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

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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. The likelihood of pregnancy decreased by a factor of 0.73 and 0.91 in cows had subclinical mastitis and for each increase in parity (from 1 to ≥4), respectively. The likelihood of pregnancy increased in cows inseminated in the mid (1.3) and late (1.7) and in cows inseminated in the autumn (1.73) and winter (1.38). 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.

Keywords: Conception rate, Subclinical mastitis, Parity, Season, Holstein dairy cow

Introduction

Reproductive performance has reduced in high producing dairy cows simultaneously with strong genetic progress for high milk production (Berry et al., 2003). Reasons for the decline in fertility are intricate 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). The risk of diseases such as mastitis was increased by high milk production (Wang et al., 2021).

The risk of mastitis is higher in multiparous or older cows than in primiparity (Silva et al., 2021). Mastitis based on its symptoms is classified into two forms, clinical and subclinical mastitis. Intramammary infection not only reduces the production, but also increases the cost of treatment and culling rates (Wolfenson et al., 2015; Cheng and Han 2020). Risk factors of subclinical mastitis included stage of lactation, management system, herd size, and season (Jarassaeng et al., 2012). Somatic cells count (SCC) in milk is highly associated with mastitis, parity (age), milk yield, stage of lactation and season; an increase in SCC above 250000 cells/ml in milk is considered as subclinical mastitis (Ruegg 2003). Schrick et al. (2001) reported that the subclinical mastitis reduce reproductive parameters of lactating cows. Days open of cows experiencing mastitis increased by 20 day compared with control cows (Santos et al., 2004), and they had 0.6 more service per conception than control cow (Schrick et al., 2001). The odds of conception were 0.85 for cows with a high scc (Pinedo et al., 2009). Lower conception rate and more days open was reported in cows with an SCC of 200,000 to 500,000 than cows with an SCC of less than 200,000 (Nguyen et al., 2011). Also, Nguyen et al. (2011) 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. Breeding risk period was defined from 3 days before to 32 days after artificial insemination by Wang et al. (2021). It has been reported that subclinical mastitis had negative effect on the conception rate during the breeding risk period

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(FuenzalidaFricke and Ruegg, 2015).

The fertility of the cows could be improved by the monitoring the number of somatic cells in the milk (Siatka et al., 2019). The disruptive effect of SCC within 30 days after first artificial insemination on the conception rate was reported (Sadeghi et al., 2021). Association of high SCC with the decline of fertility was reported by Wolfenson et al. (2015). Probability of conception was lowered by 14.5 and 20.5% in cows exhibiting moderate (from 450,000 to 10^6 cells/mL milk) and high (>10^6) SCC compared to the uninfected group (Wolfenson et al., 2015). Negative effect of elevated SCC (between d -10 to +30 from AI) on the conception rate was reported by Lavon et al. (2011). So, they recommended that the farmers can perform analysis of SCC which does not require special knowledge and SCC elevation before or after AI might predict depressed conception (Lavon et al., 2011). 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 association of subclinical mastitis in different lactation stages and parities with the conception rate of Holstein dairy cows and 2) to describe the association of SCC in different seasons with conception rate of dairy cow.

Materials and Methods

Animals and Their Conditions

A retrospective epidemiological survey was conducted using 2437 SCC data during two years 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 (±SD) lactation number was 2.7±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 (9 wk postpartum) 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 (AI) about 12 h after heat detection or timed AI in cows received synchronization programs which started at clean test.

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 (≥150 DIM) stages of lactation. We also classified parities of the cows into four groups including parities 1, 2, 3 and ≥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 was 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 subclinical mastitis in different parities was done by Chi-square test. P<0.05 was considered statistically significant. Possible effects of risk factors on conception rate of studied cows were explored using the Logistic regression analysis. The data of not-pregnant cows was used as reference. The data of inseminated cows with and without subclinical mastitis and from each stage of lactation groups, parity and season were compared by logistic regression analysis using the pregnancy as the dependent variable (0 denotes not-pregnant and 1 denotes pregnant), and subclinical mastitis, lactation number or parity, season and stage of lactation groups as independent variables. Cows without subclinical mastitis, inseminated in spring season and during early lactation were considered as references in the logistic regression model.

Results

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,

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

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) [a]	20.2 (49/242) [b]
Late lactation (n=1122)	34 (273/804)	29.6 (94/318)
Total (n=2437)	30.9 (551/1786) [a]	24.6 (160/651) [b]

[a,b] 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).

Table 4. Odds ratios of the variables included in the final logistic regression model for Conception rate of studied cows

Variable	Class	Conception rate %(n)	Odds ratio	%95 Confidence Interval	P value
Parity	Continuous		0.91	0.84-0.98	0.01
Season	Spring	25.9 (183/707)	Reference		
	Summer	24.9 (160/642)	0.93	0.73-1.19	0.5
	Autumn	38.1 (134/352)	1.73	1.31-2.28	<0.001
	Winter	31.8 (234/736)	1.38	1.10-1.75	0.006
Stages of lactation	Early	22.9 (77/336)	Reference		
	Mid	27.3 (267/979)	1.30	0.97-1.74	0.07
	Late	32.7 (367/1122)	1.70	1.27-2.26	<0.001
Subclinical mastitis*	Absence	30.9 (551/1786)	Reference		
	Presence	24.6 (160/651)	0.73	0.59-0.90	0.004

Likelihood-ratio test=2886.1, 7 d.f., P=0.0001; Hosmer and Lemeshow Goodness-of-fit test=2.8, 8 d.f., P=0.94; (the model fits).
*Cows with subclinical mastitis had somatic cell count>250000 cells/ml and without subclinical mastitis had somatic cell count ≤250000 cells/ml

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).

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).

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).

The likelihood of pregnancy decreased by a factor of 0.73 in cows had subclinical mastitis compared to that of healthy cows. The likelihood of pregnancy increased in cows inseminated in the mid and late lactation by factor of 1.3 and 1.7 compared to that of the cows inseminated in early lactation. The likelihood of pregnancy increased in cows inseminated in the autumn and winter by factor of 1.73 and 1.38, respectively, and decreased by a factor of 0.91 for each increase in parity (from 1 to ≥4) of cows (Table 4). There was no significant interaction effect of different variables on the conception rate that was explored using the logistic regression analysis.

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 SCC following the increase of lactation number. Consistent with our findings, Jarassaeng et al. (2012) showed that increase of lactation number causes increase of SCC. 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). A higher incidence of postpartum delayed first ovulation in cows with high SCC cows (> 500,000) during the first month and a higher incidence of prolonged luteal phase in cows with SCC from 200,000 to 500,000 was reported (Nguyen et al., 2011). 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. Invasion of the mammary gland lead to the release of cytokines including interleukins (IL-1β, IL-6, IL-10, IL-12, and IL-1α), interferon-β, tumour necrosis factor-**α** (TNF-α) (Hansen et al., 2004). It has been demonstrated that certain cytokines such as IFN-β 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 F2α (PGF2**α**) (Hoeben et al., 2000) and nitric oxide (NO) (Athanassakis et al., 2000). PGF2α 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 SCC 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). Positive correlation (0.3 and 0.11) was found between parity and SCC during hot and cold season, respectively (Sabuncu et al., 2013); and a higher incidence of high SCC of cows was reported in parity 5 or more than cows in the first and second parities (Nguyen et al., 2011). The odds of subclinical mastitis were 1.41 and 2.37 for cows in parities 4-6 and > 6, respectively, while it was 0.54 for cows in parities 1-3 (Jarassaeng et al., 2012). 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. 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.

Conclusion

Cows with subclinical mastitis had less conception rate compared that of cows without subclinical mastitis especially in mid lactation stage, and in the second and the third parities. 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 healthy cows. 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.

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.

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Authors' Contribution

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. 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.

Competing Interests

Author Amin Tamadon was employed by PerciaVista R&D Co. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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