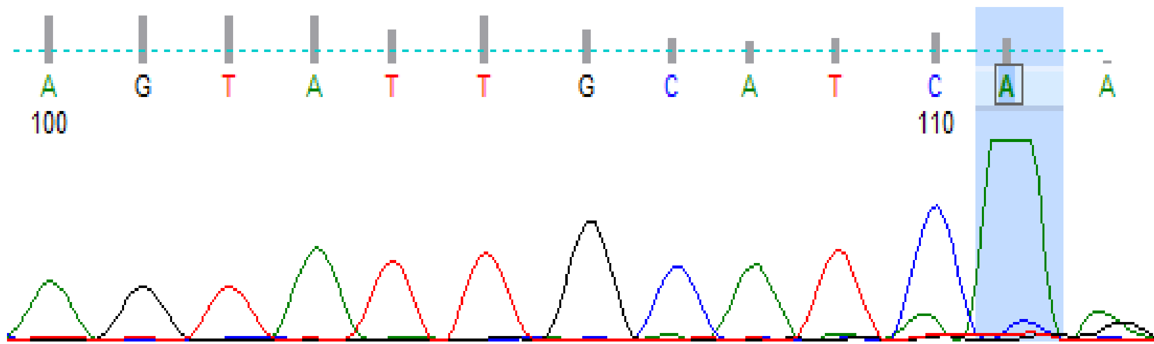

Figure 4. the variation in position 111 bp where the Guanine was replaced by Adenine

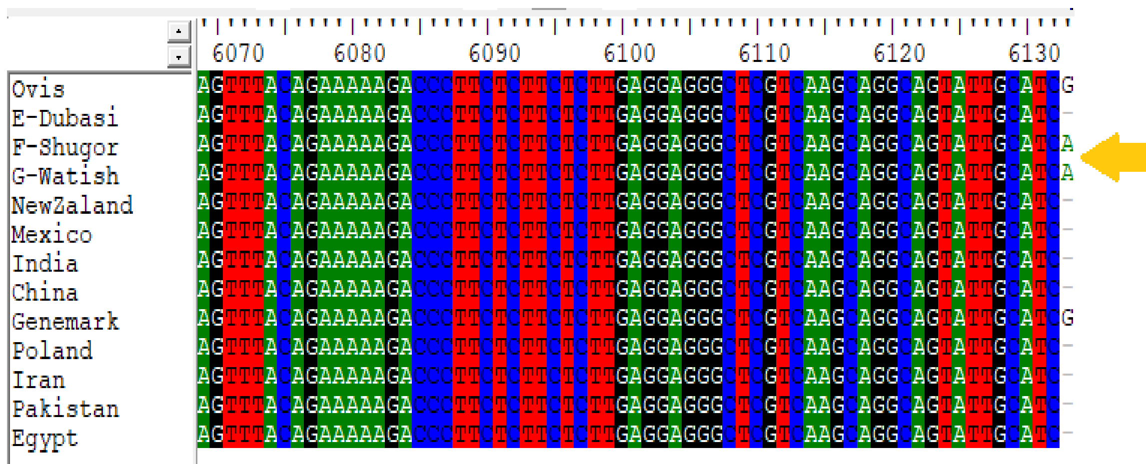

Figure 5. Similarity and identity among in the alignment of BMP15 with database and *Ovis* ref seq (Bio-Edit 7.0 software), Egypt (KT238846), New Zealand (AH009593 AF236078 AF236079), Mexico (KT853038), Iran (KT013294), Pakistan (JN655672), Poland (HQ878326), India (FJ600405) and China (EU402923).

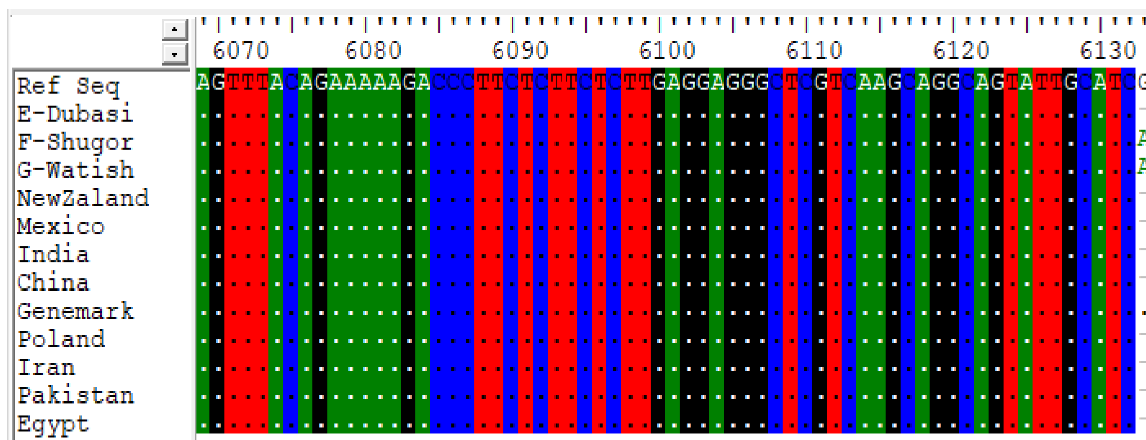

Figure 6. Conserved regions in the alignment of *BMP15* with database and ref sequences (Bio-Edit 7.0 software), Egypt (KT238846), New Zealand (AH009593 AF236078 AF236079), Mexico (KT853038), Iran (KT013294), Pakistan (JN655672), Poland (HQ878326), India (FJ600405) and China (EU402923).

There were no observable changes in the protein sequence and consequently, all amino acids were the same at residue 275 (Figure 7). This means that there is a silent mutation as a result of one nucleotide change without causing change in either the resulting protein or the phenotype of Watish and Shugor ecotypes.

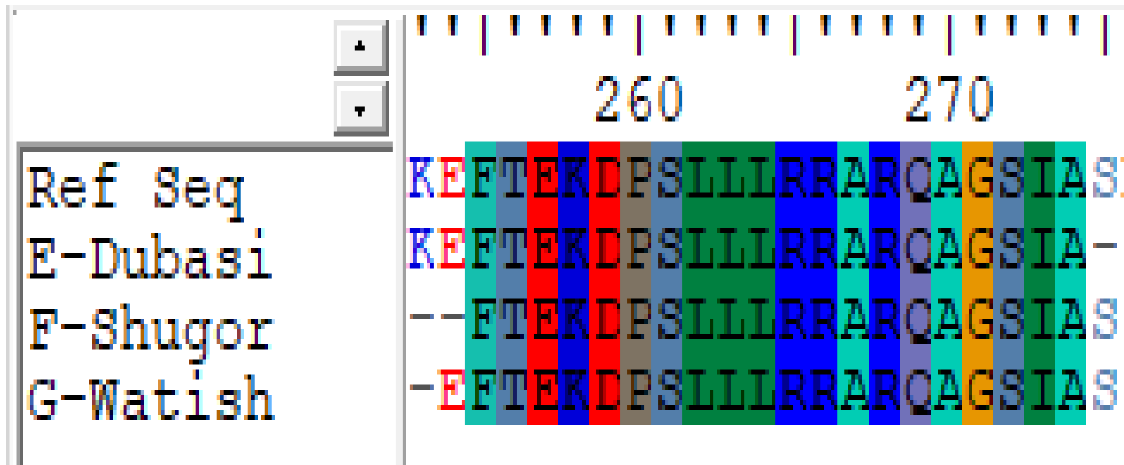

Figure 7. Amino acid alignment with the reference Sequence by using Bio-Edit 7.0 software.

Figure 8 shows the Phylogenetic tree results, Watish and Shugor breeds were out-groups, while Dubasi breed was in the same line with other database sequences (Egypt, New Zealand, Mexico, Iran, Pakistan, Poland, India, and China) and Egyptian sample is the most related to Sudanese Dubasi ecotype.

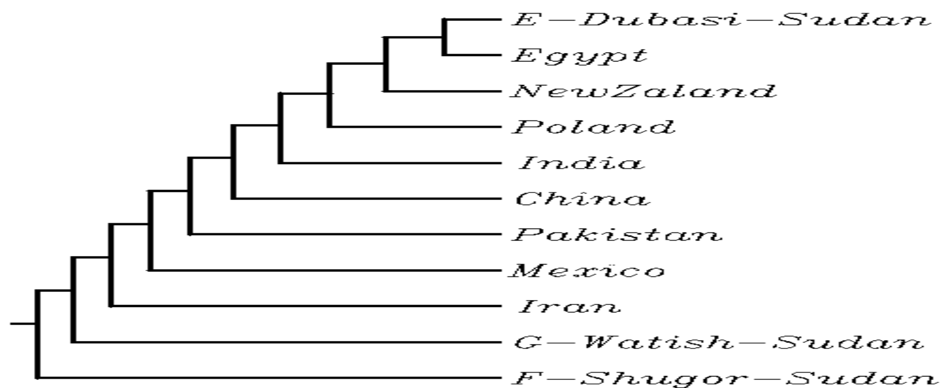

Figure 8. Global Phylogenetic tree (Multiple sequence alignment by CLUSTALW). Egypt (KT238846), New zaland (AH009593 AF236078 AF236079), Mexico (KT853038), Iran (KT013294), Pakistan (JN655672), Poland (HQ878326), India (FJ600405) and China (EU402923).

4. Discussion

In the present study, the Bone Morphogenetic Protein gene in the three ecotypes of Sudanese sheep (Dubasi, Shugor and Watish) was scanned for polymorphism aiming to detect any possible mutation(s) and test their association with litter size. The *BMP15* gene has a crucial role in the regulation of prolificacy in sheep [19]. The study was focused on the *FecX*^{G} point mutation located in exon 2 of *BMP15* gene in the above Sudanese

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sheep types. PCR-RFLP technique was used to identify nucleotide sequence polymorphism within the *BMP15* gene. The results indicated restriction sites of 111, 30 bp fragments which represented the wild type genotype. This is similar to the results found in Small Tailed Han and Hu Chinese sheep [1]. The fragments of 141, 111, 30 bp represented the heterozygous genotype. This is similar to the findings in Egyptian sheep breeds by [20]. The calculated total frequencies of BB, Bb and bb genotypes were 0.31, 0.69 and 0.00, respectively, and the total frequencies of the *B* and *b* alleles were 0.66 and 0.34, respectively. The genotypic frequency of the heterozygous genotype (Bb) in all three ecotypes study was higher than the genotypic frequency of the wild type (BB). The results confirm that *BMP15* locus is polymorphic in the studied populations. This result is in agreement with reports claimed in Small Tail Han sheep [21] and Egyptian sheep breeds [22]. Nevertheless, the results of this study are dissimilar to those reported on the $FecX^G$ locus in the North African breeds [23]; local Turkey breeds [24]; Barki and Rahmani sheep breeds [20] who reported that, the $FecX^G$ locus was monomorphic in their studied individuals.

None of the studied sheep breeds carried the $FecX^G$ mutation in the *BMP15* gene in the homozygous state. The absence of this genotype in the studied populations was also noted in other sheep breeds such as the Indian Bonpala sheep [6]. It was found that the homozygous mutant type ewes of $FecX^G$ mutation were sterile while the heterozygous ewes had improved ovulation rates and increased litter size by 0.7 lambs, as [9] reported. Based on the calculated prolificacy, our results indicated an increase in the litter size of the heterozygous ewes by about 0.17. However, the absence of mutant type individuals and the presence of only the wild type and the heterozygous individuals in our samples was probably a result of the fact that sterile ewes were culled at an early stage from flocks. However, owners did not complain of recurrent problems of infertility among Dubasi, Shugor and Watish ewes.

On the other hand, the litter size was significantly affected by $FecX^G$ point mutation of *BMP15* gene. Heterozygous (Bb) increased litter size by 0.17 lambs more than the wild types (BB) in the studied Sudanese sheep. Similar results were obtained in Belclare and Cambridge breeds [9].

Moreover, the study revealed that, the Watish ecotype demonstrated the highest prolificacy (1.305±0.047 lambs/lambing) compared to the Shugor (1.288±0.059) and Dubasi (1.270±0.054) ecotypes. This is close to our finding in the *G1* mutation of *GDF9* gene. Therefore, this suggests that the multiple birth phenomenon present in Watish ecotype is probably due to the action of both the *G1* mutation of *GDF9* gene and the $FecX^G$ mutation of the *BMP15* gene. Estimates of mean litters size of the three ecotypes are affected by the fact that sheep herders in Sudan prevent lambing in the dry season. Lambing only occurs between June and December when water and pasture are plentiful.

The results showed that, the 2nd and 4th parity had the highest litter size followed by the 3rd and 1st parities. The difference in litter size means in different parities might be due to nutritional factors leading to variation in hormone levels. Both genetic potential and environmental factors play a vital role in increasing litter size.

For the purpose of expanding and generalizing the results, three different samples were used in sequence analysis. The *BMP15* gene displays five mutations as shown in figures 2-5 ($FecX^G$, $FecX^I$, $FecX^H$, $FecX^L$, and $FecX^B$) [9]. The results did not show the presence of any of the five commonly known mutations occurring in *BMP15* gene that are associated with modulation of ovarian function in sheep [10].

Our findings showed a nucleotide substitution at the DNA level (111G>A SNP), where Guanine was replaced by Adenine at position 111 (Figure 4). This nucleotide substitution did not lead to a functional change in protein or phenotype of Watish and Shugor ecotypes as the resultant amino acid (Serine) at residue 275 did not change (Figure 9). Despite the nucleotide variation the mutation was silent.

Furthermore, the phylogenetic tree analysis showed that Iran sample (KT013294) is the main common ancestor of the Mexico sample (KT853038), Pakistan sample (JN655672), China sample (EU402923), India sample (FJ600405), Poland sample (HQ878326), NewZaland sample (AH009593) and sub group of Egypt (KT238846) and Dubasi (E). However, Watish (G) and Shugor (F) were out-grouped. According to this result, Watish and Shugor types are more related to each other. Egyptian breeds were the most related to Dubasi breed.

5. Conclusions

The results of the current study showed that the presence of one copy of $FecX^G$ point mutation of *BMP15* gene increased the litter size by 0.17 lambs in the studied Sudanese sheep ecotypes. This emphasizes the importance of using more prolific breeds as dam breeds and applying crossbreeding in the future of the sheep industry in Sudan. The selected mutant rams and wild type ewes should be used to avoid the occurrence of homozygous sterile females.

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The litter size trait in Dubasi, Shugor and Watish sheep ecotypes is probably related to some extent to the mutations of the *BMP15* (*FecX^G*) locus, in addition to the possible effect of other fecundity genes, which may be responsible for prolificacy in Sudanese sheep.

References

1. Wang, W.; Liu, S.; Li, F.; Pan, X.; Li, C.; Zhang, X.; Ma, Y.; La, Y.; Xi, R.; Li, T. Polymorphisms of the ovine BMPR-IB, BMP-15 and FSHR and their associations with litter size in two Chinese indigenous sheep breeds. *Int. J. Mol. Sci.* **2015**, *16*, 11385–11397.
2. Abir, R.; Fisch, B.; Johnson, M.H. BMP15, fertility and the ovary. *Reprod. Biomed. Online* **2014**, *29*, 525–526.
3. Zamani, P.; Nadri, S.; Saffaripour, R.; Ahmadi, A.; Dashti, F.; Abdoli, R. A new mutation in exon 2 of the bone morphogenetic protein 15 gene is associated with increase in prolificacy of Mehraban and Lori sheep. *Trop. Anim. Health Prod.* **2015**, *47*, 855–860.
4. Alakilli, S.Y.M.; Mahrous, K.F.; Salem, L.M.; Ahmed, E.S. Genetic polymorphism of five genes associated with growth traits in goat. *African J. Biotechnol.* **2012**, *11*, 14738–14748.
5. Han, H.; Lei, Q.; Zhou, Y.; Gao, J.; Liu, W.; Li, F.; Zhang, Q.; Lu, Y.; Cao, D. Association between BMP15 gene polymorphism and reproduction traits and its tissues expression characteristics in chicken. *PLoS One* **2015**, *10*, e0143298.
6. Roy, J.; Polley, S.; De, S.; Mukherjee, A.; Batabyal, S.; Pan, S.; Brahma, B.; Datta, T.K.; Goswami, S.L. Polymorphism of fecundity genes (FecB, FecX, and FecG) in the Indian Bonpala sheep. *Anim. Biotechnol.* **2011**, *22*, 151–162.
7. Zhou, M.; Pan, Z.; Cao, X.; Guo, X.; He, X.; Sun, Q.; Di, R.; Hu, W.; Wang, X.; Zhang, X.; et al. Single nucleotide polymorphisms in the HIRA gene affect litter size in Small Tail Han Sheep. *Animals* **2018**, *8*, 71.
8. Galloway, S.M.; McNatty, K.P.; Cambridge, L.M.; Laitinen, M.P.E.; Juengel, J.L.; Jokiranta, T.S.; McLaren, R.J.; Luiro, K.; Dodds, K.G.; Montgomery, G.W.; et al. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat. Genet.* **2000**, *25*, 279–283.
9. Hanrahan, J.P.; Gregan, S.M.; Mulsant, P.; Mullen, M.; Davis, G.H.; Powell, R.; Galloway, S.M. 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 (Ovis aries). *Biol. Reprod.* **2004**, *70*, 900–909.
10. Bodin, L.; Di Pasquale, E.; Fabre, S.; Bontoux, M.; Monget, P.; Persani, L.; Mulsant, P. 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* **2007**, *148*, 393–400.
11. Demars, J.; Fabre, S.; Sarry, J.; Rossetti, R.; Gilbert, H.; Persani, L.; Tosser-Klopp, G.; Mulsant, P.; Nowak, Z.; Drobik, W.; et al. Genome-wide association studies identify two novel BMP15 mutations responsible for an atypical hyperprolificacy phenotype in sheep. *PLoS Genet* **2013**, *9*, e1003482.
12. Davis, Gh. Fecundity genes in sheep. *Anim. Reprod. Sci.* **2004**, *82*, 247–253.
13. Gassoum, A.; Arbab, M.A.; Aldeaf, S.A.H.; Elhassan, L.A.; Elshibli, E.; Elhassan, A.M. Allele Frequency Of P53 Gene Arg72Pro In Sudanese Meningioma Patients And Controls. *IJSTR.* **2014**, *6*, 243–248.
14. Moradband, F.; Rahimi, G.; Gholizadeh, M. Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-1B with litter size in Iranian Baluchi sheep. *Asian-Australasian J. Anim. Sci.* **2011**, *24*, 1179–1183.
15. Frankham, R. Introduction to quantitative genetics (4th edn): by Douglas S. Falconer and Trudy FC Mackay Longman, 1996.£24.99 pbk (xv and 464 pages) ISBN 0582 24302 5 1996.
16. Johnson, M.; Zaretskaya, I.; Raytselis, Y.; Merezhuk, Y.; McGinnis, S.; Madden, T.L. NCBI BLAST: a better web interface. *Nucleic Acids Res.* **2008**, *36*, 525–526, doi:10.1093/nar/gkn201.
17. Pruitt, K.D.; Tatusova, T.; Maglott, D.R. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res.* **2007**, *35*,

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D61--D65.
18. Gasteiger, E.; Gattiker, A.; Hoogland, C.; Ivanyi, I.; Appel, R.D.; Bairoch, A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* **2003**, *31*, 3784–3788.
19. Wilson, T.; Wu, X.-Y.; Juengel, J.L.; Ross, I.K.; Lumsden, J.M.; Lord, E.A.; Dodds, K.G.; Walling, G.A.; McEwan, J.C.; O'Connell, A.R.; et al. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biol. Reprod.* **2001**, *64*, 1225–1235.
20. El-Seedy, A.S.; Shafiek, H.; Kitzis, A.; Ladevèze, V. CFTR Gene Mutations in the Egyptian Population: Current and Future Insights for Genetic Screening Strategy. *Front. Genet.* **2017**, *8*, 37.
21. Chu, M.X.; Liu, Z.H.; Jiao, C.L.; He, Y.Q.; Fang, L.; Ye, S.C.; Chen, G.H.; Wang, J.Y. Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (Ovis aries). *J. Anim. Sci.* **2007**, *85*, 598–603.
22. Barakat, I.A.H.; Salem, L.M.; Daoud, N.M.; Khalil, W.K.B.; Mahrous, K.F. Genetic polymorphism of candidate genes for fecundity traits in Egyptian sheep breeds. **2017**.
23. Vacca, G.M.; Dhaouadi, A.; Rekik, M.; Carcangiu, V.; Pazzola, M.; Dettori, M.L.; Wang, W.; Liu, S.; Li, F.; Pan, X.; et al. *Prolificacy genotypes at BMPR 1B, BMP15 and GDF9 genes in North African sheep breeds*; 2010; Vol. 88;.
24. KARSLI, T.; BALCIO\uGLU, M.S. Türkiye'de Yeti{\c{s}}tirilen Alt{\i} Yerli Koyun Irk{\i}nda BMPR-IB (Booroola) Geninde FecB Allel Varl{\i}{\u{g}}{\i}n{\i}n PCR-RFLP Yöntemiyle Ara{\c{s}}t{\i}r{\i}lmas{\i}. *Kafkas Univ. Vet. Fak. Derg.* **2011**, *17*.