

# Sub-Clinical Mastitis and Reproduction: Season, Parity and Stage of Lactation Effects on Conception Rate and Milk Somatic Cell Count

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## Original article

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# Abstract

The aim of the present study was to investigate the effect of subclinical mastitis (somatic cell count (SCC)>250000 cells/ml) on the fertility of Holstein dairy cows in different parities, lactation stages and seasons. Data of 2437 SCC in a dairy farm including number of insemination, pregnancy detection, date of calving and insemination, SCC after insemination (maximum of 30 days), and parity were evaluated. The rate of subclinical mastitis in the first, second, third and fourth and more lactation number was 18.8%, 25.7%, 26.5% and 35.4%, respectively and in spring, summer, autumn and winter was 24.5%, 19.7%, 18.5% and 33.6%, respectively. Conception rate of cows with subclinical mastitis (24.6%) was lower than cows without subclinical mastitis (30.9%;  $P=0.003$ ). In addition, the conception rate in the second and third lactation in cows with subclinical mastitis was significantly lower than in cows without subclinical mastitis. The conception rate of cows with subclinical mastitis in summer in the second lactation and in winter in the second and third lactation was significantly lower than in cows without subclinical mastitis. Therefore, subclinical mastitis in mid lactation stage and also in the second and third parities especially in summer and winter can disturb fertility more than the other conditions. Also, considering these facts during these periods of times in managing of subclinical mastitis can improve fertility outcome of the herd.

## Introduction

Reproductive performance has reduced in industrial dairy cows simultaneously with strong genetic progress for high milk production (Berry et al. 2003). Reasons for the decline in fertility are abstruse but effects of management (Chagas et al. 2007) and physiological factors (Kafi et al. 2012a; Kafi et al. 2012b; Tamadon et al. 2011b) on reproductive performance has been demonstrated and high milk yield as a physiological process caused decline in fertility in dairy cow (Tamadon et al. 2011a). On the other hand, it has been demonstrated that with increase of milk yield, the sensitivity to mastitis has increased.

Cows produced more milk and also had 2% more lactations affected by mastitis (Shanks et al. 1978). Mastitis based on its symptoms is classified into two forms, clinical and subclinical mastitis. Subclinical mastitis due to effect on production, reproduction, and its longer duration than clinical mastitis creates more economic damage. Risk factors of subclinical mastitis included stage of lactation (age), management system, herd size, and season (Jarassaeng et al. 2012). High somatic cells count (SCC) in milk is highly associated with mastitis, such a way that an increase in SCC above 250000 cells/ml in milk has associated with subclinical mastitis (Ruegg 2003). The most important factor affecting the variation of SCC in milk related to the stage of lactation, lactation number (age), season, milk yield and infection status (Ruegg 2003).

Also, it has been revealed the relationship between a high SCC in milk and subclinical mastitis on conception in dairy cows; but the results of these studies are contradictory (Nguyen et al. 2011b; Pinedo et al. 2009; Santos et al. 2004; Schrick et al. 2001). Due to the effective factors such as different stages of lactation on reproduction (Ansari-Lari et al. 2010), subclinical mastitis (Jarassaeng et al. 2012) and

SCC (Ruegg 2003); and on the other hand, effect of a high SCC and subclinical mastitis on conception, the aims of the present study were 1) to investigate the effect of subclinical mastitis in different lactation stages and parities on the conception rate of Holstein dairy cows and 2) to describe the effect of SCC in different seasons on conception rate and number of insemination in dairy cow in mid-lactation (75 to 150 days of lactation).

## **Materials And Methods**

### **Animals and their conditions**

A retrospective epidemiological survey was conducted using 2437 SCC data from a management program in a high-producing Holstein-Friesian dairy herd in Mashhad (latitude of 36° 20' N and longitude 59° 35' E, 980 m above sea level) northeast Iran. Mean ( $\pm$  SD) lactation number was  $2.7 \pm 1.5$  and ranged from 1 to 8 lactations. Throughout the year, the cows were kept under roofed structures (free-stall barns) with open sides (zero-grazing system) and washed sand for bedding. They were grouped according to their milk production and fed according to the NRC 2001. The ration (total mixed ration) included mainly alfalfa, corn silage, beet pulp, cotton seed, soybean, corn, and barley. The cows under study were non-seasonal with year-round calving. The cows were machine-milked three times daily. The mean peak milk yield of the cows was 56 kg/day. The mean size of the herd was 1542 cows during the period of study. Dry cows were kept in a separate group and transferred three weeks prior to parturition to a close-up group. All animals were tested free of tuberculosis and brucellosis. The voluntary waiting period from calving to the first artificial insemination established for this dairy herd was 45 days. All cows were artificially inseminated.

### **Data collection and sorting**

Recorded data were included number of insemination, pregnancy detection, date of calving and insemination, SCC after insemination (maximum of 30 days), and parity of the cows. Cows had SCC above 250000 cells/ml in milk considered as cows with subclinical mastitis (Ruegg 2003) and cows with SCC < 250000 cells/ml considered as cows without subclinical mastitis. We categorized different lactation stages based on the days in milk into early (0–74 days in milk, DIM), mid (75–150 DIM), and late ( $\geq$  150 DIM) stages of lactation. We also classified parities of the cows into four groups including parities 1, 2, 3 and  $\geq$  4. There are four clearly distinguishable seasons in the geographical area of the present study. Climatological information and temperature–humidity index (THI) regarding this location during the course of the study is previously described (Moosavi et al. 2014).

### **Statistical analysis**

Spearman correlation test was used for evaluation of the relationship between SCC, season, number of insemination, parity and DIM using SPSS (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). The effect of season and subclinical mastitis on conception rate and number of insemination were statistically analyzed with the Chi-square test. Moreover, the effect of subclinical mastitis in different

lactation number on conception rate was investigated using Chi-square tests. Comparing of SCC and subclinical mastitis in different times of artificial insemination was done by analysis of variance and Chi-square tests, respectively.  $P < 0.05$  was considered statistically significant.

## Results

The conception rate in the second (22.6%) and the third (19.7%) lactations of cows with subclinical mastitis was significantly lower than cows without subclinical mastitis (31.3%;  $P = 0.03$  and 32.1%;  $P = 0.01$ , respectively). While, there were no significant difference in the first and fourth lactations and higher lactation (Table 3). The conception rate in cows with subclinical mastitis in summer in the second lactation (8.3%) and in winter in the second (22.6%) and third lactation (21%) was significantly lower than in cows without subclinical mastitis (22.4%;  $P = 0.05$ , 38.3%;  $P = 0.03$  and 35.2%;  $P = 0.05$ , respectively) (Table 3). While, there was no significant difference in the other seasons. Rates of subclinical mastitis in the first lactation number (18.8%) were less than the second (25.7%), third (26.5%) and fourth and more ones (35.4%) ( $P < 0.05$ ). Moreover, rates of subclinical mastitis in the second and third lactation number were less than the fourth and more one ( $P < 0.05$ ).

We observed the significant correlation between season and SCC in different lactation stages (Table 1). In the early, mid and late lactation the correlation coefficients between season and SCC were 0.2, 0.13 and 0.17 ( $P < 0.01$ ). In the early lactation, there was no significant correlation between SCC and other variables ( $P > 0.05$ ). In the mid lactation, an increase in milk SCC from spring to winter was observed ( $P = 0.001$ ;  $r = 0.13$ ). There were significant positive correlations between SCC and parity ( $r = 0.14$ ,  $P = 0.001$ ) and number of insemination ( $r = 0.1$ ,  $P = 0.03$ ). The rates of subclinical mastitis in spring (24.5%) were more than summer (19.7%), and autumn (18.5%) and less than winter (33.6%) ( $P < 0.05$ ). Moreover, the rates of subclinical mastitis in summer (19.7%) and autumn (18.5%) were less than winter (33.6%;  $P < 0.05$ ). Mean of number of insemination ( $P = 0.02$ ) and rate of non-pregnant cows ( $P = 0.002$ ) were more in cows with subclinical mastitis. Only in the spring, increased SCC was leading to increase the number of inseminations ( $P = 0.009$ ). In winter and spring, high SCC decreased the pregnancy rates in the first insemination ( $P < 0.05$ ). In summer, high SCC decreased the pregnancy rates in the second insemination ( $P = 0.03$ ).

Table 1

Correlation coefficients (r) between somatic cell count and different parameters including season, number of insemination, parity and days in milk during different stages of lactation

Stages of lactation	Season	Number of insemination	Parity	Days in milk
Early lactation (n = 336)	0.20**	0.03	0.10*	0.07
Mid lactation (n = 979)	0.13**	0.10*	0.14**	0.05
Late lactation (n = 1122)	0.17**	0.10**	0.15**	0.10**
Total (n = 2437)	0.16**	0.10**	0.15**	0.10**
** P < 0.01; * P < 0.05				

Cows with subclinical mastitis had less conception rate compared that of cows without subclinical mastitis in mid lactation stage (20.2 vs 29.6%; P = 0.005; Table 2). Based on the analysis of pooled data of all samplings, there was a significant different in conception rate between cows with and without subclinical mastitis (24.6 vs 30.9%; P = 0.003).

Table 2

Comparison of conception rate (pregnant cows/total cows; %) between cows with subclinical mastitis (somatic cell count > 250000 cells/ml) and without subclinical mastitis (somatic cell count ≤ 250000 cells/ml) as control group during different stages of lactation

Stages of lactation	Control	Subclinical mastitis
Early lactation (n = 336)	24.5 (60/245)	18.7 (17/91)
Mid lactation (n = 979)	29.6 (218/737) <sup>a</sup>	20.2 (49/242) <sup>b</sup>
Late lactation (n = 1122)	34 (273/804)	29.6 (94/318)
Total (n = 2437)	30.9 (551/1786) <sup>a</sup>	24.6 (160/651) <sup>b</sup>
<sup>a,b</sup> Different superscript letters indicate significant difference in the same rows (P < 0.05)		

Table 3  
Conception rate (%) in cows with subclinical mastitis and without subclinical mastitis as control group in different parity

Parity	Total		Summer		Winter	
	Control	subclinical mastitis	Control	subclinical mastitis	Control	subclinical mastitis
1 (n = 658)	33.0	30.6	30.7	20.7	37.8	38.1
2 (n = 654)	31.3	22.6 *	22.4	8.3 *	38.3	22.6 *
3 (n = 441)	32.1	19.7 *	23.0	20.0	35.2	21.0 *
≥4 (n = 684)	26.9	25.2	31.2	21.3	29.0	26.0

Stars indicate significant difference between control and subclinical mastitis in each season (P < 0.05).

## Discussion

In the present study, the conception rate in cows with subclinical mastitis in the second and third lactations was lower than in cows without subclinical mastitis. Meanwhile, there was no significant difference between cows with and without subclinical mastitis in the first and fourth and higher lactation. The results of this study also indicated an increase of subclinical mastitis following the increase of lactation number. Consistent with our findings, Jarassaeng et al. (2012) showed that increase of lactation number causes increase of incidence of subclinical mastitis. These may be due to weakened immune system with aging and a higher exposure to pathogens. Moreover, conception rate in the first and fourth lactations was lower than the second and third lactations (Ansari-Lari et al. 2010). Therefore, the effect of subclinical mastitis in these lactation numbers may be defective. Sensitivity to mastitis with increase milk production has increased (Shanks et al. 1978). Consequently, control of subclinical mastitis in the second and third lactations is more important, but that control must also be considered in the other lactation numbers.

In the present study, number of insemination and rate of non-pregnant cows were higher in cows with subclinical mastitis than cows without subclinical mastitis. Consistent with our findings, it has been shown that subclinical mastitis had effects on different reproductive indices in dairy cows (Schrick et al. 2001). Cows with subclinical mastitis before the first service had increased days to the first service, days open, and services per conception compared with controls (Schrick et al. 2001). It has been demonstrated that high SCC resulted in longer intervals from calving to the first breeding and to conception (Pinedo et al. 2009), as well as in lower the first artificial insemination, conception rate and total pregnancy rate (Santos et al. 2004). Contradictory results regarding the effect of high SCC on reproductive performance have been also reported (Nguyen et al. 2011a). High SCC and lameness reduced the likelihood of ovulation, but the same was not true for animals with a high SCC and without lameness (Morris et al.

2009). It is presumed that high SCC or subclinical mastitis inhibited the release of gonadotropic hormones which delayed resumption of ovarian cyclicity postpartum. So that, Lavon et al. (2010) reported that 30% of cows with subclinical mastitis exhibited delayed ovulation. Also, Nguyen et al. (2011b) demonstrated that cows with a high SCC have a higher incidence of abnormal postpartum resumption of ovarian cyclicity, included delayed first ovulation and prolonged luteal phases. Invasion of the mammary gland lead to the release of cytokines including interleukins (IL-1 $\beta$ , IL-6, IL-10, IL-12, and IL-1 $\alpha$ ), interferon- $\beta$ , tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Hansen et al. 2004). It has been demonstrated that certain cytokines such as IFN- $\beta$  decrease the secretion of luteinizing hormone (LH) (McCann et al. 2000). IL-6 blocks the secretion of estradiol (Alpizar and Spicer 1994) which can lead to reduced LH secretion, which is responsible for ovulation. Cytokines can also cause increased secretion of other molecules such as prostaglandin F $_{2\alpha}$  (PGF $_{2\alpha}$ ) (Hoeben et al. 2000) and nitric oxide (NO) (Athanasakis et al. 2000). PGF $_{2\alpha}$  may reduce fertility by interruption to oocytes maturation and pre-mature luteolysis (Hansen et al. 2004; Soto et al. 2003). Elevated concentrations of NO have also been associated with embryonic death (Soto et al. 2003). Also, mastitis results in increased blood concentrations of cortisol, a hormone that blocks the release and the peak of LH (Hockett et al. 2000). Also, the direct association between subclinical mastitis and endometritis has been suggested by Bacha and Regassa (2010). Endometritis increased service per conception and decreased pregnancy rate. Therefore, subclinical mastitis may cause increased service per conception and decreased pregnancy rate in dairy cows by interruption in normal function of hypothalamus, pituitary, ovaries, and uterus.

It has been reported that the incidence of mastitis can increase in spring and summer (Sabuncu et al. 2013), the others reported that incidence of mastitis can increase in winter and autumn (Batra et al. 1977). Also, the effect of parity on SCC (Nguyen et al. 2011b; Sabuncu et al. 2013) and subclinical mastitis (Jarassaeng et al. 2012) has been shown. In this study, we show that the pregnancy rate in cows with subclinical mastitis in summer in the second lactation and in winter in the second and third lactations was lower than in cows without subclinical mastitis; while, there was no significant difference in the other seasons. Heat stress in summer and high humidity combined cold stress in winter could be responsible for reducing the pregnancy rate (Li et al. 2021). In the mid lactation, only in the spring, high SCC was leading to high service per conception. In winter and spring, high SCC decreased pregnancy rates in the first insemination. In summer, high SCC decreased pregnancy rates in the second insemination. So it appears that the subclinical mastitis reduced conception rate during winter and spring in the mid lactation. In this study, increasing of subclinical mastitis in winter and spring and milk production in mid-lactation could be responsible for the reduced pregnancy rate. In addition, most of inseminations were done in the mid lactation stage and it is also expected time for setting days open in dairy farms. Moosavi et al. (2014) reported that in spring and winter, occurrence of clinical mastitis in primiparous cows increased in early lactation stage and in multiparous cows increased in late lactation stage. Occurrence time of clinical mastitis was not different between three different parities (1, 2 and  $\geq 3$ ) in mid lactation stage (Moosavi et al. 2014). They concluded that the most important stage of lactation which is in high risk for mastitis occurrence depends on the parity of cows. The early lactation is critical stage for primiparous cows in winter and spring while it was important for the third parity in winter and

autumn. The late lactation is critical stage for primiparous cows in summer and autumn while it was important for the second-parity in autumn and winter but in spring for the third parity (Moosavi et al. 2014). It seems that occurrence of clinical and subclinical mastitis can be happened in mid lactation especially in the second and third parities. As a result, control of subclinical mastitis is more important in the mid lactation stage. It is also important to control the rate of subclinical mastitis in the second and third parities especially in summer and winter. However, control of subclinical mastitis in the other seasons and lactation stages and periods also must be considered.

## **Declarations**

## **Ethics approval and consent to participate**

This investigation was performed in accordance with relevant guidelines and regulations of animal studies of Ethical Committee of Shiraz University.

## **Consent for publication**

Not applicable.

## **Availability of data and material**

The data used to support the findings of this study are included within the article.

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## **Competing interests**

There is no conflict of interest.

## **Authors' contributions**

A.T., A.M., and M.G. conceived and designed the format of the manuscript. T.A., A.M., A.T. and M.G. collected the data, and drafted and edited the manuscript. A.T. drew the Figures. All the authors reviewed the manuscript and all of them contributed to the critical reading and discussion of the manuscript. All authors have read and agreed to the published version of the manuscript.

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