



Effect of Somatic Cell Count on Fertility and Milk Yield Traits During Different Lactation Periods in Holstein Cows

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ABSTRACT

The aim of this study was to evaluate the effect of somatic cell count (SCC) variation on fertility [days open (DO), number of inseminations per pregnancy (NIPP), calving interval (CI) and gestation length (GL)] and milk yield traits [daily milk yield (dMY), lactation length (LL), lactation milk yield (LMY) and 305-days milk yield (305-dMY)] during early (< 100 d), mid (100-200 d) and late lactation (> 200 d). This study was conducted with primiparous and multiparous Holstein cows at a commercial farm having an approximate herd size of 260 heads in Kırşehir, Türkiye. A total of 107 Holstein dairy cows on the farm were selected. Milk samples were collected once a month during morning milking between 30 and 240±15 d of lactation. The somatic cell counter (DCC, DeLaval, Tumba, Sweden) was used to assess SCC (cells/ml). SCC levels were categorized into three groups (< 100 × 10³ cells/mL, 100-200 × 10³ cells/mL and > 200 × 10³ cells/mL). Cows were divided into three groups according to parity: Cows with parity 1 (first group; n = 49), cows with parity 2 (second group; n = 30) and cows with parity 3 ≤ (third group; n = 28). Parity did not influence fertility traits (P>0.05). Parity significantly affected dMY and 305-dMY, but not LL or LMY. The study found that cows with SCC < 100 × 10³ cells/mL had lower DO and CI values compared to cows with SCC 100-200 × 10³ cells/mL and > 200 × 10³ cells/mL during mid-lactation, although no statistical differences were observed in the NIPP, GL, dMY, LL, LMY and 305-dMY values. A positive correlation was observed between SCC groups and DO during mid-lactation. These findings suggest that SCC can be used as an indicator in indirect selection programs to achieve shorter DO and CI in Holstein cows.

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Introduction

The primary goal of dairy farms is to provide environmental conditions that meet the needs of the farmed breed in order to maintain profitable and sustainable production. Given that cattle productivity is the result of a complex interplay between genotype and environmental conditions, it is imperative to provide suitable environmental conditions to enhance productivity (Çilek and Tekin, 2005). Sustainable and profitable production depends on one calf per year and optimal milk yield from each cow (Muller et al., 2014). Throughout the world, selection studies of dairy cows have focused primarily on increasing milk yield for many years. Thus, the increases globally in the yield characteristics of cows have been observed as a result of selection practices (Garnsworthy et al., 2008; Gutiérrez-Reinoso et al., 2020). However, most researchers have noted that this increase in milk yield

negatively affects the fertility indicators of dairy cows (Andersen-Ranberg et al., 2003; Veerkamp et al., 2003; Muller et al., 2014). Although the reasons for the decrease in fertility cannot be fully explained (Mirzaei et al., 2021), it is associated with the effects on reproductive performance of physiological factors that vary depending on management practices and increase in milk yield (Kafi et al., 2012). Also, increased metabolic velocity in high-yielding cows tends to adversely affect their immune metabolic and health status and reproductive performance (Ratwan et al., 2022; Okuyucu et al., 2023). This situation negatively affects the tolerance of cows to environmental factors. Along with the increased breeding value for milk yield in cows (Garnsworthy et al., 2008) and the acceleration of the intensification process in the dairy industry (Clay et al., 2020), some management factors

influencing reproductive performance have also varied, such as increased herd size in dairy farms and reduced labor input per cow (Muller et al., 2014). Considering the combined and complex effects of this increased metabolic load (Okuyucu et al., 2023) and changes in management factors in dairy cows (Garnsworthy et al., 2008), fertility indicators including days open (DO), number of inseminations per pregnancy (NIPP), calving interval (CI) and gestation length (GL) may deviate from ideal values and as a result, it may cause a decrease in production performances. As a result of this negative effect, cows with high breeding values are culled from the herd due to poor fertility. Therefore, poor fertility is referred to as the main limiting factor determining longevity (Wathes et al., 2008). Poor fertility reduces the number of potential replacement heifers born in a dairy herd and increases the need for heifers for the sustainability of the dairy herd. Thus, this condition results in the purchase of new replacement heifers to maintain herd size and the sustainability of production in the existing herd (Wathes et al., 2008).

The increase in milk yield on dairy farms has recently been observed and cow's sensitivity to udder diseases has increased (Mirzaei et al., 2021). Subclinical mastitis, which is the most common mammary disease can cause negative effects on lactation milk yield, milk quality and fertility traits (Malinowski and Gajewski, 2010) and thus economic losses in farms, due to both the difficulties in determining and the long treatment process. When a cow's udder is exposed to microbial contamination, it responds with somatic cells as the first defense mechanism. Therefore, somatic cells increase in the cow's bloodstream and other body fluids depending on the degree of bacterial infection, tissue damage, or other inflammatory factors (Atasever et al., 2012; Stádník and Atasever, 2017). Many authors have emphasised the association between intramammary infection and high somatic cell count (SCC) in milk (Ruegg, 2003; Aytekin and Boztepe, 2014; Erdem and Okuyucu, 2019). Although the number of studies investigating the effects of SCC on fertility traits is limited, several authors emphasized that the increase in SCC in milk adversely affects fertility traits including NIPP, DO and CI (Juozaitiene and Juozaitis 2005). Therefore, the decrease in reproductive performance as well as the increased susceptibility to mastitis due to the increase in milk yield of cows is a concern for dairy farms (Mirzaei et al., 2021).

Several studies have focused on non-genetic environmental factors influencing fertility traits (Çilek and Tekin, 2005) and milk yield traits (Kul et al., 2019) in dairy cows. However, to the best of our knowledge, reports on associations between SCC at different lactation stages and some fertility traits in Holstein cows are still limited. In this respect, revealing these relationships will add important information to the literature. Therefore, further studies are required to reveal the effects of SCC on the milk yield and fertility traits in all lactation stages of Holstein cows. We hypothesized that the SCC groups in different lactation stages are significantly associated with the milk yield [daily milk yield (dMY), lactation length (LL), lactation milk yield (LMY) and 305-days milk yield (305-dMY)] and fertility traits (DO, NIPP, CI, GL) and that cows' parity significantly affects these yield traits. The aims of this study were (i) to evaluate the effects of parity on the milk yield and fertility traits; (ii) to investigate the effects of

SCC groups on the milk yield and fertility traits; (iii) to determine the correlation coefficients between the SCC and the milk yield and fertility traits at different lactation stages in Holstein cows.

Materials and Methods

Ethical approval for the study was obtained from the local ethics committee of Yozgat Bozok University (approval number: 32-14; date: 20.04.2022). All experimental procedures, animal care and animal welfare protocols were performed according to the guidelines.

The present study was conducted with primiparous and multiparous Holstein cows on a commercial farm with a herd size of approximately 260 head in Kırşehir, Türkiye. A total of 107 Holstein dairy cows on the farm were selected. Cows without any metabolic and disease problems were included in the study. Routine management practices on the farm were not changed and the cows were milked regularly three times a day. Milking operations were performed in milking place fishbone [an automatic milking system (number of cows milked per milking: 2x20)]. All herd information such as daily milk yield, milking times, estrus behaviors, pregnancy and feeding were recorded with a computerized herd management system (Afimilk MPC, Kibbutz Afikim, Afimilk, Israel). Cows were housed in free-stall barns and fed total mixed ration (TMR). The main ingredients of TMR were corn silage, alfalfa, barley grain, cottonseed meal, soybean meal, corn flakes, wheat straw, salt and feed additives. Also, cows had free access to fresh water during the day.

The milk samples were collected once a month during the morning milking between 30 and 240±15 days of lactation. Samples were collected in 50-mL sampling bottles and kept cold at +4 °C in the lab while being processed. DeLaval Cell Counter (DCC, DeLaval, Tumba, Sweden) was used to assess SCC (cells/mL). The working principle and analysis stages of this device positioned in the laboratory are explained in the following sentences. Approximately 60 µL of milk sample is drawn into disposable fluorescence-dyed cassettes produced specifically for this device and thus the nuclei of the cells are stained with propidium iodide in the cassette. The cassette is placed in the device and operated. The device records the fluorescence signals received from the cell nuclei with the help of the light source and gives the obtained mathematical values in terms of microliters. Therefore, the existing values were multiplied by a thousand and SCC values in milliliters were obtained. DO, NIPP, CI and GL were used to evaluate reproduction traits, while dMY, LL, LMY and 305-dMY were used to evaluate milk yield traits.

The cows were divided into three groups according to parity: The cows with 1 parity (first group; n = 49), the cows with 2 parity (second group; n = 30) and the cows with parity of ≥ 3 (third group; n = 28). In addition, to examine the effects of SCC groups on milk yield and fertility traits in early (<100 d), mid (100–200 d) and late (>200 d) lactation periods of cows, SCC groups were divided into three (first group: < 100 × 10³ cells/ml, second group: 100-200 × 10³ cells/ml and third group: > 200 × 10³ cells/ml).

The following statistical model was used to examine the influence of parity on fertility and milk yield traits:

$$\gamma_{ij} = \mu + P_i + \varepsilon_{ij}$$

where γ_{ij} is the dependent factor; μ is the overall mean; P_i is the effect of parity (1, 2, 3); ε_{ij} is the random error.

To evaluate the effect of SCC values on fertility and milk yield traits, the following model was used:

$$\gamma_{ij} = \mu + SCC_i + \varepsilon_{ij}$$

where γ_{ij} is the dependent factor; μ is the overall mean; SCC_i is the effect of SCC groups (< 100 , $100-200$, $> 200 \times 10^3$ cells/ml); ε_{ij} is the random error.

Before the analysis, the normality and homogeneity of variance of the data obtained from the records related to fertility traits and milk yield traits were screened using Kolmogorov-Smirnov and Levene tests, respectively. The general linear model (GLM) procedure of the SPSS (IBM Corp. Released, 2012) software (version 21.0, SPSS Inc, Chicago, IL, USA) was used to evaluate data. Duncan's multiple range test was used to compare differences between mean values. Pearson's correlation coefficient (r) was used to calculate correlations.

Results

In the present study, the data obtained on fertility and milk yield characteristics according to parity are shown in Figure 1a,b,c,d and Figure 2a,b,c,d. The data presented in Figure 1 show that the effect of parity on the DO, NIPP, CI and GL were not statistically.

As demonstrated in Figure 2a, the dMY in the cows with parity 3 was found to be higher than that of the cows with parity 1. However, no differences between cows with parity 1 and 3 were detected in this study with regard to the mean dMY values. Similarly, the parity affected the 305-dMY values of cows (Figure 2d); the 305-dMY value also increased with an increase in the parity. However, the parity did not affect the means of LL and LMY (Figure 2b,c).

No differences between the SCC groups regarding the mean DO, NIPP, CI and GL were detected during the early lactation period. Similarly, the data presented in Table 1 show that the effect of SCC groups on the dMY, LL, LMY and 305-dMY values was not statistically significant during this lactation period.

The study showed that the DO value in cows with SCC $100-200 \times 10^3$ cells/mL and $> 200 \times 10^3$ cells/mL were higher than in cows in groups with SCC $< 100 \times 10^3$ cells/mL during mid-lactation period ($p = 0.041$; Table 2).

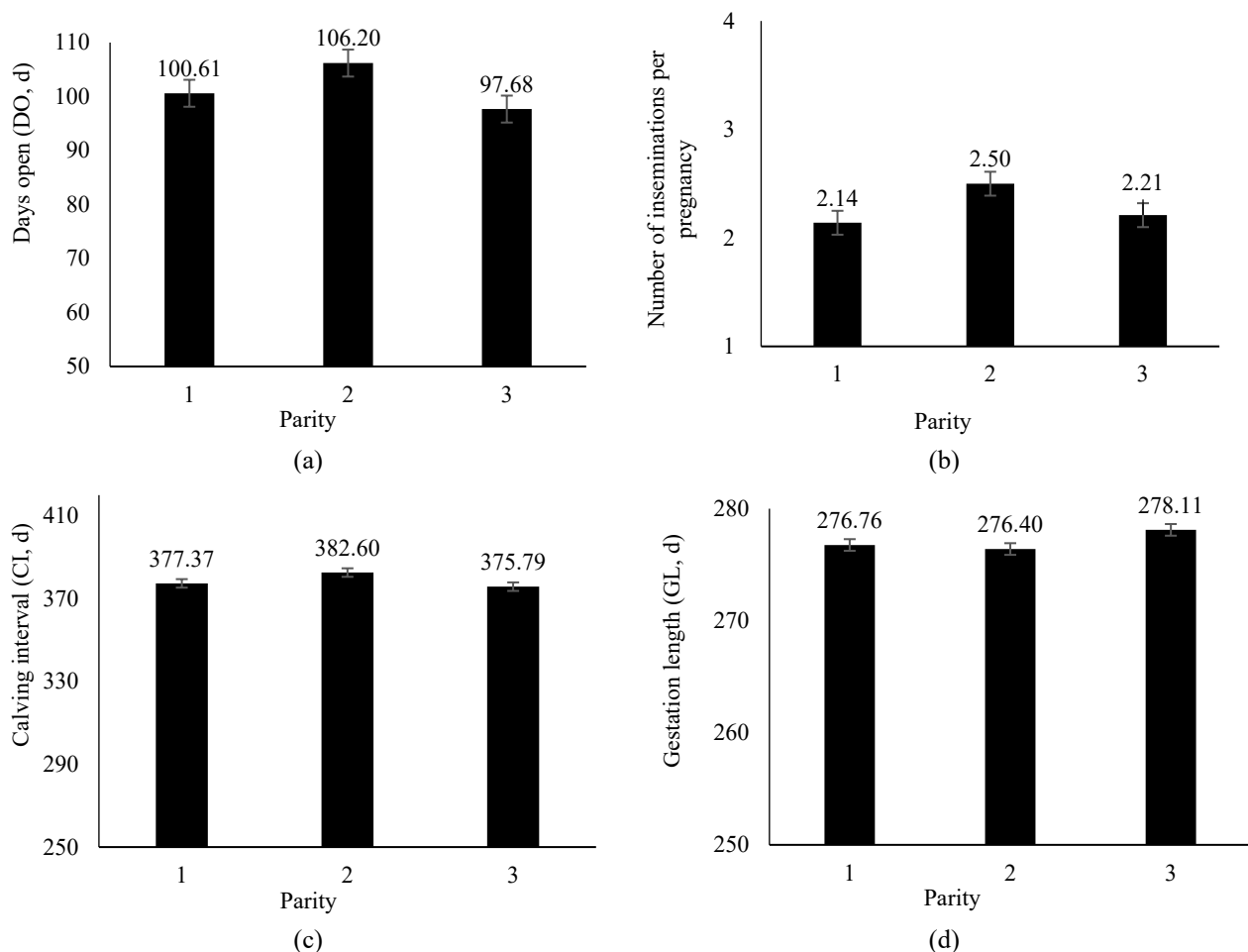


Figure 1. Changes in days open (a), number of inseminations per pregnancy (b), calving interval (c), and gestation length (d) from fertility traits ($n=107$) among the parities.

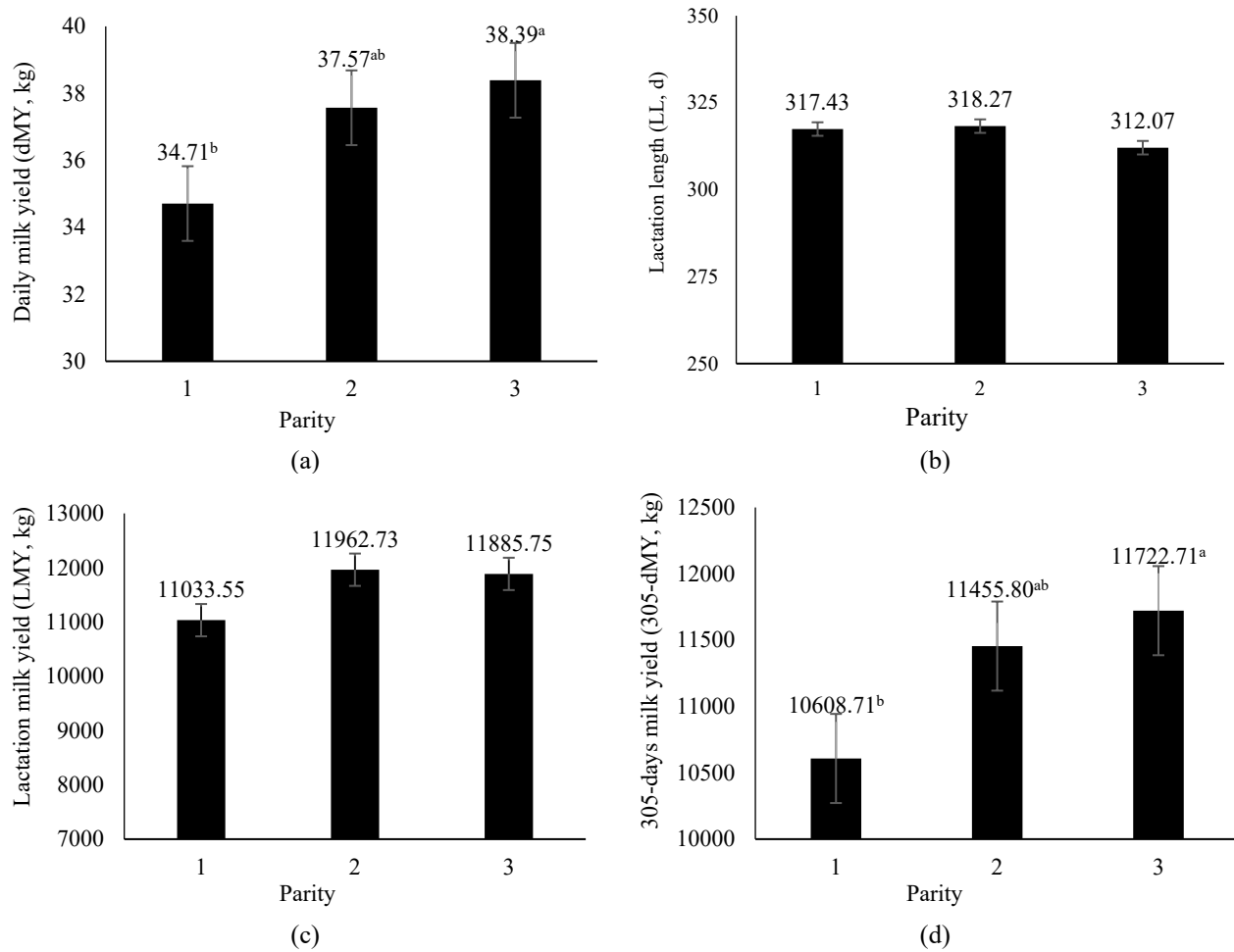


Figure 2. Changes in daily milk yield (a), lactation length (b), lactation milk yield (c) and 305-days milk yield (d) from milk yield traits (n=107) among the parity.

Table 1. Changes in the cows' fertility and milk yield traits according to SCC groups during the early lactation period

Traits	Variables	SCC Groups (cells/ml)			SEM	p-value
		< 100 × 10 ³ (n=48)	100-200 × 10 ³ (n=41)	> 200 × 10 ³ (n=18)		
Fertility	DO (d)	101.06	96.88	112.67	3.95	0.395
	NIPP	2.38	2.15	2.22	0.11	0.643
	CI (d)	378.31	373.54	389.83	3.97	0.376
	GL (d)	277.25	276.66	277.17	0.63	0.908
Milk yield	dMY (kg/d)	37.19	35.98	35.72	0.6	0.567
	LL (d)	317.31	310.61	326.33	3.96	0.390
	LMY (kg)	11744.9	11186.6	11662.4	219.2	0.494
	305-dMY (kg)	11358.8	10994.1	10875.6	182.4	0.541

SEM: Standard error of mean, DO: Days open, NIPP: Number of inseminations per pregnancy, CI: calving interval, GL: Gestation length, dMY: daily milk yield, LL: lactation length, LMY: lactation milk production, 305-dMY: 305-days milk production.

However, there was no statistical difference between the DO values of cows with $100-200 \times 10^3$ and those with $> 200 \times 10^3$ cells/mL during this period. Similarly, the CI value in cows with SCC $100-200 \times 10^3$ cells/mL and $> 200 \times 10^3$ cells/mL were higher than in cows in groups with SCC $< 100 \times 10^3$ cells/mL during the mid-lactation period ($p < 0.05$). However, the effect of SCC groups on the NIPP and GL values was not statistically significant at the mid-lactation period. In addition, the effect of SCC groups on all milk yield characteristics was found to be insignificant during this lactation period (Table 2).

As can be seen from the data in Table 3, the effect of SCC groups on all milk yield traits was found to be

insignificant ($P > 0.05$) during the late lactation period (Table 3). Similarly, the changes in cows' SCC value did not affect any fertility traits during this lactation period.

A positive and significant correlation was identified between the SCC groups and the fertility and milk yield traits of Holstein cows at various lactation stages (Table 4). The SCC groups displayed a positive correlation with the cows' DO during the mid-lactation period, but the correlations between the SCC groups and fertility and milk yield traits in other lactation periods were statistically insignificant. Although not statistically significant, the correlation between SCC and CL tends to be significant in mid-lactation ($p = 0.073$).

Table 2. Changes in the cows' fertility and milk yield traits according to SCC groups during the mid-lactation period

SCC Groups (cells/mL)						
Traits	Variables	< 100 × 10 ³ (n=38)	100-200 × 10 ³ (n=48)	> 200 × 10 ³ (n=20)	SEM	p-value
Fertility	DO (d)	88.87 ^b	107.85 ^a	108.75 ^a	3.98	0.041
	NIPP	2.03	2.40	2.40	0.11	0.289
	CI (d)	366.68 ^b	385.10 ^a	383.75 ^a	4.00	0.046
	GL(d)	277.82	277.25	275.00	0.63	0.281
Milk yield	dMY (kg/d)	37.87	35.67	35.95	0.61	0.246
	LL (d)	306.16	323.06	318.10	3.99	0.161
	LMY (kg)	11601.42	11439.63	11557.40	221.26	0.946
	305-dMY (kg)	11575.50	10890.60	10948.10	183.89	0.219

^{a,b}Different letters on the same line indicate statistically significant differences ($P < 0.05$), SEM: Standard error of mean, DO: days open, NIPP: Number of inseminations per pregnancy, CI: calving interval, GL: Gestation length, dMY: daily milk yield LL: lactation length, LMY: lactation milk production, 305-dMY: 305-days milk production.

Table 3. Changes in the cows' fertility and milk yield traits according to SCC groups during the late lactation period

SCC Groups (cells/mL)						
Traits	Variables	< 100 × 10 ³ (n=15)	100-200 × 10 ³ (n=57)	> 200 × 10 ³ (n=33)	SEM	p-value
Fertility	DO	104.47	98.96	105.24	4.02	0.758
	NIPP	2.47	2.21	2.30	0.11	0.741
	CI	386.27	374.30	383.45	4.02	0.455
	GL	281.80	275.33	278.21	0.61	0.095
Milk yield	dMY	35.80	37.47	35.36	0.61	0.269
	LL	326.20	313.21	319.36	3.97	0.508
	LMY	11763.10	11682.90	11284.60	219.14	0.679
	305-dMY	10937.47	11445.68	10791.70	184.40	0.253

SEM: standard error of mean, DO: days open, NIPP: Number of inseminations per pregnancy, CI: calving interval, GL: Gestation length, dMY: daily milk yield LL: lactation length, LMY: lactation milk production, 305-dMY: 305-days milk production.

Table 4. Correlations coefficients of the fertility and milk yield traits with SCC groups in various stages of lactation

Period	Fertility Traits				Milk production Traits			
	DO	NIPP	CI	GL	dMY	LL	LMY	305-dMY
Early	0.145	0.042	0.148	0.022	-0.075	0.132	0.025	-0.085
	(p = 0.135)	(p = 0.671)	(p = 0.127)	(p = 0.824)	(p = 0.441)	(p = 0.175)	(p = 0.795)	(p = 0.386)
Mid	0.198	0.140	0.175	-0.140	-0.102	0.152	0.019	-0.110
	(p = 0.042)	(p = 0.152)	(p = 0.073)	(p = 0.152)	(p = 0.299)	(p = 0.121)	(p = 0.850)	(p = 0.263)
Late	0.087	0.019	0.085	-0.012	-0.103	0.045	-0.053	-0.110
	(p = 0.380)	(p = 0.851)	(p = 0.391)	(p = 0.900)	(p = 0.295)	(p = 0.647)	(p = 0.589)	(p = 0.264)

DO: days open, NIPP: Number of inseminations per pregnancy, CI: calving interval, GL: Gestation length, dMY: daily milk yield, LL: lactation length, LMY: lactation milk production, 305-dMY: 305-days milk production.

Discussion

In this study, the parity did not affect any fertility traits of cows (DO, NIPP, CI and GL). The findings obtained in this study were found to be compatible with the results obtained by Çilek and Tekin (2005) and Kaya and Bardakcioglu (2016). However, Muller et al. (2014) reported that the DO increased as the parity increased. The study conducted by Şahin and Ulutas (2011) on Holstein cows determined that as the parity increased, the averages of DO, CI and NIPP decreased and there were similar changes in these characteristics. Although there was no effect of parity on fertility traits in the current study, several authors report that parity is an important environmental factor that can affect fertility (Şahin and Ulutas, 2011; Muller et al., 2014). The multiple effects, such as different geographical locations, feeding, management and housing of the Holstein cows can explain the observed variations among the obtained findings in these studies. This study determined that parity influenced milk yield traits rather than fertility traits. It was determined that dMY and 305-dMY values tended to

increase with the progress of the parity of the cows. In agreement with previous findings, we observed increases in milk yield traits with increasing parity (Şahin and Ulutas, 2011; Kul et al., 2019; Senbeta and Abebe, 2021). Therefore, the parity should be considered in establishing appropriate management and feeding practices, especially for cows in the early lactation period after calving, as the cows' age may vary in the physiological factors that support their all yield performance.

Our results did not show that increased SCC was negatively or positively associated with fertility and milk yield traits during the early lactation period. Similarly, in a study conducted by Mirzaei et al. (2021) on Holstein cows, it was reported that subclinical mastitis did not statistically affect the pregnancy rate and the number of inseminations per pregnancy in cows during this lactation period. Contrary to the findings in this study, Kul et al. (2019) emphasized that there is a negative correlation between SCC groups and test-day milk yield.

During the mid-lactation period, we determined that as the SCC value increased, the CI and DO values also increased. In this study, it is a remarkable finding that some fertility characteristics (DO and CI values) of cows with an SCC value above 200×10^3 cells/ml were adversely affected only in the mid-lactation period and this adverse effect was determined in DO and CI values rather than milk yield characteristics. Similarly, Siatka et al. (2019) reported that high SCC negatively affected CI and DO values in Polish Holstein-Friesian cows. The same authors found a similar trend of deterioration in fertility with progressive mastitis for NIPP. In a previous study conducted by other researchers (Lomander et al., 2013) on Swedish dairy cows, it was emphasized that increasing SCC causes a significant decrease in first insemination success (%60). Similarly, Nguyen et al. (2011) reported that cows' fertility traits were adversely affected when the SCC increased above 200×10^3 cells/ml. The results obtained in the present study regarding the relationships between SCC groups and some fertility traits (CI and SP values) align with the above-mentioned studies' findings. We hypothesized that when the SCC is $>200 \times 10^3$ cells/mL, the cow may adversely affect the uterus as a result of the increase in defence cells in the bloodstream and its effects on infection in the udder. Therefore, it is thought that the increase in SCC may result in an increase in DO and CI values. The statements reported by Isobe et al (2014) support our findings, with the authors emphasising that cytokines involved in mastitis may affect the function of the uterus. The same authors emphasized that this may adversely affect follicular development and ovulation, and therefore reproductive disorders may occur. Several researchers also emphasized that new chemical substances may occur in milk and blood depending on the presence of gram-negative and gram-positive pathogens that cause mastitis (Erdem et al., 2011). Erdem et al. (2011) reported that among these substances, cytokines such as interleukin, tumor necrosis factor and interferon, as well as nitric oxide and endotoxins, cause a decrease in fertility properties. Therefore, adverse environmental conditions such as improper manure management, milking operations, and feeding that may cause SCC to increase should be improved. In particular, careful monitoring and/or examination of hygiene practices in the living spaces and milking processes of cows in early lactation stages is important for improving breeding and production characteristics. However, these findings cannot be attributed solely to the relationship between SCC and fertility traits. Apart from this, the nutritional status of the cows, especially metabolic imbalances (protein-energy imbalance), may have adversely affected the fertility traits (Bertoni et al., 2009; Cheng et al., 2022). Sert et al. (2020) emphasized that high or insufficient energy intake and the ration of high-yielding cows in the early lactation period may adversely affect fertility characteristics during the lactation period. In addition, the same authors stated that various feeding manipulations should be performed to reduce the negative effect of negative energy balance in the early stages of lactation. Recently, with the increase in milk yield of cows, decreases in their immunity have been observed. This situation may adversely affect the indirect and/or direct fertility characteristics (Okuyucu et al.,

2023). Therefore, all genetic and non-genetic environmental factors affecting fertility traits should be treated cautiously. Also, all environmental factors that adversely affect fertility traits should be controlled with good herd management practices (feeding, barn hygiene, milking practices and manure management).

Conclusion

Achieving profitable production conditions in dairy farms depends on controlling environmental factors that affect fertility and milk yield characteristics throughout lactation. Therefore, it is important to investigate the effects of non-genetic environmental factors on cows' production and reproductive performance. We can conclude from the results that parity did not affect all fertility traits. However, it was determined that the 305-dMY value increased as the parity increased. In addition, it was observed that SCC values did not affect the milk yield and fertility characteristics of cows in the early and late lactation periods, while it was determined that SCC affected the DO and CI values in the mid-lactation period. Briefly, the findings of this study demonstrated that cows with an SCC value above 200×10^3 in the mid-lactation period negatively affected some fertility traits (DO and CI values) rather than milk yield traits. Therefore, the relationship between SCC and fertility traits should be carefully considered. In addition, any physiological changes that may occur in the cow's uterus should be carefully examined. Further studies are needed to evaluate the relationship between SCC and fertility function in all lactation periods.

Declarations

Ethical Approval Certificate

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee of Yozgat Bozok University, Türkiye (Approval date: 20.04.2022 and number: 32-14). All experimental procedures, animal care, and animal welfare protocols were performed according to the guidelines.

Author Contribution Statement

O.E., İ.C.O. and E.K.: Data collection, investigation, formal analysis, and writing the original draft

O.E. and E.K.: Project administration, supervision, conceptualization, methodology, review and editing

O. E., İ.C.O. and E.K.: Data collection and investigation

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