Effect of intrauterine dextrose or antibiotic therapy on reproductive performance of lactating dairy cows diagnosed with clinical endometritis

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ABSTRACT

The objectives of this study were to assess the responses to treatments (clinical cure and cow survival 14 d posttherapy) of cows with clinical endometritis (CE) that received intrauterine infusion of a hypertonic solution of 50% dextrose (DEX) or subcutaneous ceftiofur crystalline free acid (CCFA) and subsequent pregnancy per artificial insemination (P/AI) in cows with CE compared with cows without CE. Cows ($n = 760$) from 2 dairy herds were screened for CE using vaginoscopy and measurement of cervix diameters [exam 1; 26 ± 3 d in milk (DIM)]. Cows with vaginal discharge scores of 2 or 3 (scale 0–3) were stratified by parity and randomly allocated into 1 of 3 treatment groups: (1) intrauterine infusion (~ 200 mL) of 50% DEX solution ($n = 79$); (2) 6.6 mg/kg single-dose of subcutaneous administration of CCFA ($n = 75$); or (3) untreated control animals (CON, $n = 83$). Fourteen days posttherapy (at 40 ± 3 DIM), cows with CE were re-examined (exam 2; 40 ± 3 DIM) to assess the response to treatments. All cows were presynchronized with 2 injections of PGF_{2 α} given 14 d apart (starting at 26 ± 3 DIM) followed by Ovsynch (OV; GnRH-7 d-PGF-56 h-GnRH 16 h-timed-AI) 12 to 14 d later. Cows displaying signs of standing estrus any time during the protocol were inseminated, whereas the remaining cows were subjected to timed AI 16 h after the second GnRH of OV. Pregnancy diagnosis was performed via transrectal ultrasonography at 39 ± 3 d post-AI followed by pregnancy reconfirmation 30 d after the first pregnancy diagnosis. Uterine swabs revealed that *Arcanobacterium pyogenes* and *Escherichia coli* were the most predominant bacteria isolated at the time of treatments. Mortality within 14 d posttherapy was not different among treatment groups. Cows with CE had greater cervical diameter at exam 1 and decreased P/AI compared with cows without CE. Treatment with CCFA or DEX increased the proportion of

cows with clear vaginal discharge (score 0; clinical cure) 14 d posttherapy compared with CON cows. Pregnancy per AI from DEX ($29.8 \pm 4\%$) cows tended to differ from that of CON ($21.1 \pm 4\%$) or CCFA cows ($19.7 \pm 4\%$), but it resulted in similar P/AI as those cows without CE ($39.1 \pm 2\%$). The use of intrauterine DEX alone or as an adjunct of antibiotic therapy for the treatment of CE needs further investigation.

Key words: clinical endometritis, intrauterine dextrose, antibiotic, dairy

INTRODUCTION

Postpartum uterine diseases such as metritis and clinical endometritis (CE) are common disorders of lactating dairy cows that negatively affect reproductive performance (LeBlanc et al., 2002a; Gilbert et al., 2005), thus diminishing profitability of dairy operations (Overton and Fetrow, 2008). Metritis is defined as the inflammation of all layers of the uterus and is characterized by fetid red-brown uterine discharge with systemic signs of illness (i.e., fever and decreased milk yield and DMI) usually within 21 d postpartum (Sheldon et al., 2009; Dubuc et al., 2011a). Endometritis is defined as the inflammation of the endometrial lining of the uterus characterized clinically by mucopurulent or purulent vaginal discharge (or cytologically as endometrial inflammation) occurring 21 to 40 DIM (LeBlanc et al., 2002a; Gilbert et al., 2005; Dubuc et al., 2011a). Risk factors such as hygiene of the perineum at the time of calving (Schuenemann et al., 2011), peripartum metabolic status (Földi et al., 2006; LeBlanc, 2008; Konyves et al., 2009), parity (Dubuc et al., 2010b), retained fetal membranes (Paisley et al., 1986; LeBlanc et al., 2002a; Sheldon et al., 2009), delivery of twins (Földi et al., 2006), and dystocia (Paisley et al., 1986; Földi et al., 2006) have all been associated with CE in lactating dairy cows. Moreover, CE has been shown to contribute to ovarian dysfunction (e.g., smaller follicle size, lower plasma estradiol, and prolonged luteal phase; Sheldon et al., 2009), poor reproductive performance (LeBlanc et al., 2002a; Dubuc et al., 2010a), increased

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risk of culling due to reproductive failure (LeBlanc et al., 2002a,b), and reduced milk yield (Konyves et al., 2009; Dubuc et al., 2011b).

Although administration of PGF_{2α} (Heuwieser et al., 2000; Kasimanickam et al., 2005; Galvão et al., 2009b; Dubuc et al., 2011a) is often recommended to treat CE, conflicting data exist in the literature regarding the potential reproductive benefits. Antibiotic such as cephalixin is frequently prescribed to treat cows with CE (LeBlanc et al., 2002b) or vulvar discharge >13 d postpartum (McDougall, 2001) in conventional dairy herds. Ceftiofur sodium (Drillich et al., 2001), ceftiofur hydrochloride (Chenault et al., 2004; Kasimanickam et al., 2010), and ceftiofur crystalline free acid (CCFA; McLaughlin et al., 2010; Stanisiewski et al., 2010) have been shown to be effective (resulting in clinical recovery) for treatment of metritis. The use of CCFA, a long-acting antimicrobial, is currently approved for the treatment of respiratory disease in dairy cattle. It has been shown that after a single subcutaneous administration of 6.6 mg/kg of CCFA, desfuroylceftiofuracetamide (an active metabolite of CCFA) could be detected in concentrations above the reported MIC₉₀ in serum, endometrium, and lochia for relevant uterine pathogens such as *Escherichia coli* and *Arcanobacterium pyogenes* over a period of 7 d (Witte et al., 2011). Furthermore, a clinical field study found that administration of CCFA reduced the incidence of metritis in cows at high risk for uterine diseases, such as those experiencing dystocia or delivery of twins, but without retained fetal membranes (Dubuc et al., 2011a).

An in vitro study showed that mannose (a sugar monomer) inhibited the adhesion of bacteria such as *E. coli* to cultured equine endometrial epithelial cells (King et al., 2000). In addition, the use of hypertonic sucrose solutions inhibited bacterial growth such as *E. coli* from infected human wounds (Chirife et al., 1983). The emergence of multidrug resistance in *A. pyogenes* and *E. coli*, important pathogens associated with uterine infections (Sheldon and Dobson, 2004; Williams et al., 2005), has been reported in dairy cows suffering from metritis (Santos et al., 2010). Therefore, the use of pharmaceutical nonantibiotic therapy such as hypertonic solution (e.g., 50% dextrose in water) may be a viable and effective strategy for cows diagnosed with CE.

The first objective of this study was to determine the effect of CE on the reproductive performance of lactating dairy cows. The hypothesis was that CE would be detrimental to pregnancies per AI (P/AI) compared with cows without CE. The second objective was to compare the response to treatments (clinical cure and cow survival 14 d posttherapy) in lactating dairy cows diagnosed with CE and treated with an intrauterine in-

fusion of a hypertonic solution of 50% dextrose (DEX) or subcutaneous single-dose administration of CCFA compared with untreated control cows. The hypothesis was that the administration of CCFA or intrauterine DEX infusion would reduce the incidence of CE and improve clinical cure in lactating dairy cows with CE. The third objective was to determine the effect of intrauterine DEX or subcutaneous CCFA treatments in cows with CE on P/AI compared with untreated cows or cows without CE. The hypothesis was that CE would be detrimental to P/AI, but treatment with DEX or CCFA would improve P/AI in lactating dairy cows.

MATERIALS AND METHODS

Animals, Facilities, and Feeding Management

In total, 833 lactating Holstein cows (255 primiparous and 578 multiparous) from 2 commercial dairy farms located in Ohio were used in this study. Briefly, cows were housed in freestall barns and milked thrice daily at approximately 8-h intervals. The rolling herd average milk production was 10,262 kg, and the reported voluntary waiting period was 60 d. Cows were fed twice daily, in the morning and afternoon, with a TMR formulated to meet or exceed dietary nutritional requirements for lactating dairy cows (NRC, 2001). This study was conducted from September 2009 through September 2010. The procedures described below were reviewed and approved by the Institutional Animal Care Use Committee, The Ohio State University.

Diagnosis of CE, Ovarian Structures, and Treatments

Weekly, a list of cows was obtained based on their calving dates using on-farm computer records (Dairy-Comp 305, Valley Agricultural Software, Tulare, CA). Briefly, cows at 26 ± 3 DIM were sorted upon exiting the milking parlor and placed into a palpation rail for the diagnosis of CE using vaginoscopy technique and measurement of cervix diameters (cm) by ultrasonography (Kasimanickam et al., 2004; Silvestre et al., 2009). Once in the palpation rail, the uterus was massaged via transrectal palpation, the vulva was wiped off with paper towel, and a single-use vaginal speculum was introduced through the vulva. Using a light source (Mini-Maglite, Ontario, CA), the vaginal vault and cervical os were visualized and the discharge scored (vaginoscopy technique) at first gynecological examination (exam 1; Figure 1). The vaginal discharge was scored using a 0 to 3 scale (0 = normal uterine discharge, 1 = flakes of purulent exudates in the uterine discharge, 2 = >50% of the uterine discharge is made up of purulent exudates,

Table 1. Prevalence (%) of clinical endometritis (CE) at exam 1 (26 ± 3 DIM) of lactating Holstein cows ($n = 833$) using vaginoscopy scoring technique and measurement of cervical diameter by ultrasonography

Parameter	Exam 1 ¹
Clinical vaginal discharge	
0 (clear mucus)	42.9 (n = 357)
1 (mucus with flecks of pus)	26.7 (n = 223)
2 (mucopurulent)	22.5 (n = 187)
3 (brown-red foul)	7.9 (n = 66)
Cervical diameter (cm)	
<4	45.5 (n = 379)
4.0–5.0	37.3 (n = 311)
≥5.1	17.2 (n = 143)

¹Lactating dairy cows ($n = 833$) were screened for CE at 26 ± 3 DIM (exam 1) using vaginoscopy technique. The vaginal discharge was scored using a 0–3 scale (0 = normal uterine discharge, 1 = flakes of purulent exudates in the uterine discharge, 2 = >50% of the uterine discharge is made up of purulent exudates, 3 = hemorrhagic uterine discharge mixed with purulent exudates (adapted from Williams et al., 2005; Sheldon et al., 2006). Clinical endometritis was defined as any cow presenting a score of 2 or 3 (mucopurulent or worse vaginal discharge) at the time of exam 1.

3 = hemorrhagic uterine discharge mixed with purulent exudates (adapted from Williams et al., 2005; Sheldon et al., 2006). Clinical endometritis was defined as any cow presenting a score of 2 or 3 (mucopurulent or worse vaginal discharge) at the time of exam 1. Cervix diameters at exam 1 (26 ± 3 DIM) were classified as <4.0, 4.0–5.0, or >5.0 cm (Tables 1 and 2).

Immediately before the vaginoscopy examination, rectal temperatures (°C; DeltaTRAK, Pleasanton, CA) were recorded. The presence or absence of ovar-

ian structures such as corpus luteum (CL), follicles (> 3mm), or cysts were recorded via transrectal ultrasonography (Aloka 500, Tokyo, Japan) at exam 1 and at the second gynecological examination (exam 2; Figure 1). The presence of cysts was defined as a follicle-like structures >25 mm in diameter (Stevenson and Tiffany, 2004). Additionally, cows had their BCS (scale 1–5; Ferguson et al., 1994) recorded at calving and at 26 ± 3 DIM (Figure 1).

Cows with CE (uterine discharge scores of 2 or 3) were stratified by parity and randomly allocated into 1 of 3 treatment groups: (1) intrauterine infusion (~200 mL) of a 50% dextrose solution (DEX, $n = 79$; Vedco, St. Joseph, MO); (2) 6.6 mg/kg single-dose of subcutaneous administration of CCFA at the base of the ear (CCFA, $n = 75$; Excede, Pfizer Animal Health, New York, NY); or (3) untreated control cows (CON, $n = 83$; no placebo was used). The intrauterine infusions were performed using individually wrapped, single-use infusion pipettes (Continental Plastic, Delavan, WI). Two veterinarians from the research team conducted the clinical examinations and one veterinarian administered the treatments (DEX or CCFA) according to their routes of administration (intrauterine or subcutaneous). Veterinarians responsible for clinical examinations were blinded to allocation groups and the nature of treatments.

Currently, no antimicrobial agent is approved in the United States for treatment of CE in dairy cattle. The

Figure 1. Scheme of the experimental design. Lactating dairy cows ($n = 833$) were screened for clinical endometritis (CE) at 26 ± 3 DIM using vaginoscopy technique (exam 1). Cows diagnosed with CE (score 2 or 3) were randomly assigned into 1 of 3 treatment groups: (1) intrauterine infusion (~200 mL) of a 50% dextrose solution (DEX; $n = 79$); (2) 6.6 mg/kg single-dose of subcutaneous ceftiofur crystalline free acid at the base of the ear (CCFA; $n = 75$); or (3) untreated control cows (CON; $n = 83$). Cows with CE were subjected to a second gynecological exam (exam 2) 14 d later to assess the response to treatments (clinical cure). All cows (with and without CE) were subjected to the same reproductive program [Presynch followed by Ovsynch plus heat detection (HD)]. PG = prostaglandin, G = GnRH, and TAI = timed AI. Pregnancy diagnosis (PD) was performed via transrectal ultrasonography 39 ± 3 d post-AI and reconfirmation of pregnancy (RP) was made 30 ± 3 d after the first PD.

Table 2. Prevalence (%) of clinical endometritis (CE) at exam 2 (40 ± 3 DIM) in lactating Holstein cows using vaginoscopy scoring technique and measurement of cervical diameter by ultrasonography

Parameter	Exam 2 ¹			P-value
	CON (n = 83)	DEX (n = 79)	CCFA (n = 75)	
Clinical uterine discharge				
0 (clear mucus)	24.53 ^b	44.68 ^a	41.30 ^a	0.02
1 (mucus with flecks of pus)	33.96	27.66	21.74	
2 (mucopurulent)	32.08	21.28	28.26	
3 (brown-red foul)	9.43	6.38	8.70	
Cervical diameter (cm)				
<4	62.50	66.02	65.79	
4.0–5.0	31.25	29.10	23.68	
≥5.1	6.25	4.88	10.53	

^{a,b}Values with different superscript letters within a row differ significantly at $P < 0.05$.

¹Cows diagnosed with CE (at exam 1) were screened 14 d posttherapy (at exam 2) to determine the response to treatments based on vaginoscopy technique (Figure 1). A positive response to treatments (clinical cure) was defined as a cow with CE at exam 1 (26 ± 3 DIM) that scored 0 (clear mucus) at exam 2 (40 ± 3 DIM). Cows with CE were randomly assigned to 1 of 3 treatment groups: (1) intrauterine infusion (~200 mL) of a 50% dextrose solution (DEX; n = 79); (2) 6.6 mg/kg single dose of subcutaneous ceftiofur crystalline free acid at the base of the ear (CCFA; n = 75); or (3) untreated control cows (CON; n = 83).

administration of CCFA is labeled for treatment of respiratory disease in lactating dairy cows in the United States. The use of cephapirin (Metricure, Intervet International, Boxmeer, the Netherlands), labeled for treatment of CE in other countries, is not approved in the United States. Administration of prostaglandin (Dubuc et al., 2011a) or intrauterine infusion of ceftiofur hydrochloride (Galvão et al., 2009a) has been reported unsuccessful for the treatment of CE. A single subcutaneous dose of 6.6 mg/kg of CCFA to postpartum dairy cows resulted in concentration above the reported MIC₉₀ in endometrium and lochia for uterine pathogens (*A. pyogenes* and *E. coli*) over a period of 7 d (Witte et al., 2011) and was reported to decrease the incidence of metritis (Dubuc et al., 2011a). Therefore, subcutaneous administration of CCFA was investigated in an extra-label manner to treat lactating dairy cows with CE in the present study using the labeled dose and route of administration. Dairy producers and veterinarians must be aware that CCFA is not approved at this time for treatment of cows with metritis or CE in the United States.

Cows with CE were screened 14 d posttherapy (at exam 2) to assess the response to treatments (clinical cure) based on vaginoscopy technique (Figure 1). A positive response to treatments (clinical cure) was defined as a cow with CE at exam 1 (26 ± 3 DIM) that subsequently scored 0 at exam 2 (40 ± 3 DIM). Assuming that the administration of DEX or CCFA would improve the clinical cure of cows with CE (proportion of cows that scored 0 at exam 2) by 20 percentage points (e.g., from 20 to 40%) compared with CON, 75 cows per group were needed to be able to detect statis-

tical significance with 95% confidence and 80% power. In addition, the proportion of cows that died within 14 d posttherapy was assessed to determine the effect of treatments on cow survival.

Progesterone Radioimmunoassay

Blood samples (10 mL) for determination of blood progesterone (P₄) level were collected at 26 ± 3 and at 40 ± 3 DIM by coccygeal venipuncture (BD Vacutainer, Franklin Lakes, NJ) immediately before the administration of treatments to determine cyclicity status of cows (Stevenson et al., 2006). Cows were classified as cycling when the concentrations of P₄ from either blood sample was ≥ 1 ng/mL (high P₄; high-high, low-high, or high-low; Stevenson et al., 2006). Noncycling cows were identified when serum concentrations of P₄ from both blood samples were < 1 ng/mL (low P₄; low-low; Stevenson et al., 2006). Briefly, blood samples were centrifuged at $2,785 \times g$ for 20 min immediately after collection, and serum samples were stored at -20°C until assayed for P₄. Serum concentration of progesterone were determined in duplicates using a modified commercially available RIA kit (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA) as described previously (Burke et al., 2003). The intra- and interassay CV were 8.8 and 10.3%, respectively.

Breeding Management

For first postpartum services, cows were presynchronized with 2 intramuscular injections of PGF_{2 α} (25 mg, Lutalyse, Pfizer Animal Health) administered 14

d apart at 26 ± 3 and 40 ± 3 d postpartum (Figure 1). Twelve or 14 d after the second injection of PGF_{2α}, Ovsynch (OV; Pursley et al., 1995; Brusveen et al., 2008) was initiated for all cows. The initial GnRH dose (100 µg; Cystorelin, Merial, Duluth, GA) of OV was followed 7 d later by an injection of PGF_{2α} and 56 h later, cows received the second dose of GnRH followed by timed AI (TAI; 16 h after the second GnRH injection of OV; Figure 1). Following the first GnRH of OV, estrus was detected once daily and all animals presenting signs of standing estrous behavior received AI. Animals that did not display estrous behavior during the synchronization protocol were subjected to TAI 72 h after the PGF_{2α} injection of OV (Figure 1). Additionally, open cows at the time of pregnancy diagnosis were re-enrolled in an OV program as described previously. Pregnancy diagnosis (PD) was made 39 ± 3 d post-AI via transrectal ultrasonography and reconfirmation of pregnancy was made approximately 30 d after the first PD (69 ± 3 d post-AI; Figure 1).

Bacterial Growth and Identification

Uterine swab samples were collected from a subset of cows ($n = 178$) with CE for bacteriology. Briefly, individually wrapped and double guarded sterile equine swabs (Continental Plastics) were used to collect uterine samples for bacteriology. The swabs are packaged inside a sterile plastic wrapper and guarded with an outer sterile plastic sheath that is capped with a sterile, perforated rubber tip. The 3-piece swab was introduced through the vagina by parting the vaginal lips to avoid contact with the outside of the vulva. The swab device was advanced through the external cervical os and into the uterine body. The sterile swab was exposed to the uterine wall to collect the sample for bacteriology. The swab was retracted into the inner capped tube protecting it from contamination while it was removed from the uterus and vagina.

Immediately after collection, samples were placed into Stuart's transport medium (Becton Dickinson) and immediately transported to The Ohio State University Veterinary Medical Center Microbiology Laboratory. Swabs were plated on trypticase soy agar with 5% sheep blood (Becton Dickinson) and MacConkey agar plates (Becton Dickinson) followed by incubation at 35°C under aerobic conditions. Cultures were incubated for 72 h. When colony growth was observed, unique colony types were selected based on morphology, pigmentation, and hemolytic pattern. Organisms were further subcultured for purity and identified using standard biochemical methods.

Statistical Analyses

Uterine swab samples from a subset of cows with CE ($n = 178$) were collected for bacteriology, and the proportions of bacteria isolated (from the total number of identified isolates) in the 3 treatment groups (CON, DEX, or CCFA) were reported.

Data from individual lactating dairy cows (e.g., lactation number, DIM, milk yield, service number, pregnancy status) were exported from DairyComp 305 to an Excel spreadsheet (Microsoft Corp., Redmond, WA). Prior to data analyses, enrolled lactating dairy cows that met the exclusion criteria (cows that were treated but died or were sold before the AI or PD and cows that initiated lactation with an abortion) were removed from the analysis.

The distribution of cows with and without CE with respect to DIM to first service (DIMFS; d), milk yield (kg), and SCC at the closest DHIA test relative to AI as well as rectal body temperature (°C) and mean cervix diameter (cm) at exam 1 were analyzed using MIXED procedure of SAS (Table 3; SAS Institute, 2009). Additionally, the distribution of cows with and without CE with respect to BCS at exam 1 was dichotomized (≤ 2.75 or ≥ 3) and analyzed using GLIMMIX procedure of SAS (Table 3; SAS Institute, 2009). Least squares means and standard errors of the means ($\pm$ SEM) were reported. A $P < 0.05$ was considered statistically significant.

Cow survival within 14 d posttherapy, the response to treatments (clinical cure), and cycling status (presence of ovarian structures and P4) were assessed for cows with CE. Additionally, the proportions of cows that conceived to first service (P/AI) and the proportions of pregnancy losses for the 3 treatment groups (CON, DEX, or CCFA; $n = 237$) and for cows without CE ($n = 523$) were evaluated. Data were arranged in a randomized block design. Data pertaining to response to treatments (clinical cure at exam 2; Table 2), cow survival 14 d posttherapy, the proportion of pregnancy losses, and P/AI were analyzed using generalized linear mixed models (Proc GLIMMIX; SAS Institute, 2009). Cycling status of cows measured by the serum concentration of P4 (<1 ng/mL or ≥ 1 ng/mL) and presence of ovarian structures (yes or no) were dichotomized as described earlier and analyzed using the GLIMMIX procedure of SAS. A model procedure that included treatment (CON, DEX, or CCFA), parity (primiparous or multiparous), season, uterine health status, BCS at calving, milk yield, and DIM at the time of AI, sire, and SCC at the closest DHIA test relative to AI was used to compare differences among group of cows. Non-

Table 3. Distribution (LSM $\pm$ SEM) of lactating Holstein cows with and without clinical endometritis (CE) with respect to DIM to first service (DIMFS), milk yield (kg), BCS, body temperature, mean cervix diameter (cm), and SCC at the closest DHIA relative to AI, and relative to treatment

Variable	Cows with CE ¹			Cows without CE (n = 523)	P-value
	CON (n = 83)	DEX (n = 79)	CCFA (n = 75)		
DIMFS (d)	64.1 $\pm$ 0.8	61.5 $\pm$ 0.8	62.5 $\pm$ 0.8	63.1 $\pm$ 0.3	0.20
Milk yield (kg)	32.4 $\pm$ 2.4	32.9 $\pm$ 2.5	34.5 $\pm$ 2.6	35.3 $\pm$ 1	0.33
BCS (%)					
≤ 2.75	65.1 $\pm$ 5.2	68.4 $\pm$ 5.2	76.0 $\pm$ 4.9	68.3 $\pm$ 2.0	0.49
≥ 3.0	34.9 $\pm$ 5.2	31.6 $\pm$ 5.2	24.0 $\pm$ 4.9	31.7 $\pm$ 2.0	0.49
Body temperature ($^{\circ}$ C)	38.6 $\pm$ 0.1	38.7 $\pm$ 0.1	38.5 $\pm$ 0.1	38.6 $\pm$ 0.05	0.33
Mean cervix diameter (cm)	4.3 $\pm$ 0.1 ^a	4.2 $\pm$ 0.1 ^a	4.4 $\pm$ 0.1 ^a	3.6 $\pm$ 0.1 ^b	0.02
SCC ($\times 10^3$ cell/mL)	118 $\pm$ 57	246 $\pm$ 58	111 $\pm$ 110	172 $\pm$ 22	0.32

^{a,b}Values with different superscript letters within a row differ significantly at $P < 0.05$.

¹Lactating dairy cows (n = 760) were screened for CE at 26 ± 3 DIM using vaginoscopy technique. Cows with CE were randomly assigned to 1 of 3 treatment groups: (1) intrauterine infusion (~ 200 mL) of a 50% dextrose solution (DEX; n = 79); (2) 6.6 mg/kg single dose of subcutaneous ceftiofur crystalline free acid at the base of the ear (CCFA; n = 75); or (3) untreated control cows (CON; n = 83). Information from cows without CE (n = 523) were included in the analysis. Least squares means ($\pm$ SEM) were reported.

significant variables were eliminated from the logistic model one at a time using the Wald statistic backward selection criterion ($P > 0.15$). Herd was included as a random effect. The estimates (proportions of dead cows, response to treatment, cycling status, P/AI, and pregnancy losses) from the final model were reported as least squares means (Tsousis et al., 2009; Pinedo and De Vries, 2010; Bas et al., 2011). Standard errors of the means (SEM) for the binomial outcomes were computed as described by SAS and reported elsewhere (Pinedo and De Vries, 2010; Larson et al., 2011). The differences between least squares means were computed by including the PDIF option in the LSMEANS statement (Tsousis et al., 2009; Bas et al., 2011). Differences in individual least squares means were adjusted by using the Tukey-Kramer method. A $P < 0.05$ was considered statistically significant and a $P \leq 0.10$ was considered a tendency to differ.

A Cox proportional hazard model was used to assess the effect of DEX or CCFA on the time to pregnancy up to 250 DIM using the PHREG procedure (SAS Institute, 2009), controlling for the effects of parity, BCS at calving, uterine health status, and season, if significant. Data from cows without CE (n = 523) were included in the analysis. Herds were included in the STRATA statement to account for the herd effects (Dubuc et al., 2011a). Data obtained from SAS output were plotted in Excel (Microsoft Corp.) to graphically depict the proportion of cows pregnant over time (Figure 2).

RESULTS

Initially, 833 lactating dairy cows were screened for CE at 26 ± 3 DIM, of which 253 cows (30.4%; Table 1)

were diagnosed with CE (score 2 or 3) and randomly assigned into 1 of 3 treatment groups. For P/AI analysis, data from 73 cows (16 cows with CE and 57 cows without CE) were not available because they died (n = 49), were sold (n = 17), or were lost from unknown causes (n = 7) before the first AI. Therefore, 760 lactating Holstein dairy cows (240 primiparous and 520 multiparous) were available for the final P/AI analysis (Table 6).

Diagnosis of CE and Response to Treatments

The prevalence of CE at exam 1 (26 ± 3 DIM) was 30.4% (scores 2 and 3 combined; Table 1). The response to treatments was assessed 14 d posttherapy administration at exam 2 (40 ± 3 DIM; Table 2) for cows with CE. A higher proportion of cows that were treated with CCFA (41.30%) or DEX (44.68%) scored 0 (clinical cure) compared with CON cows (24.53%, $P = 0.02$; Table 2). Additionally, no significant differences ($P = 0.9$) were observed in the proportion of cows that died within 14 d posttreatment among CON (2%), DEX (1.4%), or CCFA cows (1.6%).

The cervical diameter was estimated via transrectal ultrasonography at the time of exam 1 (26 ± 3 DIM; Table 1) and at exam 2 (40 ± 3 DIM; Table 2). The distribution of cervical diameter is provided in Table 1. Cows without CE had smaller cervical diameter compared with cows diagnosed with CE ($P = 0.02$; Table 3). The distribution of cows with and without CE with respect to DIMFS, milk yield, SCC at the closest DHIA test relative to AI, rectal body temperature, BCS at exam 1, and mean cervix diameters were compared among groups and are presented in Table 3. Except for

Figure 2. Survival curves for time to pregnancy after the voluntary waiting period of Holstein cows diagnosed with clinical endometritis (CE) or without CE. Lactating dairy cows ($n = 760$) were screened for CE at 26 ± 3 DIM using vaginoscopy technique and measurement of cervical diameter and randomly assigned into 1 of 3 treatment groups: (1) intrauterine infusion (~ 200 mL) of a 50% dextrose solution (DEX; $n = 79$); (2) 6.6 mg/kg single-dose of subcutaneous ceftiofur crystalline free acid at the base of the ear (CCFA; $n = 75$); or (3) untreated control cows (CON; $n = 83$). Cows without CE ($n = 523$) were included in the survival function estimates. Adjusted hazard ratios (AHR; 95% CI) for pregnancy ($P < 0.05$) were 0.55 (0.43–0.72), 0.76 (0.58–0.98), 0.67 (0.52–0.87) for CON, DEX, and CCFA, respectively (cows without CE = referent). Median time to pregnancy (95% CI) was 112 d (107–117), 146 d (132–161), 128 d (113–143), and 147 d (133–161) for cows without CE, CON, DEX, and CCFA, respectively.

mean cervix diameters, cows with or without CE did not differ significantly for the additional parameters evaluated (Table 3).

Bacterial Growth and Identification

The proportion of uterine swab samples ($n = 178$) with positive bacterial growth was 69.6% (124 swabs; Table 4). Fifty-four swab samples (30.4%) were negative and yielded no bacterial growth. From the 124 positive cultures (69.6%), 147 isolates were identified (Table 4), and *A. pyogenes* and *E. coli* were the most predominant isolates identified across the treatment groups (Table 4). In addition, *Pasteurella* spp., *Pseudomonas* spp., *Corynebacterium* spp., *Acinetobacter* spp., and *Bacillus* spp., were occasionally isolated (Table 4).

Ovarian Structures, Cycling Status, and Effect of Treatments on P/AI

Parity, uterine health status, and BCS at calving were significantly associated ($P < 0.05$) with P/AI and remained in the final model. The proportions of ovarian structures (presence of CL, follicles, or cysts) at exam

1 (26 ± 3 DIM) and at exam 2 (40 ± 3 DIM), as well as the proportion of cows cycling (serum concentrations of $P4 \geq 1$ ng/mL), were not statistically different ($P > 0.05$) among treatment groups (Table 5). Uterine health status (with or without CE) had a detrimental effect ($P < 0.05$) on first-service P/AI (Table 6) and on time to pregnancy up to 250 DIM (Figure 2). For first service only, P/AI in DEX ($29.8 \pm 4\%$) tended to differ ($P = 0.1$) from the CON ($21.1 \pm 4\%$) and CCFA groups ($19.7 \pm 4\%$), whereas the overall P/AI in DEX cows was not significantly different from cows without CE ($39.1 \pm 2\%$; Table 6). The presence of CE was significantly associated with increased pregnancy losses compared with cows without CE (Table 6; $P = 0.03$). The proportions of pregnancy losses were lower for cows without CE compared with CON or CCFA cows (Table 6), but the percentage of pregnancy loss in DEX cows was not significantly different from that in cows without CE (Table 6).

DISCUSSION

The objectives of this study were to assess the responses to treatments (clinical cure and cow survival

Table 4. Proportion of bacteria isolated from the uterus immediately at exam 1 in lactating Holstein cows with clinical endometritis (CE)

Species	Identified isolates (%) over total (no./no.) ¹		
	CON	DEX	CCFA
<i>Arcanobacterium pyogenes</i>	60.78 (31/51)	67.39 (31/46)	64.0 (32/50)
<i>Escherichia coli</i>	29.41 (15/51)	23.91 (11/46)	30.0 (15/50)
<i>Pasteurella</i> spp.	3.92 (2/51)	6.52 (3/46)	—
<i>Pseudomonas</i> spp.	1.96 (1/51)	—	2.0 (1/50)
<i>Corynebacterium</i> spp.	1.96 (1/51)	—	2.0 (1/50)
<i>Acinetobacter</i> spp.	1.96 (1/51)	—	—
<i>Bacillus</i> spp.	—	2.17 (1/46)	2.0 (1/50)

¹From 178 swab samples submitted for bacteriology, 124 (69.6%) yielded positive bacteria growth. A total of 147 isolates were identified and reported as a proportion (%) over the total (no.) number of isolates of a particular genus from the total (no.) number of identified isolates obtained for untreated control cows (CON; n = 51), cows treated with intrauterine infusion of 50% dextrose (DEX; n = 46), and cows receiving a single dose of subcutaneous ceftiofur crystalline free acid at the base of the ear (CCFA; n = 50).

14 d posttherapy) of cows with CE that received intrauterine infusion of a hypertonic solution of 50% DEX or subcutaneous CCFA and the subsequent P/AI in cows with CE compared with cows without CE. The study showed that (1) cows with CE had greater mean cervix diameters and lower P/AI compared with cows without CE; (2) the most predominant bacteria isolated at the time of treatment from CE cows were *A. pyogenes* and *E. coli*; (3) treatment with CCFA or DEX increased the proportion of cows that scored 0 (clinical cure) 14 d posttherapy compared with CON cows; and (4) P/AI tended to increase in DEX cows compared with CCFA or CON cows.

The prevalence of CE at exam 1 was 30.4%, within the range of values cited in the literature (LeBlanc et al., 2002a; Gilbert et al., 2005; Williams et al., 2005). Postpartum CE has been defined as a presence of mucopurulent or purulent vaginal discharge using vaginoscopy at 26 DIM with a cervix diameter ≥ 7.5 cm (measured by transrectal palpation) and without systemic signs of illness (e.g., fever; LeBlanc et al., 2002a; Kasimanickam et al., 2004, 2005). In the present study, cows with CE were diagnosed according to vaginal discharges using the vaginoscopy techniques. In addition, the cervix diameters for cows with and without CE were measured by transrectal ultrasound as reported

Table 5. Proportion (%) of ovarian structures [presence of follicles, corpora lutea (CL), or cysts] and cycling status based on serum concentration of progesterone (P4) in lactating Holstein cows diagnosed with clinical endometritis (CE)

Variable	Cows with CE ¹			P-value
	CON (n = 83)	DEX (n = 79)	CCFA (n = 75)	
Cycling cows ² (%)	53.2 $\pm$ 7.2	55.8 $\pm$ 7.5	48.6 $\pm$ 7.2	0.81
Ovarian structures at 26 $\pm$ 3 DIM ³				
Follicles (%)	91.2 $\pm$ 4.1	91.8 $\pm$ 3.9	95.8 $\pm$ 2.9	0.76
CL (%)	50.9 $\pm$ 7.0	55.1 $\pm$ 7.1	56.2 $\pm$ 7.1	0.95
Cysts (%)	15.7 $\pm$ 5.1	20.4 $\pm$ 5.7	8.3 $\pm$ 3.9	0.41
Ovarian structures at 40 $\pm$ 3 DIM ⁴				
Follicles (%)	93.6 $\pm$ 3.5	89.7 $\pm$ 4.8	94.4 $\pm$ 3.8	0.87
CL (%)	80.8 $\pm$ 5.7	64.1 $\pm$ 7.6	66.7 $\pm$ 7.8	0.34
Cysts (%)	14.9 $\pm$ 5.1	23.1 $\pm$ 6.7	13.9 $\pm$ 5.7	0.71

¹CON = untreated control cows; DEX = cows treated with intrauterine infusion of 50% dextrose; and CCFA = cows receiving a single dose of subcutaneous ceftiofur crystalline free acid at the base of the ear.

²The proportion (%; LSM $\pm$ SEM) of cycling lactating dairy cows based on serum concentrations of progesterone (P4) were reported. Blood samples were collected from cows diagnosed with CE at exam 1 (26 $\pm$ 3 DIM) and exam 2 (at 40 $\pm$ 3 DIM). Cows were classified as cycling when concentrations of P4 from 1 of 2 blood samples were ≥ 1 ng/mL (high P4; high-high, low-high, or high-low). Cows were classified as non-cycling when serum concentrations of P4 from both blood samples were < 1 ng/mL (low P4; low-low).

³The proportion (%; LSM $\pm$ SEM) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at 26 $\pm$ 3 DIM in lactating dairy cows with CE.

⁴The proportion (%; LSM $\pm$ SEM) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at 40 $\pm$ 3 DIM in lactating dairy cows with CE.

Table 6. Proportions (%) of pregnancies per AI (P/AI) in lactating Holstein cows with clinical endometritis (CE) following an intrauterine dextrose infusion (DEX), ceftiofur crystalline free acid (CCFA), or untreated animal (CON)¹

Variable	Cows with CE			Cows without CE (n = 523)	P-value
	CON (n = 83)	DEX (n = 79)	CCFA (n = 75)		
First-service P/AI for cows with CE only ² (%)	21.1 ± 4	29.8 ± 4	19.7 ± 4	—	0.1
First-service P/AI for all cows ³ (%)	20.1 ± 4 ^b	30.5 ± 5 ^{ab}	19.4 ± 4 ^b	39.1 ± 2 ^a	0.0001
Pregnancy loss ⁴ (%)	11.6 ± 5 ^a	9.1 ± 4 ^{ab}	13 ± 5 ^a	5.8 ± 2 ^b	0.03

^{a,b}Values with different superscript letters within a row differ significantly at $P < 0.05$.

¹Lactating dairy cows (n = 760) were screened for CE at 26 ± 3 DIM using vaginoscopy technique. Cows with CE were randomly assigned to 1 of 3 treatment groups: (1) intrauterine infusion (~200 mL) of a 50% dextrose solution (DEX; n = 79); (2) 6.6 mg/kg single-dose of subcutaneous ceftiofur crystalline free acid at the base of the ear (CCFA; n = 75); or (3) untreated control cows (CON; n = 83).

²Proportions (%; LSM ± SEM) of first-service pregnancies per AI (P/AI) in cows with CE.

³Proportions (%; LSM ± SEM) of first-service pregnancies per AI (P/AI) in cows with and without CE (n = 523).

⁴The proportion of pregnancy losses between pregnancy diagnosis (39 ± 3 d post-AI) and reconfirmation of pregnancy approximately 30 d after the first pregnancy diagnosis (69 ± 3 d post-AI) was determined for cows with and without CE.

elsewhere (Kasimanickam et al., 2004; Silvestre et al., 2009). Although cows with CE had similar mean cervix diameters, the mean cervical diameter was greater for CE cows (>4.2 cm) compared with cows without CE (3.6 cm). Additionally, increased mean cervix diameters were associated with reduced first-service P/AI in cows diagnosed with CE compared with cows without CE. Previous studies reported that CE cows presented a cervical diameter of ≥7.5 cm at 20 DIM (based on transrectal palpation; LeBlanc et al., 2002a; Sheldon et al., 2006, 2009). The assessment of cervical diameter as a means to diagnose CE in cows may vary considerable according to the method used (e.g., transrectal ultrasonography vs. transrectal palpation). The use of different methods of measurement may explain, at least in part, the values for cervical diameter reported in the present study compared with those values reported via transrectal palpation (LeBlanc et al., 2002a; Dubuc et al., 2010a, 2011a). Furthermore, rectal temperature at the time of treatment (26 ± 3 DIM) was not different between cows with and without CE in this study, as reported elsewhere (LeBlanc et al., 2002b). The response to treatments (clinical cure and cow survival) was assessed at 14 d posttherapy. The proportion of cows with a vaginal discharge score of 0 (clinical cure) was significantly higher in DEX and CCFA cows compared with CON cows. The vaginal discharges (and cervical diameter) from CE cows are expected to decrease over time as the inflammation associated with CE resolves following treatment administration (Runciman et al., 2008). According to this study, cows with CE benefited (clinical cure) from the administration of DEX or CCFA at exam 1.

In lactating dairy cows, numerous species of bacteria can be isolated in the first 10 d postpartum (Sheldon et al., 2002). Recognized uterine pathogens (*A. pyogenes*, *Prevotella melaninogenica*, *E. coli*, *Fusobacterium nec-*

rophorum, and *Proteus* spp.), potential uterine pathogens (*Bacillus* spp. and *Pasteurella* spp.), and opportunistic uterine contaminants (*Streptococcus* spp., *Providencia* spp., *Klebsiella* spp., and *Corynebacterium* spp.) have been associated with CE and decreased fertility in dairy cows (Sheldon et al., 2002; Williams et al., 2005, 2007; Galvão et al., 2009a). Furthermore, the presence of *E. coli* and *A. pyogenes* in the bovine uterus is associated with ovarian dysfunction such as smaller follicle diameter and corpora lutea with lower concentrations of circulating estradiol and progesterone compared with the uninfected uterus (Williams et al., 2007). In this study, the most predominant bacteria isolated from CE cows were *E. coli* and *A. pyogenes*. The presence or absence of ovarian structures (follicles, CL, and cysts) and cyclicity status were assessed via transrectal ultrasonography and concentrations of serum P4 at exams 1 and 2 in cows diagnosed with CE, respectively. Neither the proportion of ovarian structures nor cycling status of CE cows differed among treatment groups.

The proportion of cows that died and the clinical response to treatments (clinical cure) 14 d posttherapy, P/AI following the administration of CCFA or DEX to CE cows, and the proportions of pregnancy losses were assessed in this field study. For cows with CE, a tendency to have a greater first-service P/AI was noted for cows in the DEX group compared with CON or CCFA cows. When cows with and without CE were considered in the analysis, reproductive performance (first-service P/AI and maintenance of pregnancy) was similar for DEX cows and cows without CE. In the present study, DEX cows had a greater first-service P/AI (by 9 or 10 percentage points) compared with untreated CON or CCFA cows, and our declaration of no difference could represent a type II statistical error (claiming that DEX treatment is the same as untreated CON animals, when actually DEX would benefit cows with CE). Therefore,

a greater number of experimental units (cows with CE) are needed to assess the efficacy of DEX on first-service P/AI and pregnancy loss. Uterine health disorders (Dubuc et al., 2010b) and anovular lactating dairy cows (Bisinotto et al., 2010) have been associated with decreased first-service P/AI and increased pregnancy losses (Ribeiro et al., 2011). Interestingly, pregnancy losses were almost 2-fold higher in cows with CE compared with cows without CE in the present study and as reported previously (Ribeiro et al., 2011). The administration of intrauterine ceftiofur hydrochloride for cows with CE has been shown unsuccessful at improving P/AI when administered in conjunction with prostaglandin (Galvão et al., 2009a). A recent field study showed that subcutaneous administration of 6.6 mg/kg of CCFA (within 24 h after parturition) for lactating dairy cows at high risk of uterine disease (having dystocia, delivery of twins, or retained fetal membranes) was unsuccessful in preventing the overall incidence of metritis (Dubuc et al., 2011a). Following a single subcutaneous administration of 6.6 mg/kg of CCFA, concentrations above the reported MIC₉₀ (serum, endometrial, and lochia) as desfuroylceftiofuracetamide (an active CCFA metabolite) for relevant uterine pathogens such as *E. coli* and *A. pyogenes* has been reported over a period of 7 d (Witte et al., 2011). In our study, the administration of CCFA as well as DEX improved the proportion of cows with CE at exam 1 that scored 0 (clinical cure) at exam 2 compared with CON cows. Administration of prostaglandin alone for cows diagnosed with CE has been reported to improve (Galvão et al., 2009b) or to have no effect (Dubuc et al., 2011a) on P/AI in lactating dairy cows. In this study, all cows were subjected to the same synchronization protocol (Presynch followed by OV with estrous detection) and reproductive performance of CCFA cows was similar to that of CON cows.

The use of intrauterine DEX in cows with CE may favor a quicker uterine recovery by inhibiting bacterial growth locally, increasing uterine tone, or by nurturing endometrial cells compared with CON or CCFA cows. Previous studies have shown the ability of sugar (hypertonic sucrose solution) to aid in wound healing through inhibition of bacterial growth such as *E. coli* (Chirife et al., 1983; Archer et al., 1990; Sharon, 2006). Sucrose has also been shown to control bacterial infections by causing an osmotic draw of fluid out of the affected area and reducing the water activity (Chirife et al., 1983) that is required for bacteria to survive. Decreased bacterial production of proteolytic enzymes (leading to less tissue damage), limiting absorption of bacterial toxins, source of energy for damaged tissues, and reducing the water activity required for bacteria to

survive were the principles behind the use of hypertonic solutions (glucose or sucrose) for the treatment of acute peritonitis in rabbits (Narat, 1923), human wounds (Chirife et al., 1983), and equine wounds (White, 1995). The osmotic draw of fluid out of tissues would aid in tissue contraction (Kilic, 2001), thereby causing increased uterine tone. Furthermore, an in vitro study has shown that mannose (a sugar monomer) inhibits the adhesion of *Pseudomonas aeruginosa* and *E. coli* to endometrial epithelial cells from equine endometrium (King et al., 2000). We propose that the intrauterine infusion of a hypertonic solution such as 50% DEX may reduce the growth rate of bacteria in the uterus, increase uterine tone, and provide energy to the natural uterine defenses (e.g., macrophages, neutrophils) to control the infection and improve the overall reproductive performance (clinical cure, P/AI, and pregnancy retention) as observed for DEX cows. Furthermore, the intrauterine infusion of DEX may have lowered the water activity (as proposed by Chirife et al., 1983) in the uterine environment, thus making water unavailable for bacteria such as *E. coli* and *A. pyogenes* to survive. Future studies should address the effect of intrauterine hypertonic solutions on water activity, concentrations of solute (e.g., dextrose), and volume and frequency of administration to cows with CE and puerperal metritis and their association with clinical recovery and reproductive performance.

CONCLUSIONS

The development of effective alternative therapies to antibiotics for the treatment of uterine diseases such as CE and puerperal metritis is needed due to the emergence of multidrug-resistant bacteria associated with uterine infections in lactating dairy cows. Under the field conditions described above, this randomized clinical study showed that the administration of DEX or CCFA in conjunction with prostaglandin improved clinical cure of cows with CE. The P/AI was lower among cows with CE than cows without CE. Although P/AI from DEX cows tended to differ from that of CON or CCFA cows, it resulted in similar P/AI as those cows without CE. However, additional research is needed with more experimental units (cows with CE) to confirm whether the use of intrauterine DEX or CCFA indeed improves P/AI compared with that in CON cows. The use of intrauterine infusion of DEX for the treatment of CE in lactating dairy cows provided useful information for the development of new hypotheses for future studies. Therefore, the use of intrauterine DEX and the underlying mechanisms by which the infection is controlled warrant further investigation.

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