Blood Selenium, Vitamin E, Vitamin A, and β-Carotene Concentrations and Udder Health, Fertility Treatments, and Fertility

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ABSTRACT

We investigated the activity of glutathione peroxidase in whole blood; concentrations of vitamin E, vitamin A, and β-carotene in serum; SCC; udder bacterial infections and the incidence of clinical mastitis; fertility treatments; and the success of first AI of 511 dairy cows for 1 yr. The mean Se content in whole blood and the concentrations of vitamin E, vitamin A, and β-carotene concentrations in serum were 191 μg/L, 5.9 mg/L, 0.39 mg/L, and 12.9 mg/L, respectively. An increase in Se concentration in whole blood was associated with a decrease in all infections, including infections by *Staphylococcus aureus*, *Actinomyces pyogenes*, and *Corynebacterium* spp. (−17.7, −31.7, and −70.6%, respectively). There was no association among the different infections or SCC and concentrations of vitamin E, vitamin A, or β-carotene, but an association existed between vitamin A concentration and SCC. The lower Se concentration in whole blood did not increase incidence of clinical mastitis. The Se concentration in whole blood (200 μg/L) was accepted as a target value to optimize udder health. The incidence of fertility disorders (anestrus, subestrus, cystic ovaries, or delayed ovulation) was 34.4%. The pregnancy rate following first insemination was 48.6%. No significant association was observed among Se in whole blood; concentrations of total vitamin E, vitamin A, or β-carotene in serum; and fertility disorders or success of first AI.
(**Key words**: udder health, selenium, vitamins, fertility)

Abbreviation key: **APC** - *Actinomyces pyogenes* or *Corynebacterium* spp., **API** = infected by any pathogen, **AS** = anestrus or subestrus, **CMT** = California mastitis test, **CNS** = coagulase-negative staphylococci, **GSHpx** = glutathione perioxidase, **ODCO** = ovulatory disorder or cystic ovaries, **SFAI** = success of first AI.

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INTRODUCTION

The favorable effect of Se and vitamin E on udder defense mechanisms has been reported in many studies using different approaches. Bone and Arthur (5) noted a decrease in the ability of neutrophilic granulocytes to kill phagocytosed *Candida albicans* in cows suffering from Se deficiency. A lack of Se also lowers the production of leucotrienes of polymorphonuclear leukocytes and thus lowers chemotaxis of neutrophils (2).

Selenium and vitamin E used separately are able to mitigate the severity of the clinical symptoms of mastitis and to shorten their effects; used together, they are even more efficacious (42). Selenium in feed has been shown to shorten the duration of mastitis and to lessen its symptoms, especially *Escherichia coli*, but not that caused by *Staphylococcus aureus* (13, 16).

Selenium and vitamin E supplementation curtail the prevalence of infections by environmental pathogens and staphylococci during calving and the incidence of clinical mastitis during the first lactation (43). Several studies (14, 34) have reported a relationship between herd SCC and blood glutathione peroxidase (**GSHpx**; EC 1.11.19) activity. High SCC was related to low GSHpx activity. The vitamin E status of herds with high SCC did not differ from that of herds with good udder health (6, 14, 46). In a study with a small range in vitamin E (6), the vitamin E status of cows with chronic mastitis was no worse than that of healthy cows; clinical mastitis did not seem to be associated with plasma vitamin E concentration (34), even though the vitamin E concentration in the plasma of mastitic cows was lower than that in healthy cows (3). In contrast, in a more recent study (47), adequate vitamin E intake reduced the incidence of clinical mastitis.

Chew et al. (8) observed an association between SCC and the concentrations of vitamin A and β-carotene in plasma. In an in vitro experiment conducted to establish the mechanism of the effect of β-carotene (9), the intracellular killing ability of phagocytic cells increased. However, an in vivo experiment (35) did not confirm these in vitro results.

Studies of the connection between Se status and reproduction initially focused on retained placenta. Julien et al. (25) obtained excellent results in reducing the number of retained placenta by the addition of Se or vitamin E to feed during the dry period. Subsequently, that finding has been confirmed (6), rejected (21), and only partially confirmed (41). Larson et al. (26) found a positive association between Se in serum and the success of AI, in agreement with the findings of Segerson et al. (41). In contrast, a comparison of herds with different fertility revealed no differences in concentrations of Se in serum, and concentrations of vitamin E were higher in herds with diminished fertility than in control herds (3).

Harrison et al. (23) reported the positive effects of Se injections administered during the dry period to prevent cystic ovaries and metritis. Addition of vitamin E to feed also reduced the incidence of retained placenta.

β-Carotene also functions independently of vitamin A in mastitis and reproduction (30). The necessity of β-carotene for reproduction has been demonstrated using cows that were first fed for a long period without β-carotene (29). Thereafter, vitamin A and β-carotene were added to the feed of the test group, and vitamin A alone was added to the feed of the control group. A positive effect was found for almost all of the external signs of estrus and fertility.

The cows in the original experiment (30) suffered from a persistent deficiency in β-carotene. Bonsembiante et al. (4) conducted an experiment in which the feed contained low amounts of β-carotene for a short period only. In that experiment (4), the addition of β-carotene to the feed had the same positive effect on fertility parameters. However, opposite results have also been obtained (17).

The present study evaluated the association between blood parameters and udder health with fertility in commercial herds.

MATERIALS AND METHODS

Thirty volunteer herds for which milk records were kept were monitored for 1 yr. The data on disease incidence for the herds were gathered using the Finnish health recording system (19). These herds were fed grass silage or wilted grass silage as roughage. Oats, barley grain, and concentrates (commercial protein feed plus grain) were fed to cows in quantities related to milk products. Grain and concentrates constituted a mean 38% (range 21 to 50%) of DM.

The cows were sampled four times: August and November 1989 and February and May 1990. The same sampling procedure was used each time. Quarter milk samples were taken before milking for bacteriological and California mastitis test (**CMT**) examination; whole milk samples were taken with a milk meter for SCC. After milking, a blood sample was taken from the jugular vein both with and without anticoagulant (EDTA).

Blood samples with EDTA from the cows were frozen immediately after sampling and stored at −18°C until analyses for GSHpx activity and Se. The serum from blood samples without anticoagulant was frozen for transportation to the laboratory and kept there at −70°C until analyses for Se, vitamin E, vitamin A, and β-carotene. Prior to treating clinical mastitis, the veterinarian always sampled the milk for bacteriological examination.

The activities of GSHpx in whole blood and red blood cells were determined using methods described by Günzler et al. (20) and Sankari (40). Selenium in whole blood and serum was measured using the fluorometric method described by Lindberg (27).

The vitamin E in the serum samples was determined with a normal phase HPLC system, a mobile phase based on heptane, and fluorescence detection; the excitation wavelength was set at 295 nm, and the emission wavelength was set at 325 nm. Pure dl-α-tocopherol was used as the external standard. This normal phase HPLC system allowed all eight natural homologues of vitamin E to be separated and quantified (36).

An HPLC method was used for the determination of vitamin A and β-carotene in the serum samples. The HPLC system consisted of a reversed-phase column, a mobile phase based on methanol, and a photodiode array detector connected in series with a fluorescence detector. The diode array detector was set at a wavelength of 453 nm for the detection of β-carotene, and the fluorescence detector was set at an excitation wavelength of 325 nm and an emission wavelength of 470 nm for the detection of vitamin A. The quantification was made using pure retinol and β-carotene as external standards.

Preparation of serum samples for the HPLC analysis of vitamin E, vitamin A, and β-carotene involved precipitation of proteins with ethanol and hexane extraction of lipid material. After evaporation of the hexane phase, the samples were redissolved in a small volume of hexane for vitamin E analysis and in a small volume of ethanol for analysis of vitamin A and β-carotene. Ascorbic acid or butyrl hydroxytoluene was present in all the extraction procedures to avoid spontaneous oxidation of the substances to be analyzed. Total lipids in serum were determined according to the method of Epstein et al. (12).

When quarter milk samples were taken, the teat ends were disinfected with 70% ethanol. Milk samples

TABLE 1. Summary of blood parameters.

Blood parameter	$\bar{X}$	SD	Range	n
Se in whole blood, μg/L[1]	191	42.4	93–305	2024
Vitamin E in serum, mg/L	5.9	2.60	1.5–15.3	496
Vitamin A in serum, mg/L	0.39	0.08	0.12–0.64	264
β-Carotene in serum, mg/L	12.9	5.2	1.2–26.6	264

[1]Estimated by a regression line between Se in whole blood and glutathione peroxidase.

for individual SCC were analyzed by the fluoro-opto-electronic method (Fossomatic; Foss Electric, Hillerød, Denmark).

Correlations among continuous variables were calculated using regression analysis. The chi-square test, Fisher's exact test, and the chi-square test for trends were used for statistical analyses. The test used and results of each calculation are given. Statistical significance was determined at $P < 0.05$.

RESULTS

Activity of GSHpx was measured on 2032 samples. Selenium concentrations in whole blood were measured on 85 samples. The correlation coefficient between Se and GSHpx was 0.78 ($P < 0.001$; $Y = 6.3 X - 63.6$, where Y = GSHpx activity in microkatals per liter, and X = Se in micrograms per liter of whole blood). The mean estimated Se concentration in whole blood was 191 μg/L ± 42.4 (range 93 to 305; n = 2032). All of the following calculations were based on GSHpx, but the corresponding Se concentrations were indicated to aid in the comparison of different studies. The analysis of GSHpx was not comparable among different laboratories.

The mean concentrations of vitamin E, retinol, and β-carotene in serum in May was 5.9 mg/L ± 2.60 (range 1.5 to 15.3; n = 496), 0.39 mg/L ± 0.08 (range 0.12 to 0.64; n = 264), and 12.9 mg/L ± 5.2 (range 1.2 to 26.6; n = 264), respectively (Table 1).

Udder Bacterial Infections

The GSHpx activities of uninfected cows, cows infected by any pathogen (**API**), and cows infected by coagulase-negative staphylococci (**CNS**) or by *S. aureus* were normally distributed. The Se concentration in whole blood that corresponded to the GSHpx activities, above which the percentage of uninfected cows numerically exceeded the percentage of cows infected by API, CNS, and *S. aureus*, was 180 μg/L. At an Se value >150 μgL of whole blood, the proportion of uninfected cows was greater than the percentage of cows infected with *Actinomyces pyogenes* or *Corynebacterium* spp. (**APC**). The whole blood Se concentrations at 150 and 180 μg/L and the recommendation of Smith et al. (46) of 200 μg/L were used as a basis for the following breakdowns.

To establish the relationship between GSHpx activity in whole blood and bacterial infections of the udder, the cows were divided into groups for the chi-square test according to their infection status and GSHpx activity. The Se concentrations in whole blood corresponding to the GSHpx activity values used were 150, 180, and 200 μg/L (Table 2). The group of uninfected cows was used as the control group. The numbers of cows that were uninfected or that were infected by API, CNS, *S. aureus*, streptococci, APC, or coliforms were 927, 808, 398, 255, 139, 38, and 13, respectively. The number of cows infected by API tended to decrease as GSHpx activity increased. Result were similar with *S. aureus*, APC, and CNS (Table 2).

Below 200 μg/L of Se in whole blood, there was a linear trend for API, *S. aureus*, and APC ($P < 0.05$; n = 533, 176, and 27, respectively). The chi-square test was used to detect the linear trend; 601 cows were uninfected. No correlation ($P > 0.05$) was significant between herd mean of GSHpx activity in whole blood and the major pathogen (*Streptococcus dysgalactiae*, *Streptococcus uberis*, *S. aureus*, coliforms) infecting the quarter (r = 0.003; n = 120).

The effects of concentrations of vitamin E, vitamin A, or β-carotene in serum on udder infections were examined by groups. Cows with <4 mg/L of vitamin E in serum were in an experimental group, and cows with >4 mg/L of vitamin E served as a control group (number of uninfected cows and number of cows infected with API, CNS, *S. aureus*, and streptococci were 252, 177, 98, 59, and 20, respectively); the breakdown was based on the recommendations of Smith et al. (45). Cows with <0.40 mg/L of vitamin A in serum were in an experimental group, and cows with >0.40 mg/L of vitamin A served as controls (number of uninfected cows and number of cows infected with API, CNS, *S. aureus*, and streptococci were 132, 74, 53, 33, and 18, respectively). A plasma vitamin A concentration >0.40 mg/L has been determined to be sufficient (30). A β-carotene concentra-

TABLE 2. Prevalence of infection in groups of cows with low and high Se.

	Pathogen[1]											
	All pathogens			CNS			SA			APC		
	no.[2]	Cows infected		no.	Cows infected		no.	Cows infected		no.	Cows infected	
		(no.)	(%)		(no.)	(%)		(no.)	(%)		(no.)	(%)
Se <150 μg/L	1735	108	55.7	1325	53	38.1	1182	34	28.3	967	11	11.3
Se >150 μg/L		700	45.7		345	29.1		221	20.8		29	3.3
			(−18)[3],*			(−24)*			(−27)**			(−71)*
Se <150 μg/L	1279	108	55.7	956	53	38.1	851	34	28.3	705	11	11.3
Se >180 μg/L		498	45.9		251	30.0		731	19.7		21	3.5
			(−18)*			(−21)*			(−31)*			(−70)*
Se <150 μg/L	825	108	55.7	624	53	38.1	544	34	28.3	453	11	11.3
Se >200 μg/L		289	45.8		143	29.5		82	19.3		14	3.9
			(−18)*			(−23)*			(−32)*			(−65)*
Se <180 μg/L	1281	310	47.7	972	147	30.2	875	111	24.6	715	19	5.3
Se >200 μg/L		289	45.8		143	29.5		82	19.3		14	3.9
			(−4)			(−2)			(−21)**			(−26)

[1]CNS = Coagulase-negative staphylococci, SA = *Staphylococcus aureus*, and APC = *Actinomyces pyogenes* or *Corynebacterium* sp.

[2]Number of cows included in high Se and low Se groups.

[3]Values in parentheses are decreases expressed as high Se relative to low Se.

*$P < 0.05$ (chi-square test).

**$P = 0.06$ (chi-square test).

tion of 3 mg/L was used to divide the material into an experimental and control group (number of uninfected cows and number of cows infected with API, CNS, *S. aureus*, and streptococci were 132, 71, 52, 32, and 17, respectively); this concentration of β-carotene has been proposed to be sufficient (28), but is not generally accepted (33). There was no significant difference among groups ($P > 0.05$; chi-square test).

SCC

To calculate the regression between SCC, activity of GSHpx in whole blood, and concentrations of vitamin E, vitamin A, and β-carotene in serum, 0.001 was added to the SCC to allow the log transformation because the data included two zero values. No significant correlation was found among the variables; the r^2 for vitamin A was 0.047 (n = 237; $P < 0.001$). The r^2 for GSHpx was 0.00002 (n = 1653; $P > 0.05$), for vitamin E was 0.004 (n = 540; $P > 0.05$), and for β-carotene was 0.0006 (n = 237; $P > 0.05$).

Clinical Mastitis

To determine the effect of GSHpx activity in whole blood on the incidence of clinical mastitis, cows were divided into groups for chi-square analysis according to whether they had been treated or had not been treated and according to their GSHpx activity prior to treatment. The numbers of untreated cows and cows with clinical cases of mastitis were 1997 and 60, respectively. The lower GSHpx activity did not expose cows to clinical mastitis ($P > 0.1$; chi-square test).

The probability of acute mastitis (n = 60) increased when the GSHpx value of 1056 μkat/L was exceeded (corresponding to 180 μg of Se/L of whole blood). Below that value, the likelihood ratio was 0.72 (mastitis:no mastitis), and the likelihood ratio >180 μg of Se/L of whole blood was 1.16. The likelihood ratio was the probability of a test result in the diseased cows divided by the probability of a test result in the healthy cows.

Fertility Treatments and Fertility

To determine the effect of whole blood GSHpx activity on the incidence of anestrus or subestrus (**AS**), the incidence of ovulatory disorder or cystic ovaries (**ODCO**), or on the success of first insemination (**SFAI**), the cows were divided into groups for the chi-square test according to whether they had been treated for AS or ODCO or not treated or according to SFAI and their GSHpx activity as presented in Table 2. The Se concentrations corresponding to the GSHpx activity values used were 150, 180, and 200 μg/L. There were 71 cows treated for AS and 143 cows not treated for AS, 81 cows treated for ODCO and 133 cows not treated for ODCO, and 144 cows conceiving at first AI and 186 cows failing to conceive. Only the difference between the AS groups was statistically significant ($P < 0.05$).

The prevalence of AS and ODCO increased when the GSHpx value in whole blood was >1056 μkat/L, corresponding to 180 μg/L of Se in whole blood. Below that value, the likelihood ratio was 0.88 (disorder:no disorder), and above that value the likelihood ratios were 1.10 and 1.11, respectively. The value set for the SFAI had no statistical significance, however.

The prevalence of fertility disorder treatments and SFAI were compared with the vitamin E concentrations in serum by dividing the material into two groups; the value for vitamin E was 4 mg/L. Anestrus or subestrus treatments were compared with vitamin E concentration for the sampling month. The control group for untreated cows contained cows that could have been treated (46 to 251 d from calving). There were six AS treatments in all and 329 cows in the control group. There was no difference among the groups ($P > 0.05$; Fisher's two-tailed exact test).

The prevalence of ODCO was analyzed in the same way. The five treatments took place 29 to 218 d after calving, and 349 untreated cows constituted the control group. There was no difference among the groups ($P > 0.05$; Fisher's two-tailed exact test).

The SFAI was compared with vitamin E concentration from the sampling month. There were 30 observations in all. No difference was found between the groups ($P > 0.05$; Fisher's two-tailed exact test).

Fertility disorder treatments were compared with vitamin A of the sampling month. The control group of untreated cows contained cows that could have been treated (29 to 251 d from calving). There were nine treatments for AS and ODCO in all, and 153 cows belonged to the control group. There was no difference among the groups ($P > 0.05$; Fisher's two-tailed exact test) when they were divided by a vitamin A value of 0.40 mg/L.

The SFAI was used as the measure of fertility. A comparison of the success of AI in May (n = 25) with vitamin A concentrations in serum in May did not reveal any differences in fertility among cows with vitamin A values of >0.40 mg/L or <0.40 mg/L ($P = 0.05$; chi-square test). In May, 45% of the group was below the limit value, and 57% were above it.

Fertility disorder treatments or SFAI could not be compared with the chosen β-carotene value (3 mg/L) because the serum concentration in β-carotene was so high.

DISCUSSION

The Se and vitamin status in cows was similar to that observed earlier for Finnish cattle fed silage (24).

Udder Health

The decrease in udder infections caused by any pathogen as Se concentrations increased was consistent with observations by Smith et al. (43). The decrease in the prevalence (−17.7%) of all infections in the present study was not as high (−42%) as reported by Smith et al. (43) when groups were compared that had equal Se concentrations (Table 2). The observation that the decrease in all infections could be explained by a decrease in the prevalence of CNS was in agreement with the study results of Smith et al. (43). They (43) observed a decrease of −42% in the prevalence of staphylococcal infection; prevalence in our study was only about half of that value (−22.7%). Conversely, Smith et al. (43) observed no effect of *Corynebacterium* sp. The large decrease (peak, −70.6%) in the infection of APC in the present study had little effect on the overall infection percentage, because these bacteria accounted for only 5.2% of all infections. The group with Se in whole blood >200 μg/L corresponded to the recommendations of Smith et al. (45). Neutrophils in cows receiving Se supplementation have been observed to kill phagocytosed *S. aureus* more effectively than neutrophils of unsupplemented cows (18). However, experimental challenge infections conducted with *S. aureus* did not show any difference in the ability of supplemented and unsupplemented cows to eliminate udder infections (16). From the results of the present study, an increase in Se concentration in whole blood to >180 μg/L could improve the defenses of a cow against *S. aureus*.

Staphylococcus aureus infections did not decrease for cows with lower concentrations of vitamin E in the present study. In the bacterial killing after phagocytosis, toxic oxygen compounds are produced inside the cell. The cells are protected from these compounds by vitamin E and β-carotene (22). A sufficient intake of vitamin A guarantees the normal function of epithelia, which might improve the defenses of the mammary gland against infections (7).

No connection was found between mean herd GSHpx activity of the herd and the infection percentage of major pathogens, contrary to the findings of Erskine et al. (14). Partly, this difference was attributed to the small number of major pathogen infections in the present study [the mean of only five herds (4%; n = 120) exceeded the infection percentage of 20%; in the study of Erskine et al. (14), half the herds exceeded the percentage]. In addition, the range of GSHpx activity was narrower, and the mean GSHpx activity value was higher, in the present study.

Many researchers (6, 34, 43, 46) have found that Se supplementation decreases the SCC in milk (44). Furthermore, Braun et al. (6) stated that other factors, such as low vitamin E intake, faulty husbandry, or milking hygiene, can lead to a high SCC as well as the low SE value. Ndiweni et al. (34) compared mean herd GSHpx activity of the herd with the SCC of the bulk tank milk and found a negative relationship. Activity of GSHpx did not affect milk SCC in the present study. In the study by Erskine et al. (14), blood Se in herds with SCC was clearly lower than that in the present study. A high SCC was determined to be >700,000 cells/ml; in this study, only one herd had >700,000 cells/ml twice, which explains the discrepancy. The relationships between SCC and vitamin E and A in serum were minimal in the present study. This result was in agreement with the observations of Erskine et al. (14), who compared concentrations of vitamin E and β-carotene in the serum of herds with different SCC. The effect of vitamin E in serum on SCC was not established in the study of Weiss et al. (47) either, but Chew et al. (8) found a negative relationship between the results of the CMT and β-carotene in plasma. In the present material, the association did not exist, possibly because of the methodological differences between the studies [SCC was used in this study, but Chew et al. (8) followed the results of the CMT on the quarter that exhibited the highest degree of gelling], and the β-carotene content in plasma was minimal (<2.2 mg/L) compared with the content in our study. Erskine et al. (14) did not observe any difference in concentrations of vitamin A in serum at different SCC. The low vitamin A concentration in serum after calving predisposed the cow to a higher SCC during lactation (Jukola et al., 1996, unpublished data); field research (35) has shown no effect of vitamin A in serum on SCC. Against this background, the r^2 of 0.22 found in the present study was surprising.

Sufficient intake of Se mitigates clinical mastitis and eases its symptoms (43, 47), but comparisons of herds with a high incidence of clinical mastitis with herds of lower disease incidence did not reveal differences in Se concentrations in the blood (35). In the present study, not all cases of clinical mastitis that were treated were correlated with low whole blood GSHpx values before infection. On the contrary, the proportion of acute mastitis cases increased as GSHpx activity in whole blood increased. This observation was probably due to the complex tissue uptake of Se and vitamin E, the connection between high Se intake and a high incidence of clinical mastitis (46), or merely a reflection of data distribution.

The correlation between the antioxidant levels studied and udder health was low. The simultaneous concentrations of different antioxidants should be considered because the metabolism of one antioxidant can interfere with that of another. The effects of Se and α-tocopherol as antioxidants are partially mutually replaceable. In addition, the antioxidant function of β-carotene may be almost the same as that of α-tocopherol (22). For the cows in this study, the combined effect of the antioxidants was enough to offset any possible momentary deficiencies of a single antioxidant. In evaluating the relationship between antioxidants and disease incidence, more attention should be paid to the relationship between the total antioxidant capacity of the system (31) and the health of the cow.

Fertility Treatments and Fertility

Activity of GSHpx in blood was associated with the incidence of AS, but GSHpx had no relationship with the incidence of ODCO and SFAI. Harrison et al. (23) succeeded in reducing the incidence of cystic ovaries by injecting Se during the dry period. Cows deficient in Se were used as the control group. Braun et al. (6) found no differences in the concentrations of Se in blood between cows with fertility disorders and healthy cows. The treatments for AS were preceded by extremely low GSHpx activity in whole blood. There may be two reasons for this observation. First, both diseases are strongly associated with energy intake. If the cow consumed so much DM that the Se in the blood was >200 μg/L, ovulatory function is likely to start normally after calving. Also, peroxidative inactivation of steroidogenic enzymes may impair reproduction (31).

The likelihood ratios of this study for fertility treatments were supported by the findings of Mohammed et al. (32), who found >169 μg/L of Se in whole blood to be a risk factor for cystic ovaries, but no risk was associated with <108 μg/L of Se in whole blood. The results of both this study and that of Mohammed et al. (32) can most likely be explained by a confounding factor, for example, herd or cow production, which was not recorded in the studies. Findings concerning the effect of production during the previous season on the onset of cystic ovaries were contradictory (11, 15) but not the effect of negative energy balance (1, 39).

The lack of association between GSHpx activity in the whole blood and SFAI of the present study was consistent with results from other studies. Selenium alone does not affect the fertilization of the ovum. Blood Se status in the test group of the study by Segerson et al. (41) equaled the mean concentration of Se of the present study, and Se status in the control group of the study was equal to the minimum values of the present material.

Vitamin E status during AI had no effect on fertility in our study. Similarly, the positive effect of sufficient vitamin E intake did not show in fertility treatments when the value of the treatment month was used as reference.

Harrison et al. (23) failed to find any decrease in incidence of cystic ovaries with vitamin E supplementation in late pregnancy, even though Daubinger and Preisinger (10) later managed to reduce the frequency of cystic ovaries with supplementation. Vitamin E concentrations in cow serum were not reported before or after the supplementation, even though the evaluation of vitamin E status in cows has to be based on serum concentrations (38).

Even high vitamin A concentrations in serum, if obtained through normal feeding, did not have an adverse effect on ovarian functions (30). Many earlier studies (6, 29) compared a group of cows with almost no intake of β-carotene with a group of cows supplemented with β-carotene. The results of those studies cannot be repeated with a cow population in which the β-carotene intake is as high as the one in this study.

CONCLUSIONS

The 180 μg of Se/L in whole blood would seem critical for all infections and for the prevention of CNS and *S. aureus*. Prevention of APC infections seemed to require almost as high a concentration of Se (150 μg/L) as did the prevention of the other pathogens. The vitamin E, vitamin A, and β-carotene concentrations in serum, >4, >0.40, and >3 mg/L, respectively, would appear to be sufficient for optimizing udder health.

The concentrations of 100 μg of Se/L in whole blood, of 4 mg of vitamin E/L, and of 0.40 mg of vitamin A/L in serum can be regarded as sufficient for AS, ODCO, and SFAI. For herds fed grass silage, there was no improvement in the fertility treatments or fertility if additional β-carotene was used.

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