



## Effects of Clinical Mastitis on Reproductive Performances in Dairy Cows

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### Abstract

The study was undertaken to study the productive and reproductive performances in clinical mastitis (CM) affected and apparently, healthy dairy crossbred cows and to analyze the effects of mastitis considering age, lactation, and severity of mastitis on reproductive performances in crossbred cows. A survey using a questionnaire was done to record different parameters of the cows during the period November 2021 to March 2022. A total of 58 lactating cows included in this study were divided into two groups named mastitis (n=24) and controlled (n=34). Cows of the mastitis groups were divided into subgroups depending on the age, lactation number and severity of mastitis. Prolactin (PRL), progesterone (P4) and estrogen (E2) levels of cows at the time of AI were analyzed. The results showed that cows of the mastitis group had substantially ( $P<0.01$ ) higher values for Days to first heat detection after parturition ( $96.16\pm6.20$ ), Days to first insemination after parturition ( $105.00\pm6.64$ ), Days open ( $140.58\pm7.40$ ), and number of services per conception ( $2.33\pm0.11$ ). The results also indicated that the greatest values were seen in the cows of Sub-group age  $>8$  for Milking Yield ( $16.21\pm2.39$  Lt), Days to first heat detection after parturition ( $109.29\pm13.57$ ), Days to first insemination following parturition ( $122.14\pm15.87$ ), and Days open ( $165.79\pm16.70$ ), and in cows of Sub-group 7-8 had for the Days of incidence of mastitis following parturition ( $51.57\pm12.73$ ). Considering the number of lactations, we observed the highest values of Milking Yield ( $16.69\pm2.20$  Lt), Days to first heat detection after parturition ( $108.75\pm11.58$ ), Days to first insemination following parturition ( $120.00\pm13.96$ ), and Days open ( $160.63\pm15.20$ ) in cows of Sub-group lactation number  $>3$  and the highest Days of occurrence of mastitis after parturition in cows of Sub-group lactation number 3. Based on the degree of clinical mastitis, it was found that cows of the severe mastitis sub-group had the greatest Milking Yield ( $24.80\pm5.40$  Liter) at a significant level ( $P<0.05$ ). This result indicates an association of severe CM with a high yield of milk. Whereas, there was no significant ( $P>0.05$ ) variation observed in different parameters in terms of reproductive performances in all sub-groups. In addition, significantly ( $P<0.01$ ) higher prolactin levels ( $16.10\pm1.17$  ng/ml) and low estrogen levels ( $13.86\pm0.57$  pg/ml) were present in CM-affected cows. This study could help veterinarians in taking strategies to minimize and control the negative effect of clinical mastitis on the reproduction of dairy cows.

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## Introduction

A farm's profitability is largely determined by the reproductive success of dairy cattle in the dairy industry. Insufficient reproductive success raises the likelihood of involuntary culling, which affects total output, the number of calves per cow, and eventually the farmer's profit. Numerous predisposing variables contribute to the appearance of infertility in cows. One important element influencing the herd's ability to reproduce is mastitis. There is a relationship between the fertility of milking dairy cows and the health of their mammary glands (Chebel et al., 2004). The relationship between mastitis and fertility is intricate and varied, arising from the interaction of the infectious agent, treatment techniques, and environmental factors.

The intensity of the immune response to bacterial intramammary infection appears to be the cause of the detrimental effects of mastitis on reproductive function. Based on retrospective data, several researchers observed the detrimental effects of mastitis on reproduction (Gunay and Gunay, 2008; Podpecan et al., 2013; Boujenane et al., 2015; Smulski et al., 2020). There are two types of mastitis observed in dairy cows, where clinical mastitis (CM) has been found to cause an increased risk of abortion or embryonic loss (Moore et al., 2005). It is reported that mastitis in dairy cattle is associated with lower rates of pregnancy, irregularities in the oestrous cycle, early embryonic death or abortions, longer days open, more services per conception, and lower rates of conception (Gunay and Gunay 2008; Hertl et al., 2010). There is limited study on the impact of CM on the ability of dairy cows to reproduce and produce milk. Therefore, the objectives were to determine the effect of CM on the reproductive and productive performances of mastitis-affected dairy cows in Bangladesh.

## Materials and Method

This research was conducted in selected medium-scale dairy farms in Tangail district in Bangladesh during the period from November 2021 to March 2022. A total of 58 lactating cows were selected and divided into two groups, groups named mastitis (n=24) and controlled (n=34). The mastitis group

comprised cows with inflammation, resulting in visible heated and swollen udder and macroscopic alterations in milk, such as clots and watery discharge (Smith et al., 2001; Smulski et al., 2020). All mastitis-affected cows were sub-grouped based on age (3-5 years, 6-8 years, >8 years lactation number( < 3, 3,>3) and severity of mastitis (Mild, Moderate, Severe).

A survey method was used in this research. Data was obtained through a face-to-face interviewing method using questionnaires that had been prepared and structured previously. The questionnaire was developed to get a reasonably accurate answer. We used a direct interviewing approach, asking respondents questions and noting their responses in a questionnaire. The animals were checked and farm records were read to get more information. Farmers were also given suggestions on the management of reproduction and data recording.

The cow's body condition was scored by visual or tactile inspection. Following the finding of Haddy et al. (1994), cows were often given a 5-point body condition score, with 1 denoting thinness and 5 denoting fatness. The severity of CM cases was rated according to the guidelines of Pinzón-Sánchez *et al.* (2011). CM with only abnormal milk is rated mild; mastitis with abnormal milk plus abnormal udder appearance (such as swelling or redness) is rated as moderate; and mastitis with systemic symptoms (such as fever, depression, or off-feed behaviour) is rated as severe. The number of services per conception, days open, and the gap between calving and the first service were noted as the reproductive performance parameters in both healthy and mastitis-affected cows.

For hormonal analysis using the Enzyme-Linked Immunosorbent Assay (ELISA) method, blood serum was taken from cows with and without mastitis. In the laboratory of the Community-Based Dairy Veterinary Foundation (CDVF), Department of Surgery and Obstetrics, BAU, the collected serum was evaluated using specific ELISA test kits of estrogen, progesterone and prolactin (Nova Tec Immunodiagnostica® GmbH, Technologio and Waldpark, Dietzenbach, Germany) and ELISA reader (SPECTRAMax® 340PC384, USA). Manufactured instructions

were followed to perform the analysis and the concentration of progesterone, estrogen and prolactin was calculated using the formula obtained from obtained from Optical density (OD) curves of standard samples.

#### *Analysis of data*

The collected survey data were compiled, classified, tabulated and analyzed using SPSS version 20.0). “Independent T” test was used to compare data of mastitis and controlled groups, whereas, major parameters of different sub-groups (Age, parity, mastitis score) were tested using one-way analysis of variance (ANOVA).  $P < 0.05$  was considered the level of significance.

### **Results and Discussion**

The study was designed to know the productive and reproductive performances in mastitis-affected and healthy cows and to analyze the effects of mastitis considering age, lactation stage, and severity of mastitis on reproductive performances in crossbred cows.

Results presented in Table 1 reveal that clinical mastitis declines milk production as we found insignificant variation in milk yield of apparently healthy cows and mastitis-affected cows. Clinical mastitis significantly increased the Days to first heat detection after parturition, Days to the first insemination after parturition, the Days open, and the number of services per conception resulting in declined reproductive performances. We observed that CM-affected cows had significantly higher Days to first heat detection after parturition, Days to the first insemination after parturition and Days open compared to clinically healthy cows. Similarly, Vasek *et al.* (2007) and Kumar *et al.* (2017) reported delayed estrus and insemination, increased days open and several services per conception in CM-affected crossbred cows. Our results on the number of services per conception, days open, days to first heat detection after parturition, and days to first insemination following parturition in cows affected by CM are consistent with those previously published (Gunay and Gunay, 2008; Nava-Trujilli *et al.*, 2010). Additionally, Hockett *et al.* (2005) found that the preovulatory period, the time of estrus, and the pregnancy rate were all lower in cows with CM.

**Table 1.** Values (Mean $\pm$  SE) of BCS, daily milk yield and reproductive parameters observed in clinical mastitis-affected dairy cows and healthy cows

Parameters	Mastitis Group	Control Group	P value
BCS	3.02 $\pm$ 0.02	3.02 $\pm$ 0.03	0.985
Daily milk yield (Liter)	14.69 $\pm$ 1.08	15.33 $\pm$ 2.03	0.772
Length of the oestrous cycle (Days)	23.16 $\pm$ 0.41	21.20 $\pm$ 0.17	0.087
Days to first post-partum heat	96.16 $\pm$ 6.20	64.67 $\pm$ 5.31	0.006
Days to first post-partum insemination	105.00 $\pm$ 6.64	66.67 $\pm$ 5.56	0.002
Days open	140.58 $\pm$ 7.40	68.13 $\pm$ 5.62	0.000
Number of services per conception	2.33 $\pm$ 0.11	1.07 $\pm$ 0.07	0.000

According to Hansen *et al.* (2004), delayed estrus manifestation in cows with CM may result from changes in the hypothalamic-pituitary hormonal axis and their impact on the commencement of cyclicity. The endotoxin produced by the mastitis-causing organisms may have local and systemic effects, leading to further deviation in reproductive indices in the clinically afflicted cows found in our research. A similar observation was reported by Hogan and Smith (2003). Mastitis in dairy cattle is linked to lower pregnancy rates, oestrous cycle irregularities, early embryonic death or abortions, longer open days, more services required for

conception, and more complications. (Gunay and Gunay 2008; Hertl *et al.*, 2010). In contrast, Gómez-Cifuentes *et al.* (2014) discovered no relationship between CM and reproductive problems, including the frequency of pregnancy or the number of services received. They evaluated the relationship between the reproductive outcomes of cows managed on pasture and clinical mastitis, and they proposed that the kind and intensity of the infection play a significant role in determining the prognosis of mastitis. According to Sikder (2018), a changed hormonal profile in cows with mastitis may be the cause of their low reproductive function,

as he observed increased prolactin levels and decreased estrogen levels at the time of AI and a strong negative relation among prolactin and P4 with E2 and estrus duration in repeat breeder cows with clinical mastitis. Therefore, it has been suggested to consider hormonal profiles during the management of mastitis to improve the reproductive performances of dairy cows (Sikder, 2018). According to Ahmadzadeh et al. (2009), mastitis by itself reduces the effectiveness of reproduction, and the presence of other illnesses has a compounding impact on mastitis.

When reproductive parameters were evaluated considering the lactation period among CM-affected cows, the highest values of days to first heat and days open were observed in cows of more than eight years of age and with more than three lactation periods (Table 2, Table 3). According to Nava-Trujillo et al. (2010), factors such as poorer energy balance, poor dry matter intake, increased body condition loss, lower blood glucose, insulin, and insulin-like growth factor-1 concentrations could be the cause of delayed heat after parturition and longer days open.

**Table 2.** Values (Mean  $\pm$  SE) of BCS, daily milk yields and the reproduction parameters in mastitis-affected dairy cows based on cow's age

Parameters	Cows affected with clinical mastitis			P value
	Age			
	3-5 years	6-8 years	>8 years	
BCS	3.06 $\pm$ 0.04	3.02 $\pm$ 0.04	2.9821 $\pm$ 0.03	0.051
Daily milk yield (Liter)	12.07 $\pm$ 1.69	15.00 $\pm$ 1.48	16.21 $\pm$ 2.39	2.656
Length of the oestrous cycle (Days)	23.23 $\pm$ 1.00	22.81 $\pm$ 0.53	23.50 $\pm$ 0.64	1.027
Days to first post-partum heat	86.15 $\pm$ 10.35	92.81 $\pm$ 8.01	109.29 $\pm$ 13.57	15.179
Days to first post-partum insemination	96.92 $\pm$ 9.09	96.56 $\pm$ 7.86	122.14 $\pm$ 15.87	16.073
Days of occurrence post-partum mastitis	23.61 $\pm$ 6.43	51.57 $\pm$ 12.73	39.07 $\pm$ 11.58	15.649
Days open	130.31 $\pm$ 10.03	126.88 $\pm$ 8.92	165.79 $\pm$ 16.70	17.337
Number of services per conception	2.15 $\pm$ 0.22	2.25 $\pm$ 0.14	2.5714 $\pm$ 0.23	0.279

**Table 3.** Values (Mean $\pm$  SE) of BCS, daily milk yields and the reproduction parameters in mastitis-affected dairy cows based on lactation number

Parameters	Cows affected with clinical mastitis			P value
	Lactation number			
	< 3	3	>3	
BCS	3.06 $\pm$ 0.04	3.01 $\pm$ 0.04	2.98 $\pm$ 0.03	0.589
Daily milk yield (Liter)	11.91 $\pm$ 1.64	14.80 $\pm$ 1.42	16.69 $\pm$ 2.20	0.485
Length of the oestrous cycle (Days)	23.33 $\pm$ 1.08	23.07 $\pm$ 0.56	23.13 $\pm$ 0.59	0.981
Days to first post-partum heat	85.83 $\pm$ 11.25	91.00 $\pm$ 8.84	108.75 $\pm$ 11.58	0.566
Days to first post-partum insemination	97.50 $\pm$ 9.86	95.00 $\pm$ 8.24	120.00 $\pm$ 13.96	0.534
Days of occurrence post-partum mastitis	21.83 $\pm$ 6.72	48.20 $\pm$ 13.26	43.38 $\pm$ 10.67	0.524
Days open	131.83 $\pm$ 10.77	126.20 $\pm$ 9.15	160.63 $\pm$ 15.20	0.440
Number of services per conception	2.25 $\pm$ 0.22	2.20 $\pm$ 0.17	2.50 $\pm$ 0.20	0.720

Results stated insignificant ( $P>0.05$ ) variations in different parameters in mastitis-affected cows of age, lactation, and severity of clinical mastitis (Table 4) sub-groups. We found the effect of mastitis on reproductive performances irrespective of age, lactation stage and severity of mastitis. When data were analyzed based on the severity of clinical mastitis, we observed Milking Yield (24.80 $\pm$ 5.40) was significantly ( $P<0.05$ ) highest in the severe mastitis Sub-group. Whereas, no

significant ( $P>0.05$ ) variation was observed in different parameters in terms of reproductive performances in all sub-groups regarding the severity of clinical mastitis. Consistent with previous findings of Kumar et al. (2017), we discovered that the majority of CM patients had mild to moderate severity. In this study, it was found that mild to medium forms of clinical mastitis occurred before the end of the involuntary waiting period and severe forms of mastitis occurred after

the end of the involuntary waiting period. In all forms of CM, the first insemination was done after mastitis affection. According to this result, we could state that cows who experienced mastitis before the end of the voluntary waiting period had more Days Open. This result is in line with the findings of Ahmadzadeh et al. (2009) and Smulski et al. (2020), which hypothesize that reproductive success may be affected if CM occurs before the

first AI. According to Lavon et al. (2011), fertility is lowered by a CM during the first 10 days before the first AI. In contrast, However, Kumar et al. (2017) found a significant impact of CM occurred before the first AI on the conception rate. They also stated that CM did have a greater detrimental effect on reproductive outcomes in terms of DO when it appeared in milk after 62 days.

**Table 4.** Values (Mean± SE) of BCS, daily milk yields and the reproduction parameters in mastitis-affected dairy cows based on the severity of clinical mastitis

Parameters	Cows affected with clinical mastitis severity of clinical mastitis			P value
	Mild	Moderate	Severe	
BCS	3.00±0.03	3.06±0.03	2.95±0.05	0.467
Daily milk yield (Liter)	13.33±1.42	13.41±1.42	24.80±2.42	0.002
Length of the oestrous cycle (Days)	23.19±0.54	23.41±0.77	22.20±0.80	0.792
Days to first post-partum heat	91.90±6.50	104.11±11.82	87.00±24.37	0.767
Days to first post-partum insemination	100.00±5.61	116.47±13.58	87.00±24.37	0.562
Days of occurrence post-partum mastitis	36.67±9.80	33.23±7.79	68.80±25.82	0.493
Days open	131.90±6.92	158.05±14.65	117.60±23.38	0.420
Number of services per conception	2.29±0.14	2.47±0.21	2.00±0.32	0.633

The effects of PRL, P4, and E2 at the time of AI on pregnancy rate were also considered and the results are shown in Table 5. In this study, mastitis-affected cows had significantly higher prolactin and lower estrogen levels than cows without mastitis at the time of AI. In contrast, Hockett et al. (2000)

found no variation in the concentration of prolactin and estradiol between mastitis and control cows. These divergent findings point to the significance of appropriate experimental design and the function of the animal's physiological state in understanding the impact of mastitis on reproduction.

**Table 5.** Values ((Mean± SE) Prolactin (PRL), progesterone (P4) and estrogen (E2) levels at the time AI in mastitis affected dairy cows and healthy dairy cows

Hormones	Control Group	Mastitis Group	P-value
Prolactin (ng/ml)	9.47±0.41	16.10 ±1.17	0.004
Progesterone (ng/ml)	0.89±0.03	0.91±0.01	0.81
Estrogen (pg/ml)	20.31±0.78	13.86±0.57	0.006

However, this study concludes that clinical mastitis significantly ( $P<0.01$ ) increases the Days to first heat detection after parturition, Days to first insemination after parturition, Days open cows and the number of services per conception in crossbred dairy cows. CM declines the reproductive performance of crossbred dairy cows irrespective of age, lactation stage and severity of clinical mastitis. Moreover, results reveal that severe clinical mastitis is associated with a high yield of milk. Due to the small number of cows and lack of records for subclinical infections in these

animals, we were unable to analyze factors like season, episode of recurrence of mastitis and subclinical mastitis infections, and the severity of adverse effects when CM occurred between post-partum insemination and pregnancy confirmation. For this reason, more research including a large sample size is required to accurately determine how CM affects crossbred dairy cow's ability to reproduce. Thorough research on the effects of various mastitis episodes on reproductive performance is essential, as the consequences of



severe instances of CM on reproduction are not as well understood. Future studies should also focus on comprehending the pathophysiology of mastitis on follicular development and the developmental competency of oocytes in cows affected by CM. Moreover, improvement of reproductive performances should be considered during designing the treatment of CM in crossbred dairy cows.

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### Conflict of Interests

The authors have no disclosure to make that qualifies as a conflict of interest.

### Statement of Author's Credit

MIA, ZM, MMH, NSJ: Conducting research, acquisition of data, and writing the manuscript. MIA. ZM: Acquisition of data and drafting the manuscript. MMH: Editing the manuscript, statistical analysis, NSJ: Designing and overall supervision of the study, finalizing the manuscript.

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