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Effect of β-carotene on Ovarium Functions and Ovsynch Success in Repeat Breeder Cows

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Summary

This study was carried out to determine the effect of β-carotene on ovarian function and pregnancy rates in repeat breeder cows. Twenty-six repeat breeder Holstein cows were randomly assigned into control (n=11) and carotene (n=15) groups. Cows were fed with diet in the form of total mixed ration (39.80% neutral detergent fiber and 16.20% crude protein of dry matter). Treatment cows received an intramuscular injection of β-carotene (1 mg/kg) simultaneously with the first GnRH injection. Largest follicle ($P<0.05$) and corpus luteum diameters ($P<0.01$) together with serum progesterone ($P<0.01$), β-carotene ($P<0.05$) and vitamin A ($P<0.01$) levels were all significantly higher in the carotene group on the day of PGF2α injection. At the second GnRH injection, largest follicle diameter ($P<0.01$), serum estradiol ($P<0.01$), β-carotene ($P<0.01$), and vitamin A ($P<0.01$) levels were again higher in the carotene group. Serum estradiol ($P<0.01$) and β-carotene ($P<0.05$) levels were still significantly higher in the carotene group at the time of AI. Ovulation response did not differ between groups after both first and second GnRH injection. The pregnancy rate was 27.2% in the control group, and 33.3% in the carotene group but this difference was not statistically significant. In conclusion, it was suggested that β-carotene did not have any significant effects on ovulation or pregnancy rates in ovsynch-treated repeat breeder cows; however it increased serum estradiol and progesterone levels due to increased follicle size and corpus luteum functionality, respectively.

Key words: *Repeat Breeding, Cow, Ovsynch, β-carotene, Ovary, Pregnancy*

Döl Tutmayan İneklerde β-karotenin Ovaryum Fonksiyonları ve Ovsynch Başarısına Etkisi

Özet

Çalışmada döl tutmayan ineklerde β-karotenin ovaryum fonksiyonları ve gebelik oranı üzerine etkisi araştırıldı. Toplam 26 baş Holstein inek, kontrol (n=11) ve karoten gruplarına (n=15) ayrıldı. İnekler tam yem (kuru maddede %39.80 nötral deterjan fiber ve %16.20 ham protein) rasyon ile beslendi. Karoten grubuna ilk GnRH enjeksiyonu ile aynı zamanda tek doz (1 mg/kg) β-karoten kas içi uygulandı. Prostaglandin enjeksiyon zamanında serum progesteron ($P<0.01$), β-karoten ($P<0.05$) ve vitamin A ($P<0.01$) düzeyleri ile birlikte en büyük follikül ($P<0.05$) ve korpus luteum çapı ($P<0.01$) karoten grubunda daha yüksek belirlendi. İkinci GnRH enjeksiyon zamanında da en büyük follikül çapı ($P<0.01$) ile serum östradiol ($P<0.01$), β-karoten ($P<0.01$), vitamin A ($P<0.01$) düzeyleri karoten grubunda daha yüksek gözlendi. Tohumlama zamanında serum östradiol ($P<0.01$) ve β-karoten ($P<0.05$) düzeyinin karoten grubunda yüksek olduğu tespit edildi. Grupların ilk ve 2.GnRH enjeksiyonları sonrasında ovulasyon oranları benzer bulundu. Gebelik oranı kontrol grubunda %27.2, karoten grubunda %33.3 olacak şekilde belirlendi, ancak farkın istatistiki önemde olmadığı gözlendi. Sonuç olarak, β-karotenin döl tutmayan ineklerde folliküllerin çapını etkileyerek plazma östrojen düzeyini artırdığı, korpus luteum çapında artış oluşturarak plazma progesteron düzeyini yükselttiği, ancak ovulasyon ve gebelik oranına etkisinin olmadığı kanısına varıldı.

Anahtar sözcükler: *Döl tutma, İnek, Ovsynch, β-karoten, Ovaryum, Gebelik*

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INTRODUCTION

Repeat breeding is one of the major reproductive problems affecting reproductive efficiency and the economy of milk production in dairy cows. Repeat breeding has been attributed to genetic predisposition, nutrition, hormonal involvement, gametic abnormalities, delayed ovulation, inadequate luteal function, infection, or managerial factors [1]. The causes of repeat breeding may originate either during the early stages of follicle maturation and/or during the preovulatory period [2,3]. Several therapies have been proposed to improve the conception rate in repeat breeder cattle, but some of these show limited success [4].

Beta-carotene plays pivotal roles as provitamin A, which is transformed into vitamin A in the intestinal wall, liver, and ovarian structures [5]. It was reported that the highest intrafollicular concentration of vitamin A was found in non-atretic follicles, and that this situation may be used as a physiological indicator of follicular quality and function in cattle [6]. Because of the blood–follicular barrier, vitamin A cannot penetrate to the follicle [7]. However, β-carotene is able to pass into follicular fluid, and its conversion to vitamin A occurs in the follicle via a controlled pathway. Hence, β-carotene is thought to play roles in cattle fertility and reproductive efficiency through mechanisms that are independent of vitamin A [8,9].

The bovine corpus luteum (CL) is very rich in β-carotene and contains about two to five times as much β-carotene as other tissues such as the liver, adipose tissue, and serum, even during times of deficiency. It has been postulated that this vitamin plays a role in luteal function of cattle [10,11]. A correlation between β-carotene levels, CL diameter, and serum progesterone level has been reported [12]. It was found that corpora lutea of heifers were less developed and cows had lower serum progesterone when dietary carotene was deficient [13]. This suggests that β-carotene may play a role in CL function, specifically in the synthesis of progesterone.

The necessity of β-carotene for reproduction has been demonstrated using cows that were fed for a long period without β-carotene [13]. Also, it was suggested that injectable β-carotene (Dalmavital®) administration may relatively increase the pregnancy rates during the postpartum period in fertil cows [14], but no studies have focused on the effects of injectable β-carotene in repeat breeding cows in the ovsynch protocol and subsequent ovulation and pregnancy rates. The aim of this study, therefore, was to evaluate the effects of injectable β-carotene on follicle and corpus luteum diameters, serum estradiol and progesterone levels during ovsynch, and ovulation and pregnancy rates after ovsynch in repeat breeder cows.

MATERIALS and METHODS

Animals, Diets and Treatments

This experiment was conducted on one commercial dairy herd (approximately 490 lactating Holstein dairy cows) located in Afyonkarahisar, Turkey. Twenty-six repeat breeder Holstein cows at the age of 3-6 years, and weighing 570±50 kg, and milk yield 27±3.0 kg/d were used. These animals (mean 167±10 d postpartum) had an average of 6.1 services. They presented good corporal condition and had calved at least once. The cows were milked two times a day and were housed in a free-stall facility.

Cows were fed with lactating dairy cow diet as a total mixed ration (TMR) following National Research Council (NRC)15 recommendations. The TMR consisted of 60% roughage, and 40% concentrate mix. Diet formulated at the form was offered twice daily and fresh water available ad libitum to each cow. The ingredient and nutrient composition of the diet is shown in *Table 1*.

Table 1. *Ingredient and nutrient composition of the diet*
Tablo 1. *Rasyon besin içeriği ve bileşenleri*

Ingredients	**% of dry matter**
Corn silage	45.10
Alfalfa hay	14.90
Grinded barley	4.00
Grinded corn	8.20
Solvent cottonseed meal	6.00
Expellers soybean meal	7.20
Whole cottonseed	8.00
Wheat bran	5.60
Dicalcium phosphate	0.10
Calcium carbonate	0.40
Salt	0.30
Vitamin-mineral premix*	0.20
Nutrient composition	**% of dry matter**
Crude protein	16.20
Crude fiber	18.75
Acid detergent fiber	25.65
Neutral detergent fiber	39.80
Calcium	0.72
Phosphorus	0.45
Net Energy Lactation,** Mcal/kg of dry matter	1.54

***** Vitamin-mineral premix provided the following per kilograms of diet: Vitamin A, 12000000 IU; Vitamin D_3, 3000000 IU; Vitamin E, 30 g; Mn, 50 g; Fe, 50 g; Zn, 50 g; Cu, 10 g; I, 0.8 g; Co, 0,1 g; Se, 0.15 g; Antioxidant, 10 g. ****** Calculated using NRC [14] values

At the start of the study, trans-rectal ultrasound to determine pregnancy was conducted with an ultrasound scanner (Falco 100, Pie Medical, Maastricht, The Netherlands) equipped with a 6-8 MHz linear-array transducer, performed after 32-35 days from last artificial insemination (AI) and nonpregnant animals were included study. The Ovsynch program was performed with 2 injections of GnRH (10µg Busereline acetate, Receptal®, Intervet Turkey, Istanbul, Turkey), 7 d before and 48 h after an injection of PGF2α (25 mg, Dinoprost tromethamine, Dinolytic®, Etkin, Istanbul, Turkey). Cows were randomly assigned to control (n=11) or β-carotene treatments (n=15). Once assigned to treatment, cows received an intramuscular injection of β-carotene (1 mg/kg, Dalmavital®, Vetaş, Istanbul, Turkey) simultaneously with the first GnRH injection. Cows were artificially inseminated 18 h after the second injection of GnRH. Ovaries of all cows were examined by transrectal ultrasonography in several planes to measure largest follicle and CL diameter at the times of GnRH and PGF2α injections (days 0, 7 and 9) and to confirm ovulation (days 1, 2 and 10, 11).

Ovulation was defined as the disappearance of a large follicle from an ovary after first and second GnRH administrations. Pregnancy diagnosis was made on days 32 to 35 by transrectal ultrasonography of the uterus.

Blood Sampling and RIA

Blood samples were collected by jugular venipuncture into 10-ml Vacutainer tubes on days 0, 7, 9, and at insemination. Blood was allowed to coagulate at room temperature and then centrifuged at 1.200 g for 30 min. Serum was harvested and stored at -20°C until analysis. Serum concentrations of estradiol and progesterone were analyzed in all samples by RIA (Immunotech Beckman Coulter Company, France). For estradiol measurements, intra- and interassay coefficients of variation were 10.1% and 11.3% respectively, while those for progesterone were 5.4% and 8.1%, respectively.

Analysis of β-carotene and Vitamin A

Serum β-carotene and vitamin A (retinol) concentrations were estimated by the method of Suzuki and Katoh [16] using a spectrophotometer. Peaks in the absorption spectrum corresponding to retinol at 325 nm and β-carotene at 453 nm were detected after the reaction of sample with an ethanol:hexane solution (1 to 1:3, respectively).

Statistical Analysis

Follicular and CL diameter, and serum estradiol, progesterone, vitamin A and β-carotene levels were compared by paired t-test between groups. Effect of treatment on ovulation and pregnancy rate was evaluated by chi-square analysis. All values are presented as mean±S.D. Group differences were declared significant at $P<0.05$.

RESULTS

Follicle diameter, CL diameter, and serum estradiol, progesterone, vitamin A, and β-carotene levels at first GnRH, PGF2α, and second GnRH injection times are shown figures 1, 2, 3 and 4, respectively. No significant differences were found among the groups for follicle and CL diameter or serum estradiol, progesterone, vitamin A, and β-carotene levels on the day of the first GnRH injection (day 0).

While largest follicle ($P<0.05$) and CL diameters ($P<0.01$) together with serum progesterone ($P<0.01$), β-carotene ($P<0.05$) and vitamin A ($P<0.01$) levels were significantly higher in the carotene group, serum estradiol did not differ between groups on the day of PGF2α injection.

On the day of the second GnRH injection, dominant follicle diameter ($P<0.01$), serum estradiol ($P<0.01$), β-carotene ($P<0.01$), and vitamin A ($P<0.01$) levels were higher in the carotene group. There were no differences in CL diameters or serum progesterone concentrations in the groups.

Follicle and CL diameters, and progesterone and vitamin A serum levels, were the same between groups at the time of AI, but serum estradiol ($P<0.01$) and β-carotene ($P<0.05$) levels were still significantly higher in the carotene group. Evaluation of preovulatory follicle diameter at AI indicated that the size of follicles in the carotene group was more narrowly distributed than control follicles. While the minimum and maximum follicle diameters in the carotene group were 1.61 and 2.17 cm, respectively, they were 1.27 and 2.31 cm in the control group.

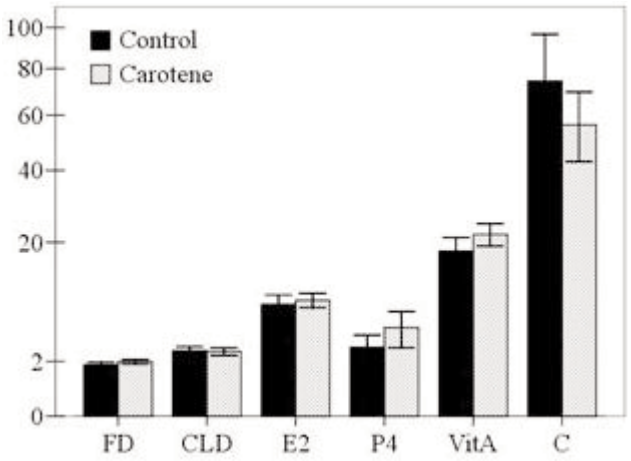

Fig 1. Follicle and CL diameters (cm), and serum estradiol (pg/ml), progesterone (ng/ml), vitamin A (µg/dL), and β-carotene (µg/dL) levels at first GnRH injection time
Şekil 1. Birinci GnRH enjeksiyon zamanında follikül, korpus luteum çapı ve serum östradiol, progesteron, vitamin A ve β-karoten düzeyleri

FD: Follicle diameter, CLD: Corpus luteum diameter, E2: Estradiol, P4: Progesterone, Vit A: Vitamin A, C: β-carotene

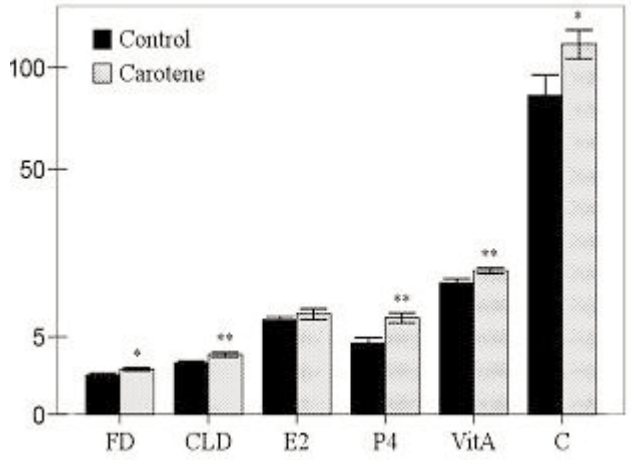

Fig 2. Follicle and CL diameters (cm), and serum estradiol (pg/ml), progesterone (ng/ml), vitamin A (µg/dL), and β-carotene (µg/dL) levels at PGF2α injection time
Şekil 2. PGF2α enjeksiyon zamanında follikül, korpus luteum çapı ve serum östradiol, progesteron, vitamin A ve β-karoten düzeyleri

FD: Follicle diameter, CLD: Corpus luteum diameter, E2: Estradiol, P4: Progesterone, Vit A: Vitamin A, C: β-carotene
* P<0.05. Differences between follicle diameter and serum β-carotene level in groups
** P<0.01. Differences among CL diameter, and serum progesterone and vitamin A levels in groups

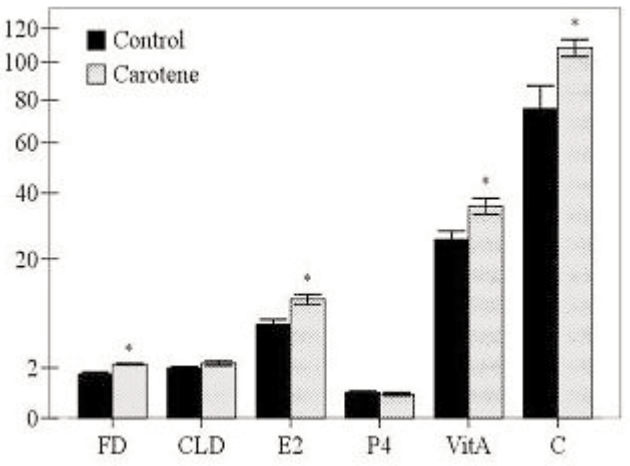

Fig 3. Follicle and CL diameters (cm), and serum estradiol (pg/ml), progesterone (ng/ml), vitamin A (µg/dL), and β-carotene (µg/dL) levels at second GnRH injection time
Şekil 3. İkinci GnRH enjeksiyon zamanında follikül, korpus luteum çapı ve serum östradiol, progesteron, vitamin A ve β-karoten düzeyleri

FD: Follicle diameter, CLD: Corpus luteum diameter, E2: Estradiol, P4: Progesterone, Vit A: Vitamin A, C: β-carotene
* P<0.01. Differences among follicle diameter, and serum estradiol, vitamin A, and β-carotene levels in groups

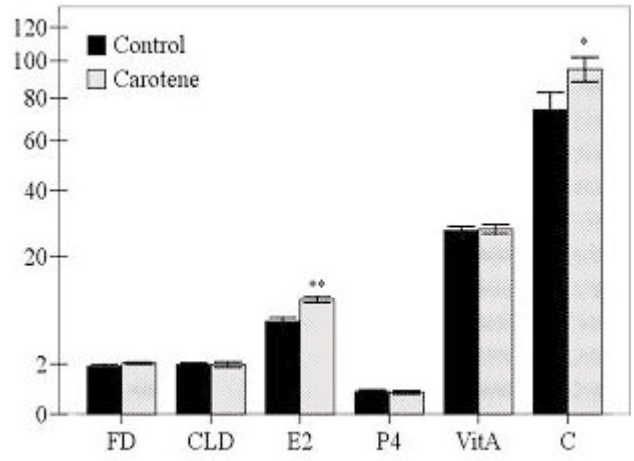

Fig 4. Follicle and CL diameters (cm), and serum estradiol (pg/ml), progesterone (ng/ml), vitamin A (µg/dL), and β-carotene (µg/dL) levels at insemination time
Şekil 4. Tohumlama zamanında follikül, korpus luteum çapı ve serum östradiol, progesteron, vitamin A ve β-karoten düzeyleri

FD: Follicle diameter, CLD: Corpus luteum diameter, E2: Estradiol, P4: Progesterone, Vit A: Vitamin A, C: β-carotene
* P<0.05. Differences in serum β-carotene level between groups
** P<0.01. Differences in serum estradiol level between groups

Mucous vaginal discharge was observed in all animals (100%) of the carotene group, but only in two animals (18.18%) of control group, during rectal examination at insemination.

It was observed that 60% and 63.6% of the animals ovulated after the first GnRH injection in the carotene and control groups, respectively. Ovulation rates after the second GnRH injection increased in the carotene group, but decreased in the control group. However, the ovulation response did not differ between groups after both first and second GnRH injection.

The pregnancy rate after AI was 27.2% (3 to 11) in the control group, and 33.3% (5 to 15) in the carotene group. Although the pregnancy rate in the carotene group was higher, the difference was not statistically significant.

DISCUSSION

This study was designed to determine the effect of intramuscular injection of β-carotene on ovsynch success in repeat breeder Holstein cows. We observed changes in follicular and CL diameters, as well as in serum estradiol and progesterone levels. Serum β-carotene concentrations were found to be similar between groups at the beginning of ovsynch (time of first GnRH application), but they were significantly higher at later sampling times in the carotene group and remained elevated throughout the study. This supports a study which reported that intramuscular injection of β-carotene increased plasma β-carotene concentrations at 24 h after injection, with the concentrations remained elevated for 13 days [17].

Carotene cleavage activity has been demonstrated in bovine ovarian follicles where enzyme activity was highly dependent on the quality of the follicles. The highest follicular cleavage activity was found in large, preovulatory follicles. These results indicate that a local conversion of β-carotene into vitamin A in follicular structures is responsible for increased intrafollicular concentrations of vitamin A during follicular development [8]. Vitamin A in follicular fluid closely correlates with the quality of the follicle and with the intrafollicular concentration of estradiol. As in follicular fluid, plasma vitamin A levels increase at proestrous and estrous stages during which the follicular activity is high [12]. Elevated vitamin A levels were observed in this study at administration of PGF2α and the second GnRH injection. The reason for the larger follicle diameters in the carotene group at PGF2α and second GnRH injections may be due directly to β-carotene or indirectly through elevated vitamin A in the follicle by local conversion of β-carotene into vitamin A.

It was observed that differences between follicle diameter of the carotene and control groups were consistent with differences in serum estradiol levels. This is compatible with previous studies which found that plasma hormone levels during the estrous cycle of the cow relate to periods of accelerated follicle growth and differ depending on follicle diameter at various cycle stages [18].

Previous studies have reported increased plasma vitamin A levels in the proestrus and estrus stages, during which follicular activity is high, and that estradiol causes increased plasma vitamin A levels [19]. It was reported that in target cells, vitamin A may be bound by a cellular retinol binding protein that is thought to act by a mechanism similar to that of steroid hormone-receptor complexes, altering gene expression in the nucleus and thus affect follicular development [20]. In the present the study, the high vitamin A levels of the carotene group at the PGF2α and second GnRH injection times may be due to a positive effect of estradiol on vitamin A synthesis.

Corpus luteum β-carotene concentrations increase during diestrus when there is maximal luteal function [12], which is consistent with the findings of Rapaport et al.[21]. According to these studies, β-carotene may play a very important role in regulating luteal functions in the cow. The CL is a steroidogenic tissue that is very rich in β-carotene. The high concentrations of β-carotene found in the CL have led many investigators [10,11,22-24], to postulate the role of this vitamin in the luteal function of cattle. The bovine CL contains about two to five times as much β-carotene as other tissues such as the liver, adipose tissue, and plasma, even when deficiency occurs [22]. In addition, the CL grows slowly and thus has small diameters in β-carotene deficient heifers, but after deficiency disappears there is a significant increase in progesterone [10]. A positive correlation between the plasma β-carotene concentration and CL size has been reported previously. In the present study, we observed that CL diameter increased in the carotene group after administration of β-carotene (day 7), but there were no changes in CL size in control during the same period.

Aslan et al.[10] suggested that CL β-carotene levels are also correlated with CL diameter and plasma progesterone level in cattle. There is increasing evidence that β-carotene may be necessary for optimal progesterone production. According to Ahlswede and Lotthammer [22], cows

fed diets deficient in β-carotene have lower amounts of progesterone in the CL. Beta-carotene may serve to protect the CL from structural and functional damage due to reactive oxygen species and thereby support optimal steroidogenic activity. Indeed, it was demonstrated that β-carotene stimulated progesterone production in bovine luteal cells in vitro [25]. In the present study, we observed that progesterone levels increased in parallel with CL diameter growth in the carotene group, which is compatible with these previous studies.

Luteal regression was induced by PGF2α injection in the present ovsynch protocol. Pursley et al.[26] reported that the first injection of GnRH causes ovulation or luteinization of any functional largest follicles, depending on the developmental stage of the dominant follicle present in the ovary and progesterone concentration, and if ovulation occurs from this initial GnRH injection, the newly induced CL or present CL appears to be responsive to PGF2α. Decreases in both CL diameter and serum progesterone in the present study indicate that luteal tissue responds both morphologically and functionally to the luteolitic effects of PGF2α.

The ability of ovsynch protocols to effectively synchronize is dependent upon the stage of follicular development at the time of initial GnRH injection [27], which represents a major limitation to their effectiveness. It was observed that cows may display standing estrus prematurely and have variable ovulation periods [28]. Because estrus observation was not performed after PGF2α injection, we did not assess the number and rate of early estrus-displaying cows, but mucus discharge from the vulva, which is an estrus symptom, was observed at rectal examination during insemination. The physical properties of cervical mucus are important in reproductive physiology, because the thin mucus secreted during estrus is favourable for sperm migration and aligns the direction of sperm migration. In contrast, the thick mucus secreted during pregnancy blocks sperm migration and seals the cervix, thus preventing microorganisms from entering the uterine cavity [29]. Glycoproteins make up much of the chemical composition of cervical mucus, and glycoprotein synthesis requires adequate vitamin A [30]. In the present study, mucus discharge was identified at higher rate in the carotene group, which may be due to higher vitamin A levels, as vitamin A levels were higher in this group prior to insemination. Thus, the higher vaginal mucus may be due to a role of vitamin A in glycoprotein synthesis.

The ovsynch protocol was developed to control the time to first and subsequent AI, thus maximizing service rate and improving overall estrus detection rate. This protocol synchronizes ovulation within a precise time after the second injection of GnRH. Precise synchrony may allow for successful AI without the detection of estrus [26]. The first injection of GnRH is intended to cause ovulation of any functional follicles present in the ovary and induce subsequent emergence of a new follicular wave, but it is reported that some cows do not respond to the first GnRH treatment [31]. Similarly to the study by Bello et al.[31], ovulation was not identified in some animals in our groups. Several features have been suggested as possible indicators of follicular maturational status before ovulation, namely, follicle size [27], follicle life span and duration of dominance [32,33], and circulating concentrations of reproductive hormones, such as estradiol and progesterone [34,35].

Despite Ovsynch's overall positive impact, variable rates of ovsynch treated cows fail to synchronize ovulation in response to final GnRH [27]. The response to the final GnRH treatment in our carotene group was similar to that observed in previous studies, but it was lower in the control group. The reason for the lower ovulation rates in the control group may be due to characteristics of the repeat breeder animals used in our study. Repeat breeder animals may be described as a heterogeneous group of subfertile animals that might exhibit various disturbances in follicular development during the periovulatory period [3]. It has also been reported that ovulatory follicle diameter in repeat breeder cows is highly variable. While some cows present very large follicular diameters (2.5 cm), relatively small values (1.2 cm) have been recorded in others [36]. In the present study, changes in follicle diameter between the second GnRH injection and insemination suggest that follicles continued to grow in the control group. However, there were no changes during the same time in the carotene group. For this reason, we speculate that β-carotene may provide for follicular homogeneity.

Programs based on GnRH and PGF2α allow

precise scheduling of AI while eliminating estrus detection. The hypothesis was that AI at observed estrus could improve conception rates after synchronization of estrus compared with a timed insemination protocol [28]. Pregnancy rates of 24 to 39 percent are achieved in dairy cows using the ovsynch protocol. We found lower pregnancy rates in control than the carotene group, in agreement with the study of Kasimanickam et al.[28], which also used repeat breeder cows. There was not statistical significance on pregnancy rate same as study of Kaçar et al.[14] that used if β-carotene. So, further studies, using a higher number of cows, are necessary to confirm if β-carotene can increased consistently pregnancy rate.

In conclusion, the data presented here suggest that while carotene did not have any significant effects on ovulation or pregnancy rates in ovsynch-treated repeat breeder Holstein cows, it increased serum estradiol and progesterone levels by affecting follicle size and supporting CL functionality.

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REFERENCES

1. **Selvaraju S, Agarwal SK, Karche SD, Srivastava SK, Majumdar AC, Shanker U:** Fertility responses and hormonal profiles in repeat breeding cows treated with insulin. *Anim Reprod Sci,* 73,141–149, 2002.
2. **Peters AR:** Embryonic mortality in the cow. *Anim Breed Abstr,* 64, 587–598, 1996.
3. **Bage R, Gustafsson H, Larsson B, Forsberg M, Rodriguez-Martinez H:** Repeat breeding in dairy heifers: follicular dynamics and oestrous cycle characteristics in relation to sexual hormone patterns. *Theriogenology,* 5, 2257-2269, 2002.
4. **Goley RR, Kadu MS:** Efficacy of prostaglandin F2 , Lutalyse, GnRH analogue, Receptal and hCG, Chorulon in treatment of repeat breeder cows. *Indian Vet J,* 72, 472–475, 1995.
5. **Weiss WP:** Requirements of fat-soluble vitamins for dairy cows: A review. *J Dairy Sci,* 81, 2493–2501, 1998.
6. **Schweigert FJ, Lutterbach A, Rambeck WA, Zucker H:** Vitamin A and β-carotene concentrations in bovine follicular fluid in relationship to follicle size. *J Vet Med A,* 33, 360, 1986.
7. **Perret BP, Parinaud J, Ribbes H, Moatti JP, Pontonnier G, Chap H, Douste-Blazy L:** Lipoprotein and phospholipid distribution in human follicular fluid. *Fert Steril,* 43, 405–409, 1985.
8. **Schweigert FJ, Zucker H:** Concentrations of vitamin A, beta-carotene and vitamin E in individual bovine follicles of different quality. *J Reprod Fertil,* 82, 575–579, 1988.
9. **Brief S, Chew BP:** Effects of vitamin A and β-carotene on reproductive performance in gilts. *J Anim Sci,* 60, 998–1004, 1985.
10. **Aslan S, Handler J, Arbeiter K:** Frühgraviditat und embryonale bzw. frühfetale Mortalität bei der Kuh-Gelbkörperdynamik, Progesteron-, Vitamin E-, Vitamin B12-, β-carotin- und Folsäurekonzentrationen im peripheren Blut. *Wien Tierarztl Mschr,* 85, 141–147 1998.
11. **Arikan S, Rodway RG:** Seasonal variation in bovine luteal concentrations of beta-carotene. *Turk J Vet Anim Sci*, 25, 165-168, 2001.
12. **Haliloglu S, Baspinar N, Serpek B, Erdem H, Bulut Z:** Vitamin A and β-carotene levels in plasma, corpus luteum and follicular fluid of cyclic and pregnant cattle. *Reprod Dom Anim,* 37, 96–99, 2002.
13. **Lotthammer KH:** Importance of β-carotene for the fertility of dairy cattle. *Feedstuffs,* 51, 6, 1979.
14. **Kaçar C, Kamiloğlu NN, Uçar Ö, Arı UÇ, Pancarcı ŞM, Güngör Ö:** İneklerde β-karoten + E vitamini uygulamasıyla kombine edilen ovsynch ve cosynch senkronizasyon programlarının gebelik oranı üzerine etkisi. *Kafkas Üniv Vet Fak Derg,* 14 (1): 45-50, 2008.
15. **National Research Council:** Nutrient requirements of dairy cattle. Seventh Revised Edition. National Academic Press, Washington, DC.,USA, 2001.
16. **Suziki J, Katoh N:** A simple and cheap methods for measuring serum vitamin A in cattle using only a spectro-photometer. *Jpn J Vet Sci,* 52, 1281-1283, 1990.
17. **Arechiga CF, Vazquez-Flores S, Ortiz O, Hernandez-Ceron J, Porras A, McDowell LR, Hansen PJ:** Effect of injection of β-carotene or vitamin E and selenium on fertility of lactating dairy cows. *Theriogenology*, 50, 65-76, 1998.
18. **Kaneko H, Watanabe G, Taya K, Sasamoto S, Hasegawa Y:** Changes in plasma concentration of immunoreactive inhibin, estradiol and FSH associated with follicular waves during the estrous cycle of the cow. *J Reprod Dev,* 41, 311-320, 1995.
19. **Maenpaa PH, Pirhonen A, Koskinen E:** Vitamin A, E and D nutrition in mares and foals during the winter seasons; effects of feeding two different vitamin-mineral concentrate. *J Anim Sci,* 66, 1424–1429, 1988.
20. **Brown JA, Eberhardt DM, Schrick FN, Roberts MP, Godkin JD:** Expression of retinol binding protein and cellular retinol-binding protein in the bovine ovary. *Mol Reprod Dev,* 64, 261–269, 2003.
21. **Rapaport R, Sklan D, Wolfenson D, Shaham-Albalancy A, Hanukoglu I:** Antioxidant capacity is correlated with steroidogenic status of the corpus luteum during the bovine estrous cycle. *Biochim Biophys Acta,* 1380, 133–140, 1998.
22. **Ahlswede L, Lotthammer KH:** Untersuchungen uber eine spezifische, vitamin A-unabhangige wirkung des β-carotins auf die fertilitat des rindes. 5. Mitt. Organunterschungen-Gewichts und Gehaltsbestimmungen. *Dtch Tierarztl Wochenschr,* 85, 7–12, 1978.
23. **Chew BP, Holpuch DM, O'Fallon JV:** Vitamin A and b-carotene in bovine and porcine plasma, liver, corpora lutea, and follicular fluid. *J Dairy Sci, 67, 1316–1322,* 1984.

24. **Graves-Hoagland RL, Hoagland TA, Woody CO:** Relationship of plasma β-carotene and vitamin A to luteal function in postpartum cattle. *J Dairy Sci,* 72, 1854–1858, 1989.
25. **Talavera F, Chew BP:** Comparative role of retinol, retinoic acid and β-carotene on progesterone secretion by pig corpus luteum in vitro. *J Reprod Fertil,* 82, 611–615, 1987.
26. **Pursley JR, Mee MO, Wiltbank MC:** Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology*, 44, 915-923, 1995.
27. **Vasconcelos JLM, Silcox RW, Rosa GLM, Pursley JR, Wiltbank MC:** Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization on different days of the estrus cycle in lactating dairy cows. *Theriogenology*, 52, 1067–1078, 1999.
28. **Kasimanickam R, Cornwell JR, Nebel RL:** Fertility following fixed-time AI or insemination at observed estrus in ovsynch and heatsynch programs in lactating dairy cows. *Theriogenology*, 63, 2550-2559, 2005.
29. **Roberts GP:** Structural on the gylicoproteins from bovine cervical mucus. *Biochem J,* 173, 941-947, 1978.
30. **Chan VT, Wolf G:** The role of vitamin A in the glycosylation reactions of glycoprotein synthesis in an in vitro system. *Biochem J,* 247, 53-62, 1987.
31. **Bello NM, Steibel JP, Pursley JR:** Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of ovsynch in lactating dairy cows. *J Dairy Sci,* 89, 3413-3424, 2006.
32. **Bleach E, Glencross CRG, Knight PG:** Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous oestrous cycles. *Reproduction*, 127, 621–629, 2004.
33. **Burns DS, Jimenez-Krassel F, Ireland J, Knight PG, Ireland JJ:** Numbers of antral follicles during follicular waves in cattle: Evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum-follicle-stimulating hormone concentrations. *Biol Reprod,* 73, 54–62, 2005.
34. **Sartori R, Haughian JM, Shaver RD, Rosa GJ, Wiltbank MC:** Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J Dairy Sci,* 87, 905–920, 2004.
35. **Souza AH, Gumen A, Silva EPB, Cunha AP, Guenther JN, Peto CM, Caraviello DZ, Wiltbank MC:** Supplementation with estradiol-17β before the last Gonadotropin-releasing hormone of the Ovsynch protocol in lactating dairy cows. *J Dairy Sci,* 90, 4623-34, 2007.
36. **Perez CC, Rodriguez I, Espana F, Dorado J, Hidalgo M, Sanz J:** Follicular growth patterns in repeat breeder cows. *Ved Med Czech,* 48, 1-8, 2003.