Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

EFFECT OF POLYMORPHISMS FOR PAIRED LIKE HOMEODOMAIN 2 GENE ON SOME REPRODUCTIVE TRAITS IN GOAT

I. A. Nayyef

College of Agricultural Engineering Science- University of Baghdad

***Correspondence to:** Inam Abdelwahed Nayyef, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.
Email: anaam.a@coagri.uobaghdad.edu.iq

Article info

Received: 2024-02-18
Accepted: 2024-03-19
Published: 2024-06-30

DOI-Crossref:
10.32649/ajas.2024.183692

Cite as:
Nayyef, I. A. (2024). Effect of polymorphisms for paired like homeodomain 2 gene on some reproductive traits in goat. Anbar Journal of Agricultural Sciences, 22(1): 16-27.

Abstract

The current research was detected at Agricultural Research Station/ ministry of Agriculture on 49 animals to clarify effect of paired like homeodomain transcription factor 2 ($pitx_2$) gene on some reproductive traits as litter size, twining rate, fertility rate and revealing of polymorphisms for this gene. This study was founded four mutations in promoter region of $pitx_2$ gene in different sites with appearance two dominant genotypes TT and GG with high effect on litter size due to each of C1141T and G1003A mutations with high litter size received to 1.48 and 1.57 born/ birth whilst G1089A and G1148A mutations not affect litter size, on another side, genotypes which refer to both mutations C1141T and G1003A influence on twining rate ($p < 0.01$). However, higher rate were for mutant genotypes CC and AA recorded 47.97% and 52.38% respectively while G1148A and G1089A and their genotypes did not affect the twining rate, moreover, genotypes within four single nucleated polymorphisms have an importance significantly ($p < 0.01$) on fertility rate which were more in local goat than Shami breed. Whereas, fertility rates were close to each other among different genotypes and lower rate belonging to GA heterozygous received 0.37 and 0.18 for native and Shami goats respectively. Values of Chi square for genetic and allelic frequencies tend up to 88.46 of GG refer to first and third mutations with higher allelic frequency for G, in the same side, frequencies were 0.50 and 0.648 due to TT and T as

arrangement in spite of allelic frequency for A was declined to 0.060, 0.068 and 0.154 to G1148A and G1089A and G1003A sequentially.

Keywords: Gene, Genotypes, Goat, paired like homeodomain 2, Reproduction.

تأثير المظاهر المتعددة لجين الاقتران الشبيه بالنطاق 2 في بعض الصفات التناسلية في الماعز

انعام عبدالواحد نايف

جامعة بغداد – كلية علوم الهندسة الزراعية

***المراسلة الى:** أ. م. انعام عبد الواحد نايف، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق.

البريد الالكتروني: anaam.a@coagri.uobaghdad.edu.iq

الخلاصة

اجري البحث الحالي في محطة البحوث الزراعية/ وزارة الزراعة على 49 حيوان لبيان تأثير جين عامل النسخ المتطابق 2 في منطقة التشغيل في بعض الصفات التناسلية مثل الخصب، نسبة التوائم، نسبة الخصوبة وعلاقتها مع تعدد المظاهر الوراثية لهذا الجين، وجدت الدراسة أربعة طفرات في مواقع مختلفة في منطقة تشغيل الجين مع G1089A وG 1148 A لم يتأثر الخصب في كلا الطفرتين TT وGG ظهور تركيبين وراثيين سائدين هما حيث وصل الى 1.48 و1.57 مولود/ البطن الواحدة G1003A وC1141T بينما تأثر بمعنوية عالية بسبب اثرت إيجابيا بصورة عالية G1003A وC1141T من جانب اخر التراكيب الوراثية ضمن الطفرتين وسجلت 47.97 AA وCC في نسبة التوائم إضافة الى نسبة الخصوبة التي كانت اعلى للتراكيب الطافرة وتراكيبها الوراثية لم تؤثر فيها، وكان للتراكيب G1089A وG A1148% و52.38% على التوالي بينما بتعدد المظاهر النيوكليوتيدية الأربعة والتي كانت لدى في نسبة الخصوبة (P< 0.01) المختلفة أهمية معنويا الماعز المحلي اعلى مما لماعز الشامي مع ان نسبة الخصوبة كانت متقاربة بين مختلف التراكيب الوراثية واقل التي وصلت الى 0.37 و0.18 لكل من الماعز المحلي والشامي على GA نسبة تعود الى التركيب الهجين التوالي من جانب اخر كانت قيم مربع كاي للتكرارات الوراثية والاليلية قد ارتفعت الى 88.46 للتركيب واكثر من ذلك كانت G التي تعود الى الطفرات الأولى والثالثة إذ ازداد تكرار الاليل GG الوراثي A من انخفاض التكرار الاليلي للاليل بنفس الترتيب بالرغم T وTT التكرارات 0.50 و0.648 عائدة الى وبنفس السياق G1003A وG1089A، G1148A للطفرات الى 0.060، 0.068 و0.154.

كلمات مفتاحية: الجين، التراكيب الوراثية، الماعز، عامل النسخ للاقتران الشبيه بالنطاق 2، التناسل.

Introduction

Environmental factors such as feeding, management, in addition, genetics influence on reproductive performance of cattle especially goat which considered economic animals with long productivity periods and survival more of that high fertility (6 and 7) also have a foundation role in animal production faculty due to their multiple purposes of breeding, this give a good chance for persons whom working to improve their economic status so that, many of researches try to develop breeding programs (23) The most crucial trait in goat and sheep production is reproduction (1), for improving projects of goat breeding these demands highlight some reproductive traits such as fertility and prolificacy (9) Goat featuring high reproductive efficiency coupled with increasing productivity plus rising litter size (2 and 16) thus, considered efficiency indicator for adaptation to harsh conditions (26) on another side, reproductive is the most crucial side at animal production faculty which includes quantitative traits such as fertility, embryonic mortality, prolificacy, twining and lambing rates (5). Raising the production carrying out traditional methods that not enough because depending upon morphological observations, at present time, using various molecular parameters like RFLP, SSR, RAPD, and DNA sequencing that helping to evaluation animals rapidly on the same side, improving this faculty, especially SNPs (single nucleotide polymorphisms) which play active markers (12 and 32) which give an idea about whole genome to selection depending on DNA level (4) $pitx_2$ a transcription factor 2 is a member of paired- like homeodomain transcription factor2, this gene was discovered firstly in persons whom suffered from Axenfeld Riger syndrome where was named RIEG which have a role in development throughout several paths as Wnt/ DVI/ B- catenin, error expression of this gene resulted several of infections (14 and 33).

In other hand, $pitx_2$ gene is responsible on pituitary gland development, however, it is member of a transcription, translation regulators group which have a significant role in control on cell differentiation moreover, regulation of transcription plus activity of Prolactin, Thyroid Stimulating Hormone (TSH), Growth Hormone Releasing Hormone (GHRH), growth hormone (11). $pitx_2$ gene encoding for protein linked with specify sites along DNA and regulates activities of other genes thus, depending on this activity it called a transcription factor in the same direction, expression of this gene reflects on several of body parts, eye, teeth, internal organs, organizing reproductive cells and blood formation during early embryonic development a symmetric between both of right and left parts of body (4). Study by 31 were illustrated that $pitx_2$ protein deficiency result in malformations in pituitary, heart, eye and teeth, on another hand, transcription factors working together, their functions various depending on location so that, they interference with occurrence of diseases (3) in other words, transcription factors are proteins associated specifically with parts of DNA through regulation transcription of genetic information from DNA to mRNA however, linkage sites are short averaged 5- 20 bp so, they can turn on or turn off RNA polymerase which perform to determine level of gene activity and association sites with promoter region, on same side, transcription factors linkage groups called enhancer and silencers, whereas, activity of transcription factors

regulating by phosphorylation. moreover, $pitx_2$ expression is controlled by two levels, the first one is transcription through the determination of the amount of mRNA produced from the gene, and the other one is regulating the translation of mRNA to a protein (15, 18 and 24), genotyping of this gene by sequencing method using for many of application including evaluation of genetic variation, breeding values, in addition clarify different of genomic fractions for genetic prediction by bio information for kinds of animals (17 and 22). A few previous studies deal with effect of genotypes due to mutations in promoter region of pitx2 gene on reproductive traits especially litter size, twining rate and fertility rate so that this study was conducted to evaluate effects of $pitx_2$ genotypes within mutations on some of reproductive traits and their distributions.

Materials and Methods

Blood samples were obtained from the Jugular vein of 49 Iraqi and Shami goat ages one to seven years, this research was conducted in the Ruminant Research Station which belongs to the ministry of Agriculture/ General Authority of Agricultural Researches, and laboratory in Baghdad was used to isolate DNA then determined isoforms of $pitx_2$ gene from 3/3/2022- 30/7/2022. Animals were kept in semi - opened barns, quantity and quality of forage various according to seasons and their availability, however, green forage was produced freely while a concentrate diet of 500 g/ animal/ day, this quantity may be increase before mating period for adult females and no grazing on the other hand, neonates stay with their mothers for suckling then began to have a little of green forage plus 100 g/ day of concentrate which given with 3% of body weight for lambs even one year meanwhile, weaning processed at ages 3-4 months, on other direction, animals undergo to protecting program by vaccinations and dipping against parasites.

DNA Extraction: Each 5ml of blood were collected in tubes with anti agglutination EDTA K_2 (produced from AFCO Jordian Company) and contained in cooled box in -4 C° then carry out for extraction by 300 µl of blood sample put in Eppendorf 1.5 ml then 900 µl of RBC lysis buffer affixed to them, Added 20 ml of proteinase and mixed up quietly for 10 minutes in room temperature for lysing blood cells, then centrifuged at 3000 r.p.m for 5 minutes, the clear was discarded without damaging the white layer and 50 µl of mixture mixed by vortex after that tubes put in incubator for 10 minutes at 60 C° with mixing every 3 minutes three times, absolute ethanol was added to tube, 600 ml of wash buffer was added to GD column and tubes located in the centrifuge for 30- 60 second with 14000 cycle/ minute also the residue was deserted, GD column was transferred to Eppendorf 1.5 ml with adding 0.75 ml of Elution buffer in bath water at 60 C° for 3- 5 minutes and centrifuged for 30 seconds at 14000 - 16000 r.p.m finally, GD column lift from Eppendorf then kept at -20 C°.

Preparation of agarose via 100 ml of IXTAE was poured into a flask. Then 1.5 g. (for 1.5%) agarose was added to the buffer, The solution was heated to boiling (using microwave) until all the gel particles were dissolved, 1 µl of Ethidium Bromide (10 mg/ml) was added to the agarose. After that the agarose was stirred in order to get mixed and to avoid bubbles, The solution was left to cool down at 50- 60 C°.

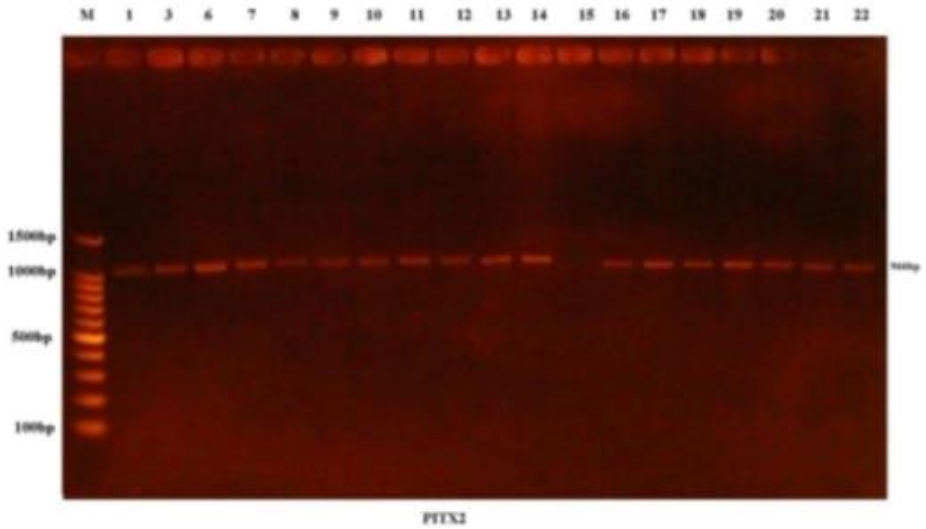

Fig. 1: Result of the amplification of pitx2 gene were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M 100 bp ladder marker.

Primer preparation: Primers were supplied by Macrogen Company in a lyophilized form, lyophilized primers were dissolved in a nuclease - free water to give a final concentration of 100 pmol/ µl as a stock solution, a working solution of these primers was prepared by adding 10 µl of primer stock solution (stored at freezer -20 C°) to 90 µl of nuclease- free water to obtain a working primer solution of 10 pmol/ µl By using DNA sequencing was resulted four mutations in promoter region which recorded in gene bank NCBI in goat.

Table 1: Primer of pitx2 gene.

	Sequence 5\ - 3\	Annealing Temp. C°	Product size (bp)
Forward	TGGTGACCTGGGTTATTT	58	966
Reverse	GAGAGGATGGCAGAAGAA		

Table 2: Numbers and mutations sites in promoter region and genotypes of Pitx2 gene.

Mutant	Heterozygous	Wild	Loci
-	GA	GG	G1148A
CC	CT	TT	C1141T
AA	GA	GG	G1089A
AA	GA	GG	G1003A

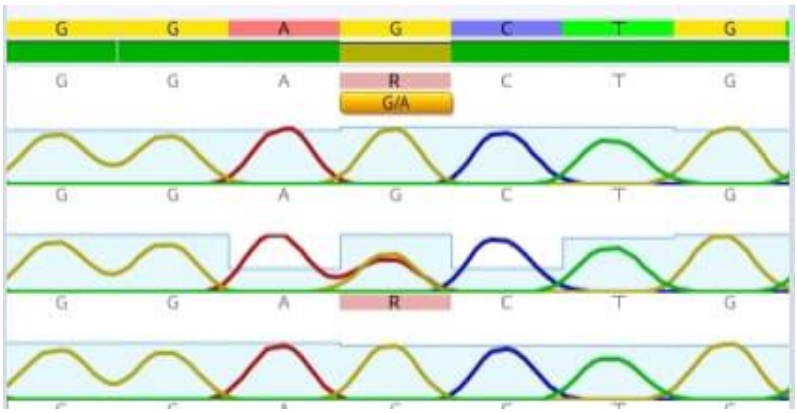

Fig. 2: Mutation site G1148 A in promoter region of pitx2 gene.

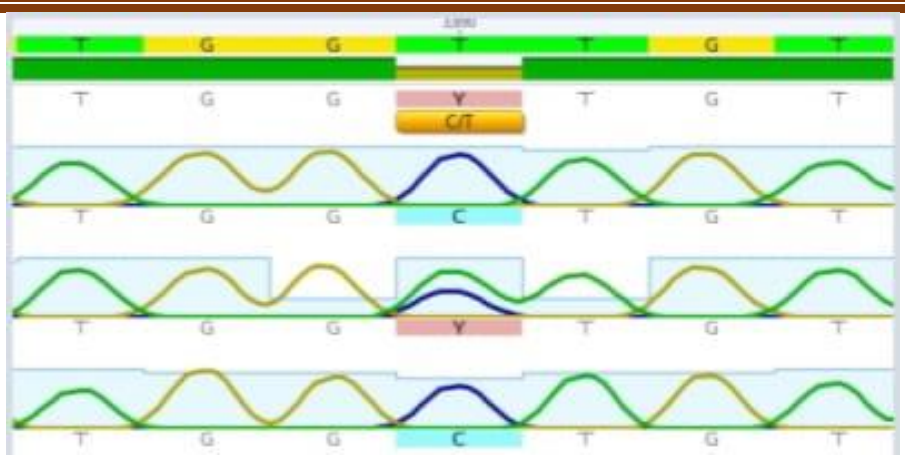

Fig. 3: Mutation site C1141 T in promoter region of pitx2 gene.

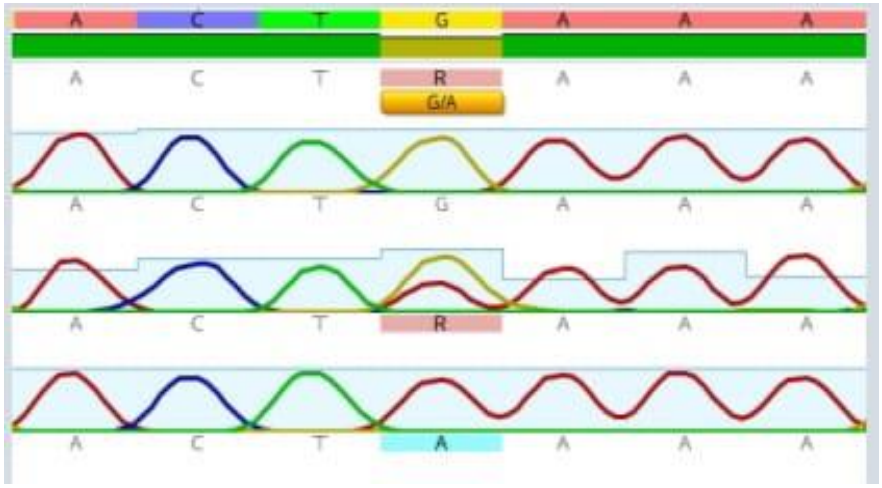

Fig. 4: Mutation site G1089A in promoter region of pitx2 gene.

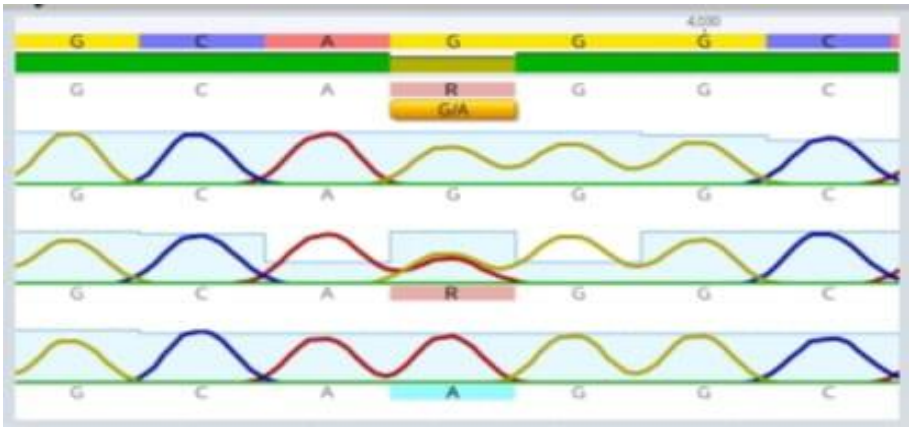

Fig. 5: Mutation site G1003A in promoter region of pitx2 gene.

Statistical Analysis: Data analysed by Statistical Analysis system (27) to study effect of genotypes for Pitx^2 gene in some reproductive traits such as litter size, fertility rate, twining rate (10) as next equations:

litter size = No. of infants born/ No. of lambing ewes ×100.

Fertility rate = No. of lambing ewes/ No. of ewes available for ram ×100.

Twining rate = No. of twin lambs/ No. of lambing ewes ×100.

Significance difference between means were comprised throughout Least square means (20).

The model used for studied traits was as following:

$Y_{i\,j\,k\,l} = \mu + G_i + T_j + B_k + e_{ijkl}$

$Y_{i\,j\,k\,l}$: Value of observation due to birth type j and breed k.

μ: General mean. G_i: Effect of genotype. T_j: Birth type. e_{ijkl}: Value of random error.

Results and Discussion

The current study indicated that a significant effect for different genotypes of $Pitx_2$ gene on litter size belonging to mutations C1141T and G1003A ($P< 0.01$), increasing litter size for individuals which have AA genotype due to G1089A and G1003A were recorded 1.50 and 1.57 neonatal/ birth further was 1.48 goat born / birth for each of GA hybrid and mutant CC within G1148A and C1141T respectively however, the least litter size were 1.38 and 1.39 born/ birth back to CT and GA for C1141T and G1089A correspondingly, on other hand, there was no effect of genotype which resulted from G1148A and G1089A mutations on litter size (Table 3). The present paper was indicated a significant impact for some of the genotypes on litter size. In another research of 8 whom reported least litter size received to 1.15- 1.19 in traditional goat flock compared with present result, in the same hand, the values of litter size which resulted from study of 29 were close to these of current research, however, these researchers were indicated that mutation 22 bp in promoter region in $Pitx_2$ gene had a real effect on litter size which associated with different genotypes significantly, reduction of litter size was 1.27 for DD whilst raised to 1.40 and 1.44 born/ birth for both of ID and II isoforms in white Cashmere goat. The significant effect of $Pitx_2$ genotypes refers to some loci in new research due to active of this gene by organizing, transcription, and expression of POUIFI, LHX_3 and PROP genes these have an important and effect on growth hormone secretion also development and reproduction furthermore, metabolism products for these genes influence on litter size (31).

Table 3: Effect of $Pitx_2$ genotypes for all mutations on litter size.

Standard error ± litter size	No.	Genotype	Mutation site
Ns 0.04 ± 1.43 a	44	GG	G > A site 1148
0.07 ± 1.48 a	5	GA	
0.07 ± 1.44 ab	26	TT	C > T site 1141
** 0.06 ± 1.38 b	15	CT	
0.05 ± 1.48 a	8	CC	
0.04 ± 1.43 a	44	GG	G > A site 1089
Ns 0.06 ± 1.39 a	4	GA	
0.11 ± 1.50 a	1	AA	
0.04 ± 1.42 b	37	GG	G > A site 1003
** 0.05 ± 1.43 ab	10	GA	
0.10 ± 1.57 a	2	AA	

Means with different letters various significantly ($p< 0.01$)**: highly significant Ns.: non significant.

Present study was indicated that C1141T and G1003A mutations significantly ($p< 0.01$) affect the twining rate which received to 47.97% and 52.38% for animals that have mutant genotypes CC and AA respectively while, no importance for G1148A

and G1089A in twining births that were induced in goat flocks despite they have a close percentage in most mutation sites which averaged 45.15, 47.67, 45.43 and 43.56% for GG and AA due to G1148A and G1089A.Whereas,were approximated for the same genotypes in site 1003 while received to 46.03 for TT and 42.79 for CT genotype (Table 4). According to twinning rate, this study agree with results of 30 whilst contrast to 7 report whom indicated to higher rate reached to 84.94% for mountain goat, in opposite side, mutation 22pb in promoter region not effect on twining rate furthermore, higher rate was 45.29% for mothers with twining that have ID while was recorded 33.33% due to DD but decreased to 21.36% for recessive II (29). The reason of significant effect of $Pitx_2$ gene in some genetic loci on twining births may be refers to that $Pitx_2$ gene have multi organizing for embryo by its influence on embryonic polarity and axis formation (10), moreover, $Pitx_2$ gene act as direct regulator for cVg1 gene expression in bird and other animals by correlation enhancers with close genes those nearest to this gene during normal development and organizing embryo to form and regulate twining (28).

Table 4: Effect of genotypes of Pitx2 gene for all mutations on twining rate.

Standard error ± twining rate %	No.	Genotype	Mutation site
Ns 2.51 ± 45.15 a	44	GG	G > A site 1148
3.96 ± 47.67 a	5	GA	
* 3.60 ± 46.03 ab	26	TT	C > T site 1141
3.38 ± 42.79 b	15	CT	
3.04 ± 47.97 a	8	CC	
Ns 2.07 ± 45.43 a	44	GG	G > A site 1089
3.54 ± 43.56 a	4	GA	
6.00 ± 52.00 a	1	AA	
* 2.53 ± 45.05 b	37	GG	G > A site 1003
3.03 ± 45.32 ab	10	GA	
5.36 ± 52.38 a	2	AA	

Means with different letters differ significantly, ($p< 0.05$): significant.

Mutations in all genetic sites influence on fertility rate for private and Shami goat ($p< 0.01$) fertility rate was 0.74 for local breed higher than 0.68 for Shami and these belong to GG genotype whereas, the lower were 0.37 and 0.18 for GA within G1148A mutation, in general, local goat predominate Shami in fertility rate for all genotypes so that, rates were close to other and more according to dominant zygosity TT was 0.71 for C1141T mutation also 0.76 and 0.75 due to GG within G1089A plus G1003A, while, rate of CT was recorded 0.75 in same side, fertility rate for mutant CC was the same in C1141T and GA for G1089A furthermore, G1003A received to 0.66 however, fertility rate ranged between 0.72 and 0.66 for both of CT and GG belong to C1141T, G1089A respectively whilst differences widely among genotypes which were 0.69, 0.75 and 100 for GG, GA and AA that for G1003A (Table 5). Compared to current study fertility rate was higher in the result of 7 which received to 80.72% in mountain goat, effect of various genotypes of Pitx2 gene on fertility rate because of action of this gene by regulating signal path Wnt/β-catenin which affect embryonic implantation (22)

Table 5: Effect of genotypes of Pitx2 gene for all mutations on fertility rate in Native and Shami breed.

Shami breed		Native breed			
SE ± fertility rate	No.	SE ± fertility rate	No.	Genotype	Mutation site
* * 0.00 ± 0.68 b	16	0.00 ± 0.74 a	25	GG	G > A site 1148
0.00 ± 0.18 d	3	0.00 ± 0.37 c	1	GA	
* 0.09 ± 0.72 b	11	0.07 ± 0.71 b	14	TT	C > T site 1141
0.16 ± 0.66 c	3	0.08 ± 0.75 b	11	CT	
0.00 ± 100 a	5	0.16 ± 0.66 c	1	CC	
* 0.04 ± 0.66 b	18	0.04 ± 0.76 b	24	GG	G > A site 1089
0.00 ± 100 a	1	0.04 ± 0.66 c	2	GA	
* 0.00 ± 0.69 ab	13	0.00 ± 0.73 b	20	GG	G > A site 1003
0.00 ± 0.75 b	4	0.00 ± 0.66 c	6	GA	
0.00 ± 100 a	2		-	AA	

Means with different letters various significantly.

Conclusions

The results of this study which concluded that genotypes GA intra G1148A and CC for C1141T have higher litter size and twining rate in particular recessive CC which had more values of reproductive traits reached to 1.48 born / birth, 47.97% twining rate plus 100% fertility rate so, recommended to including the individuals which carry this genotype in the selection program also producing more studies about relation between Pitx2 gene and reproductive diseases in future.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

All steps of the research achieved by the author.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was detected in accordance with the protocol authorized by the Ministry of Agriculture, head of the Agricultural Research Office in Abu-Graib region in Iraq.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The author is appreciate for helping of the Agricultural Research Office head which belong to Ministry of Agriculture.

References

1. Abd AL-Razak, M. J., Al-Saadi, B. Q. H., and Al-Anbari, N. N. (2019). Relationship between DIO2 (exon 2) gene polymorphism and some traits of growth and hormones in local Awassi sheep. Biochemical and Cellular Archives, 19(2): 3719-3723. DOI: 10.35124/bca.2019.19.2.3719.
2. Al-ani, H. A., S. Asker, A., & S. Hseen, N. (2023). The Effect Of Using Anabasis Articulate Extract And Melatonin Hormone Locally In Vaginal Sponges On The Reproductive Performance Of Local Iraqi Ewes. Anbar Journal Of Agricultural Sciences, 21(1), 214-223. doi: 10.32649/ajas.2023.179739
3. Abdulkareem, T. A., Senkal, R. H., Ahmed, J. R., and Mahmood, F. (2017). Association of transferrin gene polymorphism A I4037G and C14081T SNPs with the productive performance of Holstein- Frisian cows. International Journal of Science and Nature, 8(1): 117-121.
4. Ahmadi, M. M., Mirzaei, A., Sharifiyazdi, H., Hajibemani, A., and Ghasrodashti, A. R. (2015). Pituitary-specific transcription factor 1 (Pit-1) polymorphism and its association on milk production and some reproductive performance in Holstein dairy cows. Revue de Medecine Veterinaire, 166(5/6): 127-131.
5. Al-Samarai, W. I., Senkal, R. H. A., and Al-Khuzai, H. M. (2019). Estimation of breeding values and genetic variance depending on Lactoferrin gene polymorphism in Iraqi goat breed. Plant Archives, 19(1): 1257- 1260.
6. Al-Samarai, F. R., Mohammed, F. A., Al-Anbari, N. N., Al-Zaydi, F. H., and Abdulrahman, Y. K. (2015). BLUP Values of Birth to Weaning Growth Traits of Awassi Lambs in Iraq. Russian Journal of Biological Research, 5(3): 116-123. DOI: 10.13187/ejbr.2015.5.116.
7. Al-Barzinji, Y. M. (2022). Molecular analysis of fecgh gene in hamdani sheep breed in iraqi kurdistan region. Iraqi Journal of Agricultural Sciences, 53(1): 1-1. https://doi.org/10.36103/ijas.v53i1.1498.
8. Ali, I. M., and Ibrahim, N. S. (2019). Study the relationship between polymorphism of melatonin receptor type 1A (MTNR1A) gene with some reproductive performance, in local Iraqi goats. The Iraqi Journal of Veterinary Medicine, 43(2): 38-41. https://doi.org/10.30539/iraqijvm.v43i2.527.
9. Alkass, J. E., Hermiz, H. N., Baper, M. I., Akreyi, I. A., and Rashid, F. M. (2021). Productivity traits of local mountain goat and some factors affecting them. The Iraqi Journal of Agricultural Science, 52(4): 913-917. https://doi.org/10.36103/ijas.v52i4.1399.
10. Alkass, J. E., and Mayi, V. J. (2011). A survey study of sheep and goats raised under farm conditions. Journal of Duhok University, 14(1): 120-125.
11. Alkass, J. E., Hermiz, H. N., and Baper, M. I. (2021). Some aspects of reproductive efficiency in awassi ewes: A review. Iraqi journal of agricultural sciences, 52(1): 20-27. https://doi.org/10.36103/ijas.v52i1.1232.
12. Al-Khazrji, W. J., Al-Samari, W. A., and Ajil, H. M. (2014). The effect of some genetic and nongenetic factors on fertility and growth traits in Awassi sheep. Iraqi Journal of Agricultural Sciences, 45(3): 263-268.

13. Al- Saigh, M. N., and Alkass, J. E. (1992). Sheep and Goat Production. Dar Al-Hikma Press.
14. Angela, T., Mohsin, A. F., Nidia, M. M., Torlopp, A., and Oliveira, N. M. (2014). The transcription factor pitx$_2$ positions the embryonic axis and regulates twining. e Life. 3: 3743. https://doi.org/10.7554/eLife.03743.
15. Atoui, A., Carabano, M. J., and Najari, S. (2018). Evaluation of a local goat population for fertility traits aiming at the improvement of its economics sustainability through genetic selection. Spanish Journal of Agricultural Research,16(2): e0404. https://doi.org/10.5424/sjar/2018162-12604.
16. Bamerny, A. O., Alkass, J. E., and Barwary, M. S. (2022). Reproductive performance in response to different hormonal treatments in local goats. Iraqi Journal of Agricultural Sciences, 53(3): 604-610. https://doi.org/10.36103/ijas.v53i3.1570.
17. Dana, O. I., Mukhtar, R. H., Mohammed, M. O., and Dyary, H. O. (2021). Comparison of a rapid test with bPAG elisa in pregnancy diagnosis in cows. Iraqi Journal of Agricultural Sciences, 52(6): 1475-1481. https://doi.org/10.36103/ijas.v52i6.1488.
18. Dodds, K. G., McEwan, J. C., Brauning, R., Anderson, R. M., van Stijn, T. C., Kristjánsson, T., and Clarke, S. M. (2015). Construction of relatedness matrices using genotyping-by-sequencing data. BMC genomics, 16: 1-15. DOI: 10.1186/s12864-015-2252-3.
19. Duncan, D. B. (1955). Multiple range and multiple F tests. biometrics, 11(1): 1-42. https://doi.org/10.2307/3001478.
20. Hermiz, H. N., and Hadad, J. M. A. (2020). Factors affecting reproductive traits in several breeds of dairy cattle in Iraq. Iraqi Journal of Agricultural Sciences, 51(2): 629- 636. https://doi.org/10.36103/ijas.v51i2.990.
21. Jalal, B. J., & Alqaisi, M. R. M. (2024). Improving the production and quality of white button mushroom (Agaricus bisporus) by adding biochar and ash to the casing layer. Tikrit Journal for Agricultural Sciences, 24(1), 22–33. https://doi.org/10.25130/tjas.24.1.3.
22. Lan, X. Y., Pan, C. Y., Chen, H., Lei, C. Z., Hua, L. S., Yang, X. B., ... and Lun, Y. Z. (2007). DdeI polymorphism in coding region of goat POU1F1 gene and its association with production traits. Asian-Australasian journal of animal sciences, 20(9): 1342-1348. https://doi.org/10.5713/ajas.2007.1342.
23. Mohammed, A. B., Abdulwahid, A. S., & Raouf, S. M. (2022). Effect of Thymus vulgmus addition to the diet of laying hens on egg production, egg quality, biochemical and antioxidant parameters. Adv. Anim. Vet. Sci, 10(2), 427-433.
24. Mirzan, N. A. ., Khudhair, M. Y. ., & Rashid, R. M. (2024). Chemical And Oxidative Stability Of Lamb And Turkey Kaurma With Beeswax As A Fat Replacer During Cold Storage In Kurdistan-Iraq. Journal of Life Science and Applied Research, 5(1), 1–7. https://doi.org/10.59807/jlsar.v5i1.92
25. Nayyef, A. A., Eskandar, S. S., and Shekhoo, L. S. (2015). Study of enteritis morbidity among Neonatal Goats in Iraq. Iraqi Journal of Science, 56 (4B): 3057-3048.

26. Salam, L. A., Abdulla, A. N., and Sunkal, R. H. (2022). Relationship of LHX3 gene polymorphisms to fertility rate in local and Shami goats. Iraqi Journal of Market Research and Consumer Protection, 14(2): 19-25.
27. SAS. (2012). Statistical Analysis System Users guide. Statistical version 9.1th ed. SAS. Inst, Inc. Cary. N.C.U,S.A. DOI:10.12691/ edu.iq/index ajbr-5-1-1.
28. Torlopp, A., Khan, M. A., Oliveira, N. M., Lekk, I., Soto-Jiménez, L. M., Sosinsky, A., and Stern, C. D. (2014). The transcription factor Pitx2 positions the embryonic axis and regulates twinning. Elife, 3, e03743. https://doi.org/10.7554/eLife.03743.
29. Wang, X., Yang, Q., Wang, K., Zhang, S., Pan, C., Chen, H., ... and Lan, X. (2017). A novel 12-bp indel polymorphism within the GDF9 gene is significantly associated with litter size and growth traits in goats. Animal Genetics, 48(6): 735-736. DOI: 10.1111/age.12617.
30. Yan, H., Jiang, E., Zhu, H., Hu, L., Liu, J., and Qu, L. (2018). The novel 22 bp insertion mutation in a promoter region of the PITX2 gene is associated with litter size and growth traits in goats. Archives Animal Breeding, 61(3): 329-336. https://doi.org/10.5194/aab-61-329-2018, 2018.
31. Zhang, S., Zhao, H., Lei, C., Pan, C., Chen, H., Lin, Q., and Lan, X. (2020). Effects of genetic variations within goat PITX2 gene on growth traits and mRNA expression. Animal biotechnology, 31(2): 107-114. https://doi.org/10.1080/10495398.2018.1551229.
32. Zhao, H., He, S., Wang, S., Zhu, Y., Xu, H., Luo, R., ... and Sun, X. (2018). Two new insertion/deletion variants of the PITX2 gene and their effects on growth traits in sheep. Animal biotechnology, 29(4): 276-282. https://doi.org/10.1080/10495398.2017.1379415.
33. Zhu, H., Zhang, Y., Bai, Y., Yang, H., Yan, H., Liu, J., ... and Qu, L. (2019). Relationship between SNPs of POU1F1 gene and litter size and growth traits in Shaanbei white cashmere goats. Animals, 9(3): 114. https://doi.org/10.3390/ani9030114.