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Risk factors of clinical endometritis in an Iranian Holstein dairy farm

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Abstract: Clinical endometritis is one of the most common causes of declining reproductive performance in dairy cows. The purpose of this study was to investigate the effects of some risk factors including cow's parity, milk yield, dystocia, retained placenta, calf sex, calving season, abortion, and stillbirth on the incidence of clinical endometritis and the effect of clinical endometritis on open days in an Iranian dairy farm. The uterine health of 557 dairy cows was evaluated by rectal palpation and ultrasonography during 30–35 days after calving. The presence of mucopurulent secretions in the vagina and vulva as well as the ultrasonographic observation of echogenic secretions in the uterine lumen were considered endometritis. The effect of the mentioned factors on the incidence of clinical endometritis was then analyzed in univariate and multivariate logistic regression models. The chance of developing clinical endometritis in the low-producing cows was 27.2% higher than that of the high-producing cows (p=0.06). Other parameters had no significant effect on the incidence of endometritis. The incidence of clinical endometritis in the studied herd was 49.5%. Based on the results of this study, the low milk yield may be a risk factor for developing clinical endometritis in the studied herd's condition. Parameters of parity, dystocia, retained placenta, calf sex, calving season, abortion, and stillbirth were not effective in the occurrence rate of endometritis. There was no association between clinical endometritis and days open in the studied herd.
Keywords: Dairy cattle, clinical endometritis, risk factors, reproductive performance.

1. Introduction

The reproductive performance of dairy cows largely depends on the health status of the uterus after calving (Barragan et al., 2021; Moraes et al., 2017). One of the most common uterine diseases affecting the reproductive efficiency of dairy herds is endometritis (LeBlanc, 2023). Endometritis is inflammation of the endometrium that can impair the fertility of cows without general symptoms (Várhidi et al., 2024). The prevalence of the disease in herds around the world has been reported up to 70% (Nyabinwa et al., 2020b). Endometritis increases the service per conception and the interval from calving to the first insemination and consequently increases the interval from delivery to re-conception (Nyabinwa et al., 2020a). Endometritis in cattle is classified into two clinical and subclinical forms. The presence of mucopurulent discharges in the vagina from the 21th day after delivery is considered clinical endometritis, while subclinical endometritis is defined by a high percentage of polymorphonuclear cells in the uterine cytology sample (Wang et al., 2018). To prevent and control endometritis in herds, it is necessary to know the risk factors of the disease. Risk factors of clinical endometritis have been reported in Nyabinwa et al. (2021) retained placenta and calving season, in Potter et al. (2010) genital tract injuries, in Giuliodori et al. (2013) dystocia and septic metritis, and in McKay et al.(2023), parity and the presence of two or more diseases in first 50 days postpartum. Occasionally, the conflicting risk factors reported may be due to differences in management conditions (Kim & Kang, 2003). So far, the identification of the risk factors of clinical endometritis in Iranian dairy herds has not been investigated. This study aimed to investigate the relationship between some cow's related parameters and the occurrence of clinical endometritis in a dairy farm in the central part of Iran. In addition, the effect of endometritis on the "open days" index has been evaluated.

2. Material and methods

2.1. Farm and animals

This study was carried out in the industrial dairy farm of Zagros Milk and Meat Company, consisting of 4,000 Iranian Holstein dairy cows, located in Shahrekord city of Chaharmahal va Bakhtiari province of Iran (32° 19′ 32″ N, 50° 51′ 52″ E; height above sea level: 2060 m). The maximum daily temperature during the experiment was 32.9 °C, the minimum relative humidity per day was 10.4%, and the mean maximum relative humidity per day was 50.8%. The cows were housed in a free stall with a concrete floor and milked three times every day. Cattle rations were distributed as Total Mixed Ration (TMR based on milk production level) . All cows included in this study were in perfect health at the start of the study. The average open day of the herd was 140 and the average milk production of a cow during 305 days was 12500 kg. All reproductive information of the cows such as date of birth, date of estrus, date of calving, calf sex, and reproductive problems such as dystocia, retaining fetal membrane, abortion, and stillbirth as well as the amount of milk produced per day per cow were recorded.

2.2. Diagnosis of endometritis

A total of 557 post-parturient dairy cows were evaluated by rectal examination and ultrasonography (CTS-3300V, SIUI, Guangdong, China) during 30-35 days after delivery to assess uterine health. The cows with mucopurulent discharge after uterine and vaginal massage, as well as the diagnosing echogenic discharges by ultrasonography in the uterine lumen were considered to have clinical endometritis and were recorded.

2.3. Statistical analysis

For statistical analysis, regarding the parity the cows were divided into primiparous and multiparous cows, and concerning milk production they were divided into low-yielding cows (<40 kg) and high-yield cows (≥40 kg). At first, the effect of independent variables including the parity, daily milk production level, calving season, calf sex, dystocia, retained placenta, abortion, and stillbirth on the occurrence of clinical endometritis was analyzed separately in the univariate logistic regression model and the Unadjusted Odds Ratio was then calculated. The simultaneous effect of independent variables on the occurrence of clinical endometritis in the multivariate logistic regression model was then evaluated using two methods Enter (to investigate the effect of each variable on the chance of endometritis in the presence of other variables) and Backward Elimination method (to determine effective independent variables in the final model), and finally the Adjusted Odds Ratio was calculated. Interactions between variables were also evaluated. The Hosmer-Lemeshow test was used to evaluate the goodness of fit of the logistic regression model, which was non-significant (P = 0.837) and showed the appropriate fit of the logistic regression model to these data. Also, the area under the ROC curve index was calculated at 0.59, which also showed the appropriate fit of the model to the data. The Kaplan-Meier method was used to compare open days between cows with and without endometritis.

3. Results

Based on the results of the univariate logistic regression model, the effect of each of the studied parameters alone on the occurrence of clinical endometritis is shown in Table 1. None of the parameters of parity, dystocia, retained fetal membrane, calf sex in recent calving, calving season abortion, and stillbirth showed a significant effect on the incidence of clinical endometritis in the studied dairy cows. The chance of developing clinical endometritis in the high-producing cows was 2.27% lower than that of the low-producing cows (p = 0.06).

Dependent variables	cows studied (No)	Endometritis + (No)	%	P value	Odds ratio
1-parity					
primiparous	159	75	47.2%		Referent
multiparous	398	201	50.5%	0.478	1.143
2- milk production per day					
Low (40 kg>)	338	178	52.7%		Referent
High (40 kg≤)	219	98	44.7%	0.068	0.728
3- dystocia					
no	527	262	49.7%		Referent
yes	30	14	46.7%	0.745	1
4-retain placenta					
no	523	257	49.1%		Referent
yes	34	19	55.9%	0.447	1.311
5- calf sex					
female	267	130	48.7%		Referent
male	284	143	50.4%	0.906	1.014
6- calving season					
Spring	256	121	47.3%		Referent
Summer	116	61	52.6%	0.342	1.237
autumn	36	19	52.8%	0.536	1.247
winter	144	72	50%	0.599	1.116
7- abortion &still birth					
no	531	263	49.5%		Referent
yes	26	13	50%	0.963	1.019

Table 1 – The incidence of clinical endometritis in the cows according to the risk factors studied.

In the multivariate logistic regression model, out of all the variables, only the milk production level was included in the final model, which results are shown in Table 2. The incidence of clinical endometritis in the low-producing cows was 2.27% higher than those of the high-producing cows (p = 0.06).

Variables	B	Wald	P value in Wald test	Odds ratio
Milk yield				
Low (40 kg>)	0	-	-	Referent
High (40 kg≤)	-0.329	3.538	0.06	0.720

Table 2 – Multivariate logistic regression model for the risk factors affecting the incidence of clinical endometritis in the studied cows.

The Kaplan-Meier survival curves for open days in cows with endometritis and without endometritis is shown in Figure 1. Median days open was 161 for cows with endometritis and 143 for cows free of the condition which the difference was not statistically significant. (Log Rank p: 0.6)

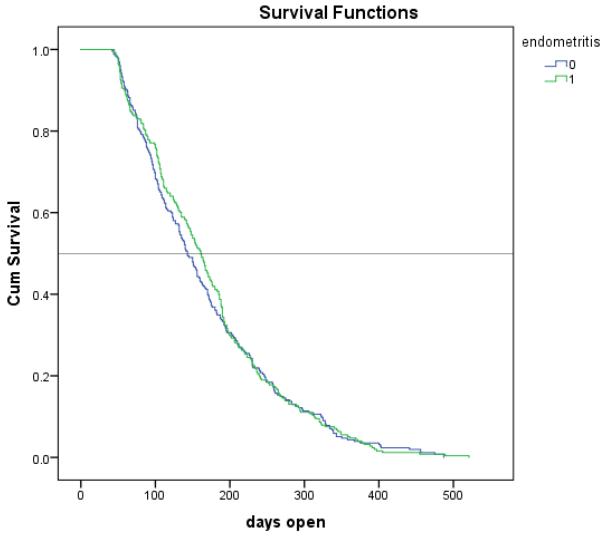

Figure 1 – Kaplan-Meier survival curve of days open in the dairy cows with endometritis and without endometritis.

4. Discussion

In the present study, no association was observed between the parity and occurrence of clinical endometritis. The primiparity has been reported to be a risk factor for clinical endometritis by Kelly et al. (2020), Giuliodori et al. (2017) and Manríquez et al. (2020). In contrast, McKay et al. (2023) proved the positive relationship between the incidence of endometritis and increased parity in Scottish dairy cattle herds. Cows are prone to dystocia and genital tract injuries at first parturition, which can predispose them to endometritis (Pinedo et al., 2020). On the other hand, in multiparous cows, the elasticity of the uterus and the rate of involution is less than in primiparous cows, and this can increase their susceptibility to endometritis (Adnane et al., 2017). However, no relationship between parity and the occurrence of clinical endometritis has been reported (Giuliodori et al., 2013; Nguyen-Kien & Hanzen, 2017), which is consistent with the results of the present study.

In the present study, the low production tends to be one of the risk factors for clinical endometritis. This finding is consistent with the results of some similar studies (Cheong et al., 2011; Somasekara, 2003). The prevalence of chronic endometritis has been reported to be significantly higher in low-producing cows than in high-yielding cows (Cheong et al., 2011; Somasekara, 2003). On the other hand, contradictory results have been reported in the study of Giuliodori et al. (2013) and Manríquez et al. (2020) that cows with endometritis had a higher milk yield than clean cows. High-yielding dairy cows are more prone to negative energy balance conditions than low-yielding cows, and this can increase their risk for uterine infections and endometritis (Adnane et al., 2017; Cheong et al., 2011). In the study of Giuliodori et al. (2013), high serum concentrations of preparturient NEFA and postpartum BHBA were associated with an increased incidence of endometritis. Negative energy balance is likely to have detrimental effects on neutrophil function, thereby affecting uterine health (Serbetci et al., 2024). In the present study the chance of developing clinical endometritis in the

low-producing cows was 1.4 times higher than the high-producing cows (p = 0.06). The reason for this relationship may be that endometritis leads to a decrease in milk production, as some studies have proven the effect of endometritis on the decrease in milk yield (Nyabinwa et al., 2020b; Sharma et al., 2019).

In many studies, dystocia has been mentioned to be as one of the most important predisposing factors for endometritis (Giuliodori et al., 2017; Kelly et al., 2020; Zahid et al., 2024). Dystocia delays the contraction of the uterus and makes it more susceptible to infection (Osawa, 2021). However, based on the results of the present study, dystocia did not increase the incidence of clinical endometritis. In the study of Kim & Kang (2003), the effect of dystocia, cesarean section, twinning and stillbirth was evaluated as abnormal parturitions on the occurrence of clinical endometritis but was not included in the final logistic regression model, which is consistent with the results of the present study.

According to numerous reports, there is a very strong relationship between retain fetal membranes and the occurrence of endometritis in dairy cows (Nguyen-Kien & Hanzen, 2017; Nyabinwa et al., 2021; Várhidi et al., 2024). The retention of fetal membranes keeps the cervix open, consequently pathogens enter and endometrium becomes infected (Kelly et al., 2020). In the present study, although the incidence of clinical endometritis in the cows with retain placenta in their recent parturition was 31.1% higher than in the other cows, this difference was not statistically significant. As a result, retain placenta is not considered as a risk factor for clinical endometritis in the studied herd. This finding is consistent with the results of cheong et al. (2011), Kelly et al. (2020) and Mckay et al. (2023).

The birth of a male calf due to larger size can increase the risk of dystocia and thus predispose to endometritis (Kelly et al., 2020; Nyabinwa et al., 2021). However, in the present study, the variable of calf sex in recent calving was not included in the final multiple logistic model and the incidence of endometritis following the birth of male and female calves was almost the same. This finding of the present study is consistent with the results of the study of McKay et al. (2023).

There was no significant difference in the incidence of clinical endometritis post-calving of the four seasons in the studied dairy cows. No difference between warm and cold seasons respected to the occurrence of endometritis was also seen. This finding is completely consistent with the results of Manríquez et al. (2020). Conversely, the effect of calving season on the incidence of clinical endometritis has been previously described by McKay et al. (2023), Giuliodori et al. (2017) and Nyabinwa et al. (2021).

In several studies, the effect of abortion or stillbirth on the occurrence of clinical endometritis has been proven (Cheong et al., 2011; Nyabinwa et al., 2021; Tayebwa et al., 2015), however; in the present study, there was no difference in the incidence of clinical endometritis between the cows that gave birth to live calves and those that had abortion or stillbirth which is consistent with the findings of McKay et al. (2023).

The incidence of clinical endometritis in the studied farm was 49.5%, which is higher than the previous reports in many studies. The incidence of endometritis has been reported in various literature from 8% to more than 70% (Manríquez et al., 2020; McKay et al., 2023; Nyabinwa et al., 2020b; Várhidi et al., 2024) It seems that the different incidence rate of clinical endometritis have been reported in the studies is related to the time (post-partum) and method of diagnosis. In the present study, clinical endometritis was being diagnosed based on not only direct observation of mucopurulent discharges in the vagina and vulva, also, ultrasonographic observation of echogenic secretions in the uterine lumen. These methods of diagnosis may have led to a high rate of endometritis in the present study.

Among the independent variables studied, only the level of production tends to be effective on the incidence of clinical endometritis. Lack of significant effect of other parameters can be due to extremely accuracy in diagnosing endometritis and consequently its high incidence in the studied farm. This means that if, like many similar studies (Dubuc et al., 2010; Giuliodori et al., 2013; Potter et al., 2010), only the observation of mucopurulent discharges was considered as clinical endometritis, the incidence of endometritis would be lower and consequently the relationship between some parameters and occurrence of endometritis was more likely to be significant. LeBlanc et al. (2002) stated that the presence of purulent or mucopurulent discharges or an increase in cervical diameter to more than 7.5 cm as a characteristic of clinical endometritis is associated with an increase in the interval between delivery and re-pregnancy. Therefore, the diagnosis should be made based on external examination, vaginoscopy and cervical palpation between 20 and 33 days after delivery, and veterinarians who use other characteristics and uterine palpation to diagnose endometritis and subsequent treatment should know that they will have many false positive cases and their treatment will not benefit the farmer (LeBlanc et al., 2002). On the other hand, the lack of significant difference in open days between cows with and without endometritis in the present study can be due to the same way of diagnosing the disease in the studied farm. In similar studies where the diagnosis of endometritis was solely based on the observation of purulent vaginal secretions, endometritis led to an increase in days open (Giuliodori et al., 2013; LeBlanc et al., 2002; McDougall et al., 2020). Contrary to our study Kelly et al. (2022) found ultrasonography to be a suitable method for diagnosing

endometritis, so that Irish grazing dairy cows that had intrauterine fluid during ultrasound examination had lower chance of pregnancy within the subsequent breeding season than normal cows. Of course, in the present study by Day 143 postpartum, 50% of the clinical endometritis-negative cows were pregnant, however, but this time was 161 days post-partum for the positive-endometritis group.

5. Conclusion

Based on the results of present study, none of the parameters of parity, dystocia, retained placenta, calf sex, calving season, abortion and stillbirth contributed to the occurrence of clinical endometritis in the dairy herd. The low milk production tend to be a risk factor for clinical endometritis in dairy cows under the condition of studied herd. It seems that there is no correlation between cases of clinical endometritis diagnosed through ultrasound examination in the studied herd and the subsequent decrease in reproductive performance.

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