African Journal of Biotechnology Vol. 11(10), pp. 2574-2580, 2 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3097
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Clinical mastitis from calving to next conception negatively affected reproductive performance of dairy cows in Nanning, China

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Accepted 28 December, 2011

The purpose of this study was to evaluate the effect of clinical mastitis between calving and next conception on reproductive performance in Chinese Holstein cows. Six hundred and three multiparous Holstein cows from a commercial dairy farm were divided into three groups respectively: cows with first clinical mastitis before first artificial insemination (AI) (MG1; n = 113), cows with first clinical mastitis between first AI and pregnancy diagnosis (MG2; n = 36) and cows without any clinical disease (CG; n = 454). Clinical cases of mastitis were identified at every milking by the trained milkers or the herd manager based on abnormal milk or signs of inflammation of the mammary gland. Number of days from calving to first AI and days from calving to conception, number of AI per conception, and the conception rate at first AI were evaluated in each group. The number of days to first AI was significantly greater for cows in MG1 than MG2 and CG ($P < 0.01$). The number of days to conception was similar for cows in MG1 and MG2 ($P > 0.05$), but they were all greater than cows in CG ($P < 0.01$). The number of services per conception was significantly greater for cows in MG1 and MG2 than CG ($P < 0.01$), and cows in MG1 had fewer number of services per conception as compared to MG2 ($P < 0.05$). Conception rate at first service was similar for cows in MG1 and MG2 ($P > 0.05$), however, conception rate for those groups were both lower than for CG ($P < 0.01$). In conclusion, clinical mastitis during early lactation markedly and negatively influenced reproductive performance of dairy cows. Therefore, reduction of clinical mastitis in early lactation should also improve reproductive performance of dairy cows. Further study is needed to better understand the mechanisms of how clinical mastitis affects reproductive performance in dairy cows which could lead to better strategies to avoid such negative effects.

Key words: Mastitis, dairy cows, reproductive performance.

INTRODUCTION

Mastitis, an inflammatory reaction of the mammary gland is the most dreaded disease of dairy farmers because of reduced milk production, increased treatment costs, labour, milk discarding following treatment, death and premature culling (Yang et al., 2011). Besides, mastitis has been recently reported to have a detrimental effect on reproductive performance in lactating dairy cows (Barker et al., 1998; Hertl et al., 2010; Nava-Trujillo et al., 2010; Santos et al., 2004; Schrick et al., 2001).

The first published report suggested that clinical mastitis were caused by Gram negative bacteria, *Escherichia coli*, which could alter the interestrous interval. Cows with clinical mastitis due to Gram negative bacteria were 1.6 times more likely to have an altered interestrous interval as compared to herdmates without clinical mastitis (Moore et al., 1991). Barker et al. (1998) indicated that cows that developed clinical mastitis during early lactation could have a markedly negative impact on the reproductive performance of dairy cows, such as the

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number of days to first artificial insemination (AI), artificial inseminations per conception and the number of days to conception. Moreover, both Gram negative and positive pathogens may act through similar mechanisms to increase inflammatory mediators, leading to reproductive failure during early lactation. Schrick et al. (2001) reported that subclinical mastitis reduced reproductive performance of lactating cows which is similar to clinical mastitis. Huszenicza et al. (2005) indicated that clinical mastitis could affect the resumption of ovarian activity in postpartum dairy cows. And Nava-Trujillo et al. (2010) suggested that clinical mastitis before first service would increase the days to first service and the days to conception in dual-purpose cows. However, Peake et al. (2011) found that cows with neither subclinical nor clinical mastitis in the first two months of lactation had a negative effect on fertility parameters.

Reproductive losses in lactating dairy cows have increased in recent years (Lucy, 2001), and these losses seems to be multifactorial. Efficient reproductive performance is essential for the maintenance of consistently high level of milk production. However, the negative effect of clinical mastitis from calving to next conception on reproductive performance in Chinese Holstein cows is generally unknown. Therefore, the purpose of this study was to determine the effects of clinical mastitis, occurring from calving to next conception, on reproductive performance in Chinese Holstein cows, under subtropical conditions.

MATERIALS AND METHODS

Animals and management

Six hundred and three multiparous Holstein dairy cows from a commercial dairy farm in Nanning were used in this study. All lactating cows in the farm were milked twice daily in a double-14 fishbone milking parlor equipped with automatic milking machine take-offs (Westfalia-Surge, Naperville, IL), with herd average 305-d milk yield between 4000 and 7,200 kg/cow. Cows stood on an elevated platform in a fishbone fashion facing away from the operator area, which exposed enough, the back half of the cow to make milking her from the side possible. Teat dipping was routinely performed before and after each milking. Milking machines were backflushed (Surge Backflush II, Westfalia- Surge) after removal from cows. Milking equipment was evaluated routinely and maintained per the recommendation of the manufacturer. Cows were housed in free-stall barns and fed via a feed alley, which were bedded in stalls with straw on rubber mats, and scrapers automatically cleaned the concrete floor 3 times daily. Within each site, all lactating cows was fed with the same diet as a total mixed ration that was formulated to meet or exceed the nutrient requirements for a lactating Holstein cow weighing 650 kg and producing 45 kg of 3.5% fat-corrected milk (NRC, 1989).

All cows were dried off approximately 7-9 weeks before expected calving, and all quarters of cows were infused with an antibiotic preparation approved for use in non-lactating cows following the last milking of lactation. And subsequently, non-lactating cows were moved into a separate pen.

Data were collected from cows that calved between September 2009 and January 2010. Respectively, cows were divided into three groups according to time of lactation when the first clinical mastitis case was identified or when there was absence of clinical mastitis: first clinical mastitis prior to first postpartum artificial insemination (AI) (MG1; n = 113); first clinical mastitis between first postpartum AI and pregnancy diagnosis (MG2; n = 36); cows that were either not diagnosed with mastitis or were diagnosed after confirmed pregnancy (CG; n = 454).

Diagnosis of clinical mastitis

At each milking, all cows were examined for symptoms of clinical mastitis by trained milkers or by the herd manager immediately before milking. Clinical mastitis cases were characterized by the presence of abnormal milk or by signs of inflammation in one or more quarters, and were treated by intramammary infusion of antibiotics according to treatment protocols established by the herd veterinarian. Periodically, milk samples from the identified quarter were obtained by the herd health veterinarian and cultured for microbiological status. Treated cows were moved to the hospital pen and the incidence, infected quarter(s) and treatment type were recorded by using computer software records (Microsoft Excel 2003).

Reproductive management

Cows were observed for estrus for 30 min at least three times daily. In addition, milkers observed cows at milking time, and all farm personnel regularly participated in estrus detection throughout the day. Cows in estrus were confined to tie stalls to minimize the chance of injury. Following calving, cows were generally subjected to a voluntary waiting period of 40 days before first AI. Pregnancy diagnosis was performed by herd veterinarians via palpating per rectum the uterus and its contents approximately 50 to 65 days after insemination. The farm maintained computerized and paper records of the reproductive performance of each cow, including the number of days to first AI and days from calving to conception, the number of AI per conception, and the conception rate at first AI.

Statistical analyses

The statistical analyses were performed using SAS/STAT software (Statistical Analyses Systems, Version 9.1.3 (SAS Institute Inc.SAS Institute Inc.)). Binomially distributed data such as the conception rate at first AI were analyzed by logistic regression (Allison, 1999) by the LOGISTIC procedure of SAS. The number of days to first AI, the number of AI per conception, and the number of days from calving to conception were analyzed according to General Linear Models (GLM) procedure of SAS (SAS system for Windows, Version 9.1.3. Data are presented as least squares mean (L.S.M.) ± standard error of mean (S.E.M). Treatment differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.01$ were considered as tendency.

RESULTS

The incidence of clinical mastitis at cow level was 24.7% (149/603) in the study period. The reproductive parameters of cows in MG1, MG2, MG1 and MG2, and CG are presented in Table 1. And the percentages of cows in each group with the times of AI per conception are shown in Table 2. In detail, the number of days to first AI was significantly greater for cows in MG1 than MG2 and CG (73.84 ± 1.23 vs. 54.98 ± 0.34; P <0.01; Figure

Table 1. Effect of clinical mastitis on reproductive parameters of lactating Holstein dairy cows (L.S.M. ± S.E.M.).

Parameter	Mastitis group 1 (n = 113)	Mastitis group 2 (n = 36)	Mastitis group 1 and 2 (n = 149)	Control group (n = 454)
The number of days to first AI	$73.84^{Aa} \pm 1.23$	$58.19^{Bb} \pm 1.69$	$70.06^{Cc} \pm 1.16$	$54.73^{Bd} \pm 0.34$
The number of days from calving to conception	$121.82^{A} \pm 5.03$	$133.31^{A} \pm 11.36$	$124.6^{A} \pm 4.69$	$89.74^{B} \pm 2.17$
The number of AI per conception	$1.88^{Aa} \pm 0.08$	$2.19^{Ab} \pm 0.16$	$1.95^{Aab} \pm 0.07$	$1.53^{Bc} \pm 0.03$
Conception rate at first AI	$38.1\%^{A}$	$27.8\%^{A}$	$35.6\%^{A}$	$54.9\%^{B}$

^{a,b,c,d}Means in a row with different superscripts lower case letters differ ($P < 0.05$). ^{A,B,C,D}Means in a row with different superscripts capital letters differ ($P < 0.01$).

Table 2. Percentages of pregnant cows with once, twice, three times and four times services per conception in MG1, MG2 and CG.

Parameter	Mastitis group 1 (n = 113)	Mastitis group 2 (n = 36)	Control group (n = 454)
Percentage of cows with once AI per conception	$38.1\%^{A}$	$27.8\%^{A}$	$54.0\%^{B}$
Percentage of cows with twice AI per conception	40.7%	36.1%	40.1%
Percentage of cows with three times AI per conception	$16.8\%^{A}$	$25.0\%^{A}$	$5.0\%^{B}$
Percentage of cows with four times AI per conception	$4.4\%^{A}$	$11.1\%^{A}$	$0.9\%^{B}$
Percentage of cows conceived at the end of the research	100%	100%	100%

^{A,B,C,D}Means in a row with different superscripts capital letters differ ($P < 0.01$).

1). For pregnant cows, the number of days from calving to conception was similar for cows in MG1 and MG2 (121.82 ± 5.03 vs. 133.31 ± 11.36; P > 0.05; Figure 2), but means for both groups were greater than for cows in CG (121.82 ± 5.03 vs. 89.74 ± 2.17 and 133.31 ± 11.36 vs. 89.74 ± 2.17; P < 0.01; Figure 2). Furthermore, the number of services per conception was significantly greater for cows in MG1 and MG2 than cows in CG (1.88 ± 0.08 vs. 1.53 ± 0.03 and 2.19 ± 0.16 vs. 1.53 ± 0.03; P < 0.01; Figure 3), and cows in MG1 had fewer number of services per conception as compared to cows in MG2 (1.88 ± 0.08 vs. 2.19 ± 0.16; P < 0.05; Figure 3). Conception rate at first service was similar for cows in MG1 and MG2 (38% vs. 28%; P > 0.05; Figure 3), however, all of them were lower than that in cows in CG (38% vs. 55 and 28% vs. 55%; P < 0.01; Figure 3).

DISCUSSION

Mastitis is one of the most costly and common diseases affecting dairy cows throughout the world (DeGraves and Fetrow, 1993). More recently, compromised reproductive performance has also been recognized as a detrimental effect of clinical mastitis (Ahmadzadeh et al., 2009; Barker et al., 1998; Gunay and Gunay, 2008; Nava-Trujillo et al., 2010; Santos et al., 2004). However, the effect of clinical mastitis from calving to next conception on reproductive performance in Chinese dairy cows under subtropical conditions is essentially unknown.

Past research on the influence of mastitis on reproductive performance focused on experimental coliform mastitis and infusion of endotoxin from Gram-negative pathogens. However, there were no differences in reproductive performance between clinical mastitis caused by Gram-negative or positive pathogens (Barker et al., 1998; Santos et al., 2004). In our study, we did not isolate the pathogens responsible for mastitis. The occurrence of clinical mastitis cases early in lactation, either before breeding or between first postpartum AI and pregnancy diagnosis resulted in detrimental effects on reproduction in Holstein cows.

In the present study, the number of days to first AI was significantly greater for cows with clinical mastitis before first AI (73.84 d) than all other

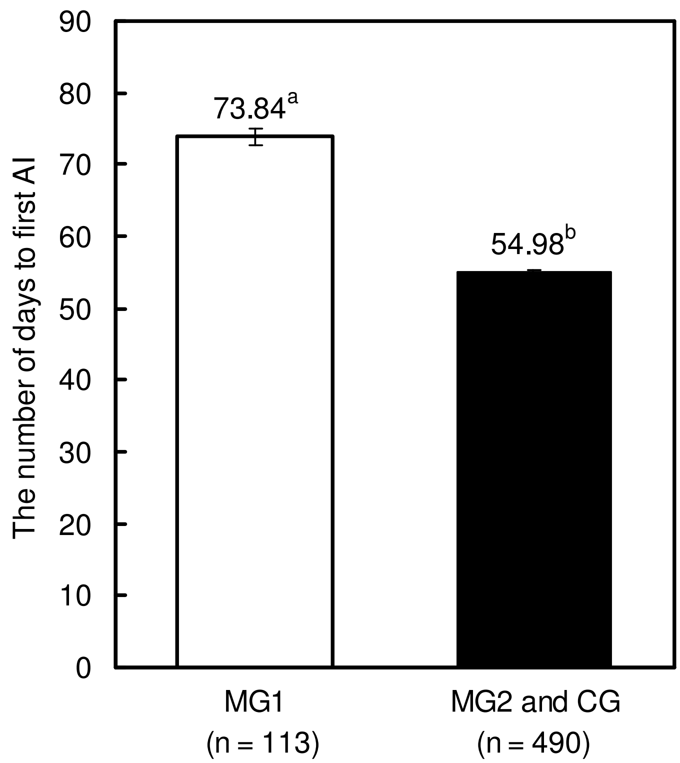

Figure 1. Influence of clinical mastitis during early lactation on the number of days to first AI of lactating Holstein dairy cows. [a,b] Different superscripts denotes differences ($P < 0.01$) between groups.

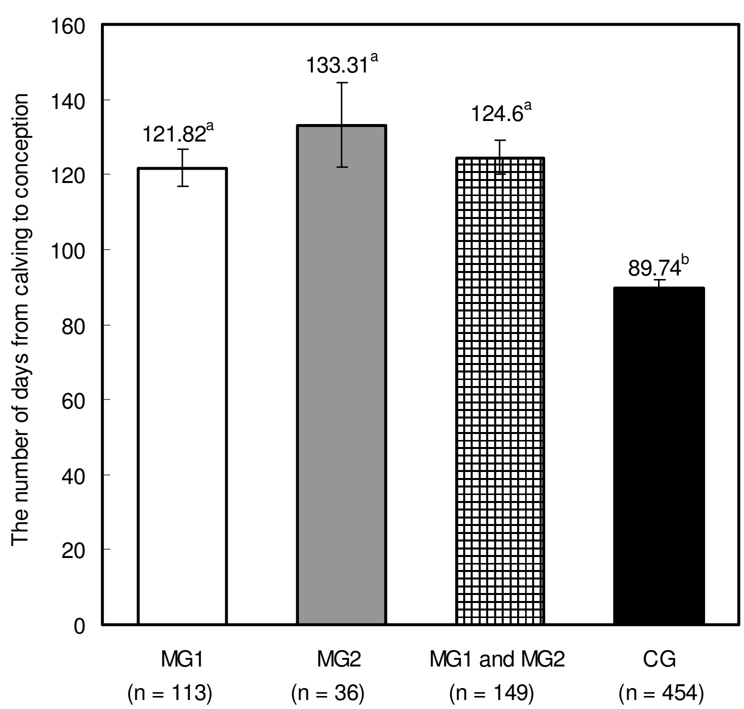

Figure 2. Influence of clinical mastitis during early lactation on the number of days from calving to conception of lactating Holstein dairy cows. [a,b] Different superscripts denotes differences ($P < 0.01$) between groups.

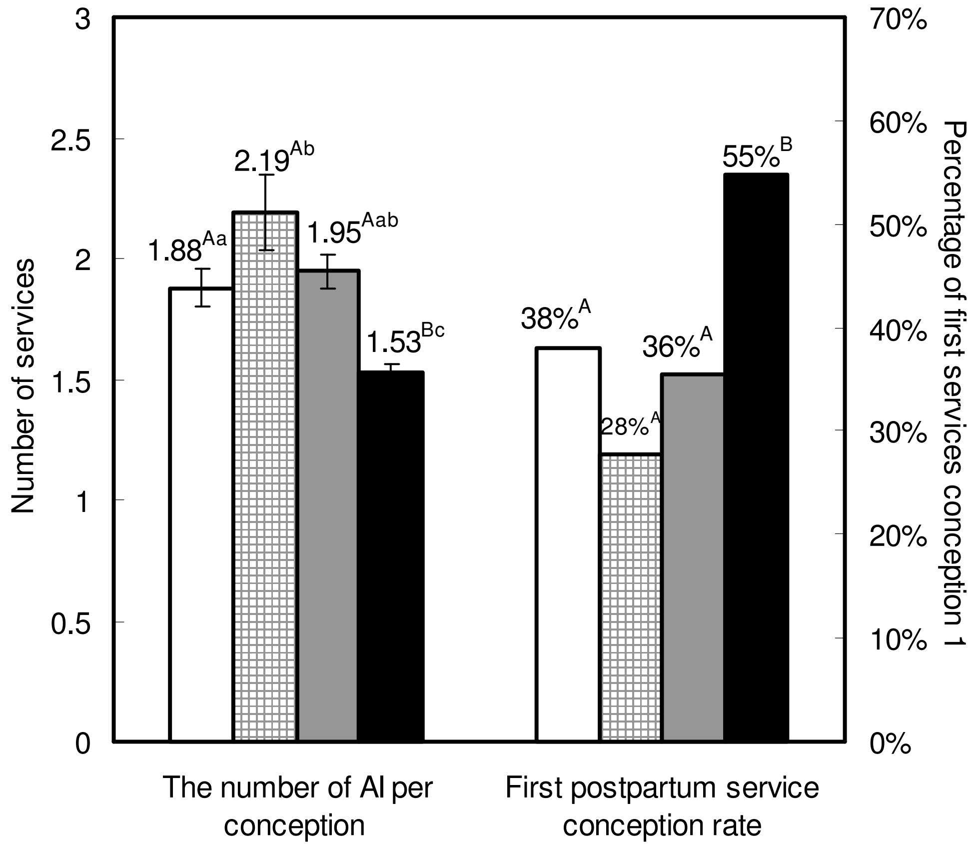

Figure 3. Influence of clinical mastitis during early lactation on the number of AI per conception and first service conception rate of lactating Holstein dairy cows. White bars represent MG1, hatched bars represent MG2, gray bars represent MG1 and MG2, and black bars represent CG. Means or percentages with different superscripts lower case letters (^{a},^{b}) within bar groups differ at $P < 0.05$, and different superscripts capital letters (^{A},^{B}) differ at $P < 0.01$.

groups (54.98 d). This finding was similar to that in the previous study (Barker et al., 1998; Santos et al., 2004; Schrick et al., 2001). It could have been due to insufficient follicular development; anovulation, resulting from blockage of the LH surge; or decreased estrogen synthesis, resulting in the loss of behavioral estrus (Schrick et al., 2001). These factors could explain the delayed first postpartum AI in cows experiencing mastitis before first AI in the current and previous studies.

We observed a much lower conception rate at first AI when cows had their clinical mastitis cases before first AI, even the effect of mastitis on first AI conception rate was exacerbated when the clinical case occurred between first AI and pregnancy diagnosis (38.1 and 27.8% vs. 54.9%), which is in accordance with the results of Santos et al. (2004) (22, 10 and 29%, respectively). The bovine mammary gland synthesizes prostaglandin $F_{2α}$ ($PGF_{2α}$), and increasing $PGF_{2α}$ was found in milk from cows with clinical mastitis (Anderson et al., 1986). This increase in plasma concentrations of $PGF_{2α}$ could induce luteolysis (Barker et al., 1998). Therefore, decreased luteal function or complete luteal regression caused by premature release of $PGF_{2α}$ induced by mastitis could potentially explain the conception rate at first AI in lactating dairy cows in MG1 and MG2. Whether the possible impacts of bacterial infections on luteal function are the exact cause of reduced conception in cows affected by clinical mastitis still remain to be proved in controlled studies.

The number of AI per conception was greater for cows experiencing clinical mastitis after the first service than cows with clinical mastitis before first service and cows with no clinical mastitis (2.19 vs. 1.88 and 1.53, respectively). Cows with clinical mastitis after first service typically had an increased number of services per conception as compared to the control group. Our findings were in agreement with the results reported by Barker et al. (1998), Santos et al. (2004) and Gunay and Gunay (2008). Elevated concentrations of serum $PGF_{2α}$ associated with mastitis may cause a decrease in embryonic development and ultimately result in an increased number of services per conception and number of days to conception. The number of days from calving to conception for cows in MG1 (121.82 days) and MG2 (133.31 d) were significantly greater than that for the control group cows (89.74 days), which is in agreement with the reports by Barker et al. (1998), Schrick et al. (2001) and Gunay and Gunay (2008). While, the days to conception for cows in MG1, MG2 and CG were all longer than Santos et al. (2004) report in the literature (165, 189 and 139 days, respectively). The longer the days to conception for cows in MG1 and MG2 than CG was mainly due to lower conception rates at first AI and additional AI per conception for cows in MG1 and MG2 than CG. These results could have been due to luteolysis, subsequent loss in progesterone, and early embryonic death.

Barker et al. (1998) reported that both Gram-negative and positive pathogens may act through similar mechanisms to increase inflammatory mediators, leading to reproductive failure during early lactation. Clinical mastitis led to the production of bioactive molecules in the reproductive tract tissues. For example, *Escherichia coli* endotoxin do not usually penetrate from the udder into the blood, but could induce massive release of cytokines. Those cytokine-mediated neural and endocrine changes played a key role in the inflammatory process (Hansen et al., 2004; Huszenicza et al., 2005). Cullor (1990) suggested that endotoxin may induce premature luteolysis and influence conception by release of inflammatory mediators. Specifically, endotoxin stimulates synthesis of $PGF_{2\alpha}$, glucocorticoids and interleukin-1 (Cort and Kindahl, 1990; Lopez-Diaz and Bosu, 1992). In addition, infusion of endotoxin resulted in a pyretic response in dairy cows (Jackson et al., 1990). Elevated body temperature was a symptom often associated with clinical mastitis. Experimentally induced *Streptococcus uberis* clinical mastitis resulted in elevated body temperatures (Hockett et al., 2000). Edwards and Hansen (1996) reported that exposure of bovine oocytes to elevated temperatures decreased blastocyst formation.

McCann et al. (1997) indicated that cytokines, released following endotoxin challenge, blocked the pulsatile secretion of luteinizing hormone (LH) but not follicle stimulating hormone (FSH) through alterations in nitric oxide production to inhibit gonadotropin releasing hormone (GnRH). Battaglia et al. (1996) reported significant inhibition of GnRH pulse amplitude and total GnRH following intravenous endotoxin infusion. Consequently, insufficient follicular development and (or) oocyte maturation could lead to insufficient estrogen production and subsequent anovulation, eventually leading to lack of behavioral estrus and failure of artificial insemination.

Results of this study demonstrated clearly that clinical mastitis either before first AI or between first AI and pregnancy diagnosis can have a markedly negative impact on the reproductive performance of dairy cows. More attention and effort needs to be assigned to prevention of this disease that decrease reproductive efficiency.

Proper dry cow treatment, adequate nutrition, housing comfort and cleanliness, and adequate milking procedures would reduce the incidence of clinical mastitis, which is expected to result in decreased days to first AI, days from calving to conception, and number of AI per conception and increase in the conception rate in lactating dairy cows.

The negative impact of mastitis on reproduction underlines the importance of avoiding mastitis early in lactation, highlighting the significance of mastitis preventive measures as the rule but not the exception. Further study is needed to better understand the mechanisms by which clinical mastitis affects reproductive performance in dairy cows and to design better strategies to avoid those negative effects.

Conclusions

In conclusion, to our knowledge, this study is the first report on the negative impact of clinical mastitis occurring from calving to pregnancy on the reproductive performance of Chinese dairy cows. Mainly delays the calving to first service interval, increases the calving to conception interval, elevates the number of AI per conception, and reduces the conception rate at first AI. Reproductive efficiency is of great concern to dairy producers. Therefore, it is important to reduce the incidence of clinical mastitis in early lactation to improve the reproductive performance of dairy cows.

ACKNOWLEDGEMENTS

The authors are grateful to the JinGuang dairy farm, its owners and staff, for providing the data for this manuscript. This work was jointly funded by '948' Project (Ministry of Agriculture of China, No. 2011-G26) and Guangxi Department of Science and Technology (No. 1123005-1 and 1123005-3).

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