Effect of Single nucleotide polymorphism T > C rs426142414 located in B4GALNT gene on lambs livability and litter size in two Iraqi sheep breeds

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Abstract

The current study was designed and done in private farm located in The study was done at private station located in the Al-Shomali district in the eastern province of Babylon – Iraq sheep breeds (Awassi and Noaimi). DNA was extracted to investigate the effect of Single nucleotide polymorphism T>C rs426142414 located in B4GALNT gene on lambs livability and litter size. Results showed a significant effect ($P \leq 0.01$) of on livability, the highest rate was recorded in lambs resulted from ewes with wild genotype while the lowest rate was noticed in lambs resulted from ewes with mutant genotype namely, 94 and 74% respectively. Results showed a significant effect ($P \leq 0.01$) of the SNP on livability of Noaimi lambs in lambing stage , the highest rate was recorded in lambs resulted from ewes with hetero genotype namely, 96%. Litter size was affected significantly by the SNP in the two breeds, the highest litter size was recorded in Awassi ewes with wild genotype (1.5 lamb / litter) while the highest litter size was recorded in Noaimi breed with hetero genotype (1.4 lamb / litter).

Key words: B4GALNT2 gene , Iraqi sheep, livability and litter size.

Introduction:

In Iraq , there are many domestic breeds of sheep which originated through long time and well adapted to poor pastures and extravagance of environmental circumstances such as Awassi, Arabi, Noaimi and Karadi. The Iraqi breeds differs in morphological and physiological traits according to surrounding environment. Awasi breeds is characterized the best sheep breed among others and the Noaimi breed is a sub group of Awassi and its originated by farming improving for a long time by framers or shepherds [1].

Markers assisted selection (MAS) nowadays began a very efficient and reliable tool to improve the low performance of local domestic animals in most of world countries and this helped by the huge developing of biotechnologies and genetic science through last decades [2][3] .

B4GALNT gene is one of many genes responsible directly of fecundity in sheep as well as other domestic animal species and many single nucleotide polymorphism were determined in this gene and related strongly with reproductive performance in sheep. B4GALNT in sheep located in 11th chromosome and its contains from 65238 nitrogen base pair and then divided in to 14 exons and 13 introns [4].

The gene is also known FecL due to existence of famous mutation located in Lacaune French sheep breed led to high prolificacy and increase of liter size [5]. Many studied referred that the B4GALNT2 gene is one of essential fecundity genes and it encoded to many glycoproteins (enzymes) which necessary for saccharides metabolism [6],[7].

The real meaning of use B4GALNT2 in this study is to searching for the genetic effect of single nucleotide polymorphism of this gene and determine the effect on livability and litter size of lambs in local sheep to employing or harnessing the information in selection program and improving enhancement.

Materials And Methods:

Experiment animals and location:

The study was done at private station located in the Al-Shomali district in the eastern province of Babylon – Iraq on samples consisting of 100 blood samples taken from 50 Awassi and 50 Noaimi ewes and lambs' weights were also measured at lambing. The genetic analyses (Laboratory part of the experiment) of blood samples were conducted at the laboratories of the Scientific Progress Company for Molecular Genetics / Al-Harithiya / Baghdad to separate the genetic material DNA and determine the genetic structures (Genotype).

Feeding and management:

Quality and quantity of animal's diets were different according the season and availability, such as clover, vetch, forage corn, and yellow corn etc. The green feed was provided from clover or yellow corn stalks or vetch or any available forage crop. The system used at the station is to use natural crevice, and a specialized veterinarian is consulted to ensure the insemination operations and score information about each ewe so that the veterinarian can treating ewes which suffering from sexual problems, pregnant ewes are deposed in sheds designated for this intend until the time of delivery in order to vindicate them from overcrowding on feed and to burke exposure to harm, which leads to slink.

Blood samples and DNA extraction:

10 ml of blood was ingather from the jugular vein of each lamb in a collection tube containing EDTAK2 anticoagulant spawn by Promega company, and transferred in a coolant to the laboratory for freezing sustentation at - 4°C and immediate processing. DNA was recovered from blood samples [8] of lambs for the purpose of molecular testing of the PPP1CC gene. DNA was extracted from blood using the kit according to the guidance provided by Promega company.

Primes design:

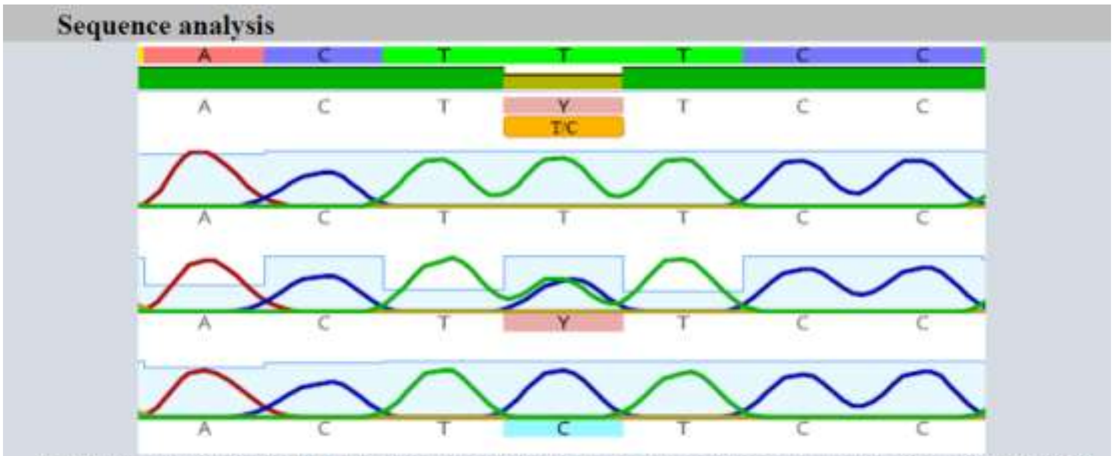
A special primer was designed to amplify the target site of B4CALNT2 gene:GAGTCCACAGTTCCGTTTAG andACTGTCAAAGAGGGAGATAGA forward and reverse respectively with annealing temperature about 55 c° and the product size was 1616 bp.

Statistical analysis:

Analyses of variance was pointed by using statistical analyses system programming computer [9]. Analyses of variance procedure according completely randomized design (C.R.D.) ONE WAY ANOVA and Duncan multiple range essay Duncan multiple range [10] was followed to determine the significance effect of several genotypes (mutant, hetero and wild) on lambs liveability and litter size in both Awassi and Noaimi sheep breeds.

Results and discussion:

The results showed a single nucleotide rs426142414 of B4GALNT2 gene in this site which alter the nitrogen base thiamin to nitrogen base cytosine (Fig-1). The SNP lead to three genotype : TT (wild), TC (hetero) and CC (mutant)



Analysis of rs426142414 SNP of B4GALNT2 gene using Sanger sequencing. Single "T" peak indicative of a T homozygous allele. Single "C" peak indicative of a C homozygous allele. Presence of the "T" and "C" peak indicative of T/C heterozygous allele.

Fig-1:analyses of rs426142414 of B4GALNT2 gene.

Results showed that the distribution of sample was differ significantly according wild and mutant alleles (Table-1) . In Awssi breed the rate of TT , TC and CC groups were 66,28 and 6% respectively while in Noaimi breed, the rate of groups were 48,30 and 22% respectively. The allele frequency of wild allele(T) was 0.80 and 0.63 for Awassi and Noaimi breeds respectively while the mutant allele frequency (C) was 0.20 and 0.37 for Awassi and Noaimire sportively.

Table -1:Distribution and allele frequency according rs426142414 SNP

Chi -square	%	No.	Genotype	Total No.	Breed
	66	33	TT		Awassi
45.688**	28	14	TC	50	
	6	3	CC		
	48	24	TT		Noaimi
14.760**	30	15	TC	50	
	22	11	CC		
	C (0.20)	T (0.80)	Awassi	Allele frequency	
	C (0.37)	T (0.63)	Noaimi		

Results represented in Fig-2 revealed a significant effect (P≤ 0.01) of on livability of Awassi lambs in lambing stage , the highest rate was recorded in lambs resulted from ewes with wild genotype (TT) while the lowest rate was noticed in lambs resulted from ewes with mutant genotype (CC) namely, 94 and 74% respectively while the livability of lambs resulted from hetero genotype was 90%.

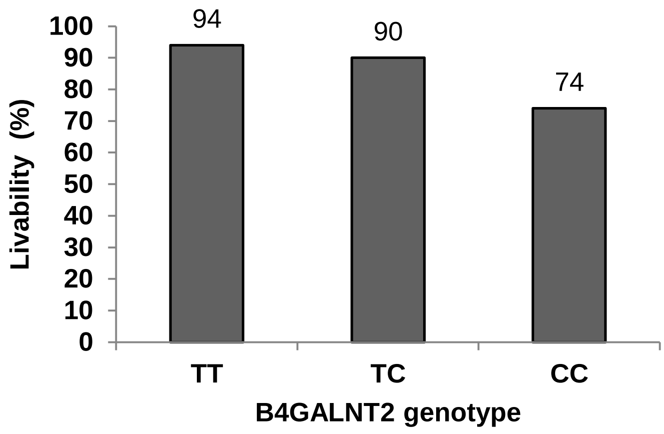


Fig 2: effect of B4GALNT2 (rs426142414) on livability in Awassi

Results represented in Fig-3 showed a significant effect (P≤ 0.01) of rs426142414 on livability of Noaimi lambs in lambing stage , the highest rate was recorded in lambs resulted from ewes with hetero genotype (TC) while the lowest rate was noticed in lambs resulted from ewes with wild genotype (TT) namely, 96 and 90% respectively while the livability of lambs resulted from mutant genotype (CC) was 92%

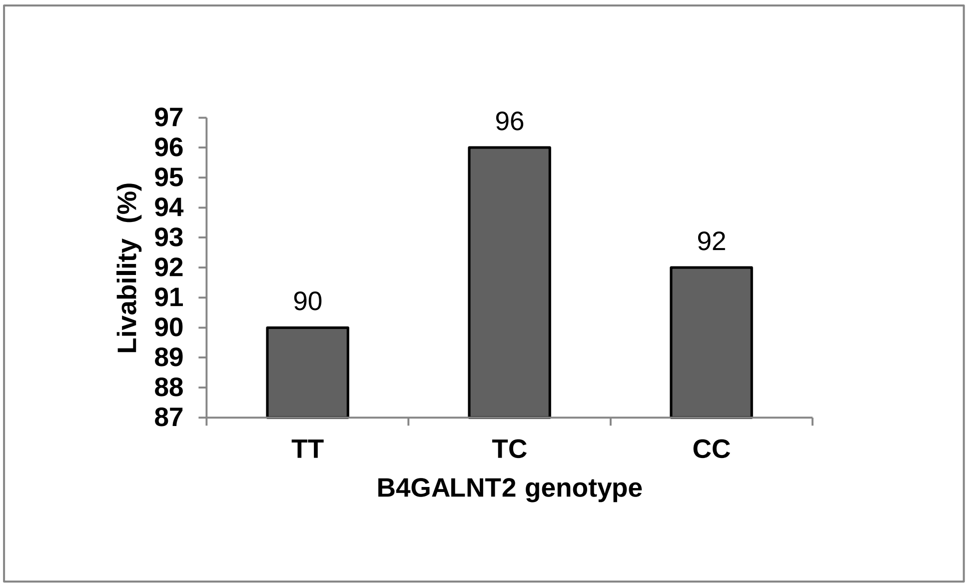


Fig 3: effect of B4GALNT2 (rs426142414) on livability in Noaimi

Results of Awassi breed sheep showed that the litter size number differ in both ewes with wild and hetero genotype significantly (P≤ 0.01with the ewes with mutant genotype (Fig-4), the highest litter size was in ewes with TT genotype (1.5 lamb / litter) while the lowest number of litter size was recorded in mutant group (1.0 lamb / litter).

In Noaimi sheep breed, results reported that the litter size differ significantly (P≤ 0.05) among ewes groups according its genotypes (Fig- 5). Litter size number was differed significantly in ewes with hetero genotype (TC) and its about 1.4 lamb / litter while no significant difference between ewes with wild or mutant genotype in litter size and the two groups were equal (1.3 lamb / litter for both).

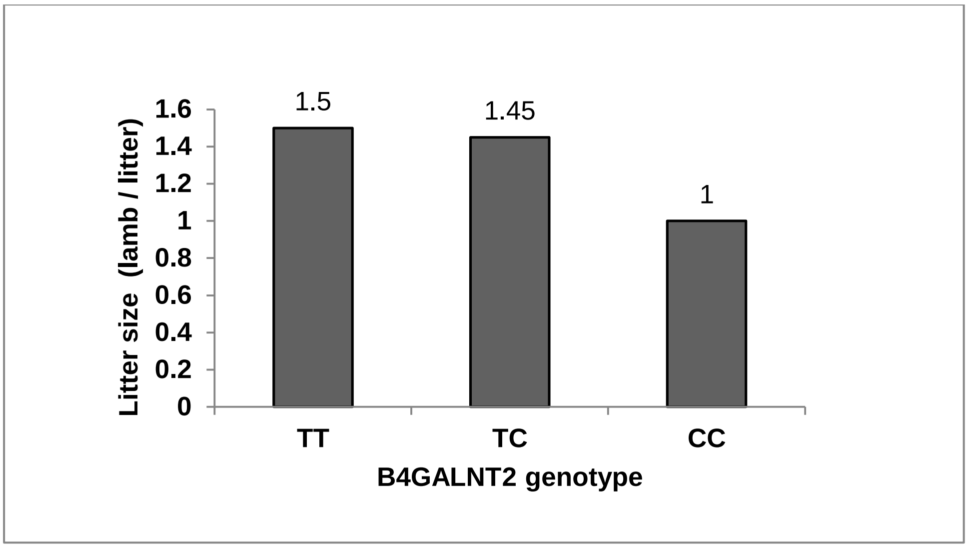


Fig 4: effect of B4GALNT2 (rs426142414) on litter size in Awassi

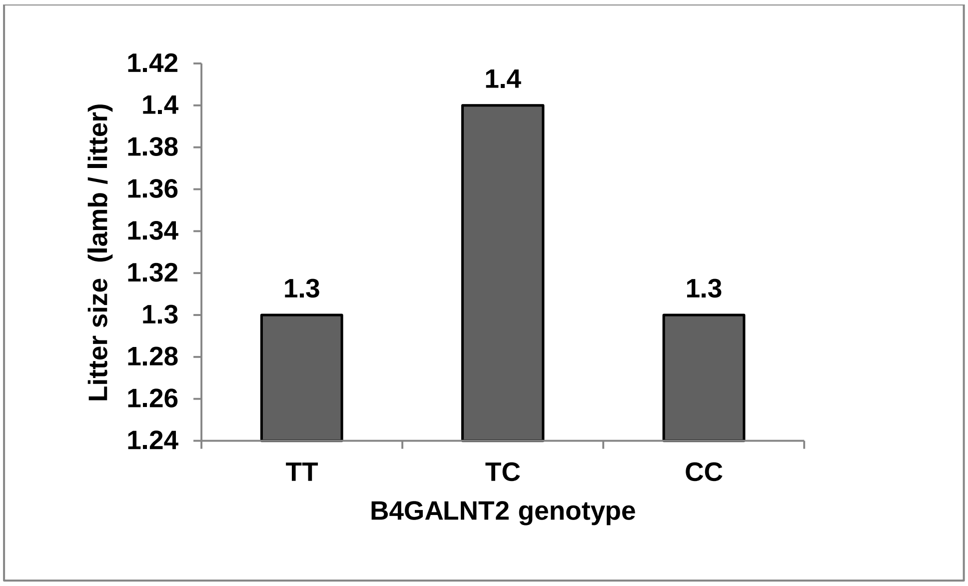


Fig 5: effect of B4GALNT2 (rs426142414) on litter size in Noaimi

Results were agreed and similar with many past studies about the importance of B4GALNT2 gene roles in reproduction and production performance in mammals,[11] recorded that the gene expressed an important protein contribute with many proteins to enhance lactose synthesis in mammary gland and the B4GALNT2 protein improve the blood function which finally reflex positively on productive and reproductive traits in mammals. The results also similar with those of Yang et.al. (2021) who reported that the B4GALNT2 protein is related tightly with the functions of FUT protein family like FUT8 and FUT6 and determined a single nucleotide polymorphism in the B4GALN2 gene (74522417 C>T) which mainly effect on growth and development in mammals. The results were similar with those of [12],[13] who determined seven important SNPs on 99 experimental sheep and all these SNPs effected significantly on reproductive performance.

reported that the B4GALNT2 protein is contribute with other similar proteins such as B3GALT1 and B4GALT3 to improve. reproduction system functions, increase litter size and enhance the immune system in goat.

Many studied indicated that the B4GALNT2 gene is a major candidate gene to improve reproductive traits such as litter size and prolificacy, recorded 25929884A>T and 25935026C >T SNPs related strongly with litter size in Mongolia and Ujimoqin sheep.

The current results agreed with the results of [14],[15] who mentioned to many SNP in this gene related with fecundity in Algerian sheep breed.

In conclusion, the B4GALNT2 gene is a good candidate gene to increase liter size and enhance livability of lambs and if we exploit the genetic variance and chose the effective alleles which resulted from mutations accrued in this gene we can improve the domestic animals to reach to super animals after a few generations.

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