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Risk factors for subclinical endometritis and its effect on reproductive performance in small-scale dairy farms

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ABSTRACT

The study aimed to evaluate the effect of subclinical endometritis (SCE) on reproductive performance and identify risk factors for this pathology in small-scale dairies. In four small-scale dairies, 608 lactations were monitored for health issues, nutritional status, and reproductive events, and SCE was diagnosed based on endometrial polymorphonuclear counting (PMN%) at 30±5 (SCE30) and 60±5 (SCE60) days postpartum. The threshold for diagnosis was established according to the quartile distribution of PMN%; 4 % and 2 % for SCE30 and SCE60. Conception rate at first service (CRFS), number of services per conception (SC), and days open (DO) were analyzed for SCE effect through Chi-square or ANOVA. Events of interest were considered in each lactation to perform a risk factor analysis using univariate logistic regression and multiple logistic regression model building. The population attributable fraction was calculated to identify the most important risk factors. Considering both sampling periods, 40.5 % of the cows were positive for SCE. SCE30-positive cows had lower CRFS (38.9 vs 53.7 %), required 0.4 more SC, and had 20.2 more DO than SCE30-negative cows ($P \leq 0.001$). SCE60-positive cows had 16 more DO than SCE60-negative cows ($P \leq 0.01$). The most important risk factors for SCE30 were retained fetal membranes (RFM) and uterine diseases, while calving season, RFM, and SCE30 status were the risk factors for SCE60. SCE could represent a major problem in small-scale dairies due to its prevalence and negative effect on reproductive performance. Furthermore, there are identifiable risk factors for SCE under these production conditions, which should be considered when designing prevention strategies.

1. Introduction

Small-scale bovine milk production is crucial to global milk production (Hemme & Otte, 2010). In Mexico, this type of dairy contributes at least 30 % of the national annual output and holds significant social value, as it promotes local and regional development and supports population retention in their places of origin Espinoza-Ortega et al. (2005); Mariscal-Aguayo et al. (2017). It has been found that reproductive performance in small-scale dairies in Mexico is suboptimal, with identified risk factors that represent potential control points for improving performance (Montiel-Olguín et al., 2019). However, the impact of the postpartum uterine diseases' puerperal metritis, clinical metritis, clinical endometritis, and subclinical endometritis, on reproductive performance and profitability of small-scale dairies has not yet been determined. According to Sheldon et al. (2006), puerperal metritis is an acute systemic disease due to bacterial infection of the uterus, occurring during the first 2 weeks postpartum, and characterized by an enlarged uterus, a fetid red-brown watery uterine discharge, and fever >39.5 °C. The condition is classified as clinical metritis if systemic signs are absent. Clinical endometritis is characterized by the presence of purulent or mucopurulent uterine discharge, detectable in the vagina after 21 days postpartum in the absence of systemic signs. Subclinical

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endometritis, in turn, is defined as an endometrial inflammation with an increased polymorphonuclear cell infiltration, and without an active bacterial infection or purulent material in the vagina.

Small-scale bovine milk production faces challenges, including limited economic resources, restricted access to technology and technical advice (Montiel-Olguín et al., 2019), and it has less intensive management than large-scale dairies. These conditions can aggravate the impact of uterine diseases, such as subclinical endometritis (SCE), which often goes unnoticed due to the absence of obvious clinical signs (Sheldon et al., 2019). Inadequate detection and management of this disease can lead to lower conception rates, prolonged calving intervals, and decreased milk production, raising production costs (Barajas-Merchan et al., 2018). In large-scale dairies, a high prevalence of SCE has been reported (Sheldon et al., 2019), with important negative effects on reproductive performance (Barajas Merchan et al., 2018; Gilbert et al., 2005). No studies have been done addressing the direct impact of SCE on dairy herd profitability, however, the negative effect of this pathology on reproductive efficiency makes it a relevant factor that could affect productivity (Inchaisri et al., 2010). The causes of SCE are still under debate, including an unspecific infection of the uterus, or more probably, a decontrolled endometrial inflammatory response after a persistent bacterial infection (Wagener et al., 2017). Negative energy balance, metabolic diseases, and uterine infections during the puerperium, as well as calving assistance and retained fetal membranes, have been identified as risk factors for this pathology in large-scale dairies (Cheong et al., 2011; Dubuc et al., 2010; Wagener et al., 2017; Yáñez et al., 2022).

In small-scale dairy herds in Mexico (Núñez et al., 2009), the prevalence of SCE, its effects on reproductive efficiency, and the risk factors that increase its prevalence are unknown. Therefore, this study aimed to identify the rate of occurrence of SCE, assess the impact of this pathology on reproductive performance, and identify risk factors predisposing its development. These findings will serve as a basis for designing reproductive improvement programs to increase the productivity and profitability of small-scale dairies.

2. Materials and methods

2.1. Location and climatic conditions

The study was conducted from October 2021 to November 2023 on four dairy farms (112 to 180 cows per dairy) in the Los Altos dairy basin in the state of Jalisco, Mexico (20°49′01″N, 102°43′59″W, 1800 mas). The climate is sub-humid to temperate, with minimum and maximum temperatures of 4.2 °C and 31.6 °C, respectively. The average annual rainfall is 881 mm, with most precipitation from June to September (Ruiz et al., 2012). The Los Altos region was selected because Jalisco is a leading milk-producing state in Mexico and because about two-thirds of the state's milk production relies on small-scale operations in this region. The farms were selected by convenience sampling looking for representativeness of typical characteristics of a small-scale dairy system (Espinoza-Ortega et al., 2005). Small-scale dairies in the Los Altos region are between 10 and 150 cows in size, they are the main source of employment for family members and incorporate intermediate levels of technology (Montiel-Olguín et al., 2019).

2.2. Herd management and selection criteria

A total of 608 lactations (first to seventh) of Holstein cows from the four selected dairies were monitored during the experimental period. The minimum sample size was determined through a power test with an alfa error ≤0.05, resulting in 450 lactations. The experimental cows were pre-selected when entering the dry period and confirmed to stay in the study at 30±5 postpartum days if they did not present clinical signs of illness at that time. If infectious or metabolic diseases occurred before the confirmation postpartum day the cows received conventional treatments until recovery or culling. Fifteen cows were culled between 30±5 to 60±5 postpartum days reducing the total to 593 lactations for statistical analysis. Mechanical milking was performed twice a day in herringbone parlors. Feed was offered twice a day, using total mixed rations consisting of 60 % corn silage and stubble and 40 % commercial concentrate for dairy cattle (88 % dry matter, 21 % crude protein, 12 % acid detergent fiber, 25 % neutral detergent fiber, and 1.8 Mcal/kg NEL). Vaccination programs for infectious diseases prevalent in the area are routinely applied. A voluntary waiting period of 60 days was used, and services were provided by fixed-time artificial insemination (AI) at 70 days postpartum after a double-ovsynch protocol. Pregnancy diagnosis was carried out by real-time ultrasonography at 45 days post-service.

2.3. Diagnosis of subclinical endometritis

To determine the presence of SCE, cytological samples of uterine epithelium were collected in cows without clinical signs of illness at 30 ±5 and 60±5 days postpartum using the cytobrush technique. The cytobrush was screwed on a modified AI device for cows, with the brush protected by a disposable plastic catheter and plastic sleeve. Once inside the uterine body, the brush was exposed and rolled along the uterine wall. After this, the brush was drawn back into the AI device before being removed from the reproductive tract to prevent contamination. Outside the cow, the brush was removed from the AI device and a cytological imprint was made by rotating it on a microscope slide (Kasimanickam et al., 2005). Thereafter the samples were fixed with alcohol and stored until stained and cell counted in the laboratory. The staining was done with a commercial dye (CAT 548 blood smear and bone marrow stain, Hy-cel®). Subsequently, by observation under the microscope at 400x magnification, the percentage of polymorphonuclear neutrophils (PMN) in a sample of 300 cells was determined, considering epithelial cells and PMN (Melcher et al., 2014). The evaluation was conducted independently by two individuals, and if there was a variation of >10 % between their determinations, the procedure was repeated. The percentage of PMN infiltration was calculated as the average of both measurements, with a sample considered positive for SCE if the percentage was equal to or greater than the third quartile limit obtained from the quartile distribution of the total observations: 4.0 % and 2.0 % infiltration for 30 (SCE30) and 60 (SCE60) days postpartum, respectively.

2.4. Events of interest and reproductive performance

The herds included in the study were supervised twice a week by an experienced veterinarian who diagnosed metabolic and infectious diseases and prescribed correspondent treatments. The visiting veterinarian compiled all the information for the study's statistical analysis. The general management and registration of events were done by the herd owner. For all cows included in the study, events during the peripartum, puerperium, and service period were recorded, and specific events of interest were established to determine risk factors for SCE (Tables 2 and 3). For the same purpose, body condition score (BCS) was determined (Ferguson et al., 1994) by the herd owner at dry-off, calving, and 30 and 60 days postpartum. The presence of metabolic (hypocalcemia and ketosis) and uterine infections (i.e., clinical metritis and endometritis) (Sheldon et al., 2009), during the first 21 postpartum days was also registered. A cow was considered positive for hypocalcemia if she exhibited prostration, muscle tremors, a neck bent to one flank, and an inability to stand up (Arechiga-Flores et al., 2022). Ketosis was diagnosed based on signs such as inappetence, acute loss of body condition, ketone-smelling breath, incoordination, and dry feces (Zhang & Ametaj, 2020). Cows diagnosed with these disorders were treated until recovery using conventional therapeutic protocols. Two calving seasons within the year were defined for statistical analysis: the rainy season, from June to October, and the dry season, from November to May. Two parity categories were also established: primiparous and multiparous (cows with two or more calving). The type of delivery was recorded as assisted when any help was required to extract the calf. Retained fetal membrane (RFM) was defined as when expulsion was not achieved in the first 12 h postpartum. The sex of the calf, occurrence of single or twin births, and cases of abortion were also recorded. The factors of interest for 60 days were the same, with the addition of the presence or absence of SCE in the first sampling. Furthermore, the conception rate at first service (CRFS), number of services per conception (SC), and days open (DO) were calculated from the data recorded.

2.5. Statistical analysis

All statistical analyses were performed with SAS version 9.4 (SAS Institute Inc. Cary, NC, USA). The effects of SCE on SC and DO were determined through analysis of variance (ANOVA) for a completely randomized design on data transformed for normality using the inverse function. The appropriateness of the transformation was tested through the Shapiro-Wilk method. The effect of SCE on CRFS was determined through Chi-Square analysis. The level of statistical significance for ANOVA and Chi-Square analysis was set at $P < 0.05$. The strategy to identify risk factors associated with SCE was carried out in two stages (Montiel-Olguín et al., 2019). In the first, univariate logistic regression was used to select the events of interest to build multiple models ($P \leq 0.35$). In the second stage, multiple logistic regression with a backward selection method and a retention criterion of $P < 0.1$ was used to build the final models. The multiple models did not include the herd effect because it was non-significant ($P > 0.40$). From these models, adjusted odd ratios, unbiased relative risks (Zhang & Yu, 1998), and population attributable fractions (PAF) (Lin & Chen, 2019) were calculated to identify the most important risk factors of SCE.

3. Results

Based on the criteria established for classifying cows as SCE-positive, 10.3 % were positive at both

30 and 60 days postpartum ($n = 61$), 14.7 % were positive only at 30 days postpartum ($n = 87$), and 15.5 % were positive only at 60 days postpartum. Overall, 40.5 % of the cows were classified as SCE-positive. No significant differences were observed in the percentage of SCE-positive cows among the herds included in the study (36.7 to 43.9, $P > 0.05$).

The effects of SCE30 and SCE60 on CRFS, SC, and DO are presented in Table 1. SCE30-positive cows had lower CRFS (38.9 % vs. 53.7 %, $P < 0.001$), required a higher number of SC (2.2 vs. 1.8 services, $P < 0.001$), and had a higher number of DO compared to negative cows (137 vs. 117 days, $P < 0.001$). In contrast, SCE60-positive cows had CRFS and SC similar to negative cows ($P > 0.10$) but had a higher number of DO (131 vs. 115 days, $P < 0.01$).

Tables 2 and 3 present selected events of interest and their effects on SCE30 and SCE60, respectively. For SCE30, parity, calving assistance (CA), abortion, RFM, metabolic disease during the first 30 days postpartum (MD30), and uterine disease during the first 30 days postpartum (UD30) were identified as events to be included in the multiple analysis. For ESC60, the selected events were calving season (CS), parity, CA, RFM, UD30, MD30, uterine disease between 31 and 60 days postpartum (UD31–60), and SCE30.

Tables 4 and 5 present the logistic regression models that include the risk factors for SCE30 and SCE60 and their relative importance based on PAF. The risk factors identified for SCE30 were CA, RFM, UD30, and MD30. For ESC60, the risk factors were CS, RFM, UD31–60, and SCE30. According to the PAF, the factors with the greatest potential impact on the development of SCE were RFM and UD30 for SCE30, and CS, RFM, and SCE30 for SCE60.

Table 1
Reproductive performance in cows negative or positive for subclinical endometritis at 30 and 60 days postpartum.

SCE diagnosis	Conception rate at first service % (95 % C.I.)	Reproductive Services for conception mean ±sd	indicator Days Open mean±sd
Negative to SCE30 ($n = 454$)	53.7 (49.5–57.6)	1.8 ± 0.05	117.1 ± 5.3
Positive toSCE30 ($n = 154$)	38.9 (34.8–42.7)	2.2 ± 0.10	137.3 ± 5.3
P	<0.001	<0.001	<0.001
Negative toSCE60 ($n = 440$)	51.5 (47.3–55.5)	1.8 ± 0.05	115±2.7
Positive toSCE60 ($n = 153$)	47.0 (42.9–51.1)	2.0 ± 0.10	131±4.1
P	0.33	0.24	<0.01

SCE, subclinical endometritis; 95 % C.I., 95 % confidence interval; P, probability value; sd, standard error of the mean.

4. Discussion

This study is the first to investigate the SCE status of cows in a small-scale production system in Mexico utilizing a large sample size that makes the results robust. According to the criteria used and considering

Table 2
Events of interest or potential risk factors and their effect on subclinical endometritis at 30 days postpartum (SCE30).

Event of interest or potential risk factor	SCE30 positive cows % (95 % C.I)	P
BCS at dry-off		
BCS ≤2.75	27.9 (20.9–34.9)	0.36
BCS >2.75	24.3 (20.1–28.4)	
BCS at calving		
BCS ≤2.75	27.8 (21.0 – 34.6)	0.34
BCS >2.75	24.2 (20.0 – 28.4)	
BCS at 30 d post calving		
BCS ≤2.75	25.8 (21.7 – 29.6)	0.54
BCS >2.75	22.7 (13.4 – 32.0)	
Calving season		
Rainy (Jun–Oct)	25.9 (20.5 – 31.3)	0.76
Dry (Nov–May)	24.8 (20.0 – 29.6)	
Parity		
Multiparous	65.0 (57.7 – 73.4)	0.07
Primiparous	57.3 (52.6 – 61.9)	
Calving assistance		
Yes	19.7 (12.8 – 26.6)	<0.001
No	4.8 (2.67 – 6.8)	
Abortion		
Yes	7.8 (3.2 – 12.3)	0.01
No	3.1 (1.3 – 4.8)	
RFM		
Yes	33.8 (25.9 – 41.5)	<0.001
No	10.6 (7.7 – 13.5)	
Calf sex		
Female	24.0 (19.2 – 28.7)	0.56
Male	26.1 (20.5 – 31.6)	
Twin birth		
Yes	1.3 (0.1 – 4.6)	0.45
No	0.7 (0.1 – 1.9)	
Uterine disease in the first 30 d postpartum		
Yes	91.4 (81.0 – 97.1)	<0.001
No	18.6 (15.0 – 21.6)	
Metabolic disease in the first 30 d postpartum		
Yes	47.4 (30.1 – 64.5)	<0.001
No	23.9 (20.2 – 27.4)	

BCS, body condition score; RFM, retained fetal membranes; 95 % C.I., 95 % Confidence Interval; P, probability value for an effect on SCE30.

Table 3
Events of interest and their effect on subclinical endometritis at 60 days postpartum (ESC60).

Event of interest or potential risk factor	SCE60 positive cows % (95 % C.I)	P
BCS at dry-off		
BCS ≤2.75	26.8 (19.7 – 33.7)	0.73
BCS >2.75	25.9 (21.1 – 29.6)	
BCS at calving		
BCS ≤2.75	27.9 (21.0 – 34.7)	0.43
BCS >2.75	24.9 (20.05 – 29.1)	
BCS at 30 d post calving		
BCS ≤2.75	25.8 (21.9 – 29.6)	0.88
BCS >2.75	22.7 (13.4 – 32.0)	
BCS at 60 d post calving		
BCS ≤2.75	25.9 (22.0 – 29.7)	0.77
BCS >2.75	24.3 (13.5 – 35.0)	
Calving season		
Rainy (Jun–Oct)	57.5 (49.3 – 65.6)	<0.001
Dry (Nov–May)	39.8 (35.0 – 44.4)	
Parity		
Multiparous	43.6 (38.8 – 48.3)	0.05
Primiparous	65.3 (57.4 – 73.2)	
Calving assistance		
Yes	14.9 (8.7 – 20.9)	0.002
No	6.2 (3.7 – 8.6)	
Abortion		
Yes	19.2 (6.5 – 39.3)	0.43
No	26.1 (22.3 – 29.8)	
RFM		
Yes	26.8 (19.4 – 34.1)	<0.001
No	12.7 (9.5 – 15.9)	
Calf sex		
Female	41.8 (33.4 – 50.1)	0.45
Male	45.3 (40.4 – 50.2)	
Twin birth		
Yes	40.0 (5.2 – 85.3)	0.47
No	25.7 (22.0 – 29.2)	
Uterine disease in the first 30 d postpartum		
Yes	53.6 (39.6 – 67.5)	<0.001
No	22.9 (19.2 – 26.5)	
Metabolic disease in the first 30 d postpartum		
Yes	43.2 (25.9 – 60.5)	0.001
No	24.6 (20.9 – 28.3)	
Subclinical Endometritis at 30 d postpartum		
Yes	39.9 (31.7 – 47.9)	<0.001
No	19.8 (15.9 – 23.6)	
Uterine disease between 31 and 60 d postpartum		
Yes	86.4 (65.0 – 97.0)	<0.001
No	23.5 (19.9 – 27.0)	
Metabolic disease between 31 and 60 d postpartum		
Yes	40.0 (5.2 – 85.3)	0.47
No	25.7 (22.0 – 29.2)	

BCS, body condition score; RFM, retained fetal membranes; P, probability value for an effect on SCE60.

the samples taken at 30 and 60 days postpartum, 40.5 % of the cows were classified as SCE-positive. This prevalence is slightly lower than the 43 % reported between 35 and 45 days postpartum in intensive milk production systems in Mexico (Barajas Merchan et al., 2018) and higher than the 21 % and 32 % observed by Ochoa (2020) and Prunner et al. (2014), around day 30 postpartum in medium and small-scale dairy farms, respectively. In contrast, in grazing dairy cows, a prevalence of SCE of 17 % was found within a range of 21 to 62 days postpartum (Madoz et al., 2013).

The prevalence of SCE reported for cows in various studies is highly variable, ranging from <10 % to >40 % (Barajas Merchan et al., 2018; Dubuc et al., 2010; Lopdell et al., 2011; Madoz et al., 2014; Ochoa., 2020; Plöntzke et al., 2010; Kasimanickam et al., 2004;). Among the factors proposed as determinants of this variability are the timing of postpartum sampling, the threshold PMN used to classify positive cases, and the production system. PMN endometrial infiltration decreases as the postpartum period progresses, regardless of whether SCE develops (Sheldon et al., 2006; Wagener et al., 2017). Therefore, the threshold for PMN endometrial infiltration for SCE diagnosis is typically adjusted downward throughout the postpartum period (Madoz et al., 2013; Wagener et al., 2017). The threshold of PMN for SCE diagnosis and the criteria used to define it vary widely (Melcher et al., 2014) indicating the absence of a universally accepted standard.

The influence of herd management or production systems on the prevalence of SCE is supported by studies that have observed differences among herds (McDougall et al., 2020; Prunner et al., 2014). Herd-level risk factors have been identified that influence the prevalence of this pathology (Cheong et al., 2011; Prunner et al., 2014), with lower prevalence in pasture-based production systems than in confined intensive systems (Madoz et al., 2014; Lopdell et al., 2011). In our study, the production system of the experimental herds was considered small-scale (Núñez et al., 2009), and there are no previous data related to SCE in this kind of herd. Thus, we chose to use the percentile distribution of endometrial PMN to diagnose SCE, as it reflects the condition of the experimental population. Additionally, because one of our objectives was to estimate the effect of ESC on reproductive performance, it was deemed inappropriate to use ROC curves for determining the diagnostic PMN threshold to avoid the circularity issue noted by McDougall et al. (2020) when using reproductive performance to diagnose SCE (ROC curve) and then evaluate the effect of SCE on reproductive performance (Barajas Merchan et al., 2018; Barlund et al., 2008; Dubuc et al., 2010; Madoz et al., 2013; Kasimanickam et al., 2004).

Nearly 40 % of SCE30-positive cows were still considered positive at 60 days postpartum, a lower rate of spontaneous recovery than that observed by Lopdell et al. (2011) and Plöntzke et al. (2010). These authors have observed significant decreases in SCE prevalence as the postpartum period progressed but did not adjust the PMN threshold at different postpartum times and worked with large-scale herds. In summary, the presence of SCE-positive cows in our study is comparable to that in large-scale dairies and is at a level that may pose a problem for reproductive and productive performance.

SCE30-positive cows had lower CRFS, needed 0.4 times more SC, and had 20.2 more DO. Conversely, SCE60-positive cows showed no difference in CRFS or SC compared to negative cows but did have 16 more DO. In both cases, the average DO exceeds the 110 to 120 days suggested as optimal for small-scale production systems in Mexico (Vera et al., 2009), potentially affecting profitability (Inchaisri et al., 2010). Other studies have also reported the negative effects of SCE on reproductive

Table 4
Logistic regression model for association between risk factors and subclinical endometritis at 30 days postpartum.

Risk Factor*	Comparison	OR	OR 95 % C.I.	RR	RR 95 % C.I.	Pe	Pe 95 % C.I.	PAF
Calving assistance	Yes vs. No	2.4	1.1–5.3	1.9	1.1–2.9	0.08	0.06–0.10	0.06
RFM	Yes vs. No	2.6	1.3–4.9	2.0	1.3–3.0	0.16	0.13–0.19	0.14
Uterine disease in the first 30 d postpartum	Yes vs. No	46.0	17.5–120.7	4.6	4.1–4.8	0.09	0.07–0.11	0.24
Metabolic disease in the first 30 d postpartum	Yes vs. No	3.4	1.6–7.2	3.6	1.5–4.0	0.06	0.04–0.08	0.08

RFM, retained fetal membranes; OR, adjusted odd ratio; 95 % C.I., 95 % confidence interval; RR, unbiased relative risk; Pe, proportion of cows exposed to each risk factor; PAF, population attributable fraction; The P value for retention in the model was ≤0.10.

Table 5
Logistic regression model for association between risk factors and subclinical endometritis at 60 days postpartum.

Risk Factor*	Comparison	OR	OR 95 % C.I.	RR	RR 95 % C.I.	Pe	Pe 95 %C.I.	PAF
Calving season	Rain vs. dry	2.1	1.4–3.1	1.8	1.3–2.4	0.44	0.40–0.48	0.22
RFM	Yes vs. No	2.0	1.2–3.3	1.7	1.2–2.4	0.16	0.13–0.19	0.10
Uterine disease between 31 and 60 d postpartum	Yes vs. No	15.8	4.4–56.5	3.7	2.5–4.3	0.04	0.02–0.06	0.07
Subclinical endometritis at 30 d postpartum	Yes vs. No	2.1	1.3–3.3	1.8	1.3–2.4	0.25	0.21–0.28	0.16

RFM, retained fetal membrane; OR, adjusted odd ratio; 95 % C.I., 95 % confidence interval; RR, unbiased relative risk; Pe, proportion of cows exposed to each risk factor; PAF, population attributable fraction; The P value for retention in the model was ≤0.10.

performance in both small (Barrio et al., 2015; Plöntzke et al., 2010) and large-scale herds (Barajas Merchan et al., 2018; Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2005). In our study, the negative effects on reproductive performance were more evident in the SCE30-positive cows than in the SCE60-positive group. The negative effects of SCE on reproductive performance might be evident in the short or medium term (Gilbert, 2011). If the medium-term effects are more significant, this could explain the greater negative effects on reproductive performance observed in SCE30-positive compared to SCE60-positive cows. The difference might also be related to the lower PMN infiltration threshold applied at 60 vs. 30 postpartum days (≥2 % and ≥4 %, respectively), suggesting that the threshold of PMN concentration required to exert negative effects is higher than our 60 postpartum threshold (Sheldon et al., 2009). This observation, although speculative, underscores the need for standardized methods for SCE diagnosis.

The occurrence of events considered in the risk factor analysis, such as abortion, CA, RFM, twin deliveries, UD, and MD, were 4 %, 8 %, 16 %, 1 %, 9 %, and 6 %, respectively (Tables 2 and 3), like findings by Montiel-Olguín et al. (2019) in small-scale dairy herds. The risk factors for SCE30 were CA, RFM, UD30, and MD30, while for SCE60, they were CS, RFM, UD31–60, and SCE30. Few studies have focused on identifying risk factors for SCE, and those that have often used different criteria for diagnosing this uterine pathology. However, conditions associated with negative energy balance (NEB) during the puerperium, such as low body condition at delivery, increased blood ketone bodies, and high milk production levels, have been identified as risk factors for SCE (Cheong et al., 2011; Dubuc et al., 2010; Yáñez et al., 2022). The alteration in uterine immune function associated with NEB during postpartum (Wathes et al., 2009) is considered a mediator that increases the prevalence of SCE.

Our study did not find a relationship between low body condition during different reproductive stages and SCE (Tables 3 and 4), which could be related to the low production levels and the depth of NEB in cows in small-scale herds (Montiel-Olguín et al., 2019). However, we found that metabolic diseases during postpartum constitute a risk factor for SCE, similar to observations by other authors (Cheong et al., 2011; Dubuc et al., 2010; Yáñez et al., 2022). Additionally, uterine infections have been identified as risk factors for SCE (Cheong et al., 2011; Dubuc et al., 2010), in agreement with our study. The relationship between these uterine pathologies and SCE seems to be associated with a persistent inflammatory condition that develops after the uterine infection (Sheldon et al., 2006) rather than the persistence of an active bacterial infection (Madoz et al., 2014; Prunner et al., 2014). In the case of CA as a risk factor for SCE, injury to the uterine epithelium during obstetric procedures may facilitate the development of uterine infections and SCE (Cheong et al., 2011; Dubuc et al., 2010). Likewise, tissue damage can initiate an inflammatory response with PMN infiltration characteristic of SCE (Mohammed et al., 2017) or generate a sequence of RFM (Montiel-Olguín et al., 2019), uterine infection (Dubuc et al., 2010; Han & Kim, 2005; T.J. Potter et al., 2010;), and SCE. However, CA and RFM were not displaced from the model by UD during the multiple regression analysis, suggesting that their effect on SCE might be independent of UD. In the case of RFM, the altered immune status predisposing subjects to its presentation (Chebel, 2021) could be the mediator for increasing the risk of SCE. The risk factors for SCE60 were similar to those identified for SCE30, including SCE30. As previously mentioned, 40 % of SCE30-positive cows remained positive at 60 days postpartum, indicating a limited recovery rate that may explain the relationship between SCE30 and SCE60. The occurrence of calvings during the rainy season increased the probability of SCE60, through factors that are difficult to explain, but which might be related to a less sanitary environment increasing the risk of uterine contamination (Sheldon et al., 2006). Other authors have not observed a relationship between CS and SCE (Prunner et al., 2014), although they did not specify whether the seasons compared were related to the rainfall patterns.

The magnitude of OR and RR for the identified risk factors partially indicates the degree of impact this risk factor has on SCE (Tables 4 and 5). However, this impact will also depend on the proportion of the risk factor in the population, a relationship that can be estimated by calculating the PAF. The PAF values in Tables 4 and 5 estimate the percentage of SCE cases that could be avoided if exposure to the corresponding risk factor is eliminated (Llorca et al., 2001). The highest PAF values for SCE30 were those corresponding to UD30 (0.24) and RFM (0.14), suggesting that prevention strategies for SCE30 should focus on reducing these risk factors. For SCE60, the strategy should aim to reduce RFM (PAF=0.10) and SCE30 (PAF=0.16). As to the effect of CS on SCE60 (PAF=0.22), an important issue should be to identify the specific factors mediating this effect before suggesting a correspondent prevention strategy. The study of SCE presents significant challenges, particularly due to the lack of a universal criterion for defining the PMN threshold and determining the optimal postpartum timing for sample collection. In this study, population-specific PMN thresholds were applied instead of standardized diagnostic criteria, and the analysis was restricted to two postpartum windows (30 and 60 days). These limitations may affect the comparability of the results with those of other studies. Additional limitations include the study's focus on a limited number of small-scale dairy farms within a specific region of Mexico, which restricts the generalizability of the findings to other regions or production systems. Moreover, critical factors such as milk production and its relationship to SCE were not evaluated, leaving a gap in understanding its influence on production efficiency. Despite these limitations, the findings of this study are consistent with results from other research and provide new evidence on potential risk factors for SCE. Continued research is essential, particularly within this production system and across a broader range of dairy farms, to validate these findings further and enhance our understanding of SCE and its management.

5. Conclusions

The prevalence of subclinical endometritis is significant in small-scale dairy herds in Mexico and negatively impacts reproductive performance. The key cow-level risk factors for this condition that should be considered when developing preventive strategies include retention of fetal membranes and uterine infections. Additionally, due to the impact of the rainy season on the occurrence of subclinical endometritis, an important issue should be to identify the specific factors mediating this effect before suggesting a correspondent prevention strategy.

CRediT authorship contribution statement

Fernando Villaseñor-González: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Luis Javier Montiel-Olguín:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mario Alfredo Espinosa-Martínez:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Marina Durán-Aguilar:** Writing – review & editing, Project administration, Funding acquisition, Formal analysis. **Feliciano Milian-Suazo:** Writing – review & editing, Validation, Formal analysis. **Héctor Raymundo Vera-Ávila:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Data availability

The database supporting the results presented in this study is available upon request to the corresponding author.

Ethics approval

The animal study protocol for studies involving animals was approved by the Bioethics Committee of the Autonomous University of Queretaro (38FCN2021).

Declaration of generative AI and AI-assisted technologies in the writing process

The writing process, devoid of generative AI and AI-assisted technologies, relies solely on human creativity and manual cognitive efforts.

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