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Associations between the clinical signs of chronic endometritis with ovarian cysts and body condition loss in German Holstein Friesian cows

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The objective of this retrospective field study was to associate the type and smell of discharge, the size of the uterus, the ovarian and treatment status, and the time to diagnosis of animals with chronic clinical endometritis (CCE) with the incidence of ovarian cysts and with a marked loss in body condition in German Holstein Friesian cows. Two hundred and sixty-four cows diagnosed with CCE from day 14 to day 42 postpartum participated in this study. In addition, 100 days milk production and the parity of the animals were included in the analysis. With the use of logistic regression, a purulent vaginal discharge (≥ 50% pus), the decision not to treat the animals for CCE and a high 100 days milk production proved to be significant factors for the incidence of ovarian cysts. Additionally, the type of discharge showed interactions with the parity and the smell of the discharge, as more animals with fetid and purulent discharge and more animals in the first lactation with a purulent discharge developed ovarian cysts. A high milk production and the parity showed associations with an excessive body condition score loss. Additionally, more animals with a diagnosis of an oversized uterus in comparison to cows with an early involution experienced a considerable reduction in their nutritional condition.

Keywords: body condition score, endometritis, ovarian cysts

Introduction

Uterine disease of dairy cattle postpartum (p.p.) influences reproductive performance and causes important financial losses [1,14,18]. There is growing evidence that not all of the clinical signs of chronic clinical endometritis (CCE) have the same effect on the reproductive parameters used in farms. For instance, LeBlanc *et al*. [16] found that only a purulent vaginal discharge or a diameter of the cervix larger than 7.5 cm had prognostic value for the diagnosis of endometritis and when vaginoscopy was applied, no diagnostic criteria based on palpation of the uterus had a predictive value for the time to pregnancy. Moreover, Williams *et al*. [32] concluded that the evaluation of the type and smell of vaginal discharge reflects the number of bacteria in the uterus. Clinical endometritis does not only have a direct effect on reproductive performance, but also seems to be associated with ovarian dysfunctions [26,31], and/or reduction of the body condition of dairy cattle [27], which in turn suppress fertility. Additionally, the effect of CCE treatment on the main fertility measures has been found to be modest [10,17].

The present study aimed to investigate if there are any associations between the clinical signs and CCE treatment with the incidence of ovarian cysts and with the reduction of the body condition score p.p. of dairy cows.

Materials and Methods

The details of the study herds and the examination protocols have been previously reported [29]. Briefly, data from seven dairy herds were gathered and evaluated. Two experienced and similarly trained veterinarians carried out all the examinations following the diagnosis and treatment. Starting from day 14 p.p. and up to day 42 p.p., all animals without peripartum problems were examined with transrectal palpation to monitor uterine involution and uterine anatomical characteristics. Deviation from the reference values for contractility and/or content of the uterus (i.e. uterus to be gathered in the hand and without fluctuation) resulted in an examination with vaginal speculum. Mean time of diagnosis was 21 ± 8 days p.p. Additionally, all animals with peripartum problems (e.g. retentio secundinarum, dystocia, fetal death, early birth, birth of twins, torsio uteri

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or abortion) were examined routinely via vaginal inspection. The animals were re-examined at 14 or 28 days intervals depending on the farm's management program and until pregnancy was confirmed, the cow was culled, or until the farmer expressed his decision not to breed the animal again.

Inclusion criteria were abnormal vaginal discharge (i.e. containing pus), no systemic disorder of the animal until day 42 p.p., and no additional genital diseases with the exception of ovarian cysts (OC) until pregnancy or the decision to cull. At the time of diagnosis, the following clinical characteristics were categorized:

・Type of discharge (TYPEDIS): Purulent (≥ 50% pus) or mucopurulent with flakes of pus (< 50% pus)

・Smell of discharge (SMELL): Fetid or neutral smell

・Size of uterus (USIZE): Uterus that could be held in the hand (normal involution) or not (oversized)

・Time of diagnosis (TIMED): ≤ 21 or > 21 days p.p.

・Presence or absence of a corpus luteum at the time of diagnosis (CL).

Two hundred and sixty-four Holstein Friesian cows with CCE were eligible for this study. The animals remained untreated (n = 166) or received treatment (n = 98) (TREAT) using routine medication, either locally (antibiotics, n = 17; disinfectants, n = 2), systemically (analogs of prostaglandin-F2-alpha, $PGF_{2\alpha}$, n = 59) or with a combination of these treatments (n = 20). The animals were classified into two parity (PARITY) groups: those of the first lactation and those past first lactation. OC were diagnosed by means of transrectal palpation as follicular structures with a diameter of at least 25 mm interfering with normal ovarian activity, which was proved in the next follow up examination. Although transrectal examinations were supported by an ultrasound device equipped with a 5 MHz transducer, no attempt was made in this study to classify into different types of OC in order to achieve adequate statistical power. The examination period extended from day 21 p.p. until the animal was diagnosed as pregnant or was culled.

Body condition score (BCS) of all animals was conducted at least once monthly throughout the lactation following the chart of Edmondson *et al.* [11]. Changes in BCS (ΔBCS) were derived from the first p.p. estimation and the lowest value before successful artificial insemination (a.i.) or culling. A ΔBCS greater or equal to one point was considered excessive. The 100 days milk production (ML100) value was derived for each animal with using Wood's lactation curves [33]. The animals were then divided into two groups according to their milk production: Those producing less than and those producing more than 3,824 kg in the first 100 days of lactation under investigation. This limit was defined by using a linear regression function between 100 days and 305 days milk production and setting as a limit 10,000 kg of milk production in the equation. Nine animals were culled before reaching 100 days of lactation.

Modelling process

Statistical analyses of OC and ΔBCS data were carried out using a linear logistic model with a binary response variable, which was modeled as a binomial random variable (y_i). The dependent variable (y_i) can have the value 1 with a probability of obtaining OC or ΔBCS π_i or the value 0 with a probability of healthy 1-π_i for observation *i*. The logistic model uses a link function g (μ_i), linking the expected value to the linear predictors η_i.

The logit link function is defined by $log\left[\frac{\pi_i}{1-\pi_i}\right] = \eta_i$ where π_i is the probability of occurrence recorded from day 21 p.p. and until the date of successful a.i. or culling.

The data were then analyzed with the SAS GLIMMIX macro, a generalized linear model, including fixed effects of TYPEDIS, SMELL, USIZE, TIMED, CL, TREAT, PARITY and ML100. All two-way interactions between the clinical characteristics, parity and treatment were tested and only significant effects among the different effects were included in the final model used for analysis. Least square means were estimated on the logit scale and then back-transformed using the inverse link function $\pi = exp(\chi\beta)/[1+exp(\chi\beta)]$ to the original scale (probability) by applying the LSMEANS statement. Significant differences between least square means were tested using a *t*-test procedure by including the PDIFF option in the LSMEANS statement. Standard errors of least square means were calculated as described by Littell *et al.* [21]. The data were analyzed using the following generalized linear model:

$$\log\left(\frac{\pi_{ijklmnop}}{1-\pi_{ijklmnop}}\right) = \varphi + D_i + S_j + T_k + P_l + U_m + CP_n + ML_o + TD_p + C_r$$

where $\pi_{ijklmnop}$ = probability of OC, φ = overall mean effect, D_i = fixed effect of TYPEDIS, S_j = fixed effect of SMELL, T_k = fixed effect of TREAT, P_l = fixed effect of PARITY, U_m = fixed effect of USIZE, CP_n = fixed effect of CL, ML_o = fixed effect of ML100, TD_p = fixed effect of TIMED, C_r = random effect of farm.

A similar model was fit for the variable ΔBCS. Stepwise forward selection was used and the levels of significance were 0.50 to enter the model and < 0.1 to remain in it. All analyses and graphic functions were conducted using SAS version 8 (SAS Institute, USA).

Results

The incidence of ovarian cysts (Table 1) proved to be substantially higher in animals not receiving any treatment for CCE ($p = 0.007$), having a purulent vaginal discharge ($p = 0.02$) and having a ML100 of more than 3,824 kg ($p = 0.001$). For OC, the SMELL by TYPEDIS and the PARITY by TYPEDIS interactions were important with p-values of 0.04 and 0.02, respectively. Namely, 27% of the animals with purulent and fetid discharge and 13% with purulent but not fetid discharge developed OC, whereas in animals with mucopurulent discharge, this percentage was below 10% (Table 1). Additionally, a purulent discharge seemed to have a detrimental effect especially in cows in the first lactation, as 26% developed OC in comparison to only 1.5% when the discharge was mucopurulent. Older cows developed OC in 14% and 11% of the cases when the discharge was purulent and mucopurulent, respectively.

Table 1. F-Values, levels of significance,and least squares means of the variables remaining in the logistic model for the incidence of ovarian cysts in Holstein Friesian cattle diagnosed with chronic clinical endometritis (CCE)

Variable	Stratum	n	Incidence	F-value	*p*-value
Corpus luteum	Absence	91	13.4 ± 5.2^{a}	3.47	0.06
	Presence	155	6.1 ± 2.8^{a}		
100 days milk yield	> 3,824 kg	59	18.7 ± 5.2^{a}	11.46	0.0008
	≤ 3,824 kg	196	4.2 ± 1.8^{b}		
Parity	1	97	6.8 ± 4.1^{a}	0.93	0.34
	> 1	167	12.2 ± 3.7^{a}		
Smell	Fetid	88	8.0 ± 3.9^{a}	0.27	0.60
	Neutral	172	10.4 ± 3.9^{a}		
Treatment	Yes	98	4.3 ± 2.7^{a}	7.52	0.007
	No	166	18.2 ± 5.2^{b}		
Type of discharge	Purulent	107	19.4 ± 5.6^{a}	5.97	0.02
	Mucopurulent	151	4.0 ± 2.6^{b}		
Smell by type of discharge	Fetid & purulent	54	27.5 ± 9.2^{a}	4.35	0.04
	Fetid & mucopurulent	33	2.0 ± 1.8^{b}		
	Neutral & purulent	54	$13.3 \pm 5.9^{a,b}$		
	Neutral & mucopurulent	117	8.0 ± 4.4^{b}		
Parity by type of discharge	> 1 Lactation & purulent	73	14.0 ± 5.0^{a}	5.38	0.02
	> 1 Lactation & mucopurulent	89	$10.7 \pm 4.8^{a,b}$		
	1 Lactation & purulent	35	26.4 ± 10.8^{a}		
	1 Lactation & mucopurulent	61	1.5 ± 1.6^{b}		

[a,b]Least squares means within variable with different superscript differ significantly ($p \leq 0.05$)

Table 2. F-Values, levels of significance,and least squares means of the variables remaining in the logistic model for the incidence of a reduction ≥ 1.0 on a five-point scale body condition score loss in Holstein Friesian cattle diagnosed with CCE

Variable	Stratum	n	Incidence	F-value	*p*-value
100 days milk yield	> 3,824 kg	59	30.3 ± 8.0^{a}	4.78	0.03
	≤ 3,824 kg	196	16.5 ± 3.5^{b}		
Parity	1	97	12.1 ± 5.0^{a}	9.80	0.002
	> 1	167	38.3 ± 4.7^{b}		
Size of uterus	Oversized	64	29.6 ± 7.9^{a}	3.74	0.05
	Involution	197	16.9 ± 3.7^{a}		

[a,b]Least squares means within variable with different superscript differ significantly ($p \leq 0.05$).

In the high milk yield group, a higher proportion of animals revealed a marked BCS loss compared with lower producing cows ($p = 0.03$, Table 2). Parity also had a significant effect, as almost 40% of the older cows experienced a severe body condition loss in comparison to only 12% of the animals in first lactation ($p = 0.002$). Additionally, more cows diagnosed with an oversized uterus experienced a considerable reduction in their nutritional condition compared to cows with an early involution, although this result was slightly over the significance level of 0.05 ($p = 0.054$). For ΔBCS no interaction between variables proved to be significant.

Discussion

There is evidence that intrauterine infections are involved in the pathogenesis of ovarian dysfunctions [8,22,23,31], probably by disrupting the normal function of the hypothalamus-pituitary-ovarian axis due to endotoxins [26]. In the present study, a purulent vaginal discharge also proved to enhance the risk of ovarian cysts compared to a mucopurulent one. A similar finding was also reported by Mateus *et al.* [23]. First-time calvers seemed to be especially affected by a purulent discharge, which could be a result of a higher frequency of assisted calvings and the resulting uterine lesions noticed in this group of animals [3]. Other authors also hypothesize that uterine trauma could be a prerequisite for the association between uterine infection and ovarian dysfunction [24]. Furthermore, a purulent discharge is associated with the presence of *Arcanobacterium pyogenes* [32], which is the microorganism with the strongest proof of causing uterine lesions [6].

On the other hand, the effect of vaginal discharge odour on reproductive performance, which is used under practical conditions quite often as a measure of the severity of CCE, has rarely been evaluated separately. Williams *et al.* [32] found that a fetid odour was associated with the presence of *A. pyogenes* and *Escherichia coli*, but not with that of *Fusobacterium necrophorum* or *Prevotella melaninogenicus*, the synergy of which is important for the pathogenesis of CCE in dairy cattle [6]. Additionally, a fetid odor was associated with higher bacterial growth densities of streptococci [32], which seem to have antagonistic effects on *A. pyogenes* [6,9]. Nevertheless, a synergic association of fetid odor and purulent discharge was significant for the incidence of ovarian cysts in this study.

It was also found that more animals with an oversized uterus demonstrated a remarkable decline in their body condition, probably resembling a situation where an afflicted organism has difficulties in coping with normal biological processes (e.g. uterine involution). Whether a nonsystemic illness further impairs the food consumption and consequently the decline in body condition, as proposed by Bell and Roberts [3], or whether a negative energy balance, which is associated with puerperal metritis [13], conflicts with the reestablishment of the genital tract to its pregravid characteristics remains to be answered by prospective research projects. In agreement with other studies [19], older cows lost more body condition than younger ones, probably because of the increase in the milk production.

The importance of a relatively early resumption of cyclicity as a predictor for good reproductive ability has often been emphasized [7,16]. Nevertheless, other authors suggest that an early luteal activity and consequently an early increase in the progesterone levels could result in an increased susceptibility to uterine infections [20] or in a prolonged calving to conception interval [28]. In this study, a prompt resumption of cyclicity showed a weak negative association with the development of ovarian cysts. The exact mechanism of this effect is not known. However, it could be a consequence of the uterine contractions during estrus resulting in the reduction of the bacterial load, as proposed by LeBlanc *et al.* [17]. On the other hand, the return to cyclicity could also be indicative of a healthy endometrium, as it is found that infected animals ovulate significantly later compared to healthy ones [25].

The impact of high milk production is a matter of controversy among researchers. An antagonistic genetic [12,30] and phenotypic [2] relationship with the reproductive measures have been reported and are generally accepted. In the present study, an association of high milk production with the occurrence of ovarian cysts and the decrease in the body condition was noticed. These findings have also been found by other authors [4,22].

Our results do suggest an association between CCE treatment with the formation of ovarian cysts. Bonnett *et al.* [5] found that a prostaglandin administration on day 26 p.p. resulted in less inflammation and fibrosis of the endometrium by day 40. Furthermore, Königsson *et al.* [15] found that cows recovered faster from *A. pyogenes* and *F. necrophorum* infections with the intrauterine application of antibiotics. A better functionality of the endometrium and a reduction in the endotoxin stress caused by the endometritis could be the explanation for the clinical results in this study.

In conclusion, we have found associations between the clinical signs of chronic endometritis and the incidence of ovarian cysts. In addition, a positive effect of CCE treatment on OC was also determined. Based on our results, animals showing a purulent discharge, a combination of purulent discharge accompanied with a fetid odor, and the first lactation cows with purulent discharge require particular veterinary attention in order to minimize the risks of ovarian cysts.

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