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Fecundity Booroola Gene (FecB) Polymorphism in some Indigenous Sheep Breeds in Iraq

Awat N. Yousif[1], Shanaz M. Abdullah[2], Ahmed S. Shaker[2*], Questan A. Ameen[1], Mohammed S. Mohammed[2] and Mohammed R. Abdalla[1]

[1] *Department of Animal Science, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan Region – F.R. Iraq.*

[2] *Animal production department, Directorate of Agricultural Research-Sulaymaniyah, Kurdistan Region – F.R. Iraq.*

Abstract

ALTHOUGH the human population of Iraq is increasing with increasing demands on meat and milk, the Iraqi sheep population is decreasing year by year. Local sheep breeds exhibit a low twin rate and fecundity, resulting in a low heritability value. Previous studies showed that FecB is one of the candidate genes related to fecundity in sheep, in this gene cause an increase in ovulation rate and litter size. The aim of this study was to identify a single nucleotide polymorphism (SNP) in the FecB gene in the Karadi and Awassi sheep breeds. 50 blood samples were collected from both breeds to extract the genomic DNA. A 140-bp DNA fragment of FecB gene was successfully amplified, and the PCR product was sequenced and compared. The DNA sequencing of the FecB gene in Karadi and Awassi sheep compared with NCBI blast. The results revealed no single nucleotide polymorphism (SNP) was found in Karadi and Awassi sheep breeds, this can explain the low level of litter size and low fecundity of these breeds. More studies on other genes related to fecundity are needed to get more potential genetic background about these indigenous breeds. In conclusion, the absence of polymorphism in the 140-bp and pattern could be the reason for low litter size and ovulation rate in these local breeds, since the absence polymorphism is significantly correlated with low litter size and ovulation rate. Further studies should be made to show the required crossbreeding between our local breeds with foreign breeds that carry the favorite genotypes of this gene which will lead to an increase in the expression of this gene in the local Iraqi sheep breeds without effect on the acclimatization traits of these breeds to the environmental conditions in Iraq.

Keywords: Awassi, FecB, Karadi, sequencing, sheep, SNP.

Introduction

Sheep are considered the most important ruminants in Iraq because of their economic production importance such as meat, milk and wool, the annual incomes of these products are about 60%, 25% and 15% respectively [1]. There are 3 main sheep breeds in Iraq and sub breeds follow them, they are considered not pure because of random crossing with other breeds, but they differ among themselves in the phenotypic and productive characteristics. The breeds are: Awassi (Naami and Shefali), Arabi, and Karadi sheep (Kurdi, Hamdani, Jaff and Dzaie), (58.2%, 21.8%, and 20% respectively). These are all fat-tailed, carpet wool production [2]. Although the human population of Iraq is increasing with increasing demands on meat and milk, the Iraqi sheep population is decreasing year by year, this decrease was recorded (-30%) from 2006 till 2009 (from 18.615 to 13.025 million) according to a report by [3]**,** this decreasing is in reaction to the successive droughts and their impact on feed wheat and barley production, also increasing the taxes an importing animals by the government are another reason for diminishing the number of animals. Therefore, any crucial and fast conservation program for the development of our local sheep breeds would be very significant. The plan should include improving the reproductive efficiency by increasing the ovulation rate and consequently litter size (The number of offspring obtained per lambing), because reproductive traits have low heritability value, the traditional selection method proved less effective for

*Corresponding authors: Ahmed S. Shaker, E-mail: dr.ahmedshaker79@gmail.com, Tel.: +9647701334900
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genetic improvement. Therefore, marker assisted selection can be used as an alternative choice. Overall genetic progress can be improved for the desired trait at a considerably higher rate [4]. High litter size or twinning is an economically important trait that enhances sheep productivity in terms of producing a higher number of lambs, meat and wool [5]. There have been many single nucleotide polymorphisms (SNPs) reported to be associated with economically important traits in livestock species. As a result, mutation, or any nucleotide substitution, is critical for improving such traits.

The booroola merino was the first breed of sheep whose litter size and ovulation rate were shown to be affected by some specific genes [6]. Fecundity gene FecB (also known as Bone morphogenetic protein receptor type 1B; BMPR1B), is a dominant autosomal gene located on chromosome 6, was the first identified gene that affecting prolificacy in sheep, which has a significant effect on ovulation rate [7]. When one copy of the gene is present, the ovulation rate increases by approximately 1.5, and when two copies are present, the litter size increases by approximately 1 and 1.5, respectively. Booroola or FecB gene plays an important role in controlling the follicular growth and development [8]; [9]. Mutation in FecB gene (A746G, p.Q249R) in sheep causes increasing the ovulation rates and litter size [10], also in some Chinese local breeds [11]; [12]. Many studies have been done to check the mechanism of action from this mutation effect, but in Iraqi local sheep breeds very little knowledge is known about fecundity gene and its mutation. We developed this project with the aim of examining the FecB gene polymorphism in Karadi and Awassi sheep breeds through sequencing methods. Our goal is to identify various genetic variants that, through the use of molecular markers, could potentially aid in advancing breeding efforts for the conservation and enhancement of Iraqi sheep breeds.

Material and Methods

Animals and sample collection

One hundred adult sheep from different breeds (Karadi and Awassi) and different locations around Sulaimani governorate (Jeshana, and Kirkuk) respectively, were used. Fifty animals from each breed from both sexes. Blood samples of 5 ml were taken from the jugular vein into EDTA containing tubes as anticoagulant. Put into a cooler box and immediately transferred to the animal biotechnology lab., at the animal science dept. college of agricultural engineering sciences at the university of Sulaimani. The collected samples from each breed were mixed to be one pooled sample per breed.

DNA extraction

Genomic DNA was extracted from whole blood samples by using DNA extraction kit. (Sinaclon, SinaPure TM DNA) based on the manufacturer`s instruction and stored at -20°C till used in the assay.

The quality and quantity of the extracted DNA were checked by Nano-spectrophotometer. All the DNA samples had the 260/280 OD ratios in the range of 1.8 to 2, indicating high purity. The concentration of each sample was adjusted to 50 ng/uL before PCR amplification and was written on each tube, later pooled samples were made from each breed to represent that breed.

Primers and PCR amplification

Fecundity Booroola (FecB) gene is located on chromosome 6 in sheep, it contains 10 exons and coded 502 amino acids with a coding sequence of 1509 bp. A pair of specific primers was designed to amplify a 140 bp fragment based on the A746G mutation of sheep BMPR-IB gene. The primer sequences were shown in (Table 1).

The polymerase chain reactions (PCRs) were done in a 25 ul reaction solution which contained: 2 µl of template DNA (50 ng), 2 µl of 10 µM primer, 400 µM of each dATP, dGTP, dCTP and dTTP, 8.5 µl of ddH2O, 2X (50 units/ml Taq DNA polymerase, and 12.5 µl PCR Master Mix. The PCR thermocycler (Labocon, U.K.) used in the amplification program started with initial denaturation for 5 min at 94 °C, followed by 35 cycles of denaturation for 1 min. at 94 °C, annealing for 1 min. at 60 °C, extension for 2 min at 72 °C, a final extension for 7 min at 72 °C. The amplification results were kept at 4°C, later run on 5% agarose gels using 1×TBE (Tris- Borate-EDTA) running buffer at 5 V/cm for 30 min. We then visualized the results by staining them with ethidium bromide. The PCR products were sent to sequencing for single nucleotide polymorphism in these two breeds. All the laboratory work was done in the biotechnology laboratory at the Department of Animal Science, College of Agricultural Engineering Sciences, University of Sulaimani, Iraq.

Data analysis

The PCR product, a 140 bp fragment, was sequenced by (sanger sequencing/ ABI 3500, Macrogen Genome Center, Republic of Korea). Sequence analysis of the FecB gene from Karadi and Awassi breeds and alignments were carried out using NCBI BLAST: Nucleotide sequence.

Results and Discussion

The aim of the current project was to study and find if there is any single nucleotide polymorphism (SNP) in FecB gene in two indigenous Iraqi sheep breeds (Karadi and Awassi). These two breeds have shown a low reproductive efficiency [13].

Heritability estimates of these traits are rather low, and reflect small genetic variation in these traits. The primary determinant of sheep meat production is the number of lambs born per birth. However, most sheep breeds typically give birth to slightly more than one lamb per birth [14]. Reproduction is a multifaceted process, and fertility traits are genetically regulated [15]. Selecting for desired traits using traditional methods can be challenging, particularly when the traits have low heritability values. For instance, fertility characteristics such as litter size and ovulation rate often have heritability values ranging from 0.06 to 0.13 [16]. Several studies have shown that the members of transforming growth factor beta (TGF-β) superfamily are the main regulators of ovulation rate and litter size in sheep, which they are bone morphogenetic protein receptor type 1B (BMPR 1 B) known as (FecB) on chromosome 6, bone morphogenetic protein 15 (BMP 15) known as (FecG) on chromosome 5 and growth differentiation factor 9 (GDF 9) known as (FecX) on chromosome X [17].

In the current study, the FecB gene was amplified in all samples. The electrophoresis of the PCR products showed the same bands of 140 bp for FecB gene in Karadi and Awassi breeds (Fig. 1). Table 2 shows the similar characters of the two bands by both breeds, which they show the same length (140 bp), molecular weight (42158, 42134 Daltons), molecular weight of double strand (85091, 85021 Daltons), GC content (47.86, 48.57 %) and AT content (52.14, 51.43 %) for Karadi and Awassi breeds respectively, this result insured the monomorphism of the FecB gene in the breeds under study.

Table 3 shows the genetic distance between the two Iraqi sheep breeds (Karadi and Awassi) and the NCBI database. However, our previous study showed that Karadi sheep is most genetically distant from Awassi sheep (0.1168) [18] but in the current study at FecB gene locus no genetic distance (0.000) was found between (Karadi and Awassi) sheep. While, the genetic distance found between (Karadi and Awassi) breeds with NCBI was 1.865, and 1.879 respectively. There is no genetic distance, which means that the genetic variation observed between those two breeds, as measured by SNPs, is very low or negligible. In other words, the genetic makeup of the individuals from those two breeds, as assessed by the SNPs examined, is highly similar or indistinguishable.

The Ovis aries sheep genome contains the FecB gene locus on chromosome 6q23-31 [7]. Structurally, it comprises 15 exons. FecB plays a significant role in signal transduction for various factors and is primarily located in the ovary of sheep, although it is also present in other tissues, contributing to follicular development [19]. Specifically, the FecB mutation, resulting in an arginine to glutamine transition (746A→G), occurs in exon 7 and is expressed in oocytes and granulosa cells [20]; [19]. This mutation is primarily responsible for the increased litter size observed in Merino sheep [21].

The Karadi and Awassi breeds lack any previous molecular data obtained through DNA sequencing. The results of the current study are the first attempt molecular identification of these Iraqi sheep breeds by using DNA sequencing. The FecB gene was amplified from Karadi and Awassi ewes using the PCR technique. Analysis, depicted in Figure 1, revealed a single band representing the PXR product, measuring 140 base pairs. The results exhibited high accuracy under optimal conditions, as demonstrated by agarose gel electrophoresis. A quantitative trait loci region was identified on chromosome six of sheep, which serves as a strong candidate locus for increasing ovulation rate and litter size in sheep breeds. DNA sequencing of the FecB gene in Karadi and Awassi sheep, compared with NCBI blast results, showed a match between the FecB locus of Karadi and Awassi ewes and that of other sheep breeds worldwide, exhibiting similar litter size FecB loci (Fig. 2). Both sheep breeds displayed a 100% match with the NCBI database for FecB gene DNA base pairs. This result indicated that these Iraqi sheep breeds have no mutated FecB locus which has no significant effect on reproductive traits for these indigenous Iraqi sheep breeds. This result is in agreement with [22] in Awassi sheep and [23] in Iraqi sheep breeds. Various studies have uncovered mutations in the FecB gene, such as the discovery of two new SNPs in exon 7 by the Iranian Mehraban breed, suggesting a potential impact on litter size at the FecB locus and affirming its role in regulating sheep reproduction [24]. In Mongolian sheep, litter size is predominantly influenced by FecB [25]. The effects of these mutations vary among different sheep breeds. For instance, in the Kendrapada breed, the mean prolificacy of non-carrier, heterozygous, and homozygous FecB mutation ewes was recorded as 1.61, 1.80, and 2.06, respectively [26]. A total of 41 polymorphisms have been identified in the FecB gene, with eight affecting litter size, notably the p.Q249R SNP mutation, which is prevalent in Asian countries and contributes significantly to sheep's exceptional productivity [27].

The current study results are also in agreement with Bulgarian sheep breeds, the mutation in FecB is absent [28], only in the Northeast Bulgarian Merino breed it was observed in heterozygous state in separate individuals [29].

Conclusion

According to the results obtained from the current study, we can conclude that there is no genetic variation observed at the SNP loci within the FecB gene across Karadi and Awassi breeds, it suggests that the gene is monomorphic. The lack of mutation observed in the 140 bp band pattern across all animals from the studied breeds We could attribute the absence of mutation in the 140 bp band pattern across all animals from the studied breeds to their low litter size. Future investigations should focus on determining the optimal crossbreeding ratio with foreign breeds carrying favorable genotypes of this gene. We expect this approach to enhance the gene expression in local Iraqi sheep breeds without compromising their adaptation to Iraqi environmental conditions.

Acknowledgment

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Competing interests

Authors have declared that no competing interests exist.

TABLE 1. Primer and PCR condition

Gene	Primer sequence (5`→3`)	Annealing temperature (°C)	Size (bp)
FecB	GTCGCTATGGGGAAGTTTGGATG	60	140
	CAAGATGTTTTCATGCCTCATCAACACGGTC		

TABLE 2. FecB gene bands charachterists obtained in Karadi and awassi sheep breeds

Traits	Karadi	Awassi
Length (bp)	140	140
Molecular weight single stand (Daltons)	42158.00	42134.00
Molecular weight Double stand (Daltons)	85091.00	85021.00
G+C content (%)	47.86	48.57
A+T	52.14	51.43

TABLE 3. The genetic distance among Karadi and Awassi Iraqi sheep breeds and the NCBI database

	NCBI	Karadi	Awassi
NCBI	0.000		
Karadi	1.865	0.000	
Awassi	1.879	0.000	0.000

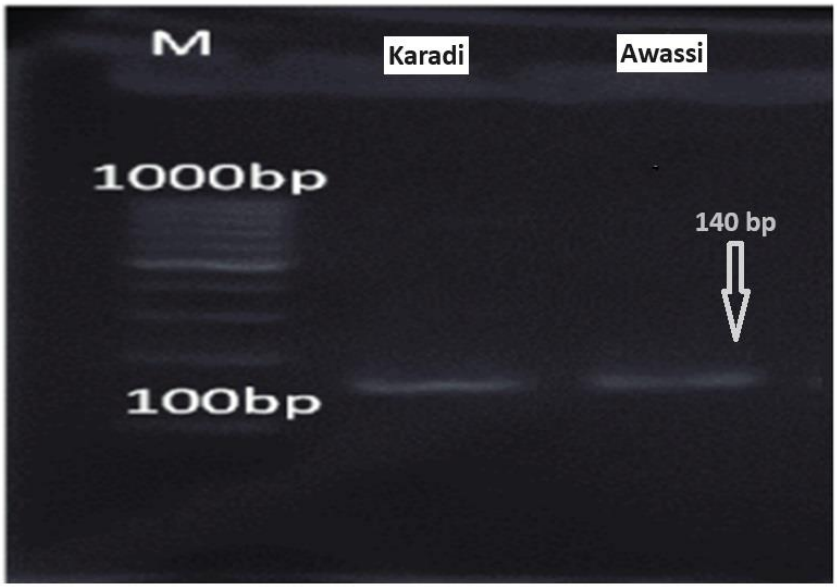

Fig. 1. Gel electrophoresis of 140 bp bands of Fec B gene, Lane 1 is Karadi sheep, Lane 2 is Awassi sheep.

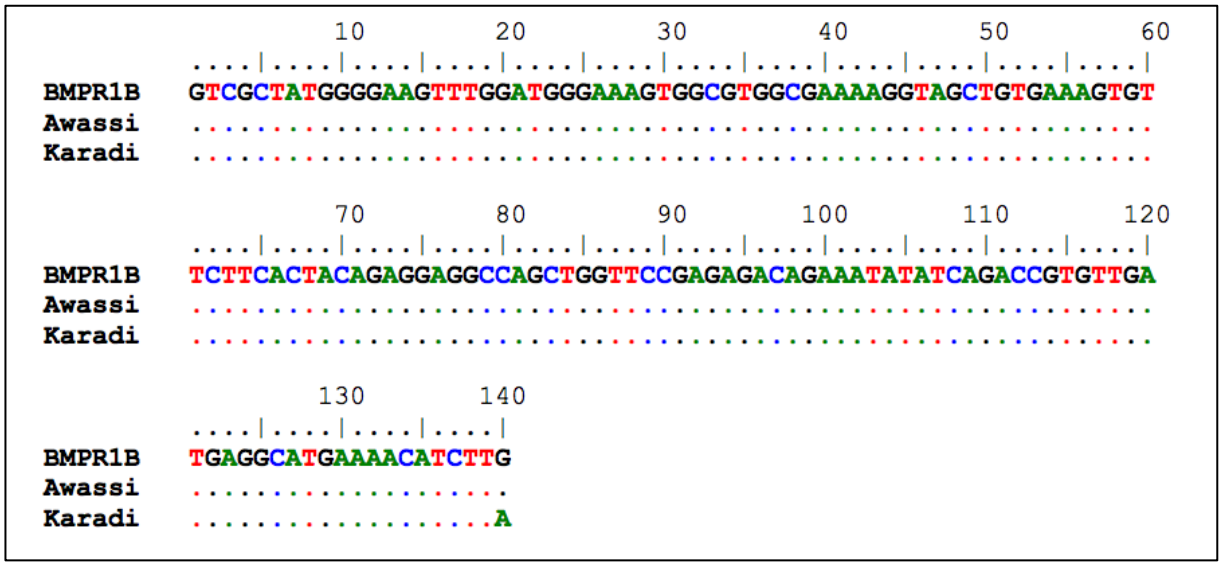

Fig. 2. The sequence of (FecB) gene in Karadi and Awassi sheep breeds comparing with the sequence of NCBI (KX896751.1).

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الخصوبة تعدد أشكال جين البورولا (FecB) في بعض سلالات الأغنام المحلية في العراق

آوات نورالدين يوسف [1]، شاناز محمد عبد الله [2]، أحمد سامي شاكر [2]، كويستان علي أمين [1]، محمد سردار محمد [2] ومحمد رسول عبد الله [1]

[1] قسم علم الحيوان، كلية علوم الهندسة الزراعية، جامعة السليمانية، السليمانية، إقليم كوردستان – العراق.

[2] قسم الانتاج الحيواني، مديرية البحوث الزراعية، السليمانية، اقليم كوردستان – العراق.

الملخص

على الرغم من أن عدد سكان العراق يتزايد مع زيادة الطلب على اللحوم والحليب، إلا أن أعداد الأغنام العراقية تتناقص سنة بعد سنة. أظهرت سلالات الأغنام المحلية مستوى منخفضاً من معدل التوائم والخصوبة مع انخفاض قيمة التوريث. أظهرت الدراسات السابقة أن FecB هو أحد الجينات المرشحة المرتبطة بالخصوبة في الأغنام، وتتسبب الطفرة في هذا الجين في زيادة معدل الإباضة وحجم المواليد. هدفت هذه الدراسة إلى التعرف على تعدد أشكال النيوكليوتيدات المفردة (SNP) في جين FecB في سلالتي الأغنام الكرادي والعواسي. تم جمع 50 عينة دم من كلا السلالتين لاستخلاص الحمض النووي منقوص الاوكسجين، وتم تضخيم جزء من الحمض النووي 140 نقطة أساس لجين FecB بنجاح، أظهرت النتائج عدم وجود تعدد أشكال النيوكليوتيدات (SNP) في سلالات الأغنام الكرادي والعواسي، وهذا يمكن أن يفسر انخفاض مستوى حجم المواليد وانخفاض الخصوبة لهذه السلالات. هناك حاجة إلى مزيد من الدراسات حول الجينات الأخرى المرتبطة بالخصوبة للحصول على المزيد من الخلفية الجينية المحتملة حول هذه السلالات الأصلية. نستنتج من ذلك أن غياب تعدد الأشكال في نمط النطاق 140 زوج أساس يمكن أن يكون السبب في انخفاض حجم المواليد ومعدل الإباضة في هذه السلالات المحلية، حيث أن غياب تعدد الأشكال يرتبط بشكل كبير بانخفاض حجم المواليد ومعدل الإباضة. يجب إجراء دراسات إضافية لبيان التهجين المطلوب بين سلالاتنا المحلية مع سلالات أجنبية تحمل التراكيب الوراثية المفضلة لهذا الجين مما سيؤدي إلى زيادة تعبير هذا الجين في سلالات الأغنام العراقية المحلية دون التأثير على صفات التأقلم لهذه السلالات للظروف البيئية في العراق.

الكلمات الدالة: العواسي، الاغنام، FecB, Karadi, sequencing