

Estimation of Genetic Variance Based on the Growth Differentiation Factor 9 (GDF9) Gene and its Association with Semen quality in Local Iraqi Goats

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Abstract

Growth differentiation factor 9 (GDF9) is a member of the TGF β superfamily that plays a critical role in females' ovarian follicular development and ovulation rate. However, its role in the testis has not been elucidated in Goats. Therefore, this study investigated the effects of GDF9 mutations on the quality of fresh semen in Bucks. Direct sequencing was used to screen possible SNP loci in the goat GDF9 exon one, one SNP locus site 2006 CTC > CTA (leu > leu) was identified positively in 15 local Iraqi Bucks. Analysis of variance revealed that significant effect of breeding value for concentration, individual ($p > 0.05$) movement, and Acrosome integrity, in All, so of dominance effects of volume, Individual movement, and Acrosome integrity. All traits have low genetic variation, which means that the environment varies high. This study is the first to show a significant association of GDF9 in the initiation or maintenance of spermatogenesis in male goats.

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Introduction

The population of Local goats is about 1.6 million in 2013 (FAO, 2013)-distributed all over the country, of which 12.5, 44.2, and 43.3 % are respectively in the southern, central, and northern parts of the country Iraq (Alkass and Juma, 2005). This vital genetic resource is raised primarily for meat (Ahmad, 2005; Al- Sahookie et al., 2016) and milk (Al-Azzawi, 2015; Al-Azzawi, 2017). At the same time, hair is of secondary importance. Yet, unlike dairy cattle and sheep, minimal work has been attempted to investigate the local goat's various performances and economic traits in Iraq. Furthermore, the goat population of the country had diminished by 13.5% percent over the period from 1999 to 2013 (FAO, 2000). Goats are also an important genetic source of genes that have been adapted thousands of years to the local Iraqi conditions used in mating and cross-breeding with foreign hybrid or pure breeds

that have good production efficiency to produce goats that are tolerant to environmental conditions with good production providing meat and milk to consumers (Khalil and Al-Azzawi, 2018). Fertility is the ability of the herd to reproduce for both males and females (Al Douri, 2002). Goats are mainly raised to produce meat and milk, therefore has increased in improving their production efficiency in the conditions of their massive population growth, which led specialists to conduct many types of research in the fields of breeding and improvement by using selection methods to improve quality traits, and crossing (Kirton and Warmington, 1990; Coffey *et al.*, 2011).

Molecular genetics techniques have been used to take advantage of the genes that resist the climatic conditions related to the reproductive traits possessed by local goats to produce individuals with high productivity in the future (Al-Khazraji et al.,2020). Genetic improvement programs

work based on knowing the breeding value of the trait to be improved so that the desired animals can be exploited about the genotypes to obtain better genetic improvement (Neopane et al., 1998; Cassell, 2001). Through molecular genetics, individual genes have been observed, that is, genes that have a significant effect, such as GDF9, which improves the productivity of goats through its contribution to molecular reproduction (Galloway et al., 2002; Hanrahan et al., 2004).

In addition, the autosomal GDF9 gene plays a significant role in controlling follicular growth by affecting the functioning of granulosa cells (Otsuka et al., 2011), and mammalian fertilization involves a series of well-organized cell-cell interaction steps between gametes, as well as between sperm and somatic cells in the male and female reproductive system (Glistler and Knight, 2003). This study aims at the effect of the GDF9 gene and its association with semen quality of local Iraqi Bucks.

Materials and Methods

Ethical approval

The Scientific Ethical Committee of the animal production department College of Agriculture University of Diyala, Iraq, approved this study (Approval no: 2021 / 3/1 5).

This study was conducted in the animal field of the Department of Animal Production at the College of Agriculture / University of Diyala to verify the presence of some genetic markers that affect some reproductive traits (semen quality and testicular dimensions). The DNA extraction process was carried out according to the steps followed by the company attached to the extraction kit (Kit) of the Taiwanese company FAVORGEN. The efficiency of the extraction process was detected by reaching the complete DNA by migrating the samples through an agarose gel. The primers were prepared to know the phenotypic polymorphism of the genes and for the molecular detection and mutations of the GDF9 gene.

Table 1. Primers sequence used in the study

Primer sequence	Primer Name	Amplicon size (pb)	Name gene	Annealing Temp C
GAAGACTGGTATGGGGAAATG F -	Exon 1	462	GDF9	61
CCAATCTGCTCCTACACACCT R -				
Based on gene sequences available in the gene bank database (Gene bank) Sequence number				

Statistical analysis

The additive (a) and dominance (d) and allele substitution and breeding values, and Genetic variation were estimated according to the equation of 1996. (Falconer and Mackay).

1- The average effect of allele $G = \alpha G = q [a+d (q - p)]$

2- The effect of substitution of alleles $\alpha = \alpha G - \alpha A$

3- The breeding values : $GG = 2 \alpha 1$, $GA = 2pqd$, $AA = 2 \alpha 2$

4- The dominant deviations: $GG = 2q^2 d$, $GA = 2pqd$, $AA = 2p^2 d$

5- The average effect of allele: $A = \alpha A = -q [a+d (q - p)]$

6- The different variances: $VA = 2pq \alpha^2$, $VD = 4 p^2 q^2 d^2$, $VG = VA + VD$

Genetic redundancy and genotype frequency were measured, significant differences in genotypes were measured using the chi-square test, and differences were not significant, i.e. (clan is balanced).

Results and Discussion

In our study, sequences in Growth differentiation factor gene (GDF9) exon one were genotyped in local Iraqi Black. Their effects on semen quality, volume, concentration, Individual activity, and Acrosomal antacy were genotyped and estimated

The genotypic and allelic frequencies were summarized in Table (2). Based on a chi-square test. At locus rs, 100860859 > A, the frequency of CC genotype (0.67) was higher than Bucks one with genotype CA (0.27) and AA (0.06), and the allele frequency were 0.8 and 0.2 for C and A. The values of the chi-square test (χ^2) showed that all genotypic frequencies in the population were in Hardy Weinberg Equi Librium ($p > 0.05$), indicating the selection pressure on this site the population was not too powerful.

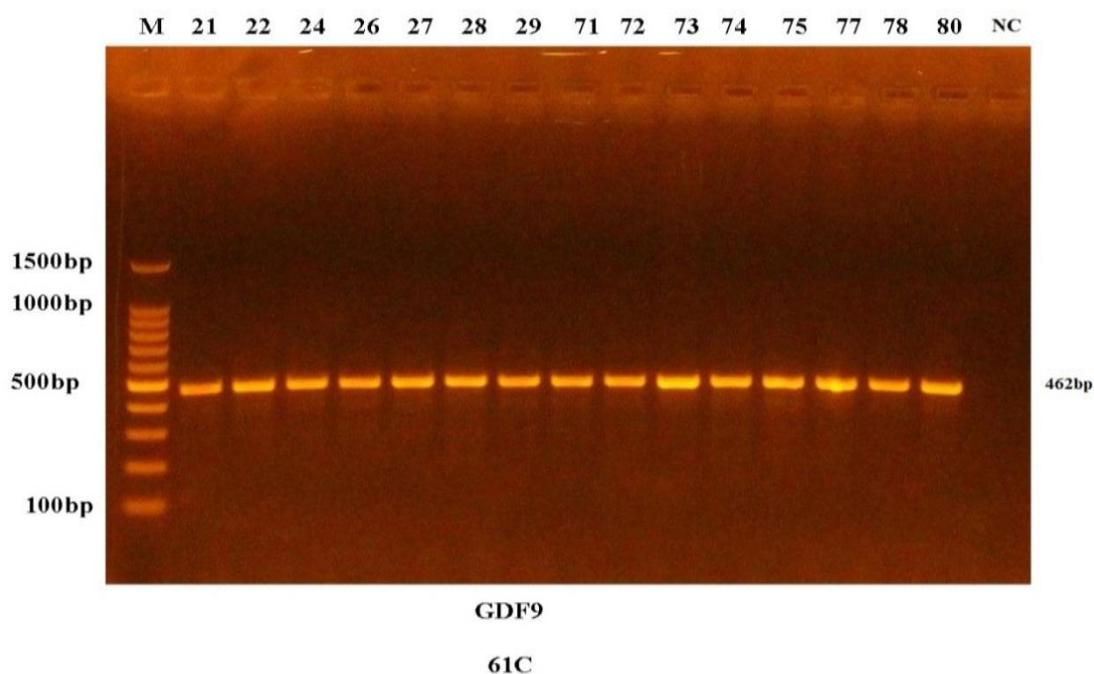


Fig 1. Electro transfer of Exon1 PCR product to GDF9 Gen at 462 bp in goats, M (Marker)

Table 2. Number and proportion of genotypes of the GDF9 rs 2006 C/A gene in a study of local Iraqi Bucks

Genotype	Replication of genotypes	Percentage %
CC	10	0.67
CA	4	0.27
AA	1	0.06
100%	15	SUM
0.42 NS	---	The value of chi-square χ^2
		Allelic Frequency
	0.8	C
	0.2	A
P > 0.05		

In table 3 there was no difference in the mean of their genotypes for volume, sperm concentration, Individual movement, and Acrosome Integrity.

Table 3. Effect of the GDF9 rs 2006 C/A polymorphism on semen quality traits in local Iraqi Bucks (mean \pm SD)

Traits	Genotype		
	g. 2006 CC	g. 2006 CA	g. 2006 AA
Vol (ml)	0.95 \pm 0.03 a	0.87 \pm 0.04 a	0.88 \pm 0.14 a
Scon($\times 10^9$ ml)	1.77 \pm 0.02 a	1.75 \pm 0.13 a	1.72 \pm 0.09 a
MoT(%)	83.39 \pm 1.50 a	82.12 \pm 2.55 a	81.66 \pm 6.50 a
A . Integrity	84.07 \pm 0.56 a	83.72 \pm 0.98 a	84.08 \pm 3.16 a

The results of Table (4) showed that the average substitution of the A allele in place of the C allele for this study is (0.3). The mean of the substitution of the C allele in place of the A allele was (-0.3), which indicates that the importance of selection is in the direction of the A allele and through the results we conclude that by using genetic markers to estimate the breeding value works to increase the genetic return as a result of reducing the generation , as for the average substitution of the A allele in place of the C allele for interval sperm concentration and the average substitution of the C allele in place of the A allele amounted to (-0.063), which indicates that

the importance of selection is in the direction of the A allele. Through the results of the current study, we conclude that the use of genetic markers to estimate the breeding value increases the genetic yield due to decreasing the generation range lower cost. The results also indicate that the average substitution of the A allele for the C allele is (0.0124) for the volume, and the mean of the C allele substituting for the A allele (-0.0048), where the selection is in the direction of the C allele. As for the integrity of the acrosome, the results showed that the average substitution of the A allele in the place of the C allele (0.228), and the average substitution of the C allele for the A allele

was (-0.228), which means that the selection is in the direction of the C allele.

Table 4. The average gene effect and the average impact of gene substitution of alleles for volume, concentration, Individual, Acrosome integrity for rs 2006 C/A for GDF9

Trait	Alleles	Average gene effect	The average effect of gene substitution
individual movement	C	- 0.06	- 0.3
	A	0.24	0.3
Sperm concentration	C	- 0.0126	- 0.063
	A	0.0504	0.063
Volume	C	0.0124	- 0.0172
	A	- 0.0048	0.0172
Acrosome integrity	C	0.0456	- 0.228
	A	- 0.1824	0.228

Table (5) shows the volume of ejaculate for the individuals carrying the CC genotype recorded the highest value compared to the CA and AA genotypes (0.95, 0.87, and 0.88, respectively). The CC genotype also scored the highest breeding value. As for the dominance deviation, the AA genotype is higher than the two genotypes (CC) and (CA). This result indicates that the (CC) genotype is the best and that the genetic variance (VG) is high compared with the dominant variance (VD). Additive variance (VA) selection for (CC) genotype is preferable; for sperm concentration compared to the CC and AA genotypes (1.825, 1.768, and 1.72, respectively). Also, the genotype AA recorded the highest breeding value, as for the CA genotype, which recorded the highest value for dominance deviation compared to the two genotypes (CC and AA). This result indicates that the (CA) genotype is the best and because the dominance variance (VD) is high compared with additive variance (VA), selection for the AA genotype is preferable.

But regarding the individual's movement carrying the CC genotype value, the highest

value compared to the CA and AA genotypes (82.97, 82.1, and 81.6, respectively). The AA genotype also recorded the highest breeding value and the highest value for dominance deviation compared with the two genotypes (CC) and (CA). This result indicates that the AA genotype is the best. Because the genetic variance (VG) is high compared with the dominant variance (VD), the selection For genotype (AA) is preferable. The results also the integrity of the acrosome recorded for the genotype AA, the highest value compared with the genotypes CC and CA (84.08, 83.70, and 84.06, respectively). As for the breeding value, the CC genotype is higher than the genotypes (AA and CA) for the breeding value. As for the dominance deviation, AA's genotype is high compared to the two genotypes (CC and CA). These results also indicate that the AA genotype is the best, the value of the genotypic variance (VG) is high compared to the additive variance (VA), and the selection for the genotype CC is the best.

Table 5. Breeding value, dominance deviation, and genetic variations genotypes in GDF9 gene rs 2006 C/A for trait volume, concentration, Individual movement, Acrosome integrity

Volume (V) ml							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	10	0.95	0.0248	0.0036	0.00153	0.00020	0.0017
CA	4	0.87	0.0076	-0.0144			
AA	1	0.88	-0.0096	0.0576			
Concentration (Con) ×10 ⁹							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	10	1.768	-0.0252	0.0064	0.00018	0.00067	0.00085
CA	4	1.825	-0.063	0.0259			
AA	1	1.72	0.1008	-0.1036			
Individual movement (Im) %							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	10	82.97	-0.12	0.0148	0.1501	0.0035	0.1536
CA	4	82.1	0.18	-0.0592			
AA	1	81.6	0.48	0.2368			
Acrosome Integrity (AI) %							
Genotype	No	Mean	BV	DD	VA	VD	VG
CC	10	84.06	0.0912	0.02976	0.0166	0.0141	0.03
CA	4	83.70	-0.1368	-0.11904			
AA	1	84.08	-0.3648	0.47616			
BV: Breeding value, DD: Dominance deviation, VA: Additive Variance, VD: Dominance variance, VG: Genetic variance							

Conclusion

The results showed that sperm concentration and individual motility are essential traits that have aggregate variation and can be passed on to the offspring's generation and benefit from it.

Conflict of interest

The authors declare that they have no competing interests.

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