Evaluation of effect of estradiol cypionate administered prophylactically to postparturient dairy cows at high risk for metritis

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Objective—To determine whether 4 mg of estradiol cypionate (ECP) administered prophylactically to high-risk postparturient dairy cows decreases incidence of postpartum metritis.

Design—Randomized, placebo-controlled, triple-masked clinical trial.

Animals—250 postparturient dairy cows in a herd with postparturient hypocalcemia, retained fetal membranes, dystocia, stillbirth, or twins.

Procedure—Cows were given 4 mg of ECP (treatment) or 2 mL of vegetable oil (control) by IM injection within 24 to 36 hours of calving. Monitoring rectal temperatures and evaluation for metritis was performed once daily for 10 days. Cows with fever ≥ 39.7°C (103.5°F) were treated with 1.5 g of ceftiofur hydrochloride.

Results—When assessed by ordinal logistic regression, there were no differences between groups in incidence of mild or severe metritis. Cows that calved during the second or third quarter of the year were at increased risk of metritis, compared with those that calved during the fourth quarter. Following stratification by lactation (first and ≥ 2), it was observed that multiparous cows that did not receive antimicrobials during the first 3 days of the postparturient period were 5 times as likely to have metritis, compared with cows treated with antimicrobials on the basis of fever or other concurrent disease.

Conclusions and Clinical Relevance—Prophylactic administration of ECP to dairy cows at high risk for metritis did not reduce risk for metritis. Treating multiparous cows with antimicrobials on the basis of fever during the early postpartum period was associated with decreased incidence of metritis. (*J Am Vet Med Assoc* 2003;223:846–851)

Metritis is a postparturient uterine disease that adversely affects dairy cow milk production and reproduction and is a risk factor for abomasal disorders and ketosis.[1-4] Traditional veterinary approaches to manage acute postparturient metritis and retained fetal membranes involve treatment of cows that have developed the disease.[5] A variety of treatments, including intrauterine administration of antimicrobials and disinfectants and systemic hormone administration, have not been effective.[5-13] Systemically active antimicrobials, such as procaine penicillin G, ampicillin, or ceftiofur, alone or in combination with intrauterine administration of antimicrobials have been suggested as useful if toxic puerperal metritis is present, but the high cost of this approach often precludes their routine use in cases that are not severe.[6-8] A variety of preventive herd-level programs have been developed to minimize postparturient problems by targeting specific risk factors before calving. These programs include the use of anionic diets; altered prepartum feeding of protein, energy, or starch; and possibly, feeding of yeast and probiotic bacteria. When properly implemented, these programs benefit periparturient cows by reducing immunosuppression, maintaining blood calcium concentration, and enhancing total feed intake.[14] Despite the adoption of these prepartum strategies, postparturient problems (eg, metritis) are common.

Implementation of routine postparturient monitoring and treatment programs has been recommended as a method to identify and manage problem postparturient cows.[15] In this approach, all postpartum cows are evaluated daily, and extra attention is given to cows in the immediate postparturient period with risk factors for metritis, such as dystocia, retained fetal membranes, twins, stillbirths, and hypocalcemia.[16-20] Cows are treated on the basis of fever or clinical signs as outlined in farm-specific protocols. Treatment of at-risk cows combines systemic antimicrobial treatment to treat toxic puerperal metritis with prophylactic treatments that may include administration of calcium, anti-inflammatory or ecbolic agents, or glucose precursors.

In many programs, cows with retained fetal membranes, fever, or vaginal discharge are treated with **estradiol cypionate** (**ECP**[a] [4 mg, IM]). Estradiol cypionate is the oil-soluble 17 β-cyclopentylpropinate ester of estradiol and has several indications for use in bovine medicine. These include treatment of anestrus in the absence of follicular cysts, persistent corpus luteum, pyometra, and retained placenta. Potentially, ECP may benefit high-risk postpartum cows by stimulating uterine contractions either directly or through maintenance of oxytocin receptor sites in the uterus,[21-24] bringing more WBCs to the site of infection by increasing blood flow to the uterus,[25] enhancing uterine immune function by increasing phagocytosis of bacteria[26-28] or movement of IgA and IgG into the uterine lumen,[29] or promoting uterine drainage by delaying closure of the cervix.

Although ECP seems to be a logical candidate as a nonantimicrobial treatment for postpartum cattle, the

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routine use of estrogen in dairy cattle raises questions about food and environmental safety and consumer acceptance.[30-31] Efficacy of use of 4 mg of ECP has only been evaluated in a single study[32] in the peer-reviewed literature. Wagner et al[32] found that prophylactic use of ECP in postparturient dairy cattle did not decrease the incidence of metritis when administered to all cattle at the time of calving. However, their study had few animals with known risk factors for metritis. The purpose of the study reported here was to determine whether postparturient administration of 4 mg of ECP to cows at high risk for metritis decreases the incidence of postpartum metritis.

Materials and Methods

Cows—Cows included in the study came from a single 2,000-cow commercial dairy in Tulare County, Calif. All cows with normal gestation length that calved from April 1 to December 11, 2000, were eligible to be included in the study. Lactating cows were housed in free-stall barns without exercise lots, and dry cows and heifers were housed in dry lots with shades. All cows were fed a total mixed ration. Lactating cows were milked 3 times daily, and herd 305-day mature equivalent milk production was approximately 11,590 kg (25,500 lb).

Experimental design—This clinical trial was reviewed and approved by the University of California–Davis Animal Use and Care Administrative Advisory Committee. The study was conducted as a triple-blind, placebo-controlled trial. The dairy was visited once daily between 6 and 9 AM by 1 or more investigators and their assistants. Cows were enrolled in the study within 24 to 36 hours of calving only if they had postparturient hypocalcemia, retained fetal membranes, assisted calving, stillbirth, twins, or any combination of these events after a gestation of normal length. The occurrence of each event was determined by examining farm records or direct observation of calving outcome by the investigators. Study cows in the hospital pen were evaluated daily for disease conditions (other than metritis) that may have affected the outcome of the project. Cows were excluded from the study if they had any clinical health event (other than metritis) during the first 10 days post partum that adversely affected the cows' ability to adequately procure feed or were culled or died prior to having metritis or achieving 10 days in lactation.

A cow was defined as having postparturient hypocalcemia if it had characteristic signs (eg, muscle fasciculations or recumbency) during or after calving and required IV administration of calcium. Retained fetal membranes were diagnosed if fetal membranes were visible at the vulva for > 12 hours after calving. Dystocia included any calving that involved the insertion of a hand or instrument into the vagina or beyond. Stillbirths were defined as calves that were born dead or died within the first 2 hours after birth, after a gestation of ≥ 260 days. Twins were defined as the delivery of more than 1 calf at parturition. All of the parturient event definitions were prepared on the basis of preexisting farm definitions that were modified slightly to improve the consistency of our data recording and match currently accepted definitions.[33]

Study cows were randomly assigned to a treatment or control group and identified with ear tags and limb bands. To maintain equal group sizes and similar temporal patterns of enrollment, assignment to groups was performed by use of a number-adaptive random allocation method, as described by Meinert.[34] Prior to enrollment, a person who was not involved with the study used a coin toss to assign group color codes, and the key for the codes was sealed until after the statistical analyses were completed. Therefore, investigators and dairy workers were unaware of group assignments throughout the study.

Cows in the treatment group were given a single 4-mg (2-mL) injection of ECP, IM, whereas control cows received a vegetable oil placebo injection with preloaded 3-mL syringes and 16-gauge, 1.5-in needles within 24 to 36 hours of calving. Because of the similarity of vegetable oil and ECP in appearance and flow through a syringe and needle, the syringes were color-coded to ensure that the correct injection was given.

Monitoring and evaluation—Approximately 4 weeks prior to calving, all potential study cows were assigned a body condition score.[35] Immediately after calving, all study cows, regardless of parity, were housed together in a single dirt pen with a bedded pack and self-lock stanchions. Monitoring began at the time of enrollment and continued for a minimum of 10 days. The investigators and assistants conducted the fresh-cow monitoring and treatment program. Rectal temperatures were obtained daily before 8:30 AM with a digital thermometer. Each cow was evaluated for metritis, general attitude, and feeding behavior. Cows with a fever (≥ 39.7°C [103.5°F]) were treated by a study member with 30 mL (1.5 g, IM) of ceftiofur hydrochloride[b] once daily for a minimum of 3 days. Additional daily ceftiofur treatments were given if the rectal temperature remained > 39.4°C (103.0°F). The principal investigator recorded body condition score during the first week after parturition. Farm personnel provided ancillary antimicrobial treatment (if necessary) to cows in the hospital pen according to the farm's treatment protocol, and these treatments were recorded in the dairy record computer system. Farm personnel moved mature cows and first-lactation cows into lactating pens as soon as the milk was salable. First-lactation cows were housed separately from mature cows when they entered the lactating groups. Monitoring continued in each respective pen for 10 days after calving or until ceftiofur administration was completed.

Trial outcomes—The outcome of interest was incidence of metritis in the first 10 days post partum. Metritis was characterized by a flaccid uterus containing fetid fluids. Palpation per rectum was used to characterize the condition of the uterus if cows had any combination of fever ≥ 39.7°C, watery vulvar discharge, or fetid odor from the perineal area. Metritis was classified as mild (cow never had rectal temperature ≥ 39.7°C during the monitoring period) or severe (rectal temperature ≥ 39.7°C on at least 1 day requiring antimicrobial treatment for metritis). Possible confounding disease processes were ruled out by use of physical examination.

Data recording—Body condition scores, calving dates, culling information, daily rectal temperatures, health events, and antimicrobial treatments administered during the postpartum period were recorded by hand on paper forms and entered into a computer spreadsheet.[c] Body condition score was evaluated as the difference between postcalving body condition score and precalving body condition score. Change in body condition score was recorded as either no change (body condition score difference ranging from –0.25 to 0.25) or loss (difference of –0.5 to –1.0). Parity and culling information were confirmed by evaluating the herd's on-farm computer record system.[d] Calving month was assigned to 1 of 3 categories: second quarter (April to June), third quarter (July to September), or fourth quarter (October to December). Each of these factors was recorded for evaluation as possible confounders during statistical analyses.

Sample size calculation—Because we were testing an inexpensive treatment with potentially large economic and

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welfare benefits, we focused on preventing type-2 errors. For sample size calculation, the α and β error were set at 0.10 and 0.20, respectively. The primary outcome for analysis was incidence of metritis conditional on treatment group. A sample size of 121 cows/group was calculated on the basis of detecting a lower incidence of metritis in the treated group (25%), compared with the control group (40%).

Statistical analyses—Entry covariates for the 2 groups were compared by use of contingency tables and χ^2 tests for homogeneity. Median retention times (in days) for retained fetal membranes were compared between groups by use of a Kruskal-Wallis test. Multivariate analyses were performed, controlling for covariates, with an ordinal logistic regression model to assess the effect of ECP treatment on incidence of metritis. Because it was believed that lactation number was an effect modifier, models were stratified by lactation (1 and ≥ 2). A value of $P < 0.30$ was required to enter the model, and $P < 0.10$ was required to remain. Treatment group was forced into all models. Odds ratios and 90% confidence intervals were calculated for all variables retained in the models.

Table 1—Distribution of risk factors for development of metritis in a clinical trial to evaluate the efficacy of prophylactic administration of 4 mg of estradiol cypionate (ECP) to dairy cows

Risk factor	ECP[1] (No. of cows [%])	Control[2] (No. of cows [%])	*P* value
Stillbirths[3]	63 (48.5)	68 (56.7)	0.19
Twins[4]	15 (11.5)	15 (12.5)	0.82
Dystocia[5]	29 (22.3)	26 (21.7)	0.93
Postparturient hypocalcemia[6]	2 (1.5)	3 (2.5)	0.59
Retained fetal membranes[7]	58 (44.6)	60 (50.0)	0.39
Parity: 1	59 (45.4)	52 (43.3)	0.74
Parity: ≥ 2	71 (54.6)	68 (56.7)	0.74
Calved April–June	36	30	0.63
Calved July–September	48	48	0.62
Calved October–December	46	42	0.95

Each covariate was evaluated for homogeneity across treatment groups by use of a χ^2 test.
[1]Cows (n = 130) received ECP (4 mg, IM) within 24 to 36 hours of calving. [2]Cows (n = 120) received vegetable oil placebo (2 mL, IM) within 24 to 36 hours of calving. [3]Calf dead at birth or died within 2 hours of birth. [4]More than 1 calf delivered at parturition. [5]Calving assistance required. [6]Cows had characteristic signs of hypocalcemia and required IV administration of calcium. [7]Retention of fetal membranes for ≥ 12 hours after calving.

Results

Group comparisons—Of the 1,284 cows that calved during the trial period, 261 (20%) were identified as having abnormal parturient events and were enrolled in the study. One hundred twenty untreated control cows and 130 estradiol-treated cows completed the 10-day monitoring period. Eleven (4%) cows were excluded from final analyses because of severe mastitis (n = 2), calving-related musculoskeletal injuries (3), displaced abomasum (3), or death (3) during the 10-day monitoring period. The number of cows that failed to complete the 10-day monitoring period was similar for both groups (5 control and 6 treated cows). Enrollment variables and parity of cows that completed the 10-day monitoring period were similar between treatment groups **(Table 1)**. A contingency table of metritis by treatment and covariates was developed **(Table 2)**; data were stratified by severity of metritis.

For the ordinal logistic regression modeling, treatment group was retained in all models because it was the outcome of interest, but treatment was not associated with incidence of metritis **(Tables 3 and 4)**. Of all the covariates considered, only calving time and antimicrobial use were significant ($P < 0.05$) effect modifiers. Spring and summer calvings were associated with an increasing risk for metritis for first lactation cows and cows with 2 or more lactations, compared with fall calvings. In addition, multiparous cows that did not receive antimicrobials during the first 3 days post partum had an odds ratio of 5.0 (90% confidence interval, 2.0 to 10.0) for metritis, compared with cows treated with antimicrobials on the basis of fever or other concurrent disease. In addition, as evaluated by the Kruskal-Wallis test, administration of ECP had no effect on median duration of retained fetal membranes.

Table 2—Distribution of outcomes (absence or development of metritis) during a 10-day monitoring period in dairy cows that were untreated or received 4 mg of ECP prophylactically

Variable	Level	No metritis (No. of cows [%])	Mild metritis[1] (No. of cows [%])	Severe metritis[2] (No. of cows [%])
ECP treatment	Yes	64 (49.2)	29 (22.3)	37 (28.5)
	No	54 (45.0)	25 (20.8)	41 (34.2)
Retained fetal membranes	Yes	45 (38.1)	32 (27.1)	41 (34.7)
	No	73 (55.3)	22 (16.7)	37 (28.0)
Multiple risk factors	Yes	27 (39.7)	20 (29.4)	21 (30.9)
	No	91 (50.0)	34 (18.7)	57 (31.3)
Early administration of antimicrobials	Yes	29 (76.3)	1 (2.6)	8 (21.0)
	No	89 (42.0)	53 (25.0)	70 (33.0)
Change in BCS	None	63 (50.0)	24 (19.1)	39 (30.9)
	Loss	55 (44.3)	30 (24.2)	39 (31.5)
Calved April–June	NA	24 (36.4)	17 (25.8)	25 (37.9)
Calved July–September	NA	39 (40.6)	16 (16.7)	41 (42.7)
Calved October–December	NA	55 (62.5)	21 (23.9)	12 (13.6)
Parity	1	49 (44.1)	30 (27.0)	32 (28.8)
	≥ 2	70 (50.4)	23 (16.5)	46 (33.1)

[1]Flaccid uterus containing fetid fluids and rectal temperature < 39.7°C (103.5°F).
[2]Flaccid uterus containing fetid fluids and rectal temperature ≥ 39.7°C.
BCS = Body condition score. NA = Not applicable.

Table 3—Results of multivariate analysis of incidence of metritis in 112 primiparous cows that were or were not treated prophylactically with ECP. Model fit was assessed comparing the final model to the model containing only the intercept term[1]

Variable	Adjusted odds ratio	90% confidence interval
ECP	1.0	0.6–1.8
Control	Referent category	NA
Calved April–June	3.4	1.6–7.2
Calved July–September	3.3	1.6–6.9
Calved October–December	Referent category	NA

[1]Log likelihood χ^2 = 10.4, 3 *df*, *P* = 0.02.
NA = Not applicable.

Table 4—Results of multivariate analysis of the incidence of metritis in 138 multiparous cows that were or were not treated prophylactically with ECP. Model fit was assessed comparing the final model to the model containing only the intercept term[1]

Variable	Adjusted odds ratio	90% confidence interval
ECP	0.7	0.4–1.3
Placebo	Referent category	NA
No antimicrobials	5.0	2.0–10.0
Antimicrobials[2]	Referent category	NA
Calved April–June	2.5	1.2–5.2
Calved July–September	3.6	1.9–7.2
Calved October–December	Referent category	NA

[1]Log likelihood χ^2 = 18.5, 4 *df*, *P* = 0.001. [2]Antimicrobials (ceftiofur hydrochloride or procaine penicillin G) were administered prior to diagnosis of metritis.
NA = Not applicable.

Discussion

The perception of efficacy of estrogen to enhance uterine defenses against infection is on the basis of the observation that cows are more resistant to uterine infections during estrus than during diestrus.[36,37] Although estrogen improves the phagocytic ability of leukocytes in gilts, mares, and cattle, the clinical importance of this finding is questionable.[26,28,38,39] Angel et al[40] and Roth et al[41] examined neutrophil function in steers after administration of estradiol implants and ECP injections, respectively, and found no significant effects.

Results of our study indicate that prophylactic IM administration of 4 mg of ECP within 24 to 36 hours of calving to dairy cattle at high risk for metritis did not decrease the incidence or severity of metritis diagnosed within the first 10 days post partum. Cows with retained fetal membranes were more likely to develop metritis than cows without this finding, regardless of treatment. This finding is consistent with findings of Erb et al.[17] Studies[16,20,42] reveal that dystocia, stillbirths, postparturient hypocalcemia, and twins are associated with metritis. These factors were not significantly associated with metritis in our study. However, all cows in the study had 1 or more risk factors. Therefore, it was not possible to assess the risk of metritis associated with each specific factor, compared with a normal parturition event. Postparturient hypocalcemia was seldom reported by the farm workers, and there were not enough cases to evaluate its relationship with metritis. Eleven cows were excluded from the final analysis because of disease or death, but these cows were evenly divided between groups and likely had no effect on the study's outcome.

Our finding of less risk of severe metritis in multiparous cows that received systemic antimicrobials during the first 3 days of the postparturient period because of fever or other mild to moderate disease was an interesting finding, although not a primary outcome. Our study was not designed to test the effectiveness or necessity of systemically active antimicrobials in postparturient cattle, but because of a belief that antimicrobial treatment might be a potential confounder in the study, its use by the study team and farm personnel was recorded. Farm personnel treated only 6 cows with antimicrobials for concurrent disease problems. Our study protocol, on the basis of similar postpartum protocols in use on many dairies, required use of antimicrobials on the first day of fever ≥ 39.7°C (103.5°F), without regard to the cow's overall appearance or condition. The magnitude of the decrease in risk of severe metritis was large (5 times), but caution should be expressed regarding the use of systemic antimicrobials in this manner. Because of the lack of a nontreated pyrexic control group, we can only speculate about the potential economic benefits of administering antimicrobials only on the basis of fever during this time period. In addition, there are unresolved concerns from the general public about potential antimicrobial resistance problems from large-scale use.

To our knowledge, our study was the first to investigate the effects of a commonly prescribed dose of ECP administered to cows at high risk for metritis. The study's strength was its design, which included daily evaluations and follow-up of cows by members of the research team, rather than relying on data input by farm workers. We followed protocols for the identification and treatment of cows with metritis that are commonly used in California dairies. There was adequate sample size to detect economically important differences in incidence of metritis between study groups over the course of 9 months.

Validity of the results for other herds or regions may be questioned because of the use of only 1 dairy, but the herd had risk-factor diseases of the expected number, and our treatment protocols reflected accepted veterinary practices in the United States. Also, the risk factors used for identification of high-risk cows were well defined and universally applicable across dairies. Unfortunately, the study did not extend over the winter season. We anticipated that the risk of metritis for cows that calved during colder temperatures would be lower, compared with the warmer times of the year, and we purposely chose the warmer months for evaluation to reduce the time required to meet our projected sample size of high-risk cows.

Warmer environmental temperatures during the second and third quarters of the year were associated with increased risk for metritis, most likely because of decreased immune function from lower prepartum and postpartum feed intake and greater numbers of pathogenic bacteria in the environment. Ingvartsen et al[43] suggested that cortisol and corticotropin-releasing factor may be associated with the decrease in postpartum feed intake that is commonly seen immediately after calving. Further decreases in feed intake may be seen during periods of heat stress, exacerbating preexisting negative energy balance and lowering immune function as a result of decreased absorption of essential nutrients.[44]

The 4-mg dose of ECP was selected after review of commonly recommended monitoring and treatment protocols in the Tulare County area and from observations by Gustafsson[45] that suggest that doses of 3 to 10 mg were appropriate. In addition, our expectation, on the basis of extrapolations of results of a study by Vynckier et al,[46] was that the 4-mg dose would achieve serum estradiol concentrations similar to normal estrus. However, no estradiol concentrations were measured during our study. Although Gustafsson[45] suggested that treatment with ECP may be repeated twice at 3-day intervals, our study used only a single dose.

Many postparturient programs in use on California dairies couple the use of ECP with administration of oxytocin 1 or 2 times daily for 2 to 4 days. The uterus' responsiveness to oxytocin quickly diminishes after calving, and use of estrogen has been suggested as a means to maintain the responsiveness of the uterus to the effects of oxytocin and improve its effect.[21] Although the possible synergistic effect of ECP and oxytocin is intriguing, our study was designed to evaluate the effectiveness of estradiol in preventing metritis and did not incorporate the use of oxytocin. To adequately evaluate the benefits of ECP with and without oxytocin would have required additional study groups and either longer study duration or a larger population of at-risk cows than we had available.

Hormone use in food animals continues to be a controversial issue. Real and perceived food safety issues must be considered when designing food animal treatment modalities. Estradiol cypionate is labeled for use in cattle at doses of ≤ 4 mg to correct anestrus, aid in treatment of pyometra, or stimulate the expulsion of retained placentas or mummified fetuses. Presently, ECP (≤ 4 mg) has no labeled withdrawal times for either meat or milk.

It is not yet known whether higher doses or repeated administration of ECP may be of benefit in preventing metritis, especially if its use decreases the severity of the disease and use of antimicrobials. At this time, however, the prophylactic use of a single 4-mg dose of ECP alone cannot be recommended for use in postparturient dairy cattle.

[a]ECP, Pharmacia & Upjohn Co, Kalamazoo, Mich.
[b]Excenel, Pharmacia & Upjohn Co, Kalamazoo, Mich.
[c]Microsoft Excel, Microsoft Corp, Redmond, Wash.
[d]DairyComp 305, Valley Ag Software, Tulare, Calif.

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