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Detection of Genetic Variation of Igf1r Gene Polymorphism and Its Relationship with Economic Traits of Goat.

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ABSTRACT

The field component of this study was conducted at the Ruminant Research Station affiliated with the Agricultural Research Department, Ministry of Agriculture, using a sample of 79 goats (32 Shami and 47 local). The laboratory phase was carried out at the Biotechnology Laboratory of the College of Agricultural Engineering Sciences, University of Baghdad, and the Advanced Scientific Laboratory for Genetic and Molecular Gene Technologies, with the aim of extracting genomic DNA. The experiment spanned from November 1, 2023, to May 1, 2024.The study focused on the IGF1R gene, specifically examining exon 12, intron 12, and exon 13, covering a 910-base-pair region. Results revealed two single nucleotide polymorphisms (SNPs) in the analyzed region: G267514A and G267782A. Analysis of the G267514A mutation in local goats identified three genotypes: wild-type (GG) at 71.1%, heterozygous (GA) at 26.67%, and mutant (AA) at 2.22%, with allele frequencies of 84% for G and 16% for A. In Shami goats, this mutation exhibited only two genotypes: wild-type (GG) at 93.75% and heterozygous (GA) at 6.25%, with allele frequencies of 97% for G and 3% for A.The study highlighted significant genetic variation between Shami and local goats regarding the G267514A mutation. This mutation influenced body dimensions, fertility rate, and milk production season length in local goats, while showing negligible effects in Shami goats. The G267782A mutation, however, showed no association with any production traits in either breed.

Key words: Body measurements, Genotypic variation, Goat genetics.

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INTRODUCTION

Goat being a small ruminant animal plays an important role in uplifting the socio-economic condition of the farmers and assures a continuous source of income (Deori et al., 2024). Another effect of these factors Heat stress led to a decrease in fertility rates and milk production in goats, confirming its negative impact on reproductive and productive performance (Adjassin et al., 2022; Danso et al., 2024; Sejian et al., 2021). Genetic maps and modern biotechnologies have provided significant opportunities to advance genetic improvement and selection processes based on genetic markers. Genetic diversity refers to the degree of genetic variation or differences among herds, populations, species, or breeds, characterized with high precision (Dodgson et al., 1997). Given the importance of genetic improvement to raise the efficiency of farm animal productivity and the inefficiency of traditional methods in genetic improvement, genetic markers were used in selection for productive traits with low heritability, economically important traits that are affected by a number of genetic sites known as quantitative trait loci,(QTL) which through them and the markers associated with them, thus genetic variation can be predicted and improved early and feasible selection programs can be developed on their basis (Abd El-Hack et al., 2018; Othman et al., 2016; Sharma et al., 2024; Talebi et al., 2022). Quantitative traits in farm animals are affected by many factors, including genetic factors, especially breed and quantitative adjective sites (Wang et al., 2017). Among these important markers are genes that have many important physiological roles, such as the insulin-like growth factor receptor 1 (IGF1R) gene is homologous to the insulin receptor gene in terms of amino acid sequences, sharing more than 50% similarity, It encodes proteins that undergo post-translational modifications to produce receptors consisting of two extracellular α subunits and two β subunits, which contain intracellular tyrosine kinase domains, Complete absence of IGF-1 receptors may result in severe or even fatal disease in humans, while less severe disruptions, such as naturally occurring abnormal mutations, lead to insulin resistance and IGF-1 resistance (Abuzzahab et al., 2003; Adams et al., 2000).IGF1R shares significant homology with the insulin receptor and activates similar signal transduction pathways (Gamaniel & Gwaza, 2017). Additionally, It can form hybrid receptors with the insulin receptor, thereby expanding its biological functions ,The binding of a ligand to IGF1R triggers auto phosphorylation of the receptor and tyrosine phosphorylation. This, in turn, leads to the phosphorylation of downstream substrates, activating two key signaling pathways PI3K/AKT/mTOR and Ras-MAPK (Slaaby et al., 2006).It plays an important role in metabolism and cell growth, it is found everywhere in animal cells and tissues, such as estrogenic cells, lymphocytes, immune B cells, endometrial cells, ovarian cells, liver cells, thyroid cells and adrenal glands (Cadoret et al., 2009; Ren et al., 2016; Yakar & Courtland, 2010). It also stimulates cell growth and proliferation and regulates apptosis (Alves et al., 2019). by mediating the transfer of metabolic signals for cell proliferation and protein synthesis in the growth hormone-like growth factor pathways (Zhang et al., 2019). Gene expression of insulin-like growth factor 1 receptor is very important for the completion of the functions of insulin-like growth factor 1 protein in cells (NCBI Gene Database, n.d.). The IGF-IR gene of goat has 21 exons with a length of 298.03 kb, and is located on chromosome 21, which encodes a polypeptide chain consisting of 1367 amino acids (Liang et al., 2010; Shen et al., 2015). It is one of the highly variable genes, which is one of the most important features of molecular markers that qualify it to be one of the genetic markers used for selection for many important economic traits (Adjassin et al., 2022).The IGFR-1 gene has a role in the development of follicle size, Increased concentration of estrogen in the follicular fluid in goats (Karaca et al., 2009). Therefore, this study aimed to detect the most important genetic variations in the IGF1R gene and their relationship to some productive traits of goat.

MATERIALS AND METHODS

The research was conducted on 79 goat (32 Shami, 45 local), with the same management and feeding conditions. Animals used were 2–5 years old, multiparous, lactating and in their first to fourth lactation Sampling: A jugular blood sample was collected from each goat using vacuum tubes with EDTA k 2 as an anticoagulant, Protein Kinase was used to isolate DNA,The pimer was designed by researchers:

forward 5'-GCTAACTTTCTTGCCTGTTTC-3'
reverse 5'-CGTATTAGTGTGCCTCTTCTC-3'

To examine the optimum annealing temperature of primer, the DNA template was amplified with the same primer pair, (Forward) (Reverse), at annealing temperatures of 55, 58, 60, 63 and 65°C. PCR amplifications were performed with 20µl volumes containing 10µl GoTaq Green Master Mix (2X); 1µl for each primer (10pmol); 6µl nuclease free water and 2µl of template DNA. PCR cycling was performed with PCR Express (Thermal Cycler, Veriti, USA) with the following temperature program: denatured at 94oC for 4 min followed by 30 cycles of denaturation at 94°C for 30 sec; annealing at 55, 58, 60, 63 or 65°C for 30 sec; and extension at 72°C for 45 sec. A final extension incubation of 7 min at 72oC was included, followed by a 10 min. Incubation at 4oC to stop the reactions. Included the were recorded. Body dimensions such as body length, high and heart girth were measured by using a tape, included the following traits Heart girth (HG) is circumferential measure taken around the chest, Withers Height (WH): This is the distance from the surface of a platform to the withers, Rump height (RH) this is the distance from the surface of a platform to the rump. Body length (BL) this is the distance from occipital protuberance to the base of the tail (Olawumi & Farinnako, 2017). Total milk yield was calculated according to equation mentioned below.

Milk yield = (one milking/day * 2) × No. of milking days

In addition to, fertility rate were calculated from equations mentioned below according to (4)

$$\text{Fertility\%} = \frac{\text{No. of doe give birth}}{\text{total no. of doe inseminated by male}} * 100$$

$$\text{Litter size} = \frac{\text{Total no. of kids}}{\text{No. of doe give birth}}$$

The DNA was extracted from frozen blood sample according to the method of by using protocol ReliaPrep™ Blood DNA Miniprep System, Promega to electrophoresis was used to confirm, as shown in the Figure (1).

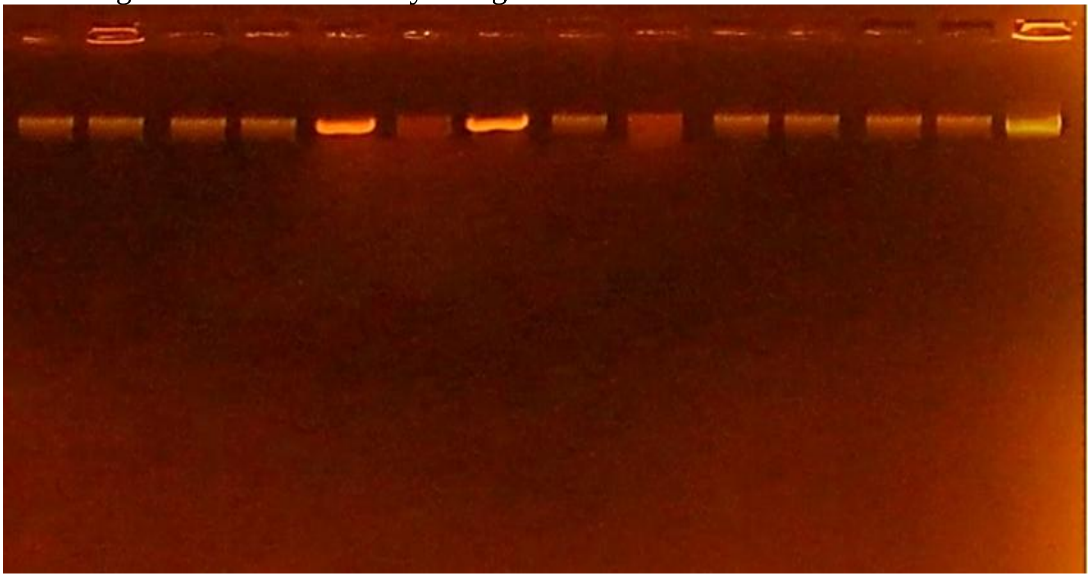

Figure1. The Electrophoresis of goat blood DNA (1% agarose gel).

The data was analyzed by Statistical Analysis System (SAS Institute, 2012) to determine the effect of IGF1R gene polymorphism under the linear model belew:

$Y_{ijkl} = \mu + G_i + A_j + B_k + e_{ijkl}$

Where: Yijk: Observed value n

μ : Overall means

Gi : Effect of IGF1R gene polymorphism

A_j : Age effect , B_k Breed effect

e_{ijkl} : Random error which distributed normally with mean = 0 and variation σ^2e.

Duncan multiple range test (Duncan, 1955) was used to detect the significant difference among means. Chi-square- χ^2 test were used to compare between the percentages of IGF1R gene polymorphism The frequency of the first allele was calculated according to the following equation (Wray & Visscher, 2015).

$$\textbf{PA} = \frac{\textbf{2 × No. of Homozygous + 1 × No. of Heterozygous}}{\textbf{2 × Total number of sample}}$$

Since: P + q = 1, the frequency of the second allele is q a = 1 – PA

The Chi-square test (Chi-square- χ2) was also used to compare the percentages of the distribution of genotypes and the allelic frequency of each of the studied SNPs.

Following PCR amplification, a 910 bp fragment spanning exon 12, intron 12, and exon 13 of the IGF1R gene was successfully generated. The amplified product was validated by 1% agarose gel electrophoresis using a 100-1500 bp molecular weight marker, confirming its precise size (Figure 2), To ensure sequence accuracy, the PCR product underwent Sanger sequencing using an ABI3730XL automated DNA sequencer (Macrogen Corporation, South Korea). The raw sequencing data, received electronically, were analyzed with Geneious software for base calling and alignment, verifying concordance with the targeted genetic region Figure (2).

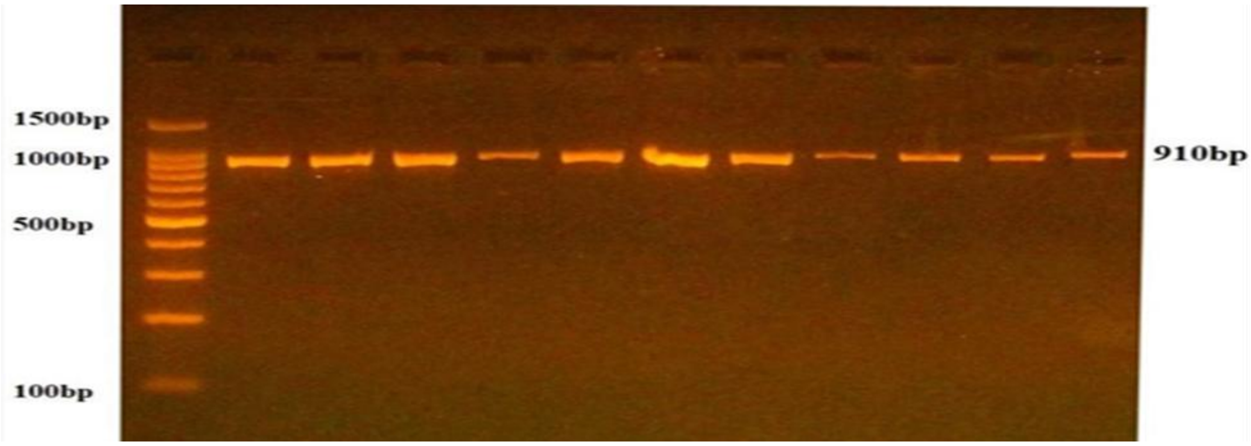

Figure 2. The result of the amplification of the studied fragmant of the IGF1R gene

RESULTS AND DISCUSSION

The results of the sequencing analysis of the studied region of the IGF1R gene showed presence of SNP in the samples of Shami and local goat in the intron 12 region, Analysis of site 267514 of IGF1R gene using Sanger sequencing, Single “G” peak indicative of a G homozygous allele, Single “A” peak indicative of a A homozygous allele, Presence of the “G” and “A” peak indicative of G/A heterozygous allele which is (G267514A) and Analysis of site 267782 of IGF1R gene using Sanger sequencing, Single “G” peak indicative of a G homozygous allele, Single “A” peak indicative of a A homozygous allele, Presence of the “G” and “A” peak indicative of G/A heterozygous allele the SNP (G267782A), as shown in Figures (3 and 4).

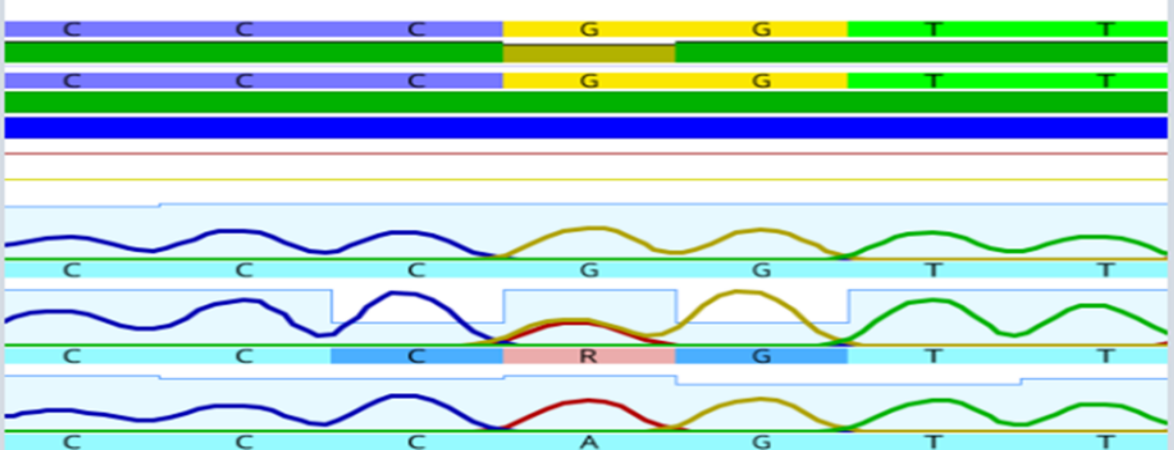

Figure 3. The sequencing of G267514A SNP in the IGF1R gene in goat

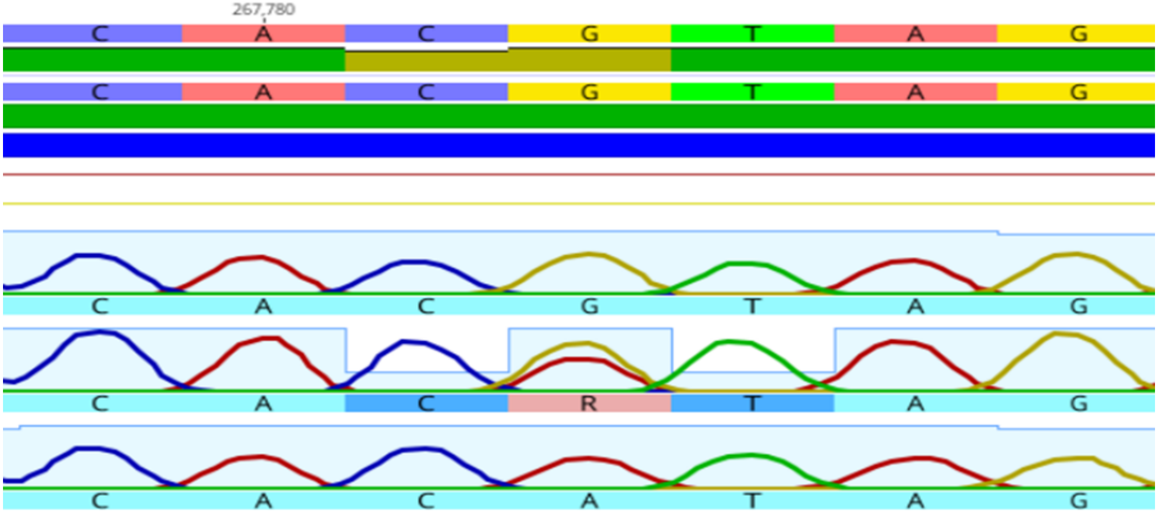

Figure 4. The Sequencing of G267782A SNP in the IGF1R gene in goat

The SNP G267514A appeared in Shami goat sample with two genotypes, wild GG and heterozygous GA (93.75 and 6.25%) respectively, while the mutant genotype AA did not appear in the Shami goat sample. The allelic frequency of the wild allele G and the mutant A were 0.97 and 0.03 respectively, and there was a high significant differences p<0.01 between genotypes distribution as well as between the allelic frequency as shown in Table 2. It is noted from the above results that the mutant allele is rare in the Shami goat sample and wild-type allele.The SNP G267514A appeared in a sample of local goat sample with three genotypes, wild GG, heterozygous GA, and mutant AA 71.11%, 26.67%, 2.22%, respectively. The allelic frequency of the wild allele G and mutant A were 0.84 and 0.16, respectively, with a high significant differences (p<0.01) between the frequency of genotypes distribution as well as between the allelic frequency as shown in Table (3), Based on these findings the wild-type G allele appears to be the most prevalent in both Shami and local goats, albeit with a significantly higher frequency in Shami goats, suggesting greater genetic stability within this breed. In contrast local goats exhibited greater genetic divers it potentially reflecting enhanced genetic resilience, these insights could inform strategic genetic improvement and selective breeding programs for goats, particularly for traits associated with this genetic locus.

Table 1. Distribution and allele frequency for the SNP G267514A in the IGF1R gene in Shami goat

Genotype	NO.	%
GG	30	93.75
GA	2	6.25
Total	32	%100
Chi-square value (χ2)	----	** 24.50
Allele		
G	frequency	
A	0.97	
Genotype	0.03	
P≤0.01)(**		

Table 2. Distribution and allele frequency for the SNP G267514A in the IGF1R gene in the local goat sample

Genotype	NO.	%
GG	32	71.11
GA	12	26.67
AA	1	2.22
Total	45	100%
Chi-square value (χ2)		52.511**
Allele	frequency	
G	0.84	
A	0.16	
(**P≤0.01)		

The results of Table (3) showed that there was no significant effect of the studied SNP G267514A on both chest circumference and body length, while there was a significant effect (p<0.05) of the SNP G267514A on both Front height and butt height in individuals that carrying the wild genotype GG (76.09, 78.12) cm compared to individuals carrying the hybrid genotype GA (73.66, 74.92) cm for both traits respectively. According to previous studies, body weight has positive correlations with body height and stature, Also individuals that carrying hybrid genotype which produced from the SNP G267514A (196.41 days) exceeded other individuals with wild genotype (171.22) days in the length of lactation period in the local goat sample, while there was no effect of this SNP was observed on both total milk production and peak, Results showed a significant effect (p<0.05) of the studied SNP G267514A on the fertility rate in females carrying the GG genotype was superior, while there was no significant effect of the SNP on the fertility rate in local goat.

The results of the analysis of the gene polymorphism of the SNP (G267782A) in the Shami goat showed that it has one genotype, which is the wild GG, at a rate of 100%, and the other genotypes did not appear in this sample. This may be attributed to the selection program in the station, and the small size of sample. given that it is the most suitable and best for survival and performance in the Shami breed. The results of the study of the genetic manifestations of the SNP G267782A show that it appears in the local goat sample with three genetic structures: the wild GG, the hybrid GA, and the mutant AA, at rates of (95.56, 2.22, and 2.22)%, respectively table

(4). The allelic frequency in the local sample for the wild allele G reached to 97% and for the mutant allele 3%. The results show highly significant differences (p<0.01) in the Chi-square values in both the comparison of the genetic structure ratios and in the comparison of the allelic frequency ratios.

Table 3. Relationship of the polymorphism of the SNP (G267514 A) to economic traits in local goat.Means with different letters within a column are significantly different from each other.

	Traits	Genotype		significant
		GG	GA	
Body dimensions (cm)	Chest circumference	77.12 ±1.05	76.58 ±1.33	N.S
	Body length	73.03 ±1.04	70.25 ±1.26	N.S
	Front height	76.09 ±0.03a	73.66 ±1.58b	*(P≤0.05)
	Butt height	78.12 ±0.91a	74.92 ±1.34b	*(P≤0.05)
Milk traits	Total milk production (kg)	162.17 ±18.96	164.51 ±30.05	N.S
	Lactation Period (day)	171.22 ±7.88 b	196.41 ±12.32 a	*(P≤0.05)
	Peak (day)	36.93 ±1.96	39.50 ±3.01	N.S
Reproductive traits	fertility%	93.75 ±0.08 a	91.67 ±0.12 b	*(P≤0.05)
	Litter size (kids/ litter)	1.31 ±0.08	1.36 ±0.15	N.S

NS: not significant

Table 4. Distribution and allele frequency for the SNP (G267782A) in the IGF1R gene in local goat

Genotype	NO.	(%)
GG	43	95.56
GA	1	2.22
AA	1	2.22
Total	45	100%
Chi-square value ($\chi 2$)	----	119.48**
Allele	frequency	
G	0.97	
A	0.03	
(**P≤0.01)		

The current results align with the significance of the IGF-IR gene in economic traits in farm animals, as indicated by previous studies. This gene has been linked to milk production in Simmental cattle (Szewczuk, 2016b). its effect on weaning weight in Angus calves (Szewczuk, 2016a). Improving meat traits in Hereford cattle (Szewczuk & Kulig, 2020). Additionally, it has been associated with milk production performance and certain growth traits in two Chinese goat breeds, where it is considered a key genetic marker for improving milk production in these breeds (Luo et al., 2019). Furthermore, the IGF-IR gene has shown a relationship with growth traits in Attappady and Malabari goats, specifically body weight at different ages, making it a valuable molecular marker for selective breeding to enhance meat production in goats (Alex et al., 2023). It has also been linked to fertility and reproductive performance in Holstein cows (Rodríguez-Borbón et al., 2023). The IGF-1R gene can be used as a genetic marker in breeding and genetic, improvement programs for certain sheep breeds through the application of marker-assisted selection (MAS) to enhance and improve meat production (Ding et al., 2022; Grochowska et al., 2021). It has an effect on growth characteristics in Akarman sheep breeds for birth weight ,It can also be used as a genetic marker for selecting growth traits in livestock (Karadag, 2022; Tang et al., 2021).

CONCLUSION

The gene IGF1R can be an important candidate gene to make as the indirect selection and partially contribute with goat improving under farming conditions and the considerable Single Nucleotide Polymorphism in this gene can related with the variance of many economical traits for domestic animals.

JOURNAL DECLARATION

The Second author (**Wasan J. Al-Khazraji**) serves as an editor for Iraqi Journal of Agricultural Sciences but was not involved in the peer review process of this manuscript beyond their role as an author. The authors declare no other conflict of interest.

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الكشف عن التنوع الجيني لجين IGF1R وعلاقته بالصفات الاقتصادية في الماعز.

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المستخلص

تم تنفيذ الجزء الحقلي من هذه الدراسة في محطة أبحاث المجترات التابعة لدائرة الأبحاث الزراعية / وزارة الزراعة، على عينة مكونة من 79 معزة (32 شامية و47 محلية)، أما الجزء المختبري فقد أُجري في مختبر التقانات الحيوية بكلية علوم الهندسة الزراعية - جامعة بغداد بالإضافة إلى مختبر التقدم العلمي لتقنيات الوراثة والجينات الجزيئية بهدف استخلاص المادة الوراثية (DNA) استمرت فترة التجربة من 2023/11/1 إلى 2024/5/1،تمت دراسة جين IGF1R وتحديدًا في الإكسون 12، الإنترون 12 والإكسون 13 بحجم 910 زوجًا قاعديًا، أظهرت النتائج وجود طفرتين نقطيتين (SNPs) في المنطقة المدروسة هما G267514A وG267782A، تم تحليل الطفرة G267514A في الماعز المحلي ظهرثلاث تراكيب وراثية البري (GG) بنسبة 71.1% ، الهجين (GA) بنسبة 26.67% و الطافر (AA) بنسبة 2.22% اذ بلغ التكرار الأليلي للأليل G 84% الأليل A 16%،في الماعز الشامي ظهرت هذه الطفرة بتركيبين وراثيين فقط البري (GG) بنسبة 93.75% و الهجين (GA) بنسبة 6.25% فقط، وبلغ التكرار الأليلي للأليل G 97% وللأليل A 3%، كشفت الدراسة عن وجود تباين وراثي بين الماعز الشامي والمحلي فيما يتعلق بالطفرة G267514A، أثرت هذه الطفرة على أبعاد الجسم، معدل الخصوبة وطول موسم انتاج الحليب في الماعز المحلي، بينما لم يكن لها تأثير يُذكر في الماعز الشامي، الطفرة G267782A لم تكن مرتبطة بأي من الصفات الإنتاجية في أي من السلالتين.

الكلمات المفتاحية: قياسات الجسم، التباين الوراثي، علم وراثة الماعز.