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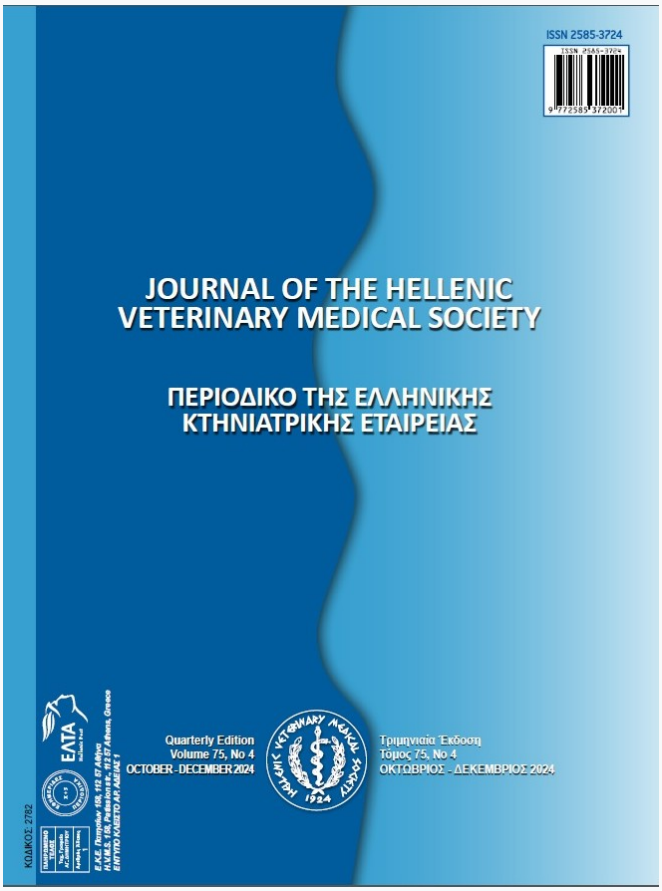

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Research article
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Associations between leptin gene polymorphism and some reproductive traits in Holstein-Friesian dairy cattle

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ABSTRACT: The aim of this study was to determine the effect of leptin gene polymorphism and other environmental factors on age at first breeding (AFB; day), age at first calving (AFC; day), service period (SP; day), number of inseminations per conception (NIPC; count), gestation period (GP; day) and calving interval (CI; day). For this purpose, whole blood samples were obtained from the *Vena coccygea* of 212 Holstein-Friesian dairy cattle reared on a private farm in Türkiye. The *Sau3A*I restriction enzyme with the PCR-RFLP method was used to determine the polymorphism of 422 base pairs (bp) in the intron 2 region of the leptin gene. The frequencies of the A and B alleles and the AA, AB and BB genotypes were determined to be 0.8821 and 0.1179, and 0.764, 0.236 and 0.000, respectively. There were no animals with the BB genotype in the population. The population of Holstein-Friesian was also at the level of Hardy-Weinberg equilibrium with regard to the leptin gene ($P>0.05$). The study found the highest direct heritability in the GP trait (0.33±0.268), while the lowest was observed in the NIPC trait (0.01±0.118). For the traits SP ($P<0.10$), NIPC ($P<0.05$), and CI ($P<0.05$), higher values were recorded in cattle with genotype AA compared to those with genotype AB. However, no significant association was found between genotypes and AFB, AFC, GP, and estimated breeding values (EBVs). This suggests that the heritability of these traits may not be strongly affected by the genotypes emphasized. As a result, the AB genotype or B allele could be used in selection for SP, NIPC, and CI, but the allele or genotype did not suggest marker-assisted selection (MAS) for AFB, AFC, and GP.

***Keywords*:** Estimated Breeding Values; Heritability; Reproductive Traits; *Sau3A*I polymorphism; Variance Components

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INTRODUCTION

In cattle breeding, the yield per animal should be at the targeted level to ensure sustainability. Since genotype and environmental factors cause yield differences between animals, it is important to implement selection programs that should be carried out to achieve the desired yield level in a herd. Both genotype and environmental variables are known to affect differences in quantitative traits, but reproductive traits are more strongly influenced by environmental factors. Although most of the milk and red meat production in Türkiye is covered by cattle, the production rates per animal are not at the desired level (FAO, 2021). Reproduction plays a crucial role, especially in dairy cows, affecting milk production traits and even longevity. For this reason, in recent years, breeders focus on improving environmental factors and do not neglect molecular methods. In fact, the generation interval has forced cattle breeders to turn to MAS in recent years (Bayraktar and Aytekin, 2021; Suchocki et al., 2010). Genes with major effects are more useful than genes with minor effects in the variation observed between individuals, and such genes are considered candidate genes in MAS. Polymorphism and association studies on candidate genes in different populations provide fundamental information for MAS approaches. One of these genes, leptin, is a protein composed of 167 amino acids with molecular weight of 16 kDa and is located on chromosome 4 (Taniguchi et al., 2002). The complete sequence of the bovine leptin gene is available in the ENSEMBL database under accession number ENSBTAG00000014911. The leptin gene is considered a candidate gene due to its pleiotropic effect on the regulation of feed intake, energy metabolism, body weight, growth, carcass composition, milk and fertility characteristics, and immune system in cattle (Taniguchi et al., 2002). Several studies, in different populations or breeds, have been performed to characterize leptin gene polymorphism (Çoban, 2015; Javanmard et al., 2005) and its association with yield related traits (Aytekin, 2011; Liefers et al., 2002; Öner et al., 2017) and health status (Ferchichi et al., 2018) in cattle. The relationships between populations for reproductive traits varied (Al-Janabi et al., 2018; Kulig, 2005; Trakovická et al., 2013). These studies conducted in different populations or breeds, highlight the diversity of leptin gene effects in cattle. Therefore, it was hypothesized that a study to be conducted in a different Holstein-Friesian population would contribute to the evaluation of the potential of the leptin gene in cattle.

The objective of the current study was to determine the leptin gene polymorphism and the effect of this polymorphism and several environmental factors on some reproductive traits in Holstein-Friesian dairy cows reared in Türkiye.

MATERIALS AND METHODS

Experimental animals and sample collection

Whole blood samples from 212 Holstein-Friesian cattle reared on a private dairy farm in Kırşehir province of Türkiye were used in the current study. The study was carried out on a single farm because no other farm could provide a sufficient data set under the prevailing conditions. Inclusion criteria required that animals had completed their first lactation without ultimately aborting any offspring, resulting in a final sample of 212 animals. Blood samples from each animal were collected in tubes containing Ethylenediaminetetraacetic acid (EDTA) from the *Vena coccygea* and stored at -18 °C until DNA extraction analysis. The reproductive traits (AFB, AFC, SP, NIPC, GP and CI) were collected from the farm's herd management program (Afifarm 5.4.2). The collection of whole blood samples as well as the animal care techniques used in the experiment, complied with the animal welfare guidelines set out in Article 9 of the Türkiye's government law.

DNA extraction and PCR-RFLP method

The commercial kit EURX (Quick Blood DNA Purification Kit) and the device QuickGene Mini 80 (Medical Expo/USA) were used to extract genomic DNA. The *Sau3A*I polymorphism in the intron 2 region of the leptin gene was determined using primers (F 5' -TGGAGTGGCTTGTTATTTTCTTCT 3' and R 5' -GTCCCCGCTTCTGGCTACCTAACT 3'F) reported by Liefers et al. (2002). The PCR reaction was carried out in a 20 µl reaction volume. The polymerase chain reaction consisted of 4 µmol L^{-1} genomic DNA, 10 µmol L^{-1} 2x HOT FIREPol® Blend Master Mix PCR Master Mix (2X; Solis BioDyne/Estonia), 0.5 µmol L^{-1} of each primer and 5 µmol L^{-1} ddH_2O in a 20 µl volume. The amplification was performed in a gradient thermal cycler (T100 Thermal Cycler/Axonia Medical/Singapore) using the following program: an initial denaturation step at 94 °C for 2 min, followed by 35 cycles at 94 °C for 60 s, 58.1 °C for 60 s and 72 °C for 60 s. The last extension was at 72 °C for 15 minutes. Then, 5 µmol L^{-1} PCR products were used for control and 15 µmol L^{-1} for digestion. The PCR products were digested with 0.5 U *Sau3A*I

restriction enzyme in a 25 µl volume (Thermo Fisher Scientific). Restriction fragments were electrophoresed on 2% agarose ethidium bromide gel in 1X TBE buffer and then visualized under UV light and scored on a gel documentation system.

Statistical analysis

The program PopGene Version 1.32 (Yeh et al., 1997) was used for the statistical analysis of the allele and genotype frequencies and the heterozygosity (Nei, 1973) of the gene region. The Chi-square (χ^2) test was performed to determine whether the population was in Hardy-Weinberg equilibrium (Düzgüneş et al., 1983). The General Linear Model (GLM) used to assess the effects of genotype and environmental factors is shown below. However, factors such as year, parity, birth type, calf sex and covariance vary in the models according to the different reproductive traits. For instance, the model $Y_{ijklm}=\mu+\alpha_i+\beta_j+S_k+G_l+Cov_{(A,B)}+\varepsilon_{ijklm}$ was used for SP. In this model; Y_{ijklm} is the observed trait in $_{ijklm}$-th animal, µ is the mean of trait for population, α_i is the effect of year (i = 2013, 2014, 2015, 2016, 2017 and 2018 years), β_j is the effect of season (j = winter, spring, summer and autumn seasons), S_k is the effect of parity (k = 1, 2, 3, 4, and 5 parity), G_l is the effect of genotype, (l = AA and AB genotypes), $Cov_{(A, B)}$ is the covariance between the continuous factor and the trait being investigated and ε_{ijklm} is the random error. Then, Tukey's Honestly Significant Difference (HSD) multiple comparison test was performed to assess differences between means that were significant as a result of analysis of variance (ANOVA). Minitab 16.1.1. package program was used for statistical analysis (Minitab, 2010).

The MTDFREML (Multiple Trait Derivative Free Restricted Maximum Likelihood) package software was utilized in the study to estimate variance components and genetic parameters using the BLUP (Best Linear Unbiased Prediction) method (Boldman et al., 1995). Genotype was not included in the statistical model used to estimate components of variance and heritability. Also, factors such as year, parity, and covariance vary in the models according to different characteristics. When estimating variance components, heritability, and EBVs, the statistically significant factors (*P<0.05, **P<0.01) were included in the model as a result of the analysis of variance (Table 1).

Below is the matrix descriptions of the models used to calculate the variance components (Mrode, 2014). The components of variance were determined using the model $y=Xb+Za+Wm+e$. In the model, y is the vector of the observations for each trait; X, Z and W are the matrices related to fixed and random factors; b is the vector of fixed effects; a is the vector of direct additive genetic effects; m is the vector of maternal effects; and e is the vector of random error. The variance-covariance structure of model was shown in the matrix $V\left|\sigma_a^2\sigma_{am}\sigma_{am}\sigma_m^2\right|$.

The equations $h_a^2=\frac{\sigma_a^2}{(\sigma¿¿a^2+\sigma_m^2+\sigma_{am}+\sigma_e^2)¿}$ and $h_m^2=\frac{\sigma_m^2}{(\sigma¿¿a^2+\sigma_m^2+\sigma_{am}+\sigma_e^2)¿}$ were used to estimate direct and maternal heritability, respectively, in the model. Using these models, direct additive genetic effect (σ_a^2), genetic covariance between maternal effect and direct additive genetic effect (σ_a^2 x σ_m^2), maternal effect (σ_m^2), environmental variance (σ_e^2), phenotypic variance (σ_p^2), and the logarithm of likelihood (-2 Log L) of variance components were estimated. The variance components and heritability were estimated

Table 1. Factors used to estimate variance components

	Factors	Discrete factors	Continuous factors
Reproductive traits	Age at first breeding (day)	Birth year**, Birth season	#
	Age at first calving (day)	Birth year, Birth season	Age at first breeding**
	Service period (day)	Parity**, Calving year, Calving season**	Peak milk yield**, Lactation milk yield**
	Number of inseminations per conception (count)	Parity**, Calving year, Calving season	Peak milk yield**, Lactation milk yield**
	Gestation period (day)	Parity, Calving year**, Calving season, Birth type**, Calf sex**	#
	Calving interval (day)	Parity**, Calving year, Calving season	Dry period, Lactation milk yield, Days in milk

**; p<0.01; #; No continuous factor

using the REML (Restricted Maximum Likelihood) technique in the MTDFREML software. Below are the models used to calculate the heritability of the direct additive genetic effect (h_a^2) and maternal effect (h_m^2). The BLUP technique was used to determine the EBVs in the MTDFREML package program. The breeding value of each animal was calculated using the equation $BV_i = h^2(P_i - P)$. In the equation, BV_i was the breeding value of the *i*-th animal; h^2 was the heritability of *i*-th animal; P_i was the phenotypic value of the *i*-th animal in terms of the trait emphasized and *P* was the population mean related to the emphasized trait. A one-way analysis of variance was performed to examine variation between genotypes with respect to EBVs. Tukey's multiple comparisons test was used to compare the means of genotypes whose effect was found to be significant by analysis of variance.

RESULTS

The descriptive statistical values, mean, standard deviation, coefficient of variation, minimum, and maximum for reproductive traits and the number of records of cows, dams, and sires were provided in Table 2.

In the present study, the mean of the AFB, AFC, SP, NIPC, GP, and CI were 438.40±36.24 days, 729.62±53.54 days, 115.47±64.35 days, 2.33±1.41 counts, 278.33±4.98 days, and 398.85±92.09 days, respectively. The coefficient of variation was high for SP, NIPC and CI, it was low for AFB, AFC and GP in Holstein-Friesian cattle (Table 2).

*Sau3A*I polymorphism in the intron 2 region of leptin gene in Holstein-Friesian dairy cattle

The influence of the leptin gene polymorphism and environmental factors on reproductive traits in Holstein-Friesian dairy cattle is investigated. PCR and restriction products are shown in Figure 1.

The results on allele and genotype frequencies and heterozygosity values are presented in the Table 3 of our previous study (Kibar and Aytekin, 2021).

Kibar and Aytekin (2021) found that the frequencies of A and B alleles and AA, AB, and BB genotypes for leptin gene polymorphism were 0.8821 and 0.1179, and 0.764, 0.236, and 0.000, respectively. Chi-square analysis showed that leptin gene polymorphism was at the *Hardy-Weinberg* equilibrium (P>0.05).

Table 2. Some descriptive statistics values for reproductive traits in Holstein-Friesian cows

Traits	AFB (day)	AFC (day)	SP (day)	NIPC (count)	GP (day)	CI (day)
Mean	438.40	729.62	115.47	2.3286	278.33	398.85
Standard deviation	36.24	53.54	64.35	1.4054	4.98	92.09
Coefficient of variation (%)	8.27	7.34	55.73	60.61	1.79	23.09
Minimum	302.00	560.00	27.00	1.0000	259.00	303.00
Maximum	631.00	1014.00	358.00	5.0000	296.00	1080.00
Number of records	212	212	430	430	425	218
Number of cows	212	212	212	212	212	212
Number of dams	191	191	191	191	190	104
Number of sires	66	66	66	66	66	54

AFB: Age at first breeding, AFC: Age at first calving, SP: Service period, NIPC: Number of inseminations per conception, GP: Gestation period, CI: Calving interval

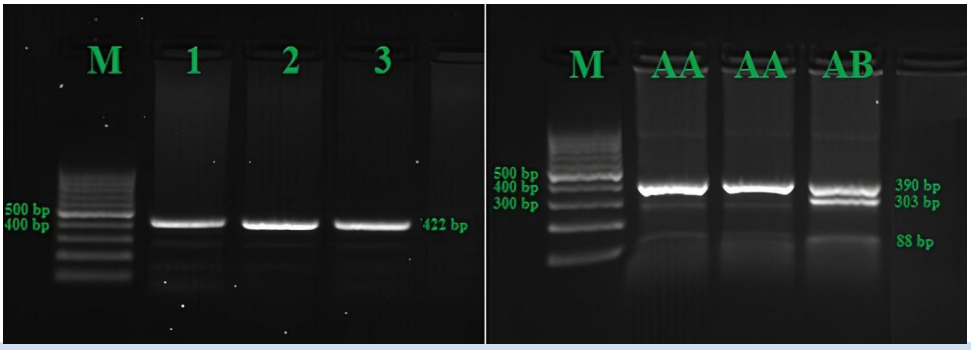

Figure 1. PCR and restriction products picture of the *Sau3A*I polymorphism; M: 100 bp Plus DNA ladder (Vivantis plus/Moldova); Left image line 1-3: PCR products and Right image line AA: 390 and 32 bp, AB: 390, 303, 88 and 32 bp (32 bp was not seen in gel)

Association analysis, variance components and EBVs

The factors influencing AFB and AFC as well as their mean values and coefficients of determination in the models are given in Table 4.

The effect of birth year (BY) on AFB was significant, while not for the genotype and birth season (BS). The effect of AFB on AFC was significant, but not for the genotype, BY, and BS. No significant difference was found between genotypes for AFB (AA: 445.14±3.01, AB: 437.11±5.22 days) and AFC (AA: 728.53±3.45, AB: 721.92±5.92 days) (Table 4). The factors affecting SP and NIPC and their means are shown in Table 5.

The effect of genotype, parity, calving season (CS), peak milk yield (PMY), and lactation milk yield (LMY) on SP was found to be significant but the effect of calving year (CY) was insignificant. The SP of cattle with genotype AA (135.58±3.30 day) was significantly higher (P<0.10) than genotype AB (127.13±4.53 day). The NIPC was considerably affected by genotype, parity, PMY and LMY, while the effects of CY and CS were insignificant. Thus, the NIPC of genotype AA (2.67±0.08 counts) was statistically higher (P<0.10) than in cattle with genotype AB (2.39±0.12 count) (Table 5). The effective factors on GP and CI, their mean values and the coefficient of determination are presented in Table 6.

Birth type (BT), calf sex, CY and CS considerably affected GP, but genotype and parity had no effect. There was no significant difference between the cattle with genotypes AA (274.46±1.07 days) and AB (274.01±1.14 days) for GP. The effect of genotype, parity, and dry period (DP) on CI was significant, while the effect of CY, CS, LMY, and DIM was insignificant. In addition, the CI of cattle with genotype AA (421.81±10.4 days) was statistically higher (P<0.05) than genotype AB (391.43±14.4 day) (Table 6). A model (direct additive-genetic effect (a) and maternal effect (m)) was used for the reproductive traits in the study, and the estimated variance components and heritability are presented in Table 7.

In the present study, direct and maternal heritability in all traits ranged from 0.01±0.118 to 0.33±0.268 and

Table 3. Allele and genotype frequencies and heterozygosity of leptin gene polymorphism in Holstein-Friesian dairy cattle

Leptin	N	Genotypes			Genotype frequencies			Allele frequencies		χ^2	H_e
		AA	AB	BB	AA	AB	BB	A	B		
Observed	**212**	162	50	0	0.764	0.236	0.000	0.8821	0.1179	3.789^{NS}	0.208
Expected	**212**	164.95	44.10	2.95	0.78	0.21	0.01				

N: Number of animals, χ^2 (HWE); Hardy-Weinberg equilibrium $\chi 2$ value, H_e: Expected heterozygosity, NS; Not significant

Table 4. The associations between leptin gene polymorphisms with AFB and AFC

Factors		N	Traits	
			Age at first breeding (day)	Age at first calving (day)
			$\underline{X} \pm S_{\underline{X}}$	$\underline{X} \pm S_{\underline{X}}$
Leptin	AA	162	445.14±3.01	728.53±3.45
	AB	50	437.11±5.22	721.92±5.92
Birth year	2011	17	454.71 ± 8.73^{AB}	716.43±9.98
	2012	32	459.35 ± 6.05^{A}	729.87±7.05
	2013	18	446.30 ± 8.35^{AB}	717.76±9.48
	2014	38	433.82 ± 5.55^{BC}	730.24±6.30
	2015	39	433.10 ± 5.60^{BC}	730.01±6.36
	2016	68	419.48 ± 4.83^{C}	727.05±5.67
Birth season	Winter	49	441.19±5.46	715.31±6.19
	Spring	32	436.14±6.36	723.93±7.21
	Summer	62	448.98±4.68	732.46±5.37
	Autumn	69	438.19±4.46	729.21±5.05
Regression			#	AFB: 1.0417±0.0797***
R^2			0.1416	0.4955

A, B: P<0.01, ***: P<0.01, N: Number of animals, R^2: Coefficient of determination, $\underline{X}$: Least mean squares, $S_{\underline{X}}$: Standard error, #: Not included in the model

Table 5. The associations between leptin gene polymorphism with SP and NIPC

Factors		N	Traits	
			Service period (day)	Number of inseminations per conception (count)
			$\underline{X} \pm S_{\underline{X}}$	$\underline{X} \pm S_{\underline{X}}$
Leptin	AA	318	135.58±3.30^{x}	2.67±0.08^{a}
	AB	112	127.13±4.53^{y}	2.39±0.12^{b}
Parity	1	212	103.44±4.19^{C}	1.98±0.11^{C}
	2	95	115.42±4.60^{C}	2.25±0.12^{BC}
	3	59	137.06±5.88^{B}	2.63±0.15^{AB}
	4	33	140.34±7.76^{AB}	2.81±0.20^{A}
	5	31	160.53±8.12^{A}	3.00±0.21^{A}
Calving season	Winter	87	130.48±5.24^{AB}	2.42±0.13
	Spring	86	143.70±4.95^{A}	2.70±0.13
	Summer	141	126.97±4.19^{B}	2.59±0.11
	Autumn	116	124.28±4.76^{B}	2.43±0.12
Calving year	2013	26	136.95±9.49	2.38±0.24
	2014	38	137.12±7.31	2.63±0.19
	2015	59	136.11±5.88	2.67±0.15
	2016	69	131.84±5.54	2.69±0.14
	2017	101	124.65±4.60	2.34±0.12
	2018	137	121.48±4.88	2.49±0.12
Regression			LMY: 0.02723±0.00117*** PMY: -4.527±0.497***	LMY: 0.000517±0.000030*** PMY: -0.0799±0.0126***
R^2			0.5927	0.4477

LMY: Lactation milk yield, PMY: Peak milk yield, A, B: P<0.01, a, b: P<0.05, x, y: P<0.10, ***: P<0.01, N: Number of animals, R^2: Coefficient of determination, $\underline{X}$: Least mean squares, $S_{\underline{X}}$: Standard error

0.00±0.00 to 0.27±0.367, respectively (Table 7). The highest and the lowest direct heritabilities were found for GP (0.33±0.268) and NIPC (0.01±0.118), respectively. However, the highest and the lowest maternal heritabilities were determined for GP (0.27±0.367) and CI (0.00±0.000), respectively. This study found that genetic covariance between cow and dam was 100% for all traits except CI. The results of one-way analysis of variance to determine the EBVs of reproductive traits by genotype are given in Table 8.

The mean EBVs of Holstein-Friesian cows with AA genotype of leptin gene *Sau3A*I polymorphism for AFB, AFC, SP, NIPC, GP, and CI were 0.24±0.74, 0.091±0.249, -0.264±0.479, -0.001±0.003, -0.196±0.167, and 0.024±0.233, respectively. For the same traits, the mean EBVs of cows with AB genotype were -1.73±1.33, -0.294±0.448, -1.380±0.861, -0.011±0.005, -0.436±0.300, and -0.484±0.420, respectively. In addition, the minimum, maximum and the mean EBVs of dams and sires were determined. In this study, the maximum and the minimum EBVs in dams and sires were found for AFC and AFB among all traits (Table 8). Although the standard error of EBVs was high, the results of this study are important from the point of view of selection, since the aim is to reduce the values of reproductive traits to the threshold.

DISCUSSION

Reproductive traits (e.g., AFB, AFC, SP, NIPC and CI) provide breeders with herd management information and directly impact the sustainability of the farm. Brzáková et al. (2019) reported that the AFB in the Czech Holstein population was 479.37±66.88 days and it was found that this value was higher than in the present study. In previous studies, the AFC was 27.2±0.06 months in Egypt Friesian cows (Salem and Hammoud, 2016), 31±3.8 months in Holstein-Friesian cattle (Ojango and Pollott, 2001), 1052.35±10.68 days in Kerala crossbred cattle (Valsalan et al., 2022), 25.3 months in five different herds of Holstein dairy cattle (Keshavarzi et al., 2020), and 39.26±9.8 months in Holstein-Friesian cattle (Wondossen et al., 2018). Regarding AFC, this study was similar to Keshavarzi et al. (2020) but different from other studies. The SP was 119.80±35.62 days in crossbred Kerala cattle (Valsalan et al., 2022), 123.6 days in five different herds of Holstein dairy cattle (Keshavarzi et al.,

Table 6. The associations between leptin gene polymorphism with GP and CI

Factors		Traits			
		Gestation period (day)		Calving interval (day)	
		N	$\underline{X} \pm S_{\underline{X}}$	N	$\underline{X} \pm S_{\underline{X}}$
Leptin	AA	313	274.46±1.07	156	421.81±10.4^{a}
	AB	112	274.01±1.14	62	391.43±14.4^{b}
Birth type	Single	404	278.34±0.37^{A}		
	Twin	18	271.61±1.20^{B}		#
	Import	3	272.75±2.82^{AB}		
Calf sex	Female	202	273.56±1.10^{B}		#
	Male	223	274.91±1.10^{A}		
Parity	1	211	273.81±1.07		#
	2	96	273.93±1.07	95	376.08±11.4^{B}
	3	58	274.82±1.20	59	389.88±14.0^{B}
	4	31	274.62±1.38	33	414.18±18.7^{AB}
	5	29	273.99±1.40	31	446.36±20.0^{A}
Calving season	Winter	84	274.80±1.20^{A}	46	403.30±16.1
	Spring	87	274.03±1.10^{AB}	48	407.69±15.5
	Summer	140	273.38±1.12^{B}	78	402.30±13.7
	Autumn	114	274.73±1.17^{A}	46	413.21±15.7
Calving year	2013	25	272.03±1.45^{C}	8	416.19±35.1
	2014	38	274.45±1.33^{ABC}	10	415.02±30.3
	2015	58	275.06±1.24^{AB}	38	422.71±16.5
	2016	68	273.95±1.18^{BC}	33	381.22±17.0
	2017	99	273.99±1.06^{BC}	58	398.80±12.5
	2018	137	275.92±1.10^{A}	71	405.82±12.3
Regression			#		DP: 1.233±0.646* LMY: 0.00612±0.00415 DIM: 0.016±0.142
R^2			0.1313		0.0814

DP: Dry period, LMY: Lactation milk yield, DIM: Days in milk, Import: Cow imported to the farm (unknown birth type), A, B: P<0.01, a, b: P<0.05, *: P<0.10, N: Number of animals, R^2: Coefficient of determination, $\underline{X}$: Least mean squares, $S_{\underline{X}}$: Standard error, #: Not included in the model

Table 7. Variance components and heritability of reproductive traits

Model	Traits					
	AFB (day)	AFC (day)	SP (day)	NIPC (count)	GP (day)	CI (day)
σ_a^2	0.02694	0.00809	0.01573	0.01255	0.00847	0.02245
σ_{am}	0.01553	0.01075	0.01082	0.03356	0.00762	0.00443
σ_m^2	0.00895	0.01428	0.00744	0.08988	0.00685	0.00089
σ_e^2	0.06581	0.12108	0.14404	0.97766	0.00235	0.77216
σ_p^2	0.11723	0.15420	0.17804	111.365	0.02529	0.79993
h_a^2	0.23±0.465	0.05±0.293	0.09±0.116	0.01±0.118	0.33±0.268	0.03±0.158
h_m^2	0.08±0.311	0.09±0.163	0.04±0.003	0.08±0.006	0.27±0.367	0.00±0.000
h_e^2	0.56±0.424	0.79±0.260	0.81±0.060	0.88±0.059	0.09±0.040	0.97±0.072
r_{am}	1.00±10.860	1.00±20.557	1.00±20.612	1.00±80.802	1.00±0.757	0.99±***
-2 Log L	-218.7477903	-179.756067	-264.9069026	513.0849805	-1706.2909380	181.4369947

AFB: Age at first breeding, AFC: Age at first calving, SP: Service period, NIPC: Number of inseminations per conception, GP: Gestation period, CI: Calving interval, σ_a^2: Direct additive genetic variance, $\sigma_a^2 x \sigma_m^2$: Covariance between additive genetic effect and maternal effect, σ_m^2: Maternal genetic variance, σ_e^2: Temporary environmental variance σ_p^2: Phenotypic variance, h_a^2: Direct heritability, h_m^2: Maternal heritability, h_e^2: Heritability of environmental effect, r_{am}: Correlation between additive genetic effect and maternal effect, -2 Log L: Log-likelihood

Table 8. EBVs of some reproductive traits according to leptin genotypes for cows

Animal	Traits	Genotype	N	Estimated breeding values						Accuracy	
				Minimum			Maximum			Mean	Range	$\underline{X} \pm S_x$
				EBV	$S_{\underline{x}}$	r_{IH}	EBV	$S_{\underline{x}}$	r_{IH}	$\underline{X} \pm S_{\underline{x}}$		
Cow	AFB (day)	AA	162	-23.3	13.0	60	50.0	13.0	61	0.24±0.74	59-66	0.63±0.02
		AB	50	-42.8	13.0	62	27.9	13.0	62	-1.73±1.33	59-65	0.62±0.01
	AFC (day)	AA	162	-7.1	8.0	40	21.7	8.0	41	0.091±0.249	35-47	0.41±0.02
		AB	50	-4.4	8.0	40	6.7	8.0	43	-0.294±0.448	38-44	0.41±0.02
	SP (day)	AA	162	-14.3	9.0	71	23.7	11.0	54	-0.264±0.479	40-71	0.51±0.08
		AB	50	-15.7	9.0	66	15.2	9.0	67	-1.380±0.861	40-68	0.53±0.09
	NIPC (count)	AA	162	-0.101	0.10	45	0.142	0.10	38	-0.001±0.003	25-49	0.33±0.06
		AB	50	-0.098	0.11	31	0.063	0.11	28	-0.011±0.005	25-46	0.34±0.07
	GP (day)	AA	162	-5.5	4.0	92	7.7	5.0	87	-0.196±0.167	47-92	0.86±0.03
		AB	50	-5.0	5.0	85	3.5	4.0	88	-0.436±0.300	83-91	0.87±0.02
	CI (day)	AA	162	-12.7	14.0	41	12.8	14.0	31	0.024±0.233	18-91	0.51±0.30
		AB	50	-5.0	5.0	85	3.5	4.0	88	-0.484±0.420	18-91	0.71±0.28
Dam	AFB (day)	#	191	-0.412	0.14	52	0.418	0.14	51	0.00±0.14	50-72	0.53±0.04
	AFC (day)		191	-0.072	0.08	42	0.212	0.08	55	0.00±0.08	39-62	0.43±0.04
	SP (day)		191	-0.138	0.10	59	0.185	0.11	52	0.00±0.11	35-67	0.44±0.09
	NIPC (count)		191	-0.137	0.09	61	0.193	0.10	52	0.00±0.10	33-68	0.44±0.10
	GP (day)		190	-0.053	0.05	83	0.047	0.05	83	0.00±0.05	81-93	0.84±0.02
	CI (day)		104	-0.081	0.14	26	0.077	0.15	25	0.00±0.15	11-26	0.16±0.04
Sire	AFB (day)	#	66	-0.09	0.16	23	0.194	0.16	24	0.00±0.15	23-69	0.33±0.13
	AFC (day)		66	-0.031	0.09	27	0.07	0.09	16	0.00±0.09	11-46	0.17±0.09
	SP (day)		66	-0.09	0.11	47	0.144	0.11	46	0.00±0.12	15-56	0.28±0.11
	NIPC (count)		66	-0.036	0.11	21	0.03	0.11	8	0.00±0.11	5-24	0.11±0.05
	GP (day)		66	-0.044	0.07	68	0.058	0.07	63	0.00±0.08	29-81	0.43±0.16
	CI (day)		54	-0.058	0.15	18	0.052	0.15	16	0.00±0.15	8-36	0.15±0.05

AFB: Age at in first breeding, AFC: Age at first calving, SP: Service period, NIPC: Number of inseminations per conception, GP: Gestation period, CI: Calving interval, N: Number of animals, EBVs: Estimated breeding values, $S_{\underline{x}}$: Standard error; S_x : Standard deviation, r_{IH} : Accuracy (%), #: Not detected

2020), and 184.54±107.55 days in Holstein-Friesian cows (Wondossen et al., 2018) and the mean SP was determined in the current study was below these values. As to NIPC and CI, the NIPC was 2.0±0.03 counts (Salem and Hammoud, 2016), 2.9 counts (Keshavarzi et al., 2020), and 2.04±1.41 counts (Wondossen et al., 2018), also the CI was 406±79 days (Ojango and Pollott, 2001), 400.08±58.74 days in the Czech Holstein population (Brzáková et al., 2019) and 473.57±124.32 days (Wondossen et al., 2018). It was determined that the current study was higher than Salem and Hammoud (2016) and Wondossen et al. (2018) in terms of NIPC and CI. Differences between studies are believed to be due to animal breed, number of animals, herd management, farm size, veterinarian, differences in the bulls, and other environmental factors (e.g., year of calving, year of season, birth type, parity). In order to know the effect of genotype (for MAS-based selection), standardization according to statistically significant factors on emphasized traits is required. The MAS would increase the speed of selection in cattle breeding. Understanding the roles of specific genes and their interactions with environmental factors is necessary accelerate breeding.

In this study, a standardization according to some environmental factors was performed and then the influence of the *Sau3A*I polymorphism on reproductive traits was determined. In relation to this topic, Valsalan et al. (2022) reported that the effect of BS on AFC was significant (P<0.01). Salem and Hammoud (2016) found that the effect of BY and BS on AFC in Holstein-Friesian cattle was important. Similar to the current study, Moussavi et al. (2006) observed no significant difference between AA and AB genotypes for AFB in Iranian Holstein-Friesian cattle. In contrast to the current study, Trakovická et al. (2013) found that the Slovak Spotted and Slovak Pinzgau cattle with the AA genotype had a lower AFC and the highest AFC was observed with the AB genotype (P<0.01). In con-

trast to the current study, Salem and Hammoud (2016) reported that the effect of CS and CY on NIPC was significant (P<0.01), but the effect of parity and AFC was not important. Valsalan et al. (2022) reported that the effect of BS on SP was significant (P<0.01). Regarding this issue, Ghazanfari et al. (2006) reported that the SP of Brown-Swiss cattle with the AA genotype was significantly shorter (P<0.05). Regarding the SP of Iran Holstein-Friesian cattle, Moussavi et al. (2006) found no significant differences between the genotypes (AA and AB). According to Trakovická et al. (2013), there was no significant association between genotypes (AA, AB and BB) and SP in Slovak Spotted and Pinzgau cattle. The present results are not in the same direction as the studies by Ghazanfari et al. (2006) and Moussavi et al. (2006). In a study on Holstein dairy heifers raised in the Marmara region of Türkiye, no significant association between NIPC and genotypes was found (Öner et al., 2017). Hunde et al. (2022) found that the effect of parity and CY on CI was important (P<0.05) and stated that CI decreased with increasing CY and parity in crossbred dairy cows. Wondossen et al. (2018) reported that the effect of CY and parity on CI was significant (P<0.001) but not on CS in Holstein-Friesian cattle. In contrast to the current study, Wondossen et al. (2018) found that the CI decreased as parity increased. Ardicli et al. (2019) found that the effect of CS on CI was important (P<0.01) but not on CY and parity in Holstein-Friesian cows. Ardicli et al. (2019) reported that the effect of CS, CY, and parity on GP in Holstein-Friesian cattle was not significant. In a previous study, Trakovická et al. (2013) found that the association between genotypes (AA, AB and BB) and CI was not significant in Slovak Spotted and Pinzgau cattle.

The direct heritability for AFB in Holstein-Friesian dairy cattle obtained in the current study was higher than previously determined by Güngör and Zülkadir (2019) 0.208±0.420, but lower than 0.363±0.237 (Öncü, 2014) and 0.42±0.15 (Öncü ,2021). Similar to the current study, Getahun et al. (2020) estimated that the direct heritability of crossbred dairy cows for AFB, AFC, SP, NIPC, and CI was 0.22±0.08, 0.30±0.08, 0.082±0.03, 0.012±0.003, and 0.071±0.03 in the model ($б_a^2$ and $б_{pe}^2$), respectively. In previous research, the direct heritability of NIPC, CI, AFC and SP was 0.006±0.022 (Salem and Hammoud, 2016), 0.047±0.020 (Ojango and Pollott, 2001), 0.172±0.029, and 0.032±0.073 (Valsalan et al., 2022) in the model ($б_a^2$ and $б_{pe}^2$). The results of the present study were higher than Salem and Hammoud (2016) and Valsalan et al. (2022) but lower than Ojango and Pollott (2001) and Valsalan et al. (2022) in relation to NIPC, SP, CI and AFC. The differences between the studies are believed to be due to the number of animals used in the study, statistical methods and models. Heritability is generally defined as the proportion of genotypic variation to phenotypic variation and is used for the selection program. According to previous studies, heritability between 0.15 and 0.40 is considered moderate (Cassell, 2009), while less than 0.30 (Düzgüneş et al., 1996) is considered low. Brzáková et al. (2019) reported that the direct heritability was 0.058, 0.031, 0.038, and 0.034 for AFB, AFC, SP, and CI and found that the additive genetic variance for all traits was sufficient to select efficient animals in Holstein cattle. This comment is at odds with the current study, as it was estimated that family selection is suitable for traits with a heritability from 0.10 to 0.25 and mass selection for traits with a heritability of more than 0.25. Thus, it was found that mass selection would only be suitable for GP. This study will contribute to the literature related to EBVs of reproductive traits as to the author's knowledge, very few studies has been published to date. Salem and Hammoud (2016) found that the minimum and the maximum EBV in Holstein cattle were -0.14±0.26 and 0.19±0.26 for NIPC and -37.9±30.5 and 64.8±33.4 for SP, respectively. In terms of the range between the minimum and maximum values, a lower value was found in the present study than in Salem and Hammoud (2016). Regarding MAS-based animal selection, cattle with the AA genotype could only be used for SP, and cattle with the AB genotype could be used for AFB, AFC, NIPC, GP, and CI.

CONCLUSION

In the current study, leptin allele or genotype did not suggest MAS-based selection for AFB, AFC, and GP traits. However, the AB genotype was found to be superior for SP, NIPC and CI, and cattle with the AB genotype or B allele could be used in selection for these traits. In the selection program for reproductive traits of this population, it is proposed that mass selection would be more appropriate for traits with a heritability value greater than 0.25 (GP), while family selection for traits with a heritability from 0.10 to 0.25 consisted of SP, AFB, AFC, NIPC, and CI. Although there was no statistically significant difference in EBVs between the AFB, AFC, SP, NIPC, and CI, the genotypes with the lowest breeding values should be used for selection studies, but with limited expectations. In GP selection studies, genotypes with breed-

ing values closer to 280 days should be selected to avoid health problems associated with early and late parturition (e.g., low weight or difficult parturition). Also, it would be more instructive that more research is needed with other genes to establish a definitive association between economic traits and gene/genes.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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