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Correlation between BMPR1B Gene Polymorphism and some Reproductive Traits in Awassi Sheep

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Abstract

The (BMPR-1B) gene is one of the main genes for ewe fertility. This study was conducted to check the association between BMPR-IB gene polymorphisms and some traits of Awassi. This study was made in Anbar Governorate - (Agricultural Research Department) Al-Dawar. Date of experience collected from 34 ewes at the age of 4-6 years. Next, DNA was extracted and a PCR reaction was performed to amplify blood samples taken from the ewes to confirm the presence of the BMPR1B gene. The sequence was determined using Sanger sequencing technology. The data were then analyzed and compared between sample sequences to determine SNPs, genotype frequencies, allele frequencies, their association with reproductive and growth traits. The results showed mutations in the heteroplasmy site rs410310346 (T>C) and rs592447725 (G>A). The results indicated that the TC genotype at the mutation site rs410310346 (T>C) was significantly superior (P≤0.05) in ewes whose rates of fertility and body length were higher compared to the CC genotype. The genotypes haven't got an important effect on the proportion of twins, fertility rate, chest circumference, front height, ewe birth weight, and lamb weight at birth. The relationship of genotype GG and GA at the mutation site rs592447725 (G > A) showed a very significant effect (P≤0.01) on the percentage fertility rate and a noteworthy result (P≤0.05) on body length, height at the front and birth weight of lambs, but there isn't a big impact. In the percentage of twins, fertility rate, chest circumference, and birth weight of ewes. Therefore, the effect of the BMPR1B gene can be used as a genetic marker to select ewes with good genotypes to improve herd performance.

Keywards: Awassi sheep- BMPR1B gene - polymorphism -,PCR\Sequencing.

الارتباط بين تعدد أشكال جين BMPR1B وبعض الصفات التناسلية في أغنام العواسي

الخلاصة

يعتبر جين مستقبل بروتين العظام من النوع B (BMPR1B1) أحد الجينات الرئيسية لخصوبة النعاج. أجريت هذه الدراسة للتحقق من صحة الارتباط ما بين تعدد أشكال جين BMPR-IB والسمات التناسلية للنعاج. أجريت هذه الدراسة في محافظة الأنبار - (قسم البحوث الزراعية) الدوار. تم جمع 34 عينة دم من اربعة وثلاثين نعجة بعمر 4-6 سنوات. بعد ذلك، تم استخلاص الحمض النووي وتم إجراء تفاعل PCR لتضخيم عينات الدم المأخوذة من النعاج لتأكيد وجود الجين BMPR1B. تم تحديد التسلسل باستخدام تقنية تسلسل سانجر. ثم تم تحليل البيانات ومقارنتها بين تسلسلات العينات لتحديد SNPs وترددات النمط الجيني وترددات الأليل وارتباطها بسمات التكاثر والنمو. وأظهرت النتائج وجود طفرات في موقع تغاير rs410310346 (T > C) و rs592447725 (G > A). أشارت النتائج إلى أن النمط الجيني لـ TC في موقع الطفرة (T> C) rs410310346 كان متفوق معنوي (P≤0.05) في النعاج التي سجلت معدلاتها نسبة خصوبة وطول جسم أعلى مقارنة بالنمط الجيني لـ CC. ولم يكن للأنماط الجينية تأثير كبير على نسبة التوائم، ومعدل الخصب، ومحيط الصدر، والارتفاع عند المقدمة، ووزن النعجة عند الولادة، ووزن الحملان عند الولادة. أظهرت علاقة النمط الجيني GG و GA في موقع الطفرة (G > A) rs592447725 تأثيرًا عالي المعنوية (P≤0.01) في النسبة المئوية لمعدل الخصوبة وتأثيرًا معنوي (P≤0.05) في طول الجسم والارتفاع عند المقدمة و وزن الحملان عند الولادة، ولكن لم يكن له تأثير كبير. في النسبة المئوية للتوائم، ومعدل الخصب، ومحيط الصدر، ووزن النعاج عند الولادة. بالتالي يمكن استخدام تأثير جين BMPR1B كعلامة وراثية لاختيار النعاج ذات الأنماط الجينية الجيدة لتحسين القطيع.

Introduction

Al- Awassi breed are considered one of the main breeds in Iraq. Their reproductive performance includes a number of economically great quantitative markers, such as fertility, twin ratio, and birth rate. Reproductive efficiency is one of the basic pillars of their productivity and breeding, which increasingly depends on the fertility of ewes (1;2). One of the most significant issues that determine the success of sheep breeding is Fertility rate , as it is the main factor determining what is produced by the herd, as well as the production of meat results (3). The BMPR1B gene is one of the genes that is responsible for fertility and reproduction which plays an important role for ovarian granulosa cells (4;5;6;7) It is one of the genes associated with an increase in the rate of ovulation and birth production as a result of a mutation resulting from this gene known as FecB (Booroola) (8;9). Generative traits in livestock species shows, in genetic studies, a single gene of great influence (known as fertility genes) (10;11). These major gene were found to participate in determining sheep fertility behavior (12). The BMPR1B gene which is the first key gene involved in reproductive control in this type of animals (13). The (BMPR1B) gene has been identified as a major gene that affects, the number of births and Fertility rate in sheep (14). Identifying SNPs for the BMPR1B gene is of great importance for improving the reproductive performance of sheep, because the BMPR1B gene enhances follicle growth and proliferation of granulosa cells in the ovary, and thus affects ovulation in mammals (15;16).This gene belongs to the superfamily of "TGF-β (Transforming Growth Factor -Beta)" (17), and it has a main part in the processes of embryonic growth, rate of ovulation, and birth preparation. The BMPR1B gene is known as (ALK6) and is located on the sixth chromosome (18). Its mutation is symbolized by FecB (Booroola). It has been found in several breeds of sheep. This mutation is considered one of the most important mutations that causes an increasing rate in fertility in sheep by enhancing the percentage of ovulation and accelerating the egg maturation process (19). It was identified for the first time in Booroola Merino ewes (20). Davis stated that one gene of the BMPR1B is increasing the ovulation amount by 1.5 and two copies that are homozygous is increasing by 3.0 in Booroola Merino ewes. Piper and Bindon (21,22) indicated that sheep Fertility rate may result in part through the effect of a only one main gene that influences ovulation rate. In 1989, the "Sheep and Goat Genetic Nomenclature Committee" named this Booroola Fertility rate gene as "FecB". This mutation as a point (A746G) lied in the region that carried a code of the BMPR1B gene (23). Previous scholarships have exposed these changes or mutations in Fertility rate genes are related to both the ovulation amount and the number of childbirths in sheep (24). Those mutations of this gene, as it was described in previous studies, as a reproductive trait specially in sheep populations. These mutations located in exon 12 of the BMPR1B gene caused the wild-type allele (+) to transform into a mutant allele (B), which had a noticeable link with the reproduction of ewes (25). However, a meanwhile research showed the prevalence of the above-mentioned mutation and its linkage with the number of births and reproductive traits in sheep populations (26). Applying traditional that is based on phenotypic data breeding methods, is a time-consuming procedure. Therefore, molecular genetics and choices are of great importance in genetic improvement of reproductive efficiency (10). This study aims to verify the genetic association of the BMPR1B gene with reproductive performance in sheep.

Materials and Methods

Animal care

Animals belonging to the ("Al-Dawar"- Agricultural Research Department) were raised in Anbar Governorate. The programs of (management, breeding, and health care) were used to manage the sheep herd by providing the needs of the sheep based on NRC (27). The preventive program for internal and external parasites was also implemented according to the preventive program of the Ministry of Agriculture/ Iraq: Field operations were also carried out, including the cutting of cloves, horns, and shearing the wool.

Animals samples

The study was conducted on 34 samples of the ewes, with ages ranging between 3-6 years and having previous births. The birth and growth data records was taken of the ewes in the (Agricultural Research Department), Anbar Governorate, at the research station. 10 rams of the Awassi breed, at the age of 4-6 years, were used for breeding purposes for the sake of ewes.

DNA extraction

Blood samples were gathered from (34 ewes) at the Agricultural Research Station in Anbar - Al-Dawar and subsequently transported to the Molecular Genetics Laboratory in Baghdad, The study was conducted between January 22th, 2023, and April 25th, 2023. That DNA was extracted according to Promega kit instruction. The samples were stored at -20c until it used for the study. DNA was distinctively showed from all genomic samples and overloaded by gel electrophoresis in 1% with band patterns showed in the results in fig (1).

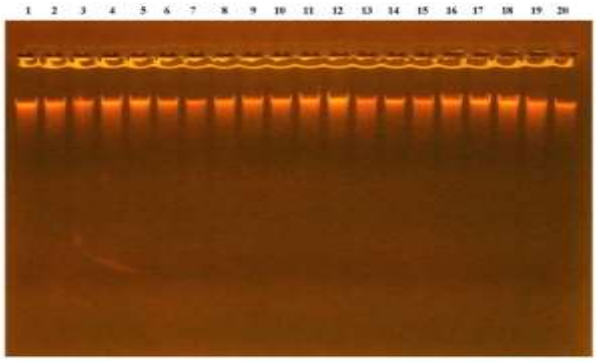

Fig (1): Electrophoresis of genomic the DNA extraction from blood, 1% agarose gel at (65 V) and (40mA)current for 1 hour.

Primer design

The table(1) explain primer designed by Macrogen Company in a lyophilized shape. Lyophilized primers were softened in a nuclease free water. This procedure was made to give a final concentration of 100pmol/µl as a stock solution. An effective solution of this solution was prepared by adding (10µl) of primer stock solution (that is saved at freezer under 20 C) to (90µl) of nuclease free water to get an effective (10pmol/µl)extracted primer solution.

PCR amplification of BMPR1B gene

PCR technology is used to augment genomic DNA. The size of the amplification fragment for the gene of BMPR1B in this study was (893 bp), and the PCR results were separated on an agarose gel by about 1.5-2% and the DNA ladder was from (100) to (1500) base pairs. All samples were positively improved. The Fig(2) shows a single group was obtained with ethidium bromide.

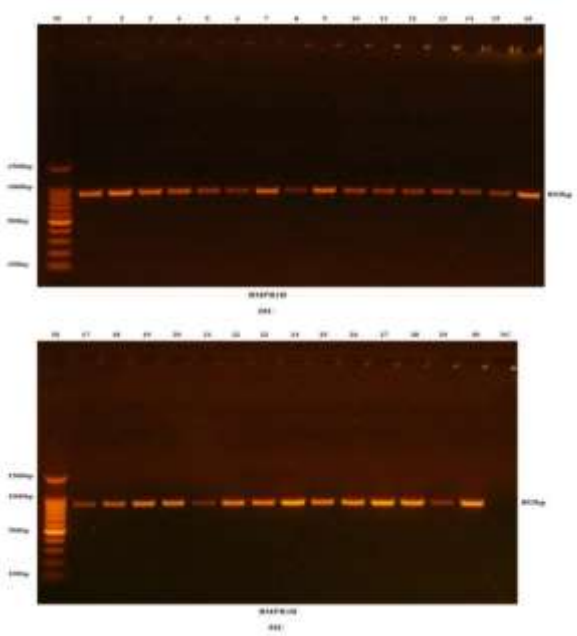

Fig (2): PCR product of the BMPR1B gene 893 bp in size. The product was electrophoresed on 1.5–2% agarose buffer at (65 V) and (40 mA) for 1 h. DNA ladder (100–1500 bp).

Statistical Analysis

Statistical Analysis System (SAS) (28), it is a way of analysis, was used to study the data and its effect on the BMPR-1B gene pleomorphism by using the General Linear Model (GLM) method. Duncan's various range test is used with the application of least square means are totally compared within to have these results by this equation.

Statistical model: (Traits on ewes) .

"Yijklm = μ + Gi + Aj + Sk + Tl + eijklm"

"Yijklm = The observed value , μ = The Overall mean of trait, Gi = The effect of gene polymorphism of dam , Aj = Adjusted to effect of Age of dam at birth , Sk = The effect of Sex , Tl = The effect of type of birth , eijklm= The random error".

Results and **Discussion**

The nitrogen base chain technique of the gene BMPR1B was adopted. The results on this study was a one face of the (BMPR1B) gene (893 bp) showed that the Single nucleotide polymorphism variant locus contains two variants rs410310346 (T>C) in the wanted region of the BMPR1B gene, the first variant appeared with three polymorphism (T>C SNP), namely TT, TC, CC, and the Single nucleotide polymorphism variant locus contains two variants rs410310346 (G>A) in the located area of the (BMPR1B) gene, with three polymorphism appearing in the second variant (G>A SNP), which is GG, GA, AA, as shown in Fig (3,4)

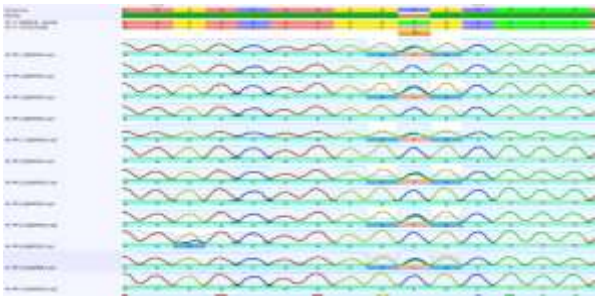

Fig (3): Variation position rs410310346 (T>C) in the BMPR1B gene of ewes.

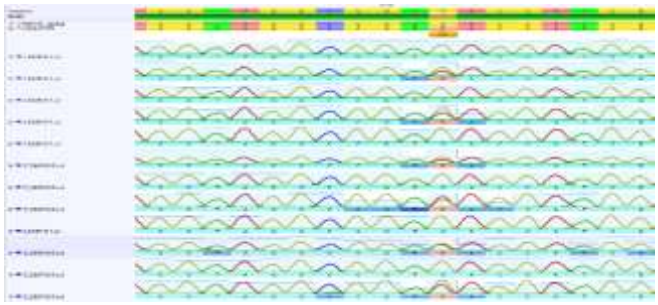

Fig (4): Variation position rs592447725 (G>A) in the BMPR1B gene of ewes.

It is clear from the results of the study according to sequencing technology that there are noticeable differences (P≤0.05) between the genotypes at SNP variation site rs410310346 (T>C), and highly significant differences (P≤0.01) between the genotypes at the SNP variation site rs592447725 (SNP)G>A). As Table (1) shows that at the SNP variation position rs410310346(T>C), ewes carrying the TC and CC genotypes were superior in fertility rates, and their rates were 81.82 and 69.56,

respectively. The numbers of sired ewes carrying the TC genotype were greater than Their counterparts carrying the CC genotype, and it is not clear from the results of this research that explained there is a superiority of ewes carrying the TC genotype and the CC gene BMPR-1B in the percentage of twins and fertility rate. As for the SNP variation position rs592447725 (G>A), it shows the superiority of ewes carrying the GG, GA genotype. Highly significant (P≤0.01) in the fertility rate, and their rates were 79.17 and 60.00, respectively, as the numbers of sired ewes carrying the GG genotype were greater than their counterparts carrying the GA genotype. It was not clear from the results of the study that there was a superiority of the ewes carrying the genotypes GG, GA in the percentage of twins and the fertility rate. These results were significantly consistent with what (15) indicated, as he showed in his study on Asian small-tailed Han Design of the(BMPR1B) gene primer.

sheep that the AG-GG genotypes had a significant effect (P≤0.05) on the number of births produced on the fertility of ewes. As for the characteristics of the twin rate and the fertility rate, the recorded differences were only mathematical, and these results (the twin rate and the fertility rate) came within the ranges indicated by (29;30;31;32) also indicated that they studied the sites of variation for BMPR1B gene mutations of the Chinese sheep breed, and their effect on ewes' fertility and offspring production. Therefore, they can be used as genetic markers in selecting high-producing animals.

Name of the Primer	Sequence 5`-3`	Annealing Temp.	(°C) roduct size (bp)
BMPR1B-F	GAGGATGTGGGACAAATGAA	55	893
BMPR1B-R	GCCACAGTCAGGAAGTAAAT		

Table(1): BMPR-1B /and rs410310346 (T> C) and rs592447725 (G> A) gene polymorphism in some reproductive traits of "Al-Awasi" sheep

SNP	Genotype	Number of ewes	Number of ewes borns	Number of births generated	Traits		
					Fertility rate(%)	Twins Ratio (%)	Fertile rate (born/ewes)
rs410310346 (T>C)	TC	11	9	9	81.82	0.00	1.00±0.00
	CC	23	16	17	69.56	6.25	1.06±0.03
	Level of significant				*	NS	NS
rs592447725 (G>A)	GG	24	19	20	79.17	5.26	1.05±0.03
	GA	10	6	6	60.00	0.00	1.00±0.00
	Level of significant				**	NS	NS
(P≤0.01),*(P≤0.05), NS: non of significan **							

The results of the study according to sequencing technology in studying the target coding area of the (BMPR1B) gene at the SNP variant locus rs410310346 (T>C) and the SNP variant locus rs592447725 (G>A) showed that there are significant differences (P≤0.05) between the genotypes at the SNP variant locus rs410310346. (T>C) and rs592447725 (G>A) with body dimensions in the number of maternity ewes, as Table (2) shows that in the position of variation rs410310346 (T>C) in the number of maternity ewes there is a significant effect (P≤0.05) on body length. The CC and TC genotypes showed a significant (P≤0.05) superiority in body length in the number of lambing ewes, and the overall average was 65.04±0.86 and 59.80±0.89, respectively. Body length in the CC and TC genotypes was significantly associated with the number of lambing ewes and was not It has a significant effect on chest circumference and body height in the front, which showed an overall average of 87.02±1.17 and 87.65±0.85, respectively, in chest circumference for ewes born with the TC and CC genotypes, while the genotypes GG and GA for the number of ewes born at the heterozygous position rs592447725 showed. (G>A) Significant level (P≤0.05) in body length and body height in the forelock. Chest circumference did not have a significant effect on the number of lambing ewes. The general average of body length and body height in the forelock, respectively, for the genotypes GG and GA was 59.66 ± 0.82 b and 63.92 ± 1.10 in body length and 78.66 ± 0.68 and 73.05 ± 0.84 in body height in the front, as the effect of the GA genotype on the number of sired ewes on body length was higher than the GG genotype, and the effect of the GG genotype on the number of sired ewes on body height in Introduction is higher than the GA genotype. This result came within the range indicated by (33), in which they explained that the average body length and body height from the front in ewes after weaning reached 45.70 and 70.0 cm, respectively. For body dimensions within a single breed, genetic influences are clear in phenotypic variation from the beginning of life and then begin to stabilize until they stabilize

after the second year of life (34). Yousif (35) also explained that in measurements of body dimensions there are There are large genetic differences between individuals of the ewes sheep breed, thus making it possible to carry out the selection process. He also indicated that there are many genetic factors that affect body dimensions in addition to the influence of fixed environmental factors. Therefore, it is preferable to follow the mixed model as an estimate of the best unbiased linear prediction. (BLUP), which is considered an effective way to increase selection efficiency.

The results of a study of the targeted coding region of the BMPR1B gene at the SNP variant locus rs410310346 (T>C) and the SNP variant locus rs592447725 (G>A) appeared with the relationship of the genotypes of the BMPR1B gene according to sequencing technology, as Table (3) shows at the SNP variant locus rs410310346. (T>C) There was no significant superiority (P≤0.05) for ewe birth weight (kg) and lamb birth weight (kg), for parent ewes carrying the TC and CC genotypes, which showed an overall average of 47.01±1.32 and 49.26±1.02 in ewe birth weight (kg) and 3.57 ± 0.11 and 3.66 ± 0.09 in lamb birth weight (kg), and at the SNP variant rs592447725(G>A) there was no significant superiority (P≤0.05) in ewe birth weight (kg) which appeared with an overall average of 48.87±1.09 and 48.20±0.96, respectively. A significant superiority (P≤0.05) was found for the ewes born to the genotypes GG and GA in the birth weight of the lambs (kg), which appeared with an overall average of 3.79±0.08 and 3.41. ±0.11, respectively, as parent ewes carrying the GG genotype outperformed their counterparts carrying the GA genotype. The reason for the discrepancy between the averages in ewe birth weight can be attributed to the difference in genetic makeup between flocks and from one country to another and in management conditions and Education, in addition to the difference in the size of the samples studied. These results were reliable with what was assumed by (36) that there was a significant variation in growth characteristics, including body-weights at birth in lambs, and this variation resulted from the distance showed in genetic factors of birthgivers and newborns.

Table (2): Correlation of BMPR-1B/rs410310346 (T>C) and rs592447725 (G>A) gene polymorphism to body dimensions in ewes.

SNP	Genotype	Number of ewes	Mean ± SE		
			Body length	Chest girth	Height at the front
rs410310346 (T>C)	TC	11	59.80±0.89b	87.02±1.17	75.40±0.92
	CC	23	65.04±0.86a	87.65±0.85	75.61±0.67
	Level of significant		*	NS	NS
rs592447725 (G>A)	GG	24	59.66±0.82b	87.58±0.81	78.66±0.68a
	GA	10	63.92±1.10a	87.10±1.19	73.05±0.84b
	Level of significant		*	NS	*
(P≤0.05), NS: non of significan *					

Table (3): Correlation of the genotypes BMPR-1B/rs410310346 (T>C) and rs592447725 (G>A) genes in the birth weight of the ewes and the newly birth weight of the ewes baby lambs.

SNP	Genotype	Number of ewes	Mean ± SE	
			Ewes weight at birth (kg)	Birth weight of lambs (kg)
rs410310346 (T>C)	TC	11	47.01±1.32	3.57±0.11
	CC	23	49.26±1.02	3.66±0.09
	Level of significant		NS	NS
rs592447725 (G>A)	GG	24	48.87±1.09	3.79± 0.08a
	GA	10	48.20±0.96	3.41± 0.11b
	Level of significant		NS	*
*(P≤0.05), NS: non of significan				

Conclusions

Point mutations (T→C) and (G→A) were detected at position 893 of the BMPR1B gene, linked to Exon 12, which had a significant impact on the fertility and reproduction of Iraqi ewes. Thus, the effect of the BMPR1B gene was reflected positively on reproductive characteristics. Body dimensions and birth weights of ewes. These mutations can be used as a molecular marker to select ewes with a good genetic makeup and in the early stages of sheep growth to improve the flock by conducting selection for ewes.

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Conflict of interest

The authors declare didn't have a conflict of interest.

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