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Influence of β-carotene and vitamin A supplementation on the ovarian activity of dairy cows with chronic fertility impairment

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ABSTRACT

The aim of this study was to determine the influence of supplemented β-carotene and vitamin A on the ovarian activity during the periovulatory period in dairy cows with chronic fertility impairment. A total of 46 non pregnant Holstein cows with fertility impairment, at the age of 5-7 years, were selected for this study. The animals in three experimental groups were supplemented with either a combination of β-carotene and vitamin A, β-carotene only or vitamin A only. Non supplemented animals served as controls. All animals included in the survey were gynecologically examined; the estrus was synchronized and inseminated. Initial blood levels of β-carotene as well as the levels at the time of insemination and in the luteal phase as well as the ovarian structures during ovulation and formation of corpus luteum and P_4 and conception rate, were monitored. Significant increases in follicular size as well as luteal tissue growth were found in animals supplemented with β-carotene and vitamin A combination and vitamin A only. Positive correlation between the serum concentrations of β-carotene at insemination and at day 7 of the luteal phase and the concentrations of progesterone and the conception rate, lead to a conclusion that periovulatory β-carotene and and vitamin A supplementation could improve follicular growth and development of the corpora lutea in cows with fertility impairement.

Key words: β-carotene, vitamin A, infertility, dairy cows

Introduction

Reproductive efficiency is a major component of economic success in dairy herds. It is reported that plasma concentrations of β-caroten and vitamin A have a beneficial effect on reproduction (ASLAN et al., 1998; GRAVES-HOAGLAND et al., 1988; HALILOĞLU et al., 2002). β-Carotene and vitamin A deficiency resulted in extended duration of oestrus,

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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 (PETHES et al., 1985). JUKOLA et al. (1996) reported decrease of the external signs of estrus and fertility in cows with β-carotene defficiency. In contrast, there are reports that the conception rate and plasma progesterone concentration were not influenced by β-carotene injection (ARÉCHIGA et al., 1998a; GOSSEN and HOEDEMAKER, 2005). LOTTHAMMER et al. (1976), which concluded that only in extreme situations β-carotene deficit, as an independent factor, could cause fertility problems.

Regardless of the axiomatic importance of vitamin A in reproductive performance (WEISS, 1998; SCHWEIGERT, 2003), there are still some controversies. ASLAN et al. (1998) reports on the beneficial effect of vitamin A on fertility in cows, however, JUKOLA et al. (1996) observed no significant association between concentration of vitamin A in serum and fertility disorders or success of first insemination. Moreover, HEMKEN and BREMEL (1982) recommended clarifying the role of vitamin A in dairy cow fertility before investigating the role of β-carotene.

Finally, De ONDARZA and ENGSTROM (2009), analyzing the results of other authors, conclude that β-carotene supplementation has beneficial effect on reproduction only in cases when cows receive sufficient amount of vitamin A. The aim of this study was to determine the role of β carotene and vitamin A supplementation in the periovulatory period, on follicle and corpus luteum development in cows with chronic functional infertility.

Materials and methods

A total of 46 non pregnant Holstein cows, aged 5-7 years, were used in this study in the period of February-April. The animals were selected randomly from one farm according to three criteria: a) non pregnant; b) more than one year open; c) more than 4 unsuccessful inseminations. The cows were fed with the same feed (a ration composed of alfalfa hay and a concentrate-mineral mix and ad libitum access to fresh water) and housed in a tie-stall confinement facility.

According to the treatment, the cows were divided in three experimental and one control group. At the time of estrus induction, using $PGF_2\alpha$ analogue, the cows in Group 1 received combination of 200 mg β carotene (Carofertin®, Alvetra GmbH, Neumünster, Germany) and 50 000 I.U. vitamin A (Oligovit, KELA Laboratoria N.V., Hoogstraten, Belgium), Group 2 received 200 mg β carotene and Group 3 received 50 000 I.U. vitamin A and mineral supplements. Control animals in the same period received injection of sterile saline. The ovarian structures were visualized and measured by ultrasound scanner Aloka 500 Micrus (Japan) equipped with 7.5 MHz linear endorectal probe at day 0 (initial exam and estrus induction), day 4 (ovulation time and A.I.), day 11 (7 days after A.I.) and at day 30 (early pregnancy) when the presence of conceptus was expected. All animals were inseminated, after detection of estrus signs and a preovulatory follicle.

Blood samples were collected from the jugular vein into evacuated 10 mL tubes for analysis of serum β carotene and 10 mL tubes containing EDTA for the hormonal analysis, before application of β carotene and vitamin A and estrus induction (day 0), at A.I. (day 4) and 7 days after A.I. (day 11). Serum and plasma were prepared by centrifugation (5000 rpm for 5 minutes at 4 °C) and frozen at -20 °C within 2-4 h for subsequent determinations of β-carotene and estradiol and progesterone concentrations, respectively. The quantitative determination of plasma progesterone (P_4) and estradiol (E_2) levels was performed using by an enzyme immunoassay method Progesterone and Estradiol serozyme (Serono Diagnostic SA - Switzerland).

The serum β-carotene concentrations were analysed by HPLC, as reported by MILLER et al. (1984).

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 SAS 9, using generalized Least Square Model (GLSM) (KENNEDY, 1989). The statistical significance of the relevant factors was analyzed by the Least significant deviation test (LSD).

Results

Mean concentrations of β-carotene, progesterone and estradiol in the period at estrus synchronization, A.I. and at day 7 after A.I. of the cows in the experimental groups and the controls are shown Table 1.

Table 1. Mean concentrations of β-carotene, progesterone and estradiol in cows that received β-carotene and vitamin A supplementation in the periovulatory period.

Blood levels	Days	Group 1	Group 2	Group 3	Control
β-carotene µg/L	Day 0	1748.9	1710.6	1748.3	1681.1
	Day 4	2056.6 [A]	1981.8 [A]	1770.1 [B]	1714.7 [B]
	Day11	1981.5 [A]	1780.9 [A]	1670.4 [B]	1600.2 [B]
Estradiol pg/mL	Day 4	15.1 [A]	14.86 [A]	14.91 [A]	12.19 [B]
Progesterone ng/mL	Day11	5.78 [a]	4.03 [c]	4.94 [b]	3.53 [c]

A vs B = $P<0.05$; a vs c = $P<0.01$; b vs c = $P<0.05$

The blood levels of β-carotene before the induction of estrus did not differ significantly in all groups. β-carotene and vitamin A supplementation in groups 1 and 2, resulted with significant increase ($P<0.05$) of β-carotene blood levels at day 4 and day 11, compared with group 3 and the controls. Estradiol levels at ovulation, were significantly higher ($P<0.05$) in all experimental groups comparing to the controls. During the luteal phase, the cows in group 3 and group 1 have shown significantly higher progesterone levels ($P<0.05$ and $P<0.01$ respectively) compared with the cows in group 2 and the controls.

The presence of follicles and their size, at ovulation time (day 4), as well as the size of functional corpora lutea during the luteal phase (day 11), were measured during ultrasonic examination. The results are shown in Table 2.

Table 2. Mean diameter of follicles in the preovulatory period and functional corpora lutea in the luteal period in experimental groups and the controls

	Group 1	Group 2	Group 3	Controls
Mean diameter of follicles (mm) ± SEM	17.85 ± 0.42^{a}	17.18 ± 0.73^{a}	17.15 ± 0.56^{a}	16.7 ± 0.31^{a}
Mean diameter of C.L. (mm) ± SEM	26.15 ± 1.29^{a}	23.71 ± 0.75^{b}	27.61 ± 1.95^{a}	22.24 ± 0.74^{b}

Significant difference between values with different superscript ($P<0.05$)

Significant increase ($P<0.05$) of preovulatory follicle size was found in groups 1, 2 and 3 comparing with the controls. On the other hand, the cows in groups 1 and 3 had significant increase of the luteal tissue ($P<0.05$), comparing with the cows in group 2 and the control cows. At day 30 after insemination, overall pregnancy rate was established as well as the pregnancy rates of each group of animals included in the experiment. The overall pregnancy rate was 46.7% and did not vary significantly from the overall pregnancy rate on the farm. However, significant differences were found between the experimental groups and the controls. The results are shown in Fig. 1.

Statistical analysis of the data revealed significant differences ($P<0.01$) between groups 1 and 3 (66.7% and 70% respectively) and the control group (13%).

The interrelations between the parameters obtained in the investigation were analyzed using the Pearson's correlation analysis. The results are shown in Table 3.

The correlation analysis showed significant correlation between the initial serum concentration of β-carotene and the serum concentrations of β-carotene at insemination and at day 7 of the luteal phase ($r = 0.4$; $P<0.01$ and $r = 0.36$; $P<0.05$ respectively). Additionally, correlation was found between the serum concentrations of β-carotene at insemination and the concentrations of Progesterone $r = 0.33$ ($P<0.05$) and the conception rate $r = 0.39$ ($P<0.01$), and the serum concentrations of β-carotene at day 7 of the luteal phase and the concentrations of Progesterone $r = 0.51$ ($P<0.01$).

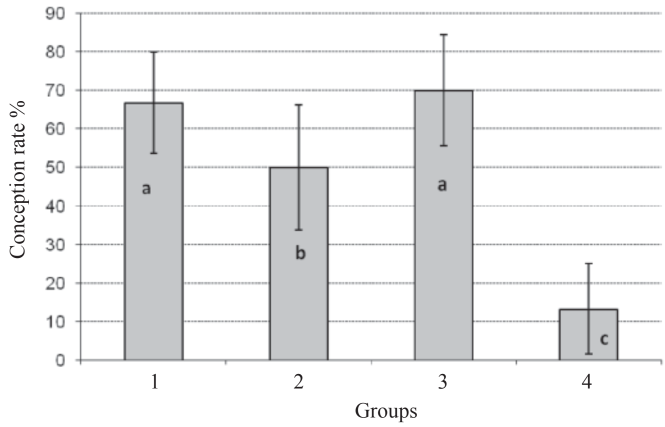

Fig. 1. Pregnancy rate of inseminated animals at day 30 after insemination.

a vs c = P<0.01; b vs c = P>0.05

Table 3. Pearson's correlation analysis of the parameters

	D0	D3	Fol	Status	E2	D11	CL	P4	D30
D0	1.00	0.40**	-0.33*	0.02	0.08	0.36*	0.25	0.26	0.29*
D3	0.40**	1.00	0.13	0.14	0.21	0.84**	0.06	0.33*	0.39**
Fol	-0.33*	0.13	1.00	0.74**	0.57**	0.19	0.18	0.34*	0.25
Status	0.02	0.14	0.74**	1.00	0.67**	0.20	0.30	0.43**	0.34*
E2	0.08	0.21	0.57**	0.67**	1.00	0.20	0.29	0.40**	0.35*
D11	0.36*	0.84**	0.19	0.20	0.20	1.00	0.26	0.51**	0.30*
CL	0.25	0.06	0.18	0.30	0.29	0.26	1.00	0.83**	0.54**
P4	0.26	0.33*	0.34*	0.43**	0.40**	0.51**	0.83**	1.00	0.78**
D30	0.29*	0.39**	0.25	0.34*	0.35*	0.30*	0.54**	0.78**	1.00

** - Statistical significance P<0.01; * - Statistical significance P<0.05; D0 - Initial serum concentration of β-carotene; D3 - Serum concentration of β-carotene at insemination; Fol - Diameter of preovulatory follicles: Status - Ovulation rate; E2 - Serum concentration of estradiol; D11 - Serum concentration of β-carotene at day 7 after insemination; CL - Diameter of functional Corpora lutea; P4 - Serum concentration of progesterone; D30 - Conception rate

Discussion

The initial analysis of β-carotene levels in cows with chronic fertility impairment have shown a mean value of 1718.25 ± 10.39 μg/L. This level was lower than the physiological levels (>2000 μg/L) in lactating dairy cows as reported by other authors (GRAVES-HOAGLAND et al., 1989; LOTTHAMMER, 1999; KATSOULOS et al., 2005) as well as by the Carofertin® manufacturer.

The intra-muscular application of 200 mg β-carotene resulted in an increase of the serum concentration to a mean level of 2000 μg/L at day 3 and a significant drop of 11% at day 11 after application in the group supplemented with β-carotene only, with positive correlation ($r=0.4$; $P<0.01$) between the initial β-carotene serum values and the β-carotene serum values at ovulation. The significant decrease of serum β-carotene levels may be a result of the transformation of the available β-carotene into vitamin A particularly in the uterus and ovaries, as proposed by SCHWEIGERT (2003).

On the other hand, significant differences were not found in the follicular diameter and the E_2 levels between the groups, nor a significant correlation was found between the β-carotene serum values and follicular dimension and E_2 levels (r = 0,12 and 0.2 respectively). Insufficient evidence could be found in the available literature on the positive influence of β-carotene on the follicular growth, with exception of KAWASHIMA et al. (2009), who reported a relationship between earlier resumption of ovarian cycle after parturition and lower number of cystic formation in cows with higher β-carotene serum values before parturition and CHEW et al. (1984) who reported positive correlation between serum and follicular concentrations of β-carotene in dairy cows.

Supplementation of β-carotene and vitamin A combination and vitamin A alone resulted in significant increases of corpora lutea size and serum progesterone ($P<0.05$ and $P<0.01$ respectively) compared to the supplementation of β-carotene only and the controls. Similar to the results of GRAVES-HOAGLAND et al. (1988), a significant positive correlation was found between the progesterone concentrations and the β-carotene levels at ovulation and during the luteal phase ($r = 0.33$; $P<0.05$ and $r = 0.51$; $P<0.01$, respectively). The similar results after supplementation of β-carotene and vitamin A, in our study could be explained with the similar effect on corpora lutea - enlargement of the luteal tissue and increased steroidogenesis (ZEROBIN, 1987; GRAVES-HOAGLAND et al., 1989).

β-Carotene and vitamin A supplementation had also significant ($P<0.01$) positive effect on the pregnancy rate, comparing to the controls, similar to the results of ARECHIGA et al. (1998b). who reported improvement of the conception by β-carotene supplementation in cows with prolonged service period (>120 days). IWAŃSKA and STRUSIŃSKA (1997). also reported positive influence of β-carotene and vitamin A supplementation on the conception rate. but on animals with normal reproductive status.

Analyzing the results of our study it is necessary to emphasize that the improvement of the reproductive functions. after β-carotene and vitamin A supplementation rest on the serious deficit in the organism. as concluded by other authors (LOTTHAMMER, 1979; BLOCK and FARMER. 1987; De KRUIF and MIJTEN, 1992). β-Carotene alone or as provitamin A has a significant role in the organism, however, it is necessary to clarify the overall mechanisms of the vitamin A action in fertility (SCHWEIGERT and ZUCKER, 1988; WEISS, 1998) before the discussion on the effects of β-carotene on fertility of dairy cows. Moreover, ACORDOR et al. (1986) concludes that β-carotene will not alleviate fertility impairment if the requirements for vitamin A are met.

Conclusion

The data presented in this study showed that sole supplementation of β-carotene could improve luteal tissue development as well as progesterone production only in animals with significant deficit of β-carotene. Moreover, vitamin A as a primary therapeutic agent in combination with β-carotene had stronger effect on the reproductive parameters than β-carotene alone. No effect on follicular growth and E_2 blood levels was observed after supplementation of β-carotene and vitamin A. The improvement of the reproductive performances in animals with chronically impaired fertility is mostly a result of the correction of the functional level of vitamin A by supplementation of either vitamin A or β-carotene. However, in spite of the results of this study, more research is needed to evaluate of the relationship between these parameters and their effects on bovine reproduction.

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SAŽETAK

Cilj ovog istraživanja bio je utvrditi utjecaj dodanog β-karotena i vitamina A na ovarijalnu aktivnost tijekom periovulatornog razdoblja u mliječnih krava s kroničnim funkcionalnim sterilitetom. Za ovo istraživanje odabrano je 46 negravidnih krava holštajnske pasmine s funkcionalnim sterilitetom, u dobi od 5 do 7 godina. Životinje su podijeljene u tri pokusne skupine, s kombinacijom β-karotena i vitamina A, samo β-karotena i samo vitamina A. Kontrolna skupina bile su krave bez dopunske terapije. Svim životinjama na početku istraživanja izvršen je ginekološki pregled, sinkronizacija estrusa i umjetno osjemenjivanje. Mjerena je početna razina β-karotena u krvi, razina u vrijeme oplodnje i tijekom luteinske faze, zatim corpus luteum i razina P4, kao i postotak koncepcije. Značajan porast folikula kao i luteinskog tkiva zabilježen je kod životinja kojima smo dali kombinaciju β-karotena i vitamina A i samo vitamin A. Pozitivna korelacija između serumske koncentracije β-karotena tijekom oplodnje i 7. dana u luteinskoj fazi i koncentracije progesterona i stope oplodnje, upućuje na zaključak da periovulatorno davanje β-karotena i vitamina A može poboljšati folikularni rast i razvitak žutog tijela u krava s funkcionalnim sterilitetom.

Ključne riječi: β-karoten, vitamin A, neplodnost, mliječne krave