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ASSOCIATION OF THE GENETICS POLYMORPHISM IN CYP19-*PVULL* GENE WITH SOME PRODUCTIVE AND REPRODUCTIVE TRAITS OF DAIRY COWS

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Abstract

This study was executed at Al-Salam Station for milk production, Private Sector, in addition to the laboratory of scientific progress for molecular genetics analyzes, to determine the genetics polymorphism in CYP19-*Pvull* gene. Besides, studying its relationship to some productive and reproductive traits in Holstein cows (40 cows), in addition to extraction ratios for distribution of the genetics polymorphism in Holstein cows and allele frequency, and the following are the most important results obtained. The distribution ratio of the genetic polymorphism of the studied position in CYP19-*Pvull* gene differed significantly ($P<0.01$) in the studied cow's sample, as they reached 87.50 and 12.50 % for each of the two genetics polymorphism AA and AG respectively, and allele frequency were 0.94 and 0.06 for both A and G alleles respectively. The results of the current study showed that there were no significant differences between the genetics polymorphism of the studied position in the CYP19-*Pvull* gene and the studied productive traits of Holstein cows (total milk production, lactation period, the period from birth to the peak of production, and the length of the peak of production). As well as, the ratios of milk components (protein, fat, lactose, non-fat solids, the specific gravity of milk). As for the relationship of the genetic polymorphism of the studied position in the CYP19-*Pvull* gene and the studied genital traits of the Holstein cows, the results showed that the services per conception and the days open were highly influenced significantly ($P <0.01$) by the difference in the genetics polymorphism of the studied position in the CYP19-*Pvull* gene. As for cows with genotype AG, the cows with the genetics polymorphism (AG) recorded the lowest services per conception to 1.00 ± 0.00 vaccinations, and the shortest with the days open was 61.80 ± 0.86 days, while cows with the genetics polymorphism (AA) recorded highest services per conception were 1.83 ± 0.10, and the longest with the days open was 89.97 ± 3.99 days. It could be concluded by studying that the relationship of the t 8 genetic polymorphism of the studied position in CYP19-*Pvull* gene with the productive traits is not significant for the Holstein cows. Nonetheless, their relationship to the reproductive traits was highly significant, so it must be checked the relationship of the genetics polymorphism in CYP19-*Pvull* gene with the productive and reproductive traits for dairy cows. As well as, confirm the results obtained by applying the study to a larger sample and other breeds, and for several productive seasons, and this enables us to obtain more accurate results in the application of the selection and exclusion strategy.

Key words : CYP19-*Pvull* gene, productive traits, reproductive traits, dairy cows.

Introduction

The successful method of cows raising and milk production depends on their fertility performance, where poor reproduction is a limiting factor in milk cattle societies to achieve rapid genetic improvement. The one that regulates estrus behavior and productivity in farm animals is the condition of ovarian hormones, most important is estrogen, which is one of the most important reproductive hormones that play a vital role in the development of anatomical, functional, and behavioral traits needed for species reproduction. In addition, many diseases and conditions that affect fertility and public health cause an imbalance in estrogen metabolism (Kumar *et al.,* 2017; Kumar, 2018). Genetic evaluation of animal reproductive performance depends on molecular techniques for gene identification and the genetics polymorphism analysis of these genes, which its products are major enzymes in metabolic pathways for important physiological processes and are associated with morphological structures of animals (Beuzen *et al.,* 2000). The main enzyme in estrogen biosynthesis is the (Aromatase cytochrome P450 enzyme), it is a protein product of the CYP19 gene, and

its role is to convert androgens into estrogens, and is necessary for reproductive physiology (Jedrzejczak *et al.,* 2011; Labrie, 2018). However, the Cytochrome P450 genes are part of a multi-gene family, which contains 27 distinct gene families, ten of them are specific to mammals (Kobylinska, 1994), including the CYP19 and CYP21 gene families. The CYP19 gene (Cytochrome P450 Aromatase Gene) has been identified in cows whose genetic maps (q2.6) are located on chromosome 10 (Vanselow *et al.,* 2000). One of the CYP19 characteristics is that it uses different promoters in tissue expression, and the products of this gene are found in the placenta, ovaries, testes, adipose tissue, and bone (Conley and Hinshelwood, 2001). (Furbass *et al.,* 2010) indicated that the expression of CYP19 gene relies on the promoter P1.1 and that the CYP19 gene selection to study reproductive performance is due to the physiological and regulatory role of estrogen in cattle reproduction (Kumar, 2018). As for the study of the productive performance represented by the characteristics of milk production, it resulted from the fact that estrogen participates indirectly in the process of Lactogenesis. Nonetheless, it affects the cells of the mammary gland by increasing numbers of prolactin hormone and growth receptors, as the level of estrogen correlates with the effectiveness of the Aromatase enzyme and gene expression of the encoding gene CYP19 (Jedrzejczak *et al.,* 2006). The role of the Aromatase enzyme and the estrogen hormone is well illustrated by the genetic differences of the CYP19 gene, which leads to a decrease or increase of the Aromatase enzyme (Praveen *et al.,* 2020). Consequently, the research aims to determine the genetics polymorphism of the CYP19-*Pvull* gene for a sample of the Holstein cows through a technique of Restriction Fragment Length Polymorphism (RFLP). As well as, the extraction of the distribution ratios of this polymorphism and their allele frequency and studying the relationship of this polymorphism in the CYP19-*Pvull* gene with some productive and reproductive traits of the dairy cows.

Materials and Methods

This study was executed at Al-Salam Station for milk production / Private Sector, located in Latifiya district (35 km south of the capital Baghdad), which is affiliated to the Mahmudiyah district (25 km south of the capital Baghdad). The main study aimed to determine associations of the genetics polymorphism in CYP19-*Pvull* gene with some productive and reproductive traits in 40 dairy cows, as well as extraction ratios for distribution of the genetics polymorphism in Holstein cows and allele frequency. Furthermore, the data was used for the 2017-2018 production season of reproductive and production performance, where the genetic analyzes of the blood were done in the laboratory of scientific progress. Polymerase Chain Reaction (PCR) technique was used to amplify the required fragment and complete the molecular detection and determine the genetics polymorphism in CYP19-*Pvull* gene and according to the restriction fragment length polymorphism technique, the primers were selected as shown below (Promoter P1.1, GenBank no. Z69241) for conducting molecular detection and identify the genetics polymorphism resulting from the presence of (Single nucleotide polymorphisms (SNP) in *Pvull* position of CYP19 gene (Vanselow *et al.,* 1999). Noting that the sequence of the studied fragment of the CYP19 gene (405 bp) was obtained according to the University of California Santa Cruz browser.

Forward = 5′-CTCTCGATGAGACAGGCTCC-3′

Reverse = 5′-ACAATGCTGGGTTCTGGACT-3′

The data were analyzed statistically using the Statistical Analysis Program - SAS software (SAS, 2012) to study the effect of the genetic polymorphism of the leptin receptor gene according to the mathematical model below. Significant differences between the averages were compared with the application of the least square mean method.

$$Yijkl = \mu\ Gi\ Pj\ Ok\ eijkl$$

Where Yijkl: view value (l) of the genetics polymorphism (i) and parity (j) and the month of birth (k), ì: the general average of the traits, Gi: effect of the genetics polymorphism in CYP19-*Pvull* gene (AA and AG). Pj: effect of parity (First and second), Ok: effect of month of birth (December, January, and February). As well as, eijkl: represent the random error that is distributed naturally at an average of zero and variance of $ó^2e$. The Chi-square- $\div^2$ test was used to compare the percentage distribution of genotypes.

Results and Discussion

DNA extraction and determination of the genetics polymorphism of CYP19-*Pvull* gene

The DNA was extracted as a first step to obtain the required fragment of CYP19 gene at 405 bp using the PCR technique, kits, the primer, and the total DNA samples, where the thermal cycler device was set as shown in Figure (1). Then, the electrophoresis of all the DNA samples was performed and photographed in order to ensure the success of the extraction process, noting that the DNA Marker ranging from (100-1500 bp DNA Ladder).

Genetic polymorphism of the Holstein cows under study was determined by studied gene fragment CYP19 (bp 405) by applying PCR-RFLP and the restriction

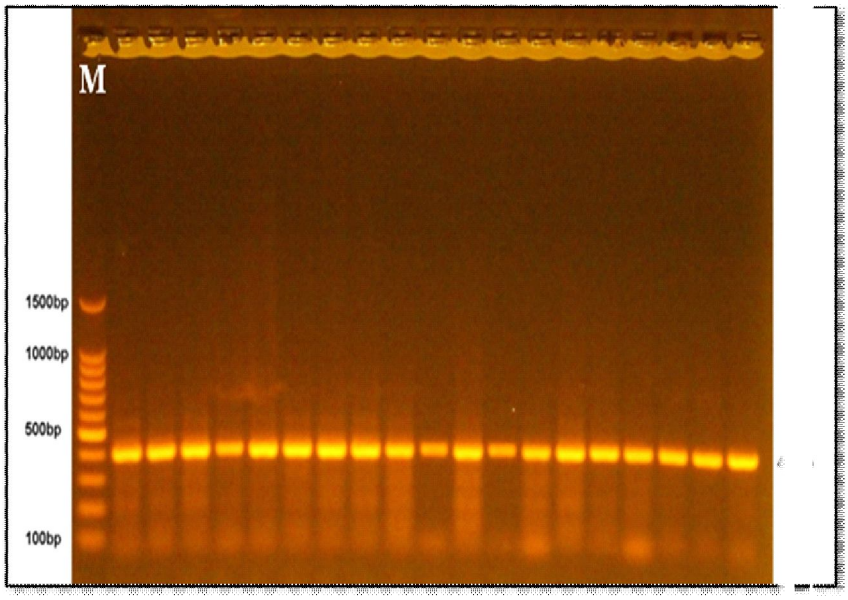

Fig. 1: Fragment Extracted of CYP19 gene (405 bp) by PCR technique.

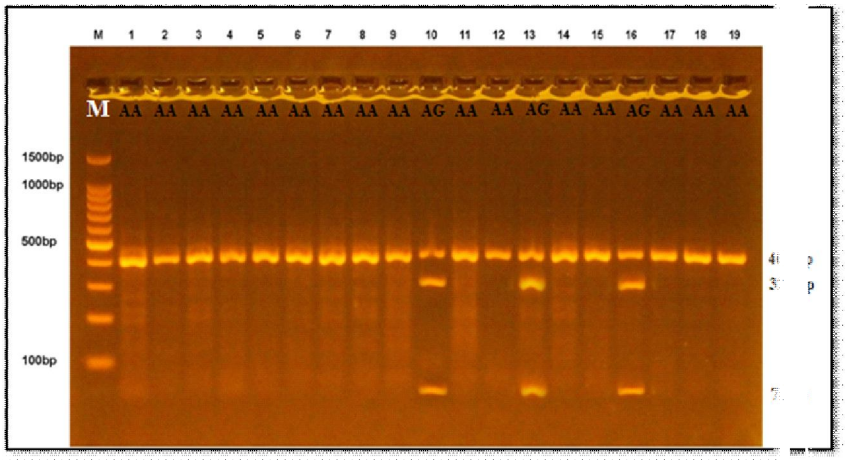

Fig. 2: The electrophoresis product for the digestion of the CYP19-*Pvull* gene fragment studied using restriction enzyme (*Pvull*).

enzyme *Pvull*. Then, the electrophoresis for all samples was performed and photographed to identify the genetics polymorphism of experiment cows according to the size and number of bands formed as per the studied position for the CYP19 gene, the marker ranging from 100 bp to 1500 bp were used as shown in (Fig. 2).

The genetics polymorphism AA (Wild): show in columns 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 17, 18, and 19, while the genetics polymorphism AG (Heterozygous): show in columns 10, 13 and 16. The process of cutting by the restriction enzyme *Pvull* within the technique of PCR-RFLP was done after identifying the sensitive position within the specific sequence of the cutting position in the studied fragment of the CYP19 gene. Therefore, two bands of each model were formed from the process of cutting, which can be compared with the fragments bands of DNA (Marker). Noting that this enzyme has a cutting position sequence (CAGCTG) in this fragment at the sequence 78 bp, as it cuts the studied fragment (bp 405) into two fragment s bp 78 and bp327 in case of presence SNP. This cutting position contains a puRine-type SNP (CAACTG) symbolized by R and is the result of substitution base A with base G (CAGCTG), where this change in the enzyme-cutting site allows the cutting process. Besides, the genetics polymorphism resulting from the presence of SNP in the studied fragment from CYP1 gene has been identified in this way and as shown in the points below. Noting that the sequence of the studied fragment CYP19 gene (405 bp) was obtained according to the (University of California Santa Cruz) UCSC as shown in (Fig. 3).

1. If the cutting does not occur in both strips, the size of the fragment will remain 405 bp, which represents two bands of the same size from both strips appear in one band, and this means that the genotype of this model is Homozygous. As well as, it represents the original genotype (AA) or wild genotype, this means without the presence of SNP.

2. If the cutting occurs in one of the two strips without the other at the sequence of 78 bp for the studied fragment, three bands of size 78, 327 and 405 bp will be formed. As the two bands (78 bp and 327 bp) consist of one of the strips due to the presence of the enzyme cutting (SNP) and the third band (405 bp) is for the other strip without cutting due to the absence of the SNP. Meaning that the genotype of this model is (Heterozygous) and it represents a hybrid genotype (AG), as the presence of SNP in one of the two strips is caused by the substitution of base A with base G. Since the presence of this SNP allows the enzyme *Pvull* to work within the specific sequence of the cutting position in the studied fragment of the CYP19 gene as a result of completing the position sequence of cutting the enzyme.

Fig. 3: The studied fragment of CYP19-*Pvull* gene by UCSC browser.

The same SNP that was analyzed within the gene sequence CYP19 recorded in (GenBank No. Z69241) was found, located at -1044 within the stimulus region of the caw gene CYP19 located on chromosome 10. Besides, this SNP is caused by the substitution of the Adenine base A with Guanine G at the sequence of 78 bp for the studied fragment 405 bp of the gene CYP19, that identified by the restriction enzyme *Pvull* (Vanselow *et al.,* 1999 and Komisarek and Dorynek, 2002).

Number and percentages of the genetics polymorphism and allele frequency in the studied CYP19-*Pvull* gene fragment for a sample of Holstein cows

(Table 1) showed the number, percentage of genetics polymorphism, and allele frequency of the CYP19-*Pvull* gene for a sample of Holstein cows. Two genetic polymorphism of Holstein cows AA and AG were distinguished, with highly significant differences ($P < 0.01$) between the percentage of the two polymorphisms, where it reached 87.50 and 12.50% for the genotypes AA (n = 35) and AG (n = 5), respectively. Meaning that there is a clear prevalence of pure AA individuals with a low percentage of the genetic polymorphism hybrid in the sample of the studied cows. These results for percentages of genetics polymorphism were very close with many previous studies of the same studied position for the gene CYP19-*Pvull* and Holstein cows. Nevertheless, at the same time similar to all studies of that the pure genetic polymorphism (AA) is dominant by high percentage with a lower percentage of hybrid polymorphism (AG) and scarcity of recessive polymorphism (GG). As (Kowalewska-Luczak *et al.,* 2009; Szatkowska *et al.,* 2011) pointed out that there were highly significant differences between the percentage of genetics polymorphism (84.00, 14.00 and 2.00%) and (84.90, 14.60 and 0.42%) for the genetics polymorphism AA, AG, and GG, respectively, during studying on the same position of gene CYP19-*Pvull* for the Polish Holstein-Friesian cattle breed. The percentages of this polymorphism were higher in the jersey breed, as they were 98.00, 2.00, and 0.00% for the genetics polymorphism AA, AG, and GG, respectively (Kowalewska-luczak *et al.,* 2013). Noting that (Keskin *et al.,* 2015) recorded similar percentages in the Holstein-Friesian cattle breed reached 94.00, 6.00, and 0.00% for the genetics polymorphism AA, AG, and GG, respectively. It can be concluded that the molecular basis for polymorphisms of the studied position in the CYP19-*Pvull* gene is the presence of SNP, and is the result of substitution the Adenine base A with Guanine G (Liefers *et al.,* 2004). In light of the results of polymorphisms percentages of the CYP19 gene in the Holstein cows, it can be concluded that pure polymorphisms (AA) is dominant by high percentage with a lower percentage of hybrid polymorphism (AG) and scarcity of recessive polymorphism (GG). The difference in the results of the genetics polymorphism percentages of the studied position for the sample of Holstein cows from some studies, as well as the difference of the breed, the size of the studied sample, and its suitability to environmental conditions. Based on the results of the probability ratio test, the position was not in the Hardy – Weinberg equilibrium, which indicating non-random mating to this position in Holstein cows. The results in Table 1 showed that the allele frequency A that belonging to the CYP19-*Pvull* gene in the studied Holstein cows sample was 0.94, while the allele frequency G was 0.06. This result reflected the dominant of allele A of this gene in the Holstein cows, as the allele A is not cutting by the restriction enzyme, but by allele G, which was similar to all previous studies that indicate the allele frequency A is the highest. The results of previous studies on the same position of the CYP19-*Pvull* gene for the Polish Holstein-Friesian cattle breed indicated that the allele frequency was 0.91 and 0.09 (Kowalewska-Luczak *et al.,* 2009), and 0.92 and 0.077 (Szatkowska *et al.*, 2011) for both A and G alleles respectively. (Kowalewska-luczak *et al.,* 2013) indicated the highest allele frequency in the jersey breed was 0.99

Table 1: Number and percentages of the genetics polymorphism and allele frequency of CYP19-*Pvull* gene.

Genetics polymorphism	Number	(%)Percentages
AA	35	87.50
AG	5	12.50
GG	0	0.00
Total	40	100 %
(X^2) Chi-Square value		83.750 **
Allele	Frequency	
A	0.94	
G	0.06	

** (P≤0.01).

and 0.01 for both alleles A and G, respectively. Whereas, (Keskin *et al.,* 2015) recorded an approximate allele frequency in the Holstein-Friesian cattle breed was 0.97 and 0.03 for both alleles A and G, respectively. Noting that the difference in allele frequency may be important in inheriting some alleles and not others through selection, as genetic diversity gives a chance for further selection.

Associations of the genetics polymorphism in CYP19-*Pvull* gene with milk production and lactation period

The results in table 2 showed that there were no significant differences in the total milk production and the lactation period between the genetics polymorphism of the CYP19 gene. As the total milk production was 1575.06 ± 49.31 kg and 1556.40 ± 107.36 kg for the genetics polymorphism AA and AG respectively, while the lactation period was 167.80 ± 2.70 days and 164.00 ± 4.78 days for the genetics polymorphism AA and AG respectively. Furthermore, (Jedrzejczak *et al.,* 2006) indicated that there was no significant difference in milk production between the genetics polymorphism of the studied position (CYP19-*PvuII*) for jersey cows and white and black cows. However, the effect of the genotype of this position (CYP19-*PvuII*) on milk production was higher in the third productive cycle and lower in the first productivity cycle (Jedrzejczak *et al.,* 2011). Moreover, (Kowalewska-luczak, 2010) reported through their studies on white and black cows that, the cows with a genetic polymorphism AA recorded the highest values ($P \leq 0.05$) for milk production in all three production cycles compared to other genetics polymorphism. However, no significant differences were observed between genetics polymorphism and milk production between jersey cows, but cows with heterozygous genotypes achieved the lowest milk production level (Kowalewska-luczak *et al.,* 2013). The similarity and difference between the results of previous studies and those of the current study may be attributed to the difference of the breed and the size of the studied sample, noting that the relationship of the genetic polymorphism of the studied position (CYP19-*PvuII*) with the lactation period was not previously studied in cows.

Table 2: Associations of the genetics polymorphism in the CYP19-*Pvull* gene with total milk production and lactation period.

Genetics polymorphism	Number of cows (Total number 40)	Mean ± SE	
		Total milk Total milk	Lactation period (day)
AA	35	1575.06±49.31	167.80±2.70
AG	5	1556.40±107.36	164.00±4.78
Significance level		NS	NS

NS: Not significant.

Table 3: Associations of the genetics polymorphism in CYP19-*Pvull* gene with the period from birth to the peak of production and the length of the peak of production.

Genetics polymorphism	Number of cows (Total number 40)	Mean ± SE	
		The period from birth to the peak of production (day)	The length of the peak of production (day)
AA	35	42.71±1.26	49.74±1.41
AG	5	41.80±0.73	44.20±1.16
Significance level		NS	NS

NS: Not significant.

Associations of the genetics polymorphism in CYP19-*Pvull* gene with the period from birth to the peak of production and the length of the peak of production

The results in table 3 showed that there were no significant differences for the period from birth to the peak of production and the length of the peak of production between the genetics polymorphism of the CYP19 gene. The period from birth to the peak of production reached 42.71 ± 1.26 days and 41.80 ± 0.73 days for the genetics polymorphism AA and AG respectively, while the length of the peak of production was 49.74 ± 1.41 days and 44.20 ± 1.16 days for the genetics polymorphism AA and AG, respectively. However, the absence of significant differences for the period from birth to the peak of production and the length of the peak of production between the genetics polymorphism is attributed to that the cows are not different by total milk production and the lactation period. Noting that the relationship of the genetic polymorphism of the studied position (CYP19-*PvuII*) with the period from birth to the peak of production and the length of the peak of production was not previously studied in cows.

Associations of the genetics polymorphism in CYP19-*Pvull* gene

Table 4: Associations of the genetics polymorphism in CYP19-*Pvull* gene with milk composition.

Genetics polymorphism	Number of cows (Total number 40) Number of samples (Total number 120)	Mean ± SE				
		Protein (%)	Fat (%)	Lactose (%) solids (%)	Non-fat of milk	Specific gravity
AA	35(105 sample)	3.09 ± 0.02	3.32 ± 0.32	4.67 ± 0.04	8.60 ± 0.07	1.031 ± 0.02
AG	5(15 sample)	3.02 ± 0.06	3.20 ± 0.83	4.58 ± 0.11	8.39 ± 0.17	1.030 ± 0.03
Significance level		NS	NS	NS	NS	NS

NS: Not significant.

with milk composition

The results in table 4 showed that there were no significant differences in the percentage of milk components between the genetics polymorphism of the CYP19-*PvuII* gene. As cows with a genetic polymorphism AA recorded component ratios of 3.09 ± 0.02%, 3.32 ± 0.32%, 4.67 ± 0.04% and 8.60 ± 0.07% and 1.031 ± 0.02 for protein, lipid, lactose, non-fat solids, and the specific gravity of milk, respectively. Furthermore, the cows with a genetic polymorphism AG recorded component ratios of 3.02 ± 0.06%, 3.20 ± 0.83%, 4.58 ± 0.11%, 8.39 ± 0.17%, 1.030 ± 0.03 for protein, fat, lactose, non-fat solids, and the specific gravity of milk, respectively. Previous studies indicated that there were no significant differences between the genetics polymorphism of the studied position (CYP19-*PvuII*) in the ratio of fat and protein that were studied for jersey cows and white and black cows (Jedrzejczak *et al.,* 2006). Nonetheless, the effect of the genotype of the position (CYP19-*PvuII*) on protein yield was higher in the third productive cycle and lower in the second productive cycle, while its effect on fat yield, fat and protein ratio was higher in the second productive cycle (Jedrzejczak *et al.,* 2011). (Kowalewska-luczak 2010) reported in their study on white and black cows, cows with a genetic polymorphism BB (GG) recorded the lowest productivity of both protein and fat yield and the ratio of protein and fat in all three-production cycles compared to other genetics polymorphism. Moreover, no significant differences were observed between genetics polymorphism and milk production characteristics between jersey cows, but the cows with heterozygous genotypes achieved the highest fat and protein ratio (Kowalewska-luczak *et al.,* 2013). Noting that the relationship of the genetics polymorphism of the studied position (CYP19-*PvuII*) with the ratio of lactose and non-fat solids and the specific gravity of milk was not previously studied in cows.

Associations of the genetics polymorphism in CYP19-*Pvull* gene with the services per conception and days open

The results in table 5 indicated the presence of highly significant differences ($P < 0.01$) with the services per conception and days open between the genetics polymorphism of the CYP19-*PvuII* gene. As the cows with a genetic polymorphism AG recorded the lowest services per conception of ± 1.00 0.00 vaccinations, and the shortest days open was 61.80 ± 0.86 days, while cows with a genetic polymorphism AA recorded the highest services per conception of 1.83 ± 0.10 vaccinations, and the longest days open was 89.97 ± 3.99 days. Previous studies on the Polish Holstein-Friesian cattle indicated a significant relationship between SNP at the studied position (CYP19-*PvuII*) with most of the studied reproductive traits. The cows with a genetic polymorphism AA recorded the longest days open compared to the heterozygous genetics polymorphism, and this difference was significant ($P \leq 0.05$) in the first and third productive cycle, while the services per conception are slightly different between genetics polymorphism but it was not significant (Szatkowska *et al.*, 2011). Finally, (Kumar, 2018) indicated during studying three breeds of cows (Sahiwal, Rathi, and Kankrej) there were no significant differences between the genetics polymorphism in the days open. It can be concluded that the services per conception and the days open are the best in cows with genetics polymorphism AG, and this indicates the advantage of allele G over allele A in determining the genetic polymorphism of genital traits even in the heterozygous genetics polymorphism. This is important in describing the molecular mechanisms that regulate reproduction, and thus identification of individuals for genetics polymorphism of optimal potential may facilitate the

Table 5: Associations of the genetics polymorphism in CYP19-*Pvull* gene with the services per conception and days open.

Genetics polymorphism	Number of cows (Total number 40)	Mean ± SE	
		The services per conception (insemination)	Days open(day)
AA	35	1.83 ± 0.10 b	89.97 ± 3.99 b
AG	5	1.00 ± 0.00 a	61.80 ± 0.86 a
Significance level		**	**

** Means having with the different letters in the same column differed significantly ($P<0.01$).

employment of a reproductive model elected by the breeder.

Conclusion

According to the results of the current study, it is necessary to verify associations between the genetics polymorphism in CYP19-*Pvull* gene with productive and reproductive traits of dairy cows and the obtained results should be confirmed by using higher animal numbers and on other breeds for representing all possible the genetics polymorphism in CYP19-*Pvull*.

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