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Comparison of ceftiofur hydrochloride and estradiol cypionate for metritis prevention and reproductive performance in dairy cows affected with retained fetal membranes

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Abstract

The objective of this study was to compare the effect of ceftiofur hydrochloride and estradiol cypionate (ECP®) administration for metritis prevention and reproductive performance in dairy cows affected with retained fetal membranes (RFMs). After parturition, 97 dairy cows affected with RFM from a single dairy herd were randomly allocated to 1 of 3 treatment groups. Cows in-group 1 ($n = 31$) were treated daily for 5 days with ceftiofur hydrochloride (2.2 mg/kg, i.m.); cows in group 2 ($n = 33$) were treated once with ECP® (4 mg, i.m.); and cows in group 3 ($n = 33$) were not treated. The proportion of cows with metritis, uterine involution patterns and the calving-to-conception interval were compared between groups. The proportion of cows that developed metritis was significantly different ($P < 0.05$) in cows treated with ceftiofur hydrochloride (13%), compared with cows treated with ECP® (42%) or cows that received no treatment (42%). Uterine involution patterns (i.e. median time to complete retraction of the uterus and mean diameter measure of cervix and uterine horns) were not significantly different between groups. Cows treated with ECP® were 0.40 times as likely to conceive as control cows ($P = 0.05$); median time to conception in cows treated with ECP® (192 days) was longer, compared to control cows (124 days). We conclude that systemic administration of ceftioufur hydrochloride is beneficial for prevention of metritis, but its effect on reproductive performance was not significantly different to that of ECP® or no treatment. In addition, administration of ECP® did not have beneficial effects on metritis prevention and reproductive performance.
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Keywords: Retained fetal membranes; Dairy cows; Ceftiofur; Estradiol cypionate; Metritis

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1. Introduction

Cows affected with retained fetal membranes (RFMs) are at a higher risk of developing postpartum metritis [1–5]. After considering the effect on reproductive efficiency, milk production, cost of medication and loses due to culling, the total cost estimate for cows affected with metritis has been reported to be US$ 106 per lactation [6]. Therefore, the primary aim in the treatment of dairy cows affected with RFM should be to prevent postpartum metritis. Studies have found that cows affected with RFM and treated with intrauterine infusions of oxytetracycline have responded favorably [5,7]. In one study [7], the incidence of postpartum fever attributed to uterine disease was significantly lower in cows affected with RFM and treated with oxytetracycline, compared to cows affected with RFM that received no antibiotic treatment. However, a major disadvantage of using oxytetracycline for treatment of RFM is the potential for antibiotic residues in milk and meat; it may also be harmful to the endometrium [5,8]. In contrast to oxytetracycline, ceftiofur hydrochloride does not result in antibiotic residues in milk when used according to label directions. In another study, dairy cows affected with metritis and treated with ceftiofur hydrochloride responded favorably [9]. Clinical observations by university veterinarians in Florida suggest that ceftiofur hydrochloride can also be used for treatment of cows affected with RFM and prevention of metritis. However, to our knowledge, the efficacy of ceftiofur hydrochloride in cows affected with RFM has not been evaluated in controlled studies.

A different approach adopted by veterinarians for treatment of dairy cows affected with RFM or metritis has been the use of estradiol cypionate (ECP®)[1] [10]. The rationale for use of ECP® in dairy cows is based upon the effects of estrogens on uterine contractility and immunity [11,12]. Estradiol cypionate may stimulate uterine involution by sensitizing the uterus to natural endogenous oxytocin release, and it may enhance uterine resistance to infection by possible mechanisms possibly including increased uterine motility and increased mucus production and flow [11,12]. Although the use of ECP® for treatment of dairy cows affected with RFM or metritis has been advocated by veterinarians, its efficacy has not been evaluated using objective research methods. Furthermore, increased public concern about hormone use in food producing animals provides a compelling reason to avoid unnecessary use of estrogens in dairy cows [12]. The purpose of the study reported here was to compare ceftiofur hydrochloride and ECP® administration for metritis prevention and reproductive performance in dairy cows affected with RFM. The proportion of cows with metritis, uterine involution patterns and rate of pregnancy by days postpartum were compared between groups.

2. Materials and methods

2.1. Cows

Holstein cows from a single herd in Florida were used in this study. The herd (1200 milk cows) was milked three times per day and had a rolling yearly herd average of 9545 kg of

[1] The 100-day contract—dairy wellness plan. Pharmacia and Upjohn, Kalamazoo, MI (V99279R2).

milk per cow. Only cows that failed to expel the placenta by at least 24 h postpartum were initially considered for inclusion in the study; cows were examined for RFM by visual examination of the perineum. Cows that had dystocia requiring veterinary assistance, cesarian section, fetotomy, or cows that had twins were excluded. Cows that were assisted at parturition by trained farm personnel were considered for inclusion.

2.2. Herd management

Parturient cows that were within 3 weeks of calving were maintained on pastured lots, fed an anionic diet and monitored for signs of calving by farm employees trained to assist with parturition. After calving, postpartum transition cows were housed in a concrete floor open-sided barn, with self-locking stanchions that provided free access to a dry lot. Diets for both parturient and postpartum transition cows were a total mixed ration formulated to meet the requirement of lactating dairy cows according to guidelines established by the National Research Council [13]. During the first 2 weeks postpartum, cows were locked in stanchions daily and monitored for appetite and rectal temperature. Cows that were not eating or had a rectal temperature >39.5 °C (103.1 °F) were examined for ketosis (pink to maroon color on the Ketostik® urine strip[2]), metritis (cows with a rectal temperature >39.5 °C with a fetid discharge from the uterus), mastitis (abnormalities of the milk or any quarter detected by milk personnel: flakes or clots in watery milk or a serum-like secretion from the affected quarter, swelling in affected quarter with systemic signs exhibited by the cow including fever) and displacement of the abomasum (cows with a tympanic sound heard over the left or right paralumbar fossa region during simultaneous percussion and auscultation of the left or right flank) according to established farm health protocols. All cows were treated with prostagladin $F_{2\alpha}$ [Lutalyse® 25 mg i.m.] 25 ± 3 and 39 ± 3 days postpartum and a 60 days voluntary waiting period was observed.

Cows were bred by natural service using bulls that had passed a breeding soundness examination, according to guidelines established by the Society for Theriogenology [14]. Pregnancy diagnosis and estimation of gestation length were performed by herd veterinarians by palpating the uterus and its contents in cows 40–60 days after being turned out with bulls at the end of the voluntary waiting period. In pregnant cows, a calving to conception interval was estimated by subtracting the estimated gestation length from the calving to pregnancy diagnosis interval.

2.3. Experimental protocol

During November 1999 to May 2000, cows were enrolled sequentially in the study as they were diagnosed with RFM. Cows affected with RFM were cows that failed to expel the placenta by 24 h postpartum. Cows affected with RFM were randomly allocated into 1 of 3 treatment groups by use of a random number table. Cows in group 1 ($n = 31$) were treated daily for 5 days with 2.2 mg/kg i.m. ceftiofur hydrochloride[3] treatment dose was based on

[2] Ketostik[0]® Bayer Corporation, P.O. Box 390, Shawnee Mission, KS 66201.
[3] Excenel® Pharmacia and Upjohn, Kalamazoo, MI.

an estimated weight of 550 and 640 kg for primiparous and multiparous cows, respectively. Cows in group 2 ($n = 33$) were treated once with 4 mg i.m. ECP®,[4] a dose used commonly in clinical practice. Cows in group 3 ($n = 33$) were not treated. Treatments started 24 h postpartum when the diagnosis of RFM was made by an attending animal health technician who was aware of treatment ingredients. All cows with a displaced abomasum were treated surgically by the attending herd veterinarian; those cows affected with mastitis and ketosis were treated following standardized treatment procedures established and supervised by the herd veterinarian. All cows that developed metritis were treated systemically with penicillin, nonsteroidal anti-inflammatory medication, and energy-calcium supplements by farm health technicians. We calculated that a sample size of 30 cows per group was adequate to detect a difference between 10 and 45% for cows that would develop metritis among treated and untreated cows, respectively (alpha = 0.05; beta = 0.20) [15].

2.4. Outcomes

The main outcome of interest was metritis. During the first 30 days postpartum, all cows were examined daily for metritis by an attending animal health technician who did not know treatment assignment of cows. Diagnosis of metritis was monitored three times weekly by one of the authors who did not know treatment assignment of cows. Other outcomes of interest were uterine involution and rates of pregnancy by days postpartum. Pregnancy diagnosis was performed by herd veterinarians by palpating the uterus and its contents in cows 40–60 days after exposure to bulls at the end of the voluntary waiting period. Uterine involution was measured by one of the authors by palpating the uterus per rectum three times weekly from Day 7 until Day 30 postpartum. Location of the uterus was classified as follows: 0 = inability to retract the uterus into the pelvis; 1 = complete retraction of the uterus into the pelvis. The cervical diameter (mm) and diameter of each uterine horn at the external bifurcation were estimated and recorded at each palpation following procedures previously described [16]. All cows that developed metritis continued to be palpated according to the schedule described above.

2.5. Statistical analyses

The null hypothesis that proportions of cows that develop metritis in each group were not different between treated and nontreated cows were tested by using a χ^2-test. In addition, median time to complete retraction of the uterus was determined using the Kaplan–Meier product limit estimator [17]. Mean uterine involution measures (diameter measure of cervix and average measure of left and right uterine horns) were compared among groups by use of repeated-measures ANOVA, followed by the Scheffe's method for pairwise comparisons [18].

Time from calving-to-conception was compared between cows treated with ceftiofur hydrochloride, or ECP® or nontreated cows by use of a Cox proportional hazards regression [17]. Cows that were culled $\leq$60 days postpartum were excluded from the

[4] ECP® Pharmacia and Upjohn, Kalamazoo, MI.

analysis (five control cows; four cows treated with ceftiofur hydrochloride; six cows treated with ECP®). Cows contributed a maximum of 200 days to the analysis. Observations were censored when a cow died or was culled or at the time the study was discontinued (i.e. 200 days after calving). In the final model, adjusted hazard ratios (HR) and 95% confidence intervals (CI) were reported. In this study, the HR was used as an epidemiological measure of association between a variable (e.g. treatment) and the outcome of interest (e.g. conception). In each variable, the reference category had an HR = 1. An assessed HR < 1.0 indicates that the probability of conceiving decreased, compared with cows in the reference category.

Baseline comparisons for body condition score loss (between calving and 30 days after calving; yes, no), calving season (Oct–Dec; Jan–Mar; Apr–Jun), assistance during parturition (yes, no), mastitis (yes or no prior to diagnosis of metritis) and culling (yes, no) were carried out to establish comparability of groups by use of a χ^2-test. In addition, parity, body condition score at calving and body condition score 30 days after calving (rank values) were compared among groups by use of the Kruskal–Wallis nonparametric test. For all tests, $P \leq 0.05$ was considered significant.

3. Results

Based on visual inspection of the perineum, all cows with RFM expelled the placenta 25–48 h after parturition. Parity, body condition score at calving, body condition score 30 days after calving, body condition score loss, calving season, number of cows assisted by farm personnel at parturition, cows with mastitis prior to diagnosis of metritis, and cows culled within 15, 30 and 60 days postpartum did not differ significantly ($P \geq 0.38$) between groups (Table 1). Proportion of cows that developed metritis was significantly different ($P < 0.05$) in cows treated with ceftiofur hydrochloride (13%), compared to cows that received no treatment (42%) or cows treated with ECP® (42%). Median time to complete retraction of the uterus (14 days) was not significantly different ($P = 0.75$) between groups (Fig. 1). Uterine involution patterns were not significantly different ($P > 0.30$) between groups (Figs. 2 and 3). In the Cox model analysis, cows treated with ECP® were 0.40 times as likely to conceive as control cows ($P = 0.05$) (Table 2). Median time to conception for cows treated with ECP® and control cows was 124 and 192 days, respectively (Fig. 4). In addition, cows treated with ceftiofur hydrochloride were 0.74 times as likely to conceive as controls; however this difference was not significant ($P = 0.36$). Median time to conception for cows treated with ceftiofur hydrochloride and control cows was 124 and 136 days, respectively (Fig. 4).

4. Discussion

This study was designed to compare the administration of ceftiofur hydrochloride and ECP® on metritis prevention and reproductive performance in dairy cows affected with RFM. All cows with RFM expelled the placenta between 25 and 48 h after calving. Because cows were not examined hourly after diagnosis of RFM, total retention time per

Table 1
Comparisons of cows affected with retained fetal membranes and treated with ceftiofur hydrochloride (2.2 mg/kg i.m., daily for 5 days), estradiol cypionate (4 mg i.m., once) or not treated (control)

Variable	Ceftiofur	Estradiol	Control	*P*
Baseline comparisons				
Parity[a]	2.6, 2 (1, 5)	2.8, 4 (1, 7)	2.7, 2 (1, 7)	0.95
BCS at calving[a,b]	3, 3.25 (2.25, 3.75)	3, 3 (2, 4.25)	3, 3 (2.25, 3.75)	0.95
BCS 30 days after calving[a]	3, 3.25 (2.25, 3.75)	3, 3 (2, 4.25)	2.8, 2.8 (1.5, 3.75)	0.86
BCS loss (%)	7/26 (27)	6/19 (31)	8/26 (30)	0.93
Calving season (%)				0.48
Oct–Dec	14/31 (31)	13/33 (39)	18/33 (55)	
Jan–Mar	13/31 (42)	18/33 (55)	11/33 (33)	
Apr–Jun	4/31 (13)	2/33 (6)	4/33 (12)	
Assistance at parturition (%)[c]	6/31 (19)	4/33 (12)	8/33 (24)	0.44
Cows with mastitis[d]	2/31 (6)	0 (0)	0 (0)	0.15
Culled cows within 15 days (%)	1/31 (3)	1/33 (3)	1/33 (3)	0.99
Culled cows within 30 days (%)	3/31 (9)	4/33 (12)	1/33 (3)	0.38
Culled cows within 60 days (%)	4/31 (13)	6/33 (18)	4/33 (12)	0.74
Cows with metritis				
Within 3–7 days (%)	2/31 (6)	8/33 (24)	8/33 (24)	0.10
Within 3–30 days (%)	4/31 (13)[a]	14/33 (42)[b]	14/33 (42)[b]	0.01

Within row, groups with different superscripts (a, b) are significantly different ($P \leq 0.05$).
[a] Data are reported as mean, median (range).
[b] BCS: body condition score.
[c] Cows that were assisted at parturition by trained farm personnel.
[d] Cows diagnosed with mastitis prior to diagnosis of metritis.

hour was not measured in each cow. It is possible that total retention time was different in each cow and may have affected the risk of developing metritis. Decaying placental tissue may exacerbate bacterial growth and production of inflammatory products [19]. In a previous study of cows affected with RFM, risk of metritis was the same during the first 6–8 h of retention, and increased slightly at 24 h of retention; however, risk of metritis did

Table 2
Results of proportional hazards analysis of days to conception for cows treated with ceftiofur hydrochloride (2.2 mg/kg i.m., daily for 5 days), estradiol cypionate (4 mg i.m., once) or untreated (control) cows

Variable	Hazard ratios	95% CI	*P*-value
Treatment			
Control	1.00	Reference	NA
Ceftiofur hydrochloride	0.74	0.39, 1.40	0.36
Estradiol cypionate	0.40	0.24, 1.01	0.05
Lactation number			
1	1.00	Reference	NA
2	0.79	0.37, 1.69	0.55
3+	0.71	0.38, 1.33	0.29

Observations were discontinued 200 days after calving.

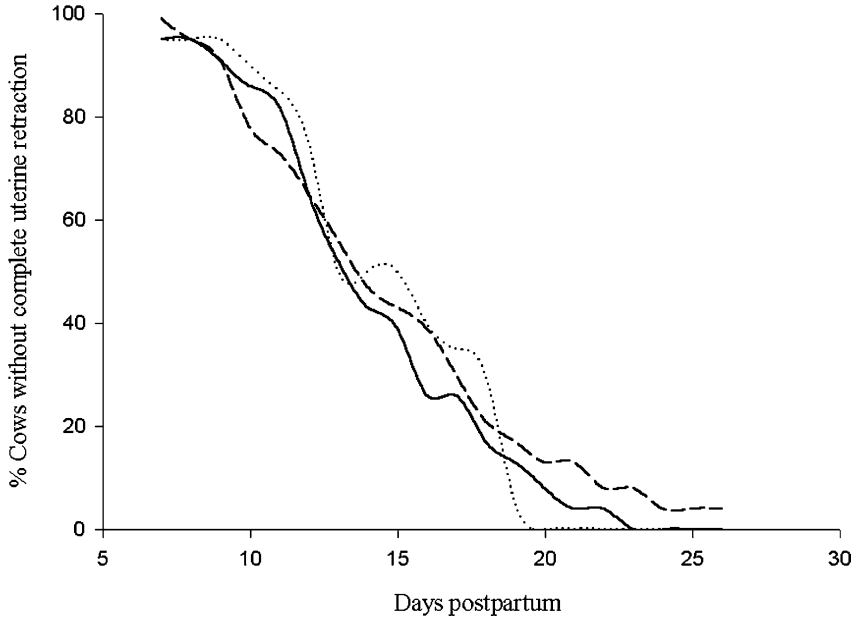

Fig. 1. Time to complete retraction of the uterus in cows affected with retained fetal membranes not treated (controls) or treated with ceftiofur hydrochloride (2.2 mg/kg i.m., daily for 5 days), or estradiol cypionate (4 mg i.m., once). Controls (—), ceftiofur hydrochloride (· · ·), estradiol cypionate (– – –).

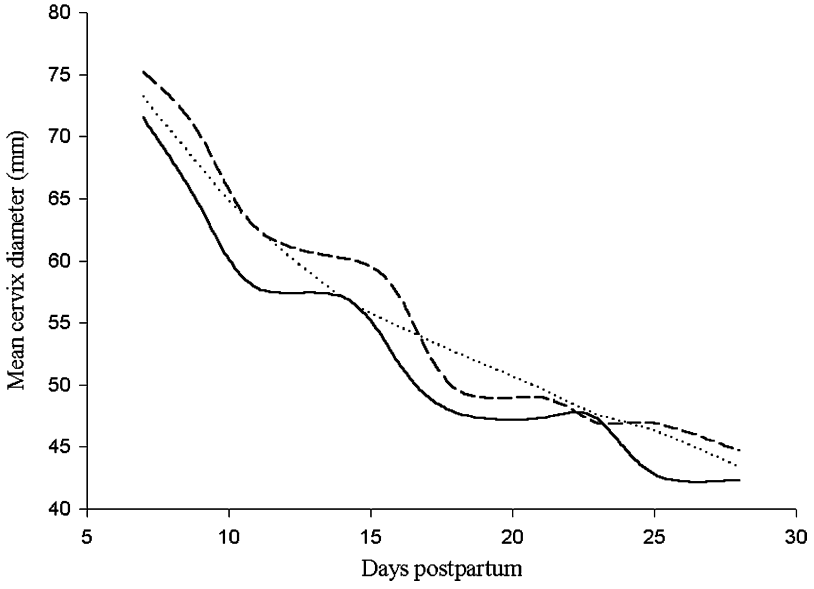

Fig. 2. Uterine cervix involution for cows affected with retained fetal membranes not treated (controls) or treated with ceftiofur hydrochloride (2.2 mg/kg i.m., daily for 5 days), or estradiol cypionate (4 mg i.m., once). Controls (—), ceftiofur hydrochloride (· · ·), estradiol cypionate (– – –).

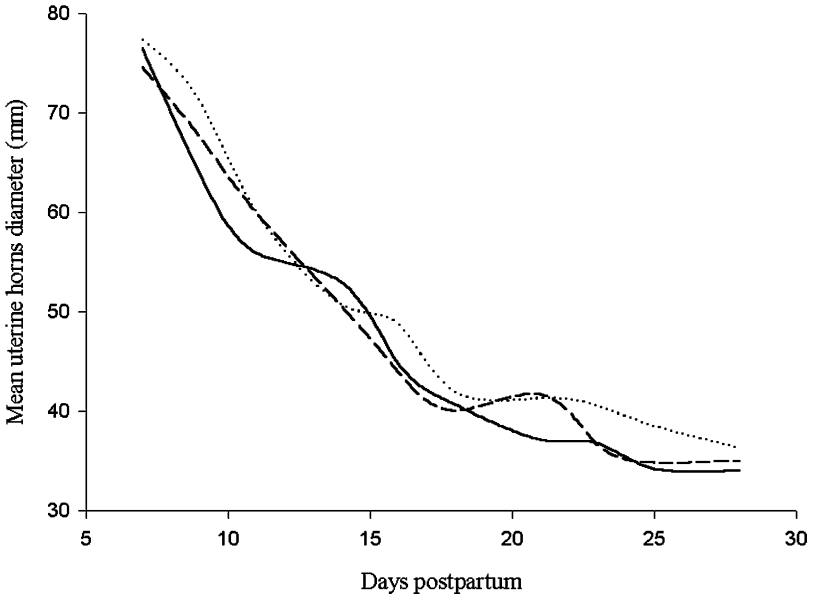

Fig. 3. Uterine horn involution for cows affected with retained fetal membranes not treated (controls) or treated with ceftiofur hydrochloride (2.2 mg/kg i.m., daily for 5 days), or estradiol cypionate (4 mg i.m., once). Controls (—), ceftiofur hydrochloride (· · ·), estradiol cypionate (– – –).

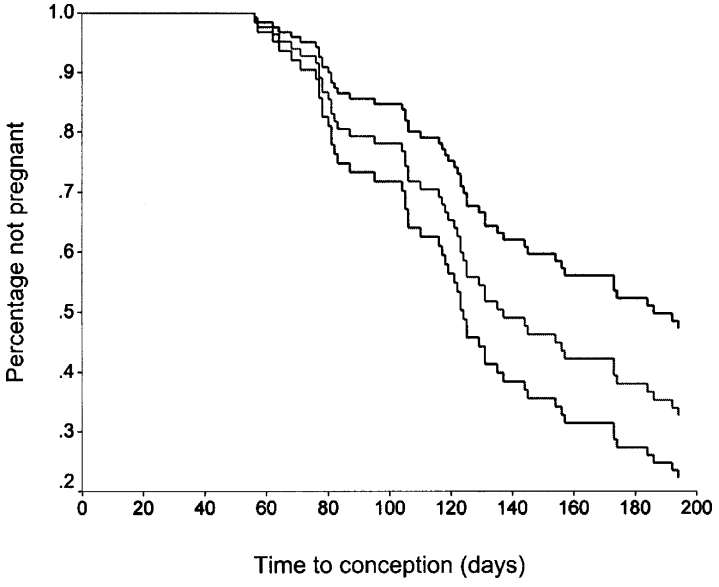

Fig. 4. Product–limit survival distribution function of days to pregnancy by 200 days postpartum in cows not treated (controls) or treated with ceftiofur hydrochloride (2.2 mg/kg i.m., daily for 5 days), or estradiol cypionate (4 mg i.m., once). Controls (bottom line), ceftiofur hydrochloride (middle line) and estradiol cypionate (top line).

not appear to increase after 24 h of retention [20]. Assuming that retention time had an effect on metritis, this source of potential bias was more likely to be nondifferential because cows with RFM were randomly allocated into 1 of the 3 treatment groups.

Analysis of results of the study reported here indicated that systemic administration of ceftiofur hydrochloride appeared significantly more effective for prevention of metritis than ECP®. Ceftiofur hydrochloride is a third generation cephalosporin with broad spectrum activity against Gram-positive organisms, beta lactamase producing bacteria, anaerobic bacteria and Gram-negative aerobic bacteria. These bacteria are found in the postpartum uterus and have been implicated in causing toxic metritis [21]. Based on pharmacokinetic principles, ceftiofur hydrochloride can reach efficacious concentration in infected tissues in vivo, which may prevent or cure metritis. Subcutaneous administration of ceftiofur hydrochloride at a dose of 1 mg/kg in dairy cows after parturition resulted in concentration of ceftiofur and active metabolites in plasma, uterine tissues, and lochial fluid that exceeded reported minimum inhibition concentrations of common pathogens involved in acute puerperal metritis [22]. Smith et al. [9] demonstrated that ceftioufur is an equally effective treatment for postpartum dairy cows affected with toxic metritis, compared with procaine penicillin G or procaine penicillin G plus intrauterine infusion of oxytetracycline.

Our study results do not support the hypothesis that administration of 4 mg of ECP® is beneficial for prevention of metritis in cows affected with RFM. Estradiol cypionate is the only estrogen approved by the Food and Drug Administration Department for use in lactating dairy cows [23]. In clinical practice, the use of ECP® in cows affected with RFM is based on the effect of estradiol and progesterone during the estrous cycle on uterine immunity. During estrus, the uterus is under the influence of estrogen and cows are less likely to develop metritis, compared to cows that are in diestrus and under the influence of progesterone [11]. However, the immunological effects of estrogen and progesterone remain unclear. In a previous study, the use of estradiol in combination with dinoprost-reduced intrauterine bacterial contamination in dairy cows, but had no effect on its own [24]. Progesterone does not have an effect on polymorphonuclear cell migration, activity, number or leukocyte response in cows [25]. The observed reduced uterine resistance seen in cows under the influence of progesterone is due to the presence of high molecular weight lymphocyte inhibitory uterine molecules, such as megasupressin which is induced by progesterone [25]. When the uterus is under the influence of estrogen during estrus, the inhibitory effect of progesterone is removed [11]. In our study, the finding that ECP® had no beneficial effect on prevention of metritis suggests the need for research into uterine immunology during the early postpartum period in dairy cows.

In this study, uterine involution patterns were not significantly different between cows affected with RFM and treated with ceftiofur hydrochloride, ECP® or not treated. Cows affected with RFM subsequently develop metritis, and a heavy leukocyte count in the uterus tends to delay uterine involution [26,27]. Because the proportion of cows with metritis was higher in cows treated with ECP® or not treated, we could have expected cows in these two groups to have experienced a delayed uterine involution. We examined uterine involution patterns in cows with or without metritis in each group, and uterine involution patterns were not different. A possible explanation for this finding is that cows with a postpartum uterine infection may have displayed longer periods of $PGF_{2\alpha}$ release from the

uterus; the correlation between higher $PGF_{2\alpha}$ release from the uterus and a faster rate of uterine involution has been reported [19,28].

The finding that ECP® had no beneficial effect on metritis or uterine involution may have been attributable to the dose of ECP® used in this study. A label dose of 10 mg of ECP® for treatment of cows affected with RFM has been reported [23]. However, to our knowledge, field trials using objective research methods to assess the efficacy of 10 mg of ECP® for treatment of cows affected with RFM have not been conducted. We chose 4 mg because it is the dose recommended in clinical practice [10]. In a previous study, coordinated myometral activity was suppressed following administration of 5 mg of ECP® 18 h postpartum, but uterine sensitivity to oxytocin was not affected and all parts of the uterus contracted simultaneously in a cervico-tubal direction [29]. The therapeutic use of ECP® using different doses in cows affected with RFM may warrant further research.

Because ceftiofur hydrochloride treatment reduced the incidence of metritis compared to control cows, improved reproductive performance in cows from this group would have been expected. It is possible that cows affected with metritis may have recovered sufficiently during the 60-day voluntary waiting period after systemic treatment with penicillin, nonsteroidal anti-inflammatory medication, and energy-calcium supplements. Furthermore, treatment with $PGF_{2\alpha}$ during the postpartum period may have restored the postpartum uterus in these cows resulting in similar responses. Administration of $PGF_{2\alpha}$ early postpartum and again 14 days later has been associated with improved fertility in cows affected with RFM, dystocia, or both [30,31].

In our study, rate of conception was significantly less among cows treated with ECP®, compared to control cows. The mechanism by which ECP® treatment may reduce fertility in cows affected with RFM is unknown to the authors, especially since the incidence of metritis in both groups was not different. In a field trial conducted in California [32], administration of 4 mg of ECP® (the same dose used in our study) resulted also in reduced fertility (HR = 0.72) in Holstein cows during the first 200 days postpartum, compared to untreated cows. A possible explanation for the detrimental effect of ECP® on fertility was not reported. Administration of ECP®, however, did not have any negative effects on days to first artificial insemination or proportion of cows pregnant after first artificial insemination. Results from both studies are difficult to compare because of differences in study design and dairy management factors. In the study conducted in California, most study animals (87%) were cows not affected with RFM, and all cows were artificially inseminated. In our study all cows were affected with RFM and were housed with bulls and bred by natural service. To our knowledge, this is the first study that has examined the effects of administration of ECP® on metritis prevention and reproductive performance in US dairy cows affected with RFM.

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