

## EFFECT OF CLINICAL MASTITIS ON REPRODUCTIVE TARGETS ACHIEVEMENT IN COWS

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### ABSTRACT

The aim of this study was to determine the effect of clinical mastitis occurring between calving and the first service on the percentage of cows that achieve two reproductive targets: be served before 70 days postpartum and be pregnant before 110 days postpartum (dpp). Relative frequency and odds ratio (OR) with the corresponding confidence interval (CI) were calculated. Fewer mastitic cows were served before 70 dpp than non-mastitic (24.86% vs 36.59% respectively  $P = 0.0137$ ); mastitic cows had lower odds to be served than non-mastitic cows (OR: 0.57; 95% CI: 0.3679-0.8938,  $P = 0.0141$ ). Fewer mastitic cows became pregnant before 110 dpp than non-mastitic cows (36.72% vs 50.73%, respectively,  $P = 0.006$ ) and mastitic cows had lower odds to become pregnant than non-mastitic cows (OR: 0.56; 95% CI: 0.3739-0.8495,  $P = 0.0062$ ). Negative effect of clinical mastitis on percentage of cows served only was observed in primiparous cows, with 4.76% of mastitic cows being served in comparison with 30.19% of non-mastitic cows ( $P = 0.0017$ ); primiparous mastitic cows had lower odds (0.11; 95% CI: 0.0249-0.5374,  $P = 0.0059$ ) to be served than non-mastitic primiparous cows. Percentage of primiparous mastitic cows pregnant before 110 dpp was lower than non-mastitic cows (16.67% vs 37.74%,  $P = 0.0245$ ) and mastitic cows had only 0.3300 odds (95% CI: 0.1234-0.8822,  $P = 0.0271$ ) to become pregnant that primiparous non-mastitic. In multiparous, 42.96% of mastitic and 55.26% of non-mastitic cows ( $P = 0.0378$ ) became pregnant before 110 dpp and mastitic cows had 0.6098 odd (95% CI: 0.3821-0.9730,  $P = 0.0380$ ) to become pregnant than non-mastitic. In conclusion, clinical mastitis reduced the possibility of cows to be served and become pregnant during the first 70 and 110 dpp respectively, and the negative impact of clinical mastitis was more harmful in primiparous cows.

**Key words:** mastitis, cows, first service, pregnancy.

## EFEITO DA MASTITE CLÍNICA NA REALIZAÇÃO DE OBJETIVOS REPRODUTIVOS EM VACAS

### RESUMO

O objetivo deste estudo foi determinar o efeito da mastite clínica que ocorre entre o parto e o primeiro serviço na porcentagem de vacas que atingem dois alvos reprodutivos: ser inseminadas antes dos 70 dias pós-parto e estar grávida antes dos 110 dias pós-parto (dpp). A frequência relativa e o odds ratio (OR) com o intervalo de confiança correspondente (CI) foram calculados. Menos vacas com mastite foram inseminadas antes de 70 dpp do que vacas sem mastite (24,86% vs 36,59%, respectivamente  $P = 0,0137$ ); as vacas com mastite apresentaram probabilidades menores de serem servidas do que as vacas sem mastite (OR: 0,57; CI 95%: 0,3679-0,8938,  $P = 0,0141$ ). Menos vacas com mastite ficaram grávidas antes de 110 dpp do que as vacas sem mastite (36,72% vs 50,73%, respectivamente,  $P = 0,006$ ) e as vacas com mastite apresentavam menores probabilidades de engravidar do que as vacas sem mastite (OR: 0,56; CI 95% 0,3739-0,8495,  $P = 0,0062$ ). O efeito negativo da mastite clínica na porcentagem de vacas inseminadas apenas foi observado em vacas primíparas, com 4.76% de vacas com mastite sendo inseminadas

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em comparação com 30.19% de vacas sem mastite ( $P = 0.0017$ ); as vacas primíparas com mastite apresentaram probabilidades menores (0.11; IC 95%: 0.0249-0.5374,  $P = 0.0059$ ) a serem servidas do que as vacas primíparas sem mastite. Percentagem de vacas primíparas com mastite grávidas antes 110 dpp foi menor do que vacas sem mastite (16,67% vs 37,74%,  $P = 0,0245$ ) e vaca com mastite tinha 0.33 probabilidades (IC 95%: 0,1234-0,8822,  $P = 0,0271$ ) para engravidar que vacas primitivas sem mastite. Em múltíparas, 42,96% de vacas com mastite e 55,26% de vacas sem mastite ( $P = 0,0378$ ) tornou-se grávida antes de 110 vacas dpp e vacas sem mastite tinham apenas 0,6098 probabilidades (IC 95%: 0,3821-0,9730,  $P = 0,0380$ ), para engravidar que vacas sem mastite. Em conclusão, a mastite clínica reduziu a possibilidade de vacas serem servidas e ficaram grávidas durante os primeiros 70 e 110 dpp, respectivamente, e o impacto negativo da mastite clínica foi mais prejudicial em vacas primíparas.

**Palavras-chave:** mastite, vacas, primeiro serviço, gravidez.

## EFFECTO DE LA MASTITIS CLÍNICA SOBRE EL LOGRO DE METAS REPRODUCTIVAS EN VACAS

### RESUMEN

El propósito de este estudio fue determinar el efecto de la mastitis clínica entre el parto y el primer servicio sobre el porcentaje de vacas que lograron dos metas reproductivas: ser inseminadas antes de los 70 días postparto y quedar preñadas antes de los 110 días postparto (dpp). Menos vacas con mastitis fueron inseminadas antes de 70 dpp (24,86% vs 36,59%,  $P = 0,0137$ ); y las vacas con mastitis tuvieron menos posibilidades de ser inseminadas que las vacas sin mastitis (odds ratio: 0.57; 95% CI: 0.3679-0.8938,  $P = 0.0141$ ). Menos vacas con mastitis quedaron preñadas antes de 110 dpp (36,72% vs 50,73%,  $P = 0,006$ ), y las vacas con mastitis tuvieron pocas posibilidades de quedar preñadas que las vacas con mastitis (0.5636; 95% CI: 0.3739-0.8495,  $P = 0.0062$ ). El efecto negativo de la mastitis clínica sobre el porcentaje de vacas inseminadas antes de 70 dpp solo se observó en las vacas primíparas, con 4,76% de estas siendo inseminadas en comparación con el 30,19% de las vacas sin mastitis ( $P = 0,0017$ ), las vacas con mastitis tuvieron menos posibilidades de ser inseminadas que las vacas sin mastitis (0.1156; 95% CI: 0.0249-0.5374,  $P = 0.0059$ ). El porcentaje de vacas primíparas con mastitis preñadas antes de 110 dpp fue menor que en las primíparas sin mastitis (16,67% vs 37,74%,  $P = 0,0245$ ) y las vacas con mastitis tuvieron menos posibilidades de quedar preñadas que las vacas sin mastitis (0.3300; 95% CI: 0.1234-0.8822,  $P = 0.0271$ ). En las vacas múltíparas, 42,96% de las vacas con mastitis y 55,26% de las vacas sin mastitis ( $P = 0,0378$ ) quedaron preñadas antes de 110 dpp y las vacas con mastitis tuvieron solo 0.6098 posibilidades (95% CI: 0.3821-0.9730,  $P = 0.0380$ ) de quedar preñadas en comparación con las vacas sin mastitis. En conclusión, la mastitis clínica reduce las probabilidades de que las vacas sean inseminadas y queden preñadas antes de 70 y 110 dpp respectivamente, con un impacto negativo más perjudicial en vacas primíparas.

**Palabras clave:** mastitis, vacas, primer servicio, preñez.

### INTRODUCTION

Mastitis has been reported to negatively affect the reproductive performance of dairy cows, altering the inter-estrus interval, increasing the days to first service, the services per conception, the days to conception, and the abortions rate (1-15). Additionally, a significant

genetic correlation between clinical mastitis and fertility of first insemination (-0.33) and with the interval between calving and the first insemination (0.29) has been observed (11).

Most the reports about the relationship mastitis-reproduction thus far have been performed with high-milk production, pure dairy breed (*Bos taurus*) cows (1,2,13). In most tropical areas, the dairy industry relies on dual-purpose crossbred cows, which have less milk production and poorer genetic merit, but similarly to *Bos taurus* pure breed dairy cows, clinical mastitis has been observed by increase the days to first service and to conception in dual-purpose cows (16).

The mechanisms behind the association between mastitis and reproduction are being studied (17-21). Pro-inflammatory mediators and bacterial endotoxins that are released during the onset of mastitis are believed to affect the endocrine pathways (hypothalamus-pituitary-ovary-uterus axis) altering the reproductive performance of cows, causing anestrus, anovulation, infertility, and other problems related to hormone imbalances.

Mastitis in cattle is considered the most important disease affecting milk production worldwide (22), in addition, clinical mastitis increasing the cost of production (23) and taking in account the negative effects on reproductive efficiency could reduce the rate of cows served and pregnant in a reasonable interval postpartum increasing the calving interval and affecting negatively the profitability. Therefore, the purpose of this study was to determine the effects of clinical mastitis, occurring between calving and the first service, on the achievement of two reproductive targets in dual-purpose cows: being served before 70 days postpartum and becoming pregnant before 110 days postpartum; these intervals are expected to achieve a calving interval of 400 days, which is acceptable in cross-bred dual-purpose cows under tropical conditions.

## MATERIALS AND METHODS

This was a 1-year-long retrospective study analyzing the reproductive records of a large herd of dual-purpose crossbred cows from a commercial farm. This commercial farm was particularly chosen as they possess a system with improved management, characterized by technology application such as computerized reproductive and health records, artificial insemination, and have genetic, nutritional, and preventive medicine standard operating procedures. This farm also has 24-h on-location veterinarians year-round. The farm is located in Machiques of Perija County, Zulia State, Venezuela. This area is a subhumid tropical forest with a mean daily temperature of 28.3 °C, 1950 mm/year of rainfall, and a relative humidity of 60–70%. The animals grazed in pasture consisting of German grass (*Echinochloa polystachya*) and Braquiaria grass (*Brachiaria humidicola*), with access to a commercial mineral mix ad libitum, as a dietary supplement.

Manual milking was performed twice a day after a short sucking by the cow's own calf to stimulate milk release. During the milking, the calf remained bound to a cow's forelimb. After milking, the cows were again suckled by their own calves to remove residual milk from the udder. Clinical mastitis was defined as abnormal milk (presence of discoloration, water-like consistency, clots, flakes, and/or blood) and inflammation of one or more quarters of the udder. Cows with clinical mastitis were identified by milking personnel during the milking routine, whom then notified the veterinarian in-charge. The veterinarian would then confirm the diagnosis and determine which cows required treatment, and maintained records of each case. Only cows with one case of clinical mastitis between calving and first service, without other concomitant diseases, were used in this study.

Estrus was detected by visual observation of cow estrus behavior over a period of 1 h each morning (6:00–7:00 am) and afternoon (6:00–7:00 pm), and using nonentry bulls (1 bull to 25 cows) for biostimulation. All cows were served after detection of the first estrus by one

technician, in accordance with the international am–pm rule. If cows did not return to estrus, an experienced veterinarian performed pregnancy diagnosis by rectal examination 45 days post-insemination. The farm maintained computerized and paper records of the reproductive performance of each cow, including day of calving, day of first service, and number of services.

The percentage of cows being served during the first 70 dpp and becoming pregnant during the 110 dpp were calculated. Cows with clinical mastitis before first service ( $n = 177$ , 42 primiparous and 135 multiparous) were compared with cows without clinical mastitis ( $n = 205$ , 53 primiparous and 152 multiparous). Data were analyzed using a commercial statistical computer software program (24). The association between clinical mastitis, parity and their interaction with the percentage of cows being served during the firsts 70 dpp and with cows becoming pregnant after 110 dpp were determined with the chi-square test, additionally odds ratio and 95% coefficient interval of mastitic cows to be served and becoming pregnant in comparison with non-mastitis cows were calculated. Differences were considered significant at  $P < 0.05$ .

## RESULTS

Percentage of non-mastitic cows being served during the first 70 dpp was 36.59% in comparison with 24.86% of mastitic cows ( $P = 0.0137$ ; Table 1). Mastitic cows had 0.5734 odds (95% CI: 0.3679-0.8938,  $P = 0.0141$ ) to be served during this interval than non-mastitic cows. Mastitic cows had lower percentage of pregnant cows at 110 dpp than non-mastitic cows (36.72% vs 50.73%,  $P = 0.006$ ) and mastitic cows had only 0.5636 odds (95% CI: 0.3739-0.8495,  $P = 0.0062$ ) to be pregnant than non-mastitis cows.

Table 1. Effects of postpartum clinical mastitis on percentage of cows served and pregnant before 70 and 110 dpp respectively.

	% Cows served $\leq 70$ dpp	Cows pregnant $\leq 110$ dpp
Non-mastitis	36.59%	50.73%
Mastitis	24.86%	36.72%
P value	0.0137	0.006
Difference	-11.73	-14.01

Negative effects of clinical mastitis on percentage of cows receiving the first insemination during the first 70 days after calving were observed only in primiparous cows, but negative effect on the percentage of cows becoming pregnant during the first 110 days after calving were observed in both primiparous and multiparous cows (Table 2). Mastitic primiparous cows had only 0.1156 odds (95% CI: 0.0249- 0.5374,  $P = 0.0059$ ) to be served than non-mastitic primiparous cows. In multiparous, mastitic cows had 0.7119 odds (95% CI: 0.4365-1.1609,  $P = 0.2117$ ) to be served than non-mastitic cows.

The 37.74% of non-mastitic primiparous cows became pregnant during the first 110 dpp in comparison with the 16.67% of primiparous mastitic cows ( $P = 0.0245$ ). Primiparous mastitic cows had 0.3300 odds (95% CI: 0.1234-0.8822,  $P = 0.0271$ ) to become pregnant during this interval that primiparous non-mastitic cows. In multiparous, 55.26% of non-mastitic and 42.96% ( $P = 0.0378$ ) became pregnant before 110 dpp, and mastitic cows had 0.6098 odds (95% CI: 0.3821-0.9730,  $P = 0.0380$ ) to become pregnant than non-mastitic cows.

Table 2. Effects of postpartum clinical mastitis and parity on percentage of cows receiving insemination  $\leq 70$  and becoming pregnant  $\leq 110$  dpp

		Non-mastitis	Mastitis	Difference	P value
Primiparous	Served	30.19%	4.76%	-25.43	0.0018
	Pregnant	37.74%	16.67%	-21.07	0.0245
Multiparous	Served	38.32%	31.11%	-7.21	0.1733
	Pregnant	55.26%	42.96%	-12.3	0.0378

## DISCUSSION

Interval to first service and conception are two important parameters to evaluate the reproductive efficiency in cows and these should be enough short to achieve a calving interval of 12-13 months (25). A high rate of cows reaching a short interval to insemination and pregnancy would improve the enterprises profitability, because cows with a short interval to conception produce more milk by day of calving interval (26,27) and more calves would be born, guaranteeing the replacement of cows and in the case of the dual-purpose cattle the sale of weaned calves and/or steers.

Several factors are affecting the reproductive efficiency in dual-purpose cows (28-31) including postpartum clinical mastitis, which increased the days to first insemination and days to conception (16) and more recently, clinical mastitis was observed by increase the days open in Sahiwal cows (*Bos indicus*), Karan Fries cows (crossbred *taurus-indicus*) and buffaloes cows (32). These results are similar to those observed in pure breed dairy cows (1,2,3,4,6,10,13,14,15).

The mechanism by which mastitis affects reproduction is complex, involving the udder, the immune system, and the hypothalamus-pituitary-ovary-uterus axis, with the consequences on reproductive performance depending on the time when mastitis is occurring relative to the stage of estrus cycle, or around the insemination (17,20,33). Mastitis has been observed by reduce LH secretion, disrupt the follicular grow, decrease androstenedione and estradiol concentration in the follicular fluid (34), increase the PGF<sub>2 $\alpha$</sub>  secretion and the cortisol (18), and in buffalo cows, it was observed than clinical and subclinical mastitis reduced the diameter of corpus luteum and the progesterone concentration (35). Additionally, clinical and subclinical mastitis has been observed by induce alteration of oocyte competence to development, disrupting nuclear and cytoplasmic maturation and affecting the expression of genes (36,37,38).

In this study, it was observed than clinical mastitis reduced the percentage of cows being served before 70 dpp (-11.73 points; Table 1); and compared with non-mastitic cows, the odds of be served was low. Most published results show the effect of clinical mastitis on interval to the first service (1,3,4,5,6,10,13,14,16). The low percentage of mastitic cows submitted to service observed in the present study, could be due to alteration in the follicular estradiol secretion (34), and to the reduction in the number of follicles with a diameter higher than 8 mm (39), affecting the follicular grow, retarding the estrus and therefore affecting the identification of cows in estrus to be served.

Clinical mastitis reduced percentage of cows (primiparous and multiparous) becoming pregnant in the first 110 dpp, and when comparing with non-mastitic cows, the probability of become pregnant in this interval are very low to both, primiparous and multiparous cows. Clinical mastitis occurring before service reduced pregnancy rate in comparison with non-mastitic cows: 38.1% vs 54.9% ( $P < 0.01$ ) (15) and 38.71% vs 61.39% ( $P < 0.05$ ) (3). In buffaloes, it was observed than pregnancy rate at 25 and 45 days after insemination were lower in buffaloes with clinical (28% and 16%) and subclinical mastitis (55.56% and 44.45%) occurring

between 15 days before and 30 days after the insemination, in comparison with buffaloes without mastitis (69.57 and 60.87%,  $P < 0.05$ ) (35). In others studies, clinical mastitis has been associated with low odd to pregnancy, especially when the case of mastitis is occurring near before or after the service or during the breeding period (6,40,41,42).

A more profound effect was observed in primiparous, with a reduction of 25.43 points in the percentage of cows being served and -21.07 points in the percentage of cows pregnant at 110 dpp, (Table 2). A greater negative impact of mastitis on primiparous cows has been observed previously. Nava-Trujillo et al. (16), observed that primiparous dual-purpose cows with mastitic needed 54 more days to first insemination and 68 days to conception than non-mastitic primiparous cows; while these differences were only 22 and 18 days in multiparous cows; while Bouamra et al. (3), observed that mastitis increased in 33 and 18 days the interval to first insemination in primiparous and multiparous dairy cows respectively; and similar results were observed by Chegini et al. (43). In addition, primiparous cows had more days to insemination from a clinical mastitis case than multiparous cows ( $110.92 \pm 8.72$  vs  $63.25 \pm 4.86$ ,  $P < 0.05$ ) (16), suggesting that clinical mastitis promote more significant hormonal alterations delaying onset of ovarian activity postpartum in primiparous cows and this higher susceptibility of primiparous cows could be due to a lesser energy balance, poor dry matter intake, greatest loss in body condition, lesser concentrations of glucose, and insulin as well as insulin-like growth factor-I (IGF-I) (44,45), and a less active mammary gland immune cells (46). However, the results of the present study reinforce previous findings on the negative effect of mastitis on the reproductive performance of *Bos indicus* and crossbred *taurus-indicus* cows (16,32), which have a lower milk yield than *Bos taurus* dairy cows, indicating that high milk yield is not necessary to observed the negative impact of mastitis on reproduction (39).

## CONCLUSIONS

Clinical mastitis affected the achievement of reproductive targets, reducing the probability of cows to be served and to become pregnant during the first 70 and 110 dpp, respectively. In addition, the negative impact of clinical mastitis was more harmful on primiparous cows than multiparous cows.

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