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Comparative study of treated and non-treated (auto-recovered) clinical endometritis: effects on dairy cows' fertility parameters

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Abstract

In this study, we evaluated the effect of treated and auto-recovered clinical endometritis on postpartum fertility. We collected data on breeding, diseases, and cyclicity. Regarding co-affections, cows auto-recovered from clinical endometritis were affected in groups 1 (n=11) and 2 (n=12). Cows with treated clinical endometritis represented groups 3 (n=08) and 4 (n=07), and cows without clinical endometritis represented Groups 5 (n=15) and 6 (n=80). Once the cyclicity recovered, cows were inseminated. Studied fertility parameters were; 1: conception rate; 2: days to first insemination; 3: success rate in the first insemination; 4: days to conception; and 5: days between first insemination and pregnancy. For days to first insemination intervals a significant difference was noted between groups 3 and 5 (p-value = 0,006), groups 3 and 1 (p-value = 0,019), and groups 3 and 6 (p-value = 0,005). However, the conception rate was significantly higher in the groups with non-treated clinical endometritis groups 1and 2 (p-value = 0.030). Concerning the other parameters, we did not find significant differences among groups. Our results showed that auto-recovered cows from clinical endometritis had better fertility outcomes.

Keywords: Dairy cows; Treated clinical endometritis; Auto-recovered clinical endometritis; Fertility parameters.

Introduction

The cow's uterine bacterial contamination is unavoidable after calving, leading to an inflammatory immune response of the uterus. Uterine diseases result from dysfunction caused by a deficiency or exasperation of the immune system. Uterine diseases can be classified as puerperal metritis, clinical metritis, clinical endometritis, and subclinical endometritis **(Sheldon, Lewis, et al., 2006)**.

Clinical endometritis (CE) prevalence has been reported by many scientists, varying significantly according to risk factors, diagnostic methods, or timing of diagnosis. The overall prevalence of endometritis during the early (15–20 days), intermediate (21–28 days), and late (29–60 days) postpartum periods was 67.8%, 40.5%, and 14.4%, respectively **(Gautam et al., 2009).** Otherwise, according to the examination time diagnosis 20 to 26 days in milk (DIM) and 27 to 33 DIM, this prevalence was 6.6 % and 23.5%, respectively **(LeBlanc et al., 2002a)**.

The prevalance was higher in cows receiving assisted calving without gloves (96.6%). In contrast to the 3.4% of farmers who used protective gloves, the CE prevalence of bull-served cows was 56.9%, while the CE prevalence of artificial insemination cows was 43.1% **(Nyabinwa, Kashongwe, Habimana, et al., 2020)**. this frequency differs from 10% to 24% depending on the used diagnostic method (vaginoscope- lavage cytology- cytobrush cytology- endometrial thickness- uterine fluid) **(Barlund et al., 2008)**.

This disease can negatively affect fertility **(Fourichon et al., 2000)** and reduce milk yield **(Coleman et al., 1985)**, **(Overton & John, 2008)**, **(Mahnani & Cabrera, 2015)**.

However, many studies have been conducted in intensively managed breeding facilities, where herds are routinely monitored and overseen by veterinarians. Notably, most of these studies did not specify whether or not affected cows by clinical endometritis received treatment. Furthermore, many treatment trials have been limited by the lack of negative controls. In some cases, cows treated for endometritis were excluded from reproductive performance analyses **(Gautam et al., 2009)**.

Le Blanc (2008) reported that only some experiments focused more on clinical and bacteriological recovery than economic aspects, such as days to conception and conception rate.

In experimental protocols, untreated sick animals are commonly used as control groups to assess treatment efficacy in comparison to treated groups, as demonstrated in the study of **LeBlanc et al. (2002)** but the comparison between the results on fertility and the economic impact remains missing in several studies.

The use of antibiotics as a treatment for clinical endometritis is a systematic practice but a few studies had showed the effect of antibiotics on hormonal activity in cows and comparative studies for fertility results and the economic impact of clinical endometritis remain missing.

Additionally, the simultaneous occurrence of clinical endometritis with other postpartum pathologies raises important questions about the potential impact of their treatments using antibiotics on fertility outcomes.

This raises the question: Is there a differential impact on fertility parameters when comparing the treatment of clinical endometritis to its spontaneous resolution?

Materials and methods

Study region

The present experimentation was realized in Batna governorate, which is an areas known for their dairy farming in eastern Algeria.

Animals

A total of133 Holstein cows participated in the study. Their stables conditions were adequate, and their nutrition was uniform, comprising concentrate, green feed, roughage, and silage. No programs of estrus or ovulation synchronization were implemented.

The milk production level of the visited farms was estimated at 6750 ± 1277 kg per cow per lactation cycle.

Data collection

The study was carried out for more than a year. In the presence of the farm veterinarians and the breeders, farms were visited every 14 days. The same investigator performed cow enrolment, clinical examination, and evaluation **(Plöntzke et al., 2010)** These visits began from day 0 (the day of calving) until the moment of the final positive or negative pregnancy diagnosis (day 90 post-artificial insemination (AI)).

Farmers and farm veterinarians closely monitored the cows between the two visits. They clearly registered disease treatment, estrus, and artificial insemination until the next visit.

During each visit, we noted findings, asked questions, and registered data for all the diagnosed diseases.

Farms did not apply any vaccination program except for the state-sponsored vaccinations against rabies and foot-and-mouth disease. Furthermore, screenings for bovine brucellosis and tuberculosis were applied. It should be emphasized that no infectious diseases were reported during the present study.

Diagnosis

The farms' veterinarians, who were monitoring the cows, diagnosed the pathologies between every two visits. As well as placental retention, milk fever, acute puerperal metritis, clinical endometritis, digestive problems, mastitis, and lameness. These pathologies were easily identified by the farms' veterinarians. Thus, diagnosing these conditions was not challenging during the study; however, we had to diagnose reproductive pathologies ourselves and verify the farm veterinarians' diagnoses.

The diagnosed clinical endometritis cases were classified following this definition: The presence of purulent or mucopurulent uterine discharge after 21 DIM; the presence of liquid in the uterus; feverless **(Sheldon, Lewis, et al., 2006)** and a cervical diameter greater than 5 cm;

using ultrasound, we found a specific image of the intra uterine fluid **(Meira et al., 2012;Barlund et al., 2008)**.

We installed a correct and controlled method of estrus detection and AI for all the cows in the study, established a fertility assessment from the monitoring sheets and calculated fertility parameters with the "Excel" software.

Examination

During each visit, we asked farmers about: the progress of calving diseases, heat, inseminations, sale, culling, reform, and mortalities. Afterward, we proceeded to

individual clinical examinations, searching for pathologies. We looked for signs of fresh discharge on the vulva, perineum, tail, or even on the ground.
Following this step, we moved to the rectal search for the presence of pneumovagina or urovagina. We had squatted adhesion, and the cervix was grasped with the hand to estimate its size in centimeters (cm). We also caught the inter-cornual bifurcation and palpated each horn to determine its diameter, length, and contents (liquid presence Then, the ovaries were probed, to determine their physiological or pathological stage.
We used ultrasound to verify the uterus and ovaries condition. Following this, we conducted a vaginoscopy. The ano-vulvar region was meticulously washed and dried to prevent the introduction of feces and germs into the vagina. Before insertion, we cleaned and disinfected speculum with a mild disinfectant. The vaginal wall was thoroughly inspected, to note its color and moisture. We also examined the uterine cervix to observe the nature of any discharge.
We recorded the conditions identified by the farms' veterinarians. Breeders and inseminators recorded heat and insemination dates by themselves.
As soon as we performed the first insemination, we stopped the practice of trans-rectal searching and vaginoscopy. But heats and inseminations continued to be recorded, noting that the conception route was AI, and all IA in this study where performed based on observed estrus.
We performed genital tract ultrasounds to confirm the uterine condition and to diagnose clinical endometritis at each visit. Between days 35 and 45 post-insemination, we performed an ultrasound check to diagnose a possible pregnancy or even cases of late embryonic mortality.
We reviewed the cow again to determine the gestational state on days 60 and 90 post-insemination.
The treatment protocol for clinical endometritis in this study was the same for all cases **(Constable et al., 2009)**:

- Antibiotics: Oxytetracycline
- Non-steroidal anti-inflammatory drugs: Flunixin Meglumine

We initiated a rigorous monitoring program for cows with untreated clinical endometritis (grp.1 and grp.2).
None of the cows had complications during the entire study.
Besides the clinical endometritis, the most noted diseases in this study were: mastitis, lameness, digestive problems, retained placenta, and milk fever (hypocalcemia).
Antibiotics were the most commonly used treatment for these diseases.

Data processing

Groups' formation

The cows suffering from clinical endometritis with or without other simultaneous diseases from the list above, which we treated, form a group apart. A single cow having acute puerperal metritis with deterioration of the general condition was recorded.
Therefore, from clinical data, we formed groups (grps) as shown under:

- Grp. 1 (n=11): Cows with non-treated clinical endometritis and other treated pathologies
- Grp. 2 (n=12): Cows with non-treated clinical endometritis and without other pathologies

- Grp. 3(n=08): Cows with treated clinical endometritis and without other pathologies
- Grp. 4 (n=07): Cows with treated clinical endometritis and other treated pathologies
- Grp. 5 (n=15): Cows without clinical endometritis and with other treated pathologies
- Grp. 6 (n=80): Healthy cows without clinical endometritis and without other pathologies

Calculated prevalence and fertility parameters

Prevalence of clinical endometritis was calculated as:

- $\text{Prevalance (\%)} = \frac{\text{total cases of clinical endométritis}}{\text{total number of cow}} \times 100$

Prevalence of non-treated clinical endometritis was calculated as:

- $\text{Prevalance (\%)} = \frac{\text{Cases of non−treated clinical endométritis}}{\text{total number of cow}} \times 100$

Once the cyclicity recovered, inseminators inseminated the cows and recorded dates. Later, we proceeded with the pregnancy diagnosis. The calculation of the fertility parameters according to the international formula **(Rethmeier et al., 2019)**; **(Rutten et al., 2016)** and **(Touchberry et al., 1959)** was as follows:

- $\text{Conception rate (\%)} = \frac{\text{Pregnant cows n°}}{\text{Inseminated cows n° in a defined time period}} \times 100 \ (01)$
- $\text{Success rate in 1st insemination (\%)} = \frac{\text{Pregnant cows after First insemination}}{\text{Inseminated cows in a defined time period}} \times 100 \ (02)$
- Days to first insemination = number of days from calving to first insemination
- Days to conception or Days open = number of days from calving to day of conception
    - First services to conception interval = number of days from the first service to pregnancy

All cows returned to their postpartum cyclicity (reproductive activity). Cows inseminated and not diagnosed for pregnancy were removed from the study. Therefore, we considered only those with positive or negative pregnancy results. We also excluded from the study all cows with missing information. Applying inclusion criteria, we eliminated from the study one batch of animals consisting of 44 cows with poor nutrition due to a poor management. Finally, only 133 cows were kept from the 557 initially introduced in the study.

Statistical analysis

The results were expressed as a mean ± standard deviation (μ ± SD) for days' intervals and as a percentage (percentage) for conception and success rates. We performed comparisons between the studied groups by using the one-way analysis of variance (ANOVA) followed by a Fisher (LSD) multiple comparisons (post-hoc) test and The Dunnett two-sided test. For conception rate and success rate in first insemination, we used the Chi-square test. A p-value equal to or less than 0.05 was considered significant.

The k-means clustering algorithm **(MacQueen, 1967)** follows an easy way to classify a given object through a certain number of fixed clusters (k). The k-means clustering results were obtained in the present study to control the data division.

We analysed the 133 cows' data set by assigning different values (1-6) to the variable k.
We performed the statistical analyses with R Software, version 3.4.2 (R Core Team, 2020).

Results
As a fact, there was no voluntary waiting period in our study. Our work focused on clinical endometritis. "Therefore, we calculated its prevalence, which was 28.57%, with 17.29% representing non-treated cases. This accounted for 60.52% of the total number of clinical endometritis cases.
Table 01 presents the results of reproductive performance. We did not detect significant differences among groups for days to conception interval, days from the first service to conception, and success rate at first insemination. However, we have noticed a significant difference between groups regarding days to first insemination and conception rate.

Table 1 Effect of treatments on reproductive parameters

Groups	Number of cows	Days to first insemination	Days to conception interval	First service to conception interval	Conception rate	Success rate in 1st insemination
Grp.1	11	75.70±38.00b	97.20±41.40a	21.45 ±25.31a	100%	45.45%
Grp.2	12	89.60±39.70ab	104.3±44.80a	14.75 ±23.02a	100%	58.33%
Grp.3	8	107.50±17.50a	115.8±30.50a	17.60 ±27.90a	62.50%	37.50%
Grp.4	7	87.10±35.80ab	87.7±34.00a	3.50 ±8.57a	85.71%	71.42%
Grp.5	15	72.50 ±23.50b	102.4±31.04a	24.11 ±20.71a	60.00%	13.33%
Grp.6	80	76.80±26.60b	91.1±38.00a	17.70±34.55a	66.25%	47.50%

95% confidence interval

Days to first insemination
Concerning the studied groups, the minimum mean recorded for days to the first insemination was 72.50 days for grp.5 with a range of 44 to 126 days. However, grp.3 had the longest days to the first insemination interval 107.5 days with a range of 82 to 130 days. Grp.1 and grp.2 had respectively recorded mean values of 75.7 and 89.6 days.
For groups 4 and 6, this parameter was 87.1 and 76.8 days respectively, with a p-value of 0.05. For Fisher (LSD) test post hoc analyses, the p-values between groups 3 and 5 (p-value = 0,006), 3 and 1 (p-value = 0,019), 3and 6 (p-value =0,005) were statistically highly significant. The Dunnett two-sided test confirmed the difference between grp.3 and grp.6 with (p-value = 0.024).

Days to conception interval

This interval was slightly longer for grp.3, with a mean of 115.8 days and a range of 82 to 157 days. However, it was not very far from the other groups: grp.1 (97.2 days) and grp.5 (102.4 days). The minimal mean recorded was 87.7 days in grp.4, with a range of 34 to 122 days. Therefore, there was no significant difference among all these groups (p-value = 0.654).

First service to conception interval

For the interval between the first service and conception, we have recorded the maximum mean of 21.45 days (grp.1) with a range of 0 to 71 days, as well the minimum mean of 3.50 days (grp.4) with a range of 0 to 21 days. For this element, we recorded a trend reversal in favour of groups 3 and 4. This situation is due to the limitations of this parameter, which hides other pieces of information, such as the negative pregnancy diagnosis. Indeed, we did not observe a significant difference among groups (p-value=0.843).

Conception rate

For the conception rate, we observed a significant difference among groups (p-value=0.03) in favour of the cows with non-treated clinical endometritis (grps.1 and 2), which had a pregnancy rate of 100%. The other groups could be classified into two tendencies; where group 4 is the first one (85.71%), and groups 3, 5, and 6 are the second one with pregnancy rate of 62.5%, 60%, and 66.25%, respectively.

Success rate in first insemination

The success rate after the first insemination varied between 13% (grp.5) and 71.42% (grp.4). No significant difference was recorded among all the groups (p-value = 0.098).

Results confirmation using K-means clustering

Figure 1 shows the obtained K-means results. As depicted, our data can be included in one or two clusters with high performance clustering results. These findings highlight the consistency of the clinical data, which supports the ANOVA results.

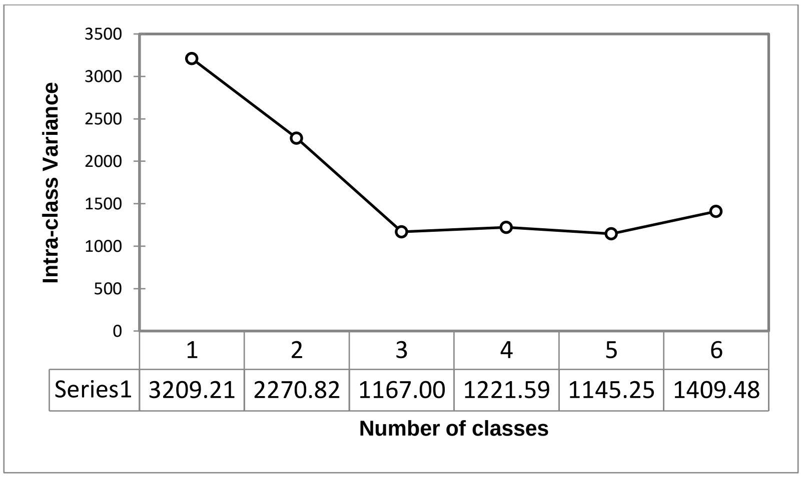

Figure 1 Clustering results using K-means

Discussion

In the present research, all the cows enrolled in the study had finally regained their cyclicity. We compared cows having clinical endometritis with healthy cows.
We noted significantly greater intervals between calving to the first AI, for treated clinical endometritis cows than the other groups, contrary to the expectations.
The study of days to first AI intervals revealed that non-treating cows with clinical endometritis did not influence the recovery of ovarian activity, as in healthy cows. On the contrary, treated cows had extended intervals between calving and first insemination, suggesting that treatment may account for this delay.
Following the cyclicity resumption , all cow exhibited similar histological profiles, with no evidence of endometrial damage **(Chapwanya et al., 2010)**. This finding may explain the lack of significant differences in our statistical analysis of both parameters: the first service to conception interval and days to conception. This outcome could also be attributed to a balanced PGF/PGE ratio, which simultaneously facilitates the expulsion of uterine contents through its uterotonic effect and restores the reproductive cycle. We can also suggest that non-treated clinical endometritis may not impact this ratio. Supporting this, LeBlanc et al., (2002)reported that moderately purulent uterine discharge up to day 33 postpartum was not associated with reduced pregnancy rates , consistent with our findings. However, an increase in purulent discharge has the most significantly negative effect on pregnancy.
Uterine diseases causing lesions on the uterus and ovaries induce a reduction in fertility **(Bonnett et al., 1993)**. Uterine inflammation leads to endometrial dysfunction **(Sheldon & Dobson, 2004)**, and blocks embryonic development **(Soto et al., 2003)**. Several studies have linked uterine diseases to lower conception rates, prolonged intervals to conception, increased culling, and significant economic losses **(Hill & Gilbert, 2008**; **Bartlett et al., 1986**; **Sheldon & Dobson, 2004**; **Gilbert et al., 2005**; **Galvão et al., 2009** ; **Overton. & Fetrow., 2008** and **Galvão, 2013)**. Other research has demonstrated that uterine diseases can negatively impact the time to first insemination and conception rates (**(Galvão, 2013)**; **(Sheldon, Noakes, & Dobson, 2002**; **Williams et al., 2008)**. This is attributed to decreasing the luteinizing hormone secretion, reduced size and growth of the first dominant follicle, and impaired follicular estradiol secretion, which ultimately affects ovulation capacity. Even after ovulation resumes, cows with uterine disease present a prolonged luteal phase **(Opsomer et al., 2000)**. However, the previously mentioned issues were investigated without specifying the real cases involved, whether puerperal metritis, clinical endometritis, or subclinical forms.
Other researcher, like **Bell and Roberts (2007)** studied the effects of uterine diseases on fertility, focusing on puerperal metritis. In our study, we focused specifically on clinical endometritis, and we did not find any effect on fertility interval parameters for non-treated clinical endometritis, which differs from all the previously stated findings. In fact, treated cows had longer days to the first AI intervals, and there was a better conception rate for non-treated clinical endometritis cases. In this work, we did not find a clear difference in the days to conception intervals between cows with treated and non-treated clinical endometritis, whether detected early or not. Our approach was to do a systematic follow-up without considering the herd's history. Unlike **Fourichon et al., (2000)**, we found that clinical endometritis may not affect fertility by increasing the calving to conception interval. Indeed, the previous study showed either slighter effects of early metritis on: days to first estrous, days to the first service, and conception at the

first service; and a comparable or higher effect of late metritis on: days to conception, and services per conception.

Reproduction management has a significant influence on dairy farm profitability. Cows with poor history of reproductive performance are more likely to be examined for metritis than their herdmates in their next reproductive cycle. Herds under a fertility program are systematically examined to promote optimal reproductive performance, and thus metritis are detected and treated early, as confirmed by its lower effects in studies reporting routine examination of cows **(Inchaisri et al., 2010)**.

In the present work, where we used moderately productive cows, we noticed that the influence of pathologies on some reproductive parameters (days to 1st insemination, days to conception, and days between first services and conception) was not obvious, reinforcing Wathes' suggestion that the most important factors affecting fertility are the metabolic more than the pathological ones **(Wathes et al., 2007)**.

Rethmeier et al., (2019) did not observe significant differences in the days to first insemination or days open in relation to milk yield, nor in conception and pregnancy rates. However, they noted a clear trend indicating that higher milk yield was associated with an increase in pregnancy rates **(Rethmeier et al., 2019)**. Nonetheless, the average production level of their study differs from ours, which does not exceed 22 kg/day. The metabolic balance of our cows, with an average of reduced production, allowed a resumption of quickly recovered energy balance, with better and more correct bodily functions like sexual and hormonal activities, earlier than that of higher-yielding cows **(Sheldon, Wathes, et al., 2006)**.

Breeds used in this research were supposed to be highly productive, with a yield of 9291 kg/lactation in Germany or 11472 kg/lactation for the USA breed **(Foksha & Konstandoglo, 2019)**. However, their average level was 6750 ± 1277 Kg, probably due to the nutritional ration insufficiency, individual variability, or herd management. This situation can be more or less contradicted by the availability and quality of food distributed to animals, either by the effect of the environment and the hotter climate comparing to the dairy breeds original countries; namely the European regions, which are visibly colder than our studied area. The last suggestion can be contradicted by the fact that dairy farms exist in the Arabian peninsula countries lime countries and have production levels similar to European farms, with the least square means of 9870 to 10146 liters for Saudi farms **(Ali et al., 2000)**.

Fourichon et al. (2000) compared the effects of clinical and puerperal metritis and concluded that puerperal metritis has a much greater negative impact on reproduction outcomes than clinical metritis **(Fourichon et al., 2000)**. Concordant to our results, **Giuliodori et al. (2013)** stated that puerperal metritis harmed pregnancy rates and increased calving to conception intervals, but not clinical metritis, which had the same results as healthy cows**(Giuliodori et al., 2013)**. Likewise, for the calving to conception interval, cows with puerperal metritis had longer intervals than cows with clinical metritis or being healthy, without specifying if cows were treated or not. This effect could have a variety of actions on fertility, including increasing days to first heat (prolonged anoestrus and luteal phases) **(Mateus et al., 2002**; **Sheldon, Noakes, Rycroft, et al., 2002)**), and causing a net disturbance in the uterine environment **(Bondurant, 1999**; **Sheldon & Dobson, 2004;Giuliodori et al., 2013)**. Unfortunately, the effective treatment options for endometritis remain limited, but the disease can persist even after treatment and recovery **(Nyabinwa, Kashongwe, Hirwa, et al., 2020)**. **Escandón et al. (2020)** used a negative control group but only for subclinical endometritis **(Escandón et al., 2020)**.

The use of antibiotics could indeed disrupt the balance of the uterine flora, promoting the development of resistant germs **(Appiah et al., 2020)**. In general, there is an agreement on using systemic antibiotics in cases of puerperal metritis (accompanied by fever). However, the treatment of clinical endometritis (without fever) using local antibiotics, prostaglandins, and non-steroidal anti-inflammatory drugs is still controversial to some extent **(Bartolome et al., 2014)**. This was reinforced by our study. **Kasimanickam et al. (2005)** had a different strategy for treatment and different results; they chose to treat cows at 20-33 DIM to allow sufficient time for uterine repair in order to increase the chance of conception. Although the untreated control cows also had an equal chance of receiving cloprostenol treatment, cows with clinical endometritis (treated once with intrauterine cephapirin 31 days before the planned spring mating) had better reproductive performance than the untreated controls **(Kasimanickam et al., 2005)**.

Our results showed a different impact on fertility because we did not use cloprostenol after clinical healing.

The use of medication during the postpartum period to treat another disease (digestive, metabolic, respiratory, locomotor, or other problems) did not affect reproductive performance. However, treating clinical endometritis may have a negative impact on the days to insemination intervals.

Despite all the observed differences, all groups remained within the recommended standard

target values for dairy farms: Conception rate (%) > 50, service rate (%) > 80, pregnancy rate (%) > 35, days to first insemination < 85, and days open < 115 days as well as : conception rate (%) > 35, service rate (%) > 60, pregnancy rate (%) > 24 proposed by **(Rethmeier et al., 2019)** . The only exceptions were for days to first insemination, where groups 2, 3, and 4 showed prolonged intervals of 89, 107, and 87 days, respectively, and the days to conception, where group 3 recorded a slight extension to 115 days. Additionally, we observed that the treated groups exhibited poorer performance overall.

There is a significant lack of scientific information on the observed phenomenon (not treating clinical endometritis has a better outcome than treating it), as well as the capacity of practitioner veterinarians in the field to estimate the correct status of the uterus. But the experience in this reflection is grounded in the same context. It is about placental retention, where veterinarians practiced manual removal and antibiotic treatment for a long time. Surprisingly; in recent years, comparisons of different treatment approaches have led to conclude that not intervening was better for the cow's health and reproductive future **(Beagley et al., 2010)**.

From these findings, new questions arise: about postpartum cow immunity in general and uterine immunity in particular; about the characterization of clinical endometritis when it occurs; about its definition as a specific case that requires special monitoring and surveillance without using antibiotics; or about the decision whether or not to treat it. The findings of our study may open the way for further research in this area.

Conclusion

Our results showed significant differences between auto-recovered cows from clinical endometritis and other groups of cows for some reproductive parameters. It clearly showed that the treatment of clinical endometritis had no benefical influence on the reproductive outcome. Unlike the expected; auto-recovered cows from clinical endometritis had better fertility results compared to the treated ones.

The benefits of not intervening for clinical endometritis would be indisputable: reduction of treatment strokes; better management of breeding; avoiding the use of antibiotics, which presents multiple risks. Therefore, this approach requires rigorous monitoring to avoid complications, generating additional costs to measure and compare with the benefits gained from this practice.

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Ethical authorization

From locale Ethical Comity of C.R.Bt (CCE): authorization reference number (03/C.R.Bt/2021).

Conflict of interest statement

All authors declare no conflict of interest in this paper. And they have no relevant financial or non-financial interests to disclose.

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