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# POLYMORPHISMS AND ALLELE FREQUENCY OF BMPR1B GENES IN LOCAL AWASSI SHEEP

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**Abstract**

This study aimed at uncovering the polymorphisms in the BMPR1B gene from the blood samples of 35 Awassi ewes at the Roundabout Agricultural Research Station - Anbar Governorate. DNA was extracted from blood samples which were tested using PCR by adopting the gene primer. Single nucleotide polymorphism (SNP) technology was used to determine the occurrence of variations for the specific alleles of each ewe. The results showed that the genotype and allele frequencies of four loci of the BMPR1B gene were significantly different (P≤0.01). The first position of rs410310346 (T > C) showed three genotypes i.e., TT, TC and CC, with percentages of 2.56, 26.88 and 70.56%, respectively, and the allelic frequency of the T alleles were - 0.16 and C - 0.84. For the second position of rs592447725 (G > A), three genotypes appeared as GG, GA, and AA at percentages of 72.26, 25.50, and 2.24%, respectively, and an allelic frequency of G 0.85 and A 0.15. For rs429006240 (T>C) the three genotypes of TT, TC and CC had percentages of 92.16, 7.68 and 0.16%, respectively, with a T allele frequency of 0.96 and C - 0.04. The fourth locus rs419457334 (G>C) showed three genotypes GG, GC, and CC with the percentages of polymorphism at 77.44, 21.12, and 1.44%, respectively

and the allelic frequency of G was 0.88 while that of C was 0.12. The results show the allelic frequency in the BMPR1B gene for four positions having polymorphisms (CC, GG, TT, and GG) that were superior to the other genotypes carried by the Awassi ewes.

**Keywords:** BMPRP1B gene, Sequencing PCR, Awassi sheep.

# التشكلات الوراثية والتكرار الاليلي لجين BMPRP1B في الاغنام العواسية المحلية

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**الخلاصة**

أجريت هذه الدراسة للكشف عن تعدد الأشكال الجين BMPR1B من عينات الدم المأخوذة من 34 نعجة عواسي في محطة البحوث الزراعية في الدوار– الانبار. تم استخلاص الحمض النووي من عينات الدم، ثم تم فحص العينات باستخدام تفاعل البلمرة المتسلسل (PCR) باستخدام بادئ الجين. تم استخدام تقنية تعدد أشكال النوكليوتيدات المفردة (SNP) لاكتشاف حدوث اختلافات للأليلات المحددة لكل نعجة.. أظهرت النتائج أن النمط الجيني وترددات الأليل لأربعة مواقع من الجين BMPR1B كانت مختلفة بشكل كبير (P≤0.01). أظهر موضع الاول (T > C) rs410310346 ثلاثة مظاهر وراثية TT وTC وCC، وكانت النسبة المئوية للمظاهر الوراثية 2.56 و26.88 و70.56٪ على التوالي، وبتردد اليلي 0.16 – T و0.84 – C على التوالي. أما بالنسبة للموضع الثاني (G > A) rs592447725، فقد اظهر ثلاثة مظاهر وراثية، GG، GA، وAA، وكانت النسبة المئوية للمظاهر الوراثية 72,26 ,25,50، و2,24%، على التوالي، بينما كان التردد الأليلي للأليلات G 0,85 و0,15 A. وأظهر الموضع الثالث (T >C) rs429006240 ثلاثة مظاهر وراثية TT وTC وCC، وبنسب مئوية 92.16 و7.68 و0.16٪ على التوالي، والتردد الأليلي للأليلات –T كان 0.96 و0.04 – C. وأظهر الموضع الرابع (G > C) rs419457334 ثلاثة مظاهر وراثية GG وGC وCC، وكانت النسبة المئوية للمظاهر الوراثية 77.44 و21.12 و1.44٪ على التوالي، وبتردد اليلي 0.88 G و0.12. C. أظهرت النتائج أن التردد الأليلي في الجين BMPR1B لأربعة مواضع أن المظاهر الوراثية CC وGG وTT وGG كانت هي المتفوقة مقارنة بالمظاهر الوراثية الأخرى التي تحملها النعاج العواسي.

**كلمات مفتاحية:** جين BMPRP1B، تسلسل PCR، أغنام العواس.

## Introduction

Awassi ewes produce meat, milk, and wool. Their reproductive performance includes economically important quantitative characteristics, such as fertility, the proportion of twins, and birth rates. Fertility is important for the productivity and breeding of the ewes (5 and 33). The possibility of using genetic compositions will lead to the development of strategies for genetic improvement and animal breeding programmes (1,4 and 28). Genetic research shows that reproductive functions are genetically systemized by the act of single crucially effective genes (known as fertility genes) (3 and 29). Genes involved in controlling reproduction were initially identified in sheep (14). The BMPR1B gene with the TGF-β (transforming growth factor beta) superfamily (8), plays a significant role in fetal change and development, the rate of ovulation and birth numbers. BMPR1B, which is a bone protein receptor (1b) is a major gene located in chromosome-6 responsible for sheep twinning and fertility (13 and 16) and was originally discovered in Booroola merino sheep (24). The A → G heterodimer encoded in exon 8 is related strongly to nucleotide 830 of the mRNA and recognised as the FecB or as the Booroola gene. Davis et al. (9) found that mutation in the BMPR1B gene increases ovulation in Booroola ewes (10 and 23). Later, the mutation spread to many other sheep breeds around the world. BMPR1B, seen in oocytes and granulosa cells, has an extra effect on the growth of granulosa cells and follicles in the ovary. It also affects the production of about 1.5 oocytes per estrus (ope) cycle in sheep, thus raising the rate of twinning and litter sizes in sheep (20 and 24). BMPR1B is known to be a receptor for different bone growth factors (BMP). Specially the BMP 2, 4 and 6 obstruct both baseline FSH production and progesterone-induced FSH production in both granulosa cells of the minor antral follicle stage, influencing ovulation in mammals in general and sheep in particular (17).

These are considered candidate markers for the BMPR1B receptors (21 and 30). The BMPR1B fertility gene responsible for reproduction in ewes mainly contributes to the production and the growth of follicles of ovarian granulosa cells in sheep (18 and 19). It is under the umbrella of the type I receptor (12). BMPs mainly appear in proliferating primordial germ cells (30). Piper and Bindon (22) stated that the special fertility gene of sheep breeds affects their ovulation rates due to the outcome of a single major gene. In 1989, the Sheep and Goat Genetic Nomenclature Committee labelled this gene the FecB Booroola fecundity. This A746G point mutation was in the coding area of the BMPR1B gene (2, 6 and 27). The BMPR1B is the main gene having significant influence over fertility and birth numbers in ewes (15 and 31). (30) stated that the BMPR1B gene promotes follicle growth and the proliferation of granulosa cells in the ovary, thus affecting ovulation in mammals. As such, evidence of SNPs existence is critical for improving the reproductive performance of sheep (25 and 32). This research sought to detect the genetic features, variations, and allelic repetitions in the BMPR1B gene from a sample of Awassi ewes in central Iraq.

## Materials and Methods

Experimental design: The study was conducted on 35 ewes aged between 3-6 years and having previous births. Their birth and growth records were taken from the research station of the Agricultural Research Department, Anbar Governorate. Ten rams of the Awassi breed aged 4-6 years were used for breeding purposes.

DNA Loading and Electrophoresis: Blood samples were collected from 35 ewes at the Agricultural Research Station at Al Dawar, Anbar from 22/1/2023 to 25/4/2023 and transported to the Progress Laboratory for Molecular Genetics in Baghdad and preserved at minus 20°C. Blood samples were treated for genomic DNA extraction using the Promega kit. The DNA was separated from blood, and all genomic DNA samples were loaded by Gel electrophoresis in 1gm agarose, with the resulting band patterns.

Primer Setting: The primer was introduced in a photophilic form, as shown in Table 1, by Macrogen™. The granulated primer was liquified in nuclease-free water to arrive at the final concentration of 100 pmol/µl as a standard solution. The setting for an active solution of these primers was prepared by adding 10 µL of a primer stock solution that was already frozen at minus 20°C to 90 µL of nuclease-free water to achieve an active primer solution of 10 pmol/Ml (7).

**Table 1: Design of the BMPR1B gene primer.**

| Primer | Sequence 5`-3` | Annealing Temp. | ($C^0$) Product size (bp) |
|---|---|---|---|
| **BMPR1B-F** | GAGGATGTGGGACAAATGAA | 55 | 893 |
| **BMPR1B-R** | GCCACAGTCAGGAAGTAAAT | | |

PCR amplification for the BMPR1B gene: PCR technology was applied to amplify the genomic DNA producing a fragment size of 893 base pairs (bp) for the BMPR1B gene. The PCR products were divided into 1.5–2% agarose gel. The DNA status was ranked from 100 to 1500 bp. All the samples were successfully amplified, and one ethidium bromide strip obtained.

Statistical analysis: A special test was used to compare the percentages of genotype distribution. The Chi-square- $\chi 2$ test was applied for each gene in the studied samples in the Statistical Analysis System (SAS) program (26). The allelic frequency, as applied by the Falconer and Mackay (1996) equation, was calculated for each gene.

PA = (2 * No. of Homozygous + 1 * No. of Heterozygous) / (2 * Total number of samples)

First allele repeat: PA
Since: P + q = 1, the frequency of the next allele is qB = 1 - PA.

## Results and Discussion

DNA Loading and Electrophoresis as shown in Figure 1.

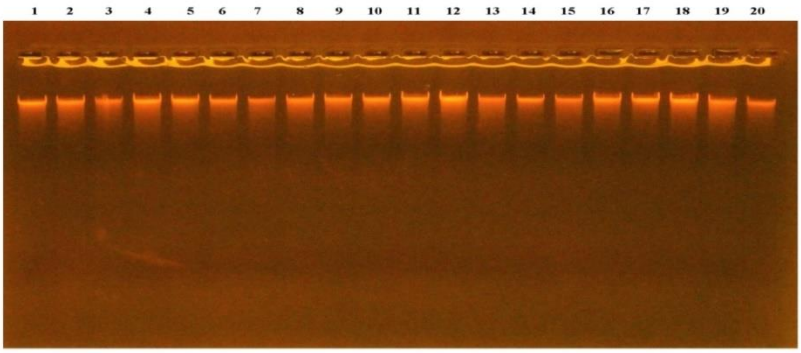


**Figure 1: Electrical relay to extract genetic DNA from blood, 1% agarose gel at 65 volts and 40 mA for an hour.**

PCR amplification for gene BMPR1B as shown in Figure 2.

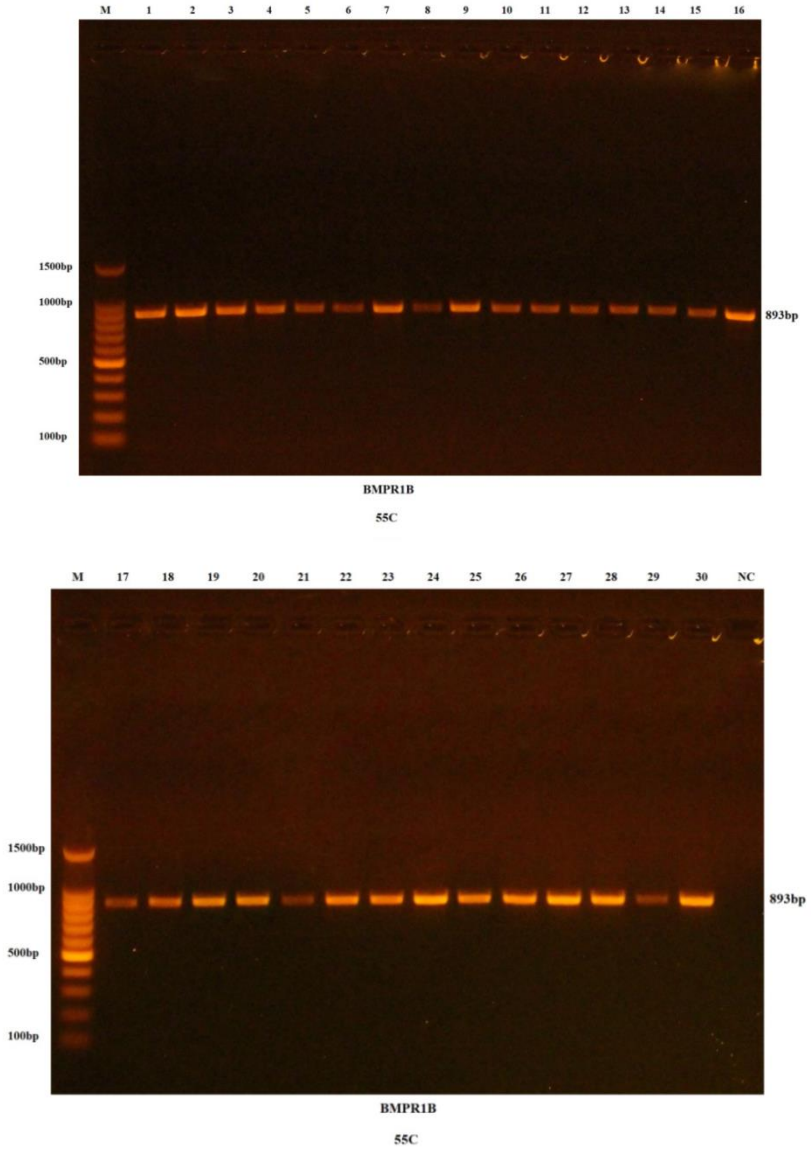


**Figure 2: A PCR product from Jane BMPR1B 893 the size of a base pair bp. The product was heated on 1.5 - 2% Agaroz at 65 V and 40 mA for 1 hour. DNA ladder (100 - 1500 bp).**

The results for the sequencing technique for the BMPR1B gene showed the part containing two variations of the gene (893 base pairs). The outcomes show 1 SNP in

the subject coding locus of the BMPR1B gene which appears to have three polymorphisms in the first heterozygosity (T>C SNP1) i.e., TT, TC, and CC. Also, the second variant (G>A SNP 2) had three polymorphisms, namely GG, GA, and AA which looked similar to the third variant (T>C SNP 3) of TT, TC, and CC. Finally, the three polymorphisms had multiple variants in the fourth variant (4 SNP G>C), which were GG, GC, and CC (see Figures 3, 4, 5, and 6).

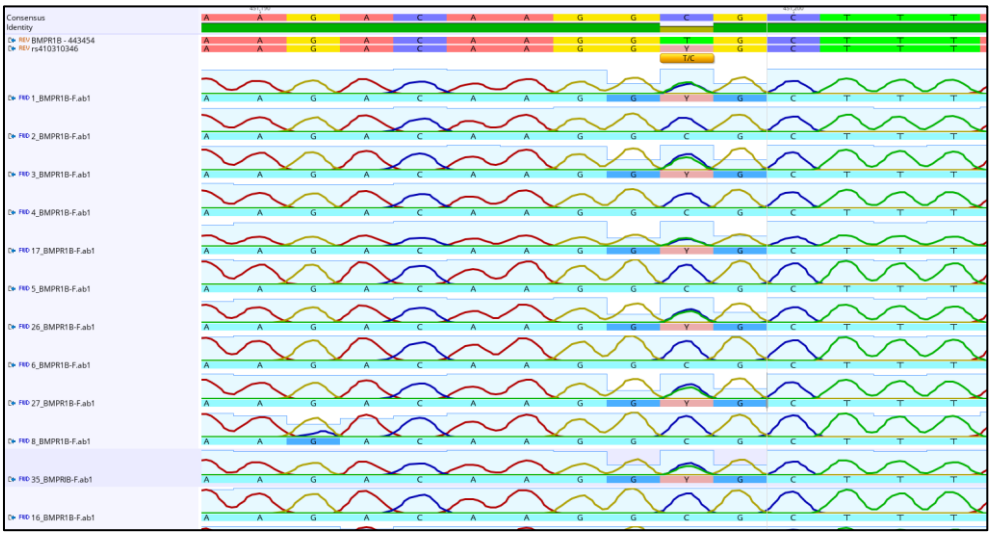

**Figure 3: The rs410310346 (T > C) variants/ SNP1.**

BMPR1B Gene Nitrogen Base Sequence (SNP1): Table 2 shows the appearance of three divisions, i.e., polymorphism, percentages of polymorphism, and the allelic frequency of the BMPR1B gene (SNP1 polymorphism). Three polymorphisms were seen in the coding area, and the outcomes of polymorphism of TT, TC, and CC were 2.56, 26.88, and 70.56%, respectively. The frequency of the T alleles was 0.16 and for C it was 0.84. Differences were noticed among the polymorphism ($P \leq 0.01$). Ewes that carry the CC genotype are superior to other genotypes, and those carrying the T and C alleles. The reason for the different proportions of genotype of this heterogeneity may be attributed to several factors, most prominent being the size of the sample, the strain, the location of the study, environmental conditions, and the technique adopted in the partial analysis.

**Table 2: Polymorphisms and the allelic frequency of BMPR-1B gene/rs410310346 (T > C) in ewes.**

| Genotype/ SNP1: rs410310346 (T>C) | Number | | Percentage | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| **TT** | 0 | 0.870 | 0.00 | 2.56 |
| **TC** | 11 | 9.14 | 32.35 | 26.88 |
| **CC** | 23 | 23.99 | 67.65 | 70.56 |
| **Total** | 34 | 34 | % 100 | % 100 |
| **P-value** | ---- | | 0.0001 | |
| **Allele** | Frequency | | | |
| **T** | 0.16 | | | |
| **C** | 0.84 | | | |

**** (P≤0.01)**

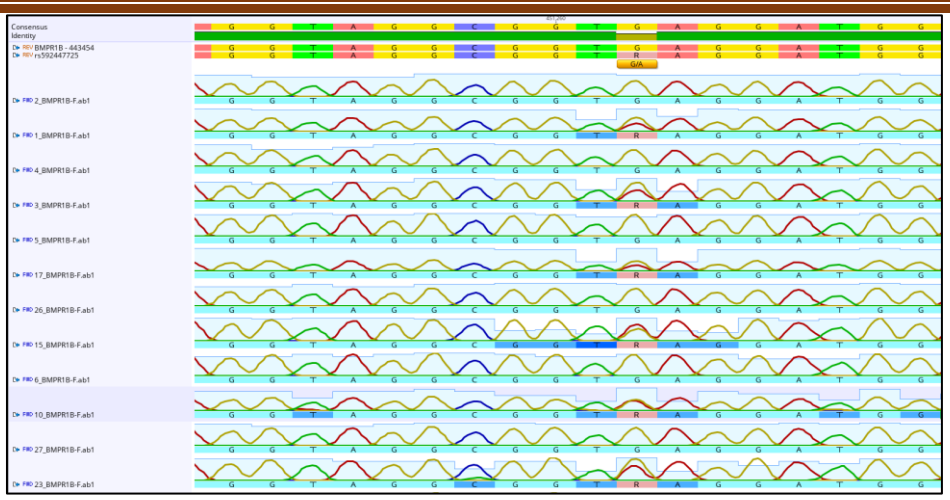

**Figure 4: The rs59244725 (G > A) variants/ SNP2.**

BMPR1B Gene Nitrogen Base Sequence (SNP2): Table 3 shows the results for the polymorphisms, their percentages, and the allelic frequencies of the BMPR1B gene (SNP2 polymorphism). The three polymorphisms give the percentages of GG, GA, and AA at 72.26, 25.50, and 2.24%, respectively. The allelic frequencies show G at 0.85 and A at 0.15, respectively. Differences were seen among the polymorphisms as $P \leq 0.01$. Ewes carrying the GG genotype become superior to other genotypes, and those carrying the G and A alleles.

**Table 3: Polymorphisms and the allelic frequency of BMPR-1B gene/rs592447725 (G > A) in ewes.**

| Genotype/ SNP2: rs592447725 (G>A) | Number | | Percentage | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| **GG** | 24 | 24.57 | 70.59 | 72.26 |
| **GA** | 10 | 8.67 | 29.41 | 25.50 |
| **AA** | 0 | 0.76 | 0.00 | 2.24 |
| **Total** | 34 | 34 | % 100 | % 100 |
| **P-value** | ---- | | ** 0.0001 | |
| **Allele** | Frequency | | | |
| **G** | 0.85 | | | |
| **A** | 0.15 | | | |

**** (P≤0.01).**

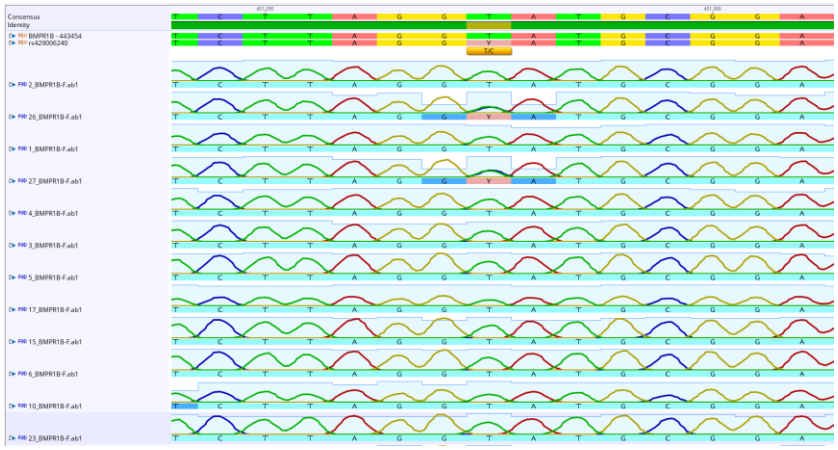

**Figure 5: The rs429006240 (T > C) variants/ SNP3.**

BMPR1B Gene Nitrogen Base Sequence (SNP3): The BMPR1B gene (SNP3 polymorphism) is shown in Table 4 together with the polymorphisms, their percentages, and the allelic frequencies. Three polymorphisms appear to have the TT, TC, and CC results with their respective percentages of 92.16, 7.68, and 0.16%, and their T and C allelic frequencies of 0.96 and 0.04, respectively. Differences were obtained among the polymorphism ($P \leq 0.01$). Ewes carrying the TT genotype are superior to other genotypes, and those carrying the T and C alleles.

**Table 4: Polymorphisms and allelic frequency of BMPR-1B gene/rs429006240 (T > C) in ewes.**

| Genotype/ SNP3: rs429006240 (T>C) | Number | | Percentage | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| TT | 31 | 31.33 | 91.18 | 92.16 |
| TC | 3 | 2.61 | 9.82 | 7.68 |
| CC | 0 | 5.44 | 0.00 | 0.16 |
| Total | 34 | 34 | % 100 | % 100 |
| P-value | ---- | | 0.0001 | |
| Allele | Frequency | | | |
| T | 0.96 | | | |
| C | 0.04 | | | |

** (P≤0.01).

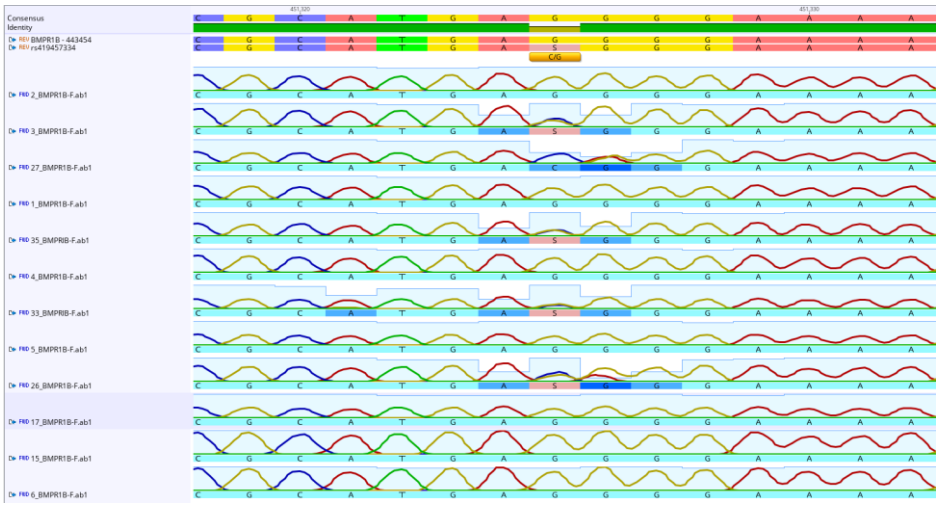

**Figure 6: The rs419457334 (G > C) variants/ SNP4.**

BMPR1B Gene Nitrogen Base Sequence (SNP4): Table 5 shows three polymorphisms with their percentages of GG, GC, and CC at 77.44, 21.12, and 1.44%, respectively of the BMPR1B gene (SNP4 polymorphism). The allelic frequencies for G were 0.88 and 0.12 for C. Differences resulted among the polymorphism ($P \leq 0.01$). Ewes carrying the GG genotype are superior to other genotypes, and those carrying the G and C alleles.

**Table 5: Polymorphisms and allelic frequency of BMPR-1B gene/rs419457334 (G > C) in ewes.**

| Genotype/ SNP4: rs419457334 (G>C) | Number | | Percentage | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| GG | 27 | 26.33 | 79.41 | 77.44 |
| GC | 6 | 7.18 | 17.65 | 21.12 |
| CC | 1 | 0.489 | 2.94 | 1.44 |
| Total | 34 | 34 | % 100 | % 100 |
| P-value | ---- | | 0.0001 | |
| Allele | Frequency | | | |
| G | 0.88 | | | |
| C | 0.12 | | | |

** (P≤0.01).

The variations in the SNP positions of the BMPR1B gene through sequencing technology studies led to variations in the sequences of the nitrogenous bases. These showed superior genotypes in four positions i.e., GG, TT, GG, and CC according to the results shown in the tables, where many genotypes appeared for the parts of the genes studied. This indicates the presence of important genetic diversity through the appearance of the Wild, Hetro, and Mutant genotypes of the studied ewes (11). Therefore, they can be used as polymorphisms for selecting high-production animals through the BMPR1B gene's effect on the reproductive traits of the Awassi ewes and for genetic improvement programs in sheep.

## Conclusions

Point mutations for the following T → C, G→A, T→C, and G→C were noticed at position 893 of the BMPR1B gene, but did not come in place of the amino acid sequence due to the SNP1 T893C and SNP2 G893A position. The SNP3 T893C and SNP4 G893C were in the exact exon-12 of the BMPR-1B gene, which did not result in amino acid changes, though the variant genotype distribution of ewes (single ewes, twin ewes) had a significant difference. The BMPR-1B exon-12 mutation has a strong link with fertility, number of litters, and pregnancy rate in ewes and can be used as a molecular marker in selecting ewes with good genotypes at the early stages of sheep. It can be used as molecular information for breeding purposes in place of the traditional selective breeding process. Over the long term they can help promote improvement programs, especially for poor genetic traits such as low birth numbers, increased misses, and low fertility.

**Supplementary Materials:**

No Supplementary Materials.

**Author Contributions:**

Author 1: methodology, writing of original draft; Authors 2 and 3: writing, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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The study was conducted in accordance with the protocols approved by the Department of Animal Production, College of Agriculture, University of Anbar.

**Informed Consent Statement:**

No Informed Consent Statement.

**Data Availability Statement:**

Ewe data was obtained from the Agricultural Research Station in Anbar governorate, Al Dawar.

**Conflicts of Interest:**

The authors declare no conflict of interest.

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