Malays. Appl. Biol. (2017) 46(2): 135–144

MOLECULAR CHARACTERISATION OF FecB, FecX AND FecGH MUTATIONS IN IRAQI SHEEP BREEDS USING RFLP-PCR TECHNIQUE

YOUSIF M.S. AL-BARZINJI* and KAMARAN M. TAHA

Animal Resource Department, College of Agriculture, Salahaddin University-Erbil
**E-mail: Yousif.noori@su.edu.krd, Y_Barzengy@yahoo.com*

Accepted 26 May 2017, Published online 27 June 2017

ABSTRACT

The TGF-β super family, (GDF9, BMP-15 and BMPR-IB) have been shown to be essential for follicular growth and ovulation in the sheep breeds. The present study aimed to test the presence mutations of the fecundity genes association with fertility, controlling ovulation rate and litter size in four Iraqi sheep breeds (Kurdi, Hamdani, Arabi and Awassi) using specific primers designed to introduce a point mutations in PCR products of FecB1, FecB2 (BMPR-IB), FecXI, FecXB, FecXG (BMP-15) and FecGH, FecGI (GDF9) belonging to the TGF-β. A total of 140 blood samples collected from individual ewes (3-7 years) were genotyped for allelic polymorphisms using PCR-RFLP screening method. The quantity and quality of DNA was checked by Nanodrop spectrophotometer and gel electrophoresis. All genes (except the FecGH) showed wild types (non carriers ++) for all breeds and results viewed to be monomorphic with a specific restriction enzyme. Whereas, FecGH showed heterozygous carriers (H+) only in Hamdani sheep breed and this result showed that the polymorphism frequencies of FecGH gene with *DedI* restriction digestion significantly imbalanced in Hamdani breed compared with other breeds and the genotype frequency of HH, H+ and ++ were (0, 0.23 and 0.77) in this type of sheep. In conclusion, results suggest new possibilities for developing a breeding system for regulating fertility in Hamdani sheep breed.

Key words: RFLP-PCR, Iraqi Sheep Breed, Ovulation Rate

INTRODUCTION

The sheep *(Ovis aries)* are a profoundly diverse species with more than 900 different breeds that shift significantly in their physiological attributes including ovulation rate and fertility (McNatty *et al*., 2005). About 629 of these sheep breeds right now breed in the 52 European populations and 233 of them were bred in the Asian and Pacific nations (FAO, 2008).

The aggregate sheep populace of Iraq in 1999 was around 9.7 million, and it contributes biggest to the collective meat production in Iraq (Al-Salihi, 2012). The majority of this populace (99.8%) is possessed by the private division and is circulated everywhere throughout the nation. The local breeds incorporate the Karadi (Kurdi, Hamdani, Jaff and Dzaie) 20%, Awassi (Naami and Shefali) 58.2% and Arabi sheep 21.8%. These are all fat-tailed, carpet-wool production (Al-Rawi *et al*., 1996).

Dickerson (1970) pointed out that efficiency of animal production is controlled by three effective purposes which are reproduction, female production and growth of offspring. Bodin *et al.* (2007) verified that genetic mutations with significant impacts on ovulation rate and litter size in sheep were as of late recognized in three genes fitting in with the TGF super family pathway called fecundity genes. In sheep, three productivity loci have been found, specifically bone morphogenetic protein receptor type IB (BMPR-IB, or activin-like kinase 6, ALK6) known as FecB (Booroola) on chromosome 6 (Souza *et al*., 2001), growth differentiation factor 9 (GDF9) known as FecG on chromosome 5 (Hanrahan *et al*., 2004), and bone morphogenetic protein 15 (BMP15) branded as FecX on chromosome X (Hanrahan *et al*., 2004 & Moradband *et al*., 2011).

Therefore, the main objective of this study is to investigate the present and absent of the fecundity genes FecB (BMPR IB), FecG (GDF9) and FecX (BMP-15) controlling ovulation rate and litter size on Iraqi sheep breeds using PCR-RFLP technique

* To whom correspondence should be addressed.

at different places in Erbil and Duhok governorates of Iraqi Kurdistan region to improve sheep production.

MATERIALS AND METHODS

Experimental Animals and Locations

This study was done on four Iraqi sheep breeds (Kurdi, Hamdani, Arabi and Awassi), 3-7 years of age, from October 2014 to February 2015. The blood samples were gathered from different locations in Erbil and Duhok governorate. An aggregate of a 115 of the chose indigenous ewes gave twins and 25 ewes producing single lamb were also examined.

Blood Collection and DNA Extraction

Blood tests were collected from 140 Iraqi ewes. Entire blood was gathered from every ewe from jugular vein into 2 (K3) vacuum blood collection tubes (AFCO- Jordan) containing the anticoagulant, ethylene diamine tetra acidic acid (EDTA) and these samples were stored at -20°C until DNA extraction was done. Genomic DNA was isolated from samples using BIONER kit (AccuPrep® Genomic DNA Extraction Unit, Korea) according to manufactures instructions. The quantity and quality of DNA was checked by Nanodrop spectrophotometer and gel electrophoresis.

PCR Primers

Polymerase chain Reaction (PCR) was occurred utilizing a modification of the forced restriction fragment length polymorphism (RFLP) strategy. The primer sequences and annealing temperatures are given in Table 1.

PCR Amplification

Thermo cycler (Applied Biosystems® Veriti® 96-Well Fast Thermal Cycler, USA) was conveyed in a final reaction volume of 25µL. A master mix for 4 polled samples (Kurdi, Hamdani, Arabi and Awassi) for every gene was readied and an aliquot of 20µL filled in every PCR tube. Five µL of DNA sample was added to each tube to make the last volume 25µL to accomplish homogeneity of reagents and decrease the risk of contamination, control reaction was situated up without genomic DNA. A Go Taq® Green Master Mix (ADM7122 00000311719, Promega-USA) incorporates with 12.5µL Taq DNA polymerase (25Units/mL, dNTPs 200µM, and MgCl2 1.5mM), 2 µL RFLP primer (forward and reveres), 5µL (50ng) of DNA template and 5.5µL DNase free water. DNA was amplified

Table 1. Primers, annealing temperature (Ta), restriction enzymes (RE) and PCR products (PP) for candidate genes

Gene	Ta	Primers		RE	PP (*bp*)	Reference
		Forward	Reverse			
FecB2	60°C	5‘-CCA GAG GAC AAT AGC AAA GCA AA-3’	5‘-CAA GAT GTT TTC ATG CCT CAT CAA CAGGTC-3’	*AvaII*	190	Davis *et al* (2002)
FecX^{G}	63°C	5’-CAC TGT CTT CTT GTT ACT GTA TTT CAA TGA GAC-3’	5’-GAT GCA ATA CTG CCT GCT TG-3’	*HinfI*	141	Hanrahan *et al* (2004)
FecX^{B}	64°C	5’-GCC TTC CTG TGT CCC TTA TAA GTA TGT TCC CCT TA-3’	5’-TTC TTG GGA AAC CTG AGC TAG C-3’	*DdeI*	153	Hanrahan *et al* (2004)
FecG^{H}	62°C	5’ATG GAT GAT GTT CTG CAC CAT GGT GTG AAC CTG A-3’	5’-CTT TAG TCA GCT GAA GTG GGA CAA C-3’	*DdeI*	139	Hanrahan *et al* (2004)
FecX^{I}	60°C	5’-GAA GTA ACC AGT GTT CCC TCC ACC CTT TTC T-3’	5’-CAT GAT TGG GAG AAT TGA GAC C-3’	*XbaI*	154	Davis *et al* (2006)
FecB1	60°C	5’-GTC GCT ATG GGG AAG TTT GGA TG-3’	5’CAA GAT GTT TTC ATG CCT CAT CAA CAC GGT C-3’	*AvaII*	140	Chu *et al* (2007)
FecG^{I}	58°C	5’-GAA GAC TGG TAT GGG GAA ATG-3’	5’-CCA ATC TGC TCC TAC ACA CCT-3’	*HhaI*	462	Moradband *et al* (2011)

Table 2. PCR products digestion components with their volume for BMPR-1B, BMP-15 and GDF9 genes

Digestion Component	Volume
Sterile deionized water	7.3µL
Reaction 10X buffer	2µL
Acetylate BSA	0.2µL
DNA (PCR product) mix by pipetting	10µL
Appropriate Reaction Enzyme	0.5µL (5U)
Final volume	20µL

initial denaturation at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 95°C for 1 min, annealing (temperatures for each primer pair are shown in Table 1) 1 min, extension at 72°C for 45 sec, with final extension 72°C for 7 min. The amplification products were size-fractionated in a 2% agarose gel containing ethidium bromide in Tris-borate EDTA buffer and visualized under UV transillumination.

PCR-RFLP Analysis

After PCR amplification PCR products were digested with the appropriate restriction enzyme (mentioned with images) for each PCR products separately to identify if a mutation is present in a DNA samples or not. All digestion component requirements are shown in Table 2 which is mixed delicately by pipetting. The reactions were incubated for 2-4 hr at the pointed out temperature for the enzyme 37°C. Ten µL of the digested samples were taken, included loading buffer, and were separated by electrophoresis on 2.5% agarose gel and visualized with ethidum bromide under UV transillumination.

RESULTS AND DISCUSSION

Detection of BMPR-IB Mutation Genes

The PCR technique was used to detect the BMPR-IB in 140 ewes depends on Davis *et al.* (2002) & Chu *et al.* (2007) PCR conditions, clear bands were observed for both 140bp and 190bp for FecB1 and FecB2 respectively. The resulted PCR products of the BMPR-IB mutations (FecB1 and FecB2) were digested with *AvaII* restriction enzyme. After that the digested product for each breed run on 2.5% agarose gel electrophoresis to detect the genotype for each breed. The results investigated that both genes from all breed samples were a wild type with (++) genotype (Figure 1 and Figure 2) respectively, which means, there were no restriction site for *AvaII* restriction enzyme were found and no mutations have occurred at both locus. These results were consistent with reports investigated in many of the previous studies on different sheep breeds (Kumar *et al*., 2006; El-Hanafy *et al*., 2009; Ghaffari *et al.*, 2009; Kasiriyan *et al*., 2009; Al-Barzinji, 2010; Abbas, 2011; Moradband *et al*., 2011; Al-Barzinji & Othman, 2013; Al-Thabhawee *et al*., 2014). On the other hand, many studies discovered this mutation point in different sheep breeds (Davis *et al*., 1991, Wilson *et al*., 2001, Davis *et al*., 2002,

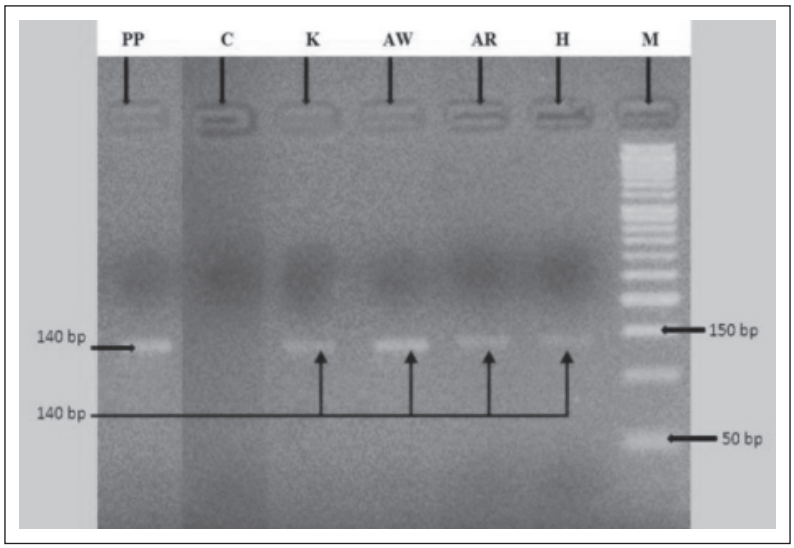

Fig. 1. Digestion of PCR product of four breeds include K=Kurdi, AW=Awassi, AR=Arabi and H=Hamdani with *AvaII* restriction enzyme of FecB1 produced 140 bp with wild type genotype (++). Lane PP is PCR product, lane C is negative control. Fragments were fractionated by 2.5% agarose gel electrophoresis (1.5 hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

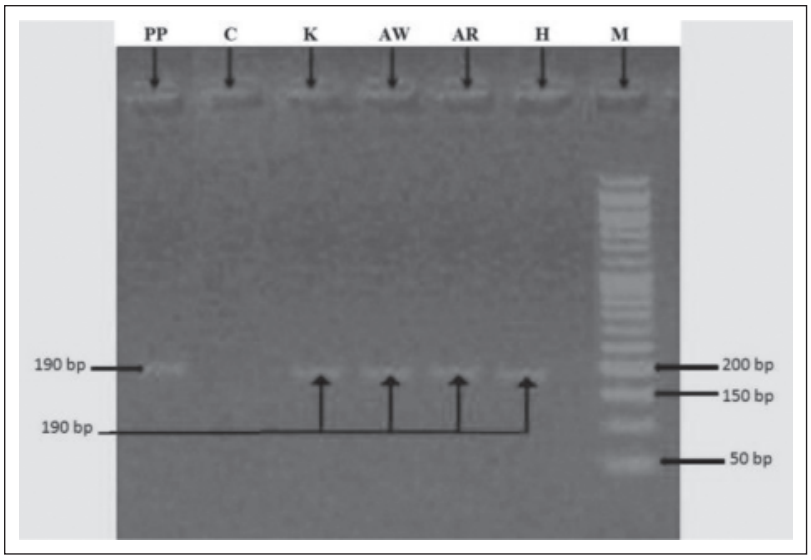

Fig. 2. Digestion of PCR product of four breeds include K=Kurdi, AW=Awassi, AR=Arabi and H=Hamdani with *AvaII* restriction enzyme of FecB2 produced 190 bp with wild type genotype (++). Lane PP is PCR product, lane C is negative control. Fragments were separated by 2.5% agarose gel electrophoresis (1.5 hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

Davis *et al*., 2006, Kumar *et al*., 2006, Feng *et al*., 2007, Kumar *et al*., 2008 & Asadpour *et al*., 2012). In the present study, the BMPR-B1 mutation genes obtained single band for FecB1 and FecB2 140 and 190 respectively in all tested Iraqi breeds.

The result obviously showed that BMPR-B1 has no effect on litter size and ovulation rate. In addition, these breeds are wild type pattern of FecB genes, it means the gene (Fec+) and genotype (Fec+ Fec+) frequency was equal to one.

Detection of BMP-15 Mutation Genes

The PCR technique was used to detect the three mutation points (FecXG, FecXB and FecXI) at BMP-15 in 140 ewes. The clear bands were obtained for FecXG, FecXB and FecXI, which was explained by Hanrahan *et al.* (2004) and Davis *et al.* (2006). Then the 2% agarose gel electrophoresis showed that all breed samples carried 141bp for FecXG, 153 bp for FecXB and 154bp for FecXI. Then to detect three mutations (FecXG, FecXI and FecXB) PCR products were digested by using HinfI, XbaI and DdeI restriction enzymes, respectively (Hanrahan *et al*., 2004, Davis *et al*., 2006). After that the digested samples run on 2.5% agarose gel electrophoresis as in (Figure 3, Figure 4 and Figure 5). As in the results both restriction enzymes HinfI and DdeI were cut all samples (Figure 3 and Figure 5).

The 1st cut samples at one point and gave two fragments (111 and 30 bp) and the 2nd (*DdeI*) gave two fragments but with different size (123 and 30 bp). The *XbaI* restriction enzyme did not cut the samples as in the Figure 4 and all fragments remained as one band (154 bp). These results showed that all breed samples for these three mutations points at BMP-15 were (monomorphic) homozygous non-carriers or wild type (++). These results agreed with reports by many researchers in different sheep breeds. The gene and genotype frequency for all three mutation points at this locus were equal to one (Davis *et al*., 2006, Hua *et al*., 2008, Vacca *et al*., 2010, Moradband *et al*., 2011, Zhang *et al*., 2011, Karsil *et al*., 2012, Dincel *et al*., 2015).

Detection of GDF-9 Mutation Genes

The PCR technique was used to detect the two mutation points (FecGI and FecGH) at GDF9 in 140 ewes. Moradband *et al.* (2011) & Hanrahan *et al.* (2004) were revealed PCR conditions of these genes, and we caught the clear bands for FecGI and FecGH. The PCR products were run on 2% agarose gel electrophoresis and all samples gave 462bp for FecGI and 139bp for FecGH. The resulted PCR products of the GDF9 mutations (FecGI and FecGH) were digested with HhaI and DdeI restriction enzyme, respectively (Moradband *et al*., 2011 & Hanrahan *et al*., 2004). After that the digested PCR products for each breed run on 2.5% agarose gel electrophoresis to detect the genotype for each breed as in Figure 6 and Figure 7. As in the results both restriction enzymes HhaI and DdeI were cut all samples (Figure 6 and Figure 7). The first cut samples at the two points gave three fragments (254, 156 and 52 bp) and showed that all breed samples

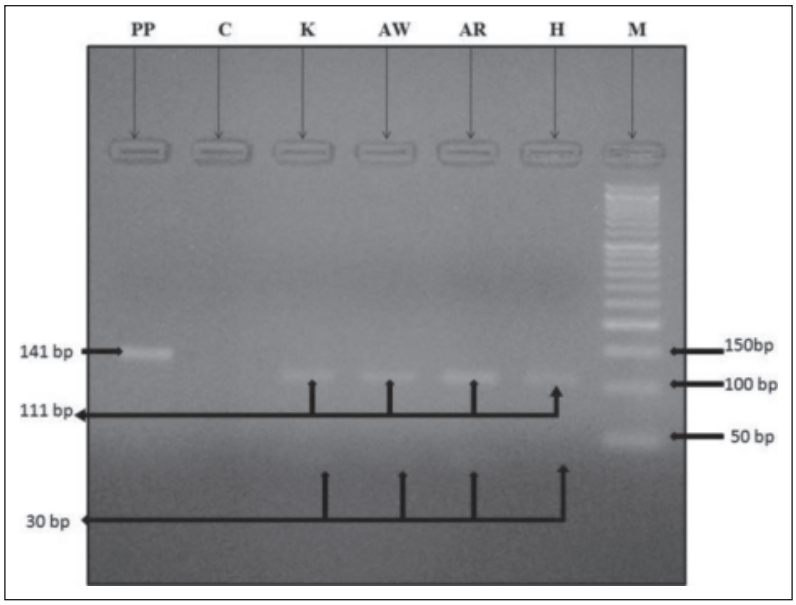

Fig. 3. Digestion of FecXG PCR product of four breeds include K=Kurdi, AW=Awassi, AR=Arabi and H=Hamdani with *HinfI* restriction enzyme produced 141 bp with wild type genotype (++). Lane PP is PCR product, lane C is negative control. Bands were separated by 2.5% agarose gel electrophoresis (1.5hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

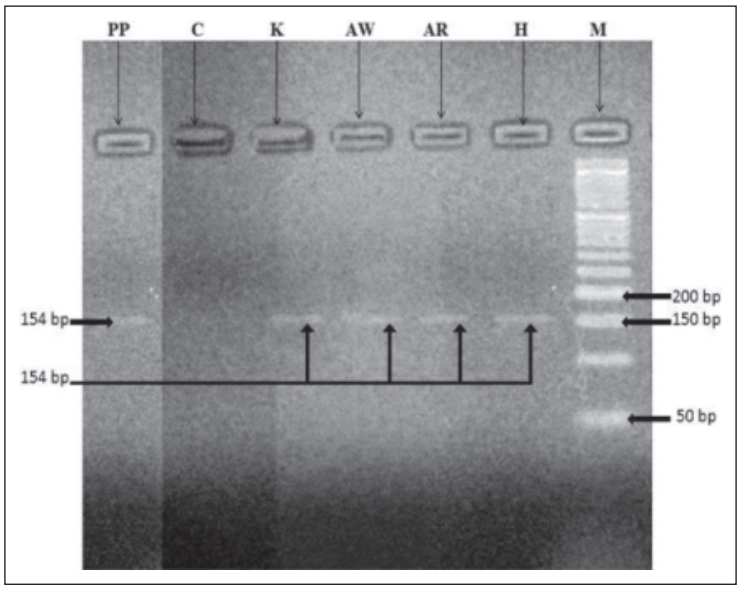

Fig. 4. Digestion of FecXI PCR product of four breeds include K=Kurdi, AW=Awassi, AR=Arabi and H=Hamdani with *XbaI* restriction enzyme produced 154 bp with wild type genotype (++). Lane PP is PCR product, lane C is negative control. Fragments were fractionated by 2.5% agarose gel electrophoresis (1.5hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

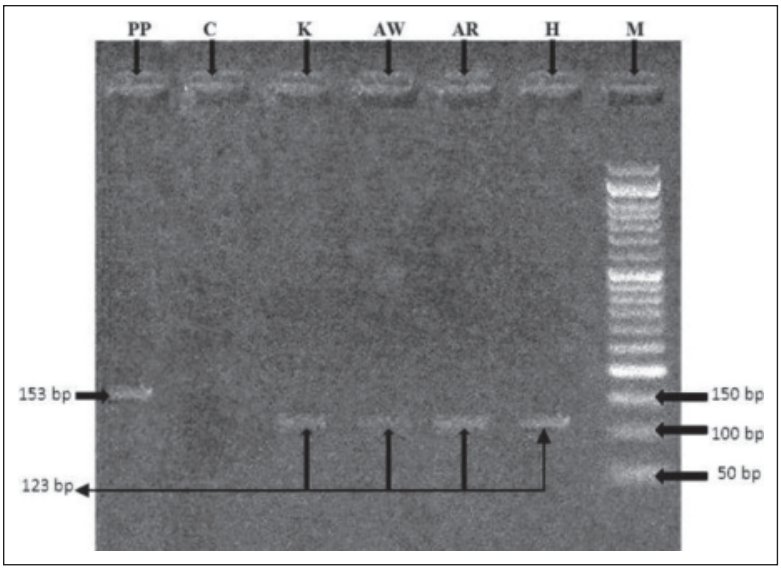

Fig. 5. Digestion of FecXB PCR product of four breeds include K=Kurdi, AW=Awassi, AR=Arabi and H=Hamdani with *DdeI* restriction enzyme produced 153 bp with wild type genotype (++). Lane PP is PCR product, lane C is negative control. Bands were separated by 2.5% agarose gel electrophoresis (1.5hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

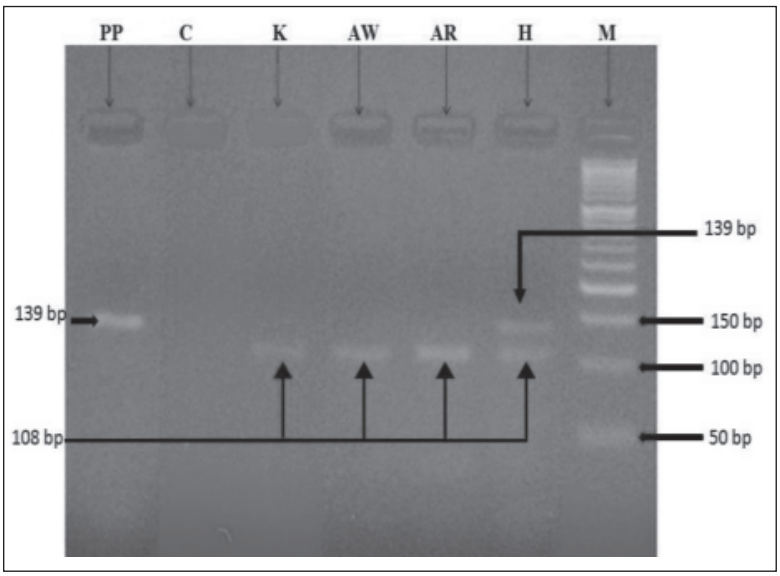

Fig. 6. Digestion of FecGI PCR product of four breeds include K=Kurdi, AW=Awassi, AR=Arabi and H=Hamdani with *HhaI* restriction enzyme produced (254,156 and 52 bp) with wild type genotype (++). Lane PP is PCR product, lane C is negative control. Fragments were separated by 2.5% agarose gel electrophoresis (1.5hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

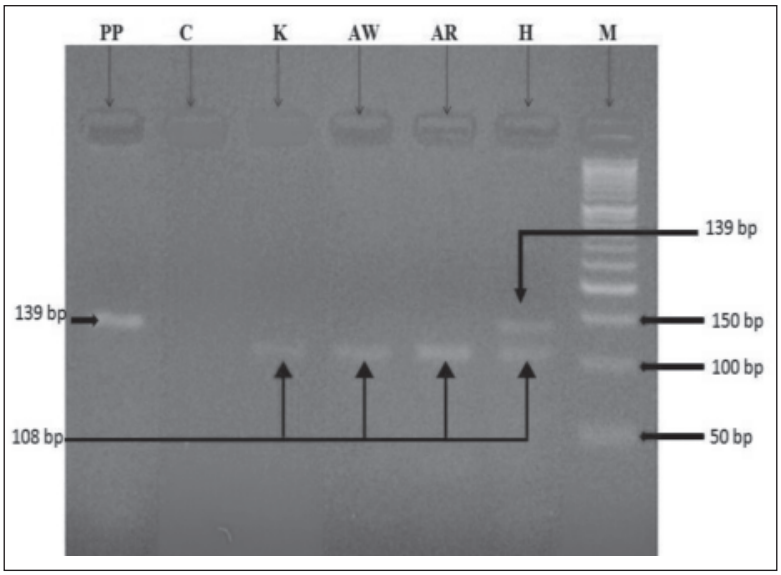

Fig. 7. Digestion of FecGH PCR product of four breeds include K=Kurdi, AW=Awassi, AR=Arabi and H=Hamdani with *DdeI* restriction enzyme produced 139 bp with wild type genotype (++) lane (Kurdi, Awassi and Arabi) were produced two fragments 139 and 108 bp with heterozygous genotype lane (Hamdani). Lane PP is PCR product, lane C is negative control. Bands were fractionated by 2.5% agarose gel electrophoresis (1.5hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

for FecGI mutation point at GDF9 were (monomorphic) homozygous non-carriers or wild type (++). Whereas, the second (*DdeI*) cut samples at one point gave two fragments (139 and 108 bp) this result showed that all breed samples for FecGH mutation point at GDF9 were (polymorphic) heterozygous carriers (H+).

In the present study, the consequences of digestive PCR products of FecGI demonstrated the same band pattern (52, 156 and 254 bp) in all breeds, including no mutation in exon one of the FecGI of GDF9 locus in four Iraqi breed ewes. On the subject of the records of twinning in these breeds it is refined that the hereditary variable controlling twinning is not related to the mutation which is expressed in the FecGI point mutation of GDF9 major gene. It perhaps will be inferred that litter size in these breeds is either not influenced by major genes or it is conceivable that other single nucleotide polymorphism (SNP) in the GDF9 gene or some other major genes control twinning in these breeds. Further research is prescribed to explore this speculation. Interestingly, digesting of FecGH with *DdeI* resulted in 139 bp, 108 and 139 bp, and 108 bp bands for HH, H+, and ++ animals, respectively. First time Hanrahan *et al.* (2004), by means of the molecular examination of GDF-9 gene in sheep of Cambridge and Belclare breeds were accounted for mutations in those genes that related to multiple births of these two strains. They uncovered new mutations in this gene and verbalized these mutations in heterozygous state relate to expanded rates of egg laying and in the homozygous state demonstrate the barrenness phenotype. The researches demonstrated that Cambridge and Belclare sheep conveying a mutation in the gene (FecGH) GDF-9 in exons 1 and 2, which builds ovulation rate in heterozygous sheep and sterility in homozygous sheep. They additionally announced that a duplicate of FecGH increases 1.4 ovulation rates in this sheep, which our outcomes affirm this gene as heterozygous in Hamdani sheep and as per containing this feature is one of indispensable characteristics in this breed and its esteem in meat production. Also, this gene is act as a growth factor which is implying from its name Growth differentiation factor 9 (GDF9), for that we can obviously see that Hamdani sheep have higher weight than other local breeds. However, Hamdani sheep which carrying this gene, the twining rate is relatively low. This is possibly because of poor environmental condition in a region where Hamdani sheep are bred. Since environmental works not in favor of reproductive performance, so that a suitable environmental circumstance must be provided due to expression of this gene and perform good reproductive performance because Hamdani breed has the reproductive gene. The gene and genotype frequencies of GDF9 mutation genes of four Iraqi breeds were imbalanced for FecGI and equal to one,

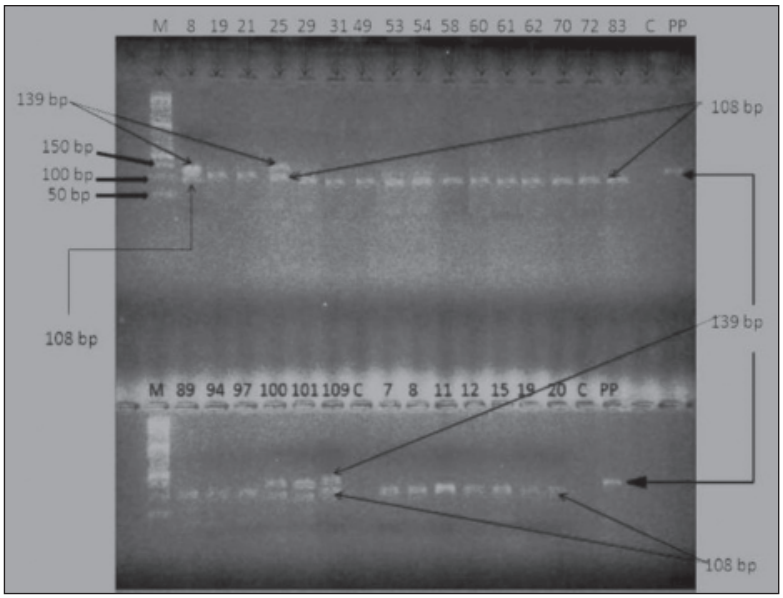

Fig. 8. Digestion of PCR product of FecGH gene of all Hamdani sheep individually, lane 8-109 is Hamdani producing twins, Lane 7-20 is Hamdani producing single lambs with *DdeI* restriction enzyme produced two genotypes (++) and (G+). Lanes 8, 25, 100,101 and 109 are heterozygous (G+) 139 bp, 108 bp, the others are homozygous wild type (++) 108 bp. Lanes C is negative controls. Lane PP is polled PCR products. Bands were separated by 2.5% agarose gel electrophoresis (1.5hr 80V 1X TBE buffer) stained with 5µL ethidium bromide.

while the genotypic frequency for FecGH of (++), (H+), and (HH) were (0.8), (0.2) and (0), respectively. Whereas, the gene and genotype frequency of GDF9 mutation genes of Hamdani breed were (++), (H+), and (HH) were (0.77), (0.23) and (0), respectively (Figure 8).

CONCLUSION

In the present study, the results of RFLP-PCR showed that all loci were wild types in Kurdi, Awassi and Arabi sheep breeds, it means the mutation factors did not make the point mutation in these loci at these types of sheep breeds on the other hand the in Hamdani sheep breed the (FecB and FecX) loci and (FecGH) locus showed the wild and mutation (Hetero genotype) types, respectively with 0.23 genotype frequencies for second one.

ACKNOWLEGEMENT

The authors are grateful to the Salahaddin University-Erbil to support this M.Sc project study.

REFERENCES

Abas, B. 2011. Identification of BMPR-IB, FecB and BMP-15 Fecundity Genes polymorphism in Awassi sheep. PhD. Thesis. Bagdad University, Iraq.

Al-Barzinji, Y.M.S. 2010. Polymorphism in Booroola (FecB) gene associated with liter size in Hamdani sheep. *Journal of Dohuk University.* **13(1)**: 413-417.

Al-Barzinji, Y.M.S. & Othman, G.U. 2013. Genetic Polymorphism in FecB Gene in Iraqi Sheep Breeds Using RFLP-PCR Technique. *IOSR-JAVS*, **2(4)**: 46-48.

Al-Rawi, A.A., Al-Haboby, A.H. & Al-Salman, M.H. 1996. Small Ruminant Breeding and Reproductive Physiology Research and Technology Transfer in Iraq (W. Mourrani and N. Haddad, ed.). ICARDA-West Asia Regional Program, Amman, Jordan.

Al-Salihi, K.A. 2012. An insight into veterinary education in Iraq. *International Development Vetinery Record*, **171**: 316-317.

Al-Thabhawee, A.H., Samaka, H.M. & Kadhim, H.M. 2014. Detections of (FecB) Gene Polymorphism in Local Sheep Breed at Different Area of Iraq. *Mirror of Research in Vetinery Science and Animal (MRVSA)*, **3(1)**: 15-20.

Asadpour, R., Jafarri-Joozani, R., ALijani, S. & Mahmodi, H. 2012. Detection of polymorphism in booroola gene (FecB) and its association with litter size in Zel sheep breed in Iran. *Slovak Journal of Animal Science*, **45(2)**: 63-66.

Bodin, L., Pasquale, E.D., Fabre, S., Bontoux, M., Monget, P., Persani, L. & 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.

Chu, M.X., Liu, Z.H., Jiao, C.L., He, Y.Q., Fang, L., Ye, S.C., Chen, G.H. & Wang, J.Y. 2007. Mutations in BMPRIB and BMP15 genes are associated with litter size in Small Tailed Han sheep (Ovis aries). *Journal of Animal Science*, **85**: 598-603.

Davis, G.H., Balakrishnan, L., Ross, I.K., Wilson, T., Galloway, S.M., Lumsden, B.M., Hanrahan, J.P., Mullen, M., Arranz, J.J. & Notter, D.R. 2006. Investigation of the Booroola (FecB) and Inverdale (FecXI) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. *Animal Reproduction Science*, **92**: 87-96.

Davis, G.H., Elsen, J.M., Bodin, L., Fahmy, M., Castonguay, F., Gootwine, E., Bor, A., Braw-Tal, R., Greef, J.C., Lengyel, A., Paszthy, G. & Cummins, L. 1991. A comparison of the production from Booroola and local breed sheep in different countries. In: Elsen, J.M., Bodin, L., Thimonier, J. (Eds.), Major Genes for Reproduction in Sheep. INRA, Paris. 315-323 pp.

Davis, G.H., Galloeay, S.M., Ross, I.K., Gregan, S.M., Ward, J., Nimbkar, B.V., Ghalsasi, P.M., Nimbkar, C., Gray, G.D., Subandriyo, I., Tiesnamurti, B., Martyniuk, E., Eythorsdottir, E., Mulsant, P., Lecerf, F., Hanrahan, J.P., Bradford, G.E. & Wilson, T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. *Biology of Reproduction*, **66**: 1869-1874.

Dickerson, G.E. 1970. Efficiency of animal production-molding the biological components. *Journal of Animal Science*, **30**: 849.

Dincel, D., Ardicil, S., Soyudal, B., Er, M., Alpay, F., Samli, H. & Balci, F. 2015. Analysis of FecB, BMP-15 and CAST Gene Mutations in Sakiz sheep. *The Journal of the Faculty of Veterinary Medicine*, **21(4)**: 483-488.

El-Hanafy, A. & El-Saadani, M.A. 2009. Finger-printing of FecB gene in five Egyptian sheep breeds. Biotech. *Animal Husbandry*, **25(3-4)**: 205-212.

FAO. 2008. Breeders hold first pageant for Saudi-bred sheep. MSNBC (Associated Press). 31.

Feng, G., Ren, L.S., Qing, S.G., Tao, A., Gan, M.D. & Guo, Y.L. 2007. Polymorphism of FecB Gene in Nine Sheep Breeds or Strains and Its Effects on Litter Size, Lamb Growth and Development. *Animal Reproduction Science*, **99**: 44-52.

Ghaffari, M., Nejati-Javaremi, A. & Rahimi, G. 2009. Detection of polymorphism in BMPR-IB gene associated with twining in Shal sheep using PCR-RFLP method. *International Journal of Agricultural & Biology,* **11(1)**: 97-99.

Hanrahan, J.P., Gregan, S.M., Mulsant, P., Mullen, M., Davis, G.H., Powell, R. & Galloway, S. 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 (*Ovis aries*). *Biology Reproduction*, **70**: 900-909.

Hua, G.H., Chen, S.L., Jun, T.A. & Yang, L.G. 2008. None of Polymorphism of Ovine Fecundity major genes FecB and fecX eas tested in Goat. *Journal of Animal Reproduction Science*, **108(3-4)**: 279-286.

Karsli, T., Şahin, E., Argun, K.B., Alkan, S. & Balcioglu, M.S. 2012. An Investigation of Mutations (FecXG, FecXI, FecXH, FecXB) On BMP-15 Gene In Some Local Sheep Breeds Raised In Turkey. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi*, **25(1)**: 29-33.

Kasiriyan, M.M., Hafezeyan, H., Sayahzadeh, H. & Jamshidi, R. 2009. Genetic polymorphism FecB and BMP15 genes and its association with litter size in Sangsari sheep breed of Iran. *JAVA*, **8(5)**: 1025-1031.

Kumar, S., Kolte, A.P., Mishra, A.K., Arora, A.L. & Singh, V.K. 2006. Identification of the FecB mutation in Garole-Malpura sheep and its effect on litter size. *Small Ruminant Research*, **64**: 305-310.

Kumar, S., Mishra, A.K., Kolte, A.P., Dash, S.K. & Karim, S. 2008. Screening for Booroola (FecB) and Galway (FecXG) mutations in Indian sheep. *Small Ruminant Research*, **80(1-3)**: 57-61.

Mcnatty, K.P., Galloway, S.M., Wilson, T., Smith, P., Hudson, L.N., Anne, O., Bibby, A.H., Heath, D.A., Davis, G.H., James, P.H. & Jenny, L.J. 2005. Physiological effects of major genes affecting ovulation rate in sheep. *Genetics Selection Evolution*, **37(1)**: 25-38.

Moradband, F., Rahimi, G. & Gholizadeh, M. 2011. Association of Polymorphisms in Fecundity Genes of GDF9, BMP15 and BMP15-1B with Litter Size in Iranian Baluchi Sheep. *Asian Australian Journal of Animal Sciences*, **24(9)**: 1179-1183.

Souza, C.J., Macdougall, C., Campbell, B.K., Mcneilly, A.S. & Baird, D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *Journal of Endocrinology*, **169**: 1-6.

Vacca, G.M., Dhaouadi, A., Rekik, M., Carcangiu, V., Pazzola, M. & Dettori, M.L. 2010. Prolificacy genotypes at BMPR1B, BMP15 and GDF9 genes in North African sheep breeds. *Small Ruminant Research*, **88**: 67-71.

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., Mcnatty, K.P. & Montgomery, G.W. 2001. 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. *Biological Reproduction*, **64**: 1225-1235.

Zhang, C.S., Geng, L.Y., Du, L.X., Liu, Z.Z. & Fu, Z.X. 2011. Polymorphic Study of FecXG, FecGH and FecB Mutation in Four Domestic Sheep Breeds in Lower Yellow River Valley of China. *Journal of Animal and Veterinary Advances*, **10(17)**: 2198-2201.