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Identification of Fecx^{H} and Fecx^{G} Mutations in Karadi Sheep Breed Using RFLP-PCR Technique in Sulaimani Province

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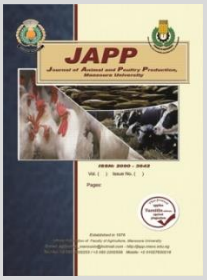

ABSTRACT

This investigation was led to recognize FecX^{H} and FecX^{G} changes in Karadi sheep. Right now Karadi sheep (4 single and 11 twining) were researched for the nearness of the FecX^{H} and FecX^{G} transformation utilizing PCR-RFLP. PCR items 240 and 141 bp was processed utilizing SpeI and HinfI limitation compound to identify FecX^{H} and FecX^{G} point Mutations, separately. The FecX^{H} and FecX^{G} DNA test demonstrated that there were no bearers for the FecX^{H} and FecX^{G} transformations in the chose Karadi sheep. These outcomes recommend that fruitfulness of this breed isn't connected to similar changes. In this way, it is important to look for different transformations or fertility qualities so as to create marker help choice methods and study the productive system of the Karadi breed.

Keywords: Karadi sheep breed, RFLP-PCR and FecX^{H} and FecX^{G} Mutations.

INTRODUCTION

The original farm animal breeds are valuable genetic reserves for adaptive and economic features, which provide a diverse genetic reserve, which can help meet future challenges. The first evidence of domestication of sheep appears to be in the regions of Iraq, Iran, and the Taurus Mountains in Turkey. The number of sheep in Iraq in 1999 was about six million. Most of this population (99.8%) is privately owned and distributed throughout the country (Al-Barzinji1 and Othman, 2013). Litter size is significant monetary incentive in sheep rearing. Profitable attributes in household domesticated animals are commonly acquired in a multigenic or quantitative way (Davis et al., 1991). The most punctual proof about sheep training is by all accounts found in the regions of Iraq, Iran and the Taurus piles of Turkey (Asadpour et al., 2012). The sheep populace in Iraq was around 7,722,372 head (Al-Alaq et al., 2011). The majority of this populace (99.8%) is claimed by the private part and is conveyed everywhere throughout the nation. The local Iraqi breeds incorporate the Karadi (Kurdi, Hamdani, Jaff and Dzaie) 20%, Awassi (Naami and Shefali) 58.2% and Arabi sheep 21.8%. These are for the most part fat-followed, cover fleece creation with some possibility to deliver milk. Despite the fact that these breeds are described by moderate development, low fruitfulness and low milk creation, their capacity to endure and duplicate under state of dry season and extraordinary atmosphere variances is noteworthy (Al-Rawi et al., 1996). The central quality shows up as a result of changes in sheep. Basic changes in the ovulation rate of extension in sheep have been found in the characteristics of the bone morphogenetic protein receptor IB (BMPR-IB), the shedding factor of the improvement factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15). The quality of Booroola (FecX^{B}) is the principle noteworthy augmentation quality recognized in sheep (Davis, 2005). The BMP-15 quality is mapped to the X sheep chromosome and contains six particular changes. These changes were perceived as "FecX^{I} (Inverdale), FecXH (Hanna), FecX^{G} (Galway) and FecX^{B} (Belclare), FecX^{R} (Taste of Aragonesa) and FecX^{L} (Lacaune)" (Galloway et al., 2000; Hanrahan et al., 2004; Monteagudo; Shows et al., 2009). These changes show a comparable phenotype; Heterozygous animals have a higher ovulation rate (+1-1.5) than their current wild species. Homozygous bearers are fruitless as a result of the progression of follicles which stop at a starting time of improvement (Galloway et al., 2002; McNatty et al., 2005). The point of the present investigation was to ID hereditary polymorphism of FecX^{H} and FecX^{G} quality change in Karadi sheep by RFLP – PCR method.

MATERIALS AND METHODS

Analysis based on PCR restriction fragment length polymorphism (RFLP), also known as excised amplified polymorphic sequence (CAPS), is a popular technique for genetic analysis. It has been applied for the detection of intraspecies, as well as the variation between species. There are several techniques that are related to PCR-RFLP and also involve gel electrophoresis, including techniques for DNA fingerprinting and expression profile.

Sampling and DNA extraction

A total of 15 (4 singlet and 11 twining) Karadi sheep chose from two diverse area in Sulaimani governorate. Blood test from every ewe was gathered from jugular vein into 10 mL vacationer tubes containing the anticoagulant, Ethylenediaminetetra-Acetic Acid (EDTA). Genomic DNA was extricated from every one of the blood tests utilizing AccuPrep® Genomic DNA Extraction Kit (Bioneer Corporation Cat. No.: K-3032 Korea). The nature of DNA was resolved utilizing 1% agarose gel electrophoresis.

PCR condition

The fundamental gathering and fortifying temperature are given in Table 1. DNA was increased by

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initial denaturation at 940°C for 5 minutes, trailed by 35 cycles including denaturation at 940°C for 30 seconds and reinforcing the temperature for each fundamental pair is showed up in Table (1) for 40 seconds, oppose 720° C for 30 seconds, with a last expansion of 720°C for 5 minutes. All examination place work is finished in the biotechnology lab at the Department of Animal Sciences, Faculty of Agricultural Sciences at Sulaimani University.

FecX^{H} change area

240 base pair segments (bp) were procured by PCR. 240 bp PCR object was prepared with impediment impetus SpeI (A/CTAGT) for 3 hours at 37°C (10 U SpeI, 2µl compound help, and 10µl PCR object) and the object was secluded by electrophoresis in 2% agarose gel and bromide envisioned with ethidium to recognize piece sizes.

FecX^{G} change recognizable proof

141 bp parts were gotten using FG and RG starters by the PCR method. 141 bp PCR object was prepared with the HinfI impediment impetus (G/ACT) for 3 hours at 37°C (10 U HinfI, 2 µl protein backing and 10µl PCR object) and the thing was detached by electrophoresis in 2% agarose gel and with ethidium envisioned bromide to perceive part evaluates.

Table 1. Primer sequencing and annealing temperature.

Mutation	Primer sequence (5…….3)	Annealing temp.ºC	Reference
FecX^{H}	F: "TATTTCAATGACACTCAGAG" R: "GAGCAATGATCCAAGTGATCCCA"	55	Hua *et al.*, 2008
FecX^{G}	F: "CACTGTCTTCTTCTTCTTACTGTATTTCAATGAGAC" R: "GATGCAATACTGCCTGCTTG"	63	Hanrahan *et al.*, 2004

RESULTS AND DISCUSSION

In the present study the FecX^{H} and FecX^{G} mutation were investigated in Karadi sheep. Fig.1and 2 showed the agarose gel electrophoresis of PCR products for FecX^{H} and FecX^{G} (240 and 141pb) gene, respectively. When PCR products of animals under study where digested SpeI and HinfI restriction enzyme, digestions resulted in 240 and 141bp band in all the animals studied revealing the absence of these restriction sites for both restriction enzymes in those animals. Hua *et al.* (2008) uncovered that FecX^{H} change produce two sections (218 bp and 22 bp), while non-transporter items stayed whole at 240 bp after absorption with SpeI. Yet, the wild sort of FecX^{G} digest by HinfI (G/ACT) with a 112 bp and 29 bp parts, and changed kind remained 112 bp (Hanrahan et al., 2004). Current results of FecX^{H} are in agreement with previous studies by (Borni et al., 2011) that detected non mutation using RFLP-PCR technique in Barbarine sheep. Gürsel et al. (2011) also observed non point mutation of FecX^{H} in indigenous sheep breeds in Turkey. In Lleyn sheep using PCR-RFLP led to the identification of 12 heterozygous carriers of *Fec*X^{G} and a single heterozygous carrier of *Fec*G^{H} (Mullen et al., 2013). However, our results are in agreement with previous results by other researchers such as (Moradband *et al.*, 2011, Karsli *et al.*, 2012 and Mullen *et al.*, 2013). These result contrasts with Han sheep for FecX^{G} mutation (Chu *et al.*, 2007), (Awassi, Chios, Imrose and Kivircik) for FecX^{G} mutation (Gürsel *et al.*, 2011), Han sheep (Zhang *et al.*, 2011).

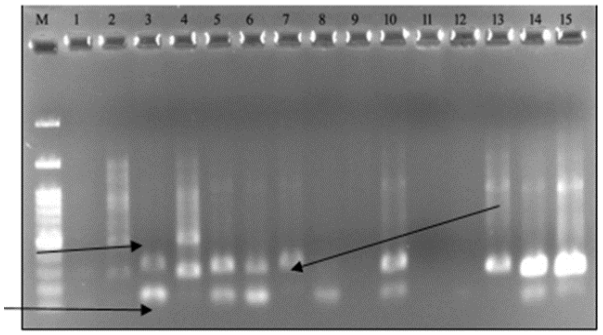

Figure 1. "Digestion of PCR product with SpeI restriction enzyme for FecX^{H} mutation. M: DNA Marker 100 bp, Lanes 1-11twin and lanes 11-15 single".

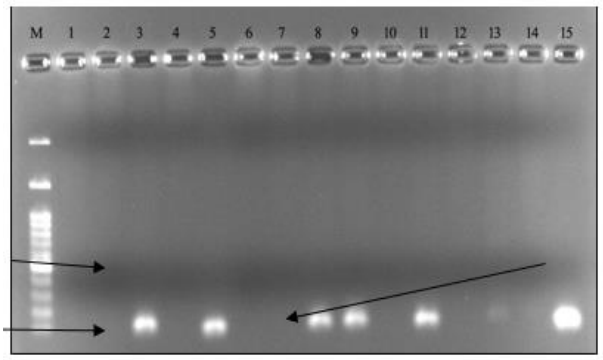

Figure 2. "Digestion of PCR product with HinfI restriction enzyme for FecX^{G} mutation. M: DNA Marker 100 bp, lanes1-11twin and lanes 11-15 single".

CONCLUSION

In conclusion, this study has highlighted the importance of further investigation for the gene(s) influencing reproductive sheep breeds in Iraq. The absence of FecX^{H} and FecX^{G} mutation in the studied animals indicates high possibility of the absence of the FecX^{H} and FecX^{G} gene mutation in Karadi sheep. Further studies regarding other genes which may influence fecundity of the Iraqi sheep should be carried out to determine the type and mode of inheritance of such genes.

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تحديد الطفرات $FecX^{G}$ and $FecX^{H}$ في الأغنام الكرادية عن طريق تقنية RFLP-PCR في محافظة السليمانية

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أجريت هذه الدراسة لتحديد طفرات على $FecX^{H}$ و $FecX^{G}$ في الاغنام الكرادية. في هذه الدراسة تم التحقق من خمسة عشر اغنام كرادي (4 فردية و 11 توأم) للتاكد من حدوث طفرة $FecX^{H}$ و $FecX^{G}$ باستخدام PCR-RFLP. تم هضم منتجات 240 bp and 141 PCR باستخدام الانزيمات القاطعة SpeI and HinfI للكشف عن نقطة الطفرات في $FecX^{G}$ and $FecX^{H}$ على التوالي. أظهر اختبار الحمض النووي ل $FecX^{G}$ and $FecX^{H}$ أنه لا توجد ناقلات لطفرات $FecX^{G}$ and $FecX^{H}$ في الاغنام الكرادية المختارة. هذه النتائج تشير إلى أن خصوبة هذا الصنف لا يرتبط مع هذه الطفرات. لذلك ، من الضروري البحث عن طفرات أخرى أو جينات خصوبة من خلال تطوير تقنيات اختيار مساعدة ودراسة آلية إنتاج سلالة الكرادي.