FULL PAPER

Theriogenology

Effect of diagnosis and treatment of clinical endometritis based on vaginal discharge score grading system in postpartum Holstein cows

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ABSTRACT. In this study, the prevalence, effectiveness of diagnosis, and treatment based on vaginal discharge score (VDS) of clinical endometritis in cattle were evaluated. To detect clinical endometritis and classify its severity, vaginoscopy was performed during 21 to 60 days postpartum in 164 Holstein cows consisting of 229 lactations. Groups were defined using the 4-point VDS scale. Study groups included the following: non-endometritis (VDS=0; no/clear mucus; NEM group; n=168); mild endometritis, no treatment (VDS=1; mucus containing flecks of white/off-white pus; NTR group; n=30); and severe endometritis, treated with PGF2α (VDS≥2; discharge containing <50% pus; and VDS=3; discharge containing >50% pus, and fluid or uterine horn asymmetry; TEM group; n=31). Cows treated with PGF2α that did not recover (VDS≥1, n=5) received intrauterine procaine penicillin and streptomycin. Prevalence of clinical endometritis (VDS≥1) was 26.6%. The NTR group required significantly more artificial inseminations per pregnancy than NEM and TEM groups (2.8 ± 1.8 vs 2.0 ± 1.3, 1.9 ± 0.8, *P*<0.05). In survival analysis, the proportion of non-pregnant cows was higher in the NTR group compared to the NEM (*P*=0.012) and TEM (*P*=0.076) groups. In the TEM group, calving to first artificial insemination interval tended to be higher in cows treated 41 to 60 days postpartum than cows treated 29 to 40 days postpartum (97.2 ± 27.1 vs 74.4 ± 19.7, *P*=0.084). Our study suggests that cows with VDS=1 may require treatment to recover fertility. Diagnosis and treatment of clinical endometritis based on a VDS grading system may improve dairy herd reproductive performance.

KEY WORDS: clinical endometritis, diagnosis, fertility, treatment, vaginoscopy

Reproductive performance is a critical factor determining the profitability of dairy enterprises [5]. Regular clinical assessment of the reproductive status of postpartum dairy cows, including the diagnosis and treatment of reproductive disorders, is essential to achieve optimal reproductive performance of dairy herds [21]. Postpartum uterine disease is a well-known cause of reduced reproductive efficiency of cattle [1, 3, 4, 7, 16, 18].

Endometritis occurs in the early postpartum period and is caused by a bacterial infection in the uterine lumen. Clinical endometritis is defined as purulent or mucopurulent uterine discharge detectable in the vagina during approximately 21 to 26 days postpartum [9, 20]. The prevalence of clinical endometritis in dairy cows 15 to 60 days postpartum ranges from 23.6 to 42.6% [3, 4, 10, 16]. Although different treatment methods have been utilized for endometritis, valid diagnostic criteria and optimal treatment timing have not been elucidated [2, 7, 8, 12, 13, 22, 23]. Rectal palpation of the genital tract is the most commonly used clinical method to detect uterine abnormalities due to its simplicity and rapid implementation in the field of veterinary practice. [15]. However, rectal palpation is one of the most insensitive and non-specific methods for detection of endometriosis [3, 11]. Vaginoscopy is an efficient diagnostic tool to diagnose clinical endometritis that is practical for use in field conditions [3, 4, 10, 17]. It is simple, inexpensive, and can be rapidly performed compared to alternative methods such as cytobrush, biopsy, and ultrasound, but is less frequently used than rectal palpation [15].

A vaginoscope, with the aid of a flashlight, is used to visually detect the presence and quality of vaginal discharge [10]. A 4-point scoring system, the vaginal discharge score (VDS), has been used to classify the nature of the vaginal discharge [10, 13, 20, 23]. Although the VDS grading system is well-known as a useful method to diagnose clinical endometritis and to estimate reproductive prognosis [3, 4, 20], information regarding the effectiveness of treatment for clinical endometritis using a VDS grading system under field conditions is lacking and controversial.

Gautam *et al*. [3, 4] studied the prevalence of clinical endometritis and its impact on subsequent reproductive performance in Japanese dairy herds. However, they did not investigate the efficacy of clinical treatments based on the VDS. Moreover, according to the clinical guidelines of the Agricultural Disaster Compensation System of the Ministry of Agriculture, Forestry, and Fisheries of Japan, the initiation date of treatment for cows diagnosed with endometritis is limited to 40 days postpartum or later [14]. Supporting scientific evidence of this limitation is unclear, and the clinical guidelines of this reference have not been revised since 1999. This guideline might cause a delay in the optimal timing of treatment of cows with clinical endometritis. Hence, we needed to clarify whether treatment for cows with endometritis before 40 days in milk (DIM) is effective or not in Japanese dairy herds.

This study was conducted in commercial dairy herds under field conditions. The objectives of this study were 1) to determine the prevalence of clinical endometritis, identified at the postpartum fresh check, and 2) to evaluate the effectiveness of strategic treatment based on a VDS grading system by vaginoscopy on subsequent reproductive performance in Holstein cows.

MATERIALS AND METHODS

Animals and management

The study was conducted using 164 Holstein Friesian cows (total 229 lactations). Cows calved from September 2013 to December 2015, a 27-month-period. The cows were located in four commercial dairy herds in the Fukuoka prefecture, Kyushu, the largest island in southern Japan, where ambient average temperature ranges from a low of 1°C during the winter to a high of 30°C during the summer. The size of the lactating herd varied from 20 to 50 cows. The herds were non-seasonal, milked twice daily, and average milk production varied between 8,100 and 10,900 kg/cow/year. Parity ranged from one to eight. Cows were managed in a free-stall barn, bedded with wood shavings (Herd A), or in a tie-stall barn with rubber mattresses (Herds B, C and D). In Herd A, the behavior of standing to be mounted was considered to be a sign of estrus, and cows were artificially inseminated 8 to 14 hr after standing estrus. In herds B, C and D, estrus detection was based on hyperemia and swelling of the vulva, mucus discharge, bellowing, and restlessness. Cows were inseminated 8 to 14 hr after observing these signs. The voluntary waiting period in each herd was set at 40 days postpartum. Cows were bred by artificial insemination (AI) performed by local technicians. Each herd was visited monthly for a clinical reproductive examination including treatment of reproductive disorders and pregnancy diagnosis. Pregnancy was diagnosed by transrectal palpation or ultrasonography 40 days after the previous insemination. For cows not inseminated within 90 days postpartum, or not pregnant at the time of pregnancy diagnosis, additional investigation and treatment were performed, such as a timed AI program using a combined injection of PGF2α and estradiol.

Clinical examination and treatment of endometritis

A monthly reproductive medical examination was performed from October 2013 to December 2015; it included vaginoscopy and the transrectal palpation of reproductive organs during the fresh check after parturition. In our study, the "fresh check" refers to the first examination of the genital tract after parturition, including the ovaries and uterus. The fresh check was conducted in all cows 21 to 60 days after parturition. First, transrectal palpation of the uterus and ovaries was performed to determine the size, location, and symmetry of the uterine horns and the presence of palpable ovarian structures. Next, the vaginoscopic examination was performed to detect endometritis and to score the appearance of the vaginal discharge. To perform vaginoscopy, the tail was held to one side by the farmer, and the vulva was washed with lukewarm water containing [mono, bis-(methylene trimethyl ammonium chloride)]-alkyl (C9-15) toluene (Pacoma®; 10% solution; Scientific Feed Laboratory Co., Ltd., Tokyo, Japan) at a 1:1,000 dilution and wiped with a clean paper towel. A stainless vaginal speculum (Fujihira Industry Co., Ltd., Tokyo, Japan; length: 270 mm, external diameter: 35 mm) was inserted into the vagina to the external cervical os. The cervix and vagina were visually examined for the presence and quality of discharge with the help of a flashlight as previously described [10]. The VDS was used to classify the discharge: 0 =no or clear mucus; 1 =mucus containing flecks of white or off-white purulent material; 2 =discharge containing less than 50% purulent material; and 3 =discharge containing more than 50% purulent material and a small amount of fluid or asymmetry of the uterine horns detected by transrectal palpation. Findings from rectal palpation and the VDS were recorded for each examination.

Cows classified with a VDS of 2 or above were treated with 25 mg of Dinoprost (PGF2α) by IM injection (Pronalgon®F; Zoetis Japan Co., Ltd., Tokyo, Japan), irrespective of transrectally palpable corpus luteum (CL) status. In contrast, because most cases of mild endometritis recover spontaneously [4], cows with a VDS of 1 or less received no further treatment or reassessment. Cows treated with PGF2α were reassessed using the same methods 27.8 ± 12.8 day after the initial treatment. A clinical cure was confirmed by no or clear mucus discharge (VDS of 0). Of the cows with treated with PGF2α, five were inseminated during a spontaneous estrus prior to re-assessment of VDS; these cows had no or clear mucus discharge confirmed by the farmer and AI technician and were defined as clinically cured. All cows that were not defined as clinically cured were treated with an intrauterine infusion of antibiotics containing an oil mixture of benzyl procaine penicillin (400,000 IU) and dihydrostreptomycin sulfate (400 mg) in 30 m*l* of ointment base (Foamingmycin for endometritis®; Riken Chikusankayaku Co., Ltd., Tokyo, Japan). No further clinical assessment or treatment was conducted after the second treatment. A flowchart of the diagnosis and treatment protocol is shown in Fig. 1.
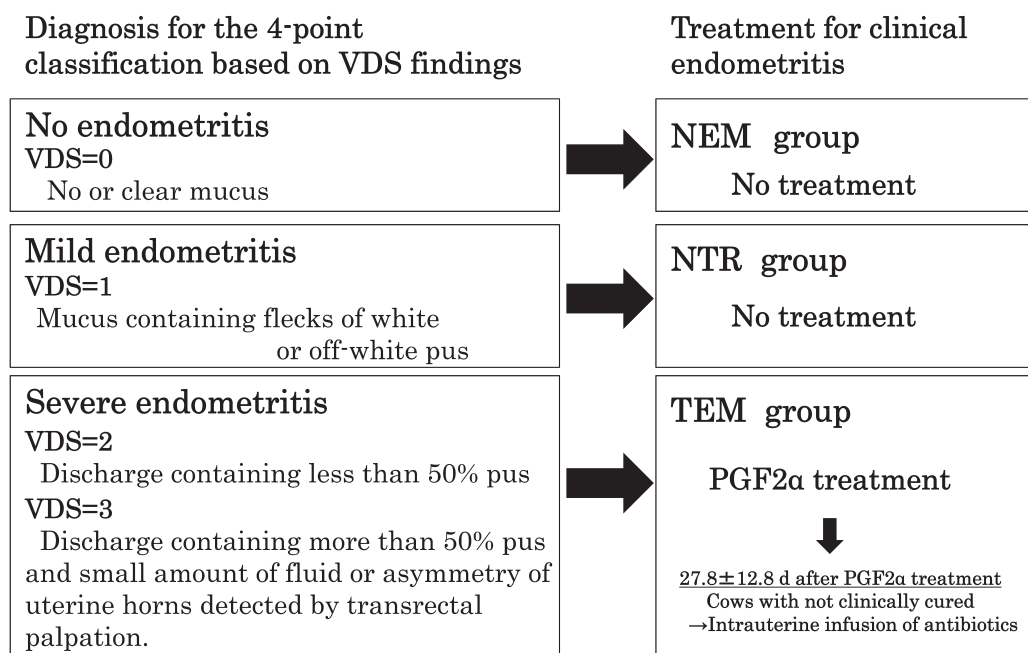
Diagnosis for the 4-point classification based on VDS findings

Treatment for clinical endometritis

| No endometritis
VDS=0
 No or clear mucus | ➡ | NEM group

No treatment |

| Mild endometritis
VDS=1
 Mucus containing flecks of white
 or off-white pus | ➡ | NTR group

No treatment |

| Severe endometritis
VDS=2
 Discharge containing less than 50% pus
VDS=3
 Discharge containing more than 50% pus
and small amount of fluid or asymmetry of
uterine horns detected by transrectal
palpation. | ➡ | TEM group

PGF2α treatment
⬇
27.8±12.8 d after PGF2α treatment
Cows with not clinically cured
→Intrauterine infusion of antibiotics |

Fig. 1. Flow chart of clinical diagnosis and treatment based on the VDS grading system.

Data collection and statistical analysis

Uterine conditions are dynamic during the postpartum period, and uterine involution in dairy cows is often completed by 28 to 40 days postpartum [1, 16, 19]. To investigate the prevalence of endometritis after parturition, the VDS data were analyzed in three DIM groups: 21 to 28, 29 to 40 and 41 to 60 days. In addition, the VDS data were analyzed for the overall DIM range (21 to 60 days). The prevalence of various degrees of endometritis, based on the VDS, was expressed as a percentage of total cows examined during the same period.

To determine the impact of a VDS-based treatment of endometritis on reproductive performance, the cows were divided into three groups: non-endometritis (NEM group, n=168; VDS=0); non-treated, mild endometritis (NTR group, n=30; VDS=1); and treated, severe endometritis (TEM group, n=31; VDS≥2). The mean days postpartum of the three groups (NEM, NTR and TEM) at the time of examination and treatment for clinical endometritis were 39.6 ± 9.1, 37.2 ± 9.4 and 36.1 ± 9.6, respectively; the difference among the three groups was not significant (P=0.84).

To evaluate the efficacy of treatment, data from the TEM group were further subdivided according to the presence or absence of a transrectally palpable CL and according to the timing of treatment (21 to 28, 29 to 40 and 41 to 60 DIM). Animals clinically cured after the initial treatment were also recorded in the TEM group. Of the 31 cows in the TEM group, two were culled before becoming pregnant. Reproductive performance data were compared among the three groups and included the following: calving to first AI (FAI) interval, successful conception after FAI, the number of AI required to become pregnant, and the number of days open. Reproductive performance data were collected until confirmation of pregnancy, culling, or death.

To evaluate the effects of reproductive management on dairy herds under farm conditions, the average monthly change in days open and the proportion of pregnant cows were analyzed.

The results are expressed as the mean ± standard error of the mean (SEM) or as a percentage. Statistical analyses were performed using Ekuseru-Toukei 2012 for Windows, version 1.11 (Social Survey Research Information Co., Ltd., Tokyo, Japan). The results for reproductive parameters of cows with or without a palpable CL in the TEM group were evaluated using the Mann-Whitney nonparametric U-test. The results for reproductive parameters of the three groups (NEM, NTR and TEM) and cows classified according to timing of treatment (21 to 28, 29 to 40 and 41 to 60 DIM) in the TEM group were analyzed using one-way analyses of variance (ANOVA) and Tukey-Kramer's post hoc test. Pregnancy rates and the clinical cure rate were compared using the χ^2 test or Fisher's exact probability test. The data on the proportion of cows from the three groups that did not become pregnant (survival rate) were visualized using Kaplan-Meier survival curves, and the differences were analyzed using a generalized Wilcoxon test. A P value <0.05 was considered statistically significant.

RESULTS

Prevalence of endometritis

The prevalence of endometritis based on the VDS grading system is shown in Table 1. The prevalence in each herd was A: 32.4% (11/34), B: 27.1% (13/48), C: 24.5% (24/98) and D: 26.5% (13/49). The percent of cows with a VDS of 1 or more, 21 to

Table 1. Prevalence of clinical endometritis at 21 to 60 days after parturition in Holstein cows

| Group | VDS | No. of cows classified based on VDS in each interval postpartum at examination (%) | | | |
		21 to 28 days (n=31)	29 to 40 days (n=102)	41 to 60 days (n=96)	Overall (n=229)
NEM	0	18 (58.1)	76 (74.5)	74 (77.1)	168 (73.4)
NTR	1	6 (19.3)	13 (12.8)	11 (11.5)	30 (13.1)
TEM	2	3 (9.7)	9 (8.8)	8 (8.3)	20 (8.7)
	3	4 (12.9)	4 (3.9)	3 (3.1)	11 (4.8)

VDS: vaginal discharge score. NEM: non-endometritis (VDS=0), NTR: non-treated, mild endometritis (VDS=1), TEM: treated, severe endometritis (VDS=2,3).

Table 2. Reproductive performance outcomes in the three groups treated for the clinical endometritis based on VDS grading system for vaginoscopy in Holstein cows

Reproductive performance	NEM group	NTR group	TEM group
Parity	2.4 ± 1.3 (n=168)	2.5 ± 1.5 (n=30)	3.0 ± 1.2 (n=31)
Days to FAI	82.6 ± 36.0 (n=165)	89.3 ± 32.9 (n=29)	85.1 ± 26.2 (n=31)
Conception of FAI	78/164 (47.6%)	9/29 (31.0%)	12/31 (38.7%)
No. of AI per pregnancy	2.0 ± 1.3[a] (n=159)	2.8 ± 1.8[b] (n=28)	1.9 ± 0.8[a] (n=29)
Days open	129.5 ± 76.7 (n=159)	155.3 ± 61.3 (n=28)	133.4 ± 66.5 (n=29)

NEM: non-endometritis (VDS=0), NTR: non-treated, mild endometritis (VDS=1), TEM: treated, severe endometritis (VDS=2,3). a,b: Significant difference ($P<0.05$) between columns.

Table 3. Clinical curing rates (%) and reproductive performance outcomes related to palpable CL or not at treatment and postpartum days at the time of treatment in TEM group in Holstein cows

| Reproductive performance | Palpable CL at treatment | | Postpartum days at treatment | | |
	Present	Absent	21 to 28 days	29 to 40 days	41 to 60 days
Clinical cure rate	22/26 (84.6%)	4/5 (80.0%)	4/7 (57.1%)	13/13 (100%)	9/11 (81.8%)
Calving to FAI interval	87.4 ± 26.5 (n=26)	84.4 ± 26.6 (n=5)	86.1 ± 30.2 (n=7)	74.4 ± 19.7 (n=13)	97.2 ± 27.1 (n=11)
Conception of FAI	11/26 (42.3%)	1/5 (20.0%)	1/7 (14.3%)	4/13 (30.8%)	6/11 (54.5%)
No. of AI	1.8 ± 0.9 (n=24)	2.0 ± 0.7 (n=5)	2.3 ± 1.0 (n=6)	1.9 ± 0.8 (n=13)	1.5 ± 0.7 (n=10)
Days open	131.3 ± 68.5 (n=24)	146.6 ± 61.7(n=5)	183.7 ± 111.6 (n=6)	119.5 ± 38.0 (n=13)	123.0 ± 53.3 (n=10)

28, 29 to 40, and 41 to 60 days postpartum, was 41.9% (13/31), 25.5% (26/102), and 22.9% (22/96), respectively. The overall prevalence of endometritis within the 60-day postpartum period was 26.6% (61/229). Thirty-one cows with a VDS of 2 or more were treated with PGF2α.

Effect of treatment of clinical endometritis categorized using a VDS grading system on reproductive performance

Reproductive performance of cows in three groups is shown in Table 2. Although conception rates, days to FAI, and days open were not different among the groups, the NTR group required a significantly higher number of AI to become pregnant as compared to either the NEM or TEM groups ($P=0.014$ and $P=0.032$, respectively).

Survival curves of the three groups are shown in Fig. 2. Pregnancy rates within 150 days postpartum were 69.6% (117/168), 64.5% (20/31), and 50.0% (15/30) in the NEM, NTR and TEM groups, respectively. In the survival analysis, the proportion of non-pregnant cows in the NTR group was significantly higher as compared to the NEM groups ($P=0.012$), and also tended to be higher than the TEM group ($P=0.076$).

Table 3 shows the clinical cure rates and reproductive performance of cows with or without a palpable CL and according to the timing of the treatment. The proportion of clinically cured cows was 83.9% (26/31). There was no association among the proportion of clinical cures, the presence or absence of a palpable CL, or the timing of treatment with PGF2α. The calving to FAI interval tended to be longer in cows treated with PGF2α at 41 to 60 days postpartum as compared to cows treated 29 to 40 days postpartum ($P=0.084$). There were no differences between groups in the status of the CL, timing of PGF2α, conception after FAI, number of inseminations, or number of days open.

The average monthly change in mean days open and the proportion of pregnant cows were not significantly different over time (Fig. 3). There was a similar variation in the proportion of pregnant cows per farm every 12 to 14 months. After the implementation of the VDS grading system at the fresh check, there was a tendency towards an increase in the proportion of pregnant cows and a decrease in number of days open.
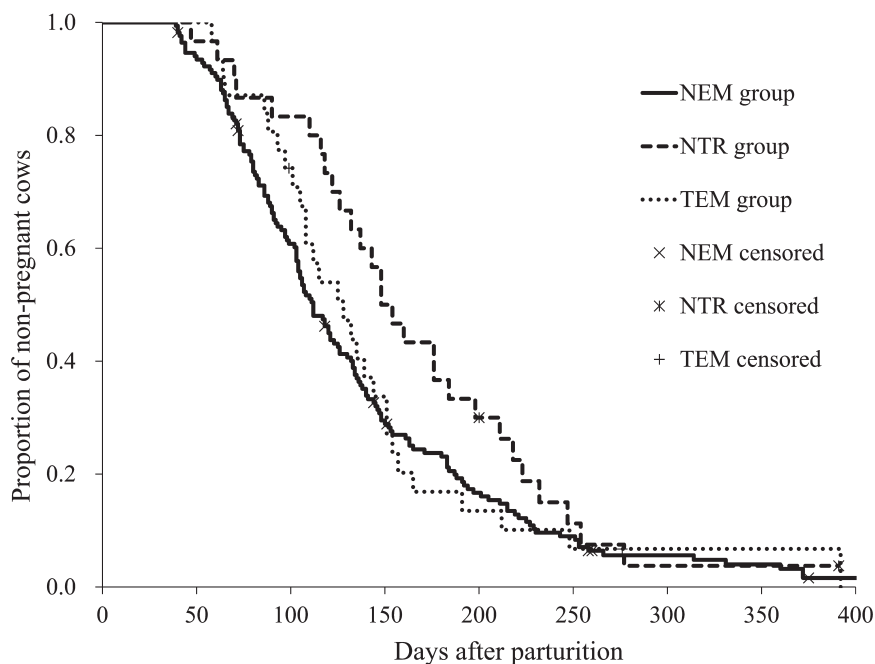
Fig. 2. Kaplan-Meier survival curves for the proportion of open cows in the three treatment groups. NEM group (VDS=0, no endometritis): n=168; median days open=112; 5.4% (n=9) censored. NTR group (VDS=1, non-treated, mild endometritis): n=28; median days open=148; 7.1% (n=2) censored. TEM group (VDS≥2, treated, severe endometritis): n=29; median days open=128; 6.9% (n=2) censored.
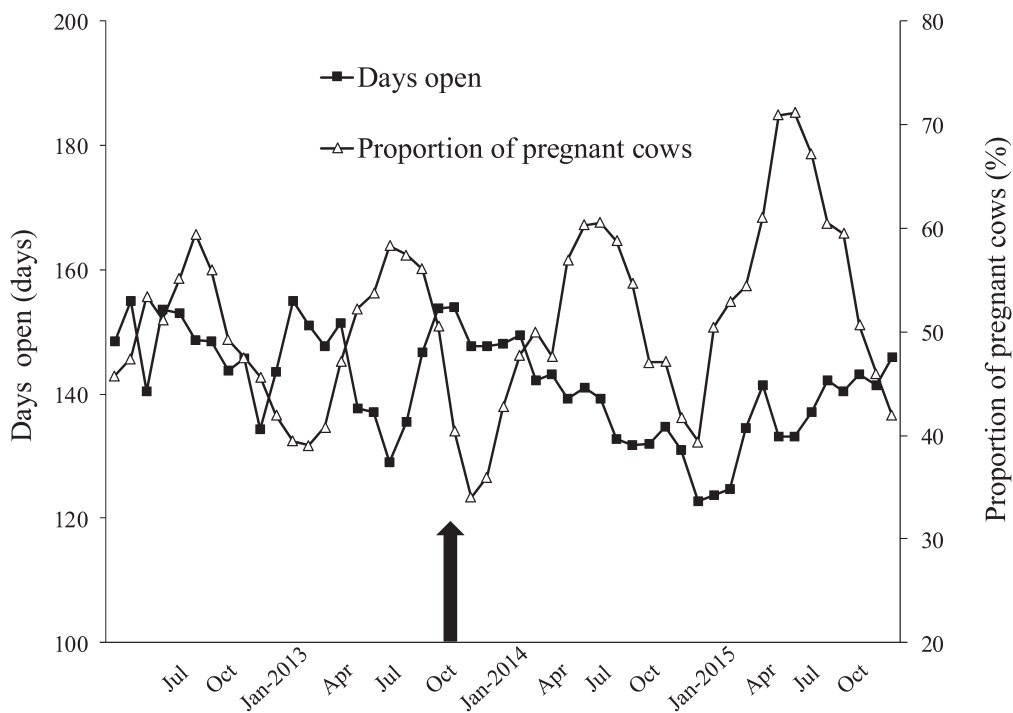
Fig. 3. Mean open period and proportion of pregnant cows in each month in four dairy farms. The arrow (↑) indicates the beginning of reproductive management at fresh check, including the vaginal discharge scoring system.

DISCUSSION

The main objectives of the present study were to investigate the prevalence of clinical endometritis and to determine the efficiency of treatment of cows diagnosed with endometritis based on a VDS grading system for vaginoscopy under field

conditions. We demonstrated that cows treated with PGF2α by clinical endometritis recovered and regained fertility more quickly; their recovery and fertility were comparable to cows without endometritis (VDS of 0). Furthermore, early treatment (29 to 40 days postpartum) may have had a positive effect in the management of dairy herds. In contrast, cows that did not receive treatment for mild endometritis (VDS of 1) showed lower reproductive efficiency.

Uterine infection within one week of parturition is identified in 40% of dairy cows. The infection rate decreases over time, and approximately 15 to 20% of cows develop clinical endometritis [1, 20]. In two field studies in Japanese dairy herds, Gautam *et al.* [3, 4] reported that the prevalence of clinical endometritis within 60 d postpartum was 32.6% (44/135) and 23.6% (104/441). In the present study, the prevalence of clinical endometritis 21 to 60 days postpartum was 26.6% (61/229), which is comparable to that reported by Gautam *et al.* [3, 4]. A previous study suggested that the presence of mild endometritis with a VDS of 1 may not have an adverse effect on reproductive performance, and that a small amount of vaginal discharge in the early postpartum period may be part of the physiologic self-cleaning process [4, 7]. It is unclear if cows with mild endometritis should be treated [4, 10, 12]. On the basis of previous reports [4, 10], we assumed that cows with mild endometritis had the ability to spontaneously recover. However, we observed that this group of cows had evidence of negative reproductive performance, such as an increase in the number of inseminations as compared to the other two groups. Thus, cows with a VDS of 1 may have had persistent mild endometritis, which impaired fertility. Our study found no difference in reproductive performance between the NEM and TEM groups. These data suggest that cows with mild endometritis require treatment to recover uterine condition and fertility, similar to cows with severe endometritis.

Vaginoscopy has been shown to have a relatively high specificity (87–96%) as compared to metricheck and ultrasound analyses (62–78%). The sensitivity of vaginoscopy is moderate (54–72%) [1]; visual assessment of VDS using a flashlight in this study may cause the error of defining a VDS of 1 or above as a 0 [10]. However, we reassessed cows with severe endometritis alone in the TEM group, and did not reassess cows with mild endometritis in the NTR group. Gautam *et al.* [4] reported that 10.6% (31/335) of cows with no endometritis on vaginoscopy during 15 to 60 days postpartum developed endometritis beyond 60 days postpartum. The cows with mild or no enodmetritis in this study might have shown severe endometritis if the second vaginoscopic examination was performed in the NEM, NTR group; hence, reassessment of uterine condition using vaginoscopy might be required in the cows with mild or no endometritis during 21 to 60 days postpartum. The definitive diagnostic method for endometritis, including subclinical disease, is a cytological assessment demonstrating the percentage of polymorphonuclear neutrophils (PMNs) at 35 to 40 days postpartum. This is because >5% PMNs generally indicates impaired reproductive performance [1]. In addition, a decrease in bacterial infection and the percentage of PMNs is reflected in a reduction of the VDS [13]. Although cytology is a very useful method for diagnosing endometritis, this method is less suitable for clinical practice because it is more costly and time consuming. Therefore, we must consider that some cows classified as having no or mild endometritis by vaginoscopy may in fact have had subclinical endometritis. Although previous reports determined that vaginoscopy is not a perfect diagnostic method for clinical endometritis, it may be better suited as a field diagnostic method in clinical practice [3, 4, 10]. In clinical practice in the field, there are no criteria available to determine if clinical endometritis should be treated. The present study suggests that mild endometritis, with a VDS of 1 based on vaginoscopy, requires reassessment and treatment to recover uterine health and fertility.

In the present study, we administered PGF2α as an initial treatment for cows with clinical endometritis, irrespective of their CL status. The proportion of subjects with clinically cured endometritis was 83.9% (26/31). This is higher than the proportion reported by previous studies with cure rates of 77 and 81.1% [9, 12]. We reassessed the VDS 29.1 ± 12.8 days after initial treatment, which is longer than the duration reported by a previous study (approximately 14 days after treatment) [2, 9, 12]. This difference may have influenced the clinical cure rate. At the time of reassessment, the cows that were not clinically cured were given a second treatment of intrauterine antibiotic infusion. Intrauterine infusion of antibiotics as the first treatment requires a 24-hr milk withdrawal period in Japan, and is associated with development of antimicrobial resistance [6]. Conversely, the administration of PGF2α does not require a milk withdrawal period and is not associated with the emergence of resistant bacteria. Strategic treatment with PGF2α during the early postpartum period efficiently treats endometritis and improves reproductive performance by accelerating uterine involution and luteolysis [8, 22]. However, Dubuc *et al.* reported that two treatments of PGF2α (administered 35 and 49 days postpartum) for endometritis diagnosed by cytological assessment did not improve reproductive performance [2]. Thus, it might be possible that only the severe cases of endometritis, and not the mild cases, benefit from treatment.

In the present study, the status of a palpable CL and the timing of the treatment did not affect the proportion of clinical cures. A previous report suggested that treatment of endometritis with PGF2α is successful if the cow has a CL (with a high progesterone concentration in the milk) at the time of treatment [17]. LeBlanc *et al.* [9] reported that cows without a palpable CL during 20 to 26 days postpartum should not be treated with PGF2α; rather, these cows should receive treatment for endometritis beyond 28 days postpartum. In contrast, Hirsbrunner *et al.* [7] reported that combined treatment of PGF2α and prostaglandin E during 21 to 35 days postpartum had a positive effect on the number of days to FAI for cows with endometritis but without a CL. Thus, the efficacy of PGF2α treatment for cows with endometritis is unclear and controversial. In the present study, although we demonstrated that PGF2α treatment during 29 to 40 days postpartum tended to shorten the calving to FAI interval as compared to treatment during 41 to 60 days postpartum, we could not clarify whether the status of CL influenced the clinical cure rate and reproductive performance after PGF2α treatment. In addition, the second treatment of five cows with severe endometritis using intrauterine antibiotic infusion in the TEM group might have a positive influence on the reproductive performance. Ovarian status and postpartum days in dairy cows are strongly associated and influence each other [17, 21]; consequently, further studies using stratified analysis are needed to avoid confounding. Senosy *et al.* [17] reported that diagnostic methods such as cytology, metricheck, and vaginoscopy were influenced by ovarian status and uterine conditions (involution, tonicity, flatus, and flaccidity). Therefore, the diagnosis and

treatment of cows with endometritis should be conducted under field conditions based on ovarian status, uterine condition, and VDS. The routine use of ultrasonography may improve reproductive efficacy and may increase the accuracy of identifying a CL and uterine abnormalities [21].

To the best of our knowledge, this is the first study on long-term regular reproductive practices, including clinical diagnosis and treatment based on the VDS grading system using vaginoscopy, which is useful and suitable for routine reproductive practice, and has a beneficial effect on the reproductive performance of dairy cows under field conditions. Conversely, cows with mild endometritis that were not treated showed a negative reproductive progression. Since the cows with mild endometritis in this study were left untreated, further controlled studies are needed to confirm whether treatment of such cows improve their reproductive performances or not. To establish a gold standard for the diagnosis and treatment of endometritis in clinical field practices, further studies are needed that consider the costs, time, required skill, and invasiveness of the method, as well as ovarian and uterine conditions.

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