

Spontaneous recovery in dairy cows with subclinical endometritis

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Abstract. The aim of this study was to evaluate the relationship between the timing of diagnosis and the prevalence of spontaneous recovery of subclinical endometritis (SE) in Holstein dairy cows. Postpartum gynecological control was performed on 512 multiparous Holstein cows, housed in three farms located in the south of the province of Santa Fe (Argentina), from April to October 2022. Experimental cows were those that were diagnosed as healthy, but after endometrial cytology, were positive to SE ($n = 56$). Two gynecological controls were performed to each cow positive to SE. Considering the moment in which the first control was made, cows with SE were separated into two groups: group M1: between 15 and 30 postpartum days (ppd) and group M2: between 31 and 60 ppd. Each group was reviewed after 15 days from the first control. After the second revision, three experimental groups were formed: SR group: cows that recovered spontaneously, SE group: cows that maintained the disease, and CE group: cows that developed clinical endometritis. A 49 % of cows were diagnosed as healthy cows. After performing endometrial cytology, 78 % of cows were healthy, and the remaining 22% of cows were positive for SE. There was a higher percentage of cows checked during M1 than during M2, and M1 had more than 60 % of spontaneous recovery to SE, although these data did not differ between the studied moments (M1 and M2, $P > 0.05$). Cows from the SR group presented an abrupt decrease in the % PMN-N found between the first and second endometrial cytology performed (12.1 ± 1.7 and 1.0 ± 0.2 % PMN, respectively; $P > 0.0001$). In conclusion, the results did not demonstrate a relationship between the timing of diagnosis and the prevalence of spontaneous recovery of subclinical endometritis in Holstein dairy cows.

Key words: uterine health, diagnosis, subclinical endometritis, spontaneous recovery

La recuperación espontánea en vacas lecheras con endometritis subclínica

Resumen. El objetivo del presente trabajo fue evaluar la relación entre el momento del diagnóstico y la prevalencia de la recuperación espontánea a endometritis subclínica (SE) en vacas lecheras. Se realizó el control ginecológico posparto a 512 vacas Holstein, multíparas, alojadas en tres establecimientos ubicados en el sur de la provincia de Santa Fe (Argentina), desde abril a octubre del 2022. Se utilizaron vacas que fueron diagnosticadas como sanas y que, tras la citología endometrial fueron positivas a SE ($n = 56$). Se realizaron dos revisiones ginecológicas a todas las vacas con SE. La totalidad de las vacas positivas a SE fueron separadas en dos grupos, considerando el momento de la realización de su primera revisión: grupo M1: entre 15 y 30 días postparto (dpp) y grupo M2: entre 31 y 60 dpp. Cada grupo, nuevamente se revisó a los 15 días después de la primera revisión. Tras la segunda revisión, se conformaron tres grupos experimentales: Grupo SR: vacas que se recuperaron de manera espontánea, Grupo SE: vacas que mantuvieron la enfermedad y Grupo CE: vacas que evolucionaron hacia una endometritis clínica. Del total de vacas revisadas, el 49 % fue diagnosticado como vaca sana. El 78 % resultaron sanas después de la citología endometrial y el restante 22 % de vacas fueron positivas a SE. Hubo un mayor porcentaje de vacas revisadas durante M1, se observó que las vacas con SE tuvieron más del 60 % de recuperación espontánea, aunque no difirieron entre ambos momentos estudiados (M1 y M2, $P > 0,05$). Las vacas del grupo SR presentaron disminución

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del % polimorfonucleares neutrófilos (PMN-N) encontrados entre la primera y la segunda citología endometrial realizadas ($12,1 \pm 1,7$ y $1,0 \pm 0,2$ % PMN, respectivamente; $P > 0,0001$). Se concluye que no hubo relación entre el momento del diagnóstico y la prevalencia de la remisión espontánea a la endometritis subclínica en vacas lecheras Holstein.

Palabras claves: salud uterina, diagnóstico, endometritis subclínica, recuperación espontánea.

Recuperação espontânea em vacas leiteiras com endometrite subclínica

Resumo. O objetivo do presente trabalho foi avaliar a relação entre o momento do diagnóstico e a prevalência da recuperação espontânea da endometrite subclínica (SE) em vacas leiteiras. Realizou-se o exame ginecológico pós-parto em 512 vacas Holstein, multíparas, alojadas em três estabelecimentos localizados na bacia sul da província de Santa Fe (Argentina), durante os meses de abril a outubro de 2022. Utilizaram-se as vacas que foram diagnosticadas como saudáveis e que, após a citologia endometrial foram positivas para SE ($n = 56$). Realizaram-se duas avaliações ginecológicas em todas as vacas com SE. Todas as vacas com SE foram separadas em dois grupos, considerando o momento da realização de sua primeira avaliação: grupo M1: entre 15 e 30 dias pós-parto (dpp) e grupo M2: entre 31 e 60 dpp. Cada grupo foi novamente avaliado aos 15 dias após a primeira avaliação. Depois da segunda avaliação, conformaram-se três grupos experimentais: Grupo SR: vacas que recuperaram de maneira espontânea, Grupo SE: vacas que mantiveram a doença e Grupo CE: vacas que evoluíram para uma endometrite clínica. Do total de vacas avaliadas, 49 % foi diagnosticado como vaca saudável. 78 % resultaram saudáveis depois de realizar a citologia endometrial e o restante 22 % das vacas foram positivas para SE. Houve maior porcentagem de vacas revisadas durante M1, observou-se que as vacas com SE tiveram mais de 60 % de recuperação espontânea, embora estes dados não tenham diferido entre ambos os momentos estudados (M1 e M2, $P > 0,05$). As vacas do grupo SR apresentaram uma diminuição de % neutrófilos polimorfonucleares (PMN-N) encontrados entre a primeira e a segunda citologia endometrial realizadas ($12,1 \pm 1,7$ y $1,0 \pm 0,2$ % PMN, respectivamente; $P > 0,0001$). Conclui-se que não houve relação entre o momento do diagnóstico e a remissão espontânea da endometrite subclínica em vacas leiteiras Holstein.

Palavras-chave: saúde uterina, diagnóstico, endometrite subclínica, recuperação espontânea.

Introduction

There are many factors that can alter the uterine environment due to the significant changes that dairy cows experience before, during and after calving. Lactating dairy cows, probably due to their own milk production and/or to the sum of metabolic changes which they go through during this period, seem to be more likely to develop uterine disease (Molina-Coto and Lucy, 2018).

Trying to solve uterine pathologies (endometritis), therapeutic protocols have included, in general, hormonal therapy and the use of intrauterine antibiotics (Sheldon *et al.*, 2019). Nowadays, infectious uterine pathologies are usually treated with antibiotics in order to prevent a decrease in milk production and fertility, also so as to reduce the suffering of the animal, by combining them with anti-inflammatory drugs (Stojkov *et al.*, 2015). However, on the other side, treatment with certain antibiotics results in the disposal of the milk, making the economic situation of the dairy farm even more complicated. In agreement with the ongoing trend in health which involves the responsible use of antimicrobials in dairy cows, animals, and humans in general, it becomes necessary to generate

new strategies of prevention and control. Furthermore, it is important to achieve a balance in the productive system to minimize man's intervention and thus improve the welfare of productive dairy cows.

Until to our knowledge, there is scarce information in dairy cows about the spontaneous recovery of the uterus with subclinical endometritis. Ghanem *et al.* (2015) showed that some cows were diagnosed as negative for bacterial isolation despite presenting a high percentage of PMN-N. Elsayed *et al.* (2020) showed that buffaloes with positive subclinical endometritis during the fifth week postpartum were able to recover from the disease with no treatment by the seventh week.

In concordance with this subject, our team's previous works have shown that the prevalence of clinical endometritis after the first postpartum gynecological revision was high in cows in extensive systems (Frana *et al.*, 2021). On the other hand, the prevalence of subclinical endometritis in the assessed area was 25.7 %, which represents intermediate values compared to other studies in similar extensive systems (Frana *et al.*, 2021).

Moreover, Plöntzke *et al.* (2010) found a 60 % spontaneous remission of SE in cows with six weeks postpartum. The spontaneous recovery in cows with SE, measured in the decrease of the % PMN-N, reached a percentage of 74 % before the end of the voluntary waiting period (Frana *et al.*, 2021).

Material and Methods

This work has the approval of the Animal Welfare and Ethic Committee (CICUAL) and the Biosecurity of the Veterinarian Faculty of the National University of Rosario, Santa Fe, Argentina (Resol. CD 192/2022).

A postpartum gynecological control was carried out to 512 Holstein multiparous cows, housed in three dairy farms in the south of Santa Fe province (Argentina), from April to October 2022.

These farms have an average of 300 lactating cows per year, an annual milk production of 24 ± 2 liters per cow per day, a system of continuous calving, using artificial insemination as reproductive system. During the experimental period, cows were fed with 22 ± 1 kg of dry matter / day, offered grazed green forage, preserved fodder and commercial concentrate. The access to clean water was *ad-libitum*. The cows involved in this work were chosen considering the following criteria:

A) Type of partum (TP): only cows with eutocic (natural) delivery, without any hormonal peripartum treatment or postpartum antibiotic were included.

B) Days in milk (DM): only cows in a postpartum period between 15 and 60 days with milk were included.

C) Both registers (TP and DM) were obtained from the digital records available in each farm.

D) Cervicovaginal mucus (CVM): in order to consider a cow as healthy (HC), the mucus had to show characteristics of CVM 0: transparent mucus and without pus, according to classification of Rinaudo *et al.* (2012). Mucus samples were obtained through the

The aim of this study was to evaluate the relationship between the timing of diagnosis and the prevalence of spontaneous recovery of subclinical endometritis (SE) in Holstein dairy cows.

MetrichickTM (Simcro Datamars, New Zealand) technique. Endometrial cytology samples were taken from all those cows having CVM 0, applying the cytobrush technique (Rinaudo *et al.*, 2012).

For this work, only dairy cows which were diagnosed as HC were used, and which -after the endometrial cytology- were found with subclinical endometritis positive ($n = 56$), for having ≥ 5 % PMN-N (Rinaudo *et al.*, 2012). All those cows with subclinical endometritis (SE), were gynecologically examined twice, with a 15-day interval in between, in order to evaluate the evolution of the disease.

All the cows with SE were separated into two groups, considering the moment of their first examination: Group M1, cows examined between 15 and 30 postpartum days (ppd) and Group M2, those examined between 31 and 60 ppd. Each group was examined again 15 days after the first examination. With the results of the second examination, considering the clinical evolution, three experimental groups were formed: Group SR: cows which showed spontaneous recovery to SE (CVM = 0 and < 4.9 % PMN-N); Group SE: cows which maintained the disease (CVM = 0 and ≥ 5 % PMN-N) and Group EC: those cows whose disease developed into a clinical endometritis (CVM > 0). The voluntary waiting period (VWP) of the herd was 60 ppd.

Dependency between the status of uterine health and the two postpartum moments used for diagnosis (M1 and M2) was evaluated with a homogeneity test of chi-squared and with a t-student. Results are expressed in percentages (%) and as mean \pm sem. Statistical analysis were carried out with R Core Team software, R 4.1.0 version (2020). The level of statistical significance was $P < 0.05$.

Results

From the total of the cows at first examination, 49 % (251/512) were diagnosed as HC for showing CVM 0. From these cows, 78 % (195/251) showed healthy after carrying out the endometrial cytology and, the remaining 22 % (56/251) were positive to subclinical endometritis.

The mean of days for the first examination was 26.7 ± 1.0 ppd for those cows examined during M1 ($n = 43$), and it was 48.1 ± 3.5 ppd for those examined during M2 ($n = 13$). Table 1 shows the evolution observed in the cows diagnosed with SE according to the moment when the first

postpartum examination was carried out. There was a higher percentage of cows which were examined during M1 than during M2; besides, it was noticed that cows diagnosed with SE had over 60 % spontaneous recovery, although no difference were found between the two studied moments (M1 y M2, $P > 0.05$).

Cows of the group SR showed an abrupt decrease of the % PMN-N found between the first and the second endometrial cytology carried out (12.1 ± 1.7 y 1.0 ± 0.2 % PMN-N, respectively; $P < 0.0001$).

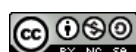


Table 1. Subclinical endometritis evolution observed in Holstein dairy cows diagnosed in two different postpartum moments (M1 and M2).

	Moment of postpartum examinations			
	M1		M2	
	Number of cows	Percentage	Number of cows	Percentage
SR	28	65.1	8	61.5
SE	6	13.9	2	15.4
CE	9	20.9	3	23.1
Total	43	100	13	100

Note: SR (there was spontaneous recovery of subclinical endometritis), SE (maintained the disease), CE (evolved into clinical endometritis). M1: first examined between 15 and 30 postpartum days (ppd) and M2: first examination between 31 and 60 ppd.

Discussion

This study showed that prevalence of subclinical endometritis was 22 %. In previous studies carried out by our group, cows examined between 21 and 56 postpartum days, showed 25.7 % of SE (Frana *et al.*, 2021) and 19 % of cases were reported by Rinaudo *et al.* (2012). On the other hand, Vallejo *et al.* (2018), who checked the cows with 30 postpartum days, reported 19.3 % of SE.

Should be highlighted that, in the present study, two different moments for the first postpartum examination were taken into account in the cows, which allowed us to report that the number of cows that presented SE when they were examined after 31 postpartum days was much lower (M2: 13/56), compared to those examined between 15 and 30 days (M1: 43/56).

The distribution of the evolution of SE was similar for both moments of the first postpartum examination (Table 1), showing that there is no relationship between the moment of the diagnosis and the prevalence of spontaneous recovery of cows with SE. Kasimanickam *et al.* (2005) agree with the fact that the prevalence of SE decreases as the time of the diagnosis is extended, until the end of the voluntary waiting period. Our results seem to indicate that there is another possibility of interpretation which contradicts these authors; since in the present work two different moments for the first postpartum examination were evaluated and it has been reported that 60 % of the cows with SE had spontaneous recovery. This could suggest that, based on the postpartum period in which these cows were examined, there might be instances of subclinical endometritis that would go undiagnosed. This might not necessarily indicate that the cows have been free from the disease; instead, it could reflect a higher rate of spontaneous recovery than reported. On the contrary, Rinaudo *et al.* (2012) showed a noticeable increase of the percentage of cows with subclinical endometritis as the postpartum days passed. However, it must be noticed that their work was carried out in a farm with an intensive system, different from the grazing systems included in this study. Additionally, our results may trigger the discussion

about which is the best moment to carry out the first postpartum examination, within the good practice recommended for professionals in dairy farms.

Several authors (Frana *et al.* 2021, Plöntzke *et al.* 2010) showed high percentages of spontaneous recovery in herd systems, which could imply that within the same dairy farm, there is a group of cows that due to their tolerance and resistance are able to recover from subclinical endometritis, spontaneously, probably becoming the most efficient cows in the herd. Failures in the tolerance and resistance of some cows would explain in better terms why antibiotics used in animals with uterine disease to eliminate pathogenic agents are hardly beneficial to fertility (Sheldon *et al.*, 2019). The high percentage of spontaneous recovery found in this study coincides with that reported by Sheldon *et al.* (2019), who concluded that there is no agreement among investigators for the treatment of subclinical endometritis since this is a multifactor disease and involves endometrial instability in tolerance and resistance of each animal.

The abrupt decrease of the % PMN-N found in the endometrial cytology of cows with spontaneous recovery, in the 15-day interval between the two postpartum examinations carried out in this work, could be explained through the capacity of the uterus to overcome infection during the first weeks postpartum. It is an expectable response of the mammals' endometrium during the uterine involution, which happens in the first weeks postpartum, where the local inflammatory response and the uterine immune system contribute to the regeneration of the physiological conditions of the endometrium (regeneration and repair) after the changes suffered due to pregnancy and partum (Ghanem *et al.*, 2015).

At the moment, the importance of improving the welfare of productive dairy cows plus the global idea of one healthy, incorporate the need to solve some situations with minimum man's intervention to a better balance to cows in the system they developed in.

Conclusion

The results obtained in this study demonstrated that there was no relationship between the moment of diagnosis and the prevalence of spontaneous recovery of subclinical endometritis in Holstein dairy cows. Therefore, new hypothesis was generated upon the spontaneous

remission and the moment in which some professional practices are carried out; with the intention of improving the animals' uterine health as well as the welfare of lactating dairy cows.

Ethic code: Authors state that this work has been carried out according to the Ethic Code for animals' experiments, following the norms: http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

Conflict of interests: The authors who sign this investigation state to have no potential conflict of personal or economic interest with other people or organizations which might inappropriately interfere with this work.

Authors' contributions: Preparation and execution: **FBE, MPR, VMI**. Development of Methodology **FBE, MPR, VMI**. Idea and design: **BI, MPR**. Edition: **FBE, MPR, VMI**. Supervision: **MPR, VMI**.

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