Antioxidant Status of Dairy Cows Supplemented Prepartum with Vitamin E and Selenium

E. BRZEZINSKA-SLEBODZINSKA,1 J. K. MILLER, J. D. QUIGLEY, III, and J. R. MOORE2
Animal Science Department
The University of Tennessee
Knoxville 37901-1071

F. C. MADSEN
Suidae Technology, Inc.
Greensburg, IN 47240

ABSTRACT
Possible relationships among dietary antioxidants, oxidative status, and placental retention were investigated in periparturient dairy cows. During 6 wk prepartum, 16 cows each were given daily by capsule 1000 IU of vitamin E, 3 mg of Se, both vitamin E and Se, or neither (control). α-Tocopherol in serum and fast-acting antioxidants in plasma increased, but, in red blood cells, thiobarbituric acid-reactive substances decreased during the last 6 wk before parturition in cows given vitamin E. These measurements were unaffected by supplementation of Se. Cows that had retained placenta ≥12 h had lower fast-acting antioxidants in plasma and glutathione peroxidase in red blood cells up to 2 wk before calving than did cows that shed fetal membranes in <12 h. Results suggest that inadequate dietary antioxidants may increase oxidative stress, production of lipid peroxides, and incidence of retained fetal membranes in dairy cows.
(Key words: dairy cows, antioxidants, vitamin E, selenium)

INTRODUCTION
The periparturient period is especially critical for health and subsequent performance of dairy cows (21). Retained fetal membranes (RFM) can, in addition to cost of treatment, lower milk yield, market value, and productive life of the cow and result in indirect costs that are difficult to quantify (14). The well-documented reduction in incidence of RFM when the nutrients used for antioxidant defense, vitamin E and Se, are supplemented prepartum (13) suggests that the etiology of this disorder may involve oxidative stress. Possible relationships between oxidative stress and periparturient disorders of dairy cows have been reviewed (16).

Oxidative stress results from an imbalance between production of oxygen-centered free radicals and their safe disposal (22). Free radicals, also referred to as reactive oxygen metabolites (ROM) (19), are products of normal metabolism and have important physiological functions when a balance between production and safe disposal of ROM is maintained (11). However, deficiencies of natural protective substances or excess exposure to substances that can stimulate ROM production may impair health and performance. Vitamin E, as the primary lipid-soluble antioxidant, and Se, as a necessary component of glutathione peroxidase (GSH-Px), are critical for the body's defense against ROM.

A relatively simple, reproducible procedure for evaluation of oxidant-antioxidant status...
that can be performed with minimum stress to the animal would be useful in relating oxidative stress to disease conditions and response to antioxidant supplementation. Glazer (8, 9) has developed a procedure for quantification of antioxidant capacity of blood plasma, or other readily accessible biological fluids, that may provide an index of an animal's ability to deal with oxidative stress. Glazer (8, 9) measured the capacity of antioxidants in biological fluids to prolong fluorescence of phycoerythrin that has been exposed to peroxo radicals generated in vitro. Two distinct fractions in blood plasma that prolong fluorescence of phycoerythrin have been identified (4): 1) a slow-acting fraction consists primarily of proteins in plasma and 2) a fast-acting fraction, remaining after precipitation of proteins, contains antioxidants, including vitamin E, ascorbate, bilirubin, and urate.

The present report investigates relationships in plasma among fast-acting antioxidants (FAA), vitamin E and Se, which are important components of antioxidant defense and RFM.

MATERIALS AND METHODS

Cows and Treatments

Sixty-four multiparous dairy cows were divided into 13 blocks of 4 Holsteins and 3 blocks of 4 Jerseys on the basis of expected calving date. Treatments, assigned randomly within block and given daily by gelatin capsule during the last 6 wk prepurum, were 1000 IU of vitamin E, as dl-α-tocopheryl acetate, per cow (group 1); 3 mg of Se, as sodium selenite, per cow (group 2); vitamin E plus Se (group 3); or neither (group 4, control). One cow assigned to Se calved >5 wk early and was removed from the experiment. Cows were kept in the same lot and fed grass hay (average, .1 mg of Se/kg of DM) for ad libitum intake plus an average of 4 kg/d of 16% protein commercial dairy concentrate, which contained .3 mg/kg of Se as sodium selenite and 33 IU/kg of vitamin E as d,l-α-tocopheryl acetate.

Collection and Preparation of Blood

Blood samples were collected at 6 wk (before initiation of treatments) and at 4, 2, and 0 wk before expected calving by venipuncture into two types of evacuated tubes (Becton Dickinson, Rutherford, NJ): heparinized tubes for red blood cells (RBC) and plasma and serum collection tubes for serum. Immediately after collection, blood was kept in an ice chest until centrifugation at 1000 × g for 20 min. Serum samples were protected from light and stored at −20°C for vitamin E assay. Plasma was separated and stored on ice for analysis the same day or frozen for analysis within 4 d for FAA. Packed RBC were resuspended in 3 ml of ice-cold .9% NaCl and centrifuged at 1000 × g for 10 min. Supernatant and buffy coat were aspirated, and the saline wash, centrifugation, and aspiration were repeated twice. Supernatants were discarded, and 1 ml of packed RBC was resuspended in 3 ml of ice-cold saline. Hematocrit was determined by capillary centrifugation so that subsequent measurements could be expressed per volume of packed RBC. The RBC were lysed by addition of 8 ml of ice-cold distilled water to 2-ml aliquots of resuspended cells. The mixture was mixed vigorously and centrifuged at 3000 × g for 10 min, and the supernatants were assayed immediately or stored at −20°C.

Analytical Procedures

Plasma FAA were measured by a phycoerythrin-based fluorescence procedure (4, 8, 9). As soon as possible after collection, .25 ml of plasma was diluted to .5 ml with .75 M phosphate buffer, pH 7. Contaminating metal ions were removed from the buffer by passage through a column of Chelex® 100 resin (100 to 200 mesh), sodium form (Bio-Rad Laboratories, Hercules, CA). A mixture of .2 ml of diluted plasma and .8 ml of saturated enzyme grade ammonium sulfate (Aldrich Chemical Co., Inc., Milwaukee, WI) was placed in ice for 90 min. After 5 min of centrifugation (Eppendorf Model 5402 microcentrifuge; Brinkman Instruments, Inc., Westbury, NY), the supernatant was decanted, and the pellet was resuspended in 1 ml of ice-cold 80% saturated ammonium sulfate and recentrifuged. The two supernatants were combined and assayed immediately or stored frozen under N for up to 4 d.

To the assay mixture was added in the following order: 1.58 ml of phosphate buffer, .2 ml of ammonium sulfate supernatant of
plasma, .02 ml of 1.7 \times 10^{-6} M \underset{B}{\text{B}} \text{phycoerythrin} (\text{Sigma Chemical Co., St. Louis, MO}), \text{and .2 ml of 40 mM } 2,2'\text{-azobis(2-amidinopropane)}\text{dihydrochloride (Polysciences, Warrington, PA). Fluorescence emission at 575 nm with excitation at 540 nm was measured at 37°C (Perkin-Elmer 650S Fluorescence Detector; Perkin-Elmer, Inc., Norwalk, CT) immediately before and at 5-min intervals for 30 min after addition of 2,2'-azobis(2-amidinopropane)dihydrochloride. Fluorescence remaining at each interval, expressed as a percentage of initial fluorescence, is proportional to the FAA content of the plasma being analyzed (4).}

\( \alpha \)-Tocopherol in serum was measured by HPLC as described by Moore (17). The RBC hemolystates were assayed for thiobarbituric acid-reactive substances (TBARS) (1), reduced glutathione (GSH) (2), and GSH-Px (15, 18).

**Statistical Procedures**

Fluorescence data were analyzed as a double split-plot design in time (7) using the model

\[
Y_{ijkl} = \mu + T_i + A_{ij} + W_k + (WT)_{ik} + W_{ik} + (MT)_{ij} + (WTW)_{ijkl} + e_{ijkl}
\]

where

- \( Y_{ijkl} \) = fluorescence,
- \( \mu \) = overall mean,
- \( T_i \) = effect of treatment i,
- \( A_{ij} \) = effect of cow j within treatment i,
- \( W_k \) = effect of week k,
- \( (WT)_{ik} \) = effect of interaction of week \( \times \) treatment,
- \( (WA)_{ijk} \) = effect of interaction of week \( \times \) cow within treatment,
- \( M_l \) = effect of minute l,
- \( (MT)_{ij} \) = effect of interaction of minute \( \times \) treatment,
- \( (MW)_{kl} \) = effect of interaction of minute \( \times \) week,
- \( (MTW)_{ijkl} \) = effect of interaction of minute \( \times \) treatment \( \times \) week, and
- \( e_{ijkl} \) = residual.

Treatment effects were tested using effects of cow within treatment and week by cow within treatment for effects of week and week \( \times \) treatment and using the residual for effects containing minute.

Other data were analyzed as a split-plot design in time using the model

\[
Y_{ijk} = \mu + T_i + A_{ij} + W_k + (WT)_{hk} + e_{ijk}
\]

where

- \( Y_{ijk} \) = dependent variable,
- \( \mu \) = overall mean,
- \( T_i \) = effect of treatment i,
- \( A_{ij} \) = effect of cow j within treatment i,
- \( W_k \) = effect of week k,
- \( (WT)_{hk} \) = effect of interaction of week \( \times \) treatment, and
- \( e_{ijk} \) = residual.

Cow within treatment was used to test effects of treatment. Orthogonal contrasts tested effects of vitamin E, Se, and interaction. Strength of association between antioxidant supplementation and indices of oxidative stress was tested by odds ratio (5) and chi-square. Unless stated otherwise, effects were considered to be significant if \( P < .05 \), a trend if \( P > .05 \) but \( P < .10 \), and nonsignificant if \( P > .10 \).

**RESULTS**

**Vitamin E or Se Supplementation and Oxidative Status**

Serum concentrations of \( \alpha \)-tocopherol averaged 2.05 \( \mu g/ml \) for all treatment groups before supplements were initiated and decreased progressively during the following 6 wk in groups that were not supplemented with vitamin E (Figure 1). In contrast, \( \alpha \)-tocopherol in serum increased approximately 75% during the first 2 wk of vitamin E supplementation and then declined at a rate paralleling that of unsupplemented groups but never fell below pretreatment concentrations. At parturition, \( \alpha \)-tocopherol in serum was higher in groups supplemented with vitamin E than in unsupplemented groups. Dietary Se had no effect on concentrations of \( \alpha \)-tocopherol in serum.

Least squares means for GSH-Px, dependent or not dependent on Se, and GSH were determined using effects of cow within treatment and week by cow within treatment for effects of week and week \( \times \) treatment and using the residual for effects containing minute.

Other data were analyzed as a split-plot design in time using the model

\[
Y_{ijk} = \mu + T_i + A_{ij} + W_k + (WT)_{hk} + e_{ijk}
\]

where

- \( Y_{ijk} \) = dependent variable,
- \( \mu \) = overall mean,
- \( T_i \) = effect of treatment i,
- \( A_{ij} \) = effect of cow j within treatment i,
- \( W_k \) = effect of week k,
- \( (WT)_{hk} \) = effect of interaction of week \( \times \) treatment, and
- \( e_{ijk} \) = residual.

Cow within treatment was used to test effects of treatment. Orthogonal contrasts tested effects of vitamin E, Se, and interaction. Strength of association between antioxidant supplementation and indices of oxidative stress was tested by odds ratio (5) and chi-square. Unless stated otherwise, effects were considered to be significant if \( P < .05 \), a trend if \( P > .05 \) but \( P < .10 \), and nonsignificant if \( P > .10 \).
Week of supplementation

Figure 1. α-Tocopherol in Serum in cows supplemented daily with 1000 IU of vitamin E (---), 3 mg of Se (--.-), neither (- - - -), or a combination (-----) during the last 6 wk. Standard error = .21 μg/ml.

calculated using as covariants the measurements on samples collected immediately before initiation of vitamin E or Se supplementation. After 6 wk, respective values of RBC from cows supplemented with vitamin E, Se, both, or neither were 41.5, 38.0, 40.3, and 39.2 U/ml of Se-dependent GSH-Px; 5.3, 5.0, 6.3, and 5.1 U/ml of Se-independent GSH-Px; and .34, .32, .34, and .33 mg/ml of GSH. When cows were supplemented with vitamin E, GSH was higher and Se-dependent GSH-Px tended to be higher, but Se effects or interactions of vitamin E × Se were not significant. Neither main effects nor interactions were significant for Se-independent GSH-Px. The lack of an effect of Se supplementation on GSH-Px suggests that basal Se intake was adequate.

More fluorescence remaining at each time increment of incubation of phycoerythrin with the free radical generator 2,2'-azobis(2-amidinopropane)dihydrochloride indicates that concentration of FAA was higher in the blood plasma assayed. Plasma from different treatment groups before supplementation with vitamin E or Se was comparable in its ability to protect phycoerythrin from degradation by ROM (Figure 2). Thereafter, plasma from cows given vitamin E, with or without Se, protected phycoerythrin against ROM more than did plasma from cows given Se only or no antioxidant supplement. A highly significant (P < .01) interaction of week × treatment × time indicated that changes of fluorescence during 30 min of the assay varied by week of supplementation. The FAA in plasma from cows supplemented with vitamin E increased over that of unsupplemented cows by 33% within 2 wk (Figure 2B), 43% by 4 wk (Figure 2C), and 61% after 6 wk (Figure 2D) and were unaffected by Se.

The TBARS in RBC remained relatively constant during 6 wk in cows given no supplements or Se only (Figure 3) but decreased 27 to 28% (P < .01) during 6 wk of vitamin E supplementation, with or without supplemental Se. The interaction of week × treatment indicated that RBC TBARS decreased (P < .01) with week of vitamin E supplementation. The TBARS were correlated (P < .01) negatively with FAA in plasma (r = -.57), α-tocopherol in serum (r = -.27), and GSH in RBC (r = -.48).

Each of 63 cows was classified as above or below the average of all cows for each of the preceding measurements immediately before calving. Oxidative stress results when ROM are produced faster than they are effectively removed (22). Decreases in components of antioxidant defense, increases in end products of ROM production, or a combination could thus be considered to be indices of oxidative stress. Numbers of cows greater than or equal to or less than the mean for six measurements related to severity of oxidative stress are in Table 1. The ability to combat oxidative stress was considered to be impaired if a cow was below the mean of all 63 cows in FAA in plasma, α-tocopherol in serum, GSH-Px in RBC, and GSH in RBC or above mean in TBARS in RBC.

Odds ratios (5) were calculated to measure the strength of the association between supplementation with vitamin E or Se and improvement of each index of oxidative stress. If 63-cow group means are considered to be representative of normal antioxidant status, a cow unsupplemented with vitamin E, compared with a cow given 1000 IU/d of vitamin E, was <1% as likely to have at least normal FAA in plasma but over 47 times more likely to have elevated TBARS in RBC (Table 1). All other measurements of antioxidant status of cows that were not supplemented with vitamin E were below average to different degrees. However, FAA in plasma (antioxidant protection) and TBARS in RBC (end products of lipid peroxidation) were most strongly influenced by vitamin E. None of these measurements was affected by supplemental Se.
ANTIOXIDANT STATUS OF COWS

Figure 2. Bovine plasma fast-acting antioxidants (FAA) after 0 (A), 2 (B), 4 (C), or 6 (D) wk of daily supplementation with 1000 IU of vitamin E (--), 3 mg of Se (--), neither (--), or a combination (-- --) during the last 6 wk of gestation. More fluorescence remaining at each interval indicates a greater amount of FAA in the plasma. Standard error = 2%.

TABLE 1. Association between ability to combat oxidative stress above (+) or below (-) the mean of 63 cows unsupplemented or supplemented with vitamin E.\(^1\)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Unsupplemented</th>
<th>Supplemented</th>
<th>Odds ratio</th>
<th>95% Confidence limits</th>
<th>(\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-Tocopherol in serum</td>
<td>11, 20</td>
<td>21, 11</td>
<td>.29</td>
<td>.09-.91</td>
<td>5.72</td>
<td>.02</td>
</tr>
<tr>
<td>FAA in plasma</td>
<td>2, 29</td>
<td>30, 2</td>
<td>.005</td>
<td>0-.04</td>
<td>48.01</td>
<td>.001</td>
</tr>
<tr>
<td>GSH-Px in RBC</td>
<td>13, 18</td>
<td>20, 12</td>
<td>.43</td>
<td>.14-1.33</td>
<td>2.67</td>
<td>.10</td>
</tr>
<tr>
<td>Se Dependent</td>
<td>14, 21</td>
<td>18, 10</td>
<td>.37</td>
<td>.12-1.16</td>
<td>3.67</td>
<td>.06</td>
</tr>
<tr>
<td>Se Independent</td>
<td>10, 17</td>
<td>22, 14</td>
<td>.37</td>
<td>.12-1.17</td>
<td>3.58</td>
<td>.07</td>
</tr>
<tr>
<td>GSH in RBC</td>
<td>4, 27</td>
<td>28, 4</td>
<td>.02</td>
<td>0-10</td>
<td>35.06</td>
<td>.001</td>
</tr>
<tr>
<td>TBARS in RBC</td>
<td>4, 27</td>
<td>28, 4</td>
<td>.02</td>
<td>0-10</td>
<td>35.06</td>
<td>.001</td>
</tr>
</tbody>
</table>

\(^1\)Below mean \(\alpha\)-tocopherol in serum, fast-acting antioxidants (FAA) in plasma, glutathione peroxidase (GSH-Px) dependent on or independent of Se, and reduced glutathione (GSH) or above mean thiobarbituric acid-reactive substances (TBARS) in red blood cells (RBC) were considered to be indicative of impaired ability to combat oxidative stress.
Figure 3. Thiobarbituric acid-reactive substances (TBARS) in red blood cells expressed as nanomoles of malondialdehyde (MDA) per gram of hemoglobin (Hb) in cows supplemented with 1000 IU of vitamin E (--), 3 mg of Se (---), neither (--), or a combination (----) during the last 6 wk of gestation. Standard error = 14.4 nmol of MDA/g of Hb.

Vitamin E or Se Supplementation and RFM

Cows retaining placenta >12 h included 4 of 16 cows fed vitamin E, 4 of 15 fed Se, 3 of 16 fed vitamin E plus Se, and 6 of 16 controls. Although incidence of RFM, compared with that in untreated controls, appeared to be reduced 33% by vitamin E, 47% by Se, and 50% by vitamin E and Se combined, a larger number of cows would be required to demonstrate significant differences among treatments. Conditions often associated with RFM (3) observed in cows that failed to shed the placenta within 12 h were difficult births requiring assistance (2 cows receiving vitamin E plus Se), calving >2 wk early (1 cow receiving vitamin E), and stillbirth (1 control cow). Twins were born to 2 unsupplemented cows, both of which had retained placenta. Three cows fed vitamin E plus Se bore twins, but only 1 cow retained her placenta. The odds ratio (5) associated with the sum of all of these problems and RFM was 8.4 (P < .025), which indicates that cows with one or more of these conditions are over eight times more likely to have RFM than cows with single, unassisted live births. In contrast, no relationship existed between concentration of α-tocopherol in serum and RFM (odds ratio = 1.0), so cows with below average α-tocopherol in serum were no more likely to retain the placenta than cows with average or above average α-tocopherol in serum.

All cows averaged 50 d from parturition to first detected estrus, 111 d from parturition to conception, and 1.9 AI. None of these measurements of reproductive performance was affected by supplementation with vitamin E or Se prepartum.

Oxidative Status and RFM

The FFA in plasma tended to be lower, and GSH-Px both dependent and not dependent on Se was lower, in cows with RFM than in cows that did not have retained placenta (Table 2). Differences appeared 2 wk before calving for FAA and Se-dependent GSH-Px. The GSH also tended to be lower (P = .09) just before calving in cows with RFM. No differences between cows with or without RFM were apparent for α-tocopherol in serum or TBARS in RBC.

DISCUSSION

Cows with RFM had a lower antioxidant status than cows that did not have retained placenta (Table 2), and cows supplemented with vitamin E were more likely than unsupplemented cows to have antioxidant status above the mean (Table 1). The number of cows used to compare effects of vitamin E on incidence of RFM was insufficient to obtain statistical significance. In Table 3, results of this report are combined with results of a comparison involving 126 cows, which we conducted 1 yr later (1992, unpublished data), and RFM was counted only when single live calves were born unassisted. Odds ratio was calculated to test the strength of the relationship between supplemental vitamin E and RFM. With the larger number of cows when 2 yr were combined, cows that were not supplemented prepartum with vitamin E tended to be more likely to retain placentas than were cows given 1000 IU/d of vitamin E (P < .06).

Of all of the measurements of oxidative status, FAA appears to be most strongly related to supplementation with vitamin E (Table 1) and incidence of RFM (Table 2). The improvement in antioxidant status with vitamin E supplementation was more consistent for FAA than for other indices of oxidative status.
α-Tocopherol in serum was also increased by vitamin E supplementation (Figure 1), but it was not lower in cows with RFM (Table 2). Like FAA, GSH-Px was lower in cows with RFM than in those without RFM, but its increase when vitamin E was supplemented was not nearly as consistent as for FAA. For GSH, increases, when vitamin E was supplemented, and decreases, when RFM was present, were only marginal. The decrease in TBARS was about as consistent as the increase in FAA in plasma when vitamin E was supplemented, but TBARS did not differ in cows with or without RFM.

Included among properties of an ideal scavenger of free radicals, as listed by Rose and Bode (20), are ease of oxidation and suitability for compartmentalization and regeneration. Antioxidants included in FAA appear to satisfy these requirements. The FAA react rapidly with ROM and terminate peroxidative chain reactions (4). Although components of FAA

<table>
<thead>
<tr>
<th>Measurement and week before calving</th>
<th>Status</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not retained</td>
<td>Retained</td>
</tr>
<tr>
<td>α-Tocopherol in serum, mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td>2.04</td>
<td>1.91</td>
</tr>
<tr>
<td>4 wk</td>
<td>2.27</td>
<td>2.45</td>
</tr>
<tr>
<td>2 wk</td>
<td>1.89</td>
<td>2.17</td>
</tr>
<tr>
<td>0 wk</td>
<td>1.59</td>
<td>1.71</td>
</tr>
<tr>
<td>FAA in plasma¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td>42.1</td>
<td>43.0</td>
</tr>
<tr>
<td>4 wk</td>
<td>45.1</td>
<td>46.6</td>
</tr>
<tr>
<td>2 wk</td>
<td>50.1</td>
<td>46.0</td>
</tr>
<tr>
<td>0 wk</td>
<td>50.5</td>
<td>44.8</td>
</tr>
<tr>
<td>GSH-Px in RBC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se Dependent²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td>39.3</td>
<td>37.3</td>
</tr>
<tr>
<td>4 wk</td>
<td>40.7</td>
<td>37.8</td>
</tr>
<tr>
<td>2 wk</td>
<td>40.6</td>
<td>35.4</td>
</tr>
<tr>
<td>0 wk</td>
<td>42.3</td>
<td>36.4</td>
</tr>
<tr>
<td>Se Independent³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td>4.7</td>
<td>4.3</td>
</tr>
<tr>
<td>4 wk</td>
<td>5.4</td>
<td>4.9</td>
</tr>
<tr>
<td>2 wk</td>
<td>5.3</td>
<td>4.9</td>
</tr>
<tr>
<td>0 wk</td>
<td>6.5</td>
<td>4.2</td>
</tr>
<tr>
<td>GSH in RBC⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td>.31</td>
<td>.32</td>
</tr>
<tr>
<td>4 wk</td>
<td>.34</td>
<td>.32</td>
</tr>
<tr>
<td>2 wk</td>
<td>.35</td>
<td>.34</td>
</tr>
<tr>
<td>0 wk</td>
<td>.35</td>
<td>.32</td>
</tr>
<tr>
<td>TBARS in RBC⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td>395</td>
<td>387</td>
</tr>
<tr>
<td>4 wk</td>
<td>362</td>
<td>355</td>
</tr>
<tr>
<td>2 wk</td>
<td>344</td>
<td>351</td>
</tr>
<tr>
<td>0 wk</td>
<td>339</td>
<td>325</td>
</tr>
</tbody>
</table>

¹Fast-acting antioxidants expressed as percentages of initial phycoerythrin fluorescence remaining after 30 min of exposure to peroxyl radicals generated in vitro.
²Selenium-dependent glutathione peroxidase (GSH-Px dependent on Se), units per milliliter of packed red blood cells (RBC).
³GSH-Px independent of Se, units per milliliter of RBC.
⁴Reduced glutathione (GSH), milligrams per milliliter of RBC.
⁵Thiobarbituric acid-reactive substances (TBARS), nanomoles of malondialdehyde per gram of hemoglobin.

TABLE 3. Data from 2-yr investigations with periparturient dairy cows testing the hypothesis that incidence of retained fetal membrane is likely to be higher when cows are unsupplemented (-) rather than supplemented (+) prepartum with vitamin E.

<table>
<thead>
<tr>
<th>Fetal membrane retained, no. cows</th>
<th>1991</th>
<th>1992</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>Not retained</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>44</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>51</td>
<td>76</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.70</td>
<td>2.32</td>
<td>2.11</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>.35-9.25</td>
<td>.83-6.84</td>
<td>91-5.04</td>
</tr>
</tbody>
</table>

The FAA protect important molecules, including protein. Control of catalytic transition elements by protein and other endogenous sequestering agents, before the hydroxyl radical can be generated (12), reduces pressure on the cell to maintain reducing equivalents for ROM control (10) because ROM reactions are stopped at the initial focal point. Oxidative stress has become severe when FAA have been exhausted, leaving only slow-acting antioxidants (protein) for protection against ROM. For these reasons, we have used FAA, which we erroneously referred to as "total antioxidants" earlier (16), as an index of oxidative stress.

CONCLUSIONS

Before calving, cows with RFM were lower in several indices of antioxidant status, including FAA in plasma, GSH-Px that is both dependent and not dependent on Se, and GSH. These antioxidants were increased by prepartum supplementation with vitamin E, but the number of cows was insufficient to also demonstrate a significant decrease in RFM. α-Tocopherol in serum was increased, and TBARS in RBC was decreased, by vitamin E supplementation, but neither differed between cows with or without RFM.

Protein, because of its relatively high concentration in plasma, appears to account for a major proportion of the total antioxidant capacity of plasma in vitro (23). However, FAA, which represent antioxidants exclusive of protein in plasma (4), probably measures more realistically an animal’s ability to deal with ROM.

ACKNOWLEDGMENTS

Appreciation is expressed to the BASF Corporation, Parsippany, NJ, for its support of our research; to A. N. Glazer, University of California, Berkeley, for helpful suggestions and for his encouragement and guidance in measurement of FAA; to C. R. Holmes and M. H. Campbell, The University of Tennessee Dairy Farm, for their cooperation; and to June Finchum for typing the manuscript.

REFERENCES

ANTIOXIDANT STATUS OF COWS
