

Negative effects of occurrence of clinical mastitis from calving to end of the voluntary waiting period on reproduction in Holstein cows

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Abstract: The objective was to evaluate the effects of clinical mastitis (CM) on reproductive performance in high-milk-producing cows before the service period. The data of Holstein Friesian cows (n = 550) in a commercial farm was retrospectively evaluated. The diagnosis of CM was made by the presence of visible abnormalities in milk or symptoms of inflammation of one or more of the quarters. Cows were checked to detect CM prior to each milking. Calving to first service interval (CFSI), calving to conception interval (CCI), and services per conception (SC) were evaluated as reproductive parameters. Calving to first service interval (83.68 ± 3.18 vs. 74.57 ± 1.08 ; $P < 0.05$) and CCI (168.48 ± 8.59 vs. 132.86 ± 3.44 ; $P < 0.001$) were prolonged by 11.77% and 26.81%, respectively, and the number of SC (3.16 ± 0.21 vs. 2.42 ± 0.09 ; $P < 0.001$) was increased by 30.58% in cows experiencing CM. The culling candidate rate in nonpregnant cows was higher in the mastitis group (4.35%; 6/132) than in the healthy group (0.48%; 2/418). Calving season, time period of CM, and lactation number were not associated with the negative effects of mastitis in reproductive performance ($P > 0.05$). In conclusion, CM in early lactation has an adverse impact on reproductive performance in high-milk-producing dairy cows. The negative effects of CM on CFSI, CCI, and SC were not interrelated with calving season, the timing of CM, or parity.

Key words: Clinical mastitis, dairy cow, reproductive performance, timing of mastitis

1. Introduction

The most important targets in dairy farms around the world are high reproductive performance and milk yield. Problems occurring in the periparturient period as a consequence of higher metabolism in high-milk-producing dairy cows, however, cause an increase in the incidence of infectious/inflammatory diseases and a decrease in reproductive performance [1,2]. Inflammatory diseases [3], low body condition scores (BCS), and negative energy balance [4] occurring prior to the insemination period are factors that reduce the rate of pregnancy. The pregnancy rates seen in the 1970s of 50%–60% at postpartum first insemination decreased to 35%–45% in the 2000s [5,6]. Infectious diseases have particular importance in reproductive performance due to their high prevalence during the early postpartum period and their direct relationship with feeding-BCS and metabolic changes [3].

It was reported that milk yield and mastitis have a positive correlation in high-yielding dairy cows [7], and

that cows are more susceptible to mastitis [8]. The fact that cows with a high milk yield tend to have a decrease in fertility suggests that the risk factors for mastitis and fertility are similar, and that there may be a relationship between them. The results of previous studies show that the effects of mastitis are related not only to milk yield/quality and udder health, but also to reproductive performance [9].

The purpose of this study is to research the effect of clinical mastitis (CM) on the reproductive performance of high-yielding dairy cows during the period from calving to the voluntary waiting period.

2. Materials and methods

2.1. Animals and management

The study was conducted in a commercial dairy farm located in the northern part of the province of İzmir, in Western Turkey. The herd consisted of Holstein (95%) and Brown Swiss (5%) cattle. The cows were housed in free-stall facilities. All cows were separated from their

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calves immediately after parturition and moved into a fresh group (<30 DIM) and high producing (≥ 30 DIM) group, respectively. The cows were fed three times daily with the total mixed ration (TMR) compatible with the National Research Council (NRC) recommendations [10]. The ration consisted of corn silage, alfalfa hay, wheat straw, commercial compound, corn flake, corn gluten, wet brewers' grain, soy hulls, and additives. Cows were milked thrice daily, and the mean milk production over 305 days of the herd was $10,488.95 \pm 125.65$ kg/per cow.

The regular health checks, including diagnosis and treatment of reproductive disorders, monitoring cyclicity and estrus, and pregnancy diagnosis, were performed once a week. Following the voluntary waiting period (VWP), all cows were subject to the reproductive management practices in breeding. A combination of visual observation and an activity meter (ALPRO™ activity meter, DeLaval International AB, Tumba, Sweden) were used for heat detection. All cows received the first insemination by Ovsynch-56. Nonpregnant cows were reinseminated according to estrus observations (standing), activity meter, and results of routine examinations. In the case that estrus could not be determined in nonpregnant cows, Ovsynch-56 protocol was used for timed-artificial insemination (AI) of cows. The same technician inseminated all cows. Pregnancy diagnosis was determined 30 days after AI (first check) with transrectal ultrasonography (a 7.5-MHz linear probe with a portable B-mode ultrasound scanner, Hasvet 838°, Hasvet, Turkey), at 60–70 days (second check), and 140–150 days (third check) after AI with rectal palpation.

2.2. Study design

Retrospective records from 550 Holstein cows from the farm were used as a subject. The cows were grouped as mastitis ($n = 132$) and healthy ($n = 418$) according to the mastitis experience from pregnancy to 60 days postpartum (pp) (voluntary waiting period). Data was obtained from herd management software (Alpro Windows® 6.93; DeLaval, Tumba, Sweden) and confirmed by herd veterinarians/technicians during routine visits from December 2014 to December 2015. Information consisted of animal identifications and records of milking, health, reproduction, feeding, and management.

2.3. Reproductive parameters and definitions

Diagnosis of CM was made by the presence of visible abnormalities in the milk (the presence of clots, flakes, blood, discoloration, or watery appearance) or through signs of inflammation (heat, pain, swelling, or redness) of one or more of the quarters. Cows were inspected to detect CM prior to each milking. When CM cases were suspected, the diagnosis was confirmed by the herd veterinarian.

Calving to first service interval (CFSI), calving to conception interval (CCI), and services per conception

(SC) were recorded and evaluated as reproductive parameters. Calving to first service interval was defined as the number of days between calving and the postpartum first insemination; CCI was defined as the number of days between calving and the insemination which resulted in a pregnancy; SC was defined as the number of inseminations that a cow required to conceive. The nonpregnant cows were defined as culling candidates until 250 DIM.

Definitions of calving seasons were: winter (Dec. 1 to Feb. 28), spring (Mar. 1 to May 31), summer (June 1 to Aug. 31), and fall (Sept. 1 to Nov. 30).

2.4. Statistical analysis

For each variable, the descriptive statistical values were calculated and presented as arithmetic mean \pm standard error. Prior to conducting significance tests, the data was analyzed with a Kolmogorov–Smirnov test to identify conformity with normal distribution and a Levene test to identify the homogeneity of variances. The significance of intergroup differences was analyzed with a Student's t-test for each researched variable, keeping in mind whether the animals have CM or are healthy, and whether they are primiparous or multiparous. The strength and direction of the relationship between the day on which the CM was diagnosed and the obtained variables were examined with Pearson's correlation coefficient. Clinical mastitis was divided into three groups: 0–20, 21–40, and 41–60, denoting the day on which CM was diagnosed in order to research the difference in terms of timing. The statistical significance of the difference between the groups was examined with a one-way analysis of variance (ANOVA). The SPSS 14.01 software package was used for all analyses. Values of $P < 0.05$ were considered significant.

3. Results

Prolonged CFSI and CCI and increased SC were observed in the mastitis group, compared to the healthy group (Table 1). According to the obtained results, for animals that experienced mastitis within the period prior to the voluntary waiting period, the CFSI, CCI, and SC increased by 11.77%, 26.81%, and 30.58%, respectively. Cull rates were 4.35% (6/132) and 0.48% (2/418) in the mastitis and healthy groups, respectively.

The negative effects of CM on reproductive parameters were not interrelated with the time of CM (Table 2), lactation number (Table 3), and calving season (Table 4; $P > 0.05$). Correlations of the reproductive parameters were shown in Table 5.

4. Discussion

The effect of CM on reproductive performance is an issue that has interested researchers since the early 2000s. Studies have revealed that CM affects reproductive

Table 1. Comparison of the parameters between the groups.

Variable*	Group	N	Mean ± SE	P
CFSI (day)	Mastitis	126	83.68 ± 3.18	0.007
	Healthy	416	74.57 ± 1.08	
CCI (day)	Mastitis	126	168.48 ± 8.59	<0.001
	Healthy	416	132.86 ± 3.44	
SC	Mastitis	126	3.16 ± 0.21	<0.001
	Healthy	416	2.42 ± 0.09	

* CFSI: Calving to first service interval; CCI: calving to conception interval; SC: services per conception.

Table 2. Comparison of the parameters according to the timing of CM.

Variable*	Pp days	n	Mean ± SE	P
CFSI (day)	0–20	50	85.68 ± 6.97	0.696
	21–40	31	79.03 ± 4.32	
	41–60	45	84.79 ± 3.78	
CCI (day)	0–20	50	167.3 ± 14.23	0.758
	21–40	31	159.35 ± 17.46	
	41–60	45	176.07 ± 13.82	
SC	0–20	50	3.04 ± 0.36	0.487
	21–40	31	2.87 ± 0.4	
	41–60	45	3.49 ± 0.34	

* CFSI: Calving to first service interval; CCI: calving to conception interval; SC: services per conception.

performance in the following ways: prolonged CFSI (longer than 22 days) and CCI (longer than 44 days) [11], increased number of services per conception [11], difficulty of estrus determination, ovulation delay [12,13], shortening of the interestrus interval [14], deterioration in oocyte development [15], decrease in the conception rate [16,17], and increased risk of abortion [18].

The adverse impact of mastitis on fertility differs depending on the length of time between insemination and the occurrence of mastitis [16,19,20], although opinions differ as to what constitutes the critical period. While some authors suggest that CM occurring in the period close to insemination has a greater adverse effect on reproductive performance [17,21], others suggest that the impacts of CM occurring during the period between insemination and the first pregnancy examination are more adverse [11]. In contrast to these views, Klaas et al. [22] states that mastitis

Table 3. Comparison of the parameters according to the calving season.

Variable*	Season	n	Mean ± SE	P
CFSI (day)	Winter	56	81.46 ± 3.29	0.546
	Spring	17	92.41 ± 14.73	
	Summer	19	93.37 ± 11.91	
	Fall	34	77.88 ± 4.26	
CCI (day)	Winter	56	158.54 ± 12.43	0.106
	Spring	17	215 ± 21.15	
	Summer	19	185.26 ± 17.8	
	Fall	34	152.21 ± 18.91	
SC	Winter	56	3.14 ± 0.35	0.543
	Spring	17	3.82 ± 0.52	
	Summer	19	3.32 ± 0.45	
	Fall	34	2.79 ± 0.4	

* CFSI: Calving to first service interval; CCI: calving to conception interval; SC: services per conception.

Table 4. Comparison of the parameters according to the parity.

Variable*	Parity	n	Mean	P
CFSI (day)	Primipar	51	86.58 ± 6.95	0.512
	Multipar	75	81.71 ± 2.52	
CCI (day)	Primipar	51	172.22 ± 14.23	0.721
	Multipar	75	165.93 ± 10.79	
SC	Primipar	51	3.3 ± 0.36	0.587
	Multipar	75	3.07 ± 0.26	

* CFSI: Calving to first service interval; CCI: calving to conception interval; SC: services per conception.

that occurs during early lactation prior to insemination has no effect on conception rates. Santos et al. [16] claims that CM occurring both in the preinsemination period and in the period between insemination and first pregnancy examination can have an adverse impact on reproductive performance. In our literature review, we encountered no study investigating the effect of CM occurring in the early postpartum period, i.e. the period up until the end of the VWP, on reproductive performance. Current studies generally investigate the effect of mastitis cases that occur in the periods both immediately before and after insemination. The results have shown that the CFSI, CCI, and SC parameters of animals that experienced mastitis

Table 5. Correlations of the parameters.

Variables*		Day of CM	CFSI	CCI	SC
Day of CM	r	1			
	P				
	n	132			
CFSI	r	-0.001	1		
	P	0.991			
	n	126	126		
CCI	r	0.07	0.312	1	
	P	0.435	<0.001		
	n	126	126	126	
SC	r	0.108	-0.086	0.835	1
	P	0.224	0.332	<0.001	
	n	126	126	126	126

* Day of CM: Timing of first clinical mastitis; CFSI: calving to first service interval; CCI: calving to conception interval; SC: services per conception.

** r: correlation coefficients

in the period prior to the VWP increased significantly. Although it is well known that diseases occurring in the early lactation period have an adverse effect on fertility, there is no precise data available on the underlying biological mechanisms [23]. That said, Ribeiro et al. [24] reported that diseases occurring in this period have a “carry over” effect, and their biological effects could last longer than 4 months.

The identified effect is possibly related to the delay in onset of ovarian activity from mastitis in the early postpartum period, or to the luteal regression from cytokine release in the early luteal period. Mastitis-related cytokines negatively regulate the secretion of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) [25]. Especially in CM, the level of cortisol in circulation increases, causing a delay or decrease in the secretion of LH and GnRH [26,27]. Accordingly, follicular development is impaired, and in this case, the level of preovulatory estradiol is low [12]. The synthesis of prostaglandins from the endometrium is under the

control of various cytokines, and endotoxins cause PGF2 α release [1]. It has also been reported by various researchers that premature luteolysis occurs in animals that contract mastitis in the diestrus stage [28,29]. The occurrence of these hormonal changes is characterized directly by the formation of an abnormal estrus/ovarian cycle. Prolonged anestrus [24] and delayed ovulation, as well as the formation of an atypical estrus cycle due to premature luteolysis [30], lead to a decrease in fertility [24]. Recently, researchers have reported that luteal activity starts early, and fertility is higher in animals with at least two luteal phases in the healthy period prior to insemination [31]. Similarly, it is suggested that mastitis has a carryover effect on follicular dynamics [32], and that the effect on both follicular dynamics and fertility may be related to the fact that cytokines secreted during mastitis maintain their presence, i.e. high concentrations in circulation, for a long time [23,33,34].

One limitation of the present study was that a pathogen isolation of the CM cases could not be performed. An evaluation of the results obtained detailing the type of pathogen that causes CM would be more useful, as there is a lack of consensus among researchers on how the type of pathogen causing mastitis affects reproductive yield. In general, it is reported that in mastitis caused by gram-negative pathogens, a decrease in fertility is more prominent [35], the interestrus interval is shortened [14], the risk of premature luteolysis is higher [36], and endocrine changes are prominent [37]. However, other researchers [11,16,18,19] have stated that the negative effect of mastitis on fertilization does not change according to the type of pathogen, and that, like gram-negative bacteria, gram-positive bacteria can also affect luteal function.

In conclusion, the CFSI and CCI are, respectively, prolonged by 11.77% and 26.81%, and the number of SC increases by 30.58% in cows experiencing CM in the early lactation period up until the VWP. It can be concluded that mastitis that occurs in the early lactation period of dairy cows can have a long-term adverse impact on fertility.

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