EFFECT OF INJECTION OF β-CAROTENE OR VITAMIN E AND SELENIUM ON FERTILITY OF LACTATING DAIRY COWS

C.F. Aréchiga, S. Vázquez-Flores, O. Ortiz, J. Hernández-Cerón, A. Porras, L.R. McDowell and P. J. Hansen

1Department of Dairy and Poultry Sciences and 2Animal Science Department, University of Florida, Gainesville FL 32611-0920

3Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Cd. Universitaria, México, D.F. 04510

Received for publication: September 5, 1997
Accepted: February 27, 1998

ABSTRACT

Experiments tested whether supplemental antioxidants improved fertility. To test effects of β-carotene, cows in a hot environment were injected with prostaglandin F2α (PGF2α) and were given 3 injections, im, of 800 mg β-carotene or saline at Days -6 and -3 before the anticipated date of insemination and at insemination (n=37-41 inseminated cows/group). There was no effect of β-carotene on the proportion of cows detected in estrus following PGF2α, timing of estrus after PGF2α injection or pregnancy rate in inseminated cows. In a second trial, cows in a temperate climate received intramuscular injections of vitamin E (500 mg) and selenium (50 mg) at 30 d post partum (n=97) or were untreated controls (n=89). Treatment did not affect interval from calving to first insemination or the proportion of cows pregnant at first service, but it increased the pregnancy rate at second service (69.8 vs 52.1%; P=0.07) and reduced services per conception (1.7 vs 2.0; P<0.05) and interval from calving to conception (84.6 vs 98.1; P<0.05). Thus, injection of vitamin E and selenium increased fertility in cattle that did not become pregnant at first service.

© 1998 by Elsevier Science Inc.

Key words: vitamin E, selenium, β-carotene, fertility, heat stress

Acknowledgments

Research was supported by the CONACyT/IIE Program (Mexico), USDA-CBAG grants 92-38420-7331 and 95-34135-1860, and Florida Milk Checkoff Funds. Authors thank Schering Plough de México (División Veterinaria) for donation of Mu-Se; Dr. Michael B. Coelho at BASF Corporation for Lucarotin; the workers and owners of La Palma Dairy (Mexico), ALC Dairy (Gainesville, Florida), Genfarm III (Bell, Florida), and the University of Florida Dairy Research Unit; Susan Gottshall, Nancy Wilkinson, Al Boning, Mary Russell and Mary Ellen Hissem. This is Journal Series No. R-05944 of the Florida Agricultural Experiment Station.

Current address: Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Zacatecas, Zacatecas, México.

Current address: ALPURA de México, Querétaro, Qro., México.

Reprint requests to Dr. P.J. Hansen, Department of Dairy and Poultry Sciences, University of Florida, Gainesville, FL 32611-0920. E-mail: Hansen@dps.ufl.edu
INTRODUCTION

Approximately 1 to 2% of metabolized oxygen is converted to a reactive oxygen species (25), and several biochemical systems exist in cells and extracellular fluid to remove these molecules. Antioxidant systems include molecules such as β-carotene and vitamin E, which act as membrane antioxidants to maintain the integrity of phospholipids against oxidative damage and peroxidation (14, 15, 36). Various enzymes also remove free radicals. Among these, glutathione peroxidase is a selenium-dependent enzyme that utilizes electrons from glutathione and other thiols to convert peroxides to water (21). Increased generation of free radicals may overwhelm antioxidant defense mechanisms and compromise cellular function (14, 23, 25). Production of free radicals could represent a source of infertility because ovarian steroidogenic tissue (12, 35), spermatozoa (1) and preimplantation embryos (24) are sensitive to free radical damage. In some studies, administration of β-carotene (7, 9, 10) or vitamin E and selenium (5, 47) improved fertility of cattle, while in other studies, providing high amounts of these antioxidants showed no beneficial effects on fertility (2, 8, 22, 26, 30, 31, 42, 44, 50).

There were 2 objectives in this study. The first was to determine whether β-carotene administered during the preovulatory period (specifically at Days -6 and -3 relative to the anticipated estrus and on the day of insemination) could increase pregnancy rates of lactating dairy cows exposed to heat stress. We hypothesized that such a treatment would be beneficial for the establishment of pregnancy for several reasons. First, the oocyte (19) and early embryo (17, 18) can be compromised by exposure to elevated temperature. The effect of elevated temperature on embryos probably involves free radical production because heat shocked cells undergo increased free radical production (33), and administration of various antioxidants to cultured embryos has been shown to reduce the effects of elevated temperature on embryonic survival (4, 17). In a previous study, it was found that administration of vitamin E at insemination did not improve the pregnancy rates of heat-stressed cows (16). It was reasoned that this scheme may have failed to provide antioxidant protection during the preovulatory period, when final follicular and oocyte development and maturation occur. Moreover, it is known that heat stress during the late preovulatory period can compromise subsequent embryonic development (41). We chose to use β-carotene for this study because of its possible additional benefit independent of antioxidant properties. In particular, injection of vitamin A (as retinol palmitate), a metabolite of β-carotene, into superovulated cows at the time FSH administrations were initiated resulted in an increase in the recovery of blastocysts but had no affect on the ovulation rate (49). Further, retinol-binding protein is present in the endometrium (51), and retinol presumably is important to the developing conceptus. Thus, perhaps metabolites of β-carotene enhance oocyte quality or embryo function in a way that improves fertilization or subsequent embryonic survival.

The second objective was to determine whether a single intramuscular injection of vitamin E and selenium at 30 d after parturition would enhance postpartum reproductive function of lactating dairy cows in a temperate climate. Previously (5), it was found that injection of vitamin E and selenium at 21 d before expected parturition reduced services per conception of lactating cows. This effect could, in part, reflect the effects of vitamin E and selenium on uterine health associated with parturition since injection of vitamin E and selenium have been shown to reduce the incidence of retained fetal membranes (5, 27). By administering vitamin E and selenium at 30 d post partum, it
was possible to determine whether these molecules enhanced fertility independently of periparturient changes in uterine and placental function.

MATERIALS AND METHODS

Experiment 1: Plasma Concentrations of β-Carotene in Cows Injected with β-Carotene

A preliminary experiment was conducted to determine the time trends for plasma concentrations of β-carotene and selected metabolites in cows receiving intramuscular injections of β-carotene. The experiment was conducted at the University of Florida Dairy Research Unit (Hague, Florida) in July and August with 10 lactating Holstein cows (70 to 147 d post partum) fed a total mixed ration formulated at each farm, composed (dry basis) of 1.5 Mcal/kg net energy for lactation (NEL), 16.4% crude protein (CP), 26.6% acid detergent fiber (ADF), 42.4% neutral detergent fiber (NDF), 0.70% Ca, 0.46% P, 0.34% Mg, 1.35% K, and 0.49% Na (diet analyzed chemically at Northeast DHIA Forage Laboratory, Ithaca, NY). Cows were randomly assigned to treatment with either β-carotene (n=4) or physiological saline solution (0.9%, w/v) as a control (n=6). At 0800 h at Days 0, 3 and 6, the cows received injections of either 800 mg β-carotene or an equivalent volume of saline (20 ml). Blood samples were collected into heparinized tubes from coccygeal vessels at Days -6, -3, 0 (before injection at 0800 h and 12 h later), 1, 3, 4, 6, 7, 12 and 19 relative to first injection. Plasma samples were analyzed for β-carotene, retinol and retinyl palmitate using high pressure liquid chromatography as described elsewhere (11, 38).

Experiment 2: Effect of β-Carotene on Fertility of Lactating Dairy Cows

To determine the effect of β-carotene on fertility, an experiment was conducted on 3 dairy farms in North Florida (Bell, Florida; 29°45'N 82°52'W, elevation 21 m; Hague, Florida; 29°46'N 82°25'W, elevation 51 m) during July through September 1994. The cows were fed a total mixed ration that was formulated at each farm. Diets were analyzed chemically at Northeast DHIA Forage Laboratory, Ithaca, New York and were composed (dry basis) of 1.5 Mcal/kg NEL, 17.4% CP, 26.4% ADF, 49.7% NDF, 0.92% Ca, 0.43% P, 0.32% Mg, 2.22% K and 0.56% Na (Farm 1); 1.5 Mcal/kg NEL, 18.7% CP, 24.8% ADF, 45.3% NDF, 0.94% Ca, 0.44% P, 0.37% Mg, 1.12% K and 0.45% Na (Farm 2); or the cows were fed the diet described for Experiment 1 (Farm 3). The dietary content of retinyl palmitate, β-carotene and retinol was evaluated in a composite sample of 4 weekly samples of the total mixed rations of each farm. Spectrophotometric analyses were performed by ABC Research Laboratories at Gainesville, Florida using Methods 941.15 and 960.45 of the Official Methods of the American Organization of Agricultural Chemists (3). Dietary content of β-carotene and retinol were 5124 and 10733 IU/kg (Farm 1); 7731 and 9285 IU/kg (Farm 2); and 4101 and 10501 IU/kg (Farm 3).

Every 2 wk, cows were identified that were either 1) not inseminated previously in the current postpartum period and were past 40 d post partum or 2) inseminated previously in the current

---

\(^d\) Lucarotin®, BASF, Parsippany, NJ.
postpartum period but subsequently diagnosed nonpregnant. These cows were given 25 mg PGF$_{2\alpha}$, i.m., to synchronize and predict estrus; they were also injected, i.m., with 3 injections of either 800 mg β-carotene or saline. These injections were given at Days -6 and -3 before anticipated date of insemination (i.e., at 3 d before PGF$_{2\alpha}$ and on the day of PGF$_{2\alpha}$ injection) and at insemination. Once assigned to treatment, cows received an intramuscular injection of 800 mg β-carotene or saline at Day 0 (i.e., Day -6 before expected estrus). At Day 3 (Day -3 before expected estrus), cows were injected with and were given a second injection of β-carotene or saline. When cows were detected in estrus, they were artificially inseminated according to the AM-PM rule (i.e., inseminated in the afternoon if observed in estrus in morning and inseminated in morning if observed in estrus the previous afternoon) and received an injection of either β-carotene or saline at insemination. Some cows came into estrus and were inseminated by herdsmen before receiving all 3 injections; consequently, they were removed from the experiment. Pregnancy rates for cows not returning to estrus following insemination were evaluated 55 to 90 d after insemination.

Cows were assigned randomly to control or β-carotene treatments. Cows that did not respond to Lutalyse and were not inseminated subsequently were used in the experiment again 2 wk after initial injection of β-carotene or saline. The cows received the same treatment (β-carotene or placebo) they had initially received. A total of 68 control and 59 β-carotene-treated cows generated 88 control and 77 β-carotene observations.

Experiment 3: Effect of Injection of Vitamin E and Selenium on Reproductive Function

This experiment utilized 186 lactating dairy cows from a commercial dairy located in Central Mexico in the vicinity of Coacalco, Estado de México (19°55' N 99º9' W, elevation of 2200 m). The region has a humid, temperate climate, with long, moderate summers (annual mean temperature varies from 12 to 18°C). Cows were used only if the uterus was diagnosed as healthy at 30 d postpartum (i.e., absence of pyometra and endometritis). Cows were randomly assigned to either an untreated control group or to a group that received a single intramuscular injection of 10 mL Mu-Se® at Day 30 ± 3 post partum. The amount of Mu-Se administered is equivalent to 500 mg vitamin E as DL-α tocopheryl acetate (680 IU) and 109.5 mg of sodium selenite (equivalent to 50 mg selenium).

Estrus detection was performed visually nearly continuously throughout the day. All cows were inseminated by one person via artificial insemination at all estrous periods after 50 d post partum. Artificial insemination was performed according to the AM-PM rule. Pregnancy was determined by palpation per rectum at 45 to 60 d after service.

Cows were milked twice a day. During each milking, the cows were fed 2 kg of alfalfa hay. Afterwards, they were given ad libitum access to fresh chopped alfalfa or alfalfa hay, and were fed ~28 kg/cow of a mixed concentrate ration based on corn silage and sorghum that had a calculated composition (dry basis) of 1.67 Mcal/kg NE$_L$, 19.1% CP, 30.7% NDF and 17.2% ADF. The ration

---

* Lutalyse®, Upjohn and Pharmacia, Kalamazoo, MI.
* Schering-Plough Animal Health Corp., Kenilworth, NJ.
for the cows was formulated to provide supplemental vitamin E (500 IU/cow/d) and selenium (0.3 ppm/cow/d).

Statistical Analysis

All data were analyzed by least squares analysis of variance using the General Linear Models Procedure of SAS (43). The mathematical model used to analyze data for Experiment 1 included the main effects of treatment, cow(treatment) and day. For Experiment 2, the main effects were treatment, farm and replicate (i.e., each 2-wk period in which treatments were initiated). Data for the proportion of cows responding to PGF$_{2\alpha}$ included some cows that were used more than one time. For this variable, the data were analyzed 2 ways: with and without cow(treatment) in the model. For Experiment 3, the main effects were treatment and parity (parity 1 vs others). Analyses were performed with all interactions in the model and were then reanalyzed after removing interactions that were nonsignificant. Effect of treatment on services per conception in Experiment 3 were also analyzed by CATMOD (43) with 4 categories of 1, 2, 3 and 4 to 5 services per conception.

RESULTS

Experiment 1: Plasma Concentrations of β-Carotene in Cows Injected with β-Carotene

Intramuscular injection of 800 mg β-carotene increased plasma concentrations of β-carotene from 2.7 ± 0.38 µg/mL before injection to 5.55 ± 0.38 µg/mL at 24 h after injection (Figure 1). The second and third injections of β-carotene resulted in slightly higher elevations in plasma β-carotene concentrations. Concentrations remained elevated until the end of the sampling period (i.e., 19 d after the first β-carotene injection and 13 d after the last injection). Retinyl palmitate concentrations were also increased from 0.40 ± 0.07 µg/mL before injection to 1.08 ± 0.07 µg/mL at 24 h after injection (Figure 1) and remained elevated until the end of the sampling period. There was no difference in retinol concentrations between treated and control cows (Figure 1).

Experiment 2: Effect of β-Carotene on Fertility of Lactating Dairy Cows

There was no effect of β-carotene on the proportion of cows that was detected in estrus after PGF$_{2\alpha}$ treatment, on the timing of estrus relative to injection of PGF$_{2\alpha}$ or on the proportion of animals that conceived to the insemination following PGF$_{2\alpha}$ (Table 1).

Experiment 3: Effect of Injection of Vitamin E and Selenium on Reproductive Function

There was no effect of injection of vitamin E and selenium on interval from calving to first insemination or on pregnancy rate at the first insemination (Table 2). However, among cows not pregnant to first service, the pregnancy rate at second service was higher (P=0.07) for the cows treated with vitamin E and Se. Similarly, there were fewer services per conception (P<0.05) for cows receiving vitamin E and selenium; the interval from calving to conception was also shorter for treated cows. The distribution of services per conception was affected by treatment (P<0.05), since there was a smaller proportion of cows requiring 4 to 5 services to achieve pregnancy in the vitamin E and selenium treatment group than in the control group (Figure 2).
Figure 1. Plasma concentrations of β-carotene, retinyl palmitate and retinol from heat-stressed dairy cows receiving 3 intramuscular injections of 800 mg β-carotene (○) or saline (●) given 3 days apart. The x axis represents time relative to the first injection of β-carotene. The arrows represent times at which injections were given. Concentrations of β-carotene were affected by treatment (P<0.001), time (P<0.001) and treatment-by-time (P<0.001). Concentrations of retinyl palmitate were affected by treatment (P<0.01), time (P<0.001) and treatment-by-time (P<0.001).
**Table 1. Effect of administration of β-carotene on estrus synchronization, interval to estrus, variation of interval to estrus, and pregnancy rate.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>β-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus within 96 hours after PGF$_{2α}$ (%)$^{a,e}$</td>
<td>46.6 ± 5.4 (41/88)</td>
<td>49.4 ± 5.7 (38/77)</td>
</tr>
<tr>
<td>Interval, PGF$_{2α}$ to estrus (days)$^{b,e}$</td>
<td>2.83 ± 0.16</td>
<td>2.90 ± 0.16</td>
</tr>
<tr>
<td>SD, interval to estrus (days)$^{c,e}$</td>
<td>1.03</td>
<td>1.07</td>
</tr>
<tr>
<td>Pregnancy rate (%)$^{d,e}$</td>
<td>24.4 ± 6.7 (10/41)</td>
<td>21.6 ± 7.0 (8/37)$^{f}$</td>
</tr>
</tbody>
</table>

$^a$ Least square means ± SEM and, in parentheses, number of animals responding/number of animals injected with PGF$_{2α}$ (Note some cows were subjected to more than one PGF$_{2α}$ injection regimen).

$^b$ Least-squares means ± SEM.

$^c$ SD=standard deviation.

$^d$ Least squares means ± SEM and, in parentheses, number of animals pregnant/number of animals inseminated at PGF$_{2α}$-induced estrus.

$^e$ All differences between control and β-carotene were nonsignificant (P>0.10).

$^f$ n=37 because one cow did not receive the 3 β-carotene injections.

**DISCUSSION**

It was hypothesized that 3 injections of β-carotene (at 6 and 3 d before anticipated estrus and again at insemination) would increase the pregnancy rate in heat-stressed cows because β-carotene would 1) eliminate some of the increased free radicals produced as a result of heat stress (33), thereby reducing damage to the oocyte and embryo, and 2) increase concentrations of vitamin A, which has
been shown to increase embryonic development in superovulated cows (49). The injection scheme was sufficient to elevate plasma concentrations of β-carotene and retinyl palmitate for at least 13 d after the day of the last injection. Nonetheless, injection of β-carotene did not improve fertility. There are several possible reasons for this. First, it is possible that early embryos are resistant to antioxidant therapy. While morulae were made more resistant to heat shock in culture by incubation with glutathione or taurine (17), no such thermoprotective effect was seen for 2-cell embryos (18). It may also be that injections of β-carotene did not elevate concentrations of β-carotene in the oviduct where the embryo resided. Recently, it was found that dietary supplementation with β-carotene had no effect on pregnancy rate of heat-stressed cows at first service but did increase pregnancy rate at 120 d postpartum if cows received β-carotene for at least 90 d (6). Therefore, it is possible that prolonged exposure to β-carotene is required to sufficiently elevate concentrations of β-carotene in the oviduct and uterus. Finally, it is possible that administration of vitamin A can increase embryonic development (49) but that either 1) these effects are exerted on embryos from superovulated cows only or 2) the increase in retinyl palmitate achieved in the present study was not of sufficient magnitude to mimic the effect of vitamin A seen in earlier studies.
The combined administration of vitamin E and selenium at Day 30 post partum increased the fertility of lactating dairy cows that were not under heat stress. In particular, administration of vitamin E and selenium increased fertility in cows receiving 2 or more services to achieve pregnancy. A single prepartum injection of vitamin E and selenium also increased fertility (5). In this earlier study (5), it was thought that vitamin E and selenium may have increased fertility by increasing uterine health due to the lower incidence of retained fetal membranes. Effects on uterine health may have been less evident in the current study because cows with uterine disorders at Day 30 were excluded from the experiment and because injection of vitamin E and selenium was delayed until 30 d after parturition. Nonetheless, given the likelihood of subclinical infections, some beneficial effects of vitamin E and selenium on fertility could have been a consequence of improved uterine health. Administration of vitamin E and/or selenium can also enhance neutrophil function (20, 37, 39, 40). Perhaps, increased neutrophil activity promotes removal of microorganisms and supports uterine tissue remodeling and involution. Prepartum treatment with selenium and vitamin E has been reported to hasten uterine involution in cows with metritis (28). It is also possible that vitamin E and selenium affect events leading to fertilization since treatment with vitamin E and selenium increased fertilization rate in cattle (47) and sheep (45). This effect was ascribed to increased sperm transport (46, 48) and may reflect actions of selenium on uterine motility (48). Experiments in ewes indicate that selenium status can also affect embryonic survival (29).

It is not surprising that a single injection of vitamin E and selenium could cause a positive effect on reproductive function several weeks after the injection because administration of these molecules has long-term effects in cattle. Intramuscular injection of vitamin E caused elevated amounts in serum for at least 28 d (13), while injection of selenium increased concentrations of selenium in whole blood and serum for 28 d and increased whole blood-glutathione peroxidase activity for at least 84 d (34). Injections of vitamin E and selenium 3 and 1.5 wk before calving increased erythrocyte GSH peroxidase in dairy cows during the first 12 wk of lactation (32).

Effects of selenium, vitamin E or their combination on fertility have been variable, with some studies reporting of an increase in fertility (5, 47) and some reporting no effect (26, 30, 31, 44, 50). Differences between studies in the amount of vitamin E and/or selenium administered, the period of administration, and nutritional status of the experimental animals with respect to vitamin E and selenium intake could explain some of these differential results. Given the effectiveness of vitamin E and selenium, as administered in the present study, and the possible importance of antioxidant status for heat-stressed cows (6), it is important to determine if injection of vitamin E and selenium increases fertility of heat-stressed cows.

REFERENCES


