

Short Communication

Increased Risk of Conception Failure in German Holstein Friesian Cows with Chronic Endometritis

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Contents

The objective of this retrospective field study was to evaluate the effect of chronic clinical endometritis (CCE) on days open in German Holstein Friesian cows. Two hundred and sixty-four cows diagnosed with CCE from day 14 to 42 postpartum were included in this study. A Cox's proportional hazards regression model with stepwise forward selection for days open was used and the variables type of vaginal discharge, ovarian cysts and a marked loss of body condition score proved to be significant with hazard ratios of 1.5, 2.0 and 1.9 respectively. Although not remaining in the model, a weak positive effect of treatment for CCE and a negative effect of high milk production could also be found in the univariate analysis. These results suggest that the suppression of fertility of animals diagnosed with CCE varies according to the different symptoms, which could be used to support decisions regarding treatment. Management factors such as a marked loss in body condition and high milk production prolonged the number of days open of animals with CCE.

Introduction

Despite the great advances in the management of dairy cattle, uterine infections postpartum (p.p.) still pose a problem causing an important financial loss at herd level (Bell and Roberts 2007). There is growing evidence that the treatment of cows with chronic clinical endometritis (CCE) has a modest effect on the main fertility measures (LeBlanc et al. 2002a). However, the main body of research does not include an untreated control group, which is an important factor when evaluating a treatment effect. The various clinical signs of endometritis seem to have different prognostic value for fertility measures (LeBlanc et al. 2002b; Williams et al. 2005). Nonetheless, their influence on days open, which is the principal component of an economic decision at farm level (Dijkhuizen et al. 1985), has rarely been estimated in a conclusive and practice-oriented way.

The aim of this retrospective study was to investigate the effect of treatment and of different clinical signs of CCE on the number of days open of dairy cows by using survival analysis.

Materials and Methods

Study herds and data collection

In the scope of this retrospective study, data from seven dairy herds of 60–100 cows were gathered and evaluated. All dairy farms participated in the herd health

management programme offered by the Production Medicine Unit of the Clinic for Cattle of the University of Veterinary Medicine Hanover. Two experienced and similarly trained veterinarians carried out all the examinations following the diagnosis and treatment protocols of the medical unit. All animals were German Holstein Friesian cows with an average of 2.9 lactations and were housed in free-stall barns with cubicles. Feeding consisted of grass and corn silage and computer-assisted supply of concentrated feeds according to milk production. The dry period was set at 6–8 weeks before parturition and the animals gave birth in individual calving pens. The average milk yield per year and farm ranged from 7860 to 9613 kg. The voluntary waiting period was set at 60 days on all farms. 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 anatomic uterine characteristics. Deviation from the reference values for contractility and/or content of the uterus resulted in an examination with vaginal speculum. Mean time of diagnosis was 21 ± 8 day p.p. Additionally, all animals with peripartum problems were examined routinely via vaginal inspection. The animals were re-examined at 14 or 28 day intervals depending on the farm's management programme and until pregnancy was confirmed or the cow was culled or until the farmer expressed his decision not to breed the animal again. Days open were calculated based on actual reproductive data as the interval from calving until the day of successful insemination.

Inclusion criteria were an abnormal vaginal discharge, 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. A prerequisite was also a good documentation of the follow-up examinations at least up to the censoring date.

At the time of diagnosis, we categorized the following clinical characteristics:

Type of discharge (TYPEDIS): Purulent ($\geq 50\%$ 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 (CL) at the time of diagnosis.

Two hundred and sixty-four Holstein Friesian cows with CCE were eligible for this study (Table 1). In 18 animals, the ovaries could not be reached at the time of diagnosis. The animals remained untreated ($n = 166$) or received treatment ($n = 98$) (TREAT) using routine medication, either locally (antibiotics, $n = 17$; disinfectants, $n = 2$), systemically (analogues of prostaglandin-F2- α , PGF_{2 α} , $n = 59$) 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 higher than first lactation animals. Ovarian cysts were diagnosed by means of transrectal palpation supported by an ultrasound device 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. No attempt was made in this study to classify into different types of OC to achieve adequate statistical power. Body condition score (BCS) of all animals was conducted at least once monthly throughout the lactation following the chart of Edmondson et al. (Edmondson et al. 1989). Changes in BCS (Δ BCS) were derived from the first p.p. estimation and the lowest value before successful artificial insemination or culling. A Δ BCS greater/equal to one point was considered excessive. The 100 days milk production (ML100) was derived for each animal by using Wood's lactation curves (Wood 1967). We then divided the animals into two groups according to their milk production: Those with and those without a production of more than 3824 kg in the first 100 days of lactation under investigation. This limit was defined by using a linear regression function between 100 and 305 days milk production and setting as a limit a production of 10 000 kg in the equation. Nine animals were culled before reaching 100 days of lactation.

Modelling process

In a first step, univariate analysis was performed using the Kaplan–Meier product limit estimates (LIFETEST procedure in SAS version 8, SAS Institute Inc. 1999). Tests of equality across strata were used to explore whether significant differences between covariates existed (Cox 1972). The log-rank test was used to derive p-values. The unwillingness of the farmer to further inseminate an animal or a spontaneously culled cow contributed to a censored observation. All variables were tested for the proportionality assumption based on the cumulative sums of martingale residuals with a Kolmogorov-type supremum test (Lin et al. 1993). Treatment was used as a time-dependent covariate. Regression analysis by the Cox proportional hazards model was used to model days open. Because of missing data, especially of the variable CL (Table 1), 234 observations were finally used for this analysis. The effect of the herd was adjusted in the model. All two way interactions could probably be significant within this model and this hypothesis was tested. None of these interactions proved to have a statistically significant effect and were dropped from the model. The derived hazard function had the following formula:

$$h(t) = h_0(t) \exp\{D_i + S_j + T_k(t) + P_l + U_m + CL_n + ML_o + OV_p + BS_q + TD_r + C_s\}$$

where $h(t)$ = Hazard function of the cow; h_0 = Cox baseline hazard function; t = days open; D_i = fixed effect of TYPEDIS; S_j = fixed effect of SMELL; T_k = time dependent fixed effect of TREAT; P_l = fixed effect of PARITY; U_m = fixed effect of USIZE; CL_n = fixed effect of CL; ML_o = fixed effect of ML100; OV_p = fixed effect of OC; BS_q = fixed

Variable	Stratum	n	Conceived	Percent censored	Median days open	95% Confidence interval	
						Lower	Upper
CL	Absence	91	69	24.2	138	116	163
	Presence	155	119	23.2	122	112	129
Δ BCS	≥ 1	61	41	32.8	185	135	215
	< 1	203	163	19.7	116	109	128
ML100	> 3824 kg	59	45	23.7	159	127	198
	≤ 3824 kg	196	159	18.8	119	111	129
OC	Yes	37	29	21.6	198	153	221
	No	227	175	22.9	118	112	128
PARITY	1	97	80	17.5	114	102	130
	> 1	167	124	25.8	134	122	156
SMELL	Fetid	88	63	28.4	125	105	162
	Neutral	172	137	20.4	127	116	140
TIMED	≤ 21 days	171	132	22.8	125	112	141
	> 21 days	93	72	22.6	129	118	150
TREAT	No	166	128	22.9	129	117	150
	Yes	98	76	22.5	118	105	140
TYPEDIS	Purulent	107	79	26.2	146	116	175
	Mucopurulent	151	119	21.2	122	115	134
USIZE	Oversized	64	48	25.0	145	129	173
	Involution	197	153	22.3	119	112	130

CL, corpus luteum; Δ BCS, BCS decline; ML100, 100d milk production; OC, ovarian cysts; TIMED, time of diagnosis; TREAT, treatment; TYPEDIS, type of discharge; USIZE, size of uterus.

Table 1. Descriptive univariate analyses of the parameters under investigation in German Holstein Friesian cattle diagnosed with CCE using the Kaplan–Meier survival function

effect of ΔBCS ; TD_r = fixed effect of TIMED; C_s = random effect of farm.

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 Inc. 1999).

Results

Univariate analysis using the Kaplan–Meier product limit estimates

The overall per cent of censored observations was 22.7%, comprising 7.9% of the animals without insemination and 14.8% that received at least one insemination. Due to infertility or other illnesses/inadequate production 5.7% and 17% of the cows in total were culled respectively. Animals with purulent vaginal discharge conceived twenty four days later than animals with mucopurulent discharge ($p = 0.01$). No other clinical sign seemed to play a statistically significant prognostic role for the median days open of animals with CCE. A weak positive treatment outcome of eleven days was evident ($p = 0.05$). Animals having OC and an extreme (≥ 1 point) reduction of their BCS had significantly lower rates of conception (both $p = 0.001$). High producers also experienced extended days open ($p = 0.05$).

Building of the Cox proportional-hazards model

In the model building, we tested the variables TYPE-DIS, SMELL, USIZE, TIMED, CL, TREAT, PARITY, OC, ΔBCS and ML100. The final model included the variables TYPEDIS, OC and ΔBCS . Cows with mucopurulent discharge, without ovarian cysts and without marked BCS loss revealed a 1.5-, 2.0- and 1.9-fold daily chance of becoming pregnant in comparison with the animals experiencing purulent discharge, OC and marked BCS loss respectively (Table 2).

Discussion

The negative effect of abnormal vaginal discharge on the fertility of dairy cattle is well documented (Bell and Roberts 2007). This retrospective field study attempted to evaluate the importance of different symptoms of CCE, separately and as a unity, on days open by using time-to-event analysis and an adequate number of untreated animals to identify cows at increased risk of reproductive failure at the time of diagnosis.

From the symptoms of CCE under investigation, statistical differences were only found for the type of

vaginal discharge. Although the structure of the variables was different, this result is in accordance with those of LeBlanc et al. (2002a) who found that a purulent uterine discharge together with a cervical diameter >7.5 cm were the only clinical signs of CCE associated with a reduced pregnancy rate. The results of McDougall (2001) are also consistent, as animals with no or mucoid discharge revealed a better reproductive performance in comparison with those with purulent discharge. A vaginal discharge with high viscosity and moderate proportion of pus in the first month postpartum probably reflects the regeneration of the genital system and a successful immune response (LeBlanc et al. 2002a). Furthermore, a purulent discharge is associated with the presence of *Arcanobacterium pyogenes* (Williams et al. 2005), which is the microorganism with the strongest proof of causing uterine lesions (Bonnett et al. 1991; Azawi et al. 2008). On the other hand, the effect of the smell of vaginal discharge on reproductive performance has rarely been evaluated separately. Williams et al. (2005) 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 (Bonnett et al. 1991). Additionally, a fetid odour was associated with higher bacterial growth densities of streptococci (Williams et al. 2005), which seem to have antagonistic effects on *A. pyogenes* (Bonnett et al. 1991).

A number of studies report a detrimental effect of BCS decline on days open in dairy cattle (Loeffler et al. 1999; Berry et al. 2003). There is evidence that a poor BCS of 2.5 points or a decline of BCS is related to the presence of metritis (Martin et al. 2006; Bell and Roberts 2007). LeBlanc et al. (2002a) found an oversized uterus horn to have a direct significant effect on conception, but only when the clinical findings of vaginoscopy were ignored and transrectal palpation was employed as the method of diagnosis. The importance of a relatively early resumption of cyclicity as a predictor for good reproductive ability has often been emphasized (Bonnett et al. 1993; LeBlanc et al. 2002a). 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 (Lewis 1997) or in a prolonged calving to conception interval (Smith and Wallace 1998). In our study, no significant differences were found for days open.

The impact of a high milk production is a matter of controversy among researchers. An antagonistic genetic (Veerkamp et al. 2001; González-Recio et al. 2006) and phenotypic (Bagnato and Oltenacu 1994) relationship with the reproductive measures have been reported and are generally accepted. Nonetheless, other authors reject the extent of this interaction (Berry et al. 2003) and attribute it either to voluntary management decisions to inseminate high producers later on (Coleman et al. 1985) or to the fact that an earlier pregnancy suppresses milk production through its energy demands (Loeffler et al. 1999). Nevertheless, only a weak negative influence of milk production on days open was apparent.

Table 2. Hazard Ratio and 95% confidence limits of the variables remaining in the Cox proportional hazards model ($n = 234$)

Variable	Chi-square	Hazard ratio	95% Hazard ratio confidence limits		Pr > chi-square
ΔBCS	12.1	1.9	1.3	2.8	0.0005
OC	9.9	2.0	1.3	3.0	0.002
TYPEDIS	5.9	1.5	1.1	2.0	0.01

ΔBCS , BCS decline; OC, ovarian cysts; TYPEDIS, type of discharge.

Regarding the treatment effect on the reproductive performance of animals diagnosed with CCE, controversy exists concerning the time and the type of treatment, and how an outcome should be measured (Hoedemaker 1998). In this study, we decided to control for the effect of treatment as an entity. LeBlanc et al. (2002b) reported an improvement in all treatment effects when a corpus luteum was present and not only with the use of PGF_{2α} as expected. Therefore, they suggested that animals that resolve cyclicity may not further benefit from treatment. Our results also did not support a strong positive treatment effect.

In this study, animals with CCE experienced prolonged calving to conception intervals to a varying extent. A purulent discharge affected the days open of dairy cows. Treatment for CCE seemed to have only a weak effect. Furthermore, the importance of the control of body condition and the prevalence of ovarian cysts was apparent, as both these factors further suppressed the fertility measures of cows with CCE. Although we have to be careful when trying to express cause–effect relationships between these kinds of traits, especially within the scopes of a retrospective field study, it is obvious that the reproductive efficiency of dairy cattle on farm level cannot be guaranteed unless follow-up controls evaluate and confront the risks of not conceiving.

Author contributions

Authors Georgios Tsousis designed and executed the study, Reza Sharifi analysed the data and Martina Hoedemaker designed and supervised the study.

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Submitted: 03 Apr 2009

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