Effects of parenteral administration of vitamin E on health of periparturient dairy cows

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Objective—To determine the effect of administration of vitamin E (D-α-tocopherol) on the incidence of retained placenta, metritis, and clinical mastitis during early lactation and on tocopherol concentrations.

Design—Prospective randomized controlled study.

Animals—420 Holstein cows.

Procedure—Vitamin E (3,000 mg, IM, once) was administered to 204 cows 8 to 14 days before expected parturition, and 216 control cows were not treated. The number of cows that had retained placenta, metritis, clinical mastitis, displaced abomasum, and clinically apparent acetonemia or hypocalcemia were recorded. Serum concentrations of tocopherol, the tocopherol:cholesterol ratio, and glutathione-peroxidase activity were determined from samples obtained before administration of vitamin E, 7 and 14 days after administration, and at 30 days after parturition from 36 treated and 36 control cows.

Results—Administration of vitamin E significantly decreased the incidence of retained placenta and metritis (13/204 [6.4%] and 8/204 [3.9%], respectively, for the vitamin E-treated group; 27/216 [12.5%] and 19/ 216 [8.8%], respectively, for the untreated group) but did not affect the incidence of clinical mastitis. Serum vitamin E concentration was significantly higher in treated than in control cattle at 7 and 14 days after administration, but serum tocopherol:cholesterol ratio was significantly higher only at 7 days after administration.

Clinical Implications—Parenteral administration of a single injection of vitamin E before parturition may decrease the incidence of retained placenta and metritis in dairy cows but will increase serum concentrations for 7 to 14 days after administration. (*J Am Vet Med Assoc* 1997;211:466–469)

Micronutrients that impart antioxidant activity, such as selenium, copper, vitamin E, and vitamin A, can improve the immune status of cattle.[1-6] Antioxidant supplementation may also beneficially affect health of neonatal calves, decrease the incidence of reproductive disorders after parturition, and decrease the incidence and severity of clinical mastitis.[7-12] Nutritional supplementation of selenium and possibly other antioxidant micronutrients may not be adequate in some dairy herds, particularly in nonlactating cattle.[12,13] Dry-matter intake often decreases in dairy cattle 1 to 2 weeks before calving, a critical period affecting resistance to mammary infections and reproductive and metabolic health of cows in early lactation.[14] Parenteral administration of vitamin E offers an alternative to overcome potential deficiencies in nutritional supplementation. However, beneficial effects of nutritional supplements or parenteral administration of vitamin E have not been universal among studies, and potentially confounding factors, such as selenium or vitamin E status at the time of administration, duration and dose, and preexisting incidence of disease within a herd before administration, can all affect the outcome of treatment.[15-19] Additionally, potential effects of parenteral administration on tissue status of cows during the periparturient period is not fully understood.

The purpose of the study reported here was to determine the efficacy of administration of vitamin E during the prepartum period on incidence and treatment losses resulting from common diseases associated with the periparturient period. A secondary objective was to determine changes in serum tocopherol concentrations resulting from parenteral administration.

Materials and Methods

Four hundred twenty Holstein cattle from 4 dairies in Michigan were used in the study. Cattle were stratified on the basis of lactation number (primiparous or multiparous), and each cow was randomly assigned to receive vitamin E (D-α-tocopherol[a]) or to serve as member of a control group (not treated). Vitamin E was administered (3,000 mg, IM) by the investigators as a single injection given 8 to 14 days before expected parturition.

To determine incidence of postpartum disease, data forms were supplied to farm managers to facilitate their participation. Farm personnel completed 1 data form for each cow in the trial, with instructions to record diseases that were evident during the first 30 days after calving. Additionally, when farm managers believed a specific episode of disease warranted drug treatment, the ensuing days during which milk was discarded to avoid residues in marketed milk was recorded. Diseases that were recorded included retained placenta (defined as a cow that had retained fetal membranes for ≥ 24 hours after calving), metritis (determined by a producer or veterinarian on the basis of an abnormal vaginal discharge, clinical signs of systemic illness, or results of transrectal palpation), acetonemia (abnormal ketone concentrations in urine[b]), displaced abomasum (diagnosed by a veterinarian), clinically apparent hypocalcemia (paralysis and recumbency), and clinical mastitis (defined as milk from ≥ 1 quarter that was apparently abnormal throughout a given milking but could include cows with signs of systemic illness). A standardized protocol for treatment of each disease on a given farm was established to reduce bias between treatment groups with respect to therapeutic decisions.

Sample collection—Serum and blood samples were obtained from 72 cattle (18 primiparous and 18 multiparous cows given vitamin E, 18 primiparous and 18 multiparous cows not given vitamin E) to determine serum concentrations of tocopherol, cholesterol, tocopherol:cholesterol

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ratio, and blood glutathione-peroxidase activity. Sample collection was divided among the 4 seasons. Twenty-four samples were obtained from each of 2 herds (3 primiparous and 3 multiparous cows/season/herd), and 12 samples were obtained from each of the 2 remaining herds (3 primiparous and 3 multiparous cows for 2 seasons/herd) because of the withdrawal of 1 herd halfway through the study, which was replaced by another herd. One of 2 schemes was used on each farm to select cows that would be used for sample collection. The first was to select 1 primiparous and 2 multiparous cows (representing the treated group) and 1 multiparous and 2 primiparous cows (representing the control group). During the subsequent sampling period 3 months later, 1 multiparous and 2 primiparous cows (representing the treated group) and 1 primiparous and 2 multiparous cows (representing the control group) were selected. Alternatively, this scheme was reversed so that during the first sampling period, 1 multiparous and 2 primiparous cows from the treated group and 1 primiparous and 2 multiparous cows from the control group were selected, with reversal of the number of primiparous and multiparous cows at the next sampling period. For each herd, including the fourth (replacement) herd, a coin toss determined the scheme that would be used.

Samples were obtained from selected cattle at 4 time points: entry into the study (8 to 14 days before expected parturition, but immediately before administration of vitamin E), 7 and 14 days after administration, and 30 days after parturition. Samples were obtained from the coccygeal vein, stored on ice during transport to the laboratory, and submitted for analysis within 4 to 6 hours after collection. Blood was collected into tubes coated with EDTA as an anticoagulant.

Clinical biochemical analysis—Vitamin E concentration in serum was determined by a modification of the high-pressure liquid chromatographic method of Widicus and Kirk.[20] Briefly, serum was thoroughly mixed with an equivalent volume of ethanol containing 0.01% butylated hydroxytoluene, and the extracted vitamin E was mixed with hexane (an amount that was twice the ethanol volume). The hexane extract was chromatographed on a 3.9 X 150-mm silica column with 10-µm particle size.[c] Elution was isocratic at 1.1 ml/min with a mobile phase of hexane:chloroform of 85:15. Detection was by absorbance at 292 nm with quantification in comparison to a single-point external standard. Cholesterol concentration was determined directly in serum by use of a commercially available enzyme-based kit with a colorimetric endpoint.[d]

Glutathione peroxidase activity was assayed essentially by the coupled-enzyme technique of Paglia and Valentine.[21] **Enzyme units** (EU) were calculated as the number of moles of reduced glutathione oxidized/min/L of blood. Values were expressed as EU/g of hemoglobin. Hemoglobin concentrations were determined colorimetrically.[22]

Statistical analysis—Logistic regression was used to determine the effect of treatment with vitamin E on disease incidence. The model included disease as the dependent variable and included herd, lactation number (primiparous or multiparous), season, and treatment group as independent variables. Because of nonnormal distribution of data, nonparametric analysis (Mann-Whitney test) was used to evaluate the difference between treatment groups for days of milk discarded per disease case. Three separate repeated-measures (split-plot) analyses were conducted to determine whether glutathione peroxidase activity, serum tocopherol concentration, and the serum tocopherol:cholesterol ratio differed between treatment groups at each of the 4 sampling periods. Lactation number (primiparous or multiparous) was also included in the 3 analyses.

Results

The incidence of retained placenta and metritis (13/204 [6.4%] and 8/204 [3.9%], respectively) for the group treated with vitamin E was significantly ($P < 0.05$) less than for the untreated group (27/216 [12.5%] and 19/216 [8.8%], respectively; Table 1). Lactation number did not have an effect on incidence of disease. Incidence of clinical mastitis did not differ between treatment groups during the 30 days after parturition. Mean (± SEM) number of days for which milk was discarded per cow affected by retained placenta or metritis (1.5 ± 0.8 days) for the treated group was not significantly different than for the untreated group (4.9 ± 1.2 days). A similar trend was observed for mean number of days for which milk was discarded per cow affected by clinical mastitis (3.4 ± 0.7 and 4.5 ± 0.9 days for the treated and untreated groups, respectively).

Blood glutathione peroxidase activity did not differ between treatment groups at any sampling period and consistently had a mean of 225 to 248 EU/g of hemoglobin at each sampling period. Serum vitamin E concentration was significantly ($P < 0.01$) higher for vitamin E-treated cattle at 7 and 14 days after administration (3.97 and 3.11 µg/ml, respectively) than for control cattle (2.68 and 2.24 µg/ml, respectively). At

Table 1—Incidence of disease in periparturient cows that were treated by administration of a single 3,000-mg dose of vitamin E, IM (n = 204), or that did not receive vitamin E treatment (n = 216)

	Lactation number					
	Multiparous		Primiparous		All cows	
Variable	Treated	Untreated	Treated	Untreated	Treated	Untreated
Retained placenta	12	18	1[a]	9[b]	13[a]	27[b]
Metritis	6	14	2	5	8[a]	19[b]
Dystocia	6	5	4	2	10	7
Acetonemia	3	4	0	1	3	5
Displaced abomasum	14	18	1	6	15	24
Hypocalcemia	3	4	0	0	3	4
Clinical mastitis ≤ 30 days after parturition	15	17	5	2	20	19
Total	142	141	62	75	204	216

[a,b]Values with different superscripts differ significantly ($P < 0.05$) between treatment groups.

30 days after parturition, vitamin E concentrations in treated cattle (4.74 µg/ml) were higher, but not significantly different, than concentrations in control cattle (4.01 µg/ml). The serum tocopherol:cholesterol ratio was significantly ($P < 0.01$) higher for vitamin E- treated cattle (5.01×10^{-3}) than for control cattle (3.69×10^{-3}) at 7 days after administration. Lactation number, herd of origin, and season did not significantly affect any of the clinical biochemical results.

Discussion

In the study reported here, administration of vitamin E before parturition reduced the incidence of reproductive disorders after parturition. In other reports,[7-9] vitamin E supplementation reduced the incidence of retained placenta and metritis. However, this has not been a consistent effect in all studies, and response can vary depending on vitamin E tissue status at the time of dietary supplementation or parenteral administration, duration of supplementation, incidence of retained placenta and metritis in the herd before supplementation or administration, and potential interactions with other nutrients, most notably selenium.[15,16,19] We did not know the incidence of retained placenta and metritis in the herds before the start of the study. However, overall incidence of retained placenta during the study was 40 of 420 (9.5%). Factors other than vitamin E treatment that may have contributed to reproductive disorders after calving such as hypocalcemia, dystocia, and birth of twins involved only 24 of 420 (5.7%) cows and were balanced between groups. Analysis of mean glutathione peroxidase activity in blood and serum vitamin E:cholesterol ratios determined at time points throughout the study indicated that the herds selected had values within reference ranges (blood glutathione peroxidase activity > 150 EU/g of hemoglobin; vitamin E:cholesterol ratio > 2.5×10^{-3}) used by the Animal Health Diagnostic Laboratory at Michigan State University. Thus, beneficial effects on postpartum reproductive health as a result of parenteral administration of vitamin E were observed despite results of clinical biochemical analysis indicative of adequate dietary supplementation.

Smith et al[10] reported that nonlactating cows fed diets supplemented with vitamin E and given a single injection of selenium before parturition had a 37% decrease in the incidence of clinical mastitis and had a duration of infection that was 62% less than those in unsupplemented control cows. Furthermore, a 57% decrease in the incidence of clinical mastitis and a 40% decrease in duration of intramammary infection were observed in selenium and vitamin E-supplemented primiparous cows, compared with unsupplemented primiparous cows.[e] In a survey of 9 dairies in Ohio, rate of clinical mastitis was negatively correlated to concentration of vitamin E in diets and to plasma selenium concentration; however, in similar surveys conducted in England or Canada,[12,17,18] there was not a relationship between mean herd plasma vitamin E concentration and incidence of clinical mastitis. In our study, vitamin E administration did not reduce the incidence of clinical mastitis or days of milk discarded because of antibiotic treatment. However, the lack of an effect of vitamin E on clinical mastitis may have resulted from adequate selenium and vitamin E status prior to administration, the fact that a 3,000-mg dose did not sustain effective concentrations for a sufficient time to enhance mammary resistance, and the incidence of clinical mastitis, particularly acute cases, was low in herds in our study, compared with other field reports.[23-25]

In our study, serum tocopherol concentrations increased in response to parenteral administration. As a result, decreases that were detected at parturition in the control group were not detected in the treated group. This is in agreement with studies in which a decrease in plasma vitamin E concentration during the periparturient period was detected and in which nutritional supplementation of vitamin E to nonlactating cows ameliorated these effects.[26,27] Additionally, results of our study agreed with those in other reports in which the importance of determining serum tocopherol in relation to lipid concentration was documented.[26-28] The apparent decrease in serum vitamin E concentration at parturition and subsequent increase 30 days after parturition were not reflected in the cholesterol:tocopherol ratio, which remained constant in the control group throughout the study period.

We concluded that a single 3,000-mg dose of vitamin E administered parenterally approximately 2 weeks before parturition decreased the incidence of retained placenta and metritis after parturition. An effect on the incidence of clinical mastitis during the first 30 days after parturition was not observed; however, the dose may not have been sufficient to affect the outcome in cows that were receiving adequate dietary supplements of vitamin E.

[a]Vital-E, Schering-Plough Animal Health, Union, NJ.
[b]Ketostix, Miles Inc, Elkhart, Ind.
[c]Waters Porasil Column, Waters Associates, Marlborough, Mass.
[d]Kit #352, Sigma Chemical Co, St Louis, Mo.
[e]Smith KL, Conrad HR, Amiet BA, et al. Effect of vitamin E and selenium dietary supplementation on mastitis in first lactation dairy cows (abstr). *J Dairy Sci* 1985;68(Suppl 1):190.

References

1. Boyne R, Arthur JR. Alterations of neutrophil function in selenium-deficient cattle. *J Comp Pathol* 1979;89:151–158.
2. Daniel LR, Chew BP, Tanaka TS, et al. ß-carotene and vitamin A effects on bovine phagocyte function in vitro during the prepartum period. *J Dairy Sci* 1991;74:124–131.
3. Grasso PJ, Scholz RW, Erskine RJ, et al. Phagocytosis, bactericidal activity, and oxidative metabolism of milk neutrophils from dairy cows fed selenium-supplemented and selenium-deficient diets. *Am J Vet Res* 1990;51:269–274.
4. Gyang EO, Stevens JB, Olson WG, et al. Effects of selenium-vitamin E injection on bovine polymorphonucleated leukocytes phagocytosis and killing of *Staphylococcus aureus*. *Am J Vet Res* 1984;45:175–177.
5. Hogan JS, Smith KL, Weiss WP, et al. Relationships among vitamin E, selenium, and bovine blood neutrophils. *J Dairy Sci* 1990;73:2372–2378.
6. Politis I, Hidiroglou M, Batra TR, et al. Effects of vitamin E on immune function of dairy cows. *Am J Vet Res* 1995;56:179–184.
7. Julien WE, Conrad HR, Moxon AL. Selenium and vitamin E and incidence of retained placenta in parturient dairy cows. II. Prevention in commercial herds with prepartum treatment. *J Dairy Sci* 1976;59:1960–1966.

8. Trinder N, Hall RJ, Renton CP. The relationship between the intake of selenium and vitamin E on the incidence of retained placenta in dairy cows. *Vet Rec* 1973;93:641–646.

9. Brzezinska-Slebodzinska E, Miller JK, Quigley JD III, et al. Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. *J Dairy Sci* 1994;77:3087–3095.

10. Smith KL, Harrison JH, Harison DD, et al. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical mastitis and duration of clinical symptoms. *J Dairy Sci* 1984;67:1293–1300.

11. Erskine RJ, Eberhart RJ, Grasso PJ, et al. Induction of *Escherichia coli* mastitis in cows fed selenium-deficient or selenium-supplemented diets. *Am J Vet Res* 1989;50:2093–2100.

12. Weiss WP, Hogan JS, Smith KL, et al. Relationships among selenium, vitamin E, and mammary gland health in commercial dairy herds. *J Dairy Sci* 1990;73:381–390.

13. Erskine RJ. Nutrition and mastitis. *Vet Clinics North Am* 1993;9:551–561.

14. Zamet CN, Colenbrander VF, Callahan CJ, et al. Variables associated with peripartum traits in dairy cows. I. Effect of dietary forages and disorders on voluntary intake of feed, body weight, and milk yield. *Theriogenology* 1979;11:229–244.

15. Kappel LC, Ingraham RH, Morgan EB, et al. Selenium concentrations in feeds and effects of treating pregnant Holstein cows with selenium and vitamin E on blood selenium values and reproductive performance. *Am J Vet Res* 1984;45:691–694.

16. Gwazdauskas FC, Bibb TL, McGilliard ML, et al. Effect of prepartum selenium-vitamin E injection on time for placenta to pass and on productive functions. *J Dairy Sci* 1979;62:978–984.

17. Batra TR, Hidiroglou M, Smith MW, et al. Effect of vitamin E on incidence of mastitis in dairy cattle. *Can J Anim Sci* 1992;72:287–297.

18. Ndiweni N, Field TR, Williams MR, et al. Studies on the incidence of clinical mastitis and blood levels of vitamin E and selenium in dairy herds in England. *Vet Rec* 1991;129:86–88.

19. Hurley WL, Doane RM. Recent developments in the role of vitamins and minerals in reproduction. *J Dairy Sci* 1989;72:784–804.

20. Widicus WA, Kirk JR. High pressure liquid chromatographic determination of vitamins A and E in cereal products. *J Assoc Off Anal Chem* 1979;62:637–641.

21. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158–169.

22. Expert Panel on Haemoglobinometry, International Committee for Standardization in Haematology (ICSH): recommendation for reference method for haemoglobinometry in human blood and specifications for international haemiglobincyanide reference preparation. 3rd ed. *Clin Lab Haematol* 1987;9:73–79.

23. Erskine RJ, Eberhart RJ, Hutchinson LJ, et al. Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. *J Am Vet Med Assoc* 1988;192:761–765.

24. Gonzalez RN, Jasper DE, Kronlund DC, et al. Clinical mastitis in two California dairy herds participating in contagious mastitis control programs. *J Dairy Sci* 1990;73:648–660.

25. Anderson KL, Smith AR, Gustafsson BK, et al. Diagnosis and treatment of acute mastitis in a large dairy herd. *J Am Vet Med Assoc* 1982;181:690–693.

26. Weiss WP, Hogan JS, Smith KL, et al. Effect of supplementing periparturient cows with vitamin E on distribution of α-tocopherol in blood. *J Dairy Sci* 1992;75:3479–3485.

27. Weiss WP, Hogan JS, Smith KL, et al. Effect of dietary fat and vitamin E on α-tocopherol and ß-carotene in blood of peripartum cows. *J Dairy Sci* 1994;77:1422–1429.

28. Herdt TH, Smith JC. Blood-lipid and lactation-stage factors affecting serum vitamin E concentrations and vitamin E:cholesterol ratios in dairy cattle. *J Vet Diagn Invest* 1996;8:228–232.

Correction: Ocular diseases of llamas: 194 cases (1980-1993)

In "Ocular diseases of llamas: 194 cases (1980-1993)" (*JAVMA*, June 15, 1997, pp 1784-1787), several symbols in the Data Analysis paragraph on page 1785 were inadvertently switched for incorrect symbols during final preparation of the manuscript for desktop publication, after the authors had examined the galley proofs. The paragraph should read as follows:

To test for significant differences in prevalences of ocular diseases among llamas, cattle, and horses, χ^2 analysis was performed, using 2-way contingency tables. Numbers of animals of each species that had ocular disease were compared with total numbers of animals of each species that were examined by the reporting hospitals. χ^2 Analysis also was used to test for differences among species in regard to prevalence of the most commonly seen general ocular disease categories (uveitis, corneal ulcers, and ocular SCC) and for differences in ocular disease prevalence among age groups (< 1 year old, ≥ 1 to < 2 years old, ≥ 2 to < 4 years old, ≥ 4 to < 7 years old, ≥ 7 years old). These age categories were chosen because they were similar to the age categories used by the VMDB. For each livestock species, the number of animals in each disease category was compared with the number of animals that had any ocular disease. For all tests, a value of $P < 0.05$ was considered significant.

The *JAVMA* regrets the error.