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RESEARCH ARTICLE

Plasma β-Carotene, Vitamin A and Vitamin C Levels in Cyclic and Pregnant Cows

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Summary

The aim of this study was to determine the plasma β-carotene, vitamin A and vitamin C levels and correlation between these parameters in cyclic and pregnant dairy cattle. A total of 101 Holstein cows used for this aim. Artificial inseminations (AI) were performed 12 h after determining the oestrus. The cows were allocated to two groups as pregnant (n=81) and nonpregnant (n=20) after the determination of pregnancy. Progesterone level was only higher on day 21 in pregnant cows than that in nonpregnant cows (P<0.05). There were variations in the mean levels of β-carotene and vitamin A in pregnant and nonpregnant cows (P<0.05) whereas vitamin C levels in nonpregnant cows did not differ during the oestrus cycle. There was a negative correlation between the plasma levels of progesterone and β-carotene (P<0.01) and progesterone and vitamin A (P<0.05) in pregnant cows, and a positive correlation between the plasma levels of β-carotene and vitamin A in both pregnant and nonpregnant cows (P<0.01), during the cycle. In conclusion, differences were determined in the levels of β-carotene, vitamin A and vitamin C in pregnant cows, and b-carotene and vitamin A in nonpregnant cows with the stages of the oestrus cycle in this study. In addition to this, more research is needed evaluating the relationship between these parameters and their effects on bovine reproduction.

Keywords: *β-carotene, Cow, Vitamin A, Vitamin C*

Siklik ve Gebe İneklerde Plasma β-Karoten, Vitamin A ve Vitamin C Seviyeleri

Özet

Bu çalışmanın amacı siklik ve gebe ineklerde plazma β-karoten, vitamin A ve vitamin C seviyelerini ve bu değerler arasındaki ilişkiyi belirlemekti. Bu amaçla toplam 101 Holstein inek kullanıldı. Östrüs tespitinden 12 saat sonra ineklere suni tohumlama uygulandı. İnekler gebeliklerinin belirlenmesinden sonra gebe (n=81) ve gebe olmayan (n=20) olarak iki gruba ayrıldı. Progesteron seviyesi yalnız 21. günde gebe ineklerde gebe olmayan ineklerden yüksek bulundu (P<0.05). Gebe olmayan ineklerde ortalama vitamin C seviyesi östrüs siklusu boyunca değişmezken gebe olan ve olmayan ineklerde β-karoten ve vitamin A seviyelerinde siklus boyunca farklılık görüldü (P<0.05). Siklus sırasında gebe ineklerde plazma progesteron ve β-karoten (P<0.01) ve progesteron ve vitamin A (P<0.05) seviyeleri arasında negatif, gebe olan ve olmayan ineklerde ise plazma β-karoten ve vitamin A seviyeleri arasında pozitif ilişki saptandı (P<0.01). Sonuç olarak gebe ineklerde β-karoten, vitamin A ve vitamin C seviyelerinde, gebe olmayan ineklerde ise β-karoten ve vitamin A seviyelerinde östrüs siklusunun evresine göre farklılıklar tespit edildi. Buna ilave olarak, bu parametrelerin arasındaki ilişkiyi ve sığırlarda reprodüksiyon üzerine etkisini araştıran daha fazla çalışmaya ihtiyaç olduğu kanısına varıldı.

Anahtar sözcükler: *β-karoten, İnek, Vitamin A, Vitamin C*

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INTRODUCTION

Reproductive efficiency is a major component of economic success in dairy herds [1] and many factors influence fertility including cyclicity, energy balance, heat stress, parity, milk production, diet and diseases [2]. In addition to these factors, it is reported that plasma concentrations of β-carotene, vitamin A and vitamin C have a marked effect on reproduction [3-7].

β-carotene is a precursor for vitamin A [8,9] and the importance of β-carotene in bovine reproduction is equivocal. Recent investigations of β-carotene and vitamin A has focused on ovarian function especially on luteal development, progesterone production and fertility [10-12]. Its deficiency resulted in extended duration of oestrus, delayed ovulation, retarded development of corpus luteum and a higher incidence of ovarian cysts which led to low conception rates and abortions in early pregnancy [13,14]. Jukola et al.[15] related the β-carotene defficiency with decrease of the external signs of estrus and fertility. In contrast, there are reports that the conception rate [16] and plasma progesterone concentration were not influenced by β-carotene injection [17]. Morover, fertility parameters did not differ between β-carotene injected and control cows, and authors emphasized that it was not recommendable to inject β-carotene for therapy or prophylaxis of fertility disorders [17].

Whereas the importance of vitamin A in reproductive performance is axiomatic [18,19], there are still some controversies about it [11]. It is reported that vitamin A has a beneficial effect on fertility in cows [3,9]. In contrast, no significant association was observed among concentration of vitamin A in serum and fertility disorders or success of first insemination [15]. Brown et al.[20] did not show any effect of vitamin A on the response of ovaries to super-ovulation in cows.

Vitamin C is not an essential dietary nutrient for adult dairy cattle because of its biosynthesis in cattles own body; however, it is an important water-soluble antioxidant [21,22]. Nevertheless, cattle are prone to ascorbic acid deficiency when ascorbic acid synthesis is impaired because exogenous supplies of this vitamin are rapidly destroyed by ruminal microflora [23]. The effect of vitamin C on reproduction has described before [24]. Vitamin C is believed to act as an antioxidant, neutralizing the oxidative by-products of cellular respiration in luteal cells, as an enzymatic cofactor in collagen synthesis and as a promotor of steroid and protein hormone synthesis [25,26]. However, no significant correlation between plasma vitamin C and progesterone levels was described in some studies [27,28].

The aim of this study was to determine the plasma β-carotene, vitamin A and C levels and correlation between these parameters in cyclic and pregnant dairy cattle.

MATERIAL and METHODS

A total of 101 healthy Holstein cows, aged 3-7 years, in September and November 2002 were used in this study. Those animals were selected taking the criterions listed as followed: *a)* no dystochia and retained fetal membranes in previous calving; *b)* no prulent discharge during vaginal examination; *c)* 50 to 90 days postpartum; *d)* have shown oestrus at least once; *e)* no artificial insemination (AI) or mating after previous calving. The cows were had the same feed (a ration composed of corn silage, alfalfa hay and a concentrate-mineral mix and ad libitum access to fresh water) and housed in a free-stall confinement facility.

Oestrus signs (visual observation of standing heat, vaginal discharge) were examined four times a day during 30 min. Oestrus was confirmed by rectal palpation of a fluctuant dominant follicle and uterine tonus. AI were performed by a single practitioner 12 h after determining the oestrus. Pregnancy was determined by rectal palpation between 60-90 days after the insemination and the cows were allocated to two groups as pregnant (n=81) and nonpregnant (n=20).

Blood samples were collected from the jugular vein into evacuated 10 ml tube containing heparin at the time of AI (day 0), at metoestrus (day 3), at dioestrus (day 12) and 21 days after AI. Plasma was prepared by centrifugation (5000 rpm for 5 minutes) and frozen at -20°C within 2-4 h for subsequent determinations of vitamins, β-carotene and progesterone concentrations. The plasma progesterone levels were determined by an enzyme immunoassay method [29]. The plasma β-carotene and vitamin A levels were analysed by spectrophotometry as reported by Suzuki and Katoh [30]. The levels of plasma vitamin C were analysed by spectrophotometry as reported by Haag [31].

The data obtained in this experiment were presented as mean±SEM. and subjected to analysis of variance (ANOVA). Associations between variables were calculated by correlation coefficients. All analyses were carried out using a statistical analysis system configured for computer (MINITAB, Release 12.1, Minitab Inc.).

RESULTS

Mean levels of progesterone are shown in *Fig. 1* and, β-carotene, vitamin A and vitamin C in pregnant and non-pregnant cows are shown in *Table 1*. There was no

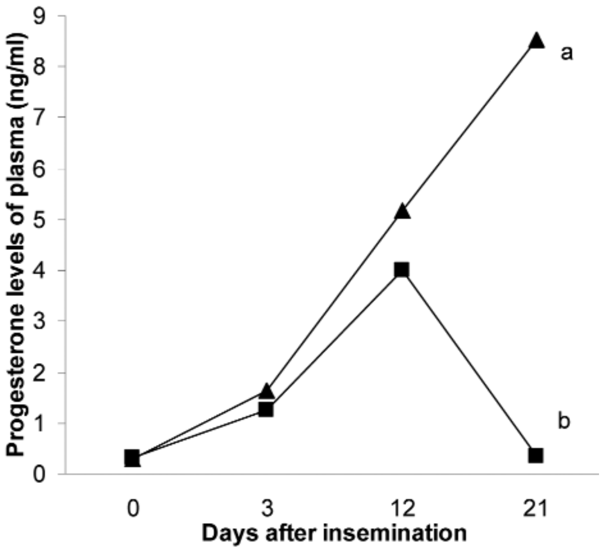

Fig 1. The mean progesterone levels in plasma (ng/ml), at the time of AI, 3, 12 and 21 days after AI in pregnant (▲) and nonpregnant (■) cows. **a,b:** Different superscripts differ significantly (P<0.05)

Şekil 1. Gebe (▲) ve gebe olmayan (■) ineklerde tohumlama, tohumlamadan 3, 12 ve 21 gün sonra ortalama progesteron seviyeleri (ng/ml). **a,b:** Farklı harf taşıyan değerler istatistiki açıdan farklıdır (P<0.05)

significant difference in progesterone levels between pregnant and nonpregnant cows at at the time of and 3 and 12 days after AI. However, progesterone levels on day 21 in pregnant cows were higher (P<0.05) than that in non-pregnant cows (0.23, 1.63, 5.18 and 8.52 ng/ml in pregnant and 0.33, 1.25, 4.0 and 0.34 ng/ml in non-pregnant cows at the time of and 3, 12 and 21 days after AI, respectively).

β-carotene levels at the time of and 3 and 21 days after AI were higher in pregnant cows (P<0.05) than that in nonpregnant cows while there was no significant difference between pregnant and nonpregnant cows 12 days after AI.

The mean vitamin A level was higher in pregnant cows than that in nonpregnant cows 21 days after AI (P<0.05), but there was no significant difference at the time of and 3 and 12 days after AI.

There was no significant difference in vitamin C levels at the time of and 21 days after AI between pregnant and nonpregnant cows. However, mean vitamin C levels were higher in pregnant cows than that in nonpregnant cows 3 and 12 days after AI (P<0.05).

The mean levels of β-carotene, vitamin A and vitamin C in pregnant cows varied during the oestrus cycle and, β-carotene and vitamin A levels were significantly higher at the time of and 3 and 12 days after AI whereas vitamin C levels were higher 12 days after AI (P<0.05). On the other hand, β-carotene levels were higher at the time of and 3 and 12 days after AI and, vitamin A levels were higher at the time of and 3 days after AI in nonpregnant cows. In addition to this, vitamin C levels in nonpregnant cows did not differ during the oestrus cycle.

Table 1. *The mean plasma levels of β-carotene (μg/dl), vitamin A (μg/dl) and vitamin C (μg/ml) at the time of AI, 3, 12 and 21 days after AI in pregnant and nonpregnant cows (±SEM)*

Tablo 1. *Gebe ve gebe olmayan ineklerde tohumlama, tohumlamadan 3, 12 ve 21 gün sonra ortalama plazma β-karoten (μg/dl), vitamin A (μg/dl) ve vitamin C (μg/ml) seviyeleri (±SEM)*

Vitamin Levels	Days	Pregnant Cows (n=81)	Nonpregnant Cows (n=20)
β-Carotene (μg/dl)	0	^{A} 136.3±15.2 ^{a}	^{A} 61.0±9.4 ^{b}
	3	^{AB} 102.6±11.4 ^{a}	^{A} 62.5±17.7 ^{b}
	12	^{B} 84.5±5.9	^{A} 68.5±9.7
	21	^{C} 52.8±4.4 ^{a}	^{B} 25.7±8.1 ^{b}
Vitamin A (μg/dl)	0	^{A} 33.7±2.2	^{A} 40.7±3.6
	3	^{A} 32.8±2.8	^{A} 29.5±4.3
	12	^{A} 37.0±5.4	^{AB} 26.1±9.4
	21	^{B} 25.2±1.9 ^{a}	^{B} 15.70±1.4 ^{b}
Vitamin C (μg/ml)	0	^{C} 2.85±0.17	2.96±0.50
	3	^{B} 3.96±0.19 ^{a}	3.04±0.30 ^{b}
	12	^{A} 4.81±0.21 ^{a}	1.67±0.44 ^{b}
	21	^{B} 3.65±0.19	3.12±0.30

^{a,b}: Different superscripts in the same row indicate significant differences (P<0.05), ^{A,B,C}: Different superscripts in the same column indicate significant differences (P<0.05)
^{a,b}: Aynı satırda farklı harf taşıyan değerler istatistiki açıdan farklıdır (P<0.05), ^{A,B,C}: Aynı sütunda farklı harf taşıyan değerler istatistiki açıdan farklıdır (P<0.05)

Table 2. *The correlation among the plasma levels of β-carotene (μg/dl), vitamin A (μg/dl) and vitamin C (μg/ml) with progesterone (ng/ml) levels at the time of AI, 3, 12 and 21 days after AI in pregnant and nonpregnant cows*

Tablo 2. *Gebe ve gebe olmayan ineklerde tohumlama, tohumlamadan 3, 12 ve 21 gün sonra plazma β-karoten (μg/dl), vitamin A (μg/dl) ve vitamin C (μg/ml) seviyeleri ile progesteron (ng/ml) seviyeleri arasındaki korelasyon*

Vitamin Levels	Days	Pregnant Cows (n=81)	Nonpregnant Cows (n=20)
β-Carotene (μg/dl)	0	-0.277 *	-0.285
	3	-0.312 **	-0.061
	12	0.154	0.885
	21	0.091	0.140
Vitamin A (μg/dl)	0	-0.052	-0.191
	3	-0.094	0.147
	12	-0.215	-0.933
	21	0.059	0.502
Vitamin C (μg/ml)	0	0.153	0.190
	3	0.045	0.426
	12	0.160	0.501
	21	0.153	-0.252

** (P<0.05), ** (P<0.01)*

The correlation among the plasma levels of β-carotene, vitamin A and vitamin C with progesterone levels at the time of AI, 3, 12 and 21 days after AI in pregnant and nonpregnant cows are shown in *Table 2*. There was a negative correlation between the plasma levels of β-carotene and progesterone at the time of AI (P<0.05) and 3 days after AI (P<0.01) in pregnant cows.

The correlation between the levels of β-carotene, vitamin A, vitamin C and progesterone during the cycle in pregnant and nonpregnant cows are shown in *Table 3*. There was a negative correlation between the plasma levels of progesterone and β-carotene (P<0.01) and progesterone and vitamin A (P<0.05) in pregnant cows, and a positive correlation between the plasma levels of β-carotene and vitamin A in both pregnant and non-pregnant cows (P<0.01).

The β-carotene concentrations of plasma were found to be variable, depending on the stages of the oestrus cycle, in this study. These findings were in agreement with the findings of others [5,34] who reported variable β-carotene concentrations of plasma during oestrus cycle.

The overall mechanism of β-carotene is not clearly understood and there are still controversies about its effect on reproduction [5]. While there are some reports that points out β-carotene has a possitive effect [32,35,36], there are also reports indicating it has no [17,37] or negative [28] effect on reproductive parameters in cows. In a study [37], β-carotene serum concentrations were not related to the incidence of retained placental fetal membranes, endometritis, ovarian cysts or the onset of cyclicity post partum, and the authors suggest that there is only a minor relationship between the β-carotene serum concentration and fertility in dairy cows. While Gossen et al.[17] do not recommend β-carotene supplementation for therapy or prophylaxis of fertility disorders, Iwańska and Strusińska [35] reported that the number of inseminations per cow was reduced and the conception rate was significantly higher in cows supplied additionally with β-carotene. In this study, there was a negative correlation between plasma β-carotene and progesterone levels at the time of and 3 days after AI while there was no correlation 12 and 21 days after AI in pregnant cows. Because we could not find any literature evaluating correlation between plasma β-carotene and progesterone levels at the time of and 3, 12 and 21 days after AI, we could not discuss our results with other studies. However, in contrast to Graves-Hoagland et al.[32] who reported a possitive correlation between plasma β-carotene and progesterone levels in dairy cows, our findings are in accord with the results of Yıldız et al.[28] who emphasized a negative correlation between serum progesterone and β-carotene levels in the pregnant

Table 3. *The correlation between the levels of β-carotene (µg/dl), vitamin A (µg/dl), vitamin C (µg/ml) and progesterone (ng/ml) during the cycle in pregnant and nonpregnant cows*

Tablo 3. *Gebe ve gebe olmayan ineklerde siklus sırasında plazma β-karoten (µg/dl), vitamin A (µg/dl), vitamin C (µg/ml) ve progesteron (ng/ml) seviyeleri arasındaki korelasyon*

Animal	Vitamin Levels	β-Carotene	Vitamin A	Vitamin C	Progesterone
Pregnant Cows	β-carotene	1.000			
	Vitamin A	0.393 **	1.000		
	Vitamin C	-0.089	-0.091	1.000	
	Progesterone	-0.281 **	-0.136 *	0.013	1.000
Nonpregnant Cows	β-carotene	1.000			
	Vitamin A	0.462 **	1.000		
	Vitamin C	-0.104	0.023	1.000	
	Progesterone	0.164	-0.105	-0.046	1.000

*** (P<0.05), **** (P<0.01)*
All data obtained at the time of AI, 3, 12 and 21 days after AI were evaluated
Tohumlama sırasında ve tohumlamadan 3, 12 ve 21 gün sonra elde edilen bütün veriler değerlendirilmiştir

DISCUSSION

In this study, plasma β-carotene levels were higher in pregnant cows than that in nonpregnant cows at the time of and 3 and 21 days after AI. These results may show that the level of plasma β-carotene had an effect on fertility. Similar to the research, Aslan et al.[6] reported higher plasma β-carotene levels in pregnant cows than that in nonpregnant cows. On the other hand, plasma β-carotene levels obtained in this study were lower than that obtained by Graves-Hoagland et al.[32] in nonpregnant cows.

One of the possible role of the β-carotene is its antioxidant effect as reported by Arechiga et al.[16]. Moreover, Arechiga et al.[33] reported higher fertility rates in β-carotene supplemented cows in another study. In the present study, β-carotene might have an antioxidant effect and, high β-carotene levels might show its antioxidant effect in pregnant cows.

cows during the oestrus cycle and, no correlation between these parameters in the nonpregnant cows. Differences between various studies may be due to the season and the characteristics of the animals like age, breed, nutrition and lactation status that have effects on plasma β-carotene and progesterone levels of cows [4,10,14,32].

It is reported that vitamin A is necessary for efficient steroid production. It might have a role in progesterone secretion by the corpus luteum and, its deficiency is detrimental to reproductive performance [11]. However, there are some controversies about vitamin A for its effect on reproduction. Moreover, the overall mechanisms of the vitamin A action in fertility is not yet clearly understood [18,19]. Several investigators failed to demonstrate positive effects of vitamin A in cattle [4,11,15,32] whereas other investigators have confirmed a beneficial effect of vitamin A on fertility [3,9,38]. In this study, the mean vitamin A levels of plasma were in agreement with the findings of Haliloğlu et al.[5]. There was no significant difference between pregnant and nonpregnant cows for plasma vitamin A levels at the time of and 3 and 12 days after AI, and there was only a difference 21 days after AI.

It is claimed that there is a negative correlation between serum progesterone and vitamin A levels during the cycle in cows [32]. Yıldız et al.[28] emphasized a negative correlation between progesterone and vitamin A levels in the pregnant cows during the oestrus cycle and, no correlation between these parameters in the nonpregnant cows. Similarly to Graves-Hoagland et al.[32] and Yıldız et al.[28], there was a negative correlation between progesterone and vitamin A levels in the pregnant cows and, no correlation between these parameters in the nonpregnant cows in this study.

β-carotene is the provitamin of vitamin A [11]. The present study showed that the level of vitamin A changed significantly with the stage of the oestrus cycle in a manner similar to changes in β-carotene in pregnant and nonpregnant cows. In addition, there was a positive correlation between β-carotene and vitamin A in both pregnant and nonpregnant cows. Johnston and Chew [39] also determined a significant correlation between plasma β-carotene and vitamin A levels during the cycle in cows.

In cows, oxidative stress has a negative impact on reproductive functions like decreased fertility and increased embryo mortality [40]. The fertilization rate after the cow has been bred is said to be about 90%, whereas the average calving rate may be some way below 50%. Much of this loss is due to embryonic mortality between 1 and 3 weeks [41], especially between day 15 and day 18 [43] after breeding. Embryo is said to be very sensitive to oxidative stress in this early stage [40,42]. In addition to this, vitamin C is shown to be an antioxidant [43] and it can reduce the effects of free radicals produced via oxidative stress [44]. In our study, plasma vitamin C levels were higher at metoestrus and dioestrus in pregnant cows than that in nonpregnant cows and also, it might have acted as an antioxidant in this early embryonic term. On the other hand, there was no correlation between vitamin C and plasma progesterone levels and, this data was consistent with Serpek et al.[27] who reported no correlation between vitamin C and plasma progesterone at various oestrus stages in Holstein cows.

In conclusion, the data presented in this study showed that there are differences in the levels of β-carotene, vitamin A and vitamin C in pregnant, and β-carotene and vitamin A in nonpregnant cows with the stages of the oestrus cycle. In addition to this, there is a negative correlation between the plasma levels of progesterone and β-carotene and progesterone and vitamin A in pregnant cows, and a positive correlation between the plasma levels of β-carotene and vitamin A in both pregnant and nonpregnant cows during the cycle. However, in spite of the results of this study, more research is needed evaluating the relationship between these parameters and their effects on bovine reproduction.

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