



## Subclinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina

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### ABSTRACT

Recently several studies have reported that subclinical endometritis impairs reproductive performance in cattle. Most of the studies were conducted in western industrialized countries under intensive housing conditions. The objective of the present study was to determine the prevalence of subclinical endometritis and its impact on reproductive performance outcomes in clinically healthy postpartum dairy cows in a pasture-based extensive dairy farming system in Argentina. Lactating Holstein cows ( $n=201$ ) at 18–38 days postpartum (dpp) from three commercial dairy farms in Buenos Aires Province, Argentina, were examined for signs of clinical endometritis by external inspection and manual vaginal examination. Only cows without signs of clinical endometritis i.e. no vaginal discharge were enrolled in this study and examined for subclinical endometritis using the cytobrush technique. Cows with  $\geq 5\%$  polymorphonuclear cells (PMN) in the cytological sample were regarded as affected by subclinical endometritis. All cows were reexamined 14 days later following the same examination protocol. Prevalence of subclinical endometritis 18–38 dpp was 38% and decreased to 19% at reexamination. The proportion of cows pregnant at first service was 29% and proportion of cows pregnant at 360 pp was 73% and 75% in cows with subclinical endometritis and those without, respectively. The probability of conception at first service, hazards of insemination and pregnancy, respectively, were not affected by subclinical endometritis. Primiparous cows had a greater chance for insemination (HR = 0.66; 95% CI = 0.47–0.92) and pregnancy (HR = 0.63; 95% CI = 0.45–0.90) than multiparous cows. In conclusion subclinical endometritis did not affect reproductive performance outcomes in a pasture-based, extensive dairy farming system in Argentina.

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### 1. Introduction

Diagnosing and monitoring subclinical diseases such as subclinical mastitis, ketosis and rumen acidosis has become a substantial part of modern health management in dairy

cattle (LeBlanc et al., 2006). In recent years, several publications have reported a negative impact of subclinical endometritis in dairy cattle on subsequent reproductive performance (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004; Lenz et al., 2007).

Subclinical endometritis is defined as an inflammation of the endometrium in the absence of clinical signs of endometritis (Sheldon et al., 2006). Diagnosis of subclinical endometritis can be performed by uterine cytology or ultrasonography. For uterine cytology, samples are obtained by lavage technique (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2005a) or cytobrush method (Barlund

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et al., 2008; Kasimanickam et al., 2004; Kasimanickam et al., 2005a,b). Cytological smears are evaluated for their proportion of polymorphonuclear cells (PMN) by endometrial cells present in a sample. An increased proportion of PMN is prognostic for impaired subsequent reproductive performance (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004). The threshold value for the proportion of PMN to define subclinical endometritis is controversial and ranges from 4% to 18% (Barlund et al., 2008; Galvao et al., 2009; Gilbert et al., 2005; Kasimanickam et al., 2004; Raab, 2003). The reported prevalence of subclinical endometritis ranges from 11% to 53% (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004) and decreases with increasing days postpartum (dpp) (Gilbert et al., 2005; Madoz et al., 2008). In previous studies, cows diagnosed with subclinical endometritis had prolonged days open and a reduced probability of conception at first AI compared with cows without subclinical endometritis (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004).

The majority of data, however, is based on studies conducted in dairy herds under confinement housing conditions. There are few data published on the occurrence of subclinical endometritis in South America. Madoz et al. (2008) reported a prevalence of subclinical endometritis of 10.1% in Argentina. It can be hypothesized that prevalence and impact of the disease vary with different housing, climatic and feeding conditions. The objective of the present study was to determine prevalence of subclinical endometritis and its impact on reproductive performance outcomes for a pasture-based, extensive, dairy farming system.

## 2. Materials and methods

### 2.1. Study farms

The study was conducted on three commercial dairy farms in Buenos Aires Province, Argentina, between September 2006 and December 2007. The land base of the dairy farms was 10,000 (farm 1), 6400 (farm 2) and 236 (farm 3) ha and the number of milking cows were 1,545, 1,200 and 96, respectively. The average daily milk yield was 29, 22 and 18 L per cow, respectively, and cows were kept on extensive grasslands, rotating for fresh pasture lots. Calving areas of approximately 0.5 ha were close to the accommodation of farm personnel. Feed was composed of mixed pastures (alfalfa, festuca, lolium, brome grass) and summer annual grasses (white and red clover, ryegrass, and soybean) and supplemented with sorghum and corn silage according to animals' requirements. Concentrates were offered twice daily during milking. Veterinarians visited the farms routinely every week or every second week for reproductive examination.

Calving occurred year round except for the hot summer months (January, February) as common for this region. The voluntary waiting period was set at 40–45 dpp. Cows observed in estrus were artificially inseminated. Cows not observed in estrus were submitted to prostaglandin F<sub>2α</sub> (Bioprost D, Biotay S.A., Grand Bourg, Argentina) and intravaginal progesterone releasing insert (CIDR, Pfizer S.

R. L., Buenos Aires, Argentina) synchronization protocols. Pregnancy diagnosis was performed by transrectal palpation of the uterus and its contents between 35 and 48 days post insemination. Relevant data were managed with herd management software (Syscord-Tamb, Lincoln, Argentina; Protambo Master, Santa Fe, Argentina).

### 2.2. Study design

Two replicates were conducted between September and December in 2006 and 2007. The owners of the farms and the veterinarians were informed about the relevant characteristics of the study and agreed with the study design. The farms were visited every 14 days. Enrolment of cows, clinical examination, sampling and evaluation were performed by the same investigator.

Cows were examined at first visit (exam 1) between 18 and 38 dpp. In each cow a clinical examination of the reproductive tract was performed by manual vaginal examination and transrectal palpation of the uterus and the ovaries. Cows with vaginal discharge were diagnosed as affected by clinical endometritis and excluded from the study. Animals without vaginal discharge were enrolled in this study and examined with the cytobrush method. Body condition of all cows was scored according to a 5-point scale (Edmonson et al., 1989). All animals were re-examined at a second visit (exam 2) 14 days later following the same examination protocol.

### 2.3. Clinical examination

Before manual vaginal examination the vulva was cleaned with water and an arm-length glove (Guantes largos descartables, Flex, Buenos Aires, Argentina) was lubricated and inserted into the vaginal lumen. Evaluation of the cervix was performed by smooth introduction of the index finger. The open hand was moved in a circular direction in the vagina, withdrawn and with spread fingers vaginal mucus was scored. Clear, transparent mucus without any particles of pus was regarded as indicative for cows not affected by clinical endometritis. In these cows uterine cytological samples were taken by the cytobrush method.

### 2.4. Cytological samples

A small brush (2.2 cm length, 0.5 cm in diameter; Medibrush, Medical Engineering Co. S.A., Buenos Aires, Argentina), mounted on the tip of a metal rod (45 cm length) and protected by a disposable catheter (Condor, Buenos Aires, Argentina) was inserted under transrectal control through the vagina, into the uterine lumen. Inside the uterine body the brush was pushed gently forward until emerging out of the catheter. Cells were collected by rotating the cytobrush while in contact with the uterine wall. For protection, the brush was retracted into the catheter during the passage through the genital tract. Smears were prepared by rolling the brush onto a microscopic slide, dried and fixed (Roby, Argencos S.A., San Martin, Argentina) immediately after collection. In the laboratory of the Institute for Theriogenology of the University of La Plata (UNLP) samples were stained (Tincion 15, Biopur S.R.L., Rosario,

Argentina) and evaluated by 400× magnification (Nikon, E200, Japan). A total of 200 endometrial cells were counted to determine the proportion of polymorphonuclear cells (PMN). The threshold value for the proportion of PMN indicating subclinical endometritis was set at 5% (Gilbert et al., 2005; Raab, 2003).

Farmers were not informed about the results of cytological examinations, to exclude bias on interventions given to enrolled cows.

### 2.5. Data management and statistical methods

All data were recorded on case report forms on farm and transferred into a spreadsheet (Excel 2003, Microsoft Corporation, Redmond, WA). Data recorded included results of the vaginal and cytological examinations. Animal specific data (parity, date of parturition), periparturient disorders (assisted calving, retained fetal membranes, hypocalcaemia, and abortion), and reproductive performance outcomes (first insemination, last insemination, number of inseminations, outcome of pregnancy exams, and date of culling) were obtained from the herd management software.

Reproductive performance outcome was characterized by days to first AI (days from parturition to first service), conception at first AI (number of cows pregnant after first AI divided by number of cows inseminated × 100), days to pregnancy (days from parturition to pregnancy), proportion of cows pregnant (number of cows documented to be pregnant at 360 dpp divided by number of cows enrolled × 100), services per pregnancy (total number of inseminations divided by number of pregnant cows) and proportion of cows culled within 360 dpp. A follow-up period was set at 360 dpp to compensate for a 2-month interruption of the breeding period in April and May, which is common in Argentina to avoid calvings during the hot summer months.

Data were analyzed using SPSS software (Version 16.0, SPSS Inc., Munich, Germany). The correlation between dpp and percentage PMN was analyzed by Spearman–Rho test. The relative risk (RR) for the diagnosis of subclinical endometritis was calculated with farm 1 (larger number of cows) as reference. In a next step, RR for the diagnosis of subclinical endometritis at 18–38 dpp and binary logistic regression models for the risk of conception at first AI as outcome variables were calculated. Survival analyses for the hazards of insemination and pregnancy within 360 dpp were performed using Kaplan–Meier survival analysis and Cox regression, censoring cows that were not inseminated and not pregnant, respectively. For calculation of RR, logistic regression models and Cox regression, subclinical endometritis (0 = no, 1 = yes), reported periparturient disorders (0 = no, 1 = yes), parity (0 = primiparous, 1 = multiparous), body condition (0 = BCS < 2.75, 1 = BCS ≥ 2.75), and replicate (0 = September to December 2006, 1 = September to December 2007) were included as factors. Adjusted RR, odds ratios, hazard ratios, confidence intervals (CI) for HR and OR, medians with interquartile ranges (IR), and *P*-values are reported. For RR, logistic

**Table 1**

Relative risk analysis for the diagnosis of subclinical endometritis in cows 18–38 dpp with periparturient disorders, parity class, body condition and study period as factors.

Factor	RR <sup>a</sup>	CI <sup>b</sup> 95%	<i>P</i>
Parity <sup>c</sup>	0.89	0.59–1.34	0.57
Periparturient disorders <sup>d</sup>	1.13	0.75–1.70	0.57
BCS group <sup>e</sup>	1.14	0.79–1.64	0.48
Replicate <sup>f</sup>	1.20	0.82–1.74	0.35

<sup>a</sup> RR: relative risk.

<sup>b</sup> CI: confidence interval.

<sup>c</sup> Parity class: 0 = primiparous, 1 = multiparous.

<sup>d</sup> Periparturient disorders: 0 = no periparturient disorder; 1 = periparturient disorder.

<sup>e</sup> BCS group: 0 = BCS 0.25–2.50, 1 = BCS 2.75–5.00.

<sup>f</sup> Replicate: 0 = September to December 2006, 1 = September to December 2007.

regression and survival analyses, CI was set at 95%. For all statistical analyses level of significance was set at  $\alpha = 0.05$ .

### 3. Results

During the two replicates, a total of 201 cows without any vaginal discharge, were enrolled in the study. Follow-up of all cows was completed 360 days after the end of the second observation period in December of 2008. Cytological samples eligible for final analyses were obtained from 194 cows. In four cows the cytobrush could not be passed through the cervix and three samples were not readable.

Spearman's correlation analysis revealed a negative correlation between dpp and percent of PMN in the cytological sample ( $r = -0.17$ ;  $P = 0.02$ ). The overall prevalence of subclinical endometritis at exam 1 was 38% (74/194). Herd prevalence was 39% (63/163), 36% (9/25) and 33% (2/6) on farms 1, 2 and 3, respectively. The likelihood for subclinical endometritis did not differ between farms, with farm 1 as reference (farm 2: RR = 0.93, 95% CI = 0.53–1.63; farm 3: RR = 0.86, 95% CI = 0.27–2.72). The overall percentage of enrolled primiparous cows was 29%. Prevalence of subclinical endometritis in primiparous and multiparous cows was 35 and 39%, respectively. The prevalence of periparturient disorders was 21% and 24% in cows with and without subclinical endometritis, respectively. Relative risk analysis did not reveal any significant effect of parity class, periparturient disorders, body condition or replicate on the prevalence of subclinical endometritis (Table 1).

At exam 2 (32–52 dpp) 114 cows were reexamined. Overall prevalence of subclinical endometritis decreased from 38% at exam 1 to 19% at exam 2. Of 43 reexamined cows with subclinical endometritis at exam 1, 21% remained ≥5% PMN at exam 2, 60% were found with <5% PMN and 19% were diagnosed with clinical endometritis. Of 71 reexamined cows negative for subclinical endometritis at first visit 80% remained with <5% PMN at exam 2, 15% were found with >5% PMN, and 4% were diagnosed with clinical endometritis.

Descriptive statistics of reproductive performance outcomes are presented in Table 2. Conception at first AI was affected by periparturient disorders (OR = 2.41; 95% CI = 1.10–5.29;  $P = 0.03$ ), but not by subclinical endometritis

**Table 2**

Descriptive reproductive performance outcomes of 194 cows examined 18–38 dpp for subclinical endometritis by uterine cytology.

Trait	Subclinical endometritis	
	Yes	No
Number of cows	74	120
Cows inseminated, % (CI <sup>a</sup> 95%)	85.1 (76.4–92.6)	86.7 (80.2–92.3)
Median days to first AI (IR <sup>b</sup> )	56.0 (50.0–62.0)	56.0 (53.0–60.0)
Conception at first AI, % (CI 95%)	28.6 (16.6–38.9)	28.8 (19.7–37.1)
Median days to pregnancy (IR)	160.0 (58.5–237.8)	113.0 (61.5–204.0)
Services per pregnancy	3.7	3.4
Cows pregnant, % (CI 95%)	73.0 (62.2–82.4)	75.0 (66.8–82.3)
Cows culled, % (CI 95%)	27.0 (16.2–36.5)	25.0 (16.8–32.3)

<sup>a</sup> CI: confidence interval.

<sup>b</sup> IR: interquartile range.

**Table 3**

Results of binary logistic regression analysis for the risk of conception at first AI, in cows examined for subclinical endometritis at 18–38 dpp by uterine cytology, including subclinical endometritis, periparturient disorders, parity, body condition and study period as covariates.

Factor	Conceiving after first AI		
	OR <sup>a</sup>	CI <sup>b</sup> 95%	P
Subclinical endometritis <sup>c</sup>	0.91	0.45–1.87	0.80
Periparturient disorders <sup>d</sup>	2.41	1.10–5.29	0.03
Parity class <sup>e</sup>	1.19	0.56–2.52	0.65
BCS group <sup>f</sup>	0.62	0.28–1.36	0.23
Replicate <sup>g</sup>	1.31	0.60–2.84	0.50
Constant	0.35		0.02

<sup>a</sup> OR = Odds ratio.

<sup>b</sup> CI = Confidence interval.

<sup>c</sup> Subclinical endometritis: 0 = no subclinical endometritis; 1 = subclinical endometritis.

<sup>d</sup> Periparturient disorders: 0 = no periparturient disorder; 1 = periparturient disorder.

<sup>e</sup> Parity class: 0 = primiparous; 1 = multiparous.

<sup>f</sup> BCS group: 0 = BCS 0.25–2.50; 1 = BCS 2.75–5.00.

<sup>g</sup> Replicate: 0 = September to December 2006; 1 = September to December 2007.

(OR = 0.91; 95% CI = 0.45–1.87; P = 0.80) at exam 1 (Table 3). The hazards for insemination and pregnancy are reported in Table 4. Parity class affected the likelihood for insemination (HR = 0.66; 95% CI = 0.47–0.92; P = 0.01) and pregnancy (HR = 0.63; 95% CI = 0.45–0.90; P = 0.01), with a lower hazard for primiparous than for multiparous cows. Subclinical endometritis did not affect the likelihood of insemination or pregnancy (Fig. 1).

**Table 4**

Results of Cox regression for the hazard of insemination and pregnancy in cows examined for subclinical endometritis 18–38 dpp by endometrial cytology.

Factor	Insemination			Pregnancy		
	HR <sup>a</sup>	CI <sup>b</sup> 95%	P	HR	CI 95%	P
Subclinical endometritis <sup>c</sup>	0.94	0.69–1.29	0.70	0.92	0.66–1.30	0.65
Periparturient disorders <sup>d</sup>	0.98	0.68–1.42	0.92	1.07	0.72–1.59	0.74
Parity class <sup>e</sup>	0.66	0.47–0.92	0.01	0.63	0.45–0.90	0.01
BCS group <sup>f</sup>	1.30	0.92–1.82	0.13	1.27	0.87–1.86	0.21
Replicate <sup>g</sup>	1.27	0.92–1.76	0.15	0.66	0.47–0.95	0.02

<sup>a</sup> HR: hazard ratio.

<sup>b</sup> CI: confidence interval.

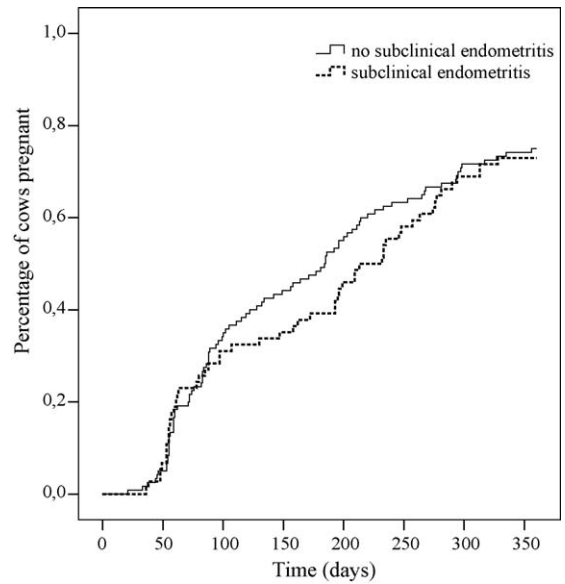
<sup>c</sup> Subclinical endometritis: 0 = no subclinical endometritis; 1 = subclinical endometritis.

<sup>d</sup> Periparturient disorders: 0 = no periparturient disorder; 1 = periparturient disorder.

<sup>e</sup> Parity class: 0 = primiparous; 1 = multiparous.

<sup>f</sup> BCS group: 0 = BCS 0.25–2.50; 1 = BCS 2.75–5.00.

<sup>g</sup> Replicate: 0 = September to December 2006; 1 = September to December 2007.



**Fig. 1.** Kaplan–Meier survival curves for proportion of cows pregnant by subclinical endometritis status. The proportion of cows censored was 27% and 25% for cows with and without subclinical endometritis, respectively.

## 4. Discussion

### 4.1. Prevalence of subclinical endometritis

The present study is one of the first studies on the prevalence of subclinical endometritis and its impact on

reproductive performance in clinically healthy postpartum dairy cows in a pasture-based extensive housing system.

The overall prevalence of subclinical endometritis in this study was 38% in a pasture-based dairy farming system. That result is in accordance with previously published data from confinement housing systems. Kasimanickam et al. (2004) diagnosed subclinical endometritis by endometrial cytology in clinically healthy dairy cows and described a prevalence of 35% and 34% for 20–33 and 34–47 dpp, respectively. Gilbert et al. (2005) reported a prevalence of 53% in cows examined 40–60 dpp. The timeframe chosen by Gilbert et al. (2005) is overlapping with the second visit of our study, for which the prevalence was 19%. The authors observed a large range of prevalence varying from 37% to 74% between five herds. In our study we did not find significant differences between the three herds. Gilbert et al. (2005) performed endometrial cytology by uterine lavage in cows without previous vaginal examination for uterine discharge. Therefore it is likely that some cows with clinical endometritis were enrolled in that study. Madoz et al. (2008) reported a prevalence of subclinical endometritis in Argentina of only 10.1%. In that study, however, cows were examined later (i.e. 21–62 dpp) with the majority of cows examined after 38 dpp and the PMN threshold used to identify cows with subclinical endometritis by endometrial cytology was 18% (21–33 dpp), 10% (34–47 dpp) and 5% ( $\geq 48$  dpp) as proposed by Kasimanickam et al. (2004). Using those thresholds in our data set prevalence of subclinical endometritis would have been 18% and 9% at first (18–38 dpp) and second (32–52 dpp) examination, respectively.

Prevalence at the second visit was less than at first visit. Several studies on dairy cows (Gilbert et al., 2005; Kasimanickam et al., 2004, 2005a), beef cows (Santos et al., 2009), and Zebu–Friesian crossbred cows (Bacha and Regassa, 2009) are in agreement with our findings that the prevalence of subclinical endometritis decreased with increasing time postpartum. Interestingly, 10% of the cows were found with signs of clinical endometritis at reexamination. Similar findings were reported by Kasimanickam et al. (2004), who diagnosed 9% of cows with uterine discharge within 24 h after a cytobrush examination. It remains unclear if these cows were affected by clinical endometritis, but vaginal discharge was not identified at first examination (Pleticha et al., 2009), or if examination by cytobrush itself caused clinical endometritis. Kaufmann et al. (2009) showed that the collection of the samples with the cytobrush did not have an effect on conception after first AI.

Relative risk analysis did not reveal an association between parity, body condition or periparturient disorders on the occurrence of subclinical endometritis. In contrast, Kasimanickam et al. (2004) reported that cows with periparturient events (i.e. twins, assisted calving, retained placenta) were three times more likely to be diagnosed with subclinical endometritis. Recently, Santos et al. (2009) described that beef cows with twin calves have a higher risk for subclinical endometritis. Additional risk factors for subclinical endometritis are decreased dry matter intake prepartum, elevated prepartum plasma NEFA concentrations, and

impaired PMN function prior to calving (Hammon et al., 2006).

Threshold values from 4% to 18% PMN have been suggested to define subclinical endometritis by uterine cytology (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2005a; Raab, 2003; Santos et al., 2009). Recent studies on endometrial mRNA expression profiles reported a dys-regulated cytokine and prostaglandin profile in cows with  $\geq 5\%$  PMN in the endometrial sample (Fischer et al., 2010; Gabler et al., 2009) supporting the assumption that 5% PMN is a suitable threshold for the diagnosis of subclinical endometritis. These results demonstrate that biomolecular techniques may be appropriate to help to define subclinical endometritis in the future.

#### 4.2. Impact on reproductive performance

In accordance with recently published findings in beef cattle (Santos et al., 2009), an impact of subclinical endometritis on reproductive performance was not observed in the present study for grazing dairy cows in an extensive housing system. Days to first service, conception at first AI, days open, proportion of cows pregnant and services per pregnancy did not differ between cows with and without subclinical endometritis. In contrast, Madoz et al. (2008) reported an increase of 52 days open and a reduced proportion of pregnant cows at 120 dpp. In that study the time frame for cytological examination varied widely (21–62 dpp), with the majority of cows being examined after 38 dpp and three PMN thresholds based on dpp (18%, 10%, 5%) were used as described by Kasimanickam et al. (2004). The same authors observed a decreased proportion of cows pregnant, increased days open (29 and 62 days), and a reduced conception at first AI for cows with subclinical endometritis kept under intensive farming conditions (Kasimanickam et al., 2004). Applying the threshold of Kasimanickam et al. (2004) to our data set, no difference was observed for time to pregnancy by Kaplan–Meier survival analysis between cows with or without subclinical endometritis (log rank test:  $\chi^2 = 0.59$ ,  $P = 0.44$ ). Similar to the present study, an effect on days to first insemination has not been reported by Kasimanickam et al. (2004).

Although the prevalence of subclinical endometritis was similar to reports from confinement housing systems a negative effect on reproductive performance outcomes as previously reported (Barlund et al., 2008; Galvao et al., 2009; Gilbert et al., 2005; Kasimanickam et al., 2004; Madoz et al., 2008) was not observed. It can be speculated that diminished bacterial load in the uterus or a more effective immune system for cows in extensive, pasture-based systems did improve self-cure rate compared to cows in confinement housing systems. Furthermore, the effects of other factors such as feeding, the design of calving areas, management practices such as synchronization of estrus should be evaluated by specifically designed studies.

#### 5. Conclusion

The prevalence of subclinical endometritis in a pasture-based, extensive dairy production system in Argentina was similar to previously published data for confinement

production systems. In this study, however, subclinical endometritis did not have a negative impact on reproductive performance outcomes.

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