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DISTRIBUTION OF PARTICULAR AWASSI GENETIC LINES TO ADDRESS JORDAN'S FOOD SAFETY CHALLENGE

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ABSTRACT

This study aimed to enhance food security in Jordan by improving meat quantity and quality in Awassi sheep through introducing two economical genomic mutations; FecB and CLPG. In this study, a group of 209 Awassi ewes were involved in the study where 10 heads were distributed to each collaborated farmer, then Half of the ewes were inseminated with mutations Carrier's rams, while the other half was inseminated with rams carrying the CLPG mutation, After the lambing season, a genomic DNA extraction and genotyping process (FRLP) for the newborns was conducted and revealed successful introgression of the mutations, resulting in lambs carrying either the FecB or CLPG mutations. Our results showed the successful introduction of the FecB and CLPG mutations in Awassi sheep, with a considerable number of lambs carrying the mutation, in Jordan the frequencies of the mutations were almost zero but now and after introducing the carrier rams to farmer's fields it has been increased to 0.41 (as heterozygous FecB genotype) and 0.50 for that CLPG heterozygous genotype (in the supported population). Ewes who were in the first parity their Prolificacy was elevated up to 2.5 lambs/ of those holds the FecB mutation. The new introduction of the mutation to farmer's fields will contribute directly to the livelihood of the farmers and enhancing food security in Jordan.

Keywords: Awassi, Callipyge gene, FecB gene, gene and genotype frequencies.

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INTRODUCTION

Sheep farming is a major part of Jordan livestock sector and plays a significant role in agrarian economy and indusial, due to its part in providing important products such as meat, milk, and their derivatives and leather, in addition to wool (Jawasreh, & Al-Amareen, 2023). The Awassi sheep is one of the major indigenous sheep breeds of Jordan, and their number is estimated at 3 million heads (FAO, 2012.). Although this breed is characterized by reduced growth and twining rates (about 1.1 lambs born per lambing) (Ajam, et al. 2019), it has a noticeable ability to keep up the extreme climate fluctuations (18). Fertility and growth are important economic traits in sheep breeding and reproduction. Recently, mutations affecting have fertility and growth rate been identified in sheep. The exploitation of these mutations in breeding strategies has the potential to significantly improve growth and fertility rates. Therefore, breeding

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objectives are needed to develop selection programs for this breed. Some studies have reported that the high prolificacy and growth in many sheep breeds around the world is the result of the found FecB and CLPG genes (DEAC, 2022, Liu, et al. 2014). Investment in livestock genetic enhancement initiatives is profit and sustained, according to knowledge in industrialized nations. Farmers continue to reap the benefits of genetic improvement year after year since it is a lasting phenomenon. Introduction of a major gene into the main breeding line by a process of backcrossing is called introgression (Smith, 1985). The well-known FecB or Booroola gene which increases prolificacy and litter size in sheep has been introgressed profitably into the Mérinos d'Arles breed in France, the Assaf breed in Palestine, the Malpura breed in Rajasthan (Teyssier, et al. 2009) and many other breeds in other countries. FecB gene is an autosomal dominant gene located on chromosome 6, responsible for increasing the ovulation rate and litter size in sheep (Yousit, et al. 2024).The B allele increases prolificacy while the + allele is the wild type. Guo et al.,(2018) and Reader et al.,(2012) found that the effect of FecB mutation is additive for ovulation rate and each copy increases ovulation rate by about 1.6 and approximately one to two extra lambs in Booroola Merinos. High fecundity in Booroola sheep is due to a non-conservative mutation in a highly conserved intracellular kinase signaling in bone morphogenetic protein receptor-1B (BMPR-1B) expressed in the ovary and granulosa cells (Gedik, 2023). The callipyge gene (CLPG), was first discovered in the 1983 by breeding of Dorset sheep in Oklahoma, is one of the well-studied genes affecting sheep muscle development and is located on ovine chromosome 18 (Cockett, 1996). The hypertrophy develops only in paternal heterozygous animals ($+^{M}/CLPG^{P}$, where M is the maternal and P is the paternal inheritance of the alleles). Animal that inherit the mutation from their maternal ($CLPG^{M}/CLPG^{P}$, or $CLPG^{M}/+^{P}$) show normal muscle development (Esen, et al. 2022). According to a number of studies (7 Esen, et al. 2022, Jawasreh, et al. 2019), CLPG lambs have superior feed efficiency, higher dressing percentages, and 42% more muscle mass compared with normal type lambs. Thus, the incorporating these genes into Awassi sheep, can made a significant increase in its productivity and the profitability The aims of this study arise to report on the impacts of the FecB and CLPG gene dissemination program implemented in local smallholder sheep flocks in Jordan, and using this analysis to contribute to the design of an extension strategy to facilitate the dissemination of the FecB and CLPG mutation beyond the current project area.

MATERIAL AND METHODS

The improved Rams with the FecB and Callipyge mutations were used in the study, which was carried out in Jordan. Twenty farmers received ten Awassi ewes each from the two hundred Awassi ewes that were part of the project. Following the estrus synchronization procedure, the ewes were either exposed to mating by the rams' semen or natural insemination in various locations. In an effort to preserve and spread the mutations to farmer flocks, either natural or artificial inseminations (using Frozen semen imported from USA; (Uta University) or fresh semen collected from the rams found at the university) were employed, depending on the farm conditions, with the goal of introducing the mutations. Pregnancy detection was carried out (using ultrasound) after insemination or mating, and the pregnant ewes received particular attention in terms of nutrition and all other aspects of health care. The ewes were isolated from the flock during lambing season. Following the recording of the lambs' and

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dams' records, blood samples were drawn from the lambs for the purposes of DNA and genotyping. Briefly, DNA samples were extracted from blood samples of 184 lambs of the FecB-Awassi collected from jugular vein into 5 mL vacationer tubes containing the anticoagulant, Ethylenediaminetetra-acetic acid EDTA), and stored under -20 °C until analysis. . DNA isolation was performed using a commercial DNA extraction kit. (OMGA-Bio-Tek, Inc., Madison., WI, USA) 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 ultraviolet light and 1.5% agarose gel electrophoresis. Fecundity Region amplification and Genotyping. The DNA amplification of the FecB and CLPG gene was achieved by polymerase chain reaction (PCR). Two primer pairs (F, 5' CCAGAGGACAATAGCAAAGCAAA-3'and R, 5'- CAAGATGTTTTCATGCCTCATCA ACACGGTC-3') that targeted a fragment of 190 bp as described by (5) were used for the identification of the FecB gene.

Callipyge region amplification

For the amplification of the CLPG gene was performed with primer pairs:

(F, 5'-TGAAAACGTGAACCCAGAAGC-3'; R, 5'-GTCCTAAATAGGTCCTCTCG-3') that targeted a fragment of 426 bp as described by (9). PCR mix (HOT FIREPol DNA Polymerase; Solis BioDyne, Estonia) was carried out in a total volume of 20 µLcontaining10µL of nuclease-free water, 2 µL of genomic DNA (100 ng/ µL) as a template, 2 µL of each primer, and 4 µL (5U/µL) of Taq DNA polymerase (Eppendorf AG, Hamburg, Germany). Primer sequences, annealing temperature, and restriction enzymes used for genotyping .The PCR reaction was carried out in the following conditions of 95 ◦C for 5 min for initial denaturation followed by 33 cycles at 95 ◦C for 30 s of denaturation, 40 s annealing and extension each at 72 ◦C, and a final extension step at 72 ◦C for 7 min. FecB and CLPG genes variants were identified by the PCR-RFLP method. The amplified FecB gene fragment (190 bp) was digested by *AvaII* restriction enzyme for 1 h at 37 °C. Restriction products were separated in a 2% agarose gel with ethidium bromide and visualized under ultraviolet (UV) light. The amplified CLPG gene fragment (426 bp) was restricted with *BsmFI* endonuclease at 37 °C for 13 h. The RFLP profile was visualized by the same way as for FecB.

Statistical analysis

The statistical analysis was condusted using SAS (OnDemand for academic online version). Gene and genotype frequencies and Hardy Weinberg Equilibrium were calculated manually as described by Jawasreh et al (1).

RESULT AND DISCUSSION

In this study, one hundred and eighty-four newborn (that resulted from inseminated with ram of FecB-Awassi and CLPG-Awassi with pure-Awassi ewes were examined to determine carrier lambs of FecB and CLPG gene by the polymerase chain reaction followed by restriction fragment length polymorphisms (PCR-RFLP) technique. Two distinct genotypic patterns were obtained from the resulting 185-base pair (bp) PCR product digested with the AvaII restriction enzyme: the wild AA homozygote showed an uncut 181 bp band, while the AB heterozygote had 185 and 186 bp bands (Figure 1). The frequency distribution of FecB mutation in Awassi sheep breeds are given in Table 1. The A or - and B or + allele frequencies of the FecB genotypes were 0.77 and 0.23, respectively, while the genotypic frequencies of AA or --, AB or -+ and BB or ++ were 0.56, 0.41 and 0.02, respectively. The frequency distribution of FecB mutation in Awassi sheep breeds in governorates are given in Table 2. Analyzing

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the genotype and allele frequencies FecB at the governorate level (Table 2), we noted that Irbid governorate exhibited the highest frequency of the specific AB or -+ genotype at 0.47, followed by Jarash AB or -+ (0.45), Balqa AB or -+ (0.38), and Ajloun AB or -+ (0.29). Regarding allele frequency B or +, Irbid had the highest frequency at 0.28, while Ajloun had the lowest at 0.14. Jarash fell in between with an allele frequency of 0.23, and Balqa had a frequency of 0.19. The PCR genotyping analysis showed that 41 of the ram lambs carried the FecB (AB or -+) mutation in the heterozygous state, including 29 female lambs. These results highlighted the significance of the project's objectives, which can be further demonstrated through a simulation. At farmer's fields, the first parity of the 29 ewe lambs born heterozygous carriers for the FecB mutation was monitored. In their first parturition, 19 out of 29 ewes gave birth to triplites achieving 2.31 lamb/lambing as opposite to 1.12 lambs /lambing for those non- carrier mothers. In addition to his significant quantification of the economic values for ewe's prolificacy, sheep with the FecB mutation have larger litters and greater ovulation rates (Guo, et al. 2018). Numerous popular sheep breeds found in Australia, New Zealand, India, China, Indonesia, and Iran have been found to carry the FecB mutation (Potki, et al. 2020). FecB raised the ovulation rate by +0.26 ova (+0.8 to +2.0) and the litter size of the born lambs by +0.67 (+0.4 to +1.3), according to the weighted mean effects of FecB in ewes with one copy (+/B). As compared to wild type (+/+) ewes, the impact of a second copy (B/B) was +3.61 for ovulation rate and +0.77 for litter size (Fogarty, 2009.). Additionally, Teyssier el al.,(2009) noted that the Booroola gene's increased ovulation rate had a significant impact on the size of the litter at birth, which in turn altered the lambs' growth and survival. It was observed in China (Hua, & Yang, 2009.) that crossbreeding some poor prolificacy breeds with the FecB mutation can economically increase the reproductive features. The body weights of the Hu-ewes genotypes (BB, B+) lambs were also greater than those of the Hu-ewes genotype (++) lambs at 90 days after birth (18.6 + 3.70 kg), (18.0 + 3.71 kg), and (15.6 + 2.22 kg), respectively ($P < 0.05$). Deccani ewes with poor prolificacy (mean litter size of 1.03) were able to rapidly increase their prolificacy when FecB was used in low-input smallholder flocks in India (Nimbkar, et al. 2009). The average number of live lambs born to heterozygous ewes in that experiment was 1.5, but the average number of live lambs born to homozygous ewes was 1.65. Compared to wild type ewes, heterozygous ewes produced 7.5 percent more lamb and had a 37–50 percent larger gross margin per breeding ewe when outreach and training efforts were combined with gradual management and nutrition changes suitable for this level of prolificacy. Moreover, Talebi et al., (2018) discovered that the FecB-carrier animals had better phenotypes and that they profited from the higher lambing rate. The ovulation rate is increased by 1.65 (Liu, et al. 2014), and the litter size is increased by about 1 and 1.5, respectively, when the Booroola gene is present in two copies. The ability of B+ ewes to produce between 50 and 65 percent more lambs worldwide was consistent across comparisons (Teyssier, et al. 2009). Callipyge mutation (CLPG): In order to identify carrier lambs of the CLPG gene, fifteen newborn lambs produced by mating the heterozygous callipyge Awassi ram (50% Awassi and 50% Rambouillet) with Awassi ewes were analyzed using the polymerase chain reaction followed by restriction fragment length polymorphisms (PCR-RFLP) technique. Targeted BsmFI CLPG gene amplification results in 426 kb

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fragments (Figure 2). The CLPG gene was genotyped by using the restriction BsmFI enzyme to digest the PCR product. Segments of 395 bp and 31 bp for mutant allele c and 278 bp, 117 bp, and 31 bp for wild type allele C were obtained from the digestion of the PCR products (Figure 3). The introduction of the CLPG mutation into Awassi sheep was the second goal of our experiment. Using imported semen that was heterozygous for the CLPG mutation, we artificially inseminated 40 ewes. Seven male and eight female lambs were among the fifteen lambs that were heterozygous for the CLPG mutation. Table 3 shows the frequency distribution of CLPG mutations in Awassi sheep breeds. The genotypic frequencies of CC, Cc, and cc were 0.63, 0.37, and 0 correspondingly, whereas the C and c allele frequencies of the CLPG genotypes were 0.81 and 0.19, respectively. Simulation the scenario of using the Males: these 7 male lambs assumed to reach maturity and become rams, each one mates with 25 ewes for the next 4 seasons, taking into account a fertility rate of 90%, a mortality rate of 5%, and a prolificacy factor of 1.15, they will produce a total of 688 offspring, consisting of 344 males and 344 females. Considering the inheritance pattern of the CLPG mutation, 172 of the male offspring will carry the mutation. These lambs will produce more meat compared to the wild-type ones. The cold carcass weight for male wild Awassi is 22 kg, while for the males carrying the CLPG mutation; it is 28 kg (Talebi, et al. 2018). Therefore, the 172 CLPG lambs will yield a total of 4816 kg of meat (172 * 28 kg), resulting in an increase of 1032 kg (27%) compared to the wild-type lambs. This increase in meat production will contribute to Jordan's food security, providing a total of 3784 kg of additional meat. According to a number of studies (Jawasreh, et al. 2016, Jawasreh, et al. 2019), this gene also provides a higher leg score and carcass percentage, larger longissimus loins, superior leanness composition, higher leg values, higher percentage of dressing percentages, and optimal feed conversion, as well as higher body weight .

Table 1. Gene and genotype frequencies of the FecB gene for Awassi ewers in Jordan

	Genotype FecB	Observed Number	Expected Number	Genotype Frequency	Allele	Allele Frequency
Jordan	AA	95	100	0.56	A	0.77
	AB	70	60	0.41	B	0.23
	BB	4	9	0.02		

Table 2. Gene and genotype frequencies of the FecB gene for Awassi ewers in Jordanian governorates that included in the study

governorate	Genotype FecB	Observed Number	Expected Number	Genotype Frequency	Allele	Allele Frequency
Irbid	AA	45	48	0.49	A	0.72
	AB	43	37	0.47	B	0.28
	BB	4	7	0.04		
Ajloun	AA	25	25.7	0.71	A	0.86
	AB	10	8.6	0.29	B	0.14
	BB	0	0.7	0		
Ajloun	AA	5	5.6	0.5	A	0.75
	AB	5	3.8	0.5	B	0.25
	BB	0	0.6	0		
Jarash	AA	20	21.1	0.62	A	0.81
	AB	12	9.8	0.38	B	0.19
	BB	0	1.1	0		

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Table 3. Gene and genotype frequencies of the callipyge (CLPG) gene for Awassi ewers in Irbid governorate

	Genotype CLPG	Observed Number	Expected Number	Genotype Frequency	Allele	Allele Frequency
Irbid	CC	25	26.4	0.63	C	0.81
	Cc	15	12.2	0.37	c	0.19
	cc	0	1.4	0		

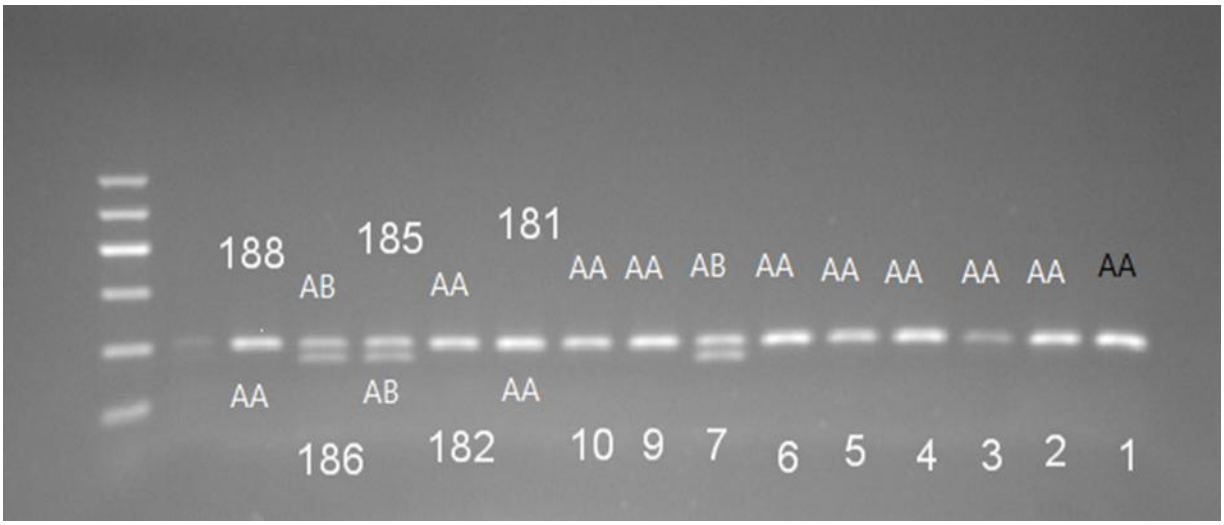

Figure 1. Agarose gel electrophoresis (2%) of the digested product of FecB mutation by Avall restriction enzyme for Jordanian Awassi lambs. Lane L: DNA marker 100bp, Lane 7, 185, 186 are heterozygous genotypes. Lane 1 to 6 wild type

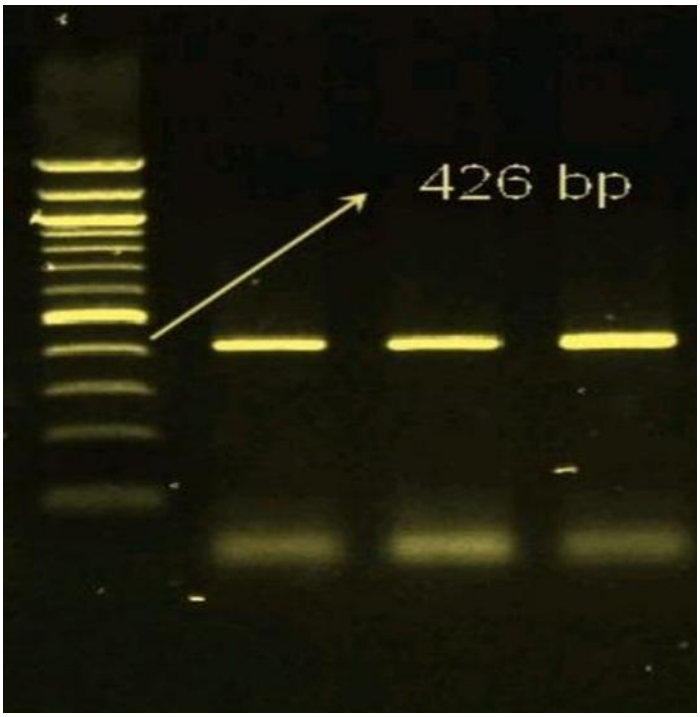

Figure 2. PCR product of the *CLPG* mutation (426 bp), L:100bp

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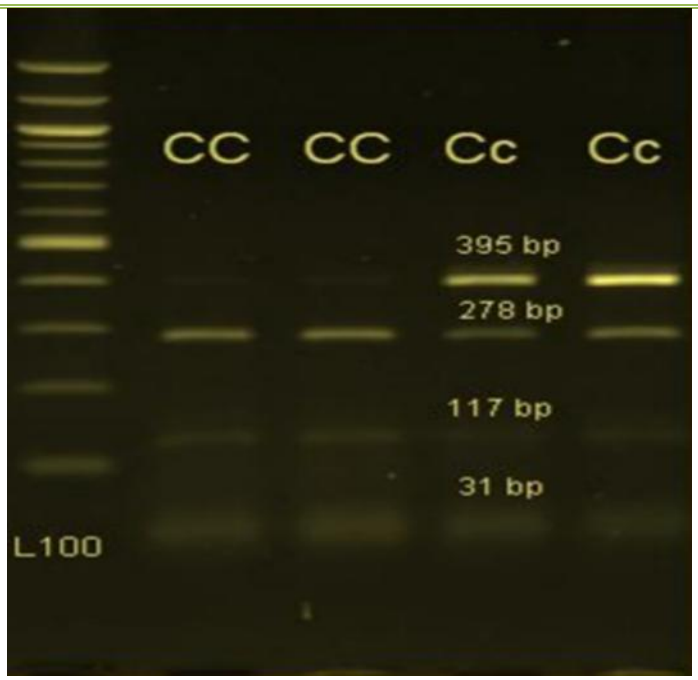

Figure 3. PCR-RFLP results for CLPG gene by BsmFI restriction enzyme on 2% agarose gel. PCR products produced segments of 395 bp and 31 bp for mutant allele c and 278 bp, 117 bp, and 31 bp for wild type allele C. L:100bp

The introgression of the Booroola gene into non-prolific sheep breeds, such as the Awassi, has the potential to significantly enhance the productivity of the sheep industry and improve the socioeconomic conditions of farmers. The successful introgression of the FecB mutation into the Awassi breed through forced PCR-RFLP followed by backcrossing technique demonstrates the feasibility of this approach. The analysis of FecB allele and genotype frequencies in various populations residing in different villages and governorates in Jordan provides useful information for future breeding programs in the country. Based on the results of this project, it can be concluded that the introduction of the FecB gene into Awassi sheep in Jordan has resulted in a moderate frequency of the FecB AB genotype (41%). This frequency is not as high as some other populations; it is still a positive result for the breeding program. The frequency of the Callipyge CLPG+ genotype is also a noteworthy result, as it indicates the presence of this gene in the population. The prolificacy rate has been genetically improved by introducing the FecB mutation and shou;d be dessiminated to the majority of local Awassi sheep for sustaining and supporting food security. the current project has contributed to the growing body of knowledge on the genetic diversity of sheep populations. The frequency of the FecB and Callipyge genes in our Awassi sheep population suggest potential for improvement in productivity and meat quality through breeding programs. However, further research's is necessary to fully understand the effects of these genes on meat quality traits and to optimize breeding strategies for Awassi sheep.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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توزيع خطوط وراثية محسنة من اغنام العواسي لمواجهة تحدي شح الغذاء في الأردن

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المستخلص

هدفت هذا الدراسة إلى تعزيز الأمن الغذائي في الاردن من خلال تحسين كمية ونوعية اللحوم أغنام العواسي عن طريق إدخال طفرتين هما طفرة الخصب (FecB) و طفرة الكاليبج (CLPG) ، شملت هذه الدراسة عدد 209 من النعاج العواسي وزعت بواقع عشرة رؤوس منها على كل مزارع، تم تلقيح نصف القطيع بالكباش المتوفرة في حقل الاغنام التابع لقسم الانتاج الحيواني/كلية الزراعة/ جامعة العلوم والتكنولوجيا الأردنية، والتي تحمل طفرة جين الخصب Awassi)، بينما تم تلقيح النصف الآخر بكباش تحمل طفرة الكاليبج وذلك بهدف نشر الطفرات الاقتصادية في أغنام العواسي. بعد موسم الولادات, تم االستخلاص المادة الوراثية الدنا من عينات الدم التي جمعت من الحملان. واستخدمت طريقة تعدد االطرز الوراثية المقيدة (RFLP) للكشف عن الطراز الجينيني لطفرتي FecB و CLPG. أظهرت نتائج الدراسة الإدخال الناجح للطفرات في أغنام العواسي في الأردن , و كانت تكرارات الطفرات الوراثية تقترب من الصفر ولكن الآن وبعد إدخال الكباش الحاملة إلى حقول المزارعين، فقد زادت إلى 0.41 (FecB) و0.50 (CLPG). في حيوانات التجربة ازداد معدل خصوبة النعاج ذات الولادة الاولى إلى 2.5 لكل الحملان الحاملة لطفرة الخصب (FecB). اوضحت نتائج الدراسة إن إدخال الطفرات الجديدة الى حقول المزارعين سيساهم بشكل مباشر في تحسين معيشة المزارعين وتعزيز الأمن الغذائي في الأردن ونوصي بالاستمرار في اكثار الحيوانات الحاملة لهذخه الطفرات الاقتصادية.

الكلمات المفتاحية: أغنام ا لعواسي , جين الخصب,جين الكاليبج, الطرز الوراثية .