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Impact of subclinical mastitis on uterine health, reproductive performances and hormonal profile of Zebu × Friesian crossbred dairy cows in and around Jimma town dairy farms, Ethiopia

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ABSTRACT

A longitudinal observational study was carried out from January 2020 to July 2021 to assess the impact of subclinical mastitis (SCM) on reproductive performance and its association with uterine health of crossbred dairy cows. The California Mastitis Test (CMT) and cytobrush technique were used to screen subclinical mastitis and subclinical endometritis, respectively. Milk samples positive for subclinical mastitis were subjected to bacteriological analysis. Data from 84 clinically healthy cows collected and analyzed. The present study revealed a prevalence of subclinical mastitis of 51.2% (43 of 84). The mean days from calving to first service interval were significantly longer in subclinical mastitis positive cows than negative (control) cows (120.51 ± 24.5 and 85.15 ± 28.3, respectively) ($P < 0.05$). The mean number of services per conception was significantly higher in positive cows (2.51 ± 0.83) than in negative cows (1.59 ± 0.81) ($P < 0.05$). Lower conception and pregnancy rates at first services were observed in subclinical mastitis cows. Risk factors analysis revealed that prevalence of subclinical mastitis significantly differed with the parity and body condition score ($P < 0.05$). The current study revealed that subclinical mastitis was significantly and directly associated with subclinical endometritis ($P < 0.05$). Subclinical mastitis significantly decreased ($P = 0.000$) progesterone concentrations and increased ($P = 0.001$) the cortisol concentrations. *Staphylococcus aureus* were the most predominant bacterial isolates from subclinical mastitic milk, followed by coagulase negative staphylococci (CNS) and streptococci. This study concludes a high prevalence of subclinical mastitis caused by *Staphylococcus aureus* could inflict harmful effects on reproductive performance of dairy cows, emphasizing the relevance of mastitis control programs in dairy farms.

1. Introduction

Reproductive performance is a key factor in determining the profitability of dairy farms. Reproductive losses are affected by many factors with the harm of mastitis being particularly critical [1]. Kumar et al. [2] also showed that mastitis during the breeding period was harmful to the reproductive capacity.

Mastitis is defined as an inflammation of the mammary gland disease that affects cattle health and wellbeing [3]. Mastitis not only affects the mammary gland, but also causes systemic immune response and endocrine changes, which has a negative impact on

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reproductive performance. Cows with mastitis have delayed oestrus, decreased pregnancy rate (PR), and increased risk of abortion [1]. Mastitis may affect the reproduction of cows by destroying follicles, affecting oocyte growth or function, and reducing ovulation ability [4]. Mastitis causes the increase in oestrus interval and decrease in the luteal phase in cows, which impair the establishment and maintenance of pregnancy and impede embryonic development [5]. The occurrence of mastitis significantly increased the mean calving to first service interval (CFSI), days open (DO), number of services per conception (NSPC), and pregnancy loss at first service [6].

Bovine mastitis can be classified into two types, namely, clinical mastitis (CM) and subclinical mastitis (SCM). Clinical mastitis is detected by the changes in physical appearance of milk, swelling, redness, and rise in temperature of udder, whereas animals with SCM do not exhibit any gross changes in milk or udder and can be detected only through laboratory tests and California Mastitis Test (CMT) [7]. Both CM and SCM reduce reproductive efficiency in dairy cattle [2]. Nava-Trujillo et al. [8] reported that postpartum CM prolonged the days of first service (DFS) and days to conception in primiparous cows.

Mastitis is caused by a wide spectrum of pathogens classified into contagious and environmental mastitis [9]. Contagious pathogens are those for which primary reservoir is the udder of infected cows. They spread from cow to cow, primarily during milking, and tend to result in chronic subclinical infections with flare-ups of clinical episodes. Contagious pathogens include: *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma* spp. and *Corynebacterium bovis* [10]. Environmental pathogens include *Klebsiella* spp*., E. coli, Strept. dysgalactiae* and *Strept. uberis* [11].

Inflammation due to mastitis occurs when bacteria enter the mammary gland, multiply and produce toxins, enzymes, and cell-wall components, which stimulate production of various inflammatory mediators [3]. Mammary gland infection causes immune responses, resulting in the abnormal secretion of cytokines and hormones, and abnormal function of the reproductive system such as the ovary, corpus luteum, uterus, and embryo. Inflammatory cytokines and bacterial endotoxins induced by mastitis can also lead to delayed oestrus, hormonal imbalances, and other related problems, resulting in the decrease of fertility [1]. Ethiopia holds large potential for dairy development due to its largest livestock population in Africa with cattle being the dominant livestock species accounting for approximately 58 million heads [12]. Despite these large numbers, milk production often does not satisfy the country's requirements due to multiple factors such as the occurrence of mastitis [13–15]. Over the years there are many well documented studies addressing the prevalence of bovine subclinical mastitis (SCM) in Ethiopia. According to the most recent published studies, the prevalence estimate falls within the range of 22.7 and 80.6% for the country [13,15–22]. Meta-analytic studies carried out in Ethiopia suggested that dystocia, retained placenta, metritis, infectious bovine Rhinotracheitis, brucellosis and metabolic disturbance occurring around parturition and postpartum negatively affected the reproductive performance of cross breed cows [23–25], however, there was no a single specific study conducted to evaluate the effect of mastitis on uterine health, reproductive performance and hormonal profile of Zebu × Friesian crossbred dairy cows. The objectives of the current study were to assess the impact of subclinical mastitis (SCM) on reproductive performance and its association with uterine health of crossbred dairy cows.

2. Materials and methods

2.1. Study population

Jimma town is located in Oromia Regional State, 352 km South-West of Addis Ababa. The region experiences annual rainfall ranging from 1400 to 1900 mm, mean maximum and minimum temperatures of 25–30 °C and 7–12 °C, respectively [13]. The area is predominantly known for its coffee, crop and livestock production.

From a total of 201 cows that calved during the study period only 84 clinically healthy Zebu × Friesian crossbred dairy cows were selected for this study. One hundred and seventeen cows that were with reproductive health problems, metabolic diseases, infectious diseases and foot problems were recorded, and excluded from the study, since these diseases can affect uterine health, reproductive performance and hormonal profiles of cows. In addition, cows with symptoms of clinical mastitis were excluded from the study, whereas cows without any sign of clinical mastitis were tested with CMT to reveal subclinical mastitis prevalence. The study was conducted on a total of 11 farms. Two of the farms were from Jimma University College of Agriculture and Veterinary Medicine and Jimma University dairy enterprise. The others 9 farms were from private dairy farms. Purposive sampling method was employed to select 11 intensive farms out of 71 active dairy farms with the same environment, and management practices. Oestrus signs were monitored by visual observation. Pregnancy was confirmed by rectal palpation 60 days after artificial insemination (AI). Hand milking was practiced in all farms, and cows were milked twice daily.

2.2. Study design

The longitudinal observational study was carried out on 84 clinically healthy crossbred dairy cows. All cows for this study were kept under the same environment, feeding regime and intensive husbandry system for the whole study period. Regular visits were carried out once per weeks for 330 days after calving to record their reproductive parameters on dairy farms.

The reproductive performance data included calving to first service interval (CFSI, days), number of services per conception, conception rate at first service (CRFS, %) and pregnancy rate at first service (PRFS, %). Calving to first service interval (CFSI) was defined as the number of days between calving and the first insemination date. The first service in each farm did occur after the voluntary waiting period, which was 50 d after calving in all herds. The number of services per conception (NSPC) was defined as the number of services that result in a conception. Conception rate at first service (CRFS, %) defined as the number of known pregnant animals divided by the number of inseminated animals with known outcomes, considering only the first AI after calving, and multiplied by 100. Pregnancy Rate at first service (PRFS, %) defined as number of known pregnant animals divided by the number of cows eligible to become pregnant, considering only the first AI after calving, multiplied by 100.

2.3. Subclinical mastitis detection and definition

The milk sample was collected from cows that were not treated early with either intramammary or systematic antimicrobials agents. Udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with cotton or gauze sponge moistened with 70% ethyl alcohol. A separate sponge was used for each teat. Scrubbing was continued until a new surface of the cotton or sponge remains clean. Sterilized screw capped test tubes were used for collecting milk samples from the individual cows and the first stream of milk from each quarter was discarded. After collection of the milk sample, all samples were clearly labelled with the appropriate identification number of the cows. The milk sample then held in an ice box for transportation to the Microbiology laboratory of Jimma University, School of Veterinary medicine. In laboratory samples were cultured immediately or stored at +4 °C [26].

The California Mastitis Test (CMT) was used to screen cows with subclinical mastitis. A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 s. The result of the test was indicated on the basis of gel formation. The CMT results were scored as 0 (negative), trace, 1(weak positive), 2 (distinct positive) and 3 (strong positive) based on gel formation. All CMT scores of 0 and trace were considered as negative while CMT scores of 1, 2, and 3 were considered indicators of subclinical mastitis. Positive cows were defined as having at least one quarter with CMT score of 1^+.

2.3.1. Bacteriological culture

Bacteriological culture was performed according to Quinn et al. [27]. For the primary isolation and identification of mastitis causing pathogens colony size, Shape, colour, pigmentation, haemolytic characteristic, Grams reaction, Oxidase test [oxidase positive in modified oxidase test and oxidative in the oxidation and fermentation test (O–F test)] were performed. After these colonies were subcultured to different media, such as MacConkey agar (Oxiod, Hampshire, England), Manitol salt agar, Edwards medium (Oxiod Hampshire, England), Eosin methylene blue medium (EMB) (Oxiod, Hampshire, England), etc. to get a pure culture. And the secondary biochemical tests such as, coagulase test, urease test, IMViC (indole, methyl red, Voges-Proskaur, and citrate) tests, sugar tests, etc. were done for bacterial species identification. Samples yielding 2 different bacterial species were grouped as "mixed culture", whereas samples yielding more than 2 different bacterial species were considered to be contaminated and rejected.

2.3.2. Subclinical endometritis

Subclinical endometritis was determined using cytobrush concomitantly with subclinical mastitis at 56 ± 3 DIM postpartum as described by Dubuc et al. [28]. Cytobrush technique was used connecting a modified human cytobrush to the plunger of an artificial insemination gun covered with plastic AI sheath. The cytobrush assembly was introduced into the uterus as for doing artificial insemination. Inside the uterus the brush was screwed gently in both directions. Immediately after removal from reproductive tract, the cytobrush was smeared on clean glass slide and stained with Giemsa stain. The slide was observed under microscope on 400× and oil immersion (for endometrial cells + polymorphnuclear cells (PMN) cells). Samples with ≥4% PMN cells were categorized as subclinical endometritis [28].

2.4. Progesterone and cortisol analysis

Blood samples were collected from cows 35 days post-first artificial insemination (AI) for plasma progesterone and cortisol profiling. Blood samples were collected from the jugular vein (10 ml) by using a vacutainer system for progesterone and cortisol analyses. Blood samples was centrifuged at 4000×*g* for 10 min, after which plasma was separated and stored in plastic tubes and frozen at −20 °C until analysis was performed. Analysis of progesterone and cortisol concentrations in blood plasma of cows were performed using **e**lectro**c**hemi**l**uminescence immunoassay "ECLIA" at Jimma specialized hospital, clinical chemistry laboratory.

2.5. Data collection

A total of 84 cows were included in this study. Based on California Mastitis Test (CMT) cows were assigned into one of two groups: (1) cows with no evidence of subclinical mastitis (control group) and (2) cows with evidence of subclinical mastitis. Data were collected using structured questionnaire format. The data were collected from primiparous (n = 48) and multiparous cows (n = 36), which calved from January 2020 to July 2021. Body condition score was assessed using a scale from 1 (emaciated), 2 (thin), 3 (average), 4 (fat) and 5 (very fat) as described by Edmonson et al. [29]. The body condition of animals was classified as poor [≤3 (emaciated and thin)] and good [>3 (fat and very fat)].

2.6. Statistical analysis

The raw data were collected and entered into MS excel program. Statistical analysis was conducted using SPSS version 20.0. Descriptive statistics were used to compute percentages, proportions and frequency distributions of the conception rates at first service (CRFS), pregnancy rate at first service (PRFS) and the prevalence of subclinical mastitis and causing bacteria. Logistic regression was used to assess the strength of association between subclinical mastitis (dependent variable) and important predictor variables such as body condition score and parity. To compute the relationship between the subclinical mastitis (independent variable) and subclinical endometritis (dependent variable) logistic regression was used. The dependent variables, calving to first service interval (CFSI, days), number of services per conception (NSPC) and the independent variable, cow subclinical mastitis status (0 = negative and 1 = positive) were compared by independent sample *t*-test. The statistical comparison between means of progesterone ($P_{4)}$ and cortisol concentrations in subclinical mastitis status were performed by the independent sample *t*-test. In a multivariable linear regression analysis the association between the dependent variables, progesterone ($P_{4)}$ and cortisol concentrations and categorical independent variables such as subclinical mastitis, body condition scores and parity were assessed. In all the cases, 95% confidence level and $P < 0.05$ was used to determine statistical significance.

3. Results

3.1. Prevalence

A total of 84 clinically healthy lactating cows were tested for subclinical mastitis using CMT, the prevalence of subclinical mastitis at the cow level was 51.2% (43 of 84).

3.2. Impact of subclinical mastitis on fertility

The results of impact of subclinical mastitis on fertility are presented in Table 1. Perusal of table showed that subclinical mastitis had statistically significant effect on CFSI, NSPC, CRFS and PRFS ($P \leq 0.05$) for all traits. The mean of days of CFSI were longer in cows with SCM compared to negative cows (120.51 ± 24.5 and 85.15 ± 28.3 days, respectively) (P = 0.000). The overall mean for the number of services per conception (NSPC) in cows with no SCM was 1.59 ± 0.81 while positive cows had 2.51 ± 0.83 (P = 0.000). Conception rate at first service (CRFS) was reduced in cows with subclinical mastitis (32.6%) compared to negative cows (56.1%) (P = 0.030). Subclinical mastitis positive cows had a lower pregnancy rate at first service (PRFS) (23.3%) compared to subclinical mastitis negative cows (46.3%) (P = 0.026).

3.3. Factors associated

Table 2 illustrates the results of factors associated with SCM. The results showed that good body conditioned cows suffered less (35.90%), whereas poor body condition cows suffered more (64.4%) with SCM and these differences were highly significant (P = 0.009). From primiparous cows examined, 30% were positive for SCM, and among multiparous cows examined, 70.5% of them were positive for SCM (P = 0.000).

3.3.1. The association between subclinical mastitis and subclinical endometritis

The results of association between SCM and subclinical endometritis are presented in Table 3. Perusal of results showed that the prevalence of subclinical endometritis in SCM positive cows was 41.9%, whereas it was 19.5% in SCM negative cows, respectively. The presence of subclinical mastitis was significantly and directly associated with subclinical endometritis [2.97, CI (1.113–7.23), P = 0.027].

3.4. Effect of subclinical mastitis on hormone profiles

The mean concentration of plasma progesterone (P_4) at 35 days post-first AI cows that exhibited subclinical mastitis were 5.7 ± 2.56 ng/ml, while in subclinical mastitis negative cows was 8.74 ± 0.95 ng/ml (Fig. 1). Cows stressed by subclinical mastitis had significantly higher mean concentrations of cortisol (65.9 ± 21.2 ng/ml) than subclinical mastitis negative cows (53.52 ± 14.9 ng/ml, Fig. 1). Risk factors like parity, body condition and presence of subclinical mastitis were considered, only subclinical mastitis was found to be significantly associated with level of progesterone hormone (P = 0.000) and cortisol hormone (P = 0.001) concentrations

Table 1
Effect of subclinical mastitis (SCM) on fertility of dairy cows.

Reproductive parameters	SCM	n	Mean ± SD	%	*t*-test	OR	95% CI	P-value
CFSI (days)	Negative	41	85.15 ± 28.3	–	6.112	–	–	0.000
	Positive	43	120.51 ± 24.5	–		–	–	
NSPC	Negative	41	1.59 ± 0.81	–	5.196	–	–	0.000
	Positive	43	2.51 ± 0.83	–	–	–	–	
CRFS (%)	Negative	41	–	56.1	–	0.38	0.16–0.92	0.03
	Positive	43	–	32.6	–			
PRFS (%)	Negative	41	–	46.3	–	0.35	0.14–0.9	0.026
	Positive	43	–	23.3	–			

CFSI = Calving to First Service Interval; CI = Confidence interval; CRFS = Conception rate at first service; n = Animals examined; NSPC = Number of Services per Conception; OR = Odds ratio; PRFS = Pregnancy rate at first service; P = Probability; SD = Standard Deviation.

Table 2
Factors associated with the occurrence of subclinical mastitis.

Independent variables	Group	n	Prevalence (%)	OR	95% CI	P-value
Body condition score	Good (>3)	39	14 (35.9)	0.309	0.13–0.76	0.009
	Poor (<3)	45	29 (64.4)	Ref		
Parity	Primiparous	40	12 (30)	Ref		0.000
	Multiparous	44	31 (70.5)	5.56	2.2–14.2	

n = Number examined, OR=Odds ratio, CI = confidence interval, P = Probability.

Table 3
The association between subclinical endometritis (SCE) and subclinical mastitis (SCM).

Subclinical mastitis	Subclinical endometritis		OR	95% CI	P-value
	Negative (%)	Positive (%)			
Negative (%)	33 (80.5)	8 (19.5)	2.97	1.113–7.93	0.027
Positive (%)	25 (58.1)	18 (41.9)			
Total	58 (69)	26 (31)			

OR = Odds ratio; CI = confidence interval; P = Probability.

(Tables 4 and 5). In this study, BCS was with P-value of 0.364 tells us that, adequate BCS has absolutely no impact on the level of progesterone, getting odds ratios of 1.51 (Table 4).

3.5. Results of bacteriological examination

The relative prevalence rates of various bacterial species isolated from the subclinical cases are shown in Table 6. These results showed that from 124 quarters of 31 cows with subclinical mastitis, 27 milk samples yielded no bacterial growth and 19 samples yielded three or more different colonies which were contaminated and then rejected. Of the remaining 78 culture positive samples, a total of 101 bacteria were isolated. Based on the number of mastitis pathogens isolations, the most frequent pathogens were *Staphylococcus aureus* (45.54%), followed by coagulase negative staphylococcus (CNS) (20.8%) and streptococci (14.85%).

4. Discussion

The outcome of this study revealed that a prevalence of bovine subclinical mastitis (SCM) of 51.2% based on CMT. Our finding agreed with reports of Zeryehun and Abera [20], in the Eastern Harrarghe zone, Tuke et al. [21] in Alage dairy farm, Abebe et al. [14] in Hawassa town, Birhanu et al. [17] in Asella dairy farms. The present finding was higher than that reported by Bedacha and Menghistu [16], in Batu district, Belina et al. [19] in North Shewa and Borana pastoral area and Kitila et al. [22] in west Wollega, western Oromia, Ethiopia. The finding of this study was lower than the results of Fesseha et al. [15] in Modjo town, Tolosa et al. [13], in Jimma town and Dabash et al. [18], in North Shewa zone of Ethiopia. These differences in mastitis prevalence between studies might indicate that the illness is multifactorial, interacting with a number of factors such as causal factors, veterinary service coverage, a lack of intramammary infusion drugs, management methods including husbandry, and the environment.

Results of this study confirm the negative effects of SCM on dairy cow fertility. The number of days from calving to first service interval (CFSI) was significantly longer in subclinical mastitis cows compared to SCM negative cows. Our finding agreed with Kirk [30] and Ranasinghe et al. [31], who reported SCM cows had an extended interval from calving to first insemination. Other authors also

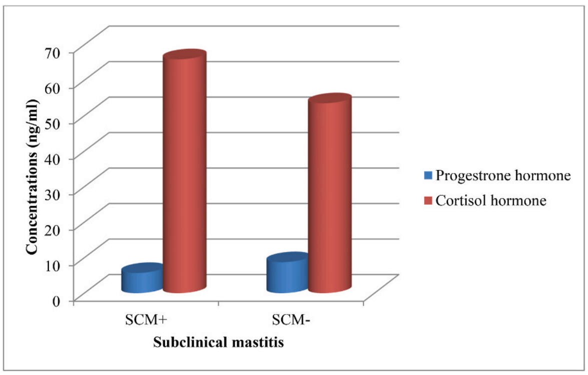

Fig. 1. Plasma Progesterone and Cortisol concentrations in subclinical mastitis positive (SCM+) and subclinical mastitis negative (SCM-) cows.

Table 4
Results of multivariable linear regression analysis of factors affecting the level of progesterone in the plasma of study animals.

Variable	Category	Coefficient	SE	OR	P- value
Subclinical Mastitis (SCM)	Positive	−2.659	0.484	0.07	0.000
	Negative	Ref			
Parity	Multiparous	−0.883	0.463	0.41	0.060
	Primiparous	Ref			
Body condition score	Score > 3	0.417	0.456	1.51	0.364
	Score ≤ 3	Ref			
Constant		8.687	0.502		0.000

CI = confidence interval; P = Probability; SE = standard error.

Table 5
Results of multivariable linear regression analysis of factors affecting the level of cortisol hormone in the plasma of study animals.

Variable	Group	Coefficient	SE	P- value
Subclinical Mastitis (SCM)	Positive	15.993	4.570	0.001
	Negative	Ref		
Parity	Multiparous	−0.18	4.378	0.968
	Primiparous	Ref		
Body condition score	Score > 3	4.41	4.313	0.310
	Score ≤ 3	Ref		
Constant		53.62	4.741	0.000

P = Probability; SE = standard error.

Table 6
The relative isolation rate of subclinical mastitis causing bacteria.

No	Bacterial species	Frequency	
		No isolated	Isolation (%)
1	*Staphylococcus aureus*	46	45.54
2	Coagulase negative staphylococci (CNS)	12	11.54
3	*Klebsella pneumonae*	4	3.96
4	*Corynebacterium* species	6	5.94
5	*Bacillus*	2	1.98
6	*Streptococci*	15	14.85
7	*Micrococcus species*	4	3.96
8	*Enterobacter aerogenes*	2	1.98
9	*Escherichia coli*	1	0.99
10	Total	101	100

pointed to the fact that cows that developed SCM had increased CFSI between 7 and 10 days compared to healthy herd mates [32].

Subclinical mastitis affected reproductive performance influencing the number of services per conception. The mean number of services per conception (NSPC) was higher in cows with SCM than in SCM negative cows. Our result agreed with Khokon et al. [33] and Dolecheck et al. [6], who reported that NSPC is expected to increase, on average, by 0.72 inseminations compared to healthy cows. Hansen et al. (2004) described that the increase in NSPC caused by mastitis was attributed to anovulation at oestrus, fertilization failure, and embryonic mortality.

A reduction in conception rate at first service has been demonstrated in cows with SCM. The results of this study revealed that Cows with SCM were less likely to conceive at first service than no SCM cows. This finding was also agreed with findings of David et al. [34] that SCM significantly lowers conception rate. A reduced conception rate in animals with mastitis may be related to the mechanism of maternal recognition of pregnancy because mastitis promotes the production of several bioactive molecules that can disrupt the functioning of the reproductive system [35].

In the present study, pregnancy rate at first service (PRFS) decreased by SCM. Previous studies have also shown the presence of a relationship between mastitis and reduced pregnancy rates [36]. In addition, Lavon et al. [37] reported a low pregnancy rate in cows with high somatic cell count (SCC) before artificial insemination (AI), this result implies that high SCC was often associated with the decline of fertility, indicating that the more severe the mastitis, the greater the impact on reproductive performance.

The present finding was in agreement with previous observations [15,16] that there was a progressive increase in the presence of SCM with increased parity and thus had poor reproductive performance. This might be due to increased chance of infection with time and the prolonged duration of infection, particularly in a herd without mastitis control program and also an increase for teat injuries [10]. A study by Dahl et al. [38] also demonstrated that a combined effect of exposure to SCM during gestation and parity ≥4 on pregnancy loss (PL) in dairy cows [38]. The uterine environment in older cows is more sensitive to a systemic inflammatory response due to mastitis because of high levels of inflammatory and IFN signalling and dysfunction of cell division, compared to younger cows as observed in vitro by Tanikawa et al. [39].

In this study, poor BCS is a risk factor for mastitis [40,41] and had poor reproductive performance. Mastitis was only associated with increased risk of pregnancy loss when cows also had a low BCS [42,43].

The current study revealed that the odds of subclinical endometritis (SCE) presence was 2.97 times higher (OR = 2.97) in cows with subclinical mastitis (SCM) compared to SCM negative cows. Cows diagnosed with SCM and SCE have a greater decrease in reproductive performance and more unstable pregnancy than those diagnosed with mastitis alone [44]. Reports from Dosogne et al. [45] showed that plasma lipopolysaccharide (LPS) increased during mastitis. The direct association between SCM and SCE could be due to transmission of bacteria and bacterial products, particularly LPS, from the udder to the uterus [46,47].

This study showed that the overall plasma progesterone levels in cows that exhibited SCM had significantly lower concentration than SCM negative cows. David et al. [34] and Pinedo et al. [48] also recorded that SCM was the cause of changes in the concentration of progesterone. Inflammatory factor TNF-α inhibits the number of LH receptors in granulosa cells, thereby reducing the response ability of LH leading to inhibition of progesterone secretion from the corpus luteum and interferes with uterine functions [1].

The results of this study also showed that cortisol concentration in plasma of cows stressed by SCM had significantly higher concentrations than SCM negative cows. Similarly, Hockett et al. [49] reported that the concentration of cortisol was increased in SCM cows. Increased cortisol concentrations decreased gonadotropin release during SCM and the inflammatory responses associated with bacterial challenges have been to cause disruption of reproductive processes [42]. David et al. [34] and Pinedo et al. [48] also reported SCM was the cause of changes in the concentration of cortisol. The concentration of cortisol in plasma increases in the luteal phase of cows with mastitis, thereby inhibiting the level of LH [1].

Bacteriology of subclinical mastitic milk samples from cattle showed that *Staphylococcus aureus*, coagulase negative staphylococcus (CNS) and streptococci were the most frequently isolated bacterial pathogen from cattle suffering mastitis. These findings were comparable to the outcomes reported by Abebe et al. [14]. The reason for the higher isolation rate of *Staphylococcus aureus* pathogen was the wide ecological distributions inside the mammary glands and skins serving as sources of infection for other healthy cows and transmitted during the milking process [10].

The isolation rate of coagulase negative staphylococcus (CNS) was much lower than the findings of Zeryehun and Abera [20] and higher than the report of Fesseha et al. [15]. This variation in the frequency of coagulase negative staphylococcus (CNS) observed from mastitic milk might be attributable to differences in laboratory analytical methodologies used and management practices. Streptococci bacteria were isolated from mastitic cows milk was comparable to previous reports of Birhanu et al. [17]. However, our result was lower than the report of Fesseha et al. [15]. This lower incidence of Streptococci might be due to the widespread utilization of Penstrip in the field for the treatment of mastitis. Intramammary infections (IMI) in dairy cows, caused by Gram-positive and Gram-negative bacteria, can decrease the reproductive performance of dairy cows [50].

There are some limitations associated with the current study. The results had shown significant effects of subclinical mastitis (SCM) on reproductive parameters. However, adjusted effects of subclinical mastitis (SCM) on the reproductive parameters after accounting for combined effects of confounding factors such as husbandry system, stage of lactation, farm types, clinical mastitis and barn hygiene could not be assessed. This was mainly due to limited number of cows. The reason for this was most of the cows that were available for this study did not fulfil the criteria set for the purpose. Some farmers were not willing to participate in the study while some farms willing have different cow environment and husbandry practices which made increasing the number of study cows difficult. Therefore, further studies, with large number of samples with suitable statistical model are necessary to understand the accurate effects of subclinical mastitis (SCM) on reproductive performance in Ethiopian Zebu × Friesian crossbred dairy cattle.

The statistics of the prevalence of SCM in this study have mainly been focused at cow level. Hence, we expect that our findings should be broadly generalizable to other farms with similar management systems and environments; further research is needed to validate this.

This study was conducted for the first time in the country. Due to this there is no similar designed studies conducted in the country to compare to our result.

5. Conclusion

The present study has shown that subclinical mastitis (SCM) is a widely prevalent disease in the dairy farms of Jimma milk shed and the subclinical occurrence of the disease remains a substantial problem for dairy farms. This study demonstrated that the occurrence of subclinical mastitis (SCM) delayed the calving to first service interval (CFSI), elevated the number of services per conception (NSPC), reduced conception rate at first services (CRFS) and decreased pregnancy rates at first services (PRFS). This study also indicated that parity and body condition were important predictors of the occurrence of SCM in dairy cows. The present study revealed that subclinical endometritis (SCE) was associated with subclinical mastitis (SCM). The SCM declined the concentration of progesterone and increased the cortisol concentration. The bacteria most commonly associated with subclinical mastitis include *Staphylococcus aureus* followed by coagulase negative staphylococcus (CNS) and streptococci. These results provide further evidence that SCM could inflict harmful effects on reproductive performance of dairy cows, emphasizing the relevance of mastitis control programs in dairy farms.

Ethics approval and consent to participate

Ethical clearance was obtained from Research and Ethics committee of Addis Abeba University College of Agriculture and Veterinary Medicine. Before conducting the research, informed consent was obtained from the owners or managers of the dairy farms used in this study.

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Author contribution statement

Nuraddis Ibrahim: Conceived and designed the experiments; Analyzed and interpreted the data; Performed the experiments; Wrote the paper.

Fekadu Regassa: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Tefera Yilma: Analyzed and interpreted the data.

Tadele Tolosa: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.***

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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