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Effect of TNF-α gene polymorphism on fertility insufficiency in Holstein cows

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***Abstract*---**The current study was conducted in private station located in the middle of Iraq by using 100 blood samples from 100 Holstein cows to isolate DNA and determine the genotypes of TNF-α gene in tow regions on reproductive performance. Results showed two alleles with co-dominance and three genotypes was detected in 1st position (4th exon), the allele frequency was 0.695 and 0.305 for A and C allele respectively. The allele frequency in 2nd region (Flank region) was 0.64 and 0.36 for A and G allele respectively. Results showed a significant effect of gene polymorphism in 4th exon on retained placenta rate which differed significantly (P≤0.01) among cow groups, the highest rate was recorded in cows with cows with AC genotype about 0.24. A significant effect of gene polymorphism in 4th exon on ovulation weakness rate, the highest rate was recorded in cows with cows with CC genotype about 0.12. Parturition difficulties rate differed significantly (P≤0.01) , the highest rate was recorded in cows with mutant genotype namely,0.18. Results showed a significant effect of gene polymorphism in flank region, retained placenta rate was differed significantly (P≤0.01) among cow groups, the highest rate was recorded in cows with GC genotype about 0.20. The highest rate of ovulation weakness was recorded in cows with wild genotype (CC) about 0.15 while the parturition difficulties differed significantly (P≤0.01), the highest rate was recorded in cows with wild genotype also namely, 0.20.

***Keywords*---**TNF-α gene, polymorphism, Holstein cows.

Introduction

Reproductive traits are considered very important traits to increase production and health in dairy cows because the success of any project and obtained economic profit is depend on the reproductive efficiency (Getahun et.al., 2019).

The increase of genetic selection for milk yield increasing lead to weakness of reproductive performance and appearance of many reproductive problems in dairy cattle because the milk production traits and reproductive traits are opposite genetically therefore, the researchers in last decades focuses on making a balance between the two types of this traits (Crowe et.al., 2018).

The results of past studies referred that the optimal range of reproductive parameters is difficult to determined exactly because the wide and multiple factors that effect on it such as genetic factors , environmental factors in addition to fixed factors such as age for example (Walish et.al., 2018). Tumor necroses factor alpha (TNF-α) is one of candidate gene that might possibly effect on reproductive traits through the single nucleotide polymorphism (SNPs) as the rare past study mentioned (Silva et.al., 2020) , therefore the current study aimed to investigate the effect of these SNPs on reproductive problems or difficulties and exploit the results as indicator to improve the cow reproductive efficiency to decrease the economic loss as possible in Holstein cows.

Materials and Methods

The current study was conducted in Taj –AL-Nahrain private station located in AL-Daggara about 20 Km north east of AL-Dewanya province center by using 100 samples of blood in addition to genetic analyses in Middle east labs which located in Baghdad (AL-harithya) during the period 2021 – 2022 in aim to isolate DNA and determine the genotypes of tumor necrosis factor gene (TNF-α) in tow position including the promoter region.

Blood samples were withdrawn from uterine vein through milking and DNA was extraction according the procedure of (Samprook and Russel, 2001) , the kit was supplemented from Promega company. Two primers were used to amplified and detection the target regions, the first region was located in 4th exon and the primer was: 5-GGG TGA CTT GCT CTA ACA CTC ATC- 3 (Forward) and 5'-AGG CCT CAC TTC CCT ACA TCC CTA-3 (Reverse) while the second region was located in flank region and the primer was : 5-TCCAAGGCTGGGGACTAGAG 3' (Forward) and 5-AGGGGCTGAAAGTAACGCT- 3 (Reverse). Three reproductive difficulties rate (problems) were measured: retained placenta, ovulation weakness and parturition difficulties for all cows (100 cows in 3rd parity).

Statistical analysis: Data were analyzed using SAS (2012) computer and Chi-square test was used to determine the significant differences among phenotypes:

$$X^2 = \sum \frac{(Observed\ No. - Expected\ No.)^2}{Expected\ No.}.$$

Results and Discussion

Results showed a significant difference of sample distribution according to alleles resulted from single nucleotide polymorphism in both position (Table – 1). Two allele with co-dominance and three genotypes was detected in 1st position (exon - 4), the allele frequency was 0.695 and 0.305 for A and C allele respectively while the rates of genotypes were 0.58 , 0.23 and 0.19 for AA, AC and CC respectively.

Table -1: Distribution of genotypes and allele frequency of TNF-α gene (exone- 4 and flank region)

Genotype (exone -4)	No.	%	Allele frequency	
AA	58	0.58	A	C
AC	23	0.23	0.695	0.305
CC	19	0.19		
X^2	27.910**			
Genotype (Flank region)	No.	%	Allele frequency	
CC	44	0.44	A	G
CG	40	0.40	0.64	0.36
GG	16	0.16		
X^2	13.909**			

**(P≤0.01)

Results showed a significant (P≤0.01) of allele and genotypes distribution in 2nd region of TNF-α gene (flank region) , the allele frequency was 0.64 and 0.36 for C and G allele respectively while the rate of genotypes were 0.44, 0.40 and 0.16 for CC, CG and GG respectively. Results showed a significant effect of gene polymorphism in 4th exon on reproductive problems in Holstein cows (Table-2). Retained placenta rate were differ significantly (P≤0.01) among cow groups, the highest rate was recorded in cows with hetero genotype (AC) about 0.24 day while the lowest rate was in cows with wild genotype (AA) about 0.05.

Table -2 effect of TNF-α gene polymorphism on reproductive difficulties in Holstein cows

Genotype (4th exon)	Reproductive problems (%)		
	R.P	O.W	P.D
AA	0.05	0.08	0.11
AC	0.24	0.10	0.03
CC	0.21	0.12	0.18
Significance	**	*	*

R.P: retained placenta, O.W: ovulation weakness, P.D: parturition difficulties **: P≤0.01 , * :P≤0.05

Results showed a significant effect (P≤0.05) of genotype on ovulation weakness rate , the highest rate was noticed in cows with mutant genotype (0.12) while the lowest rate was recorded in cows with wild genotype (AA) about 0.08. Parturition difficulties differed significantly (P≤0.05) according TNF-α gene polymorphism in 4th exon, the highest rate was recorded in cows with wild genotype namely,0.18 while the lowest period was noticed in cows with hetero genotype namely, 0.03.

Results showed a significant effect of gene polymorphism in flank region on reproductive performance in Holstein cows (Table-3). Retained placenta differed significantly (P≤0.01) among cow groups, the highest rate was recorded in cows

with hetero genotype (CG) about 0.20 while the lowest rate was in cows with wild and mutant genotypes (CC and GG) about 0.15.

Table-3: effect of TNF-α gene polymorphism on reproductive traits in Holstein cows

Genotype	Reproductive problems (%)		
	R.P		
CC	0.15	0.15	0.20
CG	0.20	0.11	0.09
GG	0.15	0.04	0.03
Significance	**	*	*

R.P: retained placenta, O.W: ovulation weakness, P.D: parturition difficulties **: P≤0.01 , * :P≤0.05

Results showed a significant effect (P≤0.05) of genotype on ovulation weakness rate, the highest rate was noticed in cows with wild genotype (0.15) while the lowest rate was recorded in cows with mutant genotype (GG) about 0.04. Parturition difficulties rate differed significantly (P≤0.05) according TNF-α gene polymorphism in flank region, the highest rate was recorded in cows with wild genotype namely,0.20 while the lowest rate was noticed in cows with mutant genotype (GG) namely,0.03.

Many past studies referred that the single nucleotide polymorphism effect significantly on reproductive activity and problems and the current results came harmonic with those results, Kawasaki et.al. (2014) mentioned that the TNF-α gene polymorphism effect significantly on cows reproduction especially on while Sakumoto et.al. (2014) referred that the single nucleotide polymorphism of this gene lead to significance variance in many of reproductive parameters in dairy cattle. Mao et.al. (2012) and Bojarojć-Nosowicz et.al. (2015) showed that many single nucleotide polymorphism in promoter region of TNF-α gene effected significantly on reproduction performance in cattle in agreement with Lendes et.al.(2021) who proved the same results. In conclusion, the results of the current study are considered a good evidence to relationship of TNF-α gene polymorphism with the reproductive efficiancy in dairy cows so it can useful as a candidate gene to be a genetic marker to indirect selection to improve the reproductive traits but this fact is need more than studies and a big data to prove confidently in future.

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