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The Role of Single Oral Dose of Excess Vitamin A and/or Vitamin E in Improving Ovarian Function Three Days Post-parturition in Primiparous Dairy Cows

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Abstract

Dairy farmers dose their cows continuously with high amount of fat-soluble vitamin supplements to improve reproductive capacity and milk quality. However, these amounts are sometimes detrimental to the health of cows. Farmers require effective methods of vitamin supplementation methods without side effects. Our study aims to ascertain the effects of a single, high-concentration oral dose of vitamin A and E supplements on the reproductive performance of dairy cows. The cows were classified based on when did their first ovulation occur: within the first three weeks postpartum (early responder: ER) or after three weeks postpartum (late responder). This is based on the notion that the first postpartum ovulation is a useful indicator of reproductive function recovery in cows. We tested whether supplementation with vitamin A, E, or both effectively induced ovulation during the early postpartum period. Three out of the eight primiparous cows that were not administered a vitamin supplement and were considered ER. All six primiparous cows that were administered a single supplement mixture containing vitamins A (20,000,000 IU) and E (2,000 mg) three days postpartum were ERs. Additionally, all primiparous cows supplied either vitamin A (n = 4) or E (n = 4) alone were also ERs. All primiparous cows treated with even half the amount of vitamins A and E used in Experiment 1 (10,000,000 IU and 1,000 mg, respectively) were designated ERs. These results strongly suggest that even a single dose of excess vitamin A and E (whether administered jointly or separately) can improve ovarian function in dairy cattle during the early postpartum period.

Discipline: Animal Science
Additional key words: dairy cattle, excess vitamins, postpartum ovulation, supplementation

Introduction

More than a century have passed since vitamins were discovered as essential micronutrients (Vandamme 2016). In 1933, the relationship between vitamin A deficiency and reproductive disturbances in dairy cattle was first reported (Hart & Guilbert 1933). Thereafter, many studies have been conducted to improve fertility through vitamin supplementation (Ikeda et al. 2005). However, efficient feeding method for cattle has not been developed to supply vitamins and improve reproductive performance. The efficacy of the administered vitamins depends on a complex combination of factors, including the potential effects of cow's physiological condition on absorbing ingested vitamins via the digestive tract (Ikeda et al. 2005). It has been reported that when blood levels in dairy heifers and cows fall below a certain threshold, β-carotene supplementation could improve fertility (Ikeda et al. 2005, Sales et al. 2008). Conversely, when β-carotene blood levels were above the threshold, β-carotene supplementation diminishes reproductive performance (Folman et al. 1987, Greenberg et al. 1986, Ikeda et al. 2005, Kaewlamun et al. 2011, Tharnish & Larson 1992). Thus, in the absence of known blood vitamin levels, supplementation with vitamins may be ineffective. Cattle usually do not undergo diagnostic testing unless they show symptoms of health problems; however, both administration of ineffective vitamin supplementation and diagnostic testing have associated costs. Therefore, dairy farmers need economical and

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effective vitamin supplement feeding methods that improve the reproductive performance of cows. Meanwhile, an increasing number of dairy farmers are continuously feeding cows excessive fat-soluble vitamins to improve mastitis and milk quality, regardless of the resultant health problems (Kayahara & Manabe 2007, Mizuno et al. 2013, Yamamoto et al. 2003).

Dairy cows have been reported to have decreased plasma vitamin A and E (AE) concentrations, and the ratio of the plasma vitamin AE concentrations in the vitamin AE intake from 0 to 1 week after calving, which may lead to increased vitamin AE requirements or decreased absorption efficiency (Aoki et al. 2014). Therefore, we hypothesized that if we fed a single dose of large amount of vitamins (with safety considerations) to dairy cows during this period of low-plasma concentrations of vitamin AE, enough to counteract the high- and low-plasma vitamin AE concentrations, we could improve early postpartum ovarian activity in dairy cows, regardless of their blood vitamin AE levels.

In this study, we experimentally administered unconventional doses of fat-soluble vitamins to non-pregnant dairy cows. We have conducted a study to ascertain the effects of a single, non continuous, high dose of oral vitamin A and E supplements on the reproductive performance of dairy cows. The dosage complies with the actual dosage written on a certain vitamin supplement for cattle. Given, our concern for both cattle and human health, we limit against conventional, continuous, and unnecessary administration of the excess fat-soluble vitamins in lactating dairy cows.

Materials and methods

1. Experimental animals

This study was conducted at the Hokkaido Agricultural Research Center of the National Agriculture and Food Research Organization (Sapporo, Japan) with a veterinarian on duty. The study was performed on primiparous Holstein cows, which calved between March 2010 and January 2015 and yielded about 10,000 kg of milk over an average of 305 days. The cows were provided with fresh water, either grass or corn silage (or both), hay, beet pulp mixture, and concentrate, lucerne, soybean flakes, and corn *ad libitum* in a free-stall barn. These feeds were sufficient for the cows' nutritional requirements according to the Japanese Feeding Standard (Agriculture, Forestry and Fisheries Research Council Secretariat 1999) and Nutrient Requirements of Dairy Cattle (Subcommittee on Dairy Cattle Nutrition 2001a). Additionally, some cows grazed on pasture during the day from May to October each year between 2010 and 2014.

All procedures used in this study were approved by the Animal Care and Use Committee of the Hokkaido Agricultural Research Center (Sapporo, Japan).

2. Experimental design

Based on the notion that the first postpartum ovulation is a useful indicator of reproductive function recovery in cows, we conducted an experiment wherein cows were sorted based on when did their first ovulation occur: within the first three weeks postpartum (early responder: ER) or after three weeks postpartum (late responder: LR) (Aoki et al. 2014). The experimental design is shown in Table 1.

Experiment 1: First, we compared the number of ERs among primiparous cows that were provided feed with and without excess vitamins. There were eight primiparous cows that were not administered any vitamins (control group). Six primiparous cows were administered a single large amount (100 g) of a commercial vitamin preparation (VIPRO SOMATIC AE1000; Nissan Gosei Kogyo Co., Japan) in powder form containing vitamin A and vitamin E (20,000,000 IU of vitamin A oil and 2,000 mg of α-tocopherol) three days postpartum (the V100 group). Next, either vitamin A liquid (10,000,000 IU of vitamin A oil, Adestar A5000; Kanematsu Shintoa Foods Co., Japan; VA group) or vitamin E (2,000 mg of α-tocopherol; Nippon Garlic Co., Japan; VE group) in powder form were administered in the same manner as the V100 group. The cows in Experiment 1 were delivered year-round, and there were no significant differences between the percentages of ERs and LRs by month of delivery.

Experiment 2: We used 10 primiparous cows and examined whether a reduced quantity of vitamins still had the same effects on the ovaries during the early postpartum period. The amount of vitamins in Experiment 1 was too large; so in Experiment 2, we halved the amount of vitamins to examine their effects. Three primiparous cows were administered the same amount of vitamins as in Experiment 1 (100 g of VIPRO SOMATIC AE1000 in powder form; V100), and three primiparous cows were administered a single dose of half the amount of vitamins (50 g of VIPRO SOMATIC AE1000 in powder form; V50) three days postpartum. Four control cows that were not administered any vitamins (control group) were delivered from January to February; therefore, they had no opportunity to eat fresh grass during the experimental period. Conversely, cows in the V100 and V50 groups were delivered from June to October, and they were often fed fresh grass in the field

Table 1. A summary of the experiments evaluating the effect of vitamins A and E on postpartum ovulation

Treatments*	Number of animals	Early Responder	Late Responder
Experiment 1			
Control	8	3	5
V 100	6	6	0
VA	4	4	0
VE	4	4	0
Experiment 2			
Control	4	NC	NC
V 100	3	3	0
V 50	3	3	0

In Experiment 1, primiparous cows were randomly assigned to one of the four groups: the control (no supplement) and three groups that received supplements (V100, both vitamin A and E; VA, vitamin A alone; and VE, vitamin E alone). The cows in each group were classified as early responders or late responders based on whether they ovulated within the first 3 weeks postpartum. In Experiment 2, primiparous cows were treated with either the same amount of vitamins A and E used in Experiment 1 (V100) or half the amount (V50).

*The animals were administered vitamins once at three days post-parturition.

V100: primiparous cows were administered 100g of a vitamin preparation containing 20,000,000 IU of vitamin A and 2,000 mg of vitamin E.

V50: primiparous cows were administered 50g of the same preparation as above containing 10,000,000 IU of vitamin A and 1,000 mg of vitamin E.

VA: primiparous cows were administered vitamin A (10,000,000IU) alone.

VE: primiparous cows were administered vitamin E (2,000mg) alone.

Control: primiparous cows were not supplied with vitamin A and/or vitamin E.

NC: not counted.

during the experimental period. Colostrum samples were obtained from three cows each from the V100, V50, and control groups.

The vitamins in powder or liquid form were suspended in approximately 500 mL of warm water at about 40°C and orally administered in whole amounts to the cows.

3. Assessment, sampling, and analysis

The cows were classified as ERs or LRs based on whether their ovaries contained a corpus luteum 25-27 days after parturition on ultrasound examination (ECHOPAL EUB-405 7.5 MHz; Hitachi Medical Co., Tokyo, Japan) as described previously by Aoki et al. (2014). All cows were individually milked twice daily (at 0730 h and 1700 h) from all quarters of the udder with a bucket milker. The colostrum samples were collected directly from the milker bucket, mixed well, and transferred into 25 mL plastic vials in the morning of postpartum days 1-6. Within 30 minutes after sampling, the colostrum samples were frozen and stored at −30°C prior to analysis. Analysis should be performed within 3 months of storage, and the thawed the colostrum samples were mixed thoroughly before assaying. Vitamin A (all-trans-retinol), vitamin E (α-tocopherol), and β-carotene concentrations in colostrum were extracted according to the method from Plozza et al. (2012) and analyzed by HPLC (AOAC international, Carotene in fresh plant materials and silages 2000, Landen Jr. 1981, Milne & Botnen 1986, Plozza et al. 2012). A mixed procedure with Tukey's multiple comparison test (SAS ver. 9.2; SAS Institute, Cary, NC, USA) was performed to compare vitamin A concentrations in colostrum.

Results and discussion

Since it was thought that the increased vitamin requirements and effects of stress during the transition period would be more apparent in primiparous cows (Neave et al. 2017), this study was conducted using primiparous cows, which generally have lower milk production than multiparous cows. In our previous report (Aoki et al. 2014), half of the 12 primiparous cows that were not administered any vitamin supplement was classified as ERs. In a similar study conducted at the Hokkaido Agricultural Research Center, three of eight primiparous cows were classified as ERs and five were classified as LRs.

Six primiparous cows were administered a large amount of a commercial vitamin preparation in Experiment 1, and all six cows were ERs. Thus, the administration of a single dose of vitamin A and vitamin E mixture in the early postpartum period could induce ovulation within the first three weeks postpartum. Since, either of the vitamins that affected ovulation was not determined, we separately administered vitamin A and vitamin E. All cows administered either vitamin A (n = 4) or vitamin E (n = 4) were ERs. These results indicate that a single dose containing a large amount of vitamin A or vitamin E can induce ovulation during the early postpartum period.

In Experiment 2, three primiparous cows were administered the same amount of vitamins as that in Experiment 1 (V100). The three primiparous cows were administered half the amount of vitamins (V50) once at three days postpartum. All cows in both groups were ERs.

The concentration of vitamin A in the colostrum was measured to verify if it was safe for newborn calves (Fig. 1). Vitamin A levels in plasma and milk were reported to be stable even when cows were administered with excess vitamin A (Squibb et al. 1949); presumably, orally administrated vitamin A is stored in the liver and only released by needed amounts for retinol homeostasis. However, in this study, the excess vitamin A was discharged through colostrum; thus, the concentration of vitamin A in the milk increased as the quantity of the vitamins administered increased. The vitamin A level in colostrum peaked a day after the vitamin was administered, but it did not exceed 800 μg/100 mL. Therefore, if a calf drinks this colostrum, its health might not be affected. However, the calf should not be given any other additional vitamin A supplement. Additionally, in some regions, colostrum curd is consumed as a regional cuisine (Kalvdans in Scandinavia, Junnu in India, Shonyū-doufu in Japan). Pregnant females, children, and infants should avoid foods made with colostrum containing high vitamin A levels.

Conversely to the observations made with vitamin

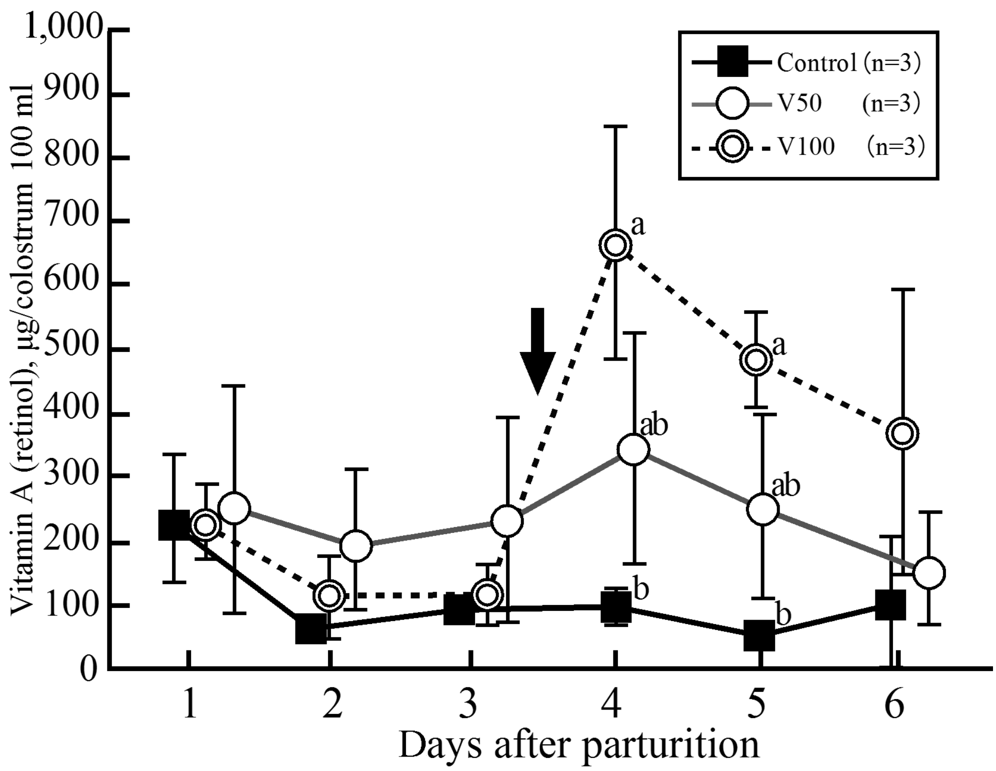

Fig. 1. Changes in vitamin A concentration in the colostrum of primiparous cows administered excess fat-soluble vitamins

Changes in vitamin A concentration in the colostrum of primiparous cows that were administered vitamins (V100 group: 20,000,000 IU of vitamin A and 2,000 mg of vitamin E; V50 group: 10,000,000 IU of vitamin A and 1,000 mg of vitamin E) once at three days post-parturition or not (Control). The arrow indicates the time at which the vitamins were administered. Control: primiparous cows were not supplied with vitamin A and/or vitamin E. The cows in the V100 and V50 groups had opportunities to eat fresh grass in fields, whereas the cows in the Control group did not. Data are presented as the mean ± SD. Different lowercase letters indicate significant differences ($P < 0.01$) between groups.

A, the concentration of vitamin E and β-carotene in colostrum did not markedly increase after administration, and there were no significant differences among the experimental groups (Figs. 2, 3). The standard deviation of vitamin E in colostrum is smaller after administration of the vitamin preparation (Fig. 2), although it is unknown if this is due to excessive vitamin E administration. While there was almost no difference in the β-carotene levels in the colostrum of cows in the control and V50 groups, variations in the β-carotene levels in the colostrum of cows in the V100 groups increased after administration (Fig. 3). This difference in the amount of β-carotene in colostrum may be due to differences in the vitamin content in the V100 and V50 preparations, individual cow β-carotene sufficiency, requirements, and absorption efficiency.

The NRC (Subcommittee on Dairy Cattle Nutrition 2001b) recommends the same vitamin A supplement for both dry and lactating cows (110 IU/kg of body weight), and the presumed safe limit for vitamin A is 66,000 IU/kg of diet for both lactating and non-lactating cattle. The quantity of vitamin A administered in this study was about 300 times larger and 6-8 times higher than the safe limit recommended by the NRC because our cows were fed approximately 40 kg-50 kg of feed (including hay, grass silage, and concentrates as fresh matter) at three days postpartum. Fortunately, no health hazards due to excess vitamin A administration were observed in any cows during the lactation period of this experiment.

We administered the excess fat-soluble vitamins only once. However, cases of health problems in cattle have been reported on a dairy farm as a result of repeated high dose administration of vitamin preparations (long-term continuous, probably exceeding our total dose) without any understanding of the side effects (Kayahara & Manabe 2007). Although informal, it was observed that cattle that were administered a higher dose of oral vitamin A (5 x 10^6 IU/kg/d; if cattle were fed

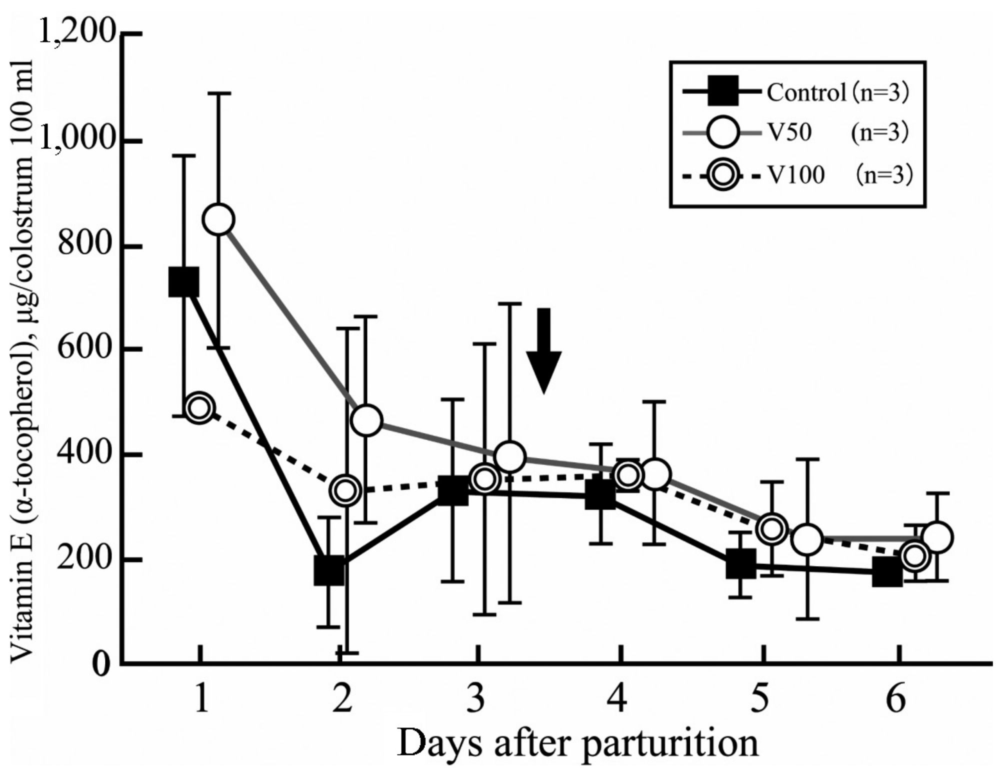

Fig. 2. Changes in vitamin E concentration in the colostrum of primiparous cows administered excess fat-soluble vitamins

Changes in vitamin E (α-tocopherol) concentration in the colostrum of primiparous cows that were administered a vitamin preparation (V100 group: 100 g containing 20,000,000 IU of vitamin A and 2,000 mg of vitamin E and V50 group: 50 g containing 10,000,000 IU of vitamin A and 1,000 mg of vitamin E) once at three days post-parturition or not (Control). The arrow indicates the time at which the cows were administered the vitamins. Control: primiparous cows were not supplied with vitamin A and/or vitamin E. The cows in the V100 and V50 groups had opportunities to eat fresh grass in the field, whereas the cows in the Control group did not. Data are presented as the mean ± SD.

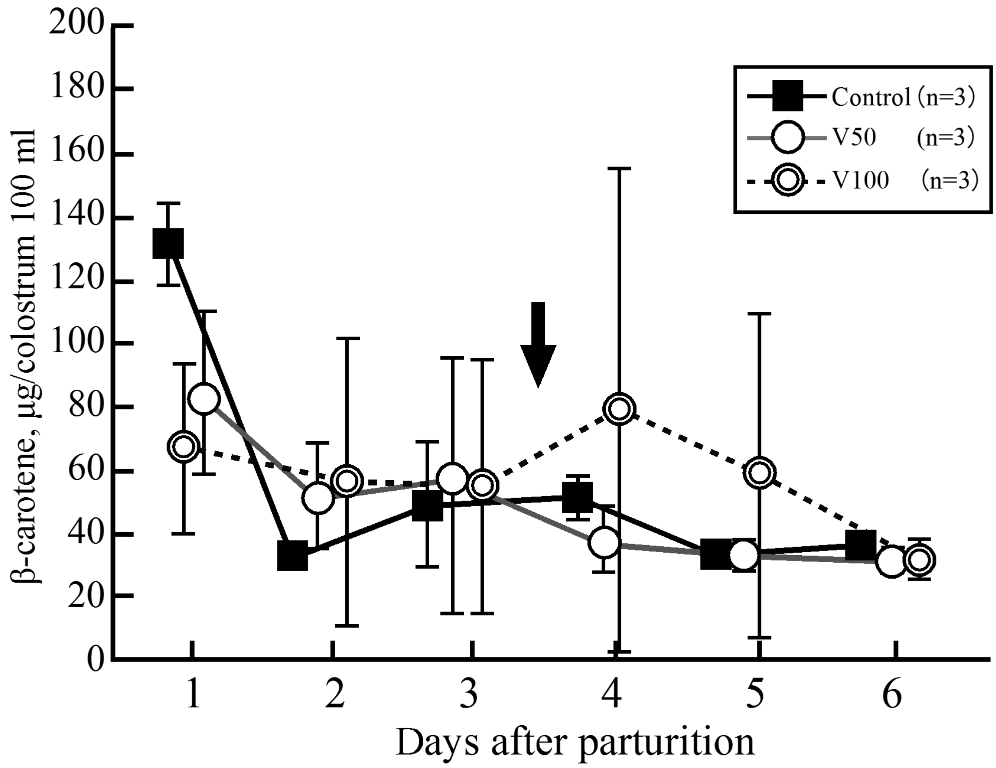

Fig. 3. Changes in β-carotene concentration in the colostrum of primiparous cows administered excess fat-soluble vitamins

Changes in β-carotene levels in the colostrum of primiparous cows that were administered a vitamin preparation (V100 group: 100 g containing 20,000,000 IU of vitamin A and 2,000 mg of vitamin E and V50 group: 50 g containing 10,000,000 IU of vitamin A and 1,000 mg of vitamin E) once at three days post-parturition or not (Control). The arrow indicates the time at which the cows were administered the vitamins. Control: primiparous cows were not supplied with vitamin A and/or vitamin E. The cows in the V100 and V50 groups had opportunities to eat fresh grass in the field, whereas the cows in the Control group did not. Data are presented as the mean ± SD.

40 kg- 50 kg of feeds, they would be given approximately 20,000,000-25,000,000 IU/head/d) continuously for three weeks and developed osteomalacia and hyena disease, even in adulthood (Yamamoto 2003). Indeed, the administration of vitamin A in pregnant cattle is contraindicated (Polin et al. 2017). Furthermore, vitamin A has an accumulative nature (Olson et al. 2021). Therefore, its continued administration in non-pregnant cattle must be conducted with extreme caution.

There were trials wherein cattle were fed the same or higher amounts of vitamin E (2,000-4,000 IU: conversions between IU and mg were as reported by Abe et al. 2016) as we did, while some cattle had benefit in health (Baldi et al. 2000, Bouwstra et al. 2008, Politis et al. 2004, Weiss et al. 1997), others had either none or had some negative effect (Bouwstra et al. 2010, LeBlanc et al. 2004, Waller et al. 2007). Previously, vitamin E, as β-carotene, was generally regarded as harmless; thus, no safe intake limit has been reported (Subcommittee on Dairy Cattle Nutrition 2001b). However, in recent years, it has been shown that excess β-carotene, and more recently, vitamin E, may cause some adverse effects on the health of various organisms (Aketagawa et al. 1994, Albanes et al. 1996, Fujita et al. 2012, Hennekens et al. 1996, Männistö et al. 2004, Omenn et al. 1996, The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group 1994). Therefore, large doses of vitamin E and β-carotene should also be administered with caution.

We suggest that a single, pinpoint, excessive dose of vitamin A or vitamin E (whether administered jointly or separately) on the third postpartum day in primiparous cows will lead to early responders. This experimental study is not a practical application; therefore, comprehensive studies are needed to determine the optimal quantity of vitamins that can be safely administered to promote ovulation while protecting the cow's health.

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