


**Association between retained fetal membranes, clinical endometritis, and reproductive performance of Holstein cows in the family dairy production system**



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**Abstract:**

In the family dairy production system, the prevalence of assisted calving and retained fetal membranes (RFM) is high; however, the extent to which these conditions affect the prevalence of uterine diseases and reproductive performance has not been determined. This study aimed to assess the presence or absence of assisted calving and RFM on the prevalence of clinical endometritis (CE) and conception rate at the first postpartum artificial insemination (1SCR) of Holstein cows. An observational retrospective cohort study was conducted to examine the relationship between assisted calving, RFM, CE, and 1SCR. The data were analyzed using descriptive statistics and logistic regression. The prevalence of assisted calving, RFM, and CE was 4.8 %, 8.3 %, and 16.8 %, respectively, and the overall 1SCR was 58.5 %. Cows with RFM had a 5.6 times higher risk of developing CE ( $P=0.001$ ), and cows with CE had a 5.4 times higher risk of not becoming pregnant at the first postpartum artificial insemination ( $P<0.001$ ). The prevalence of assisted calving and RFM observed in this study was lower than typically reported in this production system. Nevertheless, it was confirmed that RFM is a significant risk factor for CE, and this uterine condition negatively impacts reproductive performance in the family dairy production system.

**Keywords:** Calving quality, Uterine disease, Conception rate.

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## Introduction

The family dairy production system contributes to food security, job creation, and poverty alleviation, especially in families living in rural areas in developing countries<sup>(1,2)</sup>. In México, this system is characterized by the fact that operation on the farms and agricultural work to obtain forage for cattle are carried out by family members<sup>(3,4)</sup>. It has been estimated that this system contributes around 30 % of the bovine milk production in the country<sup>(5)</sup> and although it commonly faces socioeconomic and management problems, it has good potential for vertical growth based on the improvement of its production processes<sup>(6)</sup>.

The low productivity observed in family dairy farms is partially associated with inadequate management of the reproductive process<sup>(7)</sup>. Recent studies have identified that reproductive performance in these dairy farms is affected by both animal-related factors (such as body

condition score) and herd-level factors like management practices<sup>(8,9)</sup>. Regardless of the production system, poor reproductive performance negatively impacts on milk production, increases costs for reproductive treatments, and leads to losses due to involuntary culling, all of which affect the profitability of dairy farms<sup>(10-13)</sup>.

The conception rate after artificial insemination is one of the most important variables impacting the reproductive performance of cattle<sup>(14)</sup>. Studies indicate that assistance at calving and/or RFM can reduce the conception rate at first service<sup>(8,15)</sup>. In the family dairy production system, an increase in artificial insemination use<sup>(16,17)</sup>, as well as a high prevalence of assisted calving and RFM (>10%) have been observed<sup>(8)</sup>. Both, assisted calving and RFM are indicators of low-quality calving, and this condition, increases the risk of developing clinical or subclinical uterine infections<sup>(18,19)</sup>, which can affect the establishment and maintenance of pregnancy<sup>(20)</sup>. In intensive dairy farms, cows presenting clinical endometritis (CE) between days 20 and 33 postpartum have been reported to have less likely to become pregnant and more likely to be culled from the herd<sup>(21)</sup>.

The high prevalence of assisted calving and RFM in the family dairy production system in Mexico would suggest the existence of a high rate of uterine diseases and low 1SCR. However, acceptable 1SCR rates have been reported for this production system<sup>(8)</sup>, which appears contradictory. To the best of our knowledge, no studies have specifically examined the effect of calving assistance and RFM on the prevalence of uterine diseases and reproductive performance of cattle in such a system. Therefore, this study aimed to determine the effects of the presence or absence of assisted calving and RFM on the prevalence of clinical endometritis and conception rate at the first postpartum artificial insemination of Holstein cows in the family dairy production system.

## **Material and methods**

A retrospective cohort observational study was conducted using a subset of a data from a previous experiment performed within this production system in Jalisco state<sup>(8)</sup>. Information regarding CE had not been analyzed previously<sup>(8)</sup>, as it was beyond the scope of the original study. In this study, data on calving characteristics (assisted calving and RFM), the occurrence of CE, and the 1SCR of 241 Holstein cows across 22 farms were included.

Assisted calving was recorded when cows required help during the expulsion phase of their calves, regardless of the extent of help. RFM was recorded when cows failed to expel the placenta within 12 h after calving. CE was diagnosed by evaluating vaginal mucus between 28 and 35 d postpartum. Vaginal mucus was scored on a 0-to-3 scale, according to the

methodology described by Sheldon *et al*<sup>(22)</sup>. A score of 0 (clear and transparent mucus) refers to a cow with no CE problems. Scores of 1 (mucus with white or off-white flecks of pus), 2 (exudate containing  $\leq 50$  % white or off-white mucopurulent material), and 3 (exudate containing  $>50$  % purulent material, usually off-white or yellow) indicate cows with different levels of CE.

Reproductive performance was assessed using 1SCR, calculated based on the number of pregnant cows at pregnancy diagnosis following artificial insemination, which was performed by the producers after estrus detection without using any hormonal protocol for synchronization. Only inseminations from the first postpartum service across the different farms were included. Pregnancy diagnosis was conducted between 35 and 42 d post-insemination, using ultrasonography with a UMS900 unit equipped with a 5 Mhz transducer (Universal Imaging, Bedford Hills, NY, USA).

All analyses were performed using SAS 9.3 program (SAS Institute Inc. Cary, NC, USA). Descriptive statistical analysis was conducted for assisted calving (yes/no), RFM (yes/no), 1SCR (yes/no), CE including the different levels of severity (0, 1, 2 and 3) and CE without including severity levels (yes/no). A Cochran-Mantel and Haenszel analysis was used to assess associations among the evaluated factors. Since no significant associations were found ( $P > 0.1$ ), a univariate logistic regression was conducted to determine the impact of assisted calving and RFM on CE (yes/no; without severity levels). The same analysis was performed to determine the impact of assisted calving (yes/no), RFM (yes/no), and CE (yes/no) on pregnancy failure following artificial insemination. In all cases, the LOGISTIC procedure of SAS was used to calculate odds ratios and confidence intervals. Statistical significance was established at the  $P \leq 0.05$  level. None of the cows with CE at severity level 3 became pregnant, which limited the logistic regression analysis by severity level of this condition.

## Results

The prevalence of assisted calving and RFM was 4.8 % (11/231) and 8.3 % (19/230), respectively. The overall prevalence of CE was 16.7 % (39/233), with severity levels of 6.4 % (15/233), 7.3 % (17/233), and 3.0 % (7/233) for grades 1, 2, and 3, respectively (Figure 1A). The 1SCR was 51.0 % (99/154), 27.3% (3/11), 38.3 % (5/13), and 0.0% (0/5) for cows with a severity level of CE 0, 1, 2, and 3, respectively (Figure 1B). The overall 1SCR was 58.5 % (107/183); 64.3 % (99/154) for cows that did not show CE and 27.6 % (8/29) for the cows that did show CE ( $P < 0.001$ ), regardless of severity (Figure 1C).

The results of the univariate logistic regression analysis are presented in Table 1. No statistically significant association was found between assisted calving and the presence of CE ( $P=0.235$ ). However, RFM had a significant effect on the occurrence of CE ( $P<0.001$ ). The odds ratio indicates that cows with RFM were 5.6 times more likely to develop CE compared to the cows without this condition.

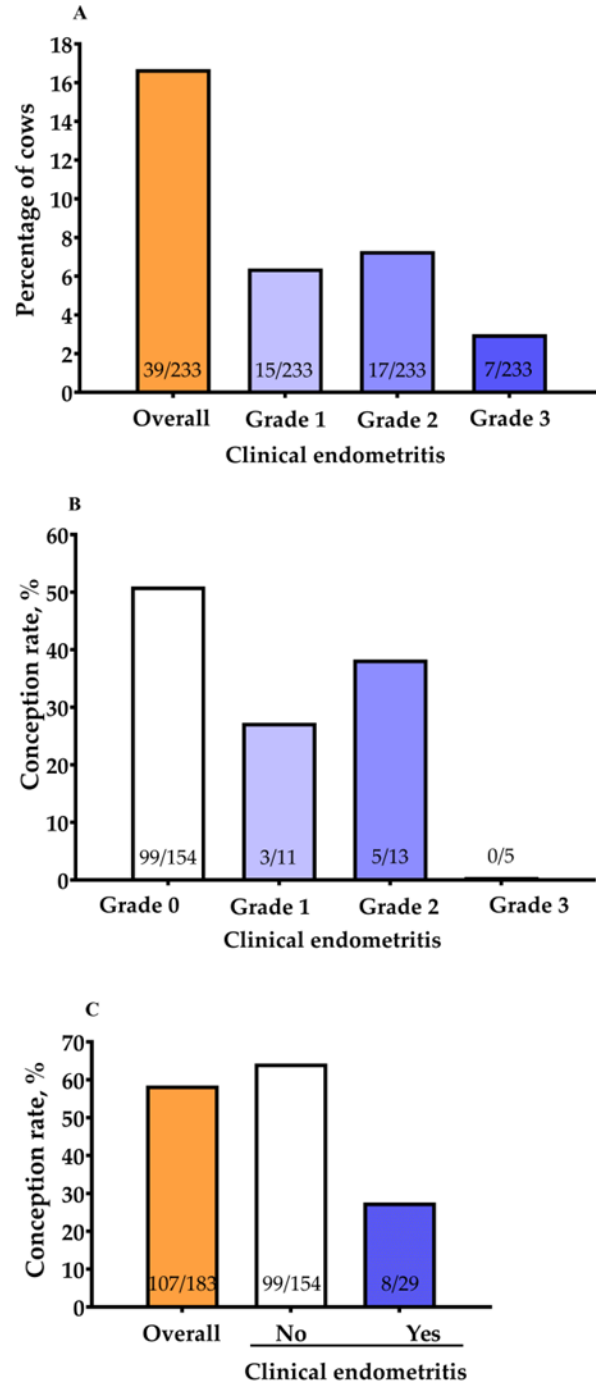
No statistically significant differences were observed for assisted calving ( $P=0.427$ ) and RFM ( $P=0.259$ ) in relation to pregnancy failure. However, there was a significant effect of CE, regardless of severity, on pregnancy failure following the first postpartum artificial insemination ( $P<0.001$ ). Cows that showed CE had a 5.4 times higher risk of not becoming pregnant after artificial insemination than cows not experiencing such uterine condition (Table 1).

**Table 1:** Impact of analyzed factors on the risk of developing clinical endometritis and pregnancy failure following the first postpartum artificial insemination

Variable	Risk factor	OR	95% CI	P-value
Clinical endometritis	Assisted calving (yes vs no)	2.3	0.57 - 9.5	0.235
	Retained fetal membranes (yes vs no)	5.6	2.0 - 15.7	0.001
Pregnancy failure	Assisted calving (yes vs no)	1.8	0.40 - 8.5	0.427
	Retained fetal membranes (yes vs no)	1.8	0.62 - 5.7	0.259
	Clinical endometritis (yes vs no)	5.4	2.17 - 13.6	0.001

OR= odds ratio; CI= confidence interval 95 %.

**Figure 1.** Prevalence of overall clinical endometritis and by severity level



(A), conception rate after the first postpartum artificial insemination according to the severity level of clinical endometritis (B), and overall conception rate after the first postpartum artificial insemination for cows with and without clinical endometritis ( $P < 0.001$ ), regardless of the degree of severity (C).

## Discussion

It was hypothesized that in the family dairy production system in Mexico, the high frequency of assisted calving and RFM would result to an elevated prevalence of CE, subsequently reducing the reproductive performance of the cows. However, the prevalence of assisted calving and RFM was considerably lower than previously reported (14.1 % and 14.9 %, respectively) in this production system<sup>(23)</sup>.

The 1SCR was similar to that reported in previous studies (~50 %) in farms under these production conditions<sup>(9,24)</sup> and higher than the rates observed in cows from intensive production systems (30-45 %) in México<sup>(25,26)</sup>. This difference is likely due to the lower production levels and nutritional demands of cows in family dairy production systems compared to those in intensive systems<sup>(27)</sup>. Additionally, it may be related to the fact that, in intensive production systems, artificial insemination is typically performed at a fixed time using ovulation synchronization protocols, whereas in the present study, inseminations were performed based on detected natural estrus, which has been linked to higher fertility rates<sup>(28)</sup>.

The percentage of cows with CE in this study was in the lower end of the range observed in studies conducted in intensive production systems<sup>(21,29,30)</sup> and it was considerably lower than the prevalence reported in small-scale dairy farms (~70 %) in other countries<sup>(31)</sup>. Assisted calving and/or RFM have been identified as important risk factors for the development of CE<sup>(18,19,32)</sup>. In this study, the occurrence of CE was influenced by RFM but not by assisted calving, which could be due to the low frequency observed (4.8 %) and could have affected the statistical power of the analysis. Additionally, but particularly dystocia, the delay in the expulsion of fetal membranes, generates a favorable environment for the rapid growth of bacteria such as *Escherichia coli* and *Arcanobacterium pyogenes*<sup>(33)</sup>, the primary microorganisms associated with postpartum uterine infections<sup>(34,35)</sup>.

On the other hand, cows that showed CE had a higher risk of failing to establish pregnancy, consistent with the results from various studies<sup>(18,21,36)</sup>. It has been observed that cows showing CE have a lower 1SCR<sup>(18)</sup>, a higher number of services per conception<sup>(36)</sup>, and an increased probability of being culled from the herd for not becoming pregnant<sup>(21)</sup>. Postpartum uterine infections negatively impact reproductive performance by disrupting the hypothalamic-pituitary-ovarian-uterus reproductive axis<sup>(37)</sup>. For example, bacteria-associated molecules such as lipopolysaccharides can alter GnRH/LH production<sup>(38,39)</sup>, reduce estradiol synthesis by granulosa cells<sup>(40,41)</sup>, affect the mRNA profile in oocytes<sup>(42)</sup>, and impair oocyte competence to develop into embryos<sup>(43)</sup>. Additionally, uterine infections induce tissue damage and trigger an inflammatory response, with perfusion of immune cells in the endometrium, which generates changes that affect its competence to establish and

maintain a pregnancy<sup>(37)</sup>. These results highlight the importance of preventive management in family dairy production systems to reduce the prevalence of uterine diseases and mitigate risk factors such as the RFM.

## Conclusions and implications

Overall, the prevalence of assisted calving and RFM was lower than what is typically observed in the Mexican family dairy production farms. However, this study confirmed that RFM constitutes a risk factor for CE, and this uterine condition negatively impacts cows' reproductive performance in this production system. Further research with a larger sample size and including other high prevalence risk factors like inadequate body condition score of cows at calving is warranted to fully understand the influence of calving conditions on reproductive indicators in the Mexican family dairy production system.

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