Iraqi Journal of Market Research and Consumer Protection
(2022) 14(2): 19-25
DOI: http://dx.doi.org/10.28936/jmracpc14.2.2022.(3)
Salam et al.,

المجلة العراقية لبحوث السوق وحماية المستهلك

RELATIONSHIP OF LHX3 GENE POLYMORPHISM TO FERTILITY RATE IN LOCAL AND SHAMI GOATS

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Received 19/ 6/ 2022, Accepted 27/ 7/ 2022, Published 31/ 12/ 2022

ABSTRACT

The study was conducted at the ruminant research station of the general commission for agricultural research/Ministry of Agriculture, as well as the laboratory of genetic resources of the department of livestock/Ministry of Agriculture and the laboratory of the college of agriculture engineering science, with the aim of determine the genotypic of the expression region (intron 2 and part of exon 3) of the LHX3 gene And its relationship to the fertility rate in local and Shami goats. For this purpose, the RFLP technique was used, and the percentages of genotypes for the LHX3 gene in the local goat sample were 29.17, 50.00, 20.83 for the TT, AT, and AA genotypes, respectively, while in the Shami goats, the genotypes of the LHX3 gene in the goat sample were 29.17, 50.00, and 20.83 Shami 44.00, 56.00 and 0.00 for the TT, AT, and AA genotypes, respectively, and it was found that there was a significant effect of the multiple genotypes of the LHX3 gene on the fertility rate in local and Shami goats. It was found that the important reproductive trait in goats is the fertility trait, which is affected by several aspects, including environmental And administrative as well as genetic influences, as I found a lot of genetic mutations, As the percentage of twins reaches 42% in goats, according to the breed, through genetic selection assisted by markers (MAS). The values of the genetic equivalent for fecundity of goats are approximately 0.02 - 0.15.

Keywords: LHX3 gene, fertility rate, genital traits.

علاقة تعدد المظاهر الوراثية لجين LHX3 بمعدل الخصب في الماعز المحلي والشامي

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الخلاصة

أجريت الدراسة في محطة أبحاث المجترات التابعة للهيئة العامة للبحوث الزراعية/ وزارة الزراعة، فضلاً عن مختبر المصادر الوراثية/ دائرة الثروة الحيوانية/ وزارة الزراعة ومختبر كلية علوم الهندسة الزراعية، بهدف تحديد التراكيب الوراثية لمنطقة التعبير (الانترون ٢ وجزء من الاكسون ٣) لجين LHX3 وعلاقته بمعدل الخصب في الماعز المحلي والشامي، وأستعمل لهذا الغرض تقنية تباين أطوال الحزم المقيدة (RFLP)، وبلغت نسب التراكيب الوراثية لجين LHX3 في عينة الماعز المحلي 29.17 و50.00 و20.83 للتراكيب الوراثية AA وAT وTT على التوالي، أما في الماعز الشامي فقد بلغت التراكيب الوراثية لجين LHX3 في عينة الماعز الشامي 44.00 و56.00 و0.00 للتراكيب الوراثية AA وAT وTT على التوالي، وأتضح وجود تأثير معنوي لتعدد المظاهر الوراثية لجين LHX3 في معدل الخصب في الماعز المحلي والشامي، وأن صفة التناسلية المهمة في الماعز هي صفة الخصب والتي تتأثر بعدة جوانب منها البيئية والادارية فضلا عن التأثيرات الوراثية، إذ وجدت الكثير من الطفرات الوراثية، حيث أن نسبة التوائم تصل الى ٤٢% في الماعز وحسب السلالة وذلك عن طريق الانتخاب الوراثي المعاون بالواسمات (MAS)، وعلى هذا الاساس يتبين أن الصفات التناسلية هي من الصفات ذات المكافئ الوراثي المنخفض، ولا يمنع هذا من أن تولي الاهتمام بالجانب الوراثي، وبينت الدراسات أن قيم المكافئ الوراثي لصفة الخصب للماعز ما يقارب ٠.٠٢ – ٠.١٥.

الكلمات المفتاحية: جين LHX3، معدل الخصوبة والصفات التناسلية.

INTRODUCTION

The LHX3 gene is located on chromosome number 11 in goats and consists of 3 exons, separated by 2 introns **(NCBI, 2021)**. Also, it was found that the Lhx3 gene in mice contains 6 exons, the first LIM domain is encoded by exon 2 and the second by exon 3 is shared by exons 4 and 5 by **Sheng *et al.* (1996)**, and that the human LHX3 gene is located on human chromosome 9 and consists of seven coding exons and six introns spanning about 8.5 kilobases **(Netchine *et al.*, 2000)** that encodes for a protein of the same name that is a factor Transcripts within the family of proteins called LIM-type Gene Regulatory protein shomeodomain) (LIM-HD) Gene-regulatory proteins, including LHX3 proteins, which have a strong activation domain at their carboxyl terminus and require activation of the expressed pituitary gland **(Li *et al.*, 2016)**, and the effect of this gene continues in the adult pituitary gland in the anterior lobe, spinal cord and medulla, and there are three types of expression for this gene: LHX3a, LHX3b, M2-LHX3, which indicates that it is subject to the process of cross-linking (alternative splicing), which is the process of producing more than one mRNA from the same gene, which allows the gene to affect more than one member or more than one trait, which has alternative amino endings, and that LHX3a has significantly higher activity on the target genes compared to the rest of the species, and M2 - LHX3 is the shortest protein from Among other species **(Sloop *et al.*, 1999)**.

MATERIALS AND METHODS

The study was carried out at the ruminant research station of the General Authority for Agricultural Research/ Ministry of Agriculture, on a sample of 50 goats (25 local and 25 Shami) with regard to the field part, while genetic analyzes were carried out in the laboratory of genetic resources of the Department of Livestock/ Ministry of Agriculture and the College of Agriculture engineering science, in order to determine the genotype of the LHX3 gene , and collect 3 ml of blood from the juglar vein, extract DNA from the blood according to the instructions of the kit supplied by Geneaiad Company.

Choose Primer and amplify PCR

To amplify the studied region (intron 2 and part of exon 3) with the sequence 7589 - 8278, the primer was selected and the PCR program was designed according to **Liu *et al.* (2011)** (Table 1), the PCR reaction was performed in a 25 µL final volume, containing 50 ng genomic DNA, 0.5 µM of each primer, 1×Buffer [including 1.5 mM $MgCl_2$], 200 µM dNTPs and 0.625 units of Taq DNA polymerase (MBI, Vilnius, Lithuania). The thermal cycling program was 5 min at 95°C, 34 cycles of 94°C for 30 sec, annealing for 30 sec, 72°C for 15~45 sec, with a final extension at 72°C for 10 min, and subsequently cooling to 4°C.

Table (1): Choose the primer according to (**Liu *et al*, 2011**) As shown in Detection of alleles of the first expression region of LHX3 gene using RFLP technique:

Gene name	exon region	Sequence	
LHX3	3	Front (F)	GGACGACTTCTTCAAGTGA
		Reverse ®	ACACGACGCAGGCGAAGCAG

The allelic difference of the resulting bundle was revealed by the use of primer enzymes. The Dar1 enzyme was used to digest the targeted LHX3 gene bundle according to a specific digestion program in (Table, 2) at a temperature of 37°C for 1 h. Then the digested samples were transported using the electrophoresis device and the resulting bundles were detected. Using a UV transiluminater to detect the cut sites and based on the resulting bundles, then determine the alleles

Table (2): The enzymatic digestion program used to study the phenotypic polymorphism of the LHX3 gene for the doubling region.

the components	Reaction volume 9 µL
Dra1 (1000 u/mL)	0.3 µL
Product PCR	4 µL
1×TBE Buffer 4	0.1 µL
DNase Free Water	4.6 µL

Fertility rate calculator

The fertility rate is defined as the number of females giving birth out of the number of goats exposed to a male, and it is calculated as a percentage as in the following equation **(Al-Sayegh & Al-Qasr, 1992)**:

$$\text{Fertility rate (\%)} = \frac{\text{Number of females giving birth}}{\text{Number of females giving birth to a male}} \times 100$$

Tatistical Analysis

The data were statistically analyzed using the SAS 2012 program to study the effect of the genetic phenotypes of the LHX3 gene on the growth characteristics of mothers and newborns in both Shami and local goats according to the mathematical model below, and the mean differences were compared using the Duncan 1955 polynomial test by applying the mean squares method. Least square means: Mathematical model: the relationship of the hereditary manifestations of the gene LHX3.

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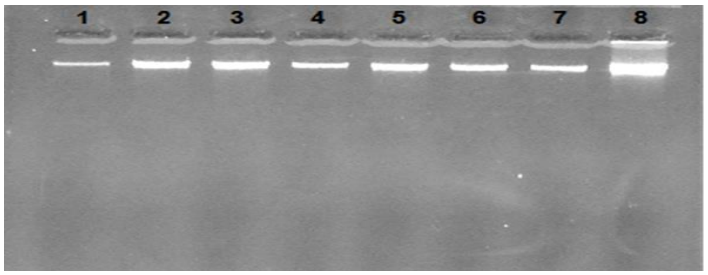
$$\text{Yijklm} = \mu + \text{SNPTA} + \text{Aj} + \text{Tk} + \text{OI} + \text{eijklm}$$

Yijklm : Viewing value m
µ: the general average of the adjective
SNPTA: Effect of the LHX3 gene phenotypes
Aj: the effect of maternal age at birth (2, 3, 4, 5 years)
Tk: the effect of the type of birth (single, twin)
O_1: influence of the month of birth (months 4, 3, 2, 1, 12
eijklm: normally distributed random error with a mean of zero and a variance of O_2e.

The Chi-square test (2χ) was also used to compare the percentages of the distribution of structures in the studied sample of Shami and local goats, and the following law was applied to calculate the allelic frequency according to Hardy Weinberg's rule.

RESULTS AND DISCUSSION

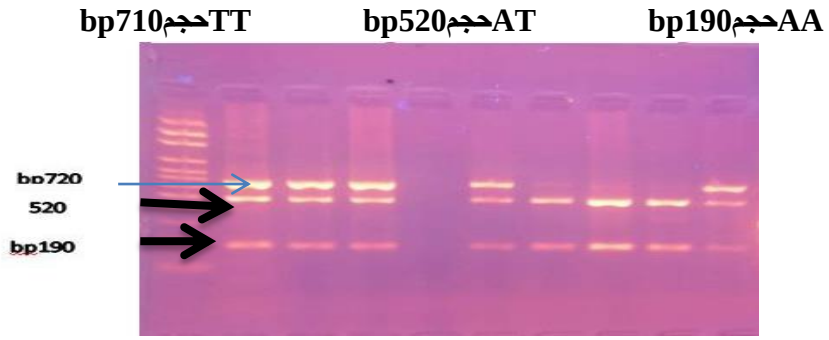
packets: It is clear from the (Picture 1) the success of the DNA extraction process in light of the electrophoresis product on the agarose gel.



Picture (1): The product of DNA electrophoresis. Agarose gel concentration: 0.1%, dye used: ethidium bromide, electric current: 60 v, amperage 50 mA, for 90 min. The numbers 1-8 refer to DNA samples from goat samples.

PCR amplification product

The (Picture 2) shows the result of PCR amplification of the studied region of the LHX3 gene on the agaros gel with a size of 710, which is the studied region located within the sequence (base pair 7589 - 8278) in the goat genome using the 100bp parameter (100bp DNA Ladder), as the results of the study showed for all samples DNA extracted from the blood of local and Shami goats and that pure wild genotype (AA) (2 bundles), hybrid genotype AT) (3 bundles), and pure mutated (TT) genotype (one bundle).



Picture (2): PCR amplification product.

Genetic lineage distribution

The results in (Table, 3) shows the distribution ratios of the genotypes for the studied region of the LHX3 gene for the local goat samples which are the pure wild genotype (AA) and the hybrid genotype AT) and the pure mutated genotype (TT), as it appears that there are no significant differences compared to the Mendelian ratios (1,2,1) and the percentages of the three genotypes, which amounted to 29, 50 and 20% for AA, AT and TT genotypes, respectively, meaning that there is a clear commonality of individuals carrying the hybrid (AT) and pure (AA) genotypes with a low percentage of genotypes pure mutant (TT) in the studied sample, the result did not agree with the study **Liu *et al.* (2011)**, which studied the same area in three Chinese goats, namely Guanzhong, Xinog sannen Inner olia white cashmer), as the proportions of the genotype were AA, respectively. (0.485, 0.482 and 0.792) for the AT genotype (0.371, 0.340 and 0.194) and for the TT genotype (0.144, 0.178 and 0.014), and as

shown in (Table, 3) the allelic frequency of the wild allele A and the mutant allele T, if the allelic frequency was 0054 and 0046 for the two alleles Wild A and mutant T, respectively, there were no significant differences between the two alleles.

Table (3): Number and percentage (%) of genotypes and allelic repeats of The LHX3 gene for a local goat sample.

Percentage (%)	Number	GenotypeGenetic makeup
29.17	7	AA
50.00	12	AT
20.83	5	TT
100%	24	Total
0.333NS	----	(chi-square value) χ^2
(Repetition) Frequency		Allele
0.54		A
0.46		T
		NS: insignificant.

Genetic lineage distribution

The results in (Table, 4) shows the presence of three genotypes of the LHX3 gene for Shami goat samples, namely, the pure wild genotype (AA), the hybrid genotype AT) and the pure mutated genotype (TT), as it appears that there are no significant differences ((P<0.05) between the percentages of The three genotypes, which amounted to 44, 56 and 0% for the AA, AT and TT genotypes, respectively, that is, there is a clear commonality for individuals carrying the hybrid (AT) and pure (AA) genotypes. The studied sample, the result did not agree with the study **Liu *et al.* (2011)** in three breeds of Chinese goats (Guanzhong, Xinog sannen, Inner olia white cashmer), as the proportions of the genotype were AA, respectively (0.485, 0.482 and 0.792) and for the genotype AT (0.371, 0.340 and 0.194) and for the TT genotype (0.144, 0.178 and 0.014), (Table 4) shows that there are no significant differences between the allelic frequency of the wild A allele and the mutant allele T, as the allelic frequency was 036 and 0063 for the two wild alleles. A and mutant T respectively.

Table (4): Number and percentage of the genotypes and allelic frequency of the LHX3 gene for the Shami goat sampl.

Percentage (%)	Number	Genotype
44.00	Total	AA
56.00	14	AT
0.00	0	TT
100%	25	
9.691**	----	(chi-square value) χ^2
Frequency		Allele
0.36		A
0.63		T
*(P<0.05) ،NS :insignificant.		

Fertility rate

The results in (Table, 5) that there was a significant effect in the LHX3 gene on the fertility rate among the genotypes of samples of local goats, as the fertility rate reached the highest average 1.42 ± 0.20 for the pure genotype (AA), followed by the mutant genotype (TT) 1.75 ± 0.25 and Hybrid genotype (AT1.27 ± 0.19) In addition, there was a significant effect of

LHX3 gene on fertility rate among the genotypes of Shami goat samples. The pure genotype (AA) was recorded 1.30 ± 0.15 higher than the average of the hybrid genotype ± 0.16 and in the hybrid 1.07 ± 0.16. Previous studies found that the important reproductive trait in goats is the fertility trait, which is affected by several aspects, including environmental and administrative, as well as genetic influences, as many genetic mutations were found, and the features of single nucleotides (SNPS) that are related to the ratio of twins, which prompted many researchers to clarify the conditions The goats are characterized by high fertility rates that increase the number of births for the herd. **Dominik (2005)** indicated that the increase in the proportion of twins through genetic selection assisted by markers (MAS), and on this basis it was found that the reproductive traits are among the traits with a low genetic equivalent, and this does not prevent this from paying attention to the genetic aspect, and studies showed that the values of the genetic equivalent for a trait The fertility of goats is approximately 0.02 - 0.15 **(García-Peniche *et al.*, 2012)**. Reproductive traits are greatly affected by the environment and must be taken care of in order to improve the productive performance in goats.

(Table 5): Relationship of the Genotypes of the LHX3 gene to the fertility rate of domestic and Shami Goats.

FERTILITY RATE ± STANDARD ERROR	GENOTYPE	GOATS
1.42± 0.20 ab	AA	the local
1.27± 0.19 b	AT	
1.75± 0.25 a	TT	
*		morale level
1.30± 0.15 a	AA	Shami
1.07± 0.16 b	AT	
*	morale level	
. The averages with different letters within the same column differ significantly between them *P<0.05.		

Conclusion

1. Increasing the composition of (AT) on the combination of (AA) for ultiplicity of manifestations on thShami goats by weight at weaning and weight gain, while there is no effect of the mese characteristics in the local goats
2. The genotypes (AA) and (AT) gave the highest significant values for body height at the forefront over the genotype (TT) in the local goats, and the composition (AA) recorded the highest significant values for the hind body height in the Shami goats.
3. The superiority of composition (AT) over genotype (AA) in Shami goats in the time required to reach peak milk production.
4. The composition of (TT) had the highest significant effect on the two structures (AA) and (AT) in the local goats for the fertility rate, while for the Shami goats, the (AA) composition was superior to the (AT) composition in it.
5. The proportion of the calculated and expected hybrid and pure structures was very close in the local goats, while the calculated pure structures were less than expected because the pure mutant composition did not appear, and the hybrid composition was the calculated value higher than the expected in the Shami goats.

6. The expected values of the allelic mixture are less than the values of the hybrid composition in the local and Shami goat samples.
7. The values of internal breeding were low, which indicates the absence of internal breeding in the herd.

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