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Occurrence of Subclinical Endometritis in Dairy Cattle and Effect on Reproductive Efficiency

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ABSTRACT

***Background*:** Subclinical endometritis (SE) have a negative impact on fertility due to an absence of clinical signs which difficult its diagnosis and treatment. The prevalence and impact of the disease on the reproductive status of dairy herds is not known in the region, the objective of the present study was to determine the prevalence of the disease and its effect on the reproductive efficiency in cows of the dairy area from Putumayo State, Colombia.

***Materials, Methods & Results*:** Cross-sectional study was made of 166 dairy cows from 30 days postpartum in the municipalities: Santiago, Sibundoy, Colon, and San Francisco. Reproductive evaluation and endometrial cytology were made to establish the SE prevalence determining subsequently the number of open days. Cow whit more than 120 days in milk (DIM) and without confirmed pregnancy was defined as "not reproductive efficiency". Association between SE and reproductive efficiency was established through Odds Ratio from contingency tables. Bias and confusion control was made through stratified analysis. Results showed 32 cows without changes in reproductive clinical evaluation and inflammatory changes in cytological evaluation (PMNn >5%) for a SE prevalence of 19.27%. Disease occurs most frequently in Colón (23.10%) but the frequency of the disease was not different among the regions ($P > 0.05$). The group of animals with the greatest days open (DO) mean (161 DIM) had a normal ovarian function and subclinical endometritis. The 6.6% of cows had a poor reproductive prognosis (subclinical endometritis, anestrous and 144 DIM). For the stratified analysis (controlling by anestrus) was estimated the crude OR (OR 5.93; $P < 0.05$; CI 95% 2.56-14.6) and adjusted OR_{MH} (OR 5.78; $P < 0.05$; CI 95% 2.39-13.9). Difference between adjusted OR_{MH} and crude OR (3%) and Wald X^2 test ($P > 0.05$) suggesting that there is no confusion and allowed to establish that "the odds of low reproductive efficiency (Days open >120) increases 5.9 times when cows suffer subclinical endometritis, than when do not have the disease".

***Discussion*:** Study prevalence was lower than the reported by other authors. Variability of the results by the different authors ratifies the multifactorial characteristic of the disease and thr differences in the immune response of the animals. Cows diagnosed with SE, normal ovarian function and 161 DIM shown that the disease increases significantly the open days of the herd reducing reproductive efficiency. These findings are consistent with other authors who report that subclinical endometritis: increased open days and reduced conception rates and increased the risk of no pregnancy at 150 days postpartum. In the study, cows with subclinical endometritis and normal ovarian function had open days mean lower to 120 and more probability to have a good reproductive performance if pregnancy is achieved, but the dairy herds of the region lack of adequate systems of detection of estro and services and have no systems that allow the diagnosis of SE and anoestrus. The 69% of the evaluated animals present some clinical characteristics (SE, anoestrous) that predispose them to low reproductive behaviour. It is recommended to routinely use endometrial cytology to diagnose SE and improving the reproductive efficiency of animals.

Keywords: cattle, infertility**,** uterine disease, anoestrous.

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INTRODUCTION

After calving, cow suffers a period of high energy requirements associated with insufficient food intake, a determinant factor in reproductive efficiency [8,20]. During this period, cow undergoes metabolic and hormonal changes, which produce immunosuppression with the predisposition to endometrium inflammation, bacterial infection and delayed in uterine involution [8,13]. This limits the development of the embryo in uterine lumen, causing infertility, prolonged calving - calving intervals, lactations with long periods and economic losses [12].

Within these postpartum uterine infections, subclinical endometritis SE reduces reproductive performance in greater proportion because the animals do not present obvious manifestations of disease, making difficult diagnosis and proper treatment which causes chronic infections [1,13].

Changes in uterine mucosa related with subfertility cannot be diagnosed through trans-rectal palpation and ultrasound, because the endometrium cannot be explored directly during a clinical evaluation [15,16]. In addition, there is no unified criterion about the definition of the disease, its diagnosis, treatment and prognosis [2,17].

Endometrial cytology allows the determination of the proportion of neutrophils in a sample collected from the uterine lumen [6]. Diagnosis of SE can be determined by a percentage of neutrophils ≥5% in a sample from 23 days postpartum [9,10].

The prevalence and impact of the disease on the reproductive status of dairy herds is not known in the region, the objective of the present study was to determine the prevalence of the disease and its effect on the reproductive efficiency in cows of the dairy area from Putumayo, Colombia.

MATERIALS AND METHODS

Animals and experimental design

A cross sectional study in dairy cattle from the 30 days postpartum was carried out in the "Alto Putumayo" which is a dairy area of Putumayo state, Colombia with four regions: Santiago, Columbus, Sibundoy, and San Francisco which presents, on average, an altitude of 2.150 m.s.n.m. [5].

The sample size was estimated according to the following criteria: the available population of adult females in the region (N = 9.373) [3], a proportion of subclinical endometritis of 24.7% [18], 7% accuracy and 95% confidence level.

Using the OpenEpi tool (Version 3.01)[1] and estimation of proportions formula [19], the sample size was estimated at 144 animals. The sample increased by 15% (app+10%) for possible data losses, animals, and information during the study. The number of cows to be evaluated was 158. Through no-probabilistic sampling, six herds by zone (with similar management conditions and representative of the area) were randomly selected. In each herd, all lactating cows were evaluated and animals selected based on the inclusion criteria.

As inclusion criteria were selected cows from specialized dairy breeds from 30 days postpartum, non-pregnant, clinically healthy to reproductive evaluation and dairy herds with similar reproductive, productive and nutritional management; easy access to the farms; correct identification of the animals and some system for recording information and the collaboration of the producers to participate in the study and provide all the information required.

Reproductive clinical evaluation and sampling for endometrial cytology

For the reproductive clinical evaluation [18] was performed: 1) external visual inspection of the cow to observe the presence of vaginal discharge; 2) rectal palpation with ultrasound (SonoScape-A5V®)[1] of the reproductive tract to determine the presence of uterine contents, pregnancy, and ovarian structures; and 3) evaluation of vaginal mucus (EVM) classified as EVM-0= Normal translucent clear, EVM-1= clear with pus flocks, EVM-2= mucopurulent without foul odor and EVM-3= purulent or brown with fetid odor [4,21].

Through ovarian ultrasound the reproductive physiological status was determined and classified as cows with normal estrus cycle or anestrus [14]. Later, the number of open days per cow (calving-conception interval) was recorded.

Endometrial samples for the cytological examination were collected by cervical catheterization, using a cytology brush (Cytobrush®)[3] technique [6,7,18]. The sample was spread on a slide (Microscope Slides®)[4] and fixed with an aerosol copolymer (SprayFix®)[5] for transport to the laboratory and subsequent staining by Wright-Giemsa (Harleco®)[6].

Case definition for subclinical endometritis and reproductive efficiency

Subclinical endometritis: cows from 30 days postpartum, no pregnant, with a normal reproductive clinical evaluation (without external vaginal discharge, EMV=0, no clinical signs to trans-rectal palpation and ultrasound) and cytological evaluation with a percentage of neutrophils ≥ 5% [2,10,11].

Reproductive efficiency: cows no pregnant, with less to 120 days in milk (DIM < 120) in the moment of the cytological sampling [13,18,20].

Statistical analysis

Subclinical endometritis was defined as the predictor variable for low reproductive efficiency (dependent variable). Reproductive physiological status was defined as the confusion variable.

Prevalence (p) of the disease was established according to the formula [19]: p= [(Cows with subclinical endometritis / total study population) x 100]. As a measure of association between subclinical endometritis occurrence and reproductive efficiency, we used the Odds Ratio (OR) and, as an effect measure, the fraction of attributable risk (AFe = (OR-1) / OR). Effects were considered significant at $P \leq 0.05$ [19].

For the control of confusion [19], through stratified analysis (determining confounding, homogeneity, interaction and adjusted OR) were performed by the variables: anestrous. The analysis was performed with Stata (Version 13.0)[7] statistical package.

RESULTS

The selected herds (24 herds) were dairy production systems, with representative characteristics of the "Alto Putumayo" dairy region like: manual milking, rotational grazing without additional supplementation, the supply of mineralized salt and natural breed to estro detected. Clinical evaluation was performed to all lactating cows on selected farms evaluating a total of 268 cows (Table 1). The 4.85% of animals presented signs related to uterine infection like uterine horn asymmetry, abnormal uterine content, vaginal discharge or MVE= 1-3 and were excluded from sampling. Pregnant cows and with less than 20 days postpartum were also excluded from the sampling.

The no pregnant cows that met the inclusion criteria (n = 166), were included in the sampling as the study population (Table 1). The evaluation of reproductive physiological status of these cows found

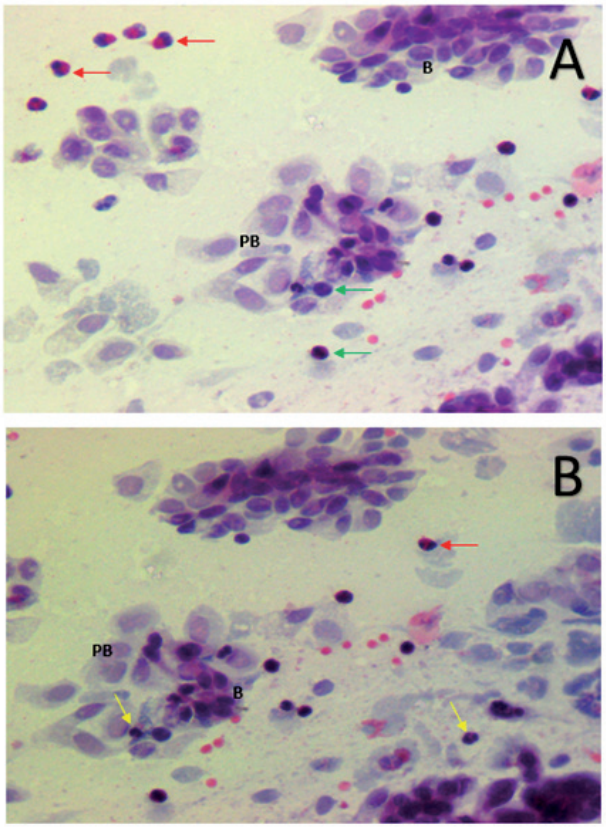

Figure 1. Endometrial cytology of cow with subclinical endometritis. Wright-Giemsa staining [Scale Bar= 40X]. Dairy cow, 119 days in milk (DIM), normal estrous cycle. Basal cells (B), Parabasal cells (PB) and inflammatory cells indicatives of an active process originate in endometrium: eosinophils (red arrow), neutrophils (green arrow) & hipper-segmented neutrophils with changes associated to cellular dead (yellow arrow).

69 cyclic cows with normal ovarian activity (presence of corpus luteum and follicles at different stages of development) and 97 non-cyclic cows with some type of non-ovulatory structure (Table 1).

Subclinical endometritis prevalence

Results showed 32 cows without changes in reproductive clinical evaluation and inflammatory changes in the cytological evaluation (Figures 1A and 1B) with a major proportion of neutrophil cell counts (PMNn >5%) in comparison to cytological samples of healthy cows (Figures 2A and 2B). The prevalence of subclinical endometritis was of 19.27% in dairy cattle from "Alto Putumayo", Colombia.

Among the regions of Putumayo dairy area (Figure 3), the disease occurs most frequently in Colón (23.10%) and less frequently in Santiago (8.70%). The Pearson Proportion Comparison Test showed with 95% confidence ($P > 0.05$) that the disease frequency of the disease is not different among the regions.

Subclinical endometritis and reproductive efficiency

To establish the impact of subclinical endometritis on reproductive efficiency, the study population was grouped based on the disease (with or without presence of endometritis) and the number of days open (DO). Animals with DO ≥120 were classified as cows with low reproductive efficiency.

The group of animals with the greatest days open mean (161 DO) had a normal ovarian function and subclinical endometritis. In this case, the uterine subclinical inflammation has a negative impact on reproductive efficiency. The 6.6% of cows had a poor reproductive prognosis: subclinical endometritis, anestrous and 144 DO. These animals are negative for the profitability of the herd and potential involuntary culling (Table 2).

In cows without subclinical endometritis, anestrous had negative impact on reproductive efficiency (123 DO) in the 51.8% of the population. The 28.9% of the study population had good reproductive performance: normal ovarian function, without the disease and 112 DO (Table 2).

Effect of subclinical endometritis in reproductive efficiency

To stablish the effect of disease in the reproductive status of the population at risk, stratified analysis for confounder control was performed (Table 3) by controlling for ovarian function (normal estrus cycle or anestrous) through Mantel & Hansel analysis:

Odds ratio - OR were estimated for anestrus cows (OR 11.6; $P < 0.05$; CI 95% 2.3-5.7) and without anestrus (OR 4.0; $P < 0.05$; CI 95% 1.34-11.87). Subsequently, was estimated the crude OR (OR 5.93; $P < 0.05$; CI 95% 2.56-14.6) and adjusted ORMH (OR 5.78; $P < 0.05$; CI 95% 2.39-13.9).

Difference between adjusted ORMH and crude OR was 3%, suggesting that there is no confusion. Homogeneity test (Wald X^2) was estimated at 1.18 ($P > 0.05$). We accept the null hypothesis of equality between the OR of both strata, therefore there is no interaction and its possible establish with a 95% confidence that, the odds of low reproductive efficiency (Days open >120) increases 5.9 times when cows suffer subclinical endometritis, than when do not have the disease. Condition its worse in anestrus cows (OR= 11.6).

The estimation of attributable risk fraction (AFe) in the study population shown that an 83% of the risk of low reproductive performance can be attributed to the presence of the disease.

Table 1. Reproductive clinical evaluation performed to all lactating cows on selected farms.

Clinical Evaluation Results	Frequency	Percentage %
Clinical Uterine Infection	13	4.85
Puerperium	8	2.99
Pregnancy	81	30.22
No pregnancy - Anoestrus	97	36.20
No pregnancy - Normal Estral Cycle	69	25.74
Total	268	100

DISCUSSION

Subclinical endometritis was estimated at 19.27% with 140 open days mean. The disease prevalence is lower than the reported by other authors: a study carried out by the authors in Nariño state, Colombia, reports an incidence of subclinical endometritis of 24.7% [18]; in dairy herds of rodeos in northern Argentina, the prevalence of subclinical endometritis are reported in 38% and 31% [4,13]; another study in Canada reported a prevalence of 41.4% [6]. A study carried out in dairy farms in Croatia reports a prevalence of 7.7% [22], lower compared with the findings of the present study.

The variability of the results by the different authors ratifies the multifactorial characteristic of the diseases in cattle, the different causes associated with postpartum uterine infection as well as differences in the immune response of the animals.

The 12.7% of the animals diagnosed with subclinical endometritis had a normal ovarian function and 161 open days which indicates that the disease increases significantly the open days of the herd reducing reproductive efficiency. These findings are consistent with other authors who report that subclinical endometritis: increased open days and reduced likelihood of conception compared to healthy cows [13,18], increased 1.9 times the risk of no pregnancy cows at 150 days postpartum [11] and, have a 17.9% lower conception rate at the first service and an average 24 days increase in open days [1].

In the study, cows with subclinical endometritis and normal ovarian function had an open day's mean <120 and more likely to have an efficient

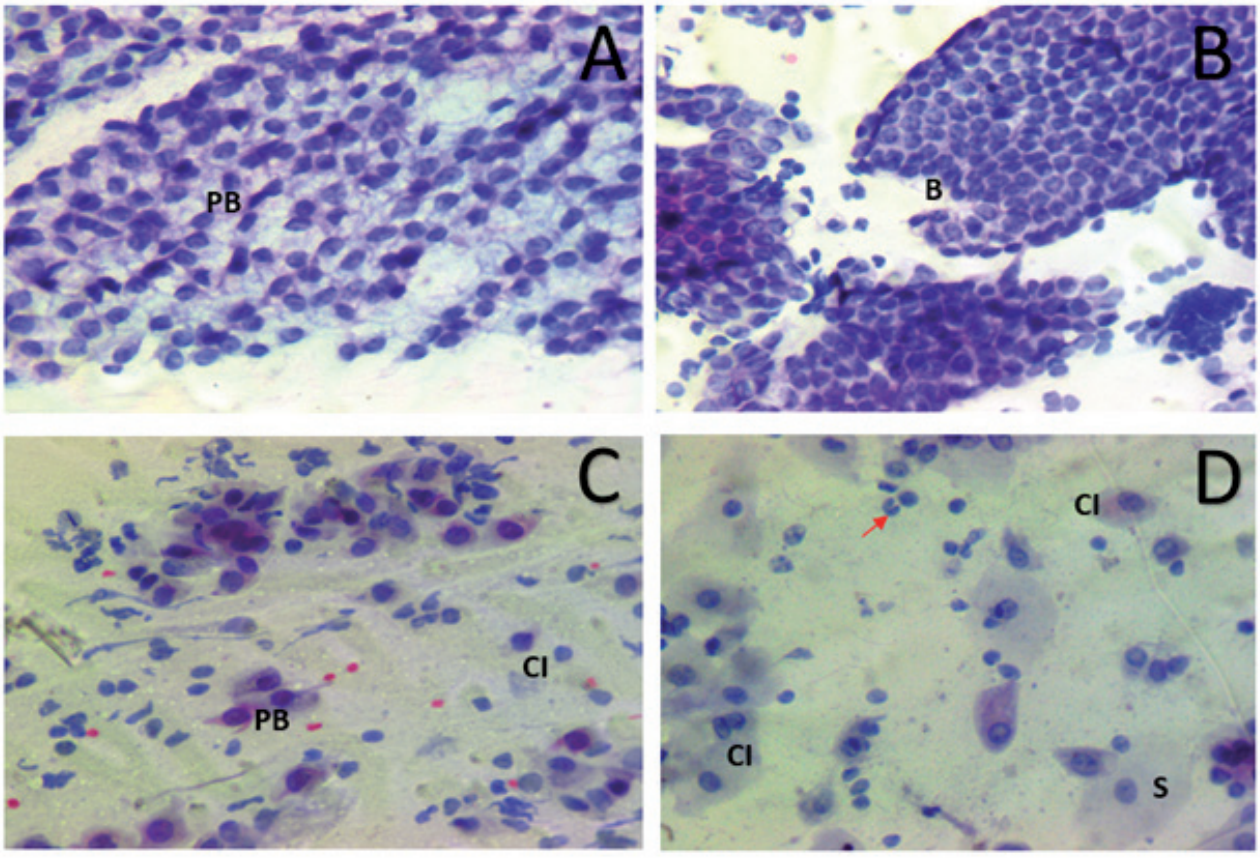

Figure 2. Endometrial cytology of cow with healthy uterus. Wright-Giemsa staining [Scale Bar= 40X]. A- Cow, 142 days in milk (DIM), anestrous; B- Cow, 131 DIM, anestrous; C- Cow, 118 DIM, normal estrous cycle and D- Cow, 121 DIM, normal estrous cycle. Basal cells (B), Parabasal cells (PB), Intermediate cells (CI) and Superficial cells (S) without the presence of inflammatory infiltrate or cellular detritus and scarce neutrophil inflammatory infiltrate (red arrow).

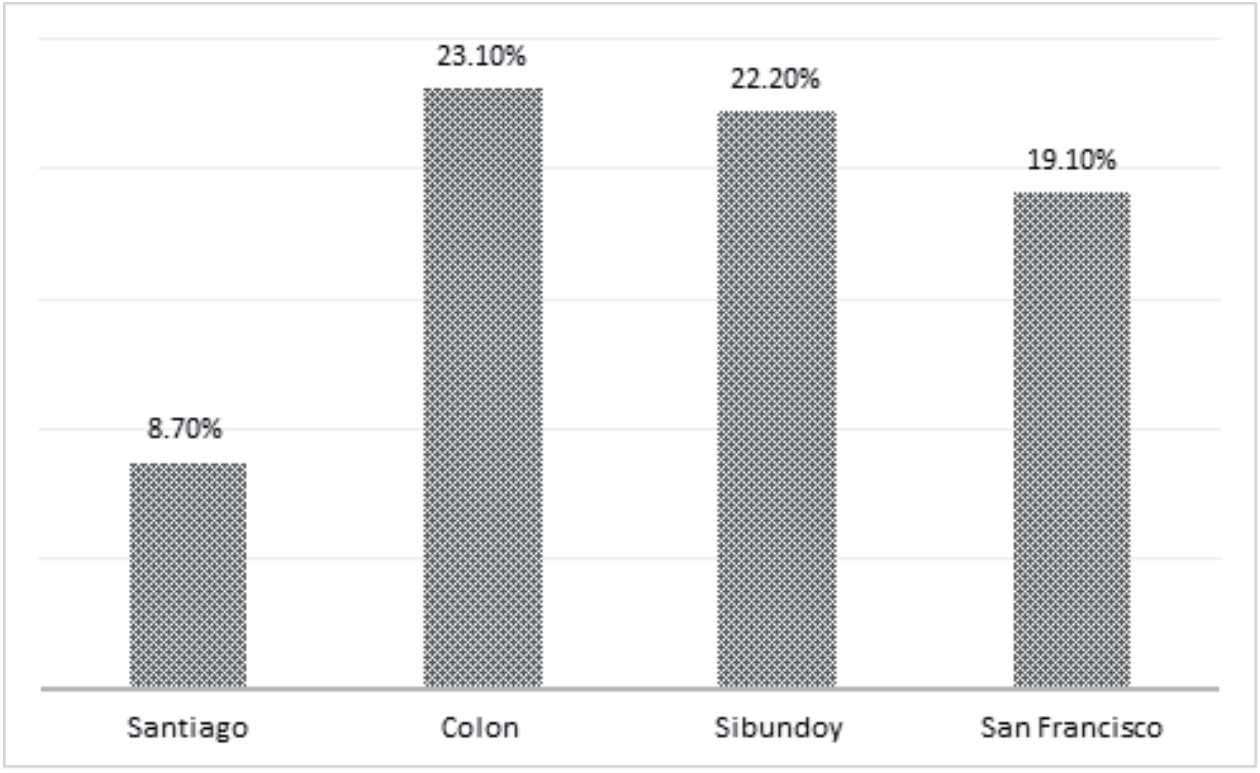

Figure 3. Subclinical endometritis prevalence per region in dairy area from Putumayo, Colombia.

Table 2. Frequency table. Ovary status, subclinical endometritis and days in milk (DIM).

Variable	Frequency	Percentage %	Days in Milk1
No pregnant, anoestrous with SE	11	0.6	144 ± 23
No pregnant, anoestrous without SE	86	51.8	123 ± 18
No pregnant, normal estral cycle, with SE	21	12.7	161 ± 4
No pregnant, normal estral cycle, without SE	48	28.9	112 ± 7
Total	166	100	135 ± 13

[1]For practical purposes, in presentation and discussion of results, Days in Milk (DIM) are equivalent to the number of open days (OD).

Table 3. Subclinical endometritis (SE) x Days in milk (DIM). Stratified analysis by anoestrous.

Stratum (Anoestrus)	Subclinical Endometritis	>120 DIM[1]	≤120 DIM	Total	Odds Ratio (OR)	X^2 Test	Confidence Interval IC 95%
Yes	Si	9	2	11			
	No	24	62	86	11.6	$P > 0.05$[a]	2.3 - 5.7[c]
Total		33	64	97			
No	Si	14	7	21			
	No	16	32	48	4.0	$P > 0.05$[b]	1.34 - 11.87[c]
Total		30	39	69			

[1]For practical purposes, in presentation and discussion of results, Days in Milk (DIM) are equivalent to the number of open days (OD). [a]Anoestrus have effect on reproductive efficiency (Increase in open days). [b]Subclinical Endometritis have effect on reproductive efficiency (Increase in open days) controlling by anoestrus. [c]Significative effect.

reproductive performance if pregnancy is achieved. The great limitation is that dairy herds of the region lack of adequate systems of detection of estro and services, do not have diagnostic systems that allow the diagnosis of SE and postpartum anoestrus continues to be a principal problem for the reproductive efficiency independent of the occurrence of ES which is reflected in that only 31% of lactating cows after 30 days postpartum are pregnant.

The 69% of the evaluated animals present some clinical characteristics (Subclinical endometritis, anoestrous) that predispose them to low reproductive behaviour. Therefore, the diagnostic system proposed in the present study based on the evaluation of uterus status (including subclinical conditions) and ovarian function allows the detection of postpartum alterations related to fertility and to estimate their impact. It is recommended to routinely use endometrial cytology for SE diagnose in postpartum dairy cattle for the purpose of improving the reproductive efficiency of animals.

CONCLUSIONS

The study allows establishing the prevalence of subclinical endometritis in "Alto Putumayo", Colombia, which is lower that the reported in other studies but has a negative impact on the fertility and reproductive efficiency of dairy cows in the region.

MANUFACTURERS

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[7]StataCorp LLC. College Station, TX, USA.

Ethical approval. The use of animals in this experiment was approved by the decision of the Curricular and Research Committee of the Veterinary Medicine Program of the University of Nariño, Pasto-Colombia (Decision No. 017 of 8 March 2015).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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